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HIV-1 integrase inhibitors as new components of antiviral therapy

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Abstract. Structural and functional features of HIV-1 integrase are considered and the state of the art in the quest for effective inhibitors of this enzyme is reported. The major classes of integrase inhibitors with known mechanisms of action as well as their *in vitro* and *in vivo* inhibitory activities are presented. The bibliography includes 80 references.

I. Introduction

Human immunodeficiency virus (HIV) affects the immune system and causes one of the most dangerous diseases, the acquired immunodeficiency syndrome (AIDS). Today, this disease is the reason of one of the most abundant epidemics. According to the estimates of the Joint United Nations Programme on HIV/AIDS (UNAIDS), in 2007, the total number of HIV-infected people all over the world was more than 33 millions. In the Russian Federation, since 1987 till now, 403 thousand of HIV-infected persons were recorded. However, the real number of HIV-infected people living in Russia is obviously higher. According to the UNAIDS estimates, the number of HIV-infected people in Russia approaches 1 million. It should also be noted that the rate of HIV infection spread in Russia continues to increase. According to the data from the Russian Federal Inspection of Consumption, in the first eight months of 2007, more than 28 974 new HIV contagion events were recorded in our country, which is 12% higher that in the analogous period of the past year.

At present, the therapy of HIV-infected people involves the use of drugs that inhibit three steps in the virus life cycle, namely, reverse transcription, proteolytic maturing and penetration into the host cell.[1, 2] The complex therapy, the so-called Highly Active Antiretroviral Therapy (HAART), includes the following three drugs: a HIV protease inhibitor or a non-nucleoside inhibitor of the viral reverse transcriptase and two nucleoside inhibitors of reverse transcriptase. In those cases where the standard therapy proves to be low effective, the only so far known inhibitor of virus penetration into the host cell, enfuvirtide (also referred to as DP-178 or T-20) is added.[2] This synthetic peptide has a sequence corresponding to the sequence of 643 – 678 amino acids of HIV-1 glycoprotein gp160. The necessity of simultaneous use of several drugs is caused by the fact that HIV rapidly evolves to produce drug-resistant viral forms. This therapy is often poorly withstood by patients. To make it effective, the patient should regularly ingest a large number of drugs and, in some cases, keep to a special diet. Moreover, the drugs included in HAART are rather toxic and their use often increases the cholesterol level in blood and leads to lipodystrophy. Finally, it should be noted that the modern complex therapy does not entirely stop the HIV replication or, in other words, merely makes its possible to prolong the life of AIDS-infected people rather than completely cure them.[1, 2] The aforesaid is associated with the peculiarities of the virus replicative cycle.

Human immunodeficiency virus belongs to the family of retroviruses with the genome represented by an RNA molecule in the virion. After the virus has penetrated into a human cell, a DNA copy of the viral genome is synthesised and incorporated (integrated) into the human cell genome. Both processes are mediated by viral enzymes, namely, reverse transcriptase and integrase, respectively. After the incorporation of the HIV genome into the host cell DNA, it is virtually impossible to stop the virus multiplication, because the further synthesis of the viral proteins and RNA in the cell is now mediated by cell enzymes. Thus, the virus replication can be completely blocked only in the early stages of its insertion into a cell, *i.e.*, reverse transcription and integration. To date, a large number of inhibitors of the HIV reverse transcriptase activity were developed and some of them are involved in the HAART therapy. However, the viral reverse transcriptase belongs to the class of polymerases so that the majority of its blockers suppress to one extent or another the action of cell enzymes of this class, which leads to considerable side effects. For this

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reason, the design of new drugs on the basis of an HIV-1 integrase inhibitor is a very important problem.

In October 2007, the USA Food and Drug Administration (FDA) approved the use of a new anti-AIDS product, Isentress (Raltegravir) manufactured by the Merck Pharmaceuticals. Attention drawn to this event is explained by the fact that Isentress belongs to a basically new class of antiviral drugs, namely, it is the first approved inhibitor of catalytic activity of HIV-1 integrase.

There are two reasons for the high therapeutic potential of integrase inhibitors.[1] First, integrase is one of the key members of the virus replicative cycle. Thus it was shown that with the inhibition of activity of this enzyme, the virus replication may continue for a certain time due to the formation of circular forms of viral DNA in the host cell nucleus; however, this episome DNA is unstable and undergoes fast degradation.[3] Second, integrase has no counterpart in the host cell and, hence, the inhibition of its activity should not disturb the normal cell metabolism processes.

To help the readers to gain deeper insight into the principles of action of integrase inhibitors and understand the problems one faces in the development of such inhibitors, this review presents brief information on the structure and functions of the enzyme itself. Comprehensive information can be found in reviews.[4, 5]

II. HIV-1 integrase: structure and functions

HIV-1 integrase represents a protein with the molecular mass of 32 kDa, which is built of 288 amino acid residues. It is assigned to the family of polynucleotide transferases, which includes retroviral integrases, transposases, recombination proteins RuvC and RuvX and some other proteins.[6] Enzymes of this family catalyse the processes that lead to the transfer of DNA or its fragments within a genome or between genomes. These processes occur without invoking any additional energy sources and, ultimately, entail no changes in the number of phosphodiester bonds. Enzymes of this family are characterised by the formation of stable complexes with the DNA-substrate; however, reactions they catalyse do not involve the formation of a covalent protein – DNA bond.[7]

To mediate integration, integrase should bind two DNA molecules at once, namely, a viral DNA (designated DNA-substrate) and a host cell DNA (DNA-target). Note that the binding of the viral DNA is sequence-specific [integrase recognises the end sequences of U5 and U3 segments in long terminal repeats (LTR) in the viral DNA (Fig. 1)], whereas the binding of the host cell DNA is independent of its nucleotide sequence. Integration includes several steps[8, 9] and begins in the cytoplasm of HIV-infected cells. There, after the completion of reverse transcription of the viral RNA genome, integrase binds the viral DNA (DNA-substrate) to form the so-called pre-integration complex (PIC). The structural arrangement of PIC and the role of its components are not yet totally clear. It is known that in addition to integrase and viral DNA, it includes viral reverse transcriptase and certain viral and host proteins.[10, 11] A catalytically active PIC can be isolated from HIV-infected cells.[12]

Following binding of the viral DNA, integrase catalyses the 3′-processing reaction, which represents the endonucleolytic cleavage of DNA leading to the elimination of GT dinucleotide from the 3′ end of each strand of the DNA-substrate. Then, PIC is transported from cytoplasm to the infected cell nucleus where integrase catalyses the strand transfer reaction. In the course of this reaction, 3′-hydroxyl groups of processed strands

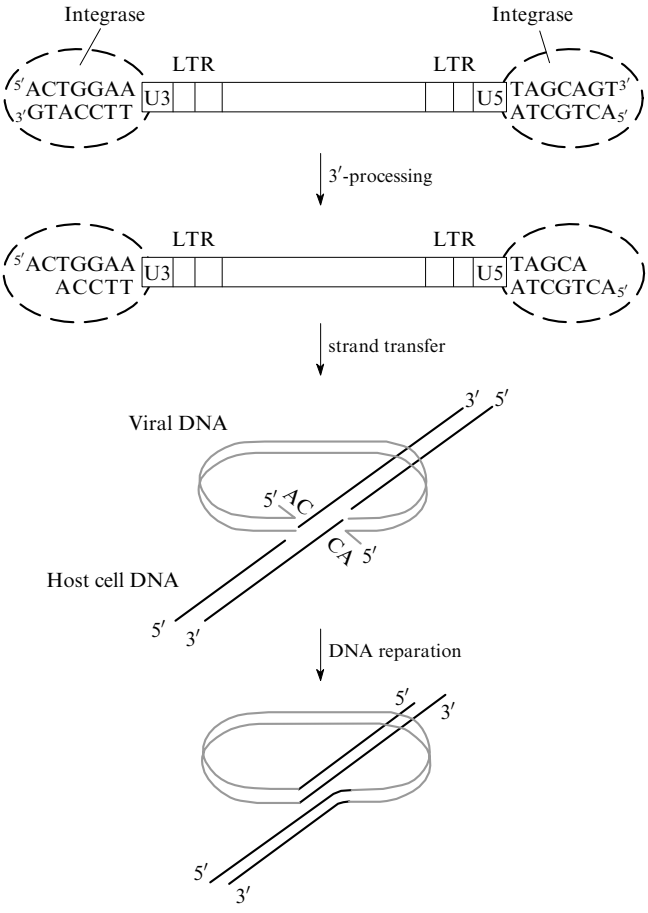


Figure 1. A scheme of integration of a viral DNA to a host cell DNA mediated by viral integrase.

of the viral DNA attack internucleotide phosphates in both strands of the cell DNA (DNA-target) to form a covalently bound product. Internucleotide bonds located in different strands of the DNA-target at a distance of five nucleotide pairs from one another undergo transesterification. The viral DNA may insert in any site of the cell DNA, but preferentially inserts in the genome sequences with the defect or weakened stacking interactions.[13]

In the HIV-1 integrase structure, three domains may be singled out, namely, N-terminus, catalytic core and C-terminus.[14, 15] Up to recently, despite repeated attempts, no precise information could be gained on the spatial structure of the full-sized enzyme. The failures were associated with the poor solubility of integrase and also with its conformational mobility and susceptibility to aggregation, which complicate the integrase crystallisation. Nonetheless, X-ray diffraction analysis and NMR spectroscopy allowed the structures of individual domains as well as of proteins that involve the N-terminal and catalytic domains of integrase to be established.[5, 16–20]

The catalytic domain has the most conservative primary structure as compared with other domains of HIV-1 integrase. Its structure resembles that of catalytic domains of other retroviral integrases and transposases.[21] The catalytic domain comprises amino acid residues from 50 to 212 of which three residues, Asp64, Asp116 and Glu152, form the active catalytic site of the enzyme. They are arranged into the so-called D,D(35)E-motif typical of catalytic domains of the majority of enzymes of the polynucleotide transferase class.[21] Muta-

tions of any amino acid residue from the catalytic triad are crucial for the enzyme activity.[22] It is assumed that HIV-1 integrase binds two metal ions and the latter are involved in the catalysis.[7] Most probably, Mg^{2+} ions play the role of the metal-cofactor in *in vivo* processes; however, in *in vitro* studies, Mn^{2+} ions were often used as the integrase cofactor.[23]

It was shown that an isolated catalytic domain can *in vitro* catalyse the disintegration reaction, *i.e.*, the reaction opposite to the strand transfer,[24] whereas 3′-processing and strand transfer require the full-sized enzyme.[14]

The primary structure of the N-terminal domain (amino acid residues 1 to 49), like the catalytic domain structure, resembles the structure of N-terminal domains of other retroviral integrases.[25] The role of N-terminus in integrase-mediated processes is the least clear. It is known that this enzyme region contains four amino acid residues, namely, His12, His16, Cys40 and Cys43, which form the so-called HHCC-motif typical of most retroviral integrases.[25] Some enzymes containing such a motif form a Zn^{2+}-binding domain of the 'zinc finger' type, which in addition to zinc ion coordination also performs the DNA-binding function.[26] It was shown[27] that HIV-1 integrase does bind the Zn^{2+} ion in the stoichiometric ratio of 1 : 1; however, no data on the integrations of its N-terminus with DNA could be found in the literature. Mutant enzymes that either lacked the N-terminus or contained replacements in their HHCC motives, lost their ability to coordinate a zinc ion and, as a consequence, to mediate the reactions of 3′-processing and strand transfer.[25,27]

Presumably, the role of the N-terminus in the integrase functioning is associated with its involvement in the formation of a catalytically active multimeric complex, because the coordination of zinc ions by integrase was shown to stimulate the multimerisation and activity of the latter.[27] Moreover, certain cellular proteins, the PIC constituents, can probably interact with the N-terminus.

The C-terminus of retroviral integrases is the least conservative part compared to the N-terminus and the catalytic domain. It is assumed to perform a function of non-specific binding of DNA.[28,29] It was shown that a segment of C-terminus that contains animo acids 220–270 can bind DNA with efficiency close to that of the full-sized enzyme.[29]

As was mentioned above, in the integration, integrase binds two DNA molecules, namely, viral and cellular. In connection with this, a question arises of whether one integrase molecule can bind two DNA molecules at once or it contains a single binding site so that binding of two DNA molecules requires at least integrase dimer. It was shown [30,31] that integrase operates as a multimer. Particularly, it was reported [30] that the integration of viral DNA into the host cell DNA requires a tetrameric organisation of integrase; however, the precise multimeric state of integrase in the 3′-processing and strand transfer reactions is still unclear. Indeed, in addition to binding two DNA molecules, this enzyme should also provide the binding and processing of two ends of the viral DNA in order to incorporate these ends into the host cell DNA at a distance of five nucleotide pairs from one another.[32]

The absence of information on the structures of a full-sized enzyme and its complex with DNA and also on the composition and operation of the pre-integration complex complicates and slows down the search for integrase inhibitors.

III. Inhibitors of HIV-1 integrase activity

Based on the notions on the mechanism of operation of HIV-1 integrase, the following several versions of its inhibition may be proposed: blocking of the viral DNA binding; inhibition of 3′-processing; action on the integrase/viral DNA complex and suppression of the strand transfer reaction; inhibition of protein–protein interactions under formation of the active multimeric form of integrase or its interaction with the other protein components of PIC.

So far, all attempts to find compounds that would prevent the binding of a viral DNA to the HIV-1 integrase into an intricate nucleoprotein complex had failed. The inhibitors of all other steps of integration are being actively developed. In the search for compounds capable of inhibiting integration, two main approaches were used, namely, the screening of libraries of chemical compounds of different classes and computer modelling of the hypothetical structure of inhibitors. In both methods, scientists face the problems that complicate the development of highly efficient and specific inhibitors of HIV integrase.

In the computer modelling of inhibitors, the absence of exact information on the structure of the full-sized enzyme and its complex with DNA is the main problem. In screening the libraries of chemical compounds, one faces the problem of inconsistency between a model system of integration used in testing the inhibitors and the real system. The integration process is reproduced *in vitro* by using a recombinant integrase and DNA duplexes that mimic the terminal sequences of the viral DNA. Due to the poor solubility of integrase and its susceptibility to aggregation, the *in vitro* studies often employ recombinant enzyme (either isolated in the presence of a detergent or containing mutations that enhance its solubility) preparations. However, recombinant integrases isolated by different procedures usually had different catalytic activities and different sensitivities with respect to ions cofactors.[31] These preparations were often inactive in the presence of the natural cofactor, the Mg^{2+} ions, but could catalyse integration in the presence of the Mn^{2+} ions. As a rule, integrases characterised by different activities in the presence of Mg^{2+} and Mn^{2+} ions, exhibited different sensitivities with respect to inhibitors.[33] Thus compounds that inhibited integrase *in vitro* in the presence of the Mn^{2+} ions were inactive in the presence of the Mg^{2+} ions and in HIV-infected cells.

1. General strategy in the development of HIV-1 integrase inhibitors

Primary screening of integrase inhibitors is carried out *in vitro* using recombinant integrase and short oligonucleotide duplexes that mimic the ends of long terminal repeats in the viral DNA.[1] The inhibition of 3′-processing and strand transfer reactions by inhibitors is studied separately and the values IC_{50}, *i.e.*, the inhibitor concentrations corresponding to the 50% reduction in the reaction efficiency, are found for each inhibitor. Then, inhibitors that exhibited activity in the first stage of tests are tested in a culture of HIV-infected cells. As a result, the drug potential in the inhibition of virus replication in cells is determined, together with its toxicity with respect to cells (cytotoxicity).

The next step in the development of an effective integrase blocker is the analysis of the structure–activity relationship (SAR) for the class of compounds under study. This kind of studies involve synthesising a library of compounds based on the structure of an already known inhibitor and studying how the changes introduced into its structure affect its activity. The SAR studies allow one to improve the pharmacological characteristics of a drug. The best inhibitors revealed by this analysis are tested in animals (mice, dogs, rhesus-monkeys) to

reveal the pharmacokinetic characteristics of this compound and its toxicity. Drugs that successfully passed the assays are later subjected to clinical trials. As was mentioned above, an integrase inhibitor Isentress has already passed clinical trials and was approved for therapy; the second inhibitor, JTK-303/GS-9137, is now in the third phase of clinical trials (see Section III.5).

Designing inhibitors is also associated with the development of new versions of complex therapy. The most promising versions make use of several drugs simultaneously that block different steps of the virus life cycle,[1] because the multiple mutations in the viral genome to acquire resistance against such a therapy weaken the virus ability to replication. In connection with this, at present, in studying the activity of inhibitors of integrase and other viral enzymes, attention is focused on verifying that the drug preferentially affects a particular step in the virus life cycle. To state that an inhibitor acts on the intergration step, at least four conditions should be fulfilled.[1]

First, in experiments where an inhibitor is added in different steps of viral infection (time-of-drug-addition experiments), the inhibitor should act in the integration step, *i.e.*, after the reverse transcription but before the maturing of viral species (between the 4th and the 16th hour after the infection) rather than in the later steps. This experiment is carried out as follows: a culture of cells is infected with a virus and then, in certain time periods, the inhibitor-containing medium is added. This allows one to identify the step in the virus life cycle in which its replication is blocked.

Second, in nuclei of infected cells treated with an inhibitor, circular viral DNA that contains two tandem copies of the LTR sequence should be accumulated and the number of integrated viral DNA should decrease. Circular DNA molecules are formed in the cyclisation of the viral DNA by host cell enzymes. Third, virus strains resistant to a given inhibitor should carry mutations in the integrase gene. Ultimately, fourth, the inhibitor should be inactive (or less active) in experiments with recombinant integrase that carries mutations revealed in resistant viral strain.

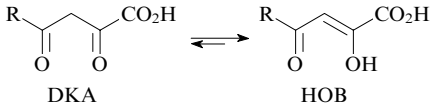
Such a strategy in the development of integrase inhibitors made it possible to reveal several classes of compounds the representatives of which demonstrated high antiviral activity in cultures of infected cells together with the low cytotoxicity and affected precisely the integration step. According to the mechanisms of their action, the following three groups can be distinguished: inhibitors of 3′-processing, inhibitors of strand transfer, and inhibitors of enzyme oligomerisation. This review considers all these groups of inhibitors. Our goal was far from describing all currently known inhibitors capable of suppressing the *in vitro* activity of HIV-1 recombinant integrase, because they form a wide spectrum of compounds with different structures and inhibition activities. Here, we show only compounds for which the mechanism of inhibition is known and the antiviral activity was demonstrated in a culture of HIV-infected cells. To find information on the whole spectrum of inhibitors of HIV-1 integrase, see reviews.[34–41]

2. Strand transfer inhibitors

Compounds that inhibit the strand transfer are considered to date as the most promising inhibitors of integration. The names of all compounds discussed in this review are taken from the original publications.

a. Derivatives of diketo compounds

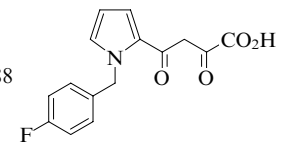
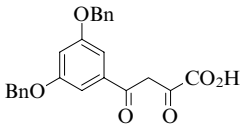
Testing a library of 250 000 compounds for the HIV-1 integrase inhibitors revealed several most active inhibitors.[42] All of them turned out to be diketo compounds, namely, derivatives of 2,4-diketobutanoic acid (DKA). Later, it was shown that in solutions, these compounds largely exist in the enol form, namely, as the derivatives of 2-hydroxy-4-oxobut-2-enoic acid (HOB);[43] however, in original publications they are always shown in the form of diketo compounds.



DKA HOB

The discovered inhibitors manifested higher activity in the inhibition of the strand transfer reaction as compared with the 3′-processing. This made it possible to gather them in a single group and speak of a new class of integrase inhibitors specific to the strand transfer.[42] For the most active members of this class of inhibitors, namely, compounds L-731,988 and L-708,906 (Table 1), the IC_{50} values in the strand transfer reactions performed by recombinant integrase were 0.05 and 0.10 μmol litre^{-1}, respectively, as opposed to 6 and 1 μmol litre^{-1} for the 3′-processing reaction.[42] These compounds also suppressed the activity of the pre-integration complex, the more so, in the same concentration range as in the inhibition of the strand transfer reaction (IC_{50} = 0.08 and 0.15 μmol litre^{-1}, respectively). Furthermore, diketo compounds suppressed the virus replication in a culture of cells, where IC_{50} values were 1 and 2 μmol litre^{-1} for L-731,988 and L-708,906, respectively.[42]

Table 1. Structure and activity of inhibitors of HIV-1 integrase — derivatives of 2,4-diketobutanoic acid.

Inhibitor		Inhibitory activity (IC_{50}) /μmol litre^{-1}		
name	structure	in strand transfer reaction	in suppression of PIC activity	in suppression of replication of HIV-infecion in cells
L-731,988		0.05	0.08	1.0
L-708,906		0.10	0.15	2.0

An assumption that it is the integrase that is affected by 2,4-diketobutanoic acid derivatives in a cell was confirmed by the following facts. First, the selection of viruses resistant against these inhibitors has shown that mutations responsible for the resistance are localised precisely in the integrase gene.

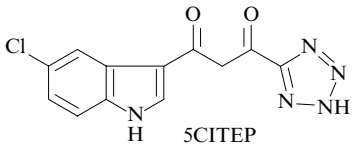
Integrase from L-731,988-resistant virus strain contained the Met154Ile substitution, and integrase from L-708,906-resistant virus strain carried the Thr66Ile and Met154Ile (or Ser153Tyr) mutations. Second, in the nuclei of cells treated with these inhibitors, circular forms of viral DNA were accumulated, an effect usually observed for cells infected with a virus defective with respect to integrase. Moreover, a decrease in the amount of integrated viral DNA was observed. And finally the third, in infected cells, the kinetics of both reverse transcription and 3′-processing did not change in the presence of these inhibitors as compared with the control experiment. This means that the diketo compounds under study do not affect the reverse transcription stage being the specific inhibitors of the strand transfer reaction. Nonetheless, it was shown that the L-708,906 inhibitor of integrase also (albeit in higher concentrations) reduces the activity of RAG 1/2 recombinase responsible for the development of a repertory of antibodies and T-cell receptors.[44] Hence, in designing integrase inhibitors, the possibility of their interplay with cellular enzymes and the appearance of concomitant cytopathogenic effects should be borne in mind.

The interaction of the inhibitor L-731,988 with integrase was studied in detail.[45] It was shown that the inhibitor does not interact with the isolated enzyme up to micromolar concentrations. Its binding with integrase requires the presence of a DNA-substrate, more exactly, the U5- or U3-terminal sequences of viral DNA. In this case, the dissociation constant of the integrase complex with L-731,988 determined in the presence of 100 nmol litre^{-1} of U5-substrate was 75 nmol litre^{-1} and approached the IC_{50} values for the strand transfer reaction.

A non-specific DNA could not mediate the interaction of the inhibitor with the enzyme. Moreover, an increase in the concentration of the substrate DNA the excess of which can act as the DNA-target, reduced the stability of the inhibitor–integrase complex. Based on the obtained data, it was assumed that the inhibitor L-731,988 suppresses the strand transfer reaction, competing with the DNA-target for the binding site; moreover, the interaction of the inhibitor with integrase requires a certain conformation of the enzyme–substrate active complex. In other words, inhibitors of this class do not bind integrase itself but only its complex with viral DNA, thus preventing further binding of cellular DNA.

A comparison of the data on the binding of the L-731,988 inhibitor to integrase with the results of the mutation assay made it possible to assume the mechanism of action of diketo compounds.[46] According to this hypothesis, these compounds coordinate the both Mg^{2+} ions involved in the catalysis and located in the enzyme active site thus suppressing the catalytic activity of integrase. The fact that integrases of virus strains resistant to this class of inhibitors contained amino acid replacements in the immediate proximity of amino acid residues Asp64 and Glu152 that belong to the integrase catalytic triad made it possible to assume that derivatives of 2,4-diketobutanoic acid interact with the enzyme active site or with its immediate surrounding.

Information on another integrase inhibitor, 1-(5-chloroindol-3-yl)-3-hydroxy-3-(2*H*-tetrazol-5-yl)propenone (5CITEP) was obtained virtually simultaneously with the information on the inhibiting effect of 2,4-diketobutanoic acid.[16]



5CITEP

X-Ray diffraction analysis of the catalytic domain of integrase crystallised in the presence of the inhibitor 5CITEP has shown that the inhibitor is located in the enzyme active site between amino acid residues of the catalytic triad, namely, Asp64, Asp116 and Glu152 (Fig. 2). According to the electron density map, 5CITEP formed several contacts with amino acid residues of integrase, which played an important role in implementation of the catalytic event and in the DNA binding. Thus Glu152 is involved in the formation of a hydrogen bond with the hydroxyl group of the enol form of 5CITEP. Moreover, hydrogen bonds can be formed between Gln148 and the nitrogen atom of the indole fragment of 5CITEP and also between Asn155, Thr66, Lys159, Lys156 and nitrogen atoms of the 5CITEP tetrazole ring. No direct contacts were observed between 5CITEP and the other two amino acid residues of the catalytic triad, namely, Asp64 and Asp116.

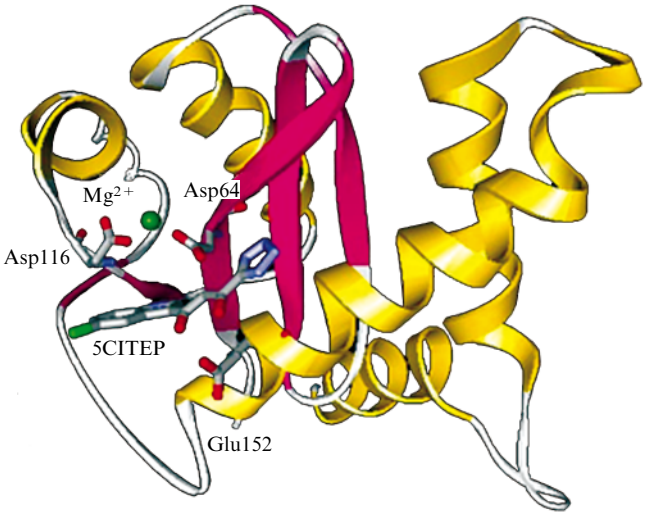


Figure 2. Structure of a complex formed by the inhibitor 5CITEP with HIV-1 integrase catalytic domain (X-ray diffraction analysis data).[16]

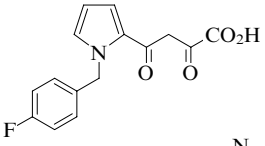
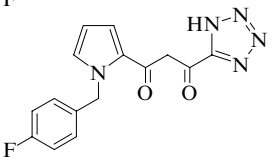
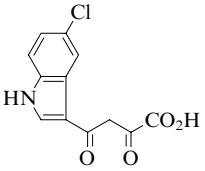
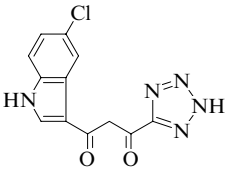
The obtained structural data were of great significance for understanding the operation mechanism of 5CITEP, L-731,988 and diketo compounds, because certain studies attested that these compounds can be assigned to one and the same class of integrase inhibitors. Thus 5CITEP, like the derivatives of 2,4-diketobutanoic acid, exhibited higher inhibitory activity in the strand transfer reaction [46,47] than in other processes. The existence of the same site responsible for binding these compounds with the enzyme was also confirmed by experiments on the competitive binding of inhibitors with integrase [46] and also by comparing the results of X-ray diffraction analysis of the complex formed by the enzyme catalytic domain with 5CITEP with the data on mutations in the integrase structure from virus strains resistant to diketo acid derivatives.

To understand the role the individual fragments of 5CITEP and L-731,988 molecules play in their binding to and inhibition of the enzyme, the activity of certain derivatives of these compounds was studied (Table 2) (see †).[33,46,47]

Studies of compounds L-731,988, 5CITEP, DKA 1 and DKA 2 made it possible to reveal two important trends. First,

† The synthesis of inhibitors DKA 1 and DKA 2, which are the analogues of inhibitors L-731,988 and 5CITEP, is described in Ref. 46.

Table 2. Structure and activity of diketo inhibitors in the presence of Mg^{2+} and Mn^{2+} cofactors.

Inhibitor		Inhibitory activity (IC_{50} /μmol litre^{-1}) in strand transfer reaction		Ref.
name	structure	Mg^{2+}	Mn^{2+}	
L-731,988		0.05	0.05	46
DKA 1		0.47	0.06	46
DKA 2		0.4 1.93	0.4 0.12	46 33
5CITEP		4.0 97.0	0.4 0.93	46 33

when the Mg^{2+} ions were used as the integrase cofactor, the carboxyl derivatives L-731,988 and DKA 2 exhibited higher inhibitory activity than the tetrazole derivatives DKA 1 and 5CITEP. Second, in the presence of different cofactors, the activities of carboxyl derivatives were the same[46] or differed not very much,[33] whereas the activities of tetrazole derivatives substantially depended on the cofactor used. Probably, the low activity of tetrazole-containing inhibitors in the presence of Mg^{2+} ions can be explained by their low antiviral activity in the cell culture (IC_{50} > 50 μmol litre^{-1}).[46]

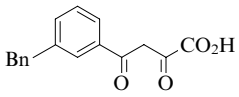
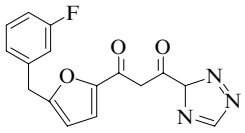
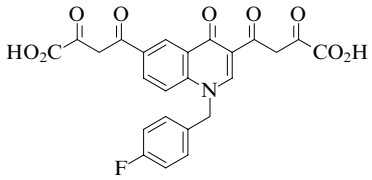
The search for the optimal activity and cytotoxicity for inhibitors of the class of diketo compounds was carried out within the framework of SAR studies (see Section III.3.a). It is clear that such studies involved the activity tests of a large number of compounds. As an example, Table 3 shows the structures of three inhibitors found in this search.

Special mention should be made of compound S-1360 that exhibited high inhibitory activity in experiments with HIV-infected cells and became the first strand transfer inhibitor that reached the stage of clinical trials (see Section III.5).

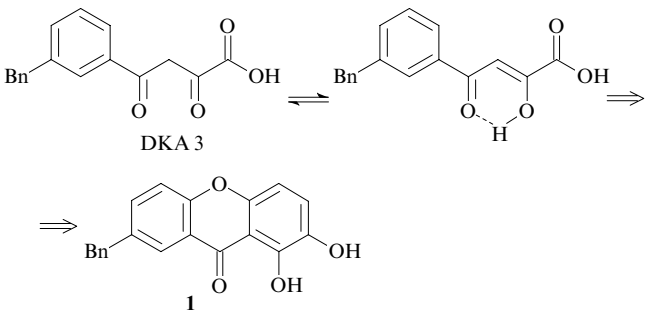
b. Naphthyridine derivatives

As follows from the above data, compounds containing the $C(O)CR_2C(O)$ fragment exhibited activity in the inhibition of HIV-1 integrase. However, biological lability of this structural fragment makes difficult the use of these compounds as chemotherapeutic agents.[50] Studies aimed at the quest for adequate substitute for diketo compounds led to a new class of compounds, *viz*., naphthyridine derivatives with the high inhibiting activity.[51] When modelling the structures of this new class of inhibitors, diketo acid DKA 3 was taken as the basis.[51]

Table 3. Structures of inhibitors of the class of diketo compounds synthesised as a result of SAR studies.[48,49]

Inhibitor		Inhibitory activity (IC_{50} /μmol litre^{-1})	
name	structure	*in vitro*	*in vivo*
DKA 3		0.01	1.11
S-1360		0.02	0.14
DKA 4		0.016	4.29

Insofar as the oxo form of DKA 3 diketo acid is in equilibrium with its enol form, in which the carboxyl group, the diketo group and the neighbouring ring lie in the same plane, it was assumed that such a structure can be adequately modelled by a derivative of 1,2-catechol **1**. This compound was synthesised and did prove to suppress the activity of integrase *in vitro* (IC_{50} = 600 nmol litre^{-1}) and virus replication in cell culture.

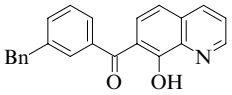
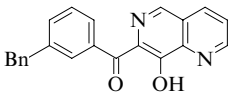
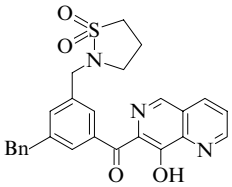
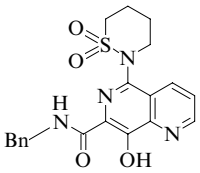
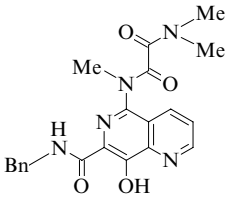


Taken in a concentration of 6 μmol litre^{-1}, compound **1** inhibited the virus replication by 95%. However, the presence of the biologically active catechol fragment in inhibitor **1** molecules dictated its further structural improvements.

According to the results of SAR studies, one of hydroxyl groups in the catechol moiety can be replaced by a hetero-aromatic nitrogen atom. Moreover, to reduce the toxicity of the model compound, the polyaromatic structure was destroyed by removing the oxygen bridge.[51] As a result of such modelling, three new inhibitors were obtained, namely, the derivatives of 8-hydroxyquinoline (compound **2**) and 8-hydroxy[1,6]naphthyridine (compounds **3** and **4**) the structures of which are shown in Table 4.

Further studies[52–54] aimed at enhancement of the inhibitory activity and improvement of pharmacological properties of naphthyridine derivatives have led to inhibitors L-870,810 and L-870,812 (see Table 4) which for a long time were considered the most promising integrase inhibitors. Compound L-870,810, like inhibitors based on 2,4-diketobutanoic acid, had the higher inhibitory activity in the strand transfer reaction as compared with the 3′-processing reaction.[52] For

Table 4. Structures of inhibitors synthesised based on 8-hydroxyquinoline and 8-hydroxy[1,6]naphthyridine.

Inhibitor		Inhibitory activity (IC_{50} /μmol litre^{-1})	
name or number	structure	in strand transfer reaction	in suppression of replication of HIV-infection in cells
2		0.37	5.0
3		0.04	6.2
4		0.01	0.39
L-870,810		0.008	0.10 [a]
L-870,812		0.04	0.25 [a]

[a] Antiviral activity was determined as IC_{95} in the presence of 50% normal human serum.

instance, depending on the DNA-target concentration (0.5 or 5 nmol litre^{-1}, respectively), IC_{50} was 8 or 15 nmol litre^{-1} in the inhibition of the strand transfer reaction and 85–250 nmol litre^{-1} in the inhibition of 3′-processing. The sensitivity of the inhibitor with respect to the DNA-target concentration suggests that in the integration complex, the inhibitor is bound to the same site as the DNA-target, while the fact that the naphthyridine derivative L-870,810 displaces diketo acid L-731,988 from the integrase complex with the DNA-substrate allows one to assume that these compounds get bound to integrase in the common or overlapping sites.

The antiviral activity of the inhibitor L-870,810 varied from 15 to 100 nmol litre^{-1} depending on the serum concentration in the cell medium (10% and 50%, respectively).[52] The action of this inhibitor on HIV-1 integrase was evidenced by the accumulation of circular viral DNA in L-870,810-treated cells and also by the reduction of the amount of integrated viral DNA. In addition to inhibition of HIV-1 integrase, this compound suppressed the replication of HIV-2 and SIV (Simian Immunodeficiency Virus).

Selection of L-870,810 resistant virus strains led to identification of three strains containing the following replacements in the amino acid sequence of integrase: Phe121Tyr/Thr125Lys, Val72Ile/Phe121Tyr/Thr125Lys or Val72Ile/Phe121Tyr/Thr125Lys/Val151Ile.[52] Viruses that carried the corresponding mutations were 4–100 times less sensitive to the action of L-870,810; however, they did not acquire resistance to diketo compounds. Similarly, viruses resistant to 2,4-diketobutanoic acid-based inhibitors were sensitive with respect to the naphthyridine-based inhibitor L-870,810. It is interesting that in the three-dimensional structure of integrase (more precisely, its catalytic domain), the amino acid residues that underwent substitutions in the selection of resistant virus strains were concentrated around the catalytic triad. Moreover, amino acid substitutions that confer resistance agaist L-870,810 and diketo compounds were located on opposite sides of the enzyme active site (Fig. 3).

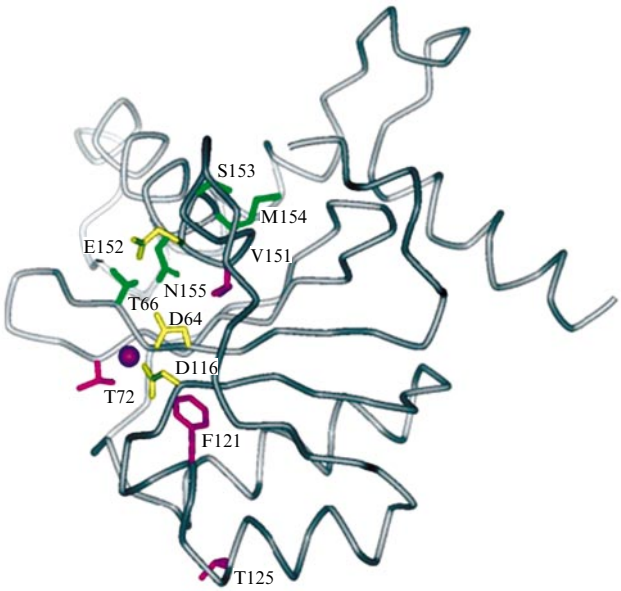


Figure 3. Localisation of amino acid substitutions in the integrase catalytic domain responsible for selection of the inhibitor-resistant virus strain.
Catalytic triad is yellow, magnesium ions coordinated by this triad are depicted lilac. Amino acid residues the substitution of which is responsible for the selection of virus strains resistant to dioxo compounds and naphthyridine derivatives are painted green and pink, respectively.

Computer simulation of binding of these compounds to the integrase active site made it possible to assume that a part of the inhibitor molecule responsible for the metal-cofactor coordination lies in the immediate proximity of the catalytic triad, whereas the orientation of the other parts of the molecule and their interaction with integrase amino acid residues arranged around the active site depends on the inhibitor structure.

Summarising the obtained data, it can be assumed that the binding of diketo acid- and naphthyridine-based inhibitors with integrase occurs in overlapping sites and these inhibitors have similar mechanisms of action.

Compound L-870,812 turned out to be optimal as regards its inhibitory activity and pharmacokinetic parameters.[54] It inhibited HIV-1 replication by 95% in a concentration of 250 nmol litre^{-1} (in the presence of 50% normal human serum). Furthermore, this compound exhibited relatively low affinity towards serum proteins (93.2% of this inhibitor was bound to the serum proteins, while for the largest part of

studied compounds this parameter was >95%). Moreover, this compound could suppress the replication of SIV the integrase of which is a close homologue of HIV-1 integrase (IC_{95} = 350 nmol litre^{-1} in the presence of 50% serum of rhesus monkey).

The next step was to study the antiviral properties of L-870,812 inhibitor in infected rhesus monkeys.[52] This study was aimed at elucidation of the role the chemotherapy and cell immune response play in suppression of the virus replication. Animals well withstood L-870,812 with no manifestation of its toxicity. Trials of the inhibitor L-870,812 in rhesus monkeys demonstrated its antiviral activity, where the most effective therapy was observed in the early stages of infection, because this allowed a specific immune response of the cell to be established. The efficiency of therapy in the late stages of infection depended on the immune status of an animal and the virus concentration level in the blood serum in the beginning of therapy. The acquired data are in favour of early therapy of HIV infection and also in favour of combined chemotherapy and vaccine treatment.

3. Inhibitors of 3′-processing

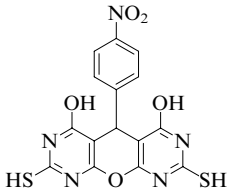
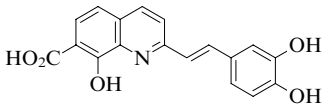
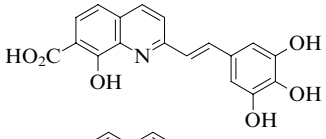
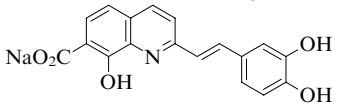
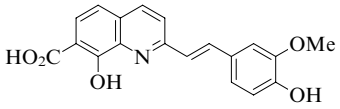
This class of inhibitors suppress both integration stages, *i.e.*, 3′-processing and strand transfer. This is explained by the fact that they interact with the active site of the enzyme itself rather than with its complex with the DNA-substrate. This class of inhibitors is the most abundant; however, the inhibition of virus replication in cell culture was observed only for the following two groups of inhibitors: 5*H*-pyrano[2,3-*d*:6,5-*d*′]dipyrimidines and styrylquinoline derivatives.

a. 5H-Pyrano[2,3-*d*:6,5-*d*′]dipyrimidines

The most active inhibitor of this group of compounds is 5-(4-nitrophenyl)-2,8-dithio-4,6-dihydroxy-5*H*-pyrano[2,3-*d*:6,5-*d*′]dipyrimidine (V-165, Table 5), which inhibits both the activity of recombinant integrase and virus replication in cell culture.[55] In contrast to inhibitors based on diketo compounds and naphthyridines, compound V-165 inhibited more effectively the 3′-processing than the strand transfer, because it prevents the binding of the DNA-substrate to integrase. The accumulation of circular DNA forms in cells and their depletion with respect to the integrated DNA confirmed the effect of the inhibitor V-165 on the integration. At the same time, the compound V-165 also suppresses the activity of reverse transcriptase, which was confirmed by both the studies with a recombinat enzyme and the time-of-drug-addition experiments. A weak correlation between the antiviral activity of the drug and the inhibition of activity of reverse transcriptase and a strong correlation between the antiviral activity and the inhibition of recombinant integrase were observed.

Recently, as a result of selection of virus strains resistant to the inhibitor V-165, a HIV-1 strain that contained mutations not only in integrase but also in the genes of proteins gp120, gp40 and reverse transcriptase was identified.[56] The fact that it is the integrase activity that is inhibited by compound V-165 was confirmed by the presence of a double mutation in its amino acid sequence, namely, Thr206Ser in the catalytic domain and Ser230Asn in the C-terminal domain. It is interesting that in contrast to mutations that arise in a virus as a result of its cultivation in the presence of strand-transfer inhibitors and are located in the Mg^{2+}-binding catalytic domain, the mutations appeared when the virus was cultivated in the presence of V-165 were situated not only in the catalytic domain but also in the C-terminus the major function of which

Table 5. Structure and activity of 3′-processing inhibitors.

Inhibitor		Inhibitory activity (IC_{50}/μmol litre^{-1})		
name	structure	in 3′-processing	in strand transfer	in suppression of replication of HIV-infection in cells
V-165		0.9	16.0	3.7–8.9
KHD161		2.4	1.0	1.3
SQ 1		0.3	0.4	1.2
SQ 2		0.8	–	20.0
FZ55		2.8	3.7	3.0

consists of DNA binding. This confirms the proposed [55] multimodal mechanism of operation of the inhibitor V-165. The presence of mutations in proteins gp120 and gp40 involved in binding of the virus to cellular receptors pointed to the possible effect of the inhibitor V-165 on this process.

Indeed, detailed studies of the action of the inhibitor V-165 on the virus replication have shown that it blocks two steps in the viral cycle, namely, the penetration of the virus into cells and its integration.[56] Thus at present, the compound V-165 is the only inhibitor of HIV-1 replication that acts *in vivo* on two virus lifecycle steps. On the one hand, such a multidirected character of the V-165 action makes it more attractive as the HIV-1 inhibitor; however, on the other hand, the ability of this compound to react with different proteins may be the reason for its toxicity. To date, the preclinical trials of the inhibitor V-165 are in progress.

b. Styrylquinolines

Taking into account the possible involvement of two Mg^{2+} ions in the catalysis, an attempt was undertaken [57] to design an inhibitor that would contain two chelating subunits connected through a spacer that would bind both metal ions and thus inhibit the integrase catalytic activity. It was proposed to use 8-hydroxyquinoline as the first chelating subunit and the polyphenol residue as the second subunit. For this purpose, a series of compounds with different substituents at different positions in the hydroxyquinoline and phenol parts were synthesised.[58] Some of them proved to be capable of suppressing the activity of recombinant integrase in the 3′-processing and strand transfer reactions. These compounds formed a new class of intergrase inhibitors, namely, styrylquinolines. Table 5 shows the structures of the most active members of this class.

Insofar as the number of bivalent ions involved in the catalysis and the direct role of each ion remain unclear, the mechanism of functioning of styrylquinoline inhibitors required additional studies. It was important to understand whether the inhibitors directly affect the catalytic act or suppress the binding of integrase to DNA. Using the fluorescence properties of compound FZ55 (see Table 5), it was shown that it interacts with integrase to form a complex the stability of which depends on the presence of the Mg^{2+} ions.[31] Moreover, it was shown that styrylquinoline FZ55 inhibits binding of integrase to DNA and reduces the integrase activity in the 3′-processing reaction in the same concentration range (IC_{50} = 1.5 and 1.2 μmol litre^{-1}, respectively). However, these two effects are manifested only when the inhibitor has been added simultaneously with the enzyme or pre-incubated with the latter. Otherwise, styrylquinoline FZ55 does not block integrase, *i.e.*, it neither destroys the preformed integrase complex with the DNA-substrate nor inhibits its 3′-processing reaction. Based on the obtained data, the competitive mechanism of action of styrylquinoline inhibitors was proposed.[31] The ability of these compounds to inhibit the disintegration reaction catalysed by the catalytic domain of the enzyme [57] and also the fact that their binding to integrase depended on the presence of the magnesium ions made it possible to assume that the binding of these inhibitors to the integrase catalytic domain is located in the vicinity of the active site.

It was found [31] that the inhibitor KHD161 (see Table 5) added to a preformed enzyme–substrate complex was inactive in the 3′-processing reaction; however, it suppressed the strand transfer reaction mediated by this complex (IC_{50} = 6 μmol litre^{-1}). To explain these facts, it was assumed that one integrase molecule has two sites for binding styrylquinoline inhibitors. According to this assumption, binding of styrylquinolines in the first site blocks the binding of viral DNA and inhibits the 3′-processing, whereas their binding in the second site blocks the reaction of viral DNA with host cell DNA, suppressing the strand transfer. Computer simulations have revealed three regions of the integrase catalytic domain as potential binding sites of styrylquinolines (Fig. 4).[31]

Presumably,[31] the first of these regions could mimic the first site of styrylquinoline binding, because it incorporates the Mg^{2+} ion and comprises certain amino acid residues, such as Lys156 and Lys159 necessary for binding viral DNA (Ref. 59). The second and third regions could mimic the second site of styrylquinoline binding. It is still unclear which of these regions should be preferred; however, only the third region contains amino acid residues that, according to biochemical studies, may be involved in DNA binding.

In addition to inhibiting recombinant integrase, styrylquinolines also suppress virus replication in cell culture. The

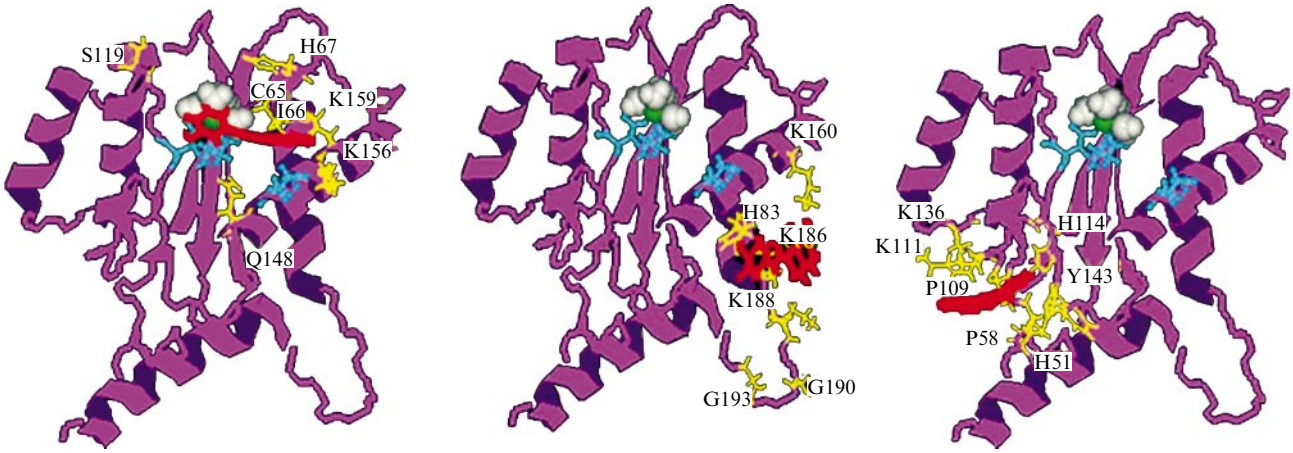


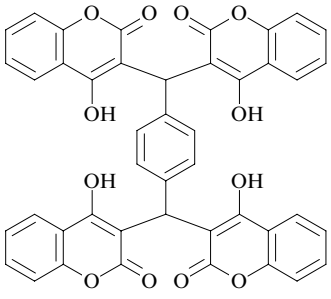
Figure 4. A computer model of interaction of the inhibitor KHD161 with the integrase catalytic domain in three different regions.[31] Pink corresponds to protein, green to magnesium ion, white to water molecules, blue to catalytic triad, red to the inhibitor, yellow to the amino acid residues that interact with this inhibitor.

following two compounds exhibited the highest antiviral activity: KHD161 and SQ1 (Ref. 58). For these inhibitors, IC_{50} values were 1–2 μmol litre^{-1}. Unfortunately, in addition to high activity, the inhibitor SQ1 also possessed considerable cytotoxicity, which ruled out its use in medicinal studies. The inhibitor KHD161 proved to be far less toxic. At present, the compound KHD161 is considered as the most promising inhibitor of this class.

There is abundant evidence to the fact that it is integrase that is affected by styrylquinolines in cells. The results of time-of-drug-addition experiments have shown that these compounds neither affect the virus penetration into cells nor act in the stages following the integration.[60] *In vitro* experiments demonstrated that styrylquinolines affect neither the binding of viral glycoprotein gp120 to the viral receptor CD4 nor fusion of membranes. Selection of styrylquinoline-resistant viruses followed by identification of mutations also confirmed that it is integrase that is the target of styrylquinolines.[60] In one of resistant viral strains, integrase carried a single amino acid substitution, namely, Cys280Tyr; in another strain, it carried a double substitution, Val165Ile/Val249Ile. It should be noted that viruses resistant to styrylquinolines were sensitive with respect to antiviral drugs that could selectively inhibit the strand transfer reaction, for example, with respect to derivatives of 2,4-dioxobutanoic acid.[60]

4. Inhibitors of integrase multimerisation

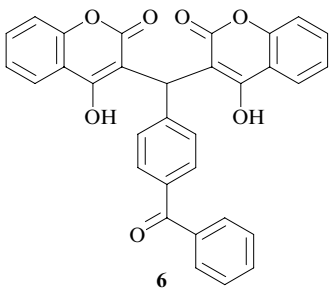
At present, the quest for compounds that have no effect on the catalytic activity of integrase but affect either its interaction with the other PIC components or its intrinsic ability to form active multimers is in progress. Compounds that suppress the multimerisation of integrase can be, *e.g.*, hydroxycoumarin derivatives. It was shown that hydroxycoumarin tetramer **5** inhibits HIV-1 replication in cell culture (IC_{50} = 11.5 μmol litre^{-1}).[61]



5

Originally, compound **5** was considered as the inhibitor of HIV-1 protease, because it inhibited the catalytic activity of this enzyme *in vitro*. However, further studies have shown that in addition to inhibiting protease, this compound is capable of suppressing *in vitro* the activity of HIV-1 integrase. The hydroxycoumarin tetramer inhibited the 3′-processing and strand transfer reactions (IC_{50} = 1.5–2.0 μmol litre^{-1}).

The ability of compound **5** to inhibit the disintegration reaction catalysed by the isolated catalytic domain of the enzyme suggests that the site responsible for binding of this inhibitor to integrase is located in the integrase catalytic domain.[61] Originally, by analogy with other hydroxylated aromatic compounds as integrase inhibitors, it was proposed that hydroxycoumarin operates in the enzyme active site affecting the coordination of the metal-cofactor. However, further studies [62] allowed proposing a different mechanism of action of this inhibitor. The covalent coupling of benzophenone derivative of hydrocoumarin **6** to integrase was carried out and the binding site of the inhibitor to the enzyme was determined.



6

MALDI mass-spectrometric studies of a tryptic hydrolysate of the covalent complex formed by inhibitor **6** with integrase made it possible to identify the peptide ^{128}AACWWAGIK^{136} to which compound **6** was attached. This peptide belonged to the integrase catalytic domain, which is consistent with conclusions drawn based on the activity of hydroxycoumarin in the disintegration reaction.

According to the results of X-ray diffraction analysis of the catalytic domain, amino acid residues of the peptide ^{128}AACWWAGIK^{136} are involved in the α3-helix and one of strands of the β-sheet.[6,16] It is known that amino acids included in the α3-helix are involved in the formation of a dimeric complex of the catalytic domain. Thus by having bound to integrase in the vicinity of the contact between two monomers, the hydroxocoumarin inhibitor prevents their dimerisation. To understand with which region of the peptide ^{128}AACWWAGIK^{136} the inhibitor interacts, three amino acid residues of integrase that are the constituents of this peptide, namely, Cys130, Trp131 and Trp132, were subjected to the site-directed mutagenesis. Mutations of Trp131 had no effect on the inhibitory activity of hydroxycoumarin derivatives, while the substitution of Ser for Cys130 and also of Gly, Ala, or Arg for Trp132 resulted in its reduction.[62] These data suggest that Cys130 and Trp132 are involved in reaction with the hydroxycoumarin inhibitor. It is interesting than one of the above mutants with respect to Trp132 was incapable of mediating the stand transfer reaction but retained activity in the 3′-processing reaction. At the same time, the enzyme in which a Tyr residue was substituted for Trp132 could mediate the strand transfer and was inhibited by hydroxycoumarin on the level of wild-type integrase.

Based on the results of mutagenesis and the X-ray diffraction analysis of the integrase catalytic domain dimer, the important role in the formation and stabilisation of the enzyme multimeric form was ascribed to the π–π-stacking interaction between the Trp132 residue in one integrase monomer and the Phe181 residue in another one. The binding of hydroxycoumarin derivatives to integrase in the vicinity of the peptide ^{128}AACWWAGIK^{136} may disturb such interactions and affect the formation and stability of the catalytically active integrase tetramer. Figure 5 shows a computer model of a complex of the hydroxycoumarin derivative with the catalytic domain dimer.

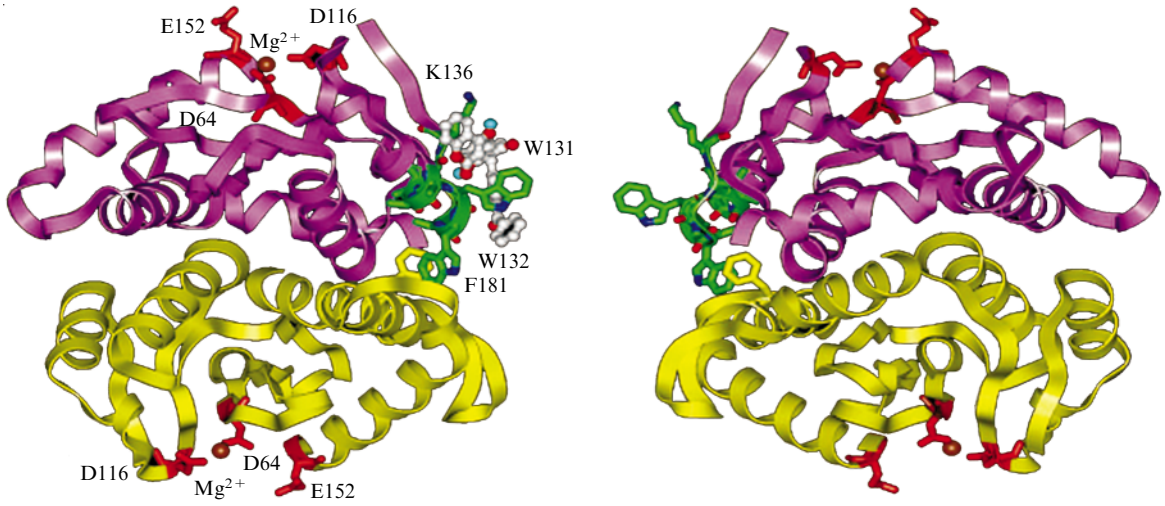


Figure 5. Hypothetical tetrameric form of integrase comprising two symmetrical dimers and the structure of hydroxycoumarin complex with the integrase catalytic domain dimer.[62]
The structure of hydroxycoumarin derivative is shown in white, amino acid residues of the peptide ^{128}AACWWAGIK^{136} are shown in green, magnesium ions are red, the catalytic triad of D,D(35)E motif is yellow and monomeric catalytic domains of integrase are pink.

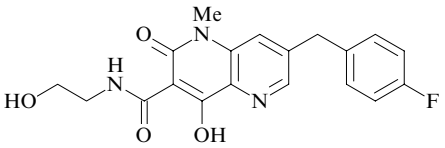
5. Inhibitors of HIV-1 integrase admitted to the stage of clinical trials

Insofar as integrase inhibitors are the components of antiviral therapy, we will separately consider those drugs that have reached the stage of clinical trials and, hence, proved to be the most effective and safe in preclinical assays. All these compounds are specific inhibitors of stand transfer.

The first inhibitor that reached the stage of clinical trials was compound S-1360 (see Table 3) synthesised by the Japanese company Shionogi.[63] It efficiently suppressed both the catalytic activity of recombinant integrase and the virus replication in cell culture, namely, EC_{50} was $\sim$200 nmol litre^{-1}, while its cytotoxicity‡ appeared only at micromolar concentrations (CC_{50} = 12 μmol litre^{-1}).[64] This drug passed the first stage of clinical trials; however, in 2003, the studies stopped due to its metabolic instability.

One of the most efficient inhibitors of the naphthyridine class, L-870,810 (see Table 4) developed by the Merck Pharmaceuticals was withdrawn from the first step of trials because of discovery of its toxicity for liver and kidneys.[64] Tests of the second drug of the same class, L-870,812 (see Table 4) are continued.[64, 65]

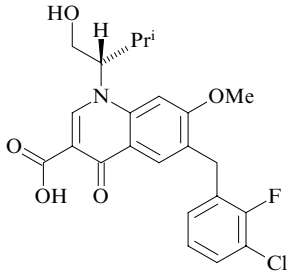
Yet another promising integrase inhibitor with the structure close to that of derivatives of 8-hydroxy[1,6]naphthyridine is the compound GSK364735, or S364735. This inhibitor is a joint development of GlaxoSmithKline and Shionogi.[66]



GSK364735

‡ CC_{50} is the cytotoxic concentration of an inhibitor that causes death of 50% of viral cells; EC_{50} is effective inhibitor concentration the response to which amounts to a half of the maximum possible effect.

This compound suppresses the activity of recombinant integrase in strand transfer reactions with $IC_{50} \approx 8$ nmol litre^{-1}. Its antiviral activity depends on the type of studied cells but does not exceed 5–7 nmol litre^{-1}, which is comparable with the activity of the best inhibitors such as Isentress that have reached the stage of clinical trials. Thus for Isentress, EC_{50} measured under the same conditions as for GSK364735 was of about 2 nmol litre^{-1}. The drug GSK364735 demonstrated low cytotoxicity (CC_{50} > 10 μmol litre^{-1}). Studies of the mechanism of inhibition of HIV-1 replication in a cell culture have shown that GSK364735 did inhibit only the integration stage; it did not affect the total amount of viral DNA, whereas the amount of circular DNA increased. The selection of virus strains resistant to GSK364735 have shown that this drug gives rise to several mutations in the integrase gene, of which the major mutation was the replacement of Gln148Arg, which was also observed when the virus was treated with the drug Elvitegravir.

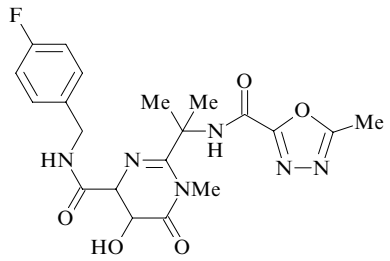


Elvitegravir (JTK-303/GS-9137)

This may point to the similarity of mechanisms of action of Elvitegravir and GSK364735; however, the latter did not lead to the mutations typical of Elvitegravir such as Thr66Ile and Glu92Gln. Moreover, recombinant proteins that carry these mutations were inhibited by GSK364735 even better that the wild-type integrase. At the same time, preclinical tests revealed that Isentress-resistant HIV-1 strains were also resistant to GSK364735. Clinical trials of GSK364735 have shown that this drug causes more than 100-fold decrease in the virus content in blood of patients; however, these tests were later stopped due to the revealed hepatotoxicity of the drug.[66]

At present, the integrase inhibitor Elvitegravir (JTK-303/GS-9137)§ is in the third phase of clinical trials. This inhibitor effectively lowers the virus level in patient's blood and entails no serious side effects. However, the necessary concentration of this drug in blood can be maintained only at the simultaneous intake of GS-9137 with Ritonavir. The latter inhibits cytochrome P450, the enzyme that plays the key role in the GS-9137 metabolism. To date, HIV-1 strains resistant to JTK-303/GS-9137 were obtained and the mutations in the integrase gene that give rise to the drug resistance were identified.[67] In total, eight amino acid substitutions in the integrase catalytic domain were revealed of which Thr66Ile and Glu92Gln located in the active site were assumed to be the major. However, it should be noted that the replication activity of the HIV-1 strains resistant to JTK-303/GS-9137 is much lower than the activity of the wild-type virus and also of its mutant forms resistant to L-870,810.

Finally, several words must be said of the Isentress (Raltegravir), which successfully passed clinical trials. This drug was developed by the Merck Pharmaceuticals and its original name was MK-0518.[63]



Raltegravir (MK-0518)

The drug was first clinically tested in 2004; in February 2006, the Phase III of its clinical trials began.[65] The inhibitor substantially reduced the virus level in patient's blood both used alone and in combination with nucleoside inhibitors of reverse transcriptase. Moreover, it proved effective in the studies with patients resistant to other antiviral drugs. This drug was well tolerated as a whole. Moreover, insofar as MK-0518 is metabolised by glucuronosyl transferase,[68] this drug, in contrast to JTK-303/GS-9137, does not require the presence of inhibitors of cytochrome P450 for maintaining the necessary concentration of inhibitor in the blood serum. The decision of the USA Food and Drug Administration to approve Isentress as a drug for curing AIDS was based on the results of clinical trials that involved 699 HIV-positive adult patients with the earlier experience of antiretroviral therapy.[65] In a group of patients who took Isentress together with other drugs, the fraction of patients with undetectable viral load was 62%. This parameter was 36% for those who took placebo in addition to the earlier used drugs. It also should be noted that some patients who took Isentress acquired drug resistance.[65] The analysis of the genome of drug-resistant HIV-1 strains has shown that in 32 cases of 41, the resistance was associated with mutations in the integrase gene as a result of Asn155His and Gln148Lys/Arg/His substitutions.

The appearance of a new class of drugs, integrase inhibitors, is especially important for those HIV-infected patients who developed resistance to other drugs. Isentress is recommended to be taken in pills of 400 mg twice a day irrespective of meals, in combination with the complex therapy.[65] At present, the drug is approved in the USA only for curing adults who developed resistance to drugs of the other classes. Obviously, certain time should pass and additional research should be carried out for this new drug to be approved for curing HIV-infected patients who never took any other drugs and also children and pregnant women.

§ Some time ago, a Japanese company Japan Tobacco have sold its developments on the JTK-303 drug to the Gilead Sciences company after which the compound name was changed for GS-9137 (see Refs 63, 65).

IV. Conclusion

To date, considerable progress is achieved in the development of HIV-1 integrase inhibitors. Besides three groups of low-molecular inhibitors of integrase (see Sections III.2–III.4) of which the most effective are compounds specifically inhibiting the strand transfer reaction, the peptide [69–71] and oligonucleotide [72–78] inhibitors are actively studied as well as the antibodies to the integrase C-terminus.[79,80] The interest in these compounds was associated with the fact that the mechanism of their action differs from that of classical low-molecular inhibitors. It is assumed that the use of drugs with mechanisms of action differing from the mechanisms of classical strand transfer inhibitors would allow curing patients resistant to JTK-303/GS-9137 or Isentress.

At the same time, further studies aimed at the development of integrase inhibitors are substantially complicated due to the deficiency of information on the structure of the full-sized protein, its multimeric nature and interactions with other PIC components and also on the nucleus import. The more detailed studies of the mechanism of action of inhibitors require carrying out the X-ray diffraction analysis of their complexes with the enzyme. At the same time, the effective design of integrase inhibitors requires carrying out a large number of SAR studies of this enzyme.

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