

# Patented HIV-1 Integrase Inhibitors (1998-2005)

Philippe Cotelle\*

Laboratoire de Chimie Organique et Macromoléculaire, UMR CNRS 8009, Université de Lille 1, 59655 Villeneuve d'Ascq, France

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**Abstract:** Combination therapy, comprising at least three anti-human immunodeficiency virus (anti-HIV) drugs, has become the standard treatment of AIDS. Since 1996, highly active antiretroviral therapy (HAART) was designed to rapidly control HIV replication. It has had a significant impact on patient health and progression of AIDS in developed countries but its success has not been complete. HAART strategy still suffers from issues of patient compliance, cost, deleterious side effects and emerging drug resistance. Therefore it is logical to look for agents that inhibit different viral targets. In addition to the fusion, reverse transcription and protein formation processes, the HIV replicative cycle offers various other events that can be considered as potential targets for chemotherapeutic intervention. Amongst them integration is a key step and integrase (IN), one of the three viral enzymes, has been rapidly identified as a rational target for many years. To date, four molecules have entered in clinical trials. The present article reviews the increasing number of patents on small molecule HIV-1 integrase inhibitors in the 1998-2005 period, from the pioneer ones (discovery of selective strand transfer inhibitors) to the last patents including the actual molecules under clinical trials.

**Keywords:** AIDS, HIV, Integrase inhibitors.

## INTRODUCTION

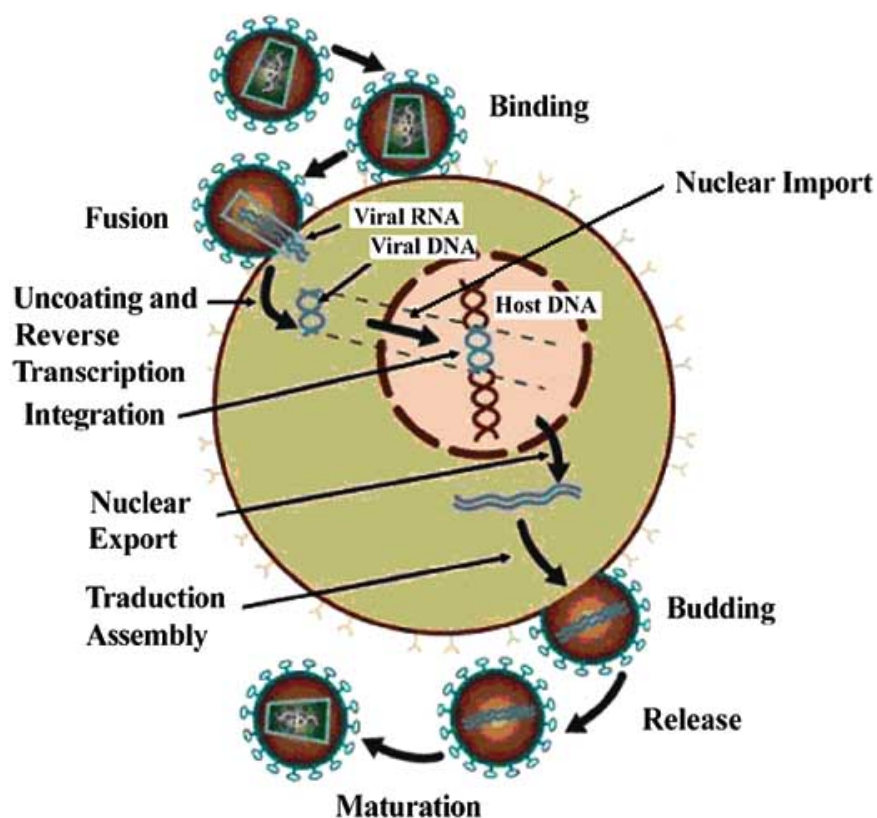
First reported in 1981 in a small number of patients, acquired immunodeficiency syndrome (AIDS) has become a major pandemic with more than 40 million people infected worldwide. Since the first clinical identifications, scientific and therapeutic progresses have been extraordinary. The replicative cycle of human immunodeficiency virus (HIV-1) (Fig. 1) is well-known and its knowledge has offered different targets that have led to spectacular therapeutic progress. Since the introduction of AZT in 1987, 21 drugs have been approved by FDA divided in four classes, i.e., nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs) and fusion inhibitors (Table 1). Development of effective therapies, early detection of HIV-positive individuals, better understanding of viral-resistance mechanisms have turned dreadful disease into chronic infection. However HAART is often not well-tolerated requires discipline and leads to multidrug resistance warranting the discovery of new drugs and new therapeutic approaches. Amongst the new approaches under clinical trials (Table 2), the inhibition of integrase (the third viral enzyme) has been proved to be a pertinent target. The two last years of the twentieth century were milestones in the research of HIV-1 integrase and the number of patents until 1998 (Fig. 2 and Fig. 3) has strongly increased leading to four molecules under clinical trial. The purpose of this article is to review the period of growing research that may lead in a near future to the first IN inhibitor approved-drug.

## I. STRUCTURE OF HIV-1 INTEGRASE (HIV-1 IN)

HIV-1 IN is a 32 kDa enzyme (288 amino acid residues), composed of three domains [1, 2], (Fig. 4). The N-terminal domain comprises residues 1-50 and contains a conserved "HHCC" motif that binds zinc cation in a 1:1 stoichiometry [3]. The catalytic domain (residues 50-212) contains a triad of invariant carboxylate residues, D64, D116 and E152 (the so-called D,D-35E motif) which are required for catalysis. The C-terminal domain, residues 212-288, resembles the Src homology 3 (SH3) fold and is known to bind DNA strongly but not specifically.

All three integrase domains are required for full catalytic activity, although the purified catalytic domain can carry out the retro reaction called disintegration. Divalent metal cations such as  $Mg^{2+}$  and  $Mn^{2+}$  are required for both 3'-processing and strand transfer reactions and for the assembly of IN onto specific viral donor DNA to form a complex competent to carry out either function [4-6]. The  $Mg^{2+}$  ion is coordinated by D64 and D116 along with water molecules whereas the third catalytic residue E152 that lies closely to D64 does not participate in metal binding [7,8]. Of particular interest is the presence of a thiol function (C65) that can be easily oxidized and acts as an efficient nucleophile and divalent metal ion chelator. Additionally, a small flexible loop (residues 140-149) faces the catalytic site and may be implicated in movements that are probably critical in the catalytic cycle [9, 10]. It must be noted that the published crystal structures concern only the core domain of IN. Whereas structural studies of IN reveal a single binding site for  $Mg^{2+}$ , the number of metal ions present and required in the active site during the process of the catalysis remains controversial. Both biochemical and structural studies suggest a plausible model in which the IN active site binds two metal ions. In Avian Sarcoma Virus (ASV) IN, an additional metal coordinated by D64 and E152 was observed

\*Address correspondence to this author at the Laboratoire de Chimie Organique et Macromoléculaire, UMR CNRS 8009, Université de Lille 1, 59655 Villeneuve d'Ascq, France; Tel: +33-320337231; Fax: +33-320336309; E-mail: philippe.cotelle@univ-lille1.fr



**Fig. (1).** Replicative cycle of HIV-1 and drug targets.

**Table 1.** Anti-AIDS Drugs Approved by the FDA

FDA approval	Brand Name	Generic name	Manufacturer
<b>Nucleoside reverse transcriptase inhibitors</b>			
March 19, 1987	Retrovir	Zidovudine (AZT)	GlaxoSmithKline
Oct. 9, 1991 Oct. 21, 2000 EC	Videx Videx EC	Didanosine (ddl)	Bristol-Myers Squibb
June 19, 1992	Hivid	Zalcitabine (ddC)	Roche Pharmaceuticals
June 24, 1994	Zerit	Stavudine (d4T)	Bristol-Myers Squibb
Nov. 17, 1995	Epivir	Lamivudine (3TC)	GlaxoSmithKline
Dec. 17, 1998	Ziagen	Abacavir	GlaxoSmithKline
Nov. 14, 2000	Trizivir	Abacavir, Lamivudine Zidovudine	GlaxoSmithKline
Oct. 21, 2001	Viread	Tenofovir DF	Gilead Sciences
July 2, 2003	Emtriva	Emtricitabine (FTC)	Gilead Sciences
Aug. 2, 2004	Truvada	Emtricitabine, Tenofovir DF	Gilead Sciences
Aug. 2, 2004	Epzicom	Abacavir, Lamivudine	GlaxoSmithKline
<b>Non-nucleoside reverse transcriptase inhibitors</b>			
June 21, 1996	Viramune	Nevirapine	Boehringer Ingelheim
April 4, 1997	Rescriptor	Delavirdine (DLV)	Pfizer
Sept. 17, 1998	Sustiva	Efavirenz	Bristol-Myers Squibb

(Table 1) Contd....

FDA approval	Brand Name	Generic name	Manufacturer
<b>Protease inhibitors</b>			
Dec. 6, 1995	Invirase	Saquinavir	Roche Pharmaceuticals
March 1, 1996	Norvir	Ritonavir	Abbott Laboratories
March 13, 1996	Crixivan	Indinavir (IDV)	Merck
March 14, 1997	Viracept	Nelfinavir	Pfizer
Nov. 7, 1997	Fortorase	Saquinavir Mesylate	Roche Pharmaceuticals
April 15, 1999	Agenerase	Amprenavir	Abbott Laboratories
Sept. 15, 2000	Kaletra	Lopinavir, Ritonavir	Abbott Laboratories
June 20, 2003	Reyataz	Atazanavir	Bristol-Myers Squibb
Oct. 20, 2003	Lexiva	Fosamprenavir	GlaxoSmithKline
<b>Fusion Inhibitors</b>			
March 13, 2003	Fuzeon	Enfuvirtide (T-20)	Roche Pharmaceuticals/ Trimeris

Table 2. Investigational Drugs in Clinical Trial (source: www.aidsinfo.nih.gov)

Classes of inhibitor	NNRTI	NRTI	Entry/Fusion	Microbicide	PI	IN	Opportunistic infections
Number of drugs	2	6	9	9	2	1	1

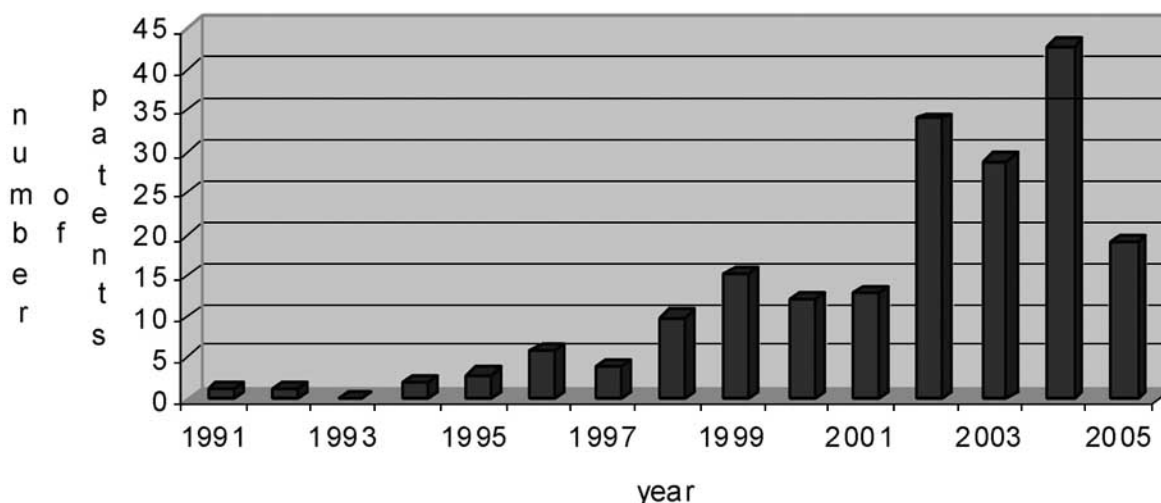


Fig. (2). Number of patents per year devoted to HIV-1 integrase inhibitors research.

by using either Zn<sup>2+</sup> or Cd<sup>2+</sup> [11]. Moreover the HIV-1 RNase H active site also contains two metals coordinated concurrently by the DDE motif [12]. These results and other studies on a variety of phosphotransferases suggest that there are two discrete metal binding sites that both sites can be occupied concurrently and that occupancy may be affected by DNA.

## II. CATALYTIC ROLES OF HIV-1 INTEGRASE (FIG. 5)

### 2.1. *In Vivo*

The viral DNA formed after reverse transcription is a double stranded linear DNA with LTR sequences at each end. As soon as the viral double-stranded DNA is synthesized, IN recognizes an intact LTR end, binds viral

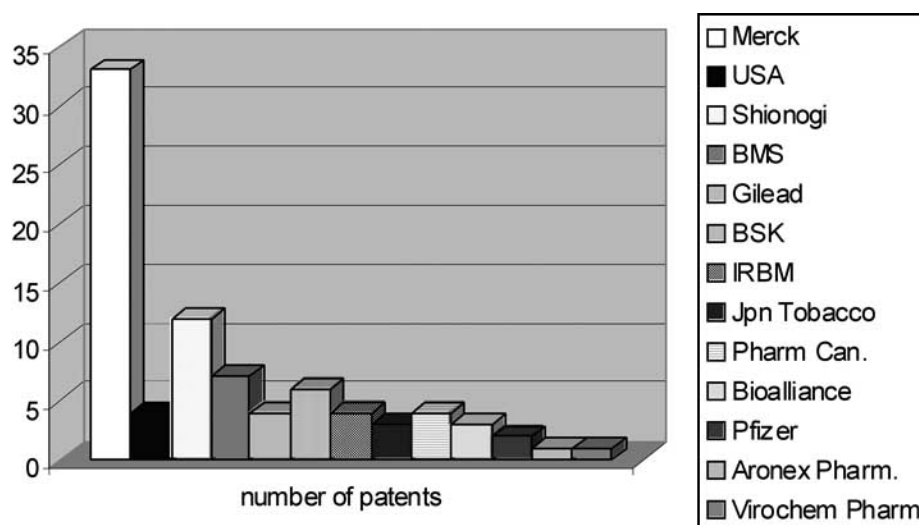


Fig. (3). Number of patents per company (in the 1998-2005 period)(USA means academic laboratories from USA).

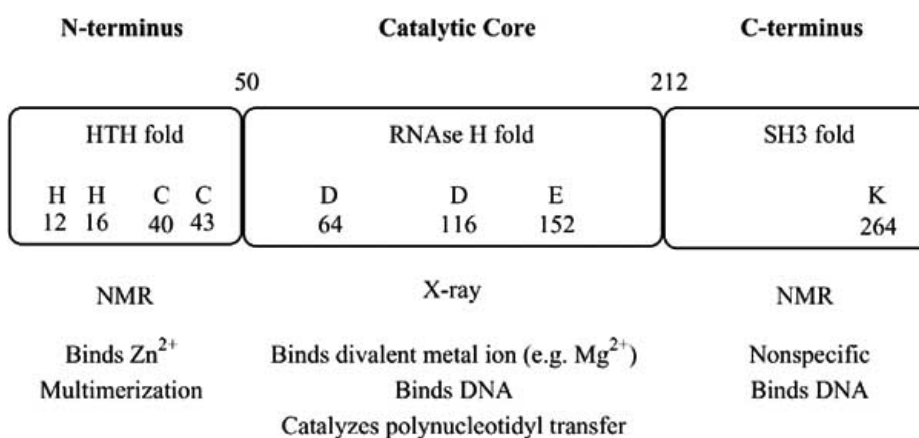


Fig. (4). Schematic representation of the three different domains of HIV-1 integrase.

DNA and exerts its catalytic activity. The first step is the removal of the terminal GT dinucleotides from each 3'-OH end. This endonucleolytic activity is referred to as the 3'-processing reaction and takes place within the pre-integration complex (PIC) before its transport to the nucleoplasm. The integrase does not exist in a free form but rather in a nucleoprotein complex along with processed viral DNA, RT, matrix and other auxiliary proteins called PIC [13-14]. The PIC is then transported into the host nucleus. The compact structure disintegrates and the second reaction, DNA strand transfer, takes place. The 3'-OH groups of the viral DNA attack phosphodiester bonds in the host DNA, covalently joining to two sites on opposite host DNA strands separated by five nucleotides. The integration reaction is completed by the removal of the two unpaired nucleotides at the 5'-end of the viral DNA and the repair of the single stranded gaps presumably by host enzymes.

## 2.2. In vitro

### 2.2.1 Disintegration

*In vitro*, IN removes two nucleotides from the 3'-end of a model substrate DNA and integrates that 3'-end into another

oligonucleotide to yield Y-shaped integration product, but purified IN can also catalyze a reversal of this reaction named disintegration [15]. One particularly simple substrate to prepare and analyze is the "dumbbell disintegration" substrate of Chow and Brown [16]. The IN core domain is also able to carry out this reaction, thus inhibition of dumbbell disintegration by IN<sup>50-212</sup> indicates that the inhibitor is acting on the central catalytic domain (or on the DNA substrates). The disintegration assay is convenient for rapid drug screening, since products appear in a single band and are easily quantified.

### 2.2.2. 3'-Processing and Strand Transfer

Unlike disintegration, 3'-processing and strand transfer require the three domains of IN. IN purified after over expression in *Escherichia Coli* can carry out DNA cleavage and joining reactions that mimic normal integration in several aspects. IN can remove two nucleotides from the 3'-end of a model viral cDNA end and then join the recessed 3'-end to a 5'-phosphate in a target DNA. Both reactions can be monitored by formation of smaller and longer model DNA strands respectively. Both assays have the advantage of

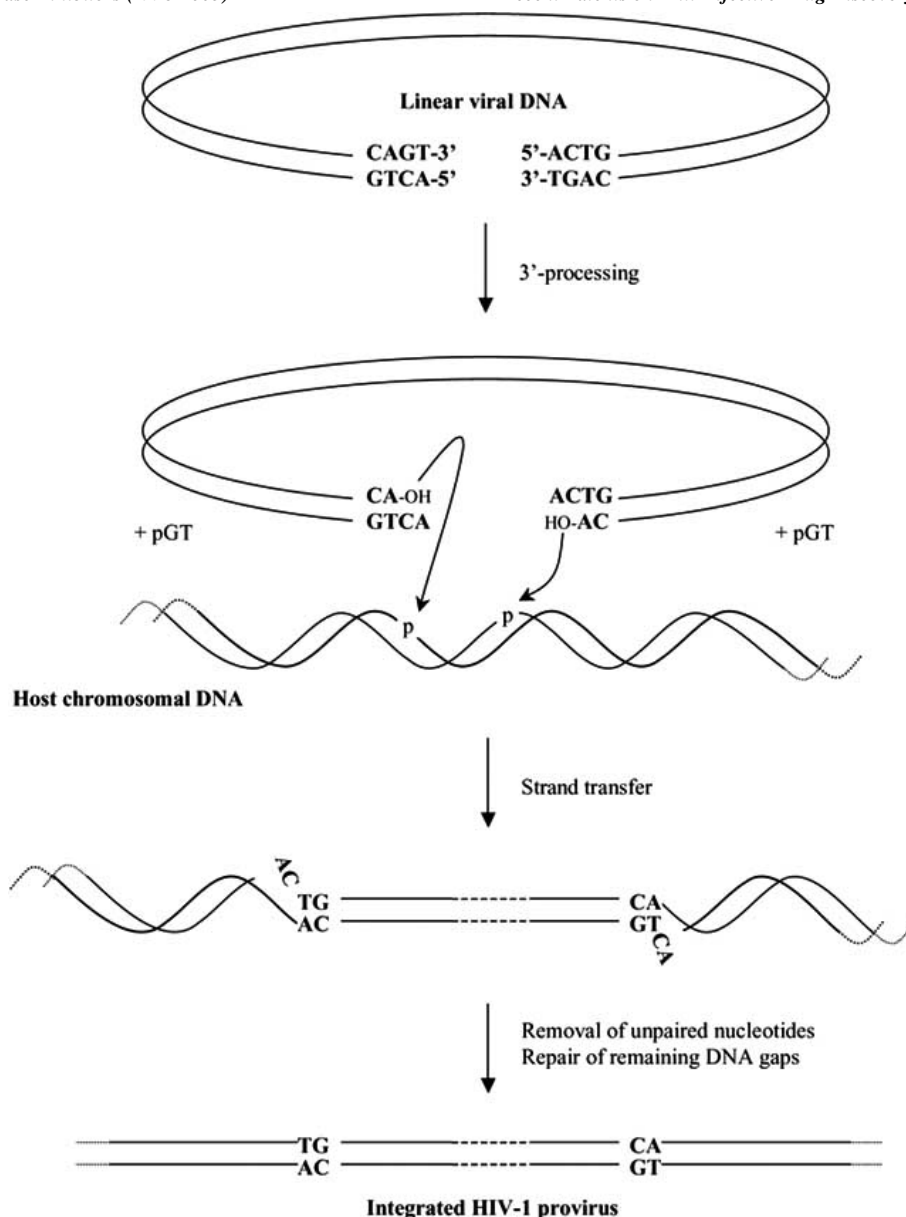


Fig. (5). The different reactions of integration.

being fast, convenient and adapted for high throughput screening of IN inhibitors [17]. Unfortunately, these purified IN based assays identify a significant number of false positives, whose IN inhibitions do not translate into antiviral activities.

### 2.2.3. $Mn^{2+}$ or $Mg^{2+}$

*In vitro*, in the absence of a target DNA substrate, IN forms stable complexes with HIV LTR sequence oligonucleotides. Assembly of a stable integrase donor substrate complex and strand transfer are processes requiring  $Mn^{2+}$  or  $Mg^{2+}$  [4-6]. Subsequent to assembly, catalysis of both 3'-processing and strand transfer requires a divalent cation cofactor and these requirements can be functionally distinguished [18].  $Mg^{2+}$  is considered to be the divalent cation cofactor *in vivo* but  $Mn^{2+}$  is frequently used in assays because IN activity is generally more robust in its presence.

As a result, many of the inhibitors were identified using  $Mn^{2+}$  but many of them were inactive in the presence of  $Mg^{2+}$ .

### 2.2.4. Preincubation

IN has a high affinity for its DNA substrate and under physiological conditions, an IN-DNA-metal complex is formed. Generally, IN is preincubated with the metal ion and the drug following which an oligonucleotide is added. But assays can be carried out by preincubating IN with oligonucleotide and metal ion prior to addition of the drug. The inhibitory activities are generally lower using this last procedure [19]. The IN-DNA complex in the presence of the metal ion can be easily monitored using an assay based on the formation of imine group (Schiff base) that is reduced using sodium borohydride [20]. IN-DNA-metal complexes

may constitute one of the possible targets for IN inhibitors that recently have been demonstrated [21].

### 2.2.5 PIC

The limited transferability of results from assays using purified IN has led to the development of PIC assays [22]. *In vitro* integration mediated by PIC is considered to resemble the *in vivo* reaction most closely. PIC, partially purified from cells infected with HIV-1, can direct joining of both ends of the viral cDNA in a coordinated fashion, giving a product resembling the gapped integration intermediate. Expectedly, many of the inhibitors that were active in purified IN assays failed to show activity in the PIC assay since IN is inaccessible to many molecules [23, 24]. However the PIC assay is labor intensive and has not been successfully optimized for high throughput screening.

### 2.2.6. Antiviral Activity

Ultimately, a candidate drug to become a therapeutic agent must inhibit propagation of HIV-1 in cells. Cell-based assays of HIV antiviral activity have been developed. HIV infected cells were incubated with the test molecule and cell viability was determined colorimetrically. Uninfected cells with the drug served as a toxicity control and infected and uninfected cells without the drug served as basic controls. Since the life cycle of HIV is multistep, a cell-based time-of-addition assay has been developed [25]. In this experiment, a compound was added at different times after infection with HIV and therefore its target could be easily deduced. For example, ritonavir, a protease inhibitor, was effective 18-19 hours after infection whereas DKAs (IN inhibitors) were active 7 hours after infection [26].

## III THE INHIBITORS OF INTEGRATION

Integration is a multipart process (at least four), i.e. an enzyme, one (or two) divalent metal ion(s), viral DNA and host DNA. The inhibition of the integration requires the intervention of a fifth one, the inhibitor: (i) an oligonucleotide can interact with IN at the binding sites of viral or host DNAs, (ii) a peptide may interact with viral or host DNAs or (iii) a low molecular-weight molecule binds the divalent metal ion at the active site of IN. Numerous reviews have been previously published on HIV-1 integrase inhibitors [27-29] including peptides [30], oligonucleotides [31] and patented small molecules [32]. Since the pioneer reports of Hazuda *et al.* [24] on DKA (DiKetoAcid), small molecules that inhibit integrase by interaction with its catalytic core domain can be divided into two families, i.e. the selective strand transfer inhibitors and the non-selective strand transfer inhibitors.

### 3.1. Selectivity for the Strand Transfer Step

Since the comparative study of Marchand *et al.* [33] (wild-type versus double-mutant IN and  $Mn^{2+}$  versus  $Mg^{2+}$ ) it is clear that assays performed using wild-type enzyme and  $Mg^{2+}$  represent the most stringent conditions with respect to drug inhibition. Whereas it was found that DKAs inhibit HIV-1 IN independently of the order of addition with wild-type enzyme and  $Mn^{2+}$ , it was not true when  $Mg^{2+}$  was used. In the case of L-708,906 (see scheme 1), the strand transfer selectivity (ST versus 3'-P) was found to be >2400 (double-mutant IN and  $Mn^{2+}$ ),  $375 \pm 139$  (wild-type IN and  $Mn^{2+}$ ) and

$41 \pm 15$  (wild-type IN and  $Mg^{2+}$ ). In our hand, when the IN-DNA complex was not preformed (addition of the drug at the same time as IN and DNA fragment) the selectivity dropped to about 7 [34]. Since divalent metal cofactor stimulates and stabilizes IN-viral DNA complexes and decreases binding of IN to target DNA [35], these results are consistent with the fact that L-708,906 interacts with  $Mg^{2+}$ .

### 3.2. The Different Classes of IN Inhibitors

According to the structural features in direct connection with their ability to bind divalent metal ions [36], the patented HIV-1 integrase inhibitors can be divided into three different classes: (i) the benzoylpyruvic acid derivatives, (ii) the 8-hydroxyquinoline derivatives and (iii) the polyphenols. A fourth one, namely miscellaneous, includes unclassified molecules.

### 3.3. A Secondary Pharmacophore

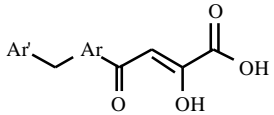
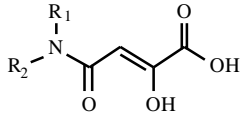
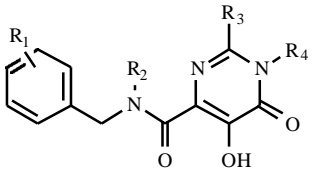
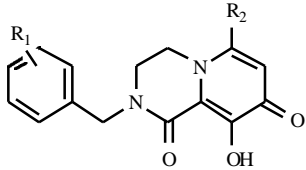
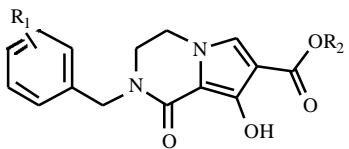
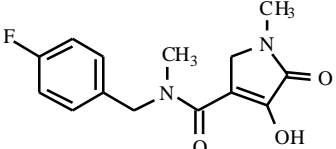
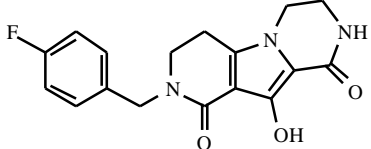
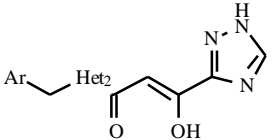
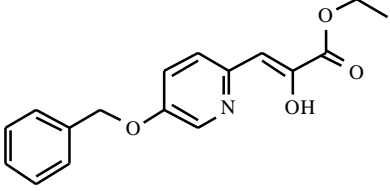
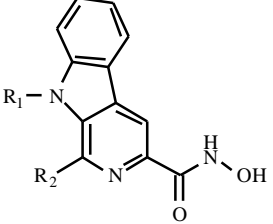
An aromatic ring separated by at least one  $sp^3$  atom to the pharmacophore responsible for the metal binding constitutes a secondary pharmacophore. The position and the substitution on this aromatic being essential for the selectivity for the strand transfer step. This recurrent benzyl group (found in the two first classes of IN inhibitors) generally substituted at the para position by a fluorine atom is expected to play a role in the ST selectivity by specific interactions in the ST pocket (N155, K156, and K159) [37]. The para-fluoro benzyl group is found in three of the four drugs that are or have been under clinical trials and is generally found in the most active molecules of a series. It is critical to define its exact role in the inhibition of the integration and in the selectivity for the strand transfer step.

## IV. THE DIFFERENT CLASSES OF IN INHIBITORS

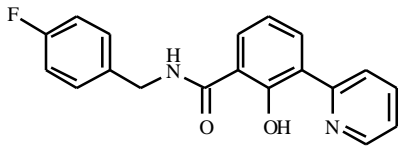
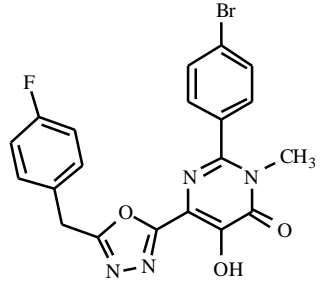
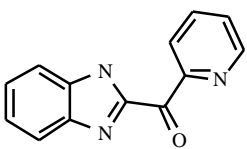
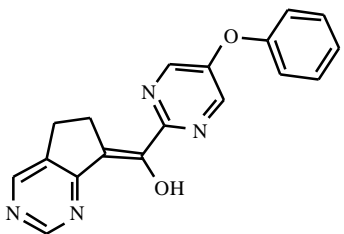
### 4.1. Benzoylpyruvic Acid Derivatives (Table 3)

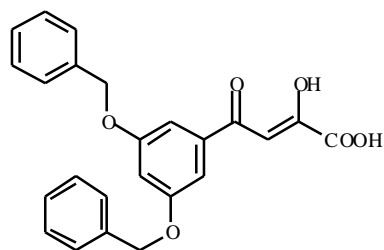
Under the inadequate acronym DKA, the first integrase inhibitors that selectively inhibit the second step catalyzed by IN, i.e. strand transfer, were patented in 1999 and published in 2000 [24]. In fact, a more adequate name may be 4-aryl-3-oxo-2-hydroxybutenoic acid since it has been clearly established that these molecules exist mainly in this form in solution [38]. In the presence of  $Mg^{2+}$  this pharmacophore can be easily deprotonated to give a dianion and may interact with two divalent ions. But it is not still sufficient to propose that DKA bind two metal ions in the active site and this binding cannot be correlated with the ST selectivity. The story of the DKA family started with the pioneer works of Merck Company in 1999 [39-41] (Table 3, first line, left column) followed by a series of Shionogi patents [42] (Table 3, fourth line, right column) where the carboxylic function is replaced by a polyaza pentacycle bioisostere. The lead DKA from Merck company were L-708,906 and L-731,988 with  $IC_{50}$  against IN of 100 and 50 nM respectively and micromolar  $EC_{50}$  values in the inhibition of the HIV-1 replication. Pommier's group (from the NIH (National Institute of Health)) has patented a series of DKAs where the phenyl ring was diversely substituted and notably by an azidomethyl group (scheme 1). S-1360 is issued from the pioneer patents from Shionogi Company and its antiviral properties will be discussed in the section V relative to the drugs under clinical trials. 5-CITEP (scheme

Table 3. Benzoylpyruvic Acid Derivatives

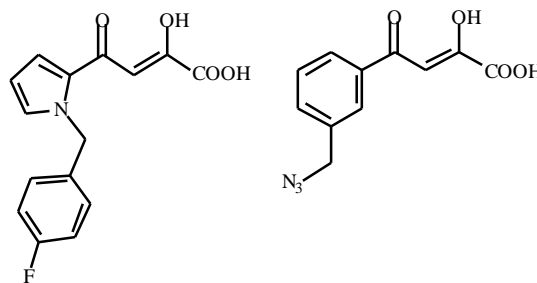
	
<p>Merck WO 9962513            WO 9962520            WO 9962897            NIH WO 2003049695</p>	<p>BMS WO 2001096283            WO 2001098248            WO 2003049690            Jpn Tobacco WO 2003016266</p>
	
<p>Merck WO 2005061501            IRBM WO 2003035076            WO 2003035077            WO 2004058756            WO 2004058757            Shionogi JP 2004244320            BMS WO 2004062613            WO 2004096128</p>	<p>Merck WO 2004024078            Jpn Tobacco WO 2005016927</p>
	
<p>Merck WO 2004047725</p>	<p>BMS WO 2004004657</p>
	
<p>Merck WO2005041664</p>	<p>Shionogi WO 2000039086            WO 2001095905            WO 2001096329            Merck WO 2001000578</p>
	
<p>Shionogi WO 2001017968            WO 2003016275</p>	<p>Pfizer WO 2004039803            WO 2004067531</p>

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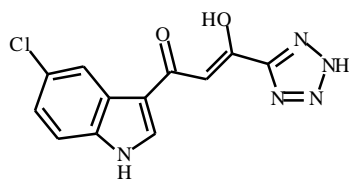
	
Virochem Pharma WO2005042524	Shionogi WO 2005061490
	
Shionogi WO 2002070491 WO 2002070486	Shionogi WO 2003047564 WO 2003016275



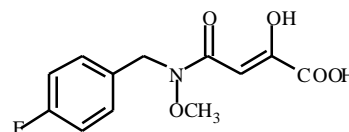
L-708,906 and L-731,988 from Merck Company



Azido derivative from Pommier's group



5-CITEP from Shionogi Company



Carbamoylhydroxyacrylic acid from BMS Company

**Scheme (1).**

1) also from Shionogi Company, has been co-crystallized in the catalytic site of the HIV-1 IN core [43]. In spite of a very modest activity against IN and the absence of antiviral properties, the crystallographic data were considered of some help for the design of new antiviral drugs. The left side aromatic moiety was then replaced by disubstituted amido groups [44-47] (Table 3, first line, right column). As an example, the Bristol-Myers Squibb compound reported on

scheme 1 exhibited 96% inhibition of recombinant HIV virus expressing luciferase in cell culture at 1.6  $\mu$ M.

The three oxygen atoms of DKA were then included into heterocycles leading to the more recent patents (in the last two years) (lines 2 and 3 and line 4 left column). The  $IC_{50}$  (against IN) are generally submicromolar and sometimes nanomolar. For example Bristol-Myers Squibb Company reported  $IC_{50}$  ranging between 2 and 200 nM [48,49].

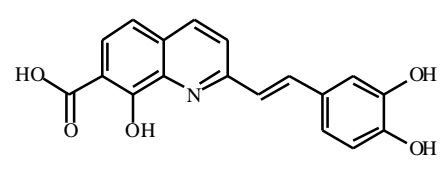
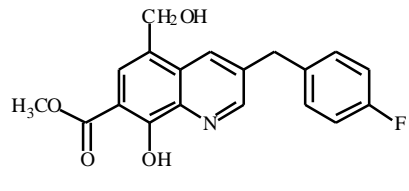
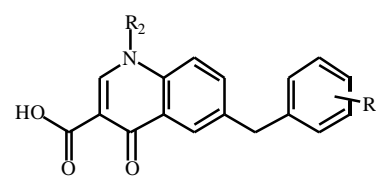
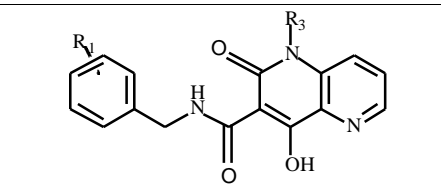
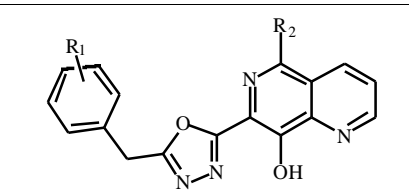
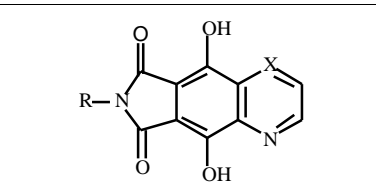
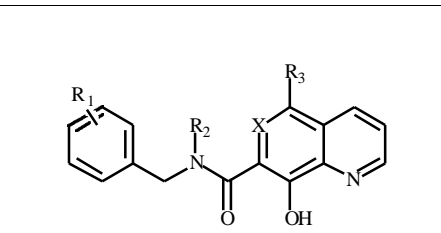
One or two oxygen atoms were replaced by a pyridine-type nitrogen atom as attested by the very different structures reported in the table 3 (Lines 5 to 7). The anti-integrase activities are good as in the last patent from Shionogi Company that reported  $IC_{50}$  ranging between 1.8 and 57 ng/mL [50].

#### 4.2. 8-Hydroxyquinoline Derivatives (Table 4)

8-Hydroxyquinoline is a well-known ligand of divalent ions and was rapidly recognized as a major pharmacophore. D'Angelo's group [51] was the first to include 8-hydroxyquinoline-7-carboxylic acid (Table 4, line1, left column) into the structure of new HIV-1 IN inhibitors with  $IC_{50}$  in the range of 0.3-4  $\mu$ M. A substitution on the right side of the molecule (mainly by hydroxyl groups) has led to the absence of selectivity towards one or the other step catalyzed by IN [52, 53]. Whereas they were identified as *in vitro* IN inhibitors, the exact *in vivo* target is still under investigation. *In vivo*, styrylquinolines were found to act prior to integration by reducing the amount of the late cDNA, suggesting for the first time that integrase targeting

molecules may affect the accumulation of DNA during reverse transcription [54]. More recently, Shionogi patented 8-hydroxyquinoline-7-carboxylic acid methyl esters substituted on position 3 by a substituted benzyl group (Table 4, line 1, central column). The example given on table 4 has an  $IC_{50}$  against IN of 200 nM. It seems that the absence of the heterocyclic nitrogen is not dramatic for the IN inhibitory activity since Jpn Tobacco Company patented 6-benzyl-3-carboxy-4-quinolones [55]. Their anti-integrase activity was evaluated to be submicromolar and the fourth and the most recent drug under clinical trials is reported in this patent. During this time, Merck Company followed its own way and apparently independently to the work of d'Angelo and Bioalliance patented 8-hydroxyquinoline-7-carboxylic amides substituted on the nitrogen of the amide function by a substituted benzyl group leading to two molecules under clinical trials (L-870,810 and L-780,812) [56] (Table 4, left column, lines 2 and 3). This structure was declined in many ways by replacing the carboxylic function by a heterocycle, including it in a new heterocycle or modified the quinoline heterocycle by adding a nitrogen atom in position 5 or 6 (Table 4, line 2, center and right

Table 4. 8-Hydroxyquinoline Derivatives

		
Bioalliance FR 20032839646 WO 2003031413 CNRS WO 9845269	Shionogi WO 2004024693	Jpn Tobacco WO 2004046115
		
Merck WO2003062204	SKB WO 2004101512	Gilead WO 2005028478 WO 2004035576 WO 2004035577 Tibotec WO 2004096807
	Merck WO 2002036734 WO 2002030426 WO 2002030930 WO 2002030931 WO 2002055079 WO 2003016294 WO 2003016309 WO 2003016315	Merck WO 2003016309 WO 2003016315 WO 2003077850 WO 2003077857 WO 2003086319 WO 2004080402 Gilead WO 2005028478 Shionogi WO 2002070486

columns). Amongst these last patents, SmithKline Beecham patented a series of molecules with anti-HIV activity in the range  $IC_{50}$  of 1-1000 nM [57].

#### 4.3. Polyphenols (Table 5)

Whereas a great number of polyphenols were identified as IN inhibitors and this class of IN recognized as the most important (in term of number of molecules), a few patents were filed and were due, at least in part, to academics. Natural polyphenols such as chicoric acid, 3,5-dicaffeoylquinic acid and lithospermic acids are found in medicinal plants (*Salvia*) or vegetables (endive, lettuce) [58]. We have proposed that an adequate diet may contribute to lighten the actual tritherapies. Other synthetic polyphenols included the structure of caffeic acid or DOPA. As in the case of Zintevir (structure is given on Table 8), chicoric acid was developed for an *in vitro* activity that is different to its *in vivo* target. Probably due to its great number of hydroxyl groups, chicoric acid has been found to inhibit the entry of the virus by interacting with gp120 [59]. Chicoric acids (L or D) inhibits 3'-P ( $IC_{50} = 0.5 - 1.0 \mu M$ ) and overall reaction ( $IC_{50} = 0.2 - 0.3 \mu M$ ) but not ST ( $> 105 \mu M$ ) and the viral cytopathic effect of HIV-1 in MT-4 cell cultures ( $IC_{50} = 1.7 - 5.2 \mu M$  and  $CC_{50} = 115 \mu M$ ). Recently Robinson *et al.* [60] reported that chicoric acid inhibits both entry and integration at similar concentrations. In deep contrast with the previous two classes, the anti-integrase activities of synthetic polyphenols from an academic laboratory from China and the Pharmacor Inc. company (Table 5, line 2) are modest ( $IC_{50} > 100 \mu M$ ).

#### 4.4. Miscellaneous

##### 4.4.1. Synthetic Molecules (Table 6)

A great structural diversity has also been reported attesting the interest for the integration as a potent target for

anti-HIV therapy. Amongst them it must be mentioned the patent of De Clercq's group where V-165 (Table 6, line 4, right column) was identified as a potential candidate for clinical trials [61]. V-165 inhibits IN activity with  $IC_{50}$  value of  $0.3 \mu M$  (overall reaction)  $16.1 \mu M$  (ST) and  $0.9 \mu M$  (3'-P). V-165 does not directly affect the catalysis of 3'-P but the IN-DNA complex formation. It blocks the viral replication in cell cultures (MT-4 cells) with an  $IC_{50}$  value of  $8.9 \mu M$  and a  $CC_{50}$  value of  $121 \mu M$  [62]. Bristol-Myers Squibb patented a series of dissymmetric fumaric amides with  $EC_{50} = 0.08-12 \mu M$  against the recombinant HIV virus expressing Renella luciferase [63]. The best results were obtained with the molecule depicted on table 6 (line 3, right column) that can be structurally related to the DKA from the same Company given on scheme 1.

##### 4.4.2. Natural Products Isolated from Cultures (Table 7)

Before and during the development of diketoacid family and related compounds, Merck Company has continued to explore the natural sources of molecules as IN inhibitors. Cultures of *Xylaria* sp., *Fusarium* sp., *Actinoplanes* sp. and *Cytonaema* have yielded a great structural diversity of IN inhibitors. However, this activity has been slowed as DKA emerged as a major progress in the IN inhibitor research.

#### V. MOLECULES UNDER CLINICAL TRIALS (TABLE 8)

To date, five integrase inhibitors were or are under preclinical or clinical trials. The first one, AR-177 also named zintevir from Aronex Pharm. [64], is in phase I. It was initially designed as IN inhibitors *in vitro* (it inhibits 3'-P and ST with  $IC_{50}$  values of 79 and 49 nM respectively) [65, 66] but was found to inhibit viral attachment (gp120) *in vivo*. Its  $EC_{50}$  and  $CC_{50}$  values in MTT assay (MT-4 cells infected with IIIB) are 600nM and  $> 125\mu M$  respectively [67].

Table 5. Polyphenols

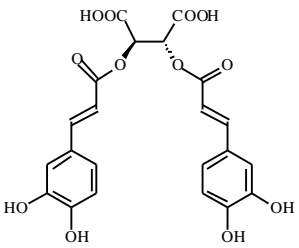
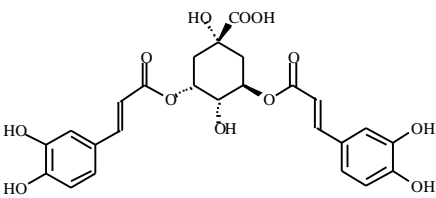
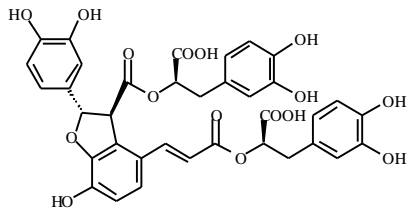
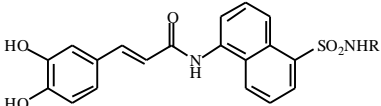
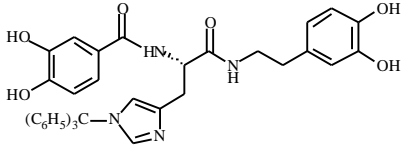
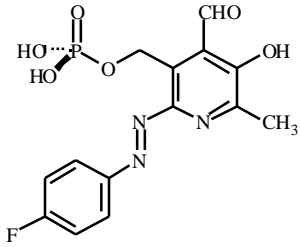
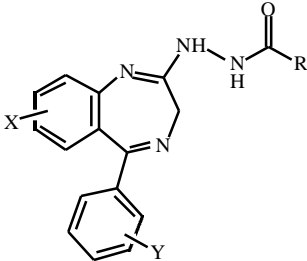
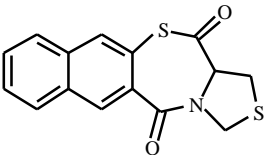
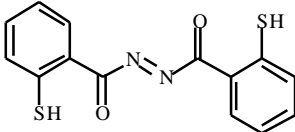
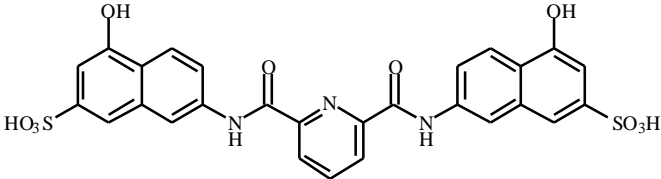
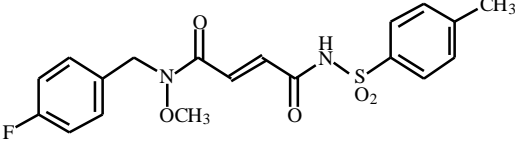
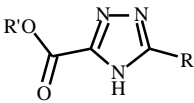
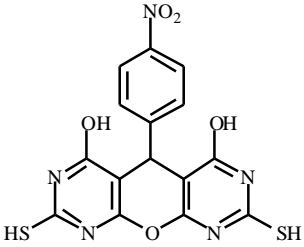
		
NIH WO 2000063152 USA WO 2005049242 WO 9948371	PPP WO 2002020006 Pharmacia US 2005026902 CN US 6331565 (2001)	USA US 6043276 (2000) WO 9966942 US 5994400 (1999)
		
CN CN 1482116 (2004)	Pharmacor Inc. WO 2002026697 WO 2000059867	

Table 6. Synthetic Molecules

	
Pharmacor WO 2003082881	Merck WO 9818473
	
NIH WO 2000068235	NIH WO 2000053577
	
USA WO 9850347	BMS WO 2004103278
	
Shionogi JP 2004018480	Stichting Rega WO 2002003971

S-1360 was developed by a Japanese company called Shionogi in partnership with GSK. S-1360 inhibits IN *in vitro* with an  $IC_{50}$  of 20nM and HIV replication with  $EC_{50}$  and  $CC_{50}$  in MTT assay of 200nM and 12 $\mu$ M respectively [68, 69]. S-1360 reached phase II, but its development was halted in 2003. Clinical data suggested involvement of a non-cytochrome P450 clearance pathway [70]. Reduction of S-1360 at the carbon linked to the triazole heterocycle generates a key metabolite in humans. This metabolic instability is probably the reason of the abandon of its development.

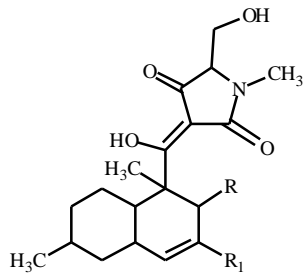
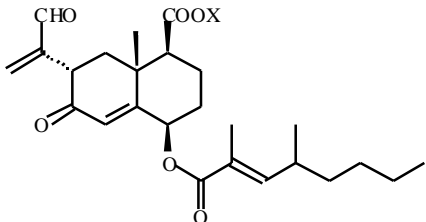
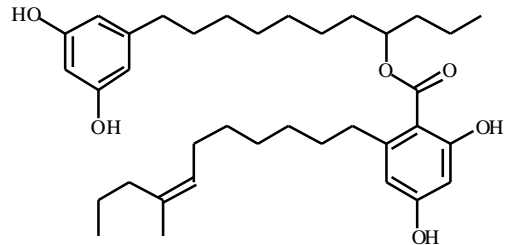
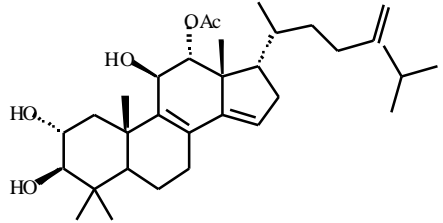
Merck Pharmaceuticals has commenced clinical trials with L-870,810. L-870,810 is a potent selective ST inhibitor with an  $IC_{50}$  value of 10nM and an antiviral  $EC_{95}$  of 19 nM ( $CC_{50} > 5 \mu$ M). In spite of promising antiviral activity,

research on L-870,810 has been stopped after unacceptable liver and kidney cell toxicity was found in dogs. However, research on the related drug L-870,812 is proceeding.

Recently Gilead Sciences acquired the development rights to JTK-303, an IN inhibitor licensed by Japan Tobacco. JTK-303 has already undergone phase I studies in Japan, and will get phase I/II trials underway in HIV-positive volunteers in 2005. Little information is known about this compound. It shows  $IC_{50}$  of 1.7 nM against integrase. Its activity is expected to be due to its ability to bind  $Mg^{2+}$  but the absence of three heteroatoms as in the four-point 3D model of Barreca *et al.* [71] may suggest that it is not a selective inhibitor of ST.

FZ41 inhibits the 3'-processing and the strand transfer reactions with  $IC_{50}$  of 0.7 and 1.7  $\mu$ M respectively. It shows

Table 7. Natural Products Isolated from Cultures

	
	
<p>Merck WO 2001027309          WO 2001009114          WO 2000036132          GB 2327674 (1999)          US 5858738 (1999)</p>	<p>Merck GB 2319026 (1998)          US 5759842 (1998)          US 9834932 (1998)          GB 2306476 (1997)</p>

antiviral EC<sub>50</sub> of 5-10 μM (CEM cells) and 1-4 μM (HeLa cells) with TC<sub>50</sub> > 100 μM. FZ-41 (Bioalliance) is under preclinical tests and is expected to enter in clinical trials in the beginning of the next year.

### CURRENT AND FUTURE DEVELOPMENTS

It was approximately a decade between the identification of IN as potent viral target and the first report (in 1999) of true antiviral inhibitors of IN (their *in vivo* mode of action was demonstrated to be IN inhibition). This date almost coincides with the beginning of the increase in patents of IN inhibitors. The fact that integrase has no counterpart in human is an advantage and a drawback: an advantage because integrase inhibitors are expected to be selective; a drawback because our knowledge's about its structure (monomer, dimer, multimer, one or two metal ions), its function and its position into the cell (intra or extra nuclear) are still fragmentary. The progresses in the research of IN inhibitors recorded at the end of the last century are at least in part due to our progresses in the knowledge on integrase and to the development of more robust assays. False positive inhibitors are now strongly reduced with assays on wild-type enzyme and Mg<sup>2+</sup> as divalent ions. In the period (1998-2005) reviewed in the present article, about two hundred patents were registered, yielding four IN inhibitors under clinical trials. To date, there is no IN inhibitor in phase III clinical trial that is the last step before FDA approval and the road is still long and full of pitfalls before the FDA approval of an integrase inhibitor. Molecules that inhibit IN on the micromolar range are numerous but there is not a great chemical diversity for the much closed group of molecules

active at nanomolar level. Therefore, new original structures are warranted to continue to feed the pipeline of drug candidates. It may not be impossible that an IN inhibitor will be on the market before the end of the present decade.

The successful development of integrase inhibitors would target an additional viral component in addition to the actually available antiretroviral combination therapy and will provide a new treatment option in the case of resistance to protease and reverse transcriptase inhibitors. It was demonstrated *in vitro* that L-chicoric acid was largely synergistic with zidovudine and synergistic with both dideoxycytidine and nelfinavir [72] but there is still no data concerning the efficiency of IN inhibitors under clinical trials as part of combination therapy.

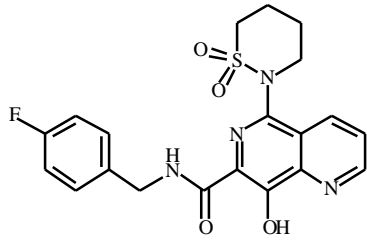
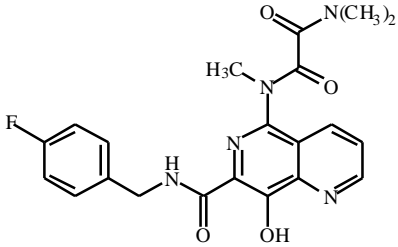
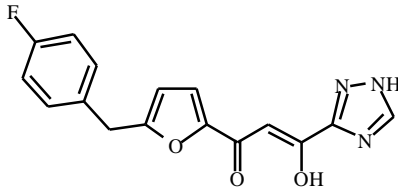
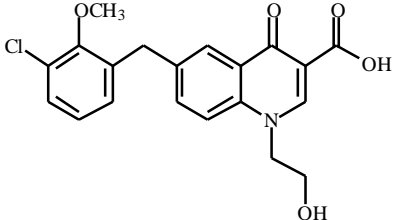
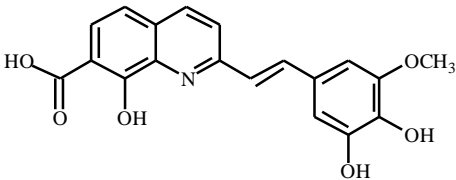
### ACKNOWLEDGMENT

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### ABBREVIATIONS

AIDS	=	Acquired ImmunoDeficiency Syndrome
ASV	=	Avian Sarcoma Virus
BMS	=	Bristol-Myers Squibb
DKA	=	Diketo Acid
FDA	=	Food and Drug Agency
gp120	=	Glycoprotein 120
GSK	=	GlaxoSmithKline

Table 8. Integrase Inhibitors Under Clinical Trials

	
L-870,810 (Merck) Phase I/II stopped after unacceptable liver and kidney cell toxicity found in dogs.	L-870,812 (Merck) preclinical tests are proceeding.
	
S-1360 (Shionogi) or GW810781 (GSK) phase II, halted in 2003.	JTK-303 (Jpn Tobacco, Gilead) under phase I/II clinical trials.
	5'-GTGGTGGGTGGGTGGGT-3'
FZ-41 (Bioalliance) under preclinical tests, will entered in clinical phase I trials in the beginning of 2006.	Zintevir or AR-177 first designed as IN inhibitor was found to inhibit viral attachment <i>in vivo</i> . Under clinical phase I trials.

HAART	=	Highly active antiretroviral therapy
HIV	=	Human Immunodeficiency Virus
IN	=	Integrase
IRBM	=	Instituto di Ricerche Biologia Molecolare, Italy
LTR	=	Long Terminal Repeat
NRTI	=	Nucleotide/Nucleoside Reverse Transcriptase Inhibitor
NNRTI	=	Non- Nucleotide/Nucleoside Reverse Transcriptase Inhibitor
3'-P	=	3'-Processing
PI	=	Protease inhibitor
PIC	=	Pre-Integration Complex
SKB	=	SmithKline Beecham
ST	=	Strand transfer
RT	=	Reverse transcriptase

## REFERENCES

- [1] Esposito D, Craigie R. In: *Advances in Virus Research*, Skalka, A.M. Ed.; Academic Press San Diego, 1999; 52: 319-333.
- [2] Asante-Appiah E, Skalka AM. In: *Advances in Virus Research*, Skalka, A.M. Ed.; Academic Press San Diego, 1999; 52: 351-369.
- [3] Zheng R, Jenkins T, Craigie R. Zinc folds the N-terminal domain of HIV-1 integrase, promotes multimerization, and enhances catalytic activity. *Proc Natl Acad Sci USA* 1996; 93: 13659-64.
- [4] Ellison V, Brown PO. A stable complex between integrase and viral DNA ends mediates human immunodeficiency virus integration *in vitro*. *Proc Natl Acad Sci USA* 1994; 91: 7316-20.
- [5] Vink C, Lutzke RAP, Plasterk RHA. Formation of a stable complex between the human immunodeficiency virus integrase protein and viral DNA. *Nucleic Acids Res* 1994; 22: 4103-10.
- [6] Wolfe AL, Felock PJ, Hastings JC, Blau UC, Hazuda DJ. The role of manganese in promoting multimerization and assembly of human immunodeficiency virus type 1 integrase as a catalytically active complex on immobilized long terminal repeat substrates. *J Virol* 1996; 70: 1424-32.
- [7] Goldgur Y, Dyda F, Hickman AB, Jenkins TM, Craigie R, Davies DR. Three new structures of the core domain of HIV-1 integrase: an active site that binds magnesium. *Proc Natl Acad Sci USA* 1998; 95: 9150-4.
- [8] Maignan S, Guilloteau JP, Zhou-Liu Q, Clément-Mella C, Mikol V. Crystal structures of the catalytic domain of HIV-1 integrase free and complexed with its metal cofactor: high level of

- similarity of the active site with other viral integrases. *J Mol Biol* 1998; 282: 359-64.
- [9] Asante-Appiah E, Seeholzer S, Skalka AM. Structural determinants of metal-induced conformational changes in HIV-1 integrase. *J Biol Chem* 1998; 273: 35078-87.
- [10] Asante-Appiah E, Skalka AM. A metal-induced conformational change and activation of HIV-1 integrase. *J Biol Chem* 1997; 272: 16196-205.
- [11] Bujacz G, Alexandratos J, Wlodawer A, *et al.* Binding of Different Cations to the Active Site of Avian Sarcoma Virus Integrase and their Effects on Enzymatic Activity. *J Biol Chem* 1997; 272 : 18161-8.
- [12] Davies JF, Hostomska Z, Hostomsky Z, Jordan SR, Matthews DA. Crystal structure of the ribonuclease H domain of HIV-1 reverse transcriptase. *Science* 1991; 252: 88-95.
- [13] Bukrinsky MI, Sharova N, McDonald TL, Pushkarskaya T, Tarpley WG, Stevenson M. Association of integrase, matrix, and reverse transcriptase antigens of human immunodeficiency virus type 1 with viral nucleic acids following acute infection. *Proc Natl Acad Sci USA* 1993; 90: 6125-9.
- [14] Heinzinger NK, Bukrinsky MI, Haggerty SA, *et al.* The Vpr protein of human immunodeficiency virus type 1 influences nuclear localization of viral nucleic acids in nondividing host cells. *Proc Natl Acad Sci USA* 1994; 91: 7311-5.
- [15] Chow SA, Vincent KA, Ellison V, Brown PO. Reversal of integration and DNA splicing mediated by integrase of human immunodeficiency virus. *Science* 1992; 255: 723-6.
- [16] Chow SA, Brown PO. Substrate features important for recognition and catalysis by human immunodeficiency virus type 1 integrase identified by using novel DNA substrates. *J Virol* 1994; 68: 3896-907.
- [17] Marchand C, Neamati N, Pommier Y. *In vitro* human immunodeficiency virus type 1 integrase assays. *Methods Enzymol* 2001; 340: 624-33.
- [18] Hazuda DJ, Felock PJ, Hastings JC, Pramanik B, Wolfe AL. Differential divalent cation requirements uncouple the assembly and catalytic reactions of human immunodeficiency virus type 1 integrase. *J Virol* 1997; 71: 7005-11.
- [19] Marchand C, Zhang X, Pais GCG, *et al.* Structural determinants for HIV-1 integrase inhibition by beta-diketo acids. *J Biol Chem* 2002; 277: 12596-603.
- [20] Mazumder A, Neamati N, Pilon AA, Sunder S, Pommier Y. Chemical trapping of ternary complexes of human immunodeficiency virus type 1 integrase, divalent metal, and DNA substrates containing an abasic site. Implications for the role of lysine 136 in DNA binding. *J Biol Chem* 1996; 271: 27330-8.
- [21] Brodin P, Pinskaya M, Buckle M, *et al.* Disruption of HIV-1 integrase-DNA complexes by short 6-oxocytosine-containing oligonucleotides. *Biochemistry* 2002; 41: 1529-38.
- [22] Farnet CM, Wang B, Lipford JR, Bushman FD. Differential inhibition of HIV-1 preintegration complexes and purified integrase protein by small molecules. *Proc Natl Acad Sci USA* 1996; 93: 9742-7.
- [23] Farnet CM, Wang B, Hansen M, *et al.* Human immunodeficiency virus type 1 cDNA integration: new aromatic hydroxylated inhibitors and studies of the inhibition mechanism. *Antimicrob Agents Chemother* 1998; 42: 2245-53.
- [24] Hazuda DJ, Felock P, Witmer M, *et al.* Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. *Science* 2000; 287: 646-50.
- [25] Pauwels R, Andries K, Desmyter J, *et al.* Potent and selective inhibition of HIV-1 replication *in vitro* by a novel series of TIBO derivatives. *Nature* 1990; 343: 470-4.
- [26] Pluymers W, Pais G, Van Maele B, *et al.* Inhibition of human immunodeficiency virus type 1 integration by diketo derivatives. *Antimicrob Agents Chemother* 2002; 46: 3292-7.
- [27] Pommier Y, Johnson AA, Marchand C. Integrase inhibitors to treat HIV/Aids. *Nature Reviews Drug Discovery*, 2005; 4: 236-48.
- [28] Witvrouw M, Van Maele B, Vercammen J, *et al.* Novel inhibitors of HIV-1 integration. *Current Drug Metabolism*, 2004; 5: 291-304.
- [29] Johnson AA, Marchand C, Pommier Y. HIV-1 integrase inhibitors: A decade of research and two drugs in clinical trial. *Curr Top Med Chem* 2004; 4: 1059-7.
- [30] Richard de Soultrait V, Desjobert C, Tarrago-Litvak L. Peptides as new inhibitors of HIV-1 reverse transcriptase and integrase. *Curr Med Chem* 2003; 10: 1765-78.
- [31] Nair V. Novel inhibitors of HIV integrase: The discovery of potential anti-HIV therapeutic agents. *Curr Pharm Design* 2003; 9: 2553-65.
- [32] Neamati N. Patented small molecule inhibitors of HIV-1 integrase: a 10-year saga. *Expert Opin Ther Patents* 2002; 12: 709-24.
- [33] Marchand C, Johnson AA, Karki RG, *et al.* Metal-dependent inhibition of HIV-1 integrase by -diketo acids and resistance of the soluble double-mutant (F185K/C280S). *Mol Pharmacol* 2003; 64: 600-609.
- [34] Maurin C, Bailly F, Mbemba G, Mouscadet JF, Cotelle P. Unpublished results
- [35] Yi J, Asante-Appiah E, Skalka AM. Divalent Cations Stimulate Preferential Recognition of a Viral DNA End by HIV-1 Integrase. *Biochemistry* 1999; 38: 8458-68.
- [36] Van Uiter LG, Fernelius WC. Studies on coordination compounds: Correlation of the solution stabilities of the chelate compounds of ligands coordinating through nitrogen and oxygen. *J Amer Chem Soc* 1954; 76: 379-83.
- [37] Dayam R, Neamati N. Active site binding modes of the beta-diketoacids: a multi-active site approach in HIV-1 integrase inhibitor design. *Bioorg Med Chem* 2004; 12: 6371-81.
- [38] Maurin C, Bailly F, Buisine E, *et al.* Spectroscopic studies of diketoacids-metal interactions. A probing tool for the pharmacophoric intermetallic distance in the HIV-1 integrase active site. *J Med Chem* 2004; 47: 5583-6.
- \*[39] Selnick, H.G., Hazuda, D.J., Egbertson, M. *et al.*: WO9962513A1 (1999).
- \*[40] Young, S.D., Egbertson, M., Payne, L.S. *et al.*: WO9962520A1 (1999).
- \*[41] Young, S.D., Wai, J.S., Embrey, M.W., Fisher, T.E.: WO9962897A1 (1999).
- \*[42] Fujishita, T., Yoshinaga, T., Sato, A.: WO0039086A1 (2000).
- [43] Goldgur Y, Craigie R, Cohen, GH, *et al.* Structure of the HIV-1 integrase catalytic domain complexed with an inhibitor: a platform for antiviral drug design. *Proc Natl Acad Sci USA* 1999; 96: 13040-3.
- [44] Walker, M.A., Johnson, T.D., Meanwell, N.A., Banville, J.: WO01096283A2, WO01096283A3 (2001) and WO01096283C2 (2002).
- [45] Walker, M.A., Johnson, T.D., Oak, K.: WO01098248A2 and WO01098248A3 (2001).
- [46] Walker, M.A., Banville, J., Remillard, R., Plamondon, S.: WO03049690A2 and WO03049690A3 (2003).
- [47] Katoh, S., Miyazaki, S., Hubuka, N.: WO03016266A1 (2003).
- [48] Walker, M.A., Gulgeze, H.B., Naidu, N.B., Sorenson, M.E., Ueda, Y., Matiskella, J.: WO04062613A2 and WO04062613A3 (2004).
- [49] Naidu, N.B., Walker, M.A., Sorenson, M.E.: WO04096128A2 and WO04096128A3 (2004).
- [50] Mikamiyama, H., Iwata, M., Taoda, Y.: WO05061490A1 (2005).
- \*[51] Mekouar, K., D'Angelo, J., Desmaele, D., Mouscadet, J.-F., Subra, F., Auclair, C.: WO9845269A1 (1998).
- [52] Mekouar K, Mouscadet JF, Desmaele D, *et al.* Styrylquinoline derivatives: a new class of potent HIV-1 integrase inhibitors that block HIV-1 replication in CEM cells. *J Med Chem* 1998; 41: 2846-57.
- [53] Zouhiri F, Mouscadet JF, Mekouar K, *et al.* Structure-activity relationships and binding mode of styrylquinolines as potent inhibitors of HIV-1 integrase and replication of HIV-1 in cell culture. *J Med Chem* 2000; 43: 1533-40.
- [54] Bonnenfant S, Thomas CM, Vita C, *et al.* Styrylquinolines, integrase inhibitors acting prior to integration: a new mechanism of action for anti-integrase agents. *J Virol* 2004; 78: 5728-36.
- \*[55] Satoh, M., Kawakami, H., Itoh, Y. *et al.*: WO04046115A1 (2004).
- \*[56] Anthony, N.J., Gomez, R.P., Young, S.D. *et al.*: WO02030931A2 and WO02030931A3 (2002).
- [57] Johns, B.A.: WO04101512A2 and WO04101512A3 (2004).
- [58] Bailly F, Cotelle P. Anti-HIV activities of natural antioxidant caffeic acid derivatives: Toward an antiviral supplementation diet. *Curr Med Chem* 2005; 12: 1811-8.

- [59] Pluymers W, Neamati N, Pannecouque C, *et al.* Viral entry as the primary target for the anti-HIV activity of chicoric acid and its tetra-acetyl esters. *Mol Pharmacol* 2000; 58: 641-8.
- [60] Reinke RA, Lee DJ, McDougall BR, *et al.* L-chicoric acid inhibits human immunodeficiency virus type 1 integration *in vivo* and is a noncompetitive but reversible inhibitor of HIV-1 integrase *in vitro*. *Virology* 2004; 326: 203-19.
- [61] De Clercq, E., Debyser, Z., Pluymers, W., Pannecouque, C., Witvrouw, M.: WO02003971A2 and WO02003971A3 (2002).
- [62] Pannecouque C, Pluymers W, Van Maele B, *et al.* New class of HIV integrase inhibitors that block viral replication in cell culture. *Curr Biology* 2002; 12: 1169-1177.
- [63] Walker, M.A., Gulgeze, H.B., Banville, J., Remillard, R., Corson, D.: WO04103278A2 and WO04103278A3 (2004).
- [64] Rndo, R.F., Fennewald, S., Zendegui, J.G.: WO97039971 (1997).
- [65] Mazumder A, Neamati N, Ojwang JO, Sunder S, Rando RF, Pommier Y. Inhibition of the human immunodeficiency virus type 1 integrase by guanosine quartet structures. *Biochemistry* 1996; 35: 13762-71.
- [66] Cherepanov P, Este JA, Rando RF, *et al.* Mode of interaction of G-quartets with the integrase of human immunodeficiency virus type 1. *Mol Pharmacol* 1997; 52: 771-80.

- [67] Este JA, Cabrera C, Schols D, *et al.* Human immunodeficiency virus glycoprotein gp120 as the primary target for the antiviral action of AR177 (Zintevir). *Mol Pharmacol* 1998; 53: 340-5.
- [68] Billich A. S-1360 Shionogi-GlaxoSmithKline. *Curr Opin Invest Drugs* 2003; 4: 206-9.
- [69] Yoshinaga T, Sato A, Fujishita T, Fujiwara T. S-1360: *In vitro* activity of a new HIV-1 integrase inhibitor in clinical development. 9<sup>th</sup> Conference on Retroviruses and Opportunistic Infections. Seattle, USA (Feb. 24<sup>th</sup>-28<sup>th</sup>, 2002).
- [70] Rosemond MJ, St John-Williams L, Yamaguchi T, Fujishita T, Walsh JS. Enzymology of a carbonyl reduction clearance pathway for the HIV integrase inhibitor, S-1360: role of human liver cytosolic aldo-keto reductases. *Chem Biol Interact.* 2004; 147:129-39.
- [71] Barreca ML, Rao A, De Luca L, *et al.* Efficient 3D database screening for novel HIV-1 IN inhibitors. *J Chem Inf Comput Sci* 2004; 44: 1450-5.
- [72] Beale KK, Robinson WE Jr. Combinations of reverse transcriptase, protease, and integrase inhibitors can be synergistic *in vitro* against drug-sensitive and RT inhibitor-resistant molecular clones of HIV-1. *Antiviral Res* 2000; 46: 223-32.