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Novel Herpes Simplex Virus and Human Immunodeficiency Virus Inhibitors Based on Phosphonate Nucleoside Analogs

A. N. Korovina[a], **M. V. Yasco**[b], **A. V. Ivanov**[a, b], **A. L. Khandazhinskaya**[a],
E. V. Karamov[c], **G. V. Kornilaeva**[c], **and M. K. Kukhanova**[a]
[a] *Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, 119991 Russia*
[b] *Centre for Medical Studies in Russia, University of Oslo, Moscow, Russia*
[c] *Ivanovsky Research Institute of Virology, Russian Academy of Medical Sciences, Moscow, 123098 Russia*
e-mail: lhba-imb@mail.ru.

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Abstract—Two types of novel nucleoside analogs have been synthesized: acyclic (*Z*)- and (*E*)-isomers of 9-[3-(phosphonometoxy)prop-1-en-1-yl]adenine and a carbocyclic isosteric analog of guanosine monophosphate. The (*Z*)- and (*E*)-isomers inhibit the replication of herpes simplex virus (HSV) and human immunodeficiency virus (HIV) and are nontoxic for cells. The (*Z*)-isomer activities against both viruses are higher than the (*E*)-isomer activities. Diphosphates of these compounds display substrate activities towards recombinant HSV DNA polymerase and HIV reverse transcriptase (RT). Diphosphate of the carbocyclic guanosine analog has no substrate activity towards HSV DNA polymerase but is active as a RT substrate.

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Nucleoside phiosphonates and their acyclic analogs (ANPs) in which the phosphonate group is linked to the purine or pyrimidine base via an aliphatic residue are key precursors of antiviral drugs. Several classes of ANPs have been shown to be active against various viruses [1, 2]. Analogs containing an unsaturated acyclic chain are a special class of ANPs [3–5]. Nucleoside phosphonate are metabolized by cellular enzymes to the corresponding 5'-diphosphates, which are substrates of DNA or RNA viral polymerases and, after incorporation into the viral DNA or RNA chain, terminate its synthesis [6, 7].

We synthesized two novel types of phosphonate derivatives of nucleosides: (*Z*)- and (*E*)-9-[3-(phosphonometoxy)prop-1-en-1-yl]adenines (PMEAs) and a carbocyclic isosteric analog of guanosine monophosphate, as well as their diphosphates. Their activities against human immunodeficiency virus (HIV) and herpes simplex virus (HSV) were estimated in a cell culture. Diphosphates were tested as substrates of HSV DNA polymerase and HIV-1 reverse transcriptase (RT). The antiviral activities of the diphosphates were found to be correlated with their substrate activities towards the corresponding viral enzymes.

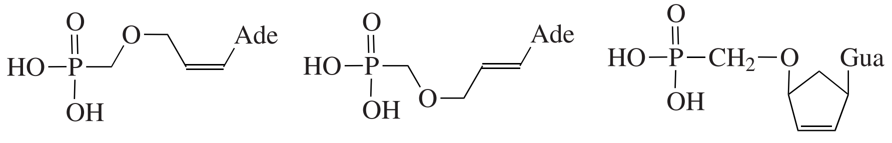
EXPERIMENTAL

The Vero and CEMss cell cultures, HIV-1 (EVK), HSV-1/L2, and the acyclovir resistant strain HSV-1/ACV^R were obtained from the Ivanovsky Research Institute of Virology of the Russian Academy of Medical Sciences (Russia); [γ-^{32}P]ATP (6000 Ci/mM) was from Izotop (Russia). We used synthetic oligonucleotides from Litech (Russia), HIV-1 RT and T4 polynucleotide kinase from Amersham BioSciences (United Kingdom), and [5'-^{32}P]-labeled primer–template complex obtained as described in [8]. The antiviral activity and cytotoxicity of the substances were determined as described earlier [9, 10].

HSV DNA polymerase was expressed in a baculovirus system as described below. The HIV RT and DNA polymerase α activities were determined as described in [11, 12]. The synthesis of nucleoside analogs and their characteristics are described elsewhere [10]; the corresponding diphosphates were synthesized as described in [13]. The compounds were isolated by chromatography using a DEAE–Toyeperl column and by HPLC using a Lichrosorb RP-18 column.

Diphosphate of the (E)-isomer: λ_{max} = 262 nm; ^{1}H NMR (D_2O): 8.22 (1H, s, H8), 8.08 (1H, s, H2), 7.13 (1H, d, $^3J_{1',2'}$ 14.3 Hz, H1'), 6.35 (1H, dt, $^3J_{2',3'}$, 6.2 Hz, H2'), 4.23 (2H, d, H3'), 3.74 (2H, d, $^2J_{CH_2,P}$ 8.7 Hz, PCH_2), 2.99 (18H, q, $^3J_{CH_2,CH_3}$, 7.5 Hz, $CH_2(Et_3NH)$), 1.09 (27H, t, $CH_3(Et_3NH)$); ^{31}P NMR (D_2O): 8.59 (1P, d, $^2J_{P_\alpha,P_\beta}$ 25.4 Hz, P_α), –5.87 (1P, br s, P_γ), –21.96 (1P, br s, P_β).

Diphosphate of the (Z)-isomer: λ_{max} = 261 nm; ^{1}H NMR (D_2O): 8.10 + 8.07 (2H, 2s, H8 + H2), 6.84 (1H, d, $^3J_{1',2'}$ 8.7 Hz, H1'), 5.99 (1H, dt, $^3J_{2,3'}$, 6.5 Hz, H2'),



Ia **Ib** **II**

The structures of the synthesized compounds

4.14 (2H, d, H3'), 3.65 (2H, d, $^2J_{CH_2,P}$ 9.0 Hz, PCH_2), 3.04 (18H, q, $^3J_{CH_2,CH_3}$, 7.5 Hz, $CH_2(Et_3NH)$), 1.12 (27H, t, $CH_3(Et_3NH)$); ^{31}P NMR (D_2O): 8.50 (1P, d, $^2J_{P_\alpha,P_\beta}$ 25.4 Hz, P_α), –8.59 (1P, br s, P_γ), –22.52 (1P, br s, P_β).

RESULTS

The scheme shows the structures of the obtained compounds. The antiviral activities and toxicities of the compounds in the cell culture are shown in the table. As evident from these data, both isomers, **Ia** and **Ib**, inhibited the replication of both HIV and HSV in the cell culture, the (*Z*)-isomer being more active than the (*E*)-isomer and comparable with the control compound, PMEA, with respect to the activity; the (*Z*)-isomer was also active against the acyclovir resistant strain HSV-1/ACV^R.

Diphosphates of the compounds were tested in one-substrate reactions of primer elongation catalyzed by HIV RT and human and HSV DNA polymerases α expressed in a baculovirus system (Fig. 1). The HSV DNA polymerase gene was cloned with the use of polymerase chain reaction (PCR) from the HSV genome and incorporated into the pFastBac HT B plasmid so that the sequence encoding a hexahistidine fragment was located at the N-end of the DNA polymerase gene. This fragment allowed us to purify the enzyme using an affine column. The resultant plasmid was used to transform *Escherichia coli* cells containing the baculovirus shuttle vector. We selected clones containing the DNA polymerase gene and transfected Sf9 insect cells; DNA polymerase was isolated chromatographically using Ni–NTA–agarose and heparin–agarose columns. The characteristics of the enzymes corresponded to those described in the literature.

Figure 2 shows the incorporation of the obtained diphosphates of the compounds studied into the 3'-end of the primer with the use of HSV and human DNA polymerases α (Fig. 2a) and HIV RT (Fig. 2b). Comparison of the intensities of the bands located above the primer at the same concentration of the substrates shows that the **Ia** diphosphate is a more effective substrate than **Ib** diphosphate for both viral enzymes but is not a substrate of human DNA polymerase α. The bands located lower than the primer, in experiments with HSV DNA polymerase, resulted from the 3' ⟶ 5' exonuclease activity of the enzyme. The diphosphate of the carbocyclic analog of guanosine (**III**) proved to be inactive towards HSV DNA polymerase (data not shown) but was a substrate of HIV RT (Fig. 2b). Selective inhibition of viral, but not cellular, enzymes is a necessary condition for the use of the tested substances in medicine.

DISCUSSION

Acyclic nucleoside diphosphonates display a wide spectrum of activity against viral and retroviral DNA [1, 2]. Upon entering a cell, they are phosphorylated by cellular kinases to the corresponding diphosphates, which interact with viral enzymes and, after incorpora-

Antiviral activity of compounds **Ia** and **Ib** in cell cultures

Compound	Anti-HIV activity in CEMss cells, µM		Anti-HSV activity in Vero cells, µM		
			HSV-1/L_2		HSV-1/ACV^R
	CD_{50}	ID_{50}	CD_{50}	ID_{50}	ID_{50}
(*Z*)-isomer(**Ia**)	>3300	81	>3100	95	106
(*E*)-isomer(**Ib**)	>3300	660	>3100	387	437
PMEA	2860	182	>344	80	77

Note: CD_{50} and ID_{50} were used as measures of the cytotoxicity and activity of the compounds, respectively.

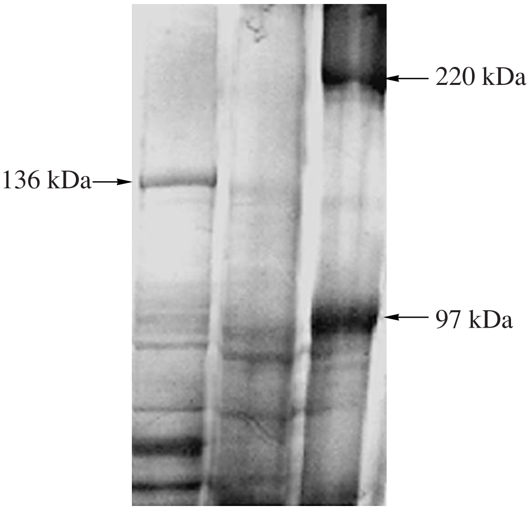


Fig. 1. Expression of HSV-1 DNA polymerase in a baculovirus system. Lanes: (1) DNA polymerase (MM = 136 kDa); (2) cell extract without the virus; (3) markers.

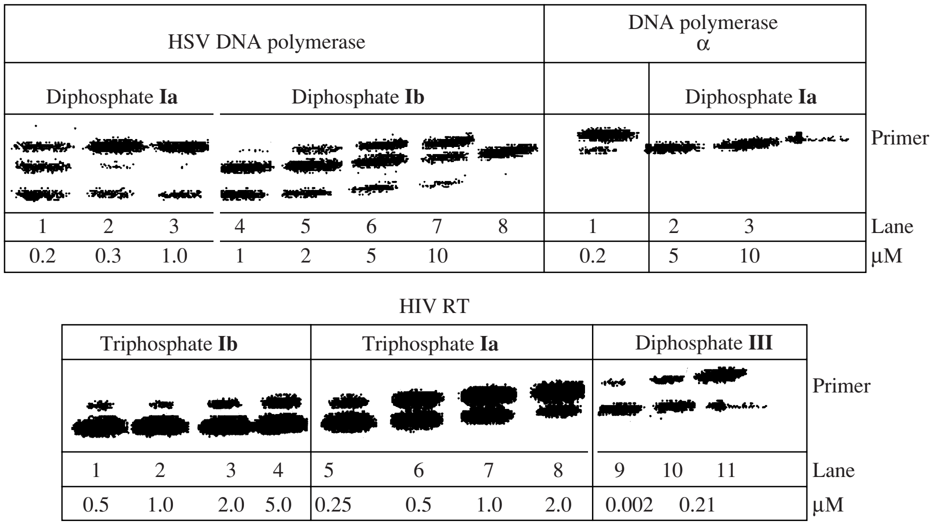


Fig. 2. Autoradiographs of the gels after PAAG electrophoresis of the products of incorporation of the synthesized compounds to the 3'-end of the [5'-^{32}P] primer–template complex catalyzed by (a) HSV DNA polymerase (lanes 1–7), DNA polymerase α (lanes 11–13) (lane 8 shows the position of the primer), and (b) HIV RT. The components of the primer–template complex for incorporation of diphosphates **Ia** and **Ib** were the following: primer, 5'-CCG TCA ATT CCT GTA GTC TCG-3'; template, 3'-GGC AGT TAA GGA CAT CAG AGC TCG GAA-5'.

tion into the viral DNA or RNA, terminate its synthesis. An advantage of these compounds is their stability in the blood serum and the capacity for inhibiting viruses independently of the cellular nucleoside kinase. The (*Z*)-isomer studied here inhibits the replication of both HIV and HSV, which often accompanies HIV infection. In addition, the (*Z*)-isomer has a low cytotoxicity, which is a necessary condition for potential drugs. The antiviral activities of **Ia** and **Ib** are correlated with the efficiency of the incorporation of their diphosphates into DNA (after the reaction catalyzed by viral enzymes) and termination of DNA synthesis.

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