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Review

Retroviral integrase inhibitors year 2000: update and perspectives

Yves Pommier *, Christophe Marchand, Nouri Neamati

Laboratory of Molecular Pharmacology, Division of Basic Sciences, National Cancer Institute, Bethesda, MD 20892-4255, USA

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Abstract

HIV-1 integrase is an essential enzyme for retroviral replication and a rational target for the design of anti-AIDS drugs. A number of inhibitors have been reported in the past 8 years. This review focuses on the recent developments in the past 2 years. There are now several inhibitors with known sites of actions and antiviral activity. The challenge is to convert these leads into drugs that will selectively target integrase in vivo, and can be added to our antiviral armamentarium. © 2000 Published by Elsevier Science B.V.

Keywords: Retroviruses; Integrase inhibitors; Resistance; Structure of integrases

1. Introduction

Combination of anti-retroviral therapy has recently changed the course and prognosis of acquired immuno deficiency syndrome (AIDS). It can suppress virus titers to undetectable levels. However, the virus persists in reservoirs such as peripheral blood mononuclear cells or resting T-lymphocytes. Emergence of resistant virus is also a limiting factor to reverse transcriptase and protease inhibitors. Because combination chemotherapy is an established procedure to limit the emergence of resistance to drugs (cf. treatment of tuberculosis and other systemic bacterial infections), it is logical to look for other antiviral targets. Integrase is such a target (Table 1).

Integration of the retroviral genome into cellular chromosomes takes place after reverse transcription. It is critical for retroviral gene expression and virus replication. Integration requires both the presence of the viral integrase and the integrity of the retroviral DNA ends. The structure, enzymology of integrase (IN) and inhibitors were recently reviewed in a series of manuscripts edited by Skalka (1999)) and in a review by (Neamati et al., 2000). This article will focus on the recent developments in the integrase

* Present address: National institute of health, Rm. 5D02, Bldg. 37, National Institutes of Health, Bethesda, MD 20892-4255, USA..

E-mail address: pommier@nih.gov (Y. Pommier).

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field with the perspective of antiviral drug development.

Discovery of retroviral integrase inhibitors is facilitated by the availability of recombinant enzyme, which can be assayed in vitro for inhibitors using oligonucleotides that mimic the retroviral DNA ends, which correspond to the tips of the LTR's (long terminal repeats) (Table 1), and by the existing structural information regarding the enzyme (see below). The use of preintegration complexes has recently been emphasized for the testing and discovery of integrase inhibitors (Hansen et al., 1999). They contain several proteins in association with integrase, which probably play a role in nuclear uptake of the proviral DNA-retroviral protein complexes, and possibly in the integration reaction.

2. Recent developments in determining the structure of retroviral integrases

Integrase proteins are highly conserved among retroviruses (Pommier et al., 1997; Esposito and Craigie, 1999; Wlodawer, 1999). Based on partial proteolysis experiments, the enzyme can be divided into three domains. The N-terminal domain (residues 1–50 for HIV-1 IN) contains 2 histidines and 2 cysteines that bind Zn^{2+}. The core domain (residues 50–212 for HIV-1 IN) contains the endonuclease and polynucleotidyl transferase site with its three acidic residues (Asp64, Asp116 and Glu152 for HIV-1 IN). These residues are highly conserved in the integrase superfamily and polynucleotide transferases. They bind divalent metals, Mn^{2+} or Mg^{2+} and are commonly referred to as the 'DDE motif'. The C-terminus domain (residues 212–288 for HIV-1 IN) contains basic amino acid residues and binds DNA. Its overall structure is comparable to that of the src-homology 3 (SH3) domain that can mediate protein–protein interactions. High resolution structure of each domain has been solved. The N- and C-terminus domains had been resolved by nuclear magnetic resonance (NMR) (reviewed in Esposito and Craigie, 1999), and the core domain by X-ray crystallography (reviewed in Wlodawer, 1999). Although each of these domains forms a dimer, it is not clear how the full size enzyme could be accommodated without invoking the formation of a tetra- and, possibly, an octamer.

Table 1
HIV-1 integrase as a therapeutic target

HIV-1 integrase is a rationale target because
It is essential for viral replication
There is no cellular equivalent
HIV integrase is a testable target because
Rapid and sensitive assays exist for testing enzyme activity and inhibitors
Sufficient enzyme can be produced by recombinant DNA technology
Oligonucleotides that mimic the retroviral long terminal repeats (LTR) facilitate drug discovery
Preintegration complexes have recently been emphasized as a target for inhibitors
Crystal and NMR structures are available for rational drug design

Recently, the combined structures of the core and C-terminus domain polypeptides were reported for SIV (Chen et al., 2000) and ASV integrase (Yang et al., 2000). The core dimer interface and structures are consistent for both structures and are in agreement with the previously reported core dimer structures. However, these new structures raise a number of questions. First, the C-terminal domain dimerization motif differs from those previously deduced from the NMR data. This apparent discrepancy raises the possibility that integrase C-terminal domains might associate in more than one way, which would be consistent with the formation of integrase multimers where the C-terminal domains would provide alternative multimerization motifs. Second, the relationship between the core and C-terminal domains is different in both structures, which again might indicate alternative associations between protein domains inside the integrase multimers. Finally, each study (Chen et al., 2000; Yang et al., 2000) proposes a different model for DNA binding to the enzyme. Thus, it will be important to resolve the structure of full-size integrase complexes, possibly in the presence of DNA.

Table 2
Inhibitors for which a possible site of action has been reported

Compound	References
Nucleotide binding site (Fig. 1)	
5-N_3-AZTMP	Drake et al. (1998)
Guanosine quartets	Jing et al. (2000a)
Other IN binding sites:	
Naphthalene disulfonate (Y3)	Lubkowski et al. (1998)
Diketo acids (5CITEP, see Fig. 2)	Goldgur et al. (1999)
Metal binding site (*polyhydroxylated aromatics*, Fig. 3)	
Granulatine	Pommier and Neamati (1999)
Salicylhydrazides	Neamati et al. (1998b)
Styrylquinolines	Zouhiri et al. (2000)
LTR DNA	
DNA minor groove binders; lexitropsins	Neamati et al. (1998a)
MAP30	Wang et al. (1999)

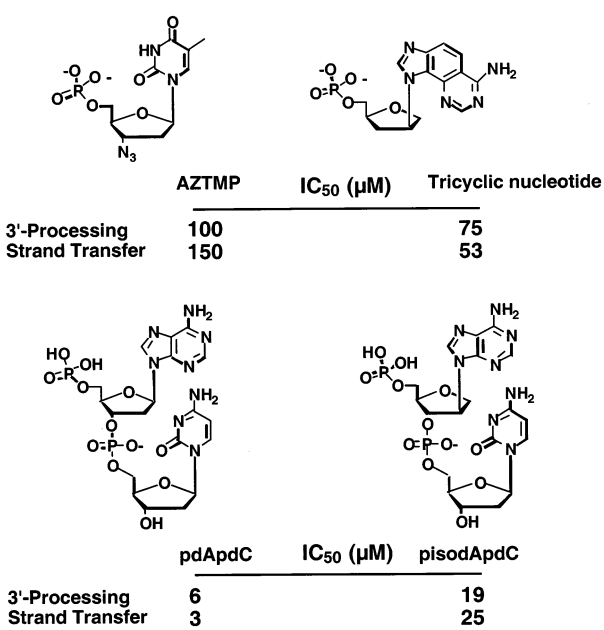

Fig. 1. Modified nucleotides inhibitors of HIV-1 integrase (for further references, see Drake et al., 1998; Zhang et al., 1998; Taktakishivli et al., 2000).

3. Recent developments in determining the sites of actions of integrase inhibitors

The inhibitors with proposed sites of action have been summarized in Table 2. Mononucleotides were the first inhibitors for which molecular interaction was proposed. Using radiolabeled AZT-mononucleotide derivatives and proteolytic mapping, it was demonstrated that 3′,5-diazido-2′,3′-dideoxyuridine 5′-monophosphate (5-N_3-AZTMP) binds to the α-4 helix of the HIV-1 core domain (Drake et al., 1998). Docking and site-directed mutagenesis showed three lysines (K156, 159 and 160) as a mononucleotide binding site in HIV-1 integrase (Drake et al., 1998). Simultaneously, Craigie and coworkers (Esposito and Craigie, 1998) found that the highly conserved lysine 159 was critical for the binding of DNA to integrase. It is likely that dinucleotides, such as, the pIsopdApdC (Taktakishivli et al., 2000; Fig. 1) and the guanosine quartets (Jing et al., 2000a) bind to the nucleotide site and compete with the DNA substrate. A high affinity interaction of HIV-1 integrase with single-stranded short oligonucleotides was recently reported (Caumont et al., 1999). Thus, further studies with modified oligonucleotides might lead to more effective integrase inhibitors.

Crystallographic studies have revealed the binding site of two classes of inhibitors outside the nucleotide binding site. First, a naphtalene disulfonate, that was discovered using a pharmacophore search (Nicklaus et al., 1997) was found to bind as a dimer to ASV integrase, and to affect the geometry of the DDE domain (Lubkowski et al., 1998). Recently, a diketo acid derivative was cocrystallized with the HIV-1 integrase core domain and was also found to bind and span both the DDE motif and the mononucleotide binding site (Goldgur et al., 1999; Fig. 2).

Chelation of the divalent metal cofactor (Mg^{2+} or Mn^{2+}) within the enzyme catalytic site has been proposed for hydroxylated aromatics (Fig. 4). However, direct evidence remains to be established for such a possibility.

The substrate DNA is another site of action for interfering with integrase activity. We first reported this possibility when we found that DNA intercalators were effective integration inhibitors (Fesen et

al., 1993). Auclair and coworkers re ported that DNA ligands forming triple helices in specific regions of the LTR were also inhibitory (Mouscadet et al., 1994; Bouziane et al., 1996; Brodin et al., 1999). More recently, we found that antiviral AT-specific minor groove ligands — the lexitropsins — inhibited integration by binding to a conserved AT stretch present and conserved in HIV-1 LTR's (Neamati et al., 1998a). We are currently investigating the conservation of the HIV LTR sequences by aligning the available HIV genomes (Feng and Pommier, unpublished). If these sequences are highly conserved, it might be possible to target them using new polyamines with hairpin-like structure that can recognize DNA sequences with high selectivity (White et al., 1998).

Polypeptides can also bind to the DNA and interfere with integrase binding and activity. This could be the mechanism for the anti-integrase and antiviral activity of MAP30 (Wang et al., 1999). MAP30 belongs to the family of ribosome inactivating proteins (RIP's). In addition to its anti-integration activity, it has DNA glycosylase/apurinic lyase activity (Wang et al., 1999). A recent report describes two new members of the RIP family — luffin and saporin — as potent inhibitors of HIV-1 integrase (Au et al., 2000). Whether interference with integrase is the key mechanism for the antiviral activity of MAP30 and other RIP's remains to be determined.

4. Update on the recent integrase inhibitors

The inhibitors reported in the past 2 years are as summarized in Table 3 Recently reported diketo acids represent a novel and selective class of HIV-1 integrase inhibitors with antiviral activity (Goldgur et al., 1999; Hazuda et al., 2000; Fig. 2). An important feature that sets these compounds apart from other inhibitors is their selectivity for the second step of the integrase reaction, the strand transfer step (Hazuda et al., 2000).

Highly modified mono- and di-nucleotides have been prepared and tested against HIV-1 integrase in vitro (Taktakishivli et al., 2000). These studies showed that in contrast to reverse transcriptase, HIV-1 integrase could accommodate bulky and highly modified nucleotides. Moreover, isodinucleotides are equally potent as normal dinucleotides against HIV-1 integrase (Taktakishivli et

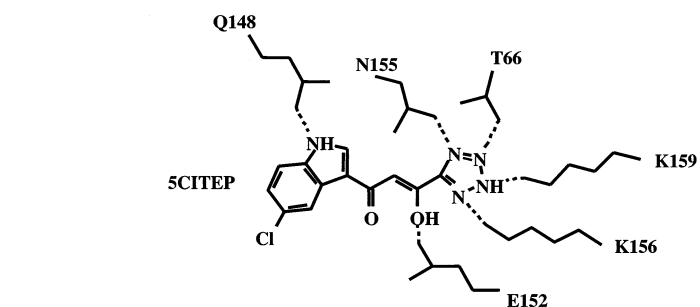

Fig. 2. Diketo acid integrase inhibitors. CITEP (1-(5-chloroindol-3-yl)-3-hydroxy-3-(2*H*-tetrazol-5-yl)-propenone was reported in Goldgur et al. (1999). The tetrazole ring is isosteric to carboxylic acid (masked carboxylic acid). The keto–enol is tautomeric with the diketo functionality. L-731 988 was reported in Hazuda et al. (2000).

Table 3
Recent inhibitors of retroviral integrases[a]

Compound	References
Diketo acids (Fig. 2)	
Merck derivatives (L-731,988, …)	Hazuda et al. (2000)
5CITEP	Goldgur et al. (1999)
Nucleotides (*see* Fig. 1):	
Tricyclic nucleotides	Zhang et al. (1998)
PisopdApdC	Taktakishivli et al. (2000)
Guanosine quartets	Jing et al. (2000a)
Thiazolothiazepines (see Fig. 3)	Neamati et al. (1999)
Chicoric acid derivatives; caffeoylquinic acids	Neamati et al. (1997a,b), Robinson (1998), King and Robinson (1998), King et al. (1999), Lin et al. (1999), Zhu et al. (1999)
Integric acid derivatives	Singh et al. (1999)
Equisetin, phomasetin	Hazuda et al. (1999), Singh et al. (1999)
Lamellarin α-20-sulfate	Reddy et al. (1999a)
Salicylhydrazides (see Fig. 4)	Neamati et al. (1998b)
Polyhydroxylated styrylquinolines	Mekouar et al. (1998), Zouhiri et al. (2000)
RIP's: MAP30, luffin, saporin	Wang et al. (1999), Au et al. (2000)

[a] For comprehensive reviews on previously reported inhibitors, see Neamati et al. (1997d), Pommier and Neamati (1999), Neamati et al. (2000).

al., 2000). Improved stability of isonucleotides toward nucleases warrants synthesis of more potent analogs. Longer oligonucleotides that form four-stranded guanosine quartet structures have been further investigated (Jing et al., 2000b). Based on studies using guanosine quartet of various lengths, we have recently been able to suggest that the integrase interaction involves the terminal loop structure of the oligonucleotides (Jing et al., 2000a).

Thiazolothiazepines were discovered by screening antiviral compounds from the NCI antiviral screen for anti-integrase activity in our in vitro screen (Neamati et al., 1999). A more extensive set of derivatives was synthesized in collaboration with Dr Nacci (University of Sienna, Italy). Three of these derivatives are shown in Fig. 3. By contrast to polyphenols, thiazolothiazepines are active in Mg^{2+}-based assays, which are potentially important for drug development because Mg^{2+} rather than Mn^{2+} has been proposed to be the physiological divalent metal cofactor for integrase in vivo (Hazuda et al., 1997). Thiazolothiazepines are active in preassembled integrase-DNA complexes, which suggests that they might bind to and inactivate these complexes. Thiazolothiazepines are antiviral, although at relatively high concentrations (Fig. 3), and they represent a new lead class for developing antiviral inhibitors of integrase.

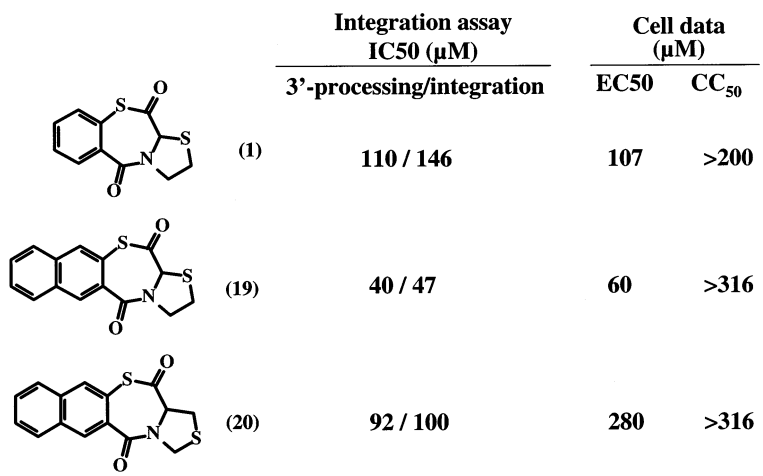

Fig. 3. Structure and activities of thiazolothiazepines. For each derivative, the number in parenthesis corresponds to the one used in the reference from Neamati et al. (1999).

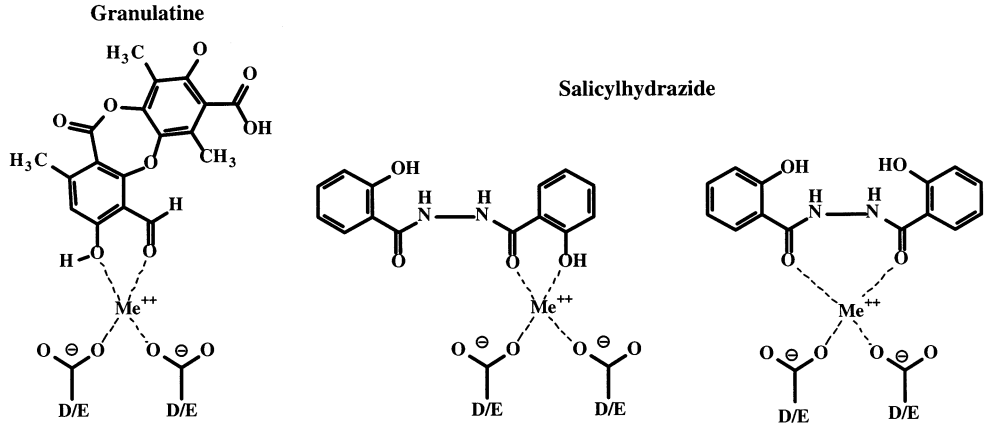

Fig. 4. Hypothetical chelation of the metal cofactor in the integrase catalytic site by hydroxylated aromatics. Granulatine is shown on the left (Neamati et al., 1997a; Pommier and Neamati, 1999). Two potential chelation models are shown for salicylhydrazides (Neamati et al., 1998a). Any one of the three acidic residues (D/E) (Asp64, Asp116 and Glu152) involved in metal binding could be implicated in the enzyme/drug/metal complex formation.

Dicaffeoylquinic acids were reported to bind to HIV-1 integrase irreversibly (Zhu et al., 1999). This observation is not surprising and in fact was reported earlier with a prototype hydroxylated aromatic, CAPE, and other hydroxylated aromatics (Stanwell et al., 1996). Extensive structure–activity studies with chicoric acids revealed that the catechol moieties or their acetylated substituents and the carboxyls are essential for anti-integrase and antiviral activity (King et al., 1999; Lin et al., 1999).

Lamellarin α-20-sulfate, an ascidian alkaloid, was recently obtained from screening of marine natural products and inhibited HIV-1 integrase with IC_{50} values in the low μM range (Reddy et al., 1999a). In cell-based assays, lamellarin α-20-sulfate inhibits the early steps of HIV-1 replication (Reddy et al., 1999a).

Other novel inhibitors of integrase were identified from screening microbial extracts of natural product libraries. These include equisetin and phomasetin (Hazuda et al., 1999; Singh et al., 1999) and integric acid derivatives (Singh et al. 1999). All these compounds inhibit HIV-1 integrase in the low micromolar range. However, at present, their selectivity for integrase and their antiviral properties are not established.

Styrylquinolines (Mekouar et al., 1998; Zouhiri et al., 2000) are recent examples of polyhydroxylated aromatics. They inhibit HIV-1 integrase in vitro within similar range as other previously reported polyhydroxylated salicylic acids (Neamati et al., 1997a). Some styrylquinolines showed activities against HIV-1 replication in CEM cells using a 3-day cytoprotection assay (Zouhiri et al., 2000). These conditions are different from the standard NCI antiviral screening protocol, where viral infection (MOI = 0.1) is evaluated after 6 days. Under these conditions, polyhydroxylated aromatics generally show considerable cytotoxicity, which obscures their potential antiviral activity (Pommier and Neamati, 1999).

5. Is integrase the target of reported antiviral inhibitors?

The integrase inhibitors that are also antiviral are listed in Table 4, which also lists the known target(s) for each of these compounds. As discussed above, the only class of inhibitors which appear to selectively target integrase are the diketo acids (Hazuda et al., 2000). Whether thiazolothiazepines and polyhydroxylated styrylquinolines selectively target integrase remains to be demonstrated.

Finding an integrase inhibitor with antiviral activity cannot be translated into the assumption

that integrase is the target of the compound. Counter-screening with other retroviral targets, such as, reverse transcriptase, protease, and Gp120, are useful. However, negative data can only provide indirect evidence. Time–course experiments have also been used to infer the drug target. By adding the drug at various times after infection, it is possible to infer at which stage the drug targets the HIV replication. Integrase inhibitors should act after virus entry and reverse transcription but before retroviral gene expression. Recently, two criteria were established to suggest that integrase is the target of the drug, i.e. (1) selection of drug-resistant virus with integrase mutation; and (2) accumulation of circular DNA forms in drug-treated cells (Hazuda et al., 2000). Regarding the first criterion, obtaining a drug-resistant viral strain with an integrase mutation is not sufficient to demonstrate that integrase is the drug target. For instance, in the case of the chicoric acid, an integrase point mutation was found at residue 140 (King and Robinson, 1998). However, we recently found that integrase point mutants bearing this mutation are not resistant to chicoric acid derivatives in vitro, which suggests that integrase is not the only target of these drugs (Pluymers et al., 2000). In agreement with this possibility, we found that chicoric acids do not inhibit the replication of virus strains that are resistant to polyanionic and polycationic compounds (Pluymers et al., 2000). Furthermore, HIV-1 strains resistant to chicoric acid contain multiple mutations in the envelope glycoprotein Gp120 (Pluymers et al., 2000). Thus, it is important to develop cellular assays to show unambiguously that integrase is the target of an antiviral drug. Recently, cell lines have been engineered containing an inducible integrase gene. Expression of wild-type sequence integrase kills cells while expression of integrase-point mutants that are catalytically inactive have no effect (Parissi et al., 2000). This approach should be useful to look for selective inhibitors of integrase that are not cytotoxic to uninfected and host cells.

Table 4
HIV-1 integrase inhibitors with antiviral activity: is integrase the target[a]

	Target(s)	References
Nucleotides	RT+IN?	Carroll et al. (1994), Drake et al. (1998)
Guanosine quartets	Gp120+IN?	Este et al. (1998), Jing et al. (2000a)
Lexitropsins	RT+IN?	Filipowsky et al. (1996), Neamati et al. (1998a), Reddy et al. (1999b)
Temacrizine	?	Turpin et al. (1998)
Cosalane derivative	Gp120	Cushman et al. (1995)
Biscoumarins	PR+IN?	Mazumder et al. (1996)
Flavones	Multiple?	Fesen et al. (1994)
Dicaffeoylquinic acid, chicoric acid	Gp120+IN?	Pluymers et al. (2000)
Hypericin	Fusion+IN?	Farnet et al. (1998)
Depsides (granulatine)	IN?	Neamati et al. (1997a)
Tetracycline derivatives	IN?	Neamati et al. (1997b)
2-Mercaptobenzene-sulfonamides	IN?	Neamati et al. (1997c)
Thiazolothiazepines	IN?	Neamati et al. (1999)
Polyhydroxylated styrylquinolines	IN?	Mekouar et al. (1998), Zouhiri et al. (2000)
Diketo acids	IN	Goldgur et al. (1999), Hazuda et al. (2000)

[a] RT, reverse transcriptase; IN, integrase; Gp120, glycoprotein 120; PR, protease.

6. Why is it taking so much time to identify antiviral drugs that target integrase?

First, one has to realize that integrase has a low turnover rate. Indeed, the 3′-processing reaction that removes the terminal nucleotides following the conserved AC residues takes place in the cytoplasm of the infected cell, immediately after reverse transcription. Following this reaction, integrase remains bound to the proviral DNA inside the preintegration complexes and is transferred to the nucleus where it catalyzes the second step in the integration reaction, the 3′-end-joining (integration) step, which insert the 3′-ends into a host chromosome. These two reactions are separated

by several hours and the enzyme remains bound to the DNA all this time. Thus, the enzyme acts in a single-turn over reaction, and inhibitors have to be very tightly bound to be effective in vivo.

Secondly, because integrase has no known cellular equivalent (which is an advantage for selective drug design), no lead compound was known when the search for inhibitors was initiated. Almost 10 years later, a large number and variety of lead compounds have been identified (Neamati et al., 1997d; Pommier and Neamati, 1999; Pommier et al., 1997). However, turning these lead compounds into inhibitors takes time.

Thirdly, the number of research groups both in the academic institutions and pharmaceutical companies focused on integrase remains limited, and discovery of integrase inhibitors would probably be accelerated if more resources were devoted to this effort. To our knowledge, the only identified retroviruses that are relevant to human health presently are the HIV's and the HTLV's. This relatively narrow spectrum might limit the involvement of drug companies to tackle these research areas. However, we believe that it is a matter of time before antiviral integrase inhibitors will be available.

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References

Au, T.K., Collins, R.A., Lam, T.L., Ng, T.B., Fong, W.P., Wan, D.C., 2000. The plant ribosome inactivating proteins luffin and saporin are potent inhibitors of HIV-1 integrase. FEBS Lett. 471, 169–172.

Bouziane, M., Cherny, D.I., Mouscadet, J.F., Auclair, C., 1996. Alternate strand DNA triple helix-mediated inhibition of HIV-1 U5 long terminal repeat integration in vitro. J. Biol. Chem. 271, 10359–10364.

Brodin, P., Pinskaya, M., Volkov, E., Romanova, E., Leh, H., Auclair, C., Mouscadet, J., Gottikh, M., 1999. Branched oligonucleotide-intercalator conjugate forming a parallel stranded structure inhibits HIV-1 integrase. FEBS Lett. 460, 270–274.

Carroll, S.S., Geib, J., Olsen, D.B., Stahlhut, M., Shafer, J.A., Kuo, L.C., 1994. Sensitivity of HIV-1 reverse transcriptase and its mutants to inhibition by azidothymidine triphosphate. Biochemistry 33, 2113–2120.

Caumont, A., Jamieson, G., de Soultrait, V.R., Parissi, V., Fournier, M., Zakharova, O.D., Bayandin, R., Litvak, S., Tarrago-Litvak, L., Nevinsky, G.A., 1999. High affinity interaction of HIV-1 integrase with specific and non-specific single-stranded short oligonucleotides. FEBS Lett. 455, 154–158.

Chen, Z., Yan, Y., Munshi, S., Li, Y., Zugay-Murphy, J., Xu, B., Witmer, M., Felock, P., Wolfe, A., Sardana, V., Emini, E.A., Hazuda, D., Kuo, L.C., 2000. X-ray structure of Simian immunodeficiency virus integrase containing the core and C-terminal domain (residues 50–293) — an initial glance of the viral DNA-binding platform. J. Mol. Biol. 296, 521–533.

Cushman, M., Golebiewski, W.M., Pommier, Y., Mazumder, A., Reymen, D., De Clerq, E., Grahm, L., Rice, W.G., 1995. Cosalane analogues with enhanced potencies as inhibitors of HIV-1 protease and integrase. J. Med. Chem. 38, 443–452.

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

Esposito, D., Craigie, R., 1998. Sequence selectivity of viral end DNA binding by HIV-1 integrase reveals critical regions for protein-DNA interactions. EMBO J. 17, 5832–5843.

Esposito, D., Craigie, R., 1999. HIV integrase structure and function. In: Skalka, A.M. (Ed.), Advances in Virus Research, vol. 52. Academic Press, San Diego, pp. 319–333.

Este, J.A., Cabrera, C., Schols, D., Cherepanov, P., Gutierrez, A., Witvrouw, M., Pannecouque, C., Debyser, Z., Rando, R.F., Clotet, B., Desmyter, J., De Clercq, E., 1998. Human immunodeficiency virus glycoprotein Gp120 as the primary target for the antiviral action of AR177 (Zintevir). Mol. Pharmacol. 53, 340–345.

Farnet, C.M., Wang, B., Hansen, M., Lipford, J.R., Zalkow, L., Robinson, W.E., Siegel, J., Bushman, F., 1998. Human immunodeficiency virus type 1 cDNA integration: new aromatic hydroxylated inhibitors and studies of the inhibition mechanism. Antimicrob. Agents Chemother. 42, 2245–2253.

Fesen, M.R., Kohn, K.W., Leteurtre, F., Pommier, Y., 1993. Inhibitors of human immunodeficiency virus integrase. Proc. Natl. Acad. Sci. USA 90, 2399–2403.

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

Filipowsky, M.E., Kopka, M.L., Brazil-Zison, M., Lown, J.W., Dickerson, R.E., 1996. Linked lexitropsins and the in vitro inhibition of HIV-1 reverse transcriptase RNA-directed DNA polymerization: a novel induced-fit of 3,5 *m*-pyridyl bisdistamycin to enzyme-associated template-primer. Biochemistry 35, 15397–15410.

Goldgur, Y., Craigie, R., Cohen, G.H., Fujiwara, T., Yoshinaga, T., Fujishita, T., Sugimoto, H., Endo, T., Murai, H., 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. USA 96, 13040–13043.

Hansen, M.S.T., Smith, G.J.I., Kafri, T., Molteni, V., Siegel, J.S., Bushman, F.D., 1999. Integration complexes derived from HIV vectors for rapid assays in vitro. Nat. Biotechnol. 17, 578–582.

Hazuda, D.J., Felock, P.J., Hastings, J.C., Pramanik, B., Wolfe, A.L., 1997. Differential divalent cation requirements uncouple the assembly and catalytic reactions of human immunodeficiency virus type 1 integrase. J. Virol. 71, 7005–7011.

Hazuda, D., UncapherBlau, C., Felock, P., Hastings, J., Pramanik, B., Wolfe, A., Bushman, R., Farnet, C., Goetz, M., Williams, M., Silverman, K., Lingham, R., Singh, S., 1999. Isolation and characterization of novel human immunodeficiency virus integrase inhibitors from fungal metabolites. Antiviral Chem. Chemother. 10, 63–70.

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

Jing, N., Marchand, C., Liu, J., Mitra, R., Hogan, M.E., Pommier, Y., 2000a. Mechanism of inhibition of HIV-1 integrase by G-tetra forming oligonucleotides. J. Biol. Chem. 275, 21460–21467.

Jing, N., de Clercq, E., Rando, R.F., Pallansch, L., Lackman-Smith, C., Lee, S., Hogan, M.E., 2000. Stability–activity relationships of a family of G-tetrad forming oligonucleotides as potent HIV inhibitors: a basis for anti-HIV drug design. J. Biol. Chem. 275, 3421–3430.

King, P.J., Robinson, W.E., 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.

King, P.J., Ma, G., Miao, W., Jia, Q., McDougall, B.R., Reinecke, M.G., Cornell, C., Kuan, J., Kim, T.R., Robinson, W.E. Jr, 1999. Structure–activity relationships: analogues of the dicaffeoylquinic and dicaffeoyltartaric acids as potent inhibitors of human immunodeficiency virus type 1 integrase and replication. J. Med. Chem. 42, 497–509.

Lin, Z., Neamati, N., Zhao, H., Kiryu, Y., Turpin, J.A., Aberham, C., Strebel, K., Kohn, K., Witvrouw, M., Pannecouque, C., Debyser, Z., De Clercq, E., Rice, W.G., Pommier, Y., Burke, T.R. Jr, 1999. Chicoric acid analogues as HIV-1 integrase inhibitors. J. Med. Chem. 42, 1401–1414.

Lubkowski, J., Yang, F., Alexandratos, J., Wlodawer, A., Zhao, H., Burke, T.R., Neamati, N., Pommier, Y., Merkel, G., Skalka, A.M., 1998. Structure of the catalytic domain of avian sarcoma virus integrase with a bound HIV-1 integrase-targeted inhibitor. Proc. Natl. Acad. Sci. USA 95, 4831–4836.

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

Mekouar, K., Mouscadet, J.F., Desmaele, D., Subra, F., Leh, H., Savoure, D., Auclair, C., d'Angelo, J., 1998. Styrylquinoline derivatives: a new class of potent HIV-1 integrase inhibitors that block HIV-1 replication in CEM cells. J. Med. Chem. 41, 2846–2857.

Mouscadet, J.F., Carteau, S., Goulaouic, H., Subra, F., Auclair, C., 1994. Triplex-mediated inhibition of HIV DNA integration in vitro. J. Biol. Chem. 269, 21634–21638.

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

Neamati, N., Hong, H., Sunder, S., Milne, G.W.A., Pommier, Y., 1997. 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.

Neamati, N., Mazumder, A., Sunder, S., Owen, J.M., Schultz, R.J., Pommier, Y., 1997. 2-Mercaptobenzenesulfonamides as novel inhibitors of human immunodeficiency virus type 1 integrase and replication. Antiviral Chem. Chemother. 8, 485–495.

Neamati, N., Sunder, S., Pommier, Y., 1997. Design and discovery of HIV-1 integrase inhibitors. Drug Discovery Today 11, 487–498.

Neamati, N., Mazumder, A., Sunder, S., Owen, J.M., Tandon, M., Lown, J.W., Pommier, Y., 1998. Highly potent synthetic polyamines, bis-distamycins and lexitropsins as inhibitors of human immunodeficiency virus type 1 integrase. Mol. Pharmacol. 54, 280–290.

Neamati, N., Hong, H., Owen, J.M., Sunder, S., Winslow, H.E., Christensen, J.L., Zhao, H., Burke, T.R., Milne, G.W.A., Pommier, Y., 1998. Salicylhydrazine-containing inhibitors of HIV-1 integrase: implication for a selective chelation in the integrase active site. J. Med. Chem. 41, 3202–3209.

Neamati, N., Turpin, J.A., Winslow, H.E., Christensen, J.L., Williamson, K., Orr, A., Rice, W.G., Pommier, Y., Garofalo, A., Brizzi, A., Campiani, G., Fiorini, I., Nacci, V., 1999. Thiazolothiazepine inhibitors of HIV-1 integrase. J. Med. Chem. 42, 3334–3341.

Neamati, N., Marchand, C., Pommier, Y., 2000. HIV-1 integrase inhibitors: past, present and future. Adv. Pharmacol. 49, 147–165.

Nicklaus, M., Neamati, N., Hong, H., Mazumder, A., Sunder, Y., Chen, J., Milne, G.W.A., Pommier, Y., 1997. HIV-1 integrase pharmacophore: discovery of inhibitors through 3-D database searching. J. Med. Chem. 40, 920–929.

Parissi, V., Caumont, A.B., deSoultrait, V.R., Calmels, C., Pichuantes, S., Litvak, S., Dupont, C.H., 2000. Selection of amino acid substitutions restoring activity of HIV-1 inte-

grase mutated in its catalytic site using the yeast *Saccharomyces cerevisiae*. J. Mol. Biol. 295, 755–765.

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

Pommier, Y., Neamati, N., 1999. Inhibitors of human immunodeficiency virus integrase. Adv. Virus Res. 52, 427–458.

Pommier, Y., Pilon, A.A., Bajaj, K., Mazumder, A., Neamati, N., 1997. HIV-1 integrase as a target for antiviral drugs. Antiviral Chem. Chemother. 8, 463–483.

Reddy, M.V., Rao, M.R., Rhodes, D., Hansen, M.S., Rubins, K., Bushman, F.D., Venkateswarlu, Y., Faulkner, D.J., 1999. Lamellarin α-20-sulfate, an inhibitor of HIV-1 integrase active against HIV-1 virus in cell culture. J. Med. Chem. 42, 1901–1907.

Reddy, B.S.P., Sondhi, S.M., Lown, J.W., 1999. Synthetic DNA minor groove-binding drugs. Pharmacol. Ther. 84, 1–111.

Robinson, W.E., 1998. L-chicoric acid, an inhibitor of human immunodeficiency virus type 1 (HIV-1) integrase, improves on the in vitro anti-HIV-1 effect of zidovudine plus a protease inhibitor (AG1350). Antiviral Res. 39, 101–111.

Singh, S.B., Zink, D., Polishook, J., Valentino, D., Shafiee, A., Silverman, K., Felock, P., Teran, A., Vilella, D., Hazuda, D.J., Lingham, R.B., 1999. Structure and absolute stereochemistry of HIV-1 integrase inhibitor integric acid. A novel eremophilane sesquiterpenoid produced by a *Xylariai* sp. Tetrahedron Lett. 40, 8775–8779.

Skalka, A.M., 1999. Retroviral integration. In: Maramorosh, K., Murphy, F., Shatkin, A.J. (Eds.), Advances in Virus Research, vol. 52. Academic Press, San Diego, pp. 271–475.

Stanwell, C., Ye, B., Yuspa, S.H., Burke, T.R., 1996. Cell protein-rosslinking by erbstatin and related compounds. Biochem. Pharmacol. 52, 475–480.

Taktakishivli, M., Neamati, N., Pommier, Y., Nair, V., 2000. Recognition and inhibition of HIV integrase by novel dinucleotides. J. Am. Chem. Soc. 122, 5671–5677.

Turpin, J.A., Buckheit, R.W. Jr, Derse, D., Hollingshead, M., Williamson, K., Palamone, C., Osterling, M.C., Hill, S.A., Graham, L., Schaeffer, C.A., Bu, M., Huang, M., Cholody, W.M., Michejda, C.J., Rice, W.G., 1998. Inhibition of acute-, latent-, and chronic-phase human immunodeficiency virus type 1 (HIV-1) replication by a bistriazoloacridone analog that selectively inhibits HIV-1 transcription. Antimicrob. Agents Chemother. 42, 487–494.

Wang, Y.X., Neamati, N., Jacob, J., Palmer, I., Stahl, S.J., Kaufman, J.D., Huang, P.L., Winslow, H.E., Pommier, Y., Wingfield, P.T., Lee-Huang, S., Bax, A., Torchia, D.A., 1999. Solution structure of anti-HIV-1 and anti-tumor protein MAP30: structural insights into its multiple functions. Cell 99, 433–442.

White, S., Szewczyk, J.W., Turner, J.M., Baird, E.E., Dervan, P.B., 1998. Recognition of the four Watson–Crick base pairs in the DNA minor groove by synthetic ligands. Nature 391, 468–470.

Wlodawer, A., 1999. Crystal structure of catalytic core domains of retroviral integrases and role of divalent cations in enzymatic activity. In: Skalka, A.M. (Ed.), Advances in Virus Research, vol. 52. Academic Press, San Diego, pp. 335–350.

Yang, Z.N., Mueser, T.C., Bushman, F.D., Hyde, C.C., 2000. Crystal structure of an active two-domain derivative of Rous Sarcoma virus integrase. J. Mol. Biol. 296, 535–548.

Zhang, J., Neamati, N., Nair, V., 1998. Inhibition of HIV integrase by novel nucleotides bearing tricyclic bases. Biorg. Med. Chem. Lett. 8, 1887–1890.

Zhu, K., Cordeiro, M.L., Atienza, J., Robinson, W.E. Jr, Chow, S.A., 1999. Irreversible inhibition of human immunodeficiency virus type 1 integrase by dicaffeoylquinic acids. J. Virol. 73, 3309–3316.

Zouhiri, F., Mouscadet, J.F., Mekouar, K., Desmaele, D., Savoure, D., Leh, H., Subra, F., Le Bret, M., Auclair, C., d'Angelo, J., 2000. 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. 43, 1533–1540.