

Antiviral and Antimicrobial Nucleoside Derivatives: Structural Features and Mechanisms of Action¹

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Abstract—The emergence of new viruses and resistant strains of pathogenic microorganisms has become a powerful stimulus in the search for new drugs. Nucleosides are a promising class of natural compounds, and more than a hundred drugs have already been created based on them, including antiviral, antibacterial and antitumor agents. The review considers the structural and functional features and mechanisms of action of known nucleoside analogs with antiviral, antibacterial or antiprotozoal activity. Particular attention is paid to the mechanisms that determine the antiviral effect of nucleoside analogs containing hydrophobic fragments. Depending on the structure and position of the hydrophobic substituent, such nucleosides can either block the process of penetration of viruses into cells or inhibit the stage of genome replication. The mechanisms of inhibition of viral enzymes by compounds of nucleoside and non-nucleoside nature have been compared. The stages of creation of antiparasitic drugs, which are based on the peculiarities of metabolic transformations of nucleosides in humans body and parasites, have been considered. A new approach to the creation of drugs is described, based on the use of prodrugs of modified nucleosides, which, as a result of metabolic processes, are converted into an effective drug directly in the target organ or tissue. This strategy makes it possible to reduce the general toxicity of the drug to humans and to increase the effectiveness of its action on cells infected by the virus.

Keywords: biosynthesis of nucleosides, antibacterial activity, antiviral activity, antiprotozoal activity, RNA viruses, target enzymes, hydrophobic derivatives of nucleosides

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INTRODUCTION

Rapidly mutating RNA viruses pose a serious threat to humanity. The COVID-19 pandemic caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 2020 exposed a shortage of universal antiviral agents. Viruses are obligate parasites, which genomes encode the minimum set of

enzymes and proteins necessary for the replication and assembly of viral particles. For the biosynthesis of building blocks (monomers), cell enzymes are used, so the reproduction of a virus is possible only inside a cell it has infected. There are two main types of antiviral drugs: the first type prevents the penetration of the virus into the cell, the second type disrupts the replication of the viral genome inside the cell. Nucleoside analogs can be active antiviral agents, and the mechanism of their action depends on the type and position of the substituents. The huge interest in the creation of antiviral drugs is not limited to the consideration of classical approaches, and as new technologies appear, new approaches are also being developed [1–5].

Nucleoside-based drugs are also used to treat infectious diseases caused by bacteria, protozoa and fungi. The emergence of drug resistance in pathogenic microorganisms is a serious problem for humanity due to the widespread, not always justified, use of antibiotics. That is why the development of new antimicrobial agents has been, and remains, relevant. In this review, we also discuss the strategy of searching for antiprotozoal compounds for the treatment of socially significant infectious diseases [6–9].

¹ This review is dedicated to the memory of the Doctor of Chemical Sciences, Professor Sergey Nikolaevich Mikhailov (1949–2020), an enthusiastic scientist and teacher. The entire scientific life of S.N. Mikhailov was associated with the Institute of Molecular Biology of the Russian Academy of Sciences. From 1995 to 2020, he headed the laboratory of design and synthesis of biologically active compounds. He made a great contribution to the development of enzymology and nucleoside chemistry. The last years of his life he devoted to the design of new antiviral agents based on nucleosides and their analogs.

Abbreviations: NNRTI, non-nucleoside reverse transcriptase inhibitors; RT, reverse transcriptase; PRPP, 5-phosphoribosyl-1-pyrophosphate; CMV, cytomegalovirus; HBV, hepatitis B virus; HCV, hepatitis C virus; HSV, herpes simplex virus; IMPDH, inosine-5'-monophosphate dehydrogenase; MERS-CoV, Middle East respiratory syndrome coronavirus; PIPNP, *Plasmodium falciparum* purine-nucleosidephosphorylase; RdRp, RNA-dependent RNA polymerase; SARS-CoV, severe acute respiratory syndrome coronavirus; VZV, varicella zoster virus.

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Table 1. Concentrations of metabolites involved in the biosynthesis of purine nucleosides^a

Metabolite	C ^b , μ M
ATP	2102
GTP	305
ADP	137
GDP	36
AMP	82
GMP	32
Ado	0.5
Guo	0.9
Ade	0.4
Gua	97

^a The table summarizes the data presented by T.W. Traut [12].

^b Nucleotide concentrations measured in human blood cells, base and nucleoside concentrations measured in extracellular fluid.

The creation of drugs based on natural compounds is a well proven classic approach. To date, about a hundred drugs have been created on the basis of nucleosides, half of which are antiviral and a quarter antitumor [10]. Natural nucleosides have a diverse structure; they are part of nucleotides, DNA, RNA, and coenzymes. More than 140 minor nucleosides were isolated from tRNA, and about 100 disaccharide nucleosides and 200 nucleoside antibiotics, in the structure of which there are additional functional groups and hydrophobic residues, were isolated from various natural sources. The library of natural nucleosides contains about 600 compounds, which serve as the basis for the creation of new biologically active compounds [11].

In this review, the strategies for creating antiviral and antiparasitic drugs are analyzed in the context of the peculiarities of nucleoside metabolism in various organisms. The structural features of nucleoside analogs that determine the mechanism of drug action are considered in details.

Nucleoside Metabolism is the Key to Understanding the Mechanism of Action of Nucleoside Drugs

Unlike most drugs, nucleosides and their analogs in the human body are first exposed to a number of enzymes of cellular metabolism, which convert them into active forms (nucleotides). The metabolic scheme of various modified nucleosides at the initial stage after entering the body is rather conservative. These compounds usually enter cells and, as a result of enzymatic phosphorylation, are sequentially converted into nucleoside-5'-mono-, di-, and triphosphates (NMP, NDP, and NTP, respectively). These metabolites then inhibit one or more enzymes of DNA or RNA biosynthesis (including viral polymerases). In a cell, the ratio of nucleoside phosphates (NMP : NDP : NTP) is vari-

able for different derivatives of nucleosides. It depends on the rate of phosphorylation of the parent nucleoside. The first stage of phosphorylation (NMP formation) is usually rate-limiting and, therefore, the most crucial for activation. In general, the nucleoside equilibrium (N) \leftrightarrow NTP in the cell is shifted towards the formation of triphosphates. It should also be noted that the concentration of NTP in the cell significantly exceeds the concentration of NMP, NDP, and nucleosides involved in metabolic processes necessary for growth, maintenance of vital functions of the organism, and reproduction (Table 1) [12]. These transformations are inseparably linked with the biosynthesis of nucleosides, nucleotides, and nucleic acids in the cell; therefore, knowledge of the peculiarities of nucleotide biosynthesis is the key to understanding the mechanism of action of biologically active nucleoside derivatives.

Nucleoside Biosynthesis Pathways

Purine and pyrimidine nucleosides and nucleotides (Fig. 1) are synthesized in living organisms in two ways. The main pathway (de novo) is a complex, multistep and energy-consuming process of the formation of heterocyclic bases in the presence of various donors of carbon and nitrogen, followed by the formation of nucleosides from these bases. An additional pathway for nucleoside biosynthesis, salvage pathways, allows cells to reuse free nitrogenous bases, which are formed as a result of enzymatic cleavage of nucleosides in metabolic processes. This makes nucleoside metabolism more economical.

The key reaction of de novo biosynthesis of purine nucleotides is the formation of phosphoribosylamine (5PRA) as a result of the amide group of glutamine to phosphoribosyl pyrophosphate (PRPP) under the action of amidophosphoribosyltransferase (ATase). Next, a purine heterocyclic base is assembled based on 5PRA with the participation of various carbon and nitrogen donors to form inosine-5'-monophosphate (IMP), a metabolic precursor of purine nucleotides. IMP is metabolized to AMP and GMP through the formation of intermediates: adenylosuccinate (sAMP) and xanthosine monophosphate (XMP), followed by phosphorylation by specific nucleoside-monophosphate and nucleoside diphosphate-kinases to active triphosphate forms (ATP and GTP) [13] (see "Purine biosynthesis", Fig. 1).

In contrast to the synthesis of purines, the pyrimidine heterocyclic base is initially synthesized from glutamine, CO₂ and ATP with the formation of carbamoyl phosphate. During the series of transformations, orotic acid, formed from carbamoyl phosphate, binds to ribose 5'-phosphate under the action of orotate phosphoribosyltransferase with the formation of orotidine 5'-monophosphate (OMP), the initial substrate for the de novo biosynthesis of pyrimidine nucleotides (see Fig. 1). Further decarboxylation leads to the for-

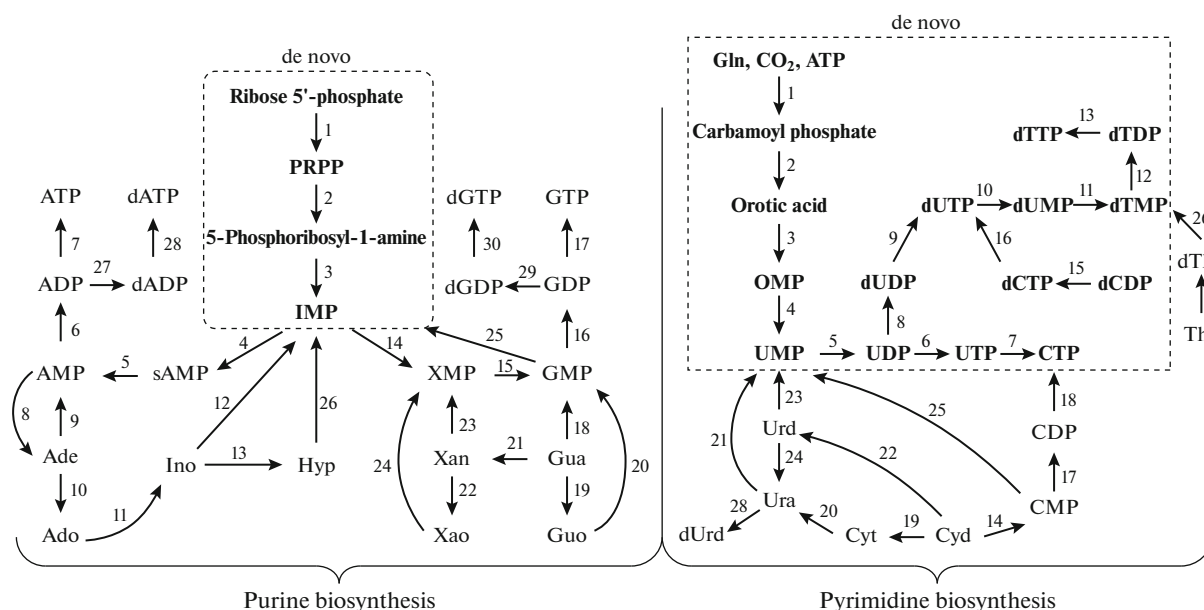


Fig. 1. Biosynthesis of purine and pyrimidine nucleosides/nucleotides. Enzymes are indicated by numbers. Purine biosynthesis: (1) phosphoribosyl pyrophosphate synthetase (EC 2.7.6.1); (2) amidophosphoribosyltransferase (EC 2.4.2.14); (3) phosphoribosylamine-glycine ligase (EC 6.3.4.13), phosphoribosylglycinamide formyltransferase (EC 2.1.2.2), phosphoribosylformylglycinamide synthetase (EC 6.3.5.3), phosphoribosylformylglycinamide cyclo-ligase (EC 6.3.3.1), phosphoribosylaminoimidazole carboxylase (EC 4.1.1.21), 5-(carboxyamino)imidazole ribonucleotide mutase (EC 5.4.99.18), phosphoribosylaminoimidazole-succinocarboxamide synthetase (EC 6.3.2.6), adenylosuccinate lyase (EC 4.3.2.2), phosphoribosylaminoimidazolecarboxamideformyltransferase (EC 2.1.2.3); (4) adenylosuccinate synthetase (EC 6.3.4.4); (5) adenylosuccinate lyase (EC 4.3.2.2); (6) adenylate kinase (EC 2.7.4.3); (7) nucleoside diphosphate kinase (EC 2.7.4.6); (8) adenylate nucleosidase (EC 3.2.2.4); (9) adenine phosphoribosyltransferase (EC 2.4.2.7); (10) purine nucleoside phosphorylase (EC 2.4.2.1); (11) adenosine deaminase (EC 3.5.4.4); (12) guanosine-inosine kinase (EC 2.7.1.73); (13) purine nucleoside phosphorylase (EC 2.4.2.1); (14) inosine-5'-monophosphate dehydrogenase (EC 1.1.1.205); (15) guanylate synthetase (EC 6.3.5.2); (16) guanylate kinase (EC 2.7.4.8); (17) nucleoside diphosphate kinase (EC 2.7.4.6); (18) hypoxanthine guanine phosphoribosyltransferase (EC 2.4.2.8); (19) purine nucleoside phosphorylase (EC 2.4.2.1); (20) guanosine-inosine kinase (EC 2.7.1.73); (21) guanine deaminase (EC 3.5.4.3); (22) purine nucleoside phosphorylase (EC 2.4.2.1); (23) xanthine-guanine phosphoribosyltransferase (EC 2.4.2.22); (24) guanosine-inosine kinase (EC 2.7.1.73); (25) guanosine-5'-monophosphate reductase (EC 1.1.1.7); (26) hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8); (27) ribonucleoside diphosphate reductase (EC 1.17.4.1); (28) nucleoside diphosphate kinase (EC 2.7.4.6); (29) ribonucleoside diphosphate reductase (EC 1.17.4.1); (30) nucleoside diphosphate kinase (EC 2.7.4.6). Pyrimidine biosynthesis: (1) carbamoyl phosphate synthase (EC 6.3.5.5); (2) aspartate carbamoyltransferase (EC 2.1.3.2), dihydroorotase (EC 3.5.2.3), dihydroorotate dehydrogenase (EC 1.3.99.11); (3) orotate phosphoribosyltransferase (EC 2.4.2.10); (4) orotidine 5'-phosphate decarboxylase (EC 4.1.1.23); (5) uridylylase kinase (EC 2.7.4.22); (6) nucleoside diphosphate kinase (EC 2.7.4.6); (7) cytidine 5'-triphosphate synthase (EC 6.3.4.2); (8) ribonucleoside diphosphate reductase (EC 1.17.4.1); (9) nucleoside diphosphate kinase (EC 2.7.4.6); (10) deoxyuridine triphosphate (dUTP) diphosphatase (EC 3.6.1.23); (11) thymidylate synthase (EC 2.1.1.45); (12) thymidylate kinase (EC 2.7.4.9); (13) nucleoside diphosphate kinase (EC 2.7.4.6); (14) uridine-cytidine kinase (EC 2.7.1.48); (15) nucleoside diphosphate kinase (EC 2.7.4.6); (16) deoxycytidine triphosphate (dCTP) deaminase (EC 3.5.4.13); (17) cytidylate kinase (EC 2.7.4.14); (18) nucleoside diphosphate kinase (EC 2.7.4.6); (19) nucleoside hydrolyase (EC 3.2.2); (20) cytosine deaminase (EC 3.5.4.1); (21) uracil phosphoribosyltransferase (EC 2.4.2.9); (22) cytidine deaminase (EC 3.5.4.5); (23) uridine-cytidine kinase (EC 2.7.1.48); (24) uridine phosphorylase (EC 2.4.2.3); (25) cytidine deaminase (EC 3.5.4.5); (26) thymidine kinase (EC 2.7.1.21); (27) thymidine phosphorylase (EC 2.4.2.4); (28) thymidine phosphorylase (EC 2.4.2.4). Abbreviations: PRPP, phosphoribosyl pyrophosphate; IMP, inosine monophosphate; OMP, orotidine 5'-monophosphate; sAMP, adenylosuccinate; XMP, xanthosine-5'-monophosphate; Xao, xanthosine, Xan, xanthine; Thy, thymine; Thd, thymidine, Hyp, hypoxanthine. A simplified scheme of biosynthesis was compiled on the basis of data on metabolic transformations of nucleosides/nucleotides [12–14].

mation of an intermediate biosynthesis product, uridine-5'-monophosphate (UMP), which, as a result of sequential phosphorylation, is converted into active uridine-5'-triphosphate (UTP). UTP is involved in the formation of cytidine-5'-triphosphate (CTP) under the action of cytidine-5'-triphosphate synthase, which catalyzes the amidation of UTP by ATP-dependent exchange reaction of the ketone group of uracil with the amide group of glutamine [13, 14].

Deoxyribonucleotides (dATP, dGTP, and dCTP) included in DNA are formed de novo from ribonucleoside-5'-diphosphates under the action of ribonucleoside-diphosphate reductases and nucleoside-diphosphate kinases (Fig. 1). Thymidine 5'-triphosphate (dTTP) is formed in a series of enzymatic reactions including the conversion of deoxyuridine 5'-triphosphate (dUTP) to deoxyuridine-5'-monophosphate (dUMP) by deoxyuridine triphosphatase, methylation

of dUMP to dTMP catalyzed by the thymidylate synthase, and sequential phosphorylation of dTMP by nucleotide kinases. Most of the dUTP (about 75%) used for dTTP synthesis is formed as a result of dCTP deamination by deoxycytidine triphosphate deaminase, rather than directly from UDP under the action of ribonucleotide reductase and nucleoside diphosphate-kinase [13].

The “re-utilization” pathway of biosynthesis is based on the attachment of the PRPP molecule to the free nitrogenous base (formed as a result of the decomposition of nucleic acids and subsequent enzymatic cleavage of nucleotides), meanwhile the PRPP-dependent phosphoribosylation of purines is catalyzed by adenine phosphoribosyltransferase, which is responsible for the formation of AMP from adenine, and hypoxanthine-guanine phosphoribosyltransferase (HGPRT), which catalyzes the formation of IMP and GMP from hypoxanthine and guanine, respectively. For the re-utilization pathway, one of the main stages, accompanied by phosphorolysis, is the cleavage of the *N*-glycosidic bond of purine nucleosides by purine nucleoside phosphorylase, and pyrimidine nucleosides, by pyrimidine nucleoside phosphorylase and uridine phosphorylase. All these enzymes catalyze the reversible cleavage of the glycosidic bond of the corresponding ribo- and deoxyribonucleosides in the presence of inorganic phosphate with the formation of a heterocyclic base and ribose/deoxyribose-1-phosphate. The thermodynamic equilibrium of reactions is shifted towards the synthesis of nucleosides [15], and this is more significant in the case of purine nucleosides [16]. The key enzyme for the phosphorolysis of pyrimidine nucleosides is uridine phosphorylase, which catalyzes the reversible cleavage of uridine, 2'-deoxyuridine and thymidine to the corresponding heterocyclic bases and ribose-1-phosphate. Further conversion of nucleosides to nucleotides is catalyzed by uridine-cytidine kinase, which phosphorylates uridine and cytidine to 5'-monophosphate forms with equal efficiency [17].

An additional biosynthetic pathway for pyrimidine deoxyribonucleotides consists in the phosphorylation of thymidine to dTMP by thymidine kinase. The phosphorolytic cleavage of deoxyuridine and thymidine by thymidine phosphorylase also plays a key role; in this case, the thermodynamic equilibrium is shifted towards the formation of nucleosides [13].

Thus, the metabolism of nucleosides/nucleotides is a complex multistep process that includes a large set of enzymes and reactions that convert nucleotides, nucleosides, and nitrogenous bases. Possessing structural similarity to natural substrates, drug compounds of a nucleoside nature can affect different stages of nucleoside biosynthesis.

*The Main Differences in de novo Biosynthesis of Nucleosides in Different Organisms.
The Principles of Creating Antiparasitic Drugs*

All living organisms of all three domains, bacteria, archaea, and eukaryotes, have de novo purine and pyrimidine biosynthesis [18]. The pathways of biosynthesis in bacteria and eukaryotes are practically the same, while in archaea they differ [19]. In the genomes of bacteria and archaea, clusters of genes encoding de novo biosynthetic enzymes are organized differently [20, 21]. The regulation of transcription of these genes also differs [19, 20]. But the enzymes involved in biosynthesis are the same in bacteria and archaea, while a number of enzymes of de novo biosynthesis differ in some eukaryotes [18]. Thus, in the yeast *Saccharomyces cerevisiae*, the active sites of carbamoyl phosphate synthetase (CPSase; EC 6.3.5.5) and aspartate carbamoyltransferase (ATCase; EC 2.1.3.2) are incorporated into the total polypeptide chain, which also contains an inactive variant of dihydroorotase (DHOase; EC 3.5.2.3). For pyrimidine biosynthesis, mammalian cells use a trifunctional protein, dihydroorotase synthetase, which contains three active centers in one polypeptide chain, which alternately perform the functions of CPSase, ATCase, and DHOase [18, 20], and a bifunctional protein, in the polypeptide chain of which are the active sites of orotate phosphoribosyltransferase (EC 2.4.2.10) and orotodin 5'-phosphate decarboxylase (OMPdecase; EC 4.1.1.23) [20]. Enzymes of pyrimidine biosynthesis in lower eukaryotes are located in mitochondria, and in mammals, in the cytosol of cells [18, 20], which ensures the maximum compactness of the reaction space during de novo synthesis in mammalian cells. In cells 95% of de novo biosynthesis is provided by IMP and UMP [18]. Viruses and phages, as infectious agents that reproduce only in living cells, use in their infectious cycle nucleosides/nucleotides synthesized by the host [22].

During evolution, some organisms lost some of the genes necessary for purine or pyrimidine synthesis de novo. These are auxotrophs, their existence is possible only if there are purine/pyrimidine derivatives in the nutrient medium. They can parasitize in other organisms, in particular in the human body, causing various diseases. The re-utilization pathway of nucleoside biosynthesis is of key importance for them. Consequently, enzymes catalyzing reactions of an additional pathway of nucleotide metabolism (among them nucleoside phosphorylases) can be considered as targets for the creation of antibacterial and antiprotozoal drugs [23–26]. Purine auxotrophs include such organisms as the helminth *Schistosoma mansoni*, which causes an endemic disease in humans, schistosomiasis [25, 26], and the malaria plasmodium, *Plasmodium falciparum*, which is the causative agent of a severe form of malaria [23, 24]. The pyrimidine auxotrophs include the flagellar protist *Giardia lamblia* [27–29], which can parasitize in the small intestine of

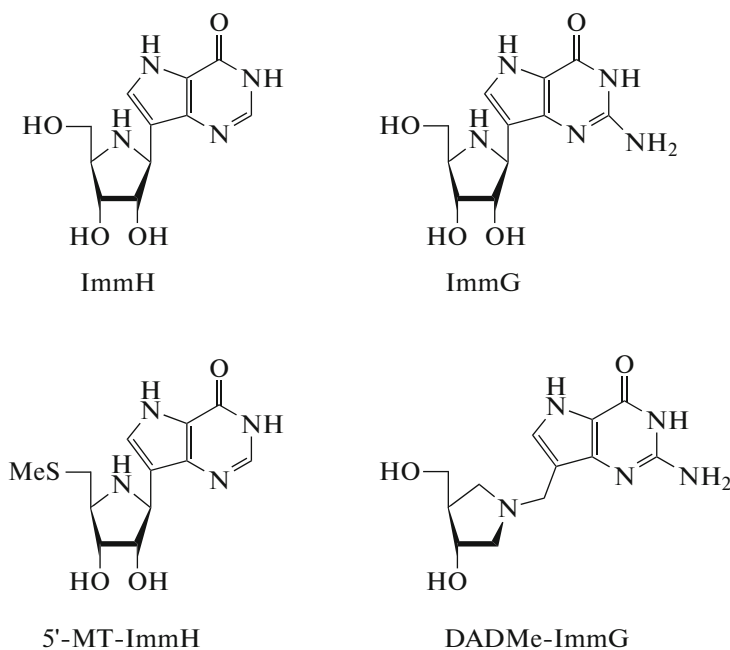


Fig. 2. Structures of immunocillins.

mammals and birds, causing giardiasis. The search for effective and highly specific inhibitors of nucleoside phosphorylases is one of the strategies for creating drugs for the treatment of diseases caused by these parasites. Certain results have already been achieved on this path. Derivatives of immunocillin were studied in detail (Fig. 2), the conformation of which is close to the assumed transitional conformation of the substrate of purine nucleoside phosphorylases [30, 31]. Earlier reported that 5'-methylthio-immucillin-H (5'-MT-ImmH) binds to the purine nucleoside phosphorylase (PfPNP) of *Plasmodium falciparum* 112 times more efficiently than to the purine nucleoside phosphorylase of human erythrocytes (K_d 2.7 and 303 nM, respectively). This is most likely due to the different structure of the active sites of these two purine nucleoside phosphorylases [31]. 5'-MT-ImmH has been proposed as a PfPNP inhibitor for the treatment of malaria in humans [9, 31, 32]. Another immunocillin derivative, DADMe-Immucillin-G (BCX4945), is currently in preclinical trials. It has already been demonstrated to be highly effective in treating malaria in a monkey model [33]. The structure of its complex with purine nucleoside phosphorylase of human erythrocytes was studied using NMR spectroscopy [34].

MECHANISM OF ANTI-VIRAL ACTION OF NUCLEOSIDES

Polymerase Inhibitors

The mechanism of biological activity of most anti-viral drugs based on nucleosides and their analogs is associated with their intracellular transformation into

5'-mono-, di- and triphosphates under the action of viral and cellular kinases with subsequent inclusion of triphosphates into nucleic acids, which leads to inhibition of viral genome replication [3]. The genome of a virus encodes a number of proteins and enzymes necessary for the multiplication of the virus in the infected cell, while maintenance of the life cycle of a virus is carried out through the metabolic pathways of the host cell with the involvement of the corresponding enzymes.

The viral enzymes involved in the replication of viral nucleic acids are RNA-dependent RNA polymerase (RNA-containing viruses), RNA-dependent DNA polymerase (retroviruses), and DNA-dependent DNA polymerase and DNA-dependent RNA polymerase for DNA containing viruses. Herpes simplex viruses (HSV), in addition to DNA polymerase as the main replication enzyme, encode thymidine kinase, which carries out 5'-monophosphorylation of nucleosides and their analogs in the cell [35]. Viral enzymes are usually less specific than the enzymes of the host cell, which leads to a high rate of viral reproduction and the rapid development of drug resistance.

The action of nucleosides and their analogs can be aimed at inhibiting the synthesis of viral nucleic acids, increasing the frequency of lethal mutations of viral genomes (lethal mutagenesis) and other possible mechanisms of action aimed at inhibiting the biosynthesis of nucleosides [4]. In viruses, enzymes that differ from cellular enzymes are involved in the synthesis of nucleic acids these are: DNA-dependent DNA polymerase, RNA-dependent DNA polymerase (reverse transcriptase, RT), and RNA-dependent

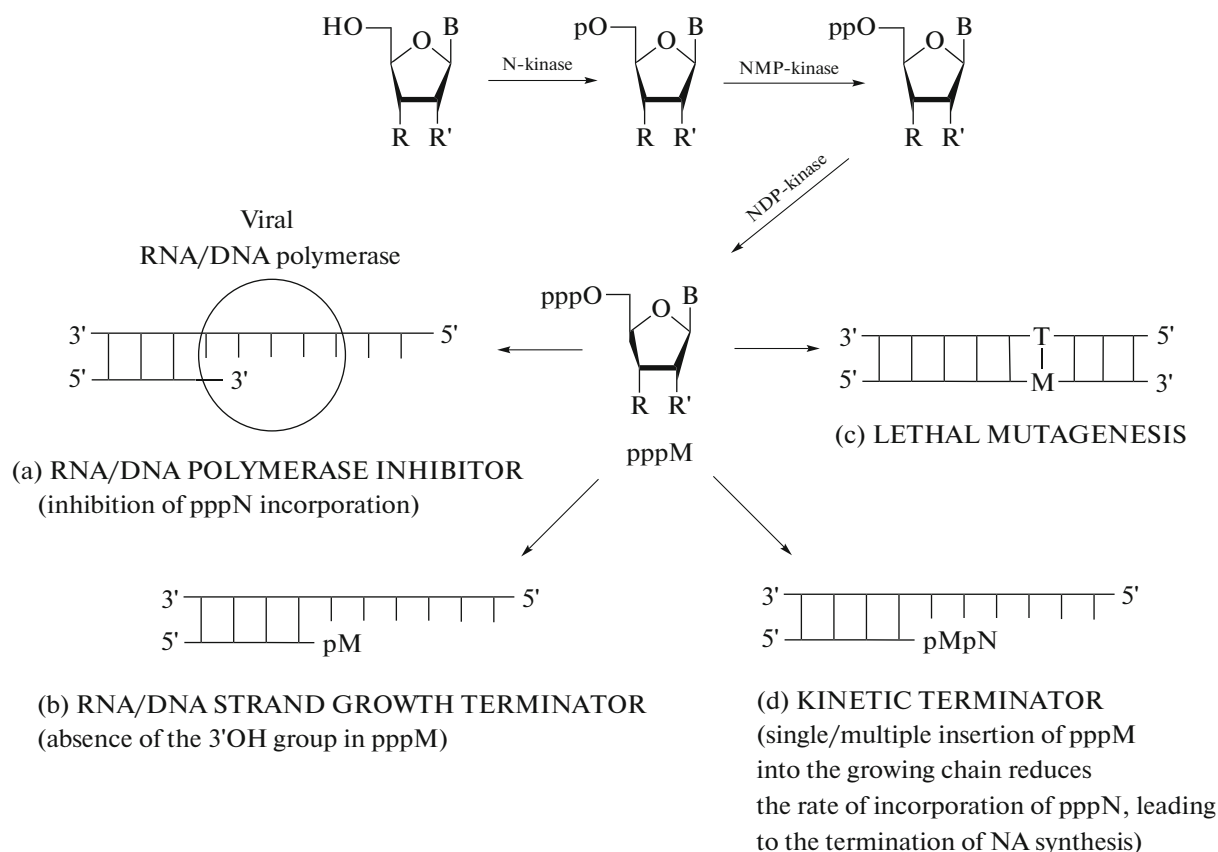


Fig. 3. The main mechanisms of action of nucleoside drugs on the synthesis of viral RNA/DNA. The triphosphate form of a nucleoside analog can act as an RNA/DNA polymerase inhibitor (a), an RNA/DNA chain growth terminator (b), a kinetic terminator (c), and by the mechanism of lethal mutagenesis (d). *Abbreviations:* N-kinase, nucleoside kinase; NA, nucleic acid; M, mutation; pppM, nucleoside 5'-triphosphate analog; pppN, natural nucleoside 5'-triphosphate; B, heterocyclic base; R = H, OH; R' = H, OH.

RNA polymerase. Nucleoside analogs in the form of NTP are aimed at suppressing viral RNA / DNA synthesis in various ways, which are shown in Fig. 3.

Any modified nucleoside 5'-triphosphate can inhibit the synthesis of nucleic acids either as a competitive inhibitor of the incorporation of natural NTP into the growing nucleic acid chain (Fig. 3a), or as a terminator of chain growth due to the incorporation of nucleoside 5'-triphosphate not containing a hydroxyl group in the 3'-position, into the growing RNA/DNA strand (Fig. 3b). Nucleoside-5'-triphosphates containing a sterically inaccessible for the enzymatic reaction hydroxyl group in the 3'-position can also act as terminators of chain growth. For example, 3'-methyl-UTP, which is the termination substrate of *Escherichia coli* RNA polymerase, can be used for nucleic acid sequencing. In this compound, the 3'-methyl group, which replaces the proton, significantly reduces the reactivity of the 3'-hydroxyl group [36, 37].

Kinetic termination of the growth of the nucleic acid chain is also possible: in this case the modified nucleoside 5'-triphosphate can be incorporated into the growing chain, but the rate of its incorporation and

subsequent chain elongation are greatly slowed down in comparison with natural NTP. Kinetic termination can be caused by intramolecular or intermolecular sterically nonoptimal interactions of the enzyme with modified nucleoside 5'-triphosphate, as well as disruption of the hydrogen bonds between the matrix and primer (Fig. 3d). As a result, the synthesis of the nucleic acid chain stops. This phenomenon was first described in the late 1980s into the 90s [37–39]. Under the conditions of a single-substrate reaction, when the *D*-allo-diastereomer or *L*-talo-diastereomer of 5'-methyl-UTP (5'-aMe-UTP and 5'-tMe-UTP) was added to the presynthesized primer RNA together with natural UTP, 5'-aMe-UTP was incorporated approximately 100 times less efficiently, and 5'-tMe-UTP was incorporated 1000 times less efficiently than the natural substrate of UTP [38, 39]. In the absence of UTP, a natural substrate of RNA polymerase, no more than two 5'-C-methyluridine residues were attached to the presynthesized RNA in this case, that is, the efficiency of the subsequent incorporation of analogs decreased even more compared to the conditions in which the

mixture of UTP and 5'-aMe-UTP or 5'-tMe-UTP was added to the enzyme [38, 39].

Another mechanism of antiviral activity of nucleoside-based compounds is lethal mutagenesis. Mutations upon the inclusion of a modified nucleoside in the RNA/DNA chain can lead to the formation of non-viable virus strains (Fig. 3c) [40]. In addition, the presence of modified nucleosides in the cell, as well as the corresponding NMP, NDP, and NTP, can inhibit the biosynthesis of nucleic acids by changing the concentrations of natural NMP, NDP, and NTP and their ratios. The main mechanisms of action of a number of known drugs are discussed below.

Most of the nucleoside/nucleotide analogs of drugs used as antiviral agents act as inhibitors and terminators of DNA or RNA synthesis [2, 5].

The clinically approved drugs acyclovir, ganciclovir, and penciclovir (Table 2), which are acyclic analogues of nucleosides, as well as their prodrugs that increase oral bioavailability (valacyclovir, valganciclovir and famciclovir), are active against DNA-containing viruses: Herpes simplex viruses (HSV) and Cytomegaloviruses (CMV) [1]. These drugs inhibit HSV and CMV polymerases and terminate replication by acting in the form of triphosphates. The genomes of viruses of the *Herpesviridae* family encode a number of enzymes of nucleic acid metabolism: thymidine kinase, thymidylate synthase, deoxyuridine triphosphatase, and ribonucleotide reductase [35]. Of greatest interest, from the point of view of intracellular activation of nucleosides and their analogs, is thymidine kinase, which catalyzes the first, rate-limiting step: monophosphorylation. Thymidine kinase of HSV does not belong to highly specific enzymes; therefore, it can phosphorylate not only thymidine, but also purine nucleoside analogs. Acyclovir and penciclovir, medicines approved by the US Food and Drug Administration (FDA) for the treatment of HSV, are phosphorylated by viral thymidine kinases after penetration into the cell to the corresponding monophosphates [41–45]. Subsequent two-step phosphorylation by the host cell enzymes guanylate kinase and nucleoside-diphosphate kinase, leads to the formation of their triphosphorylated forms, which compete with the natural substrate, dGTP, for incorporation into DNA blocking its synthesis [41]. Ganciclovir-5'-triphosphate and valganciclovir-5'-triphosphate are incorporated into viral DNA and block viral replication, working as kinetic terminators [43].

The purine nucleoside analog, vidarabine (Table 2), targeting HSV and varicella-zoster virus (VZV), in the active 5'-triphosphate form stops viral DNA replication by competitive inhibition of viral DNA polymerase, replacing dATP [46].

It should be noted that along with the main mechanism of action of nucleoside analogs, other pathways and targets are known (ribonucleoside reductase, RNA polyadenylation, and S-adenosyl homocysteine

hydrolase) [43]. The mechanism of the antiviral action of 2'-deoxyuridine analogs (idoxuridine, trifluridine; Table 2) has not been studied in details. It is assumed that these compounds inhibit the DNA synthesis of herpes viruses. After mono-phosphorylation with specific viral thymidine kinases and then cellular kinases to 5'-triphosphate forms, these drugs compete with dTTP for insertion into viral DNA [47]. After mono-phosphorylation with viral thymidine kinases, 5-(trifluoromethyl)-2'-deoxyuridine-5'-monophosphate irreversibly inhibits thymidylate synthase, and its triphosphate competes with dTTP for incorporation into the DNA strand synthesized by DNA polymerase [43]. The mechanism of action of another analogue of 2'-deoxyuridine, brivudine (the drug is approved for the treatment of the herpes virus in a number of European countries), is kinetic termination of the DNA synthesis of HSV-1 and VZV [47–50].

Acyclic nucleoside phosphonate analogs (cidofovir, adefovir dipivoxil, tenofovir disoproxil fumarate; Table 2) also act as viral replication terminators, whose mechanism of action is based on the presence of a phosphonate bond (–PC–) instead of the usual phosphate bond (–POC–). This is a characteristic feature of nucleotide analogs active against CMV polymerases, HIV, and hepatitis B virus (HBV) [51]. These drugs are initially phosphorylated by cellular kinases to phosphono-diphosphate forms, since their phosphonate group mimics 5'-monophosphate in nucleotides. As in the case of other modified nucleoside 5'-triphosphates, they competitively inhibit the incorporation of the natural substrate into the growing DNA strand and, as a consequence, the activity of DNA polymerase. The phosphonate bond, in contrast to the phosphate bond, cannot be cleaved by phosphodiesterase, which ensures the high stability of these compounds in the cell [52, 53].

The drugs adefovir and tenofovir are able to bypass the first limiting stage of phosphorylation in the intracellular metabolism of nucleoside analogs. In diphosphorylated forms, adefovir and tenofovir act as alternative substrates and chain terminators in the DNA synthesis reaction catalyzed by HIV reverse transcriptase (HIV RT) [54–56]. The inhibitory effect of adefovir and tenofovir on HBV replication may be related to the termination of viral DNA chain growth in the presence of RT. Adefovir and tenofovir in the diphosphate form apparently have higher affinity for HIV RT (and HBV RT) than for cellular DNA polymerases α , β , γ , δ , and ϵ [57, 58], and this may explain their selectivity to retroviruses and hepadnaviruses.

All nucleoside HIV RT inhibitors, known as 2',3'-dideoxynucleoside analogs (zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, emtricitabine; Table 2), in the form of 5'-triphosphates terminate viral DNA synthesis. Their mechanism of action is to compete with natural substrates for incorporation into viral DNA synthesized by HIV RT. Once incor-

Table 2. Antiviral drugs of nucleoside nature [1, 2, 41–45, 47, 50, 51, 54–59, 61, 62, 65–101]

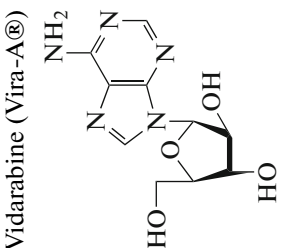
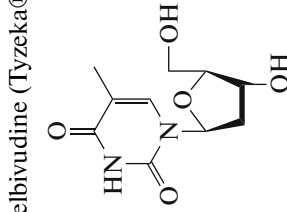
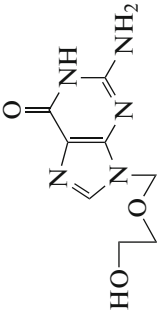
No.	Virus	Drug	Molecular target IC ₅₀ (K _i or K _d) ^a	EC ₅₀ ^b	Toxicity and side effects	Status of the drug
<i>I. Competitive inhibitors of viral DNA polymerase</i>						
1	HSV, VZV	Vidarabine (Vira-A®) 	DNA polymerase	62.7 ± 8.6 μM ^c [90]	34.7 ± 2.4 μM (HSV-1/Vero) [90] 42.1 ± 1.8 μM (HSV-2/Vero) [90] 1.6 ± 0.4 μM (VZV/HEL) [91]	Approved in 1976
			Thymidine kinase	–		
2	HBV	Telbivudine (Tyzeka®) 	HBV polymerase	243 ± 9 nM ^e [61]	8.950 ± 4.803 nM [61]	Approved in 2006
<i>II. Competitive inhibitors of viral DNA polymerase and chain growth terminators</i>						
3	HSV, VZV	Acyclovir (Acyclovir®) 	DNA polymerase	K _d 6 ± 1 μM ^e (HSV) [92]	<0.56–3.3 μM (HSV/Vero) [85] 3.78 ± 0.58 μM (HSV-1/Vero) [90] 8 ± 0.44 nM (VZV/HEL) [91]	Approved in 1982
			Thymidine kinase	–		

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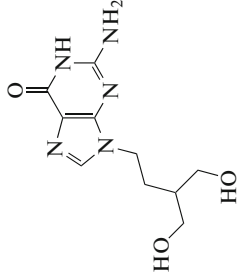
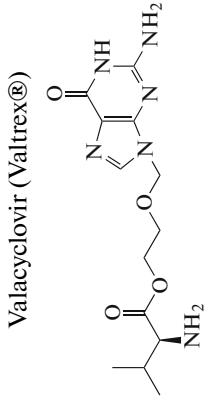
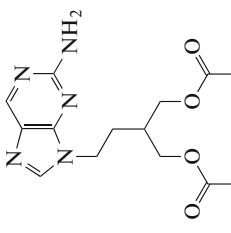
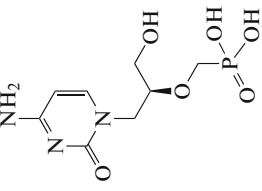
No.	Virus	Drug	Molecular target	IC ₅₀ (K _i or K _d) ^a	EC ₅₀ ^b	Toxicity and side effects	Status of the drug
4	HSV	Penciclovir (Denavir®) 	DNA polymerase, Thymidine kinase	–	0.16–1.97 μM (HSV-1/WISH), 0.79–2.37 μM (HSV-1/MRC-5), 0.39–3.16 μM (HSV-2/WISH), 3.55–8.29 μM (HSV-2/MRC-5) [93]	Headache and epigastric pain, increased serum lipase levels, nausea, dyspepsia, dizziness, hyperbilirubinemia [86]	Approved in 1993
5	HSV, VZV, CMV	Valacyclovir (Valtrex®) 	DNA polymerase, Thymidine kinase	–	0.09–60 μM (HSV-1) 0.04–44 μM (HSV-2) 0.53–48 μM (VZV) [85]	LD ₅₀ = 903.5 mg/kg (rats) [86]	Approved in 1995
6	HSV, VZV	Famciclovir (Famvir®) 	DNA polymerase, Thymidine kinase	–	1.2–2.6 μM (HSV-1/2) 6.7–71 μM (VZV) [93]	–	Approved in 1994
7	HSV, CMV (in patients with AIDS)	Cidofovir (Vistide®) 	DNA polymerase	6.6 ± 0.8 μM ^f (CMV) [94]	0.82–1.29 μM (HSV/Vero) [95]	Carcinogenesis, erythropenia, lymphopenia, nephrotoxicity, teratogenicity [88]	Approved in 1996
			Thymidine kinase	–			

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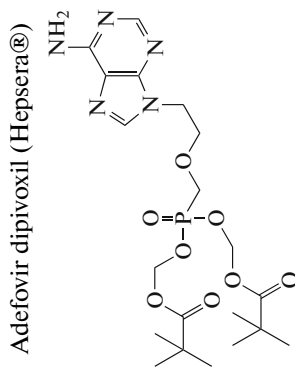
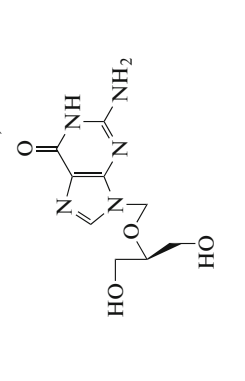
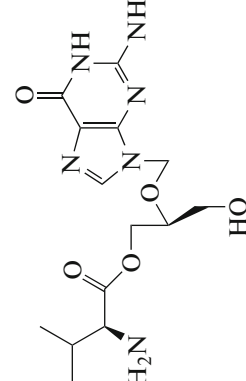
No.	Virus	Drug	Molecular target IC ₅₀ (K _i or K _d) ^a	EC ₅₀ ^b	Toxicity and side effects	Status of the drug
8	HBV	Adefovir dipivoxil (Hepsera®) 	HBV polymerase 31 ± 2.7 nM ^r [61]	2.636 ± 1.549 nM (HBV/HepG2) [61]	Nephrotoxicity [86]	Approved in 2002
<i>III. Competitive inhibitors of viral RNA/DNA polymerase and kinetic chain terminators</i>						
9	CMV	Ganciclovir (Zirgan®, Cytovene®, Vitrasert®) 	DNA polymerase, Thymidine kinase —	0.08–13.6 μM [93]	Pancytopenia, deterioration of the gastrointestinal tract, acute renal failure [86]	Approved in 1989
10	CMV	Valganciclovir (Valcyte®) 	DNA polymerase, Thymidine kinase —	0.08–22.94 μM [93]	Nephrotoxicity [86]	Approved in 2001

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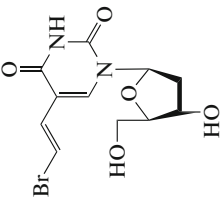
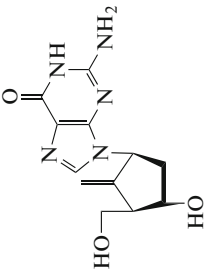
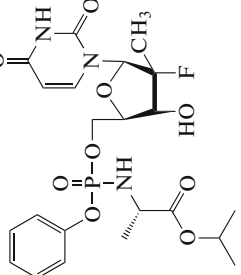
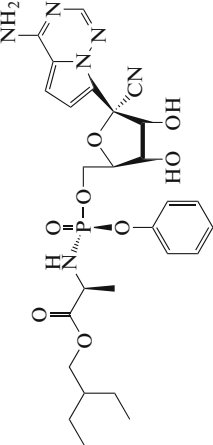
No.	Virus	Drug	Molecular target	IC ₅₀ (K _i or K _d) ^a	EC ₅₀ ^b	Toxicity and side effects	Status of the drug
11	VZV	Brivudine ^b (Zostex®, Brivirac®) 	DNA polymerase, Thymidine kinase	–	3.00–9.00 mM (VZV) [50]	–	Approved in 2000
12	HBV	Entecavir (Baraclude®) 	HBV polymerase	0.5 ± 0.1 nM ^c [61]	5.3 ± 2.5 nM (HBV/HepG2) [61]	Not described [86]	Approved in 2005
13	HCV	Sofosbuvir (Sovaldji®) 	RNA-dependent RNA polymerase (NS5B)	0.7–2.6 μM ^e [85]	0.014–0.11 μM [85]	Headache, fatigue, bradycardia (rare) [86]	Approved in 2013
14	EBOV, MERS-CoV, SARS-CoV-1/2	Remdesivir (Veklury®) 	RNA-dependent RNA polymerase	0.032 μM ^f (MERS-CoV) [85]	9.9 nM (SARS-CoV-2/HAE), 280 nM (SARS-CoV-2/CALU) [85]	Not described [86]	Approved for emergency use in the US (SARS-CoV-2) in 2020

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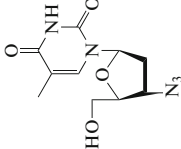
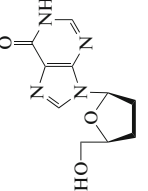
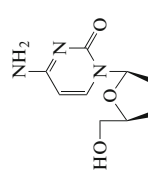
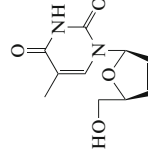
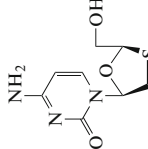
No.	Virus	Drug	Molecular target	IC ₅₀ (K _i or K _d) ^a	EC ₅₀ ^b	Toxicity and side effects	Status of the drug
<i>IV. Competitive HIV RT inhibitors and chain growth terminators</i>							
15	HIV	Zidovudine, Azidothymidine (Azidothymidine, Retrovir®) 	HIV RT, telomerase RT (TERT)	270 ± 3.7 nM ^e [96]	0.01–0.49 μM (monocytes, lymphocytes) [93]	LD ₅₀ = 3,084 mg/kg (oral administration, mice); fatigue, headache, nausea, vomiting [86]	Approved in 1987
16	HIV	Didanosine (Videx®) 	HIV RT	–	2.5–10 μM (lymphoblasts) 0.01–0.1 μM (monocytes, macrophages) [93]	Pancreatitis, peripheral neuropathy, diarrhea, hyperuricemia, liver failure [86]	Approved in 1991
17	HIV	Zalcitabine (Hivid®) 	HIV RT	–	30–500 nM (lymphoblasts, monocytes, lymphocytes) [85]	Overdose > 1.5 mg/kg; rash, fever [86]	Approved in 1992
18	HIV	Stavudine (Zerit®) 	HIV RT	0.0083–0.032 μM ^e [85]	0.009–4 μM (monocytes, lymphoblasts, mononuclear cells) [85]	Peripheral neuropathy, burning, numbness, pain in the arms or legs [86]	Approved in 1994
19	HIV, HBV	Lamivudine (Epivir®) 	HIV RT, HBV polymerase	6.2 ± 4.4 nM ^e (HBV) [61]	1.491 ± 1.033 nM (HBV) [61]	Headache, nausea, malaise, fatigue, diarrhea, cough [86]	Approved in 1995

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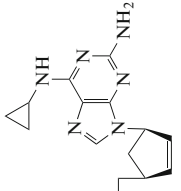
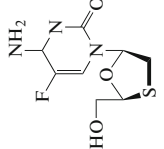
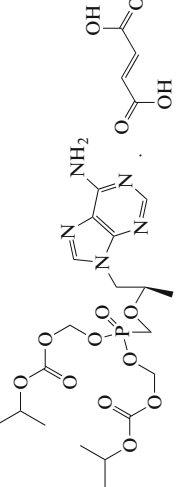
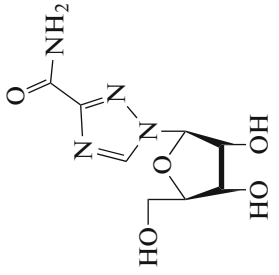
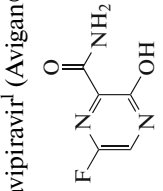
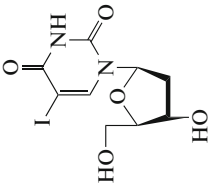
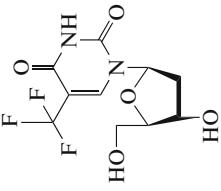
No.	Virus	Drug	Molecular target	IC ₅₀ (K _i or K _d) ^a	EC ₅₀ ^b	Toxicity and side effects	Status of the drug
20	HBV	Abacavir (Ziagen®) 	HIV RT	—	0.26 ± 0.18 μM (lymphoblasts, monocytes) [93]	Headache, malaise, fatigue, nausea, vomiting, sleep disturbance [86]	Approved in 1995
21	HIV, HBV	Emtricitabine (Emtriva®) 	HIV RT	—	0.0013–0.64 μM (lymphoblasts, MAGI-CCR5, peripheral blood mononuclear cells) [93]	Hepatotoxicity, acidosis [86]	Approved in 2003
22	HIV, HBV	Tenofovir disoproxil fumarate (Viread®) 	HIV RT, HBV polymerase	—	0.04–8.5 μM (HIV/lymphoblasts, monocytes, macrophages, lymphocytes) [93] 2.482 ± 1.938 nM (HBV) [61]	Carcinogenesis [86]	Approved in 2001 (HIV) and 2008 (HBV)
<i>V. Competitive inhibitors of viral RNA polymerase + inhibitors of IMPDH + lethal mutagenesis</i>							
23	HCV, RSV ^k , hantavirus, influenza virus	Ribavirin (Copegus®, Rebetol®, Virazole®) 	IMPDH	K _i 200 μM ^g [98]	8.4 μM (HCV/Huh-7.5) [99]	LD ₅₀ = 2700 mg/kg (rats) LD ₅₀ = 1300 mg/kg (mice) Erythropenia, sleep disturbance, irritability, anemia, liver dysfunction (HIV patients) [86]	Approved in 1985
				—			

Table 2. (Contd.)

No.	Virus	Drug	Molecular target IC ₅₀ (K _i or K _d) ^a	EC ₅₀ ^b	Toxicity and side effects	Status of the drug
24	Influenza virus, SARS-CoV-2	Favipiravir ^l (Avigan®) 	RNA-dependent RNA polymerase	0.43–0.61 μM (influenza A virus (H1N1)/MDCK) 1.34–1.97 μM (influenza A virus (H3N2)/MDCK) [100]	LD ₅₀ > 2000 mg/kg (rats) LD ₅₀ > 1000 mg/kg (dogs, monkeys) Not described in humans; in animals: inhibition of hematopoiesis, erythropenia, effect on the function of hepatocytes, teratogenicity [86]	Approved in Japan in 2014 (influenza A, B, C); in Russia and China in 2020 (SARS-CoV-2)
<i>VI. Inhibitors of viral DNA synthesis; mechanism not fully understood</i>						
25	HSV, VZV	Idoxuridine (Herplex®) 	DNA polymerase	2.8 μM (HSV-1) [101] 8.5–11.2 μM (BV) [101]	LD ₅₀ = 3080 mg/kg (mice); hypersensitivity of the eyes to light [86]	Approved in 1963
26	HSV	Trifluridine (Viroptic®) 	Thymidylate synthase	<1.7 μM (HSV-1) [101] 1.7–3.4 μM (BV) [101]	LD ₅₀ = 2 946 mg/kg (rats, intravenously), LD ₅₀ > 4379 mg/kg (oral administration); suppression of bone marrow function [86]	Approved in 1980

^a IC₅₀—concentration of 50% inhibition of enzyme activity in the presence of NTP; K_i—inhibition constant, K_d—dissociation constant. The virus/cell culture on which the specified study was carried out is indicated in brackets. ^b EC₅₀—concentration of 50% inhibition of virus reproduction in cell cultures in the presence of a nucleoside analog. ^c Thymidine kinase deficient strain of HSV-1 (B2006, mutant thymidine kinase gene). ^d LD₅₀—dose that causes the death of 50% of a laboratory animal population. ^e 5′-triphosphate form. ^f 5′-diphosphate form. ^g Methyl ester of ribavirin-5′-phosphate. ^h The drug has received approval for the treatment of HSV/VZV in the European Union, but is not approved by the FDA [59, 86]. ⁱ The drug received FDA approval for 'emergency use' in the United States for the treatment of COVID-19 in November 2020. ^j IMPDH — inosine-5′-monophosphate dehydrogenase. ^k RSV—respiratory syncytial virus. ^l The drug was approved for the treatment of influenza A, B, C viruses in Japan in 2014, approved in the Russian Federation and China, but not approved by FDA. ^mThymidine kinase of herpes B virus (monkey B virus).

porated, they terminate chain growth due to the absence of a 3'-OH group [59].

Recently, it was reported that RT of retroviruses and hepadnaviruses have a similar structure nucleotide-binding pocket [60]. This is why nucleoside analogs that inhibit HIV reproduction, such as lamivudine and emtricitabine, are also effective for treating patients with hepatitis B.

The mechanism of action of other nucleoside drugs (telbivudine and entecavir; Table 2) against HBV is also *via* the triphosphorylation cascade of the parent nucleosides, which is followed by inhibition of HBV polymerase. Although telbivudine contains a 3'-OH group, which provides the elongation of the viral DNA chain, an alternative mechanism of action may be to terminate the growth of the DNA chain [2]. Entecavir-5'-triphosphate inhibits reverse transcription by HBV polymerase in three ways: (1) it prevents the formation of a covalent complex of HBV polymerase with the growing viral DNA (–) chain (inhibition of the priming activity of HBV polymerase); (2) it kinetically terminates the synthesis of the viral DNA (–) chain, integrating into it instead of dGTP; (3) it kinetically terminates the synthesis of the viral DNA (+) chain on the template (–) chain, inserting instead of dGTP. Molecular modeling and study of the enzymatic activity suggest that the unique property of entecavir to inhibit three types of HBV polymerase activity at once is associated with the optimal correspondence to the spatial arrangement of side groups of amino acid residues in the tail of the NTP binding site while maintaining all key interactions characteristic of the natural substrate, dGTP [61, 62]. Entecavir-5'-triphosphate inhibits DNA polymerase of HBV (K_i 0.0012 mM), being a weak inhibitor of cellular DNA polymerases α , β , δ (K_i 18–40 mM) and mitochondrial DNA polymerase ($K_i > 160$ mM) [63], while telbivudine-5'-triphosphate almost does not inhibit polymerase of human cells [64]. Unlike other inhibitors of HBV polymerase (lamivudine, adefovir dipivoxil), entecavir contains a 3'-OH group, which provides the inclusion of several more nucleotides into the viral DNA strand synthesized by the polymerase (kinetic termination).

Sofosbuvir is an inhibitor of the RNA-dependent RNA polymerase (NS5B) of the hepatitis C virus (HCV). Sofosbuvir undergoes rapid intracellular metabolism in the liver with the formation of an active analogue of UTP, which binds to the active site of NS5B and then acts through a chain termination mechanism of the synthesized viral RNA [65–69]. The amino acid sequence of the active site of NS5B RNA polymerase is highly conserved, which ensures high selectivity and low toxicity of sofosbuvir according preclinical and clinical studies [67].

In addition to the HBV polymerase inhibitor, entecavir, kinetic terminators of viral nucleic acid synthesis also include the HSV polymerase inhibitor, brivudine, and the drug remdesivir which is active against a num-

ber of RNA viruses: Ebola, Middle East respiratory syndrome coronavirus (MERS-CoV), and severe coronavirus respiratory syndrome (SARS-CoV-1 and SARS-CoV-2) (Table 2) [70–74].

Another drug, ribavirin (Table 2), inhibits certain stages of de novo nucleotide biosynthesis, which reduces the rate of viral replication in the cell. Historically, it was believed that the main mechanism of action of ribavirin against a number of RNA viruses (HCV, Respiratory Syncytial Virus (RSV)) is the inhibition of inosine-5'-monophosphate dehydrogenase and the associated decrease in the level of GTP [75] which is required for translation, transcription and replication of the genome of RNA-viruses. However, competitive inhibition of RNA polymerase, as well as lethal mutagenesis [76], in which no instant inhibition occurs, and viral genomes containing erroneous nucleotides are generated, may be important alternative mechanisms of the antiviral activity of ribavirin 5'-triphosphate. Ribavirin-5'-triphosphate is incorporated into RNA instead of ATP and GTP and forms complementary pairs with uridine and cytidine, which sharply increases the frequency of mutations leading to the emergence of non-viable populations of RNA viruses [77–79].

The drug favipiravir (Table 2), originally approved in Japan in 2014 for the treatment of influenza, has been shown to be effective against SARS-CoV-2 [80]. There are several opinions about the mechanism of action of this compound. Some researchers believe that human hypoxanthine guanine phosphoribosyltransferase plays a key role in the activation of favipiravir as an analogue of the nitrogenous base, converting favipiravir into a 5'-phosphoribosylated metabolite, which is phosphorylated by kinases to active 5'-triphosphate and then inhibits viral RNA polymerase, competing with its native substrates GTP and ATP [81, 82]. Other studies have shown that favipiravir can act via lethal mutagenesis [83, 84]. Favipiravir, like ribavirin, carries a carboxamide group attached to the triazole residue that freely rotates around the C–C bond, so it can mimic both guanine and adenine. Accordingly, the concept of lethal mutagenesis, first described for ribavirin [79], may be valid for favipiravir as well.

Thus, nucleoside analogs represent a class of antiviral agents with a broad spectrum of biological activity. Their effectiveness has already been proven against a number of RNA viruses, including the HIV retrovirus, and picorno-, toga-, rhabdo-, pox- and herpes viruses.

Prodrug Forms of 5'-Nucleoside Phosphates

The specificity of kinases that catalyze the successive stages of 5'-phosphorylation of nucleosides in the cell limits the possibilities of using nucleosides for the treatment of infectious diseases. The use of 5'-phos-

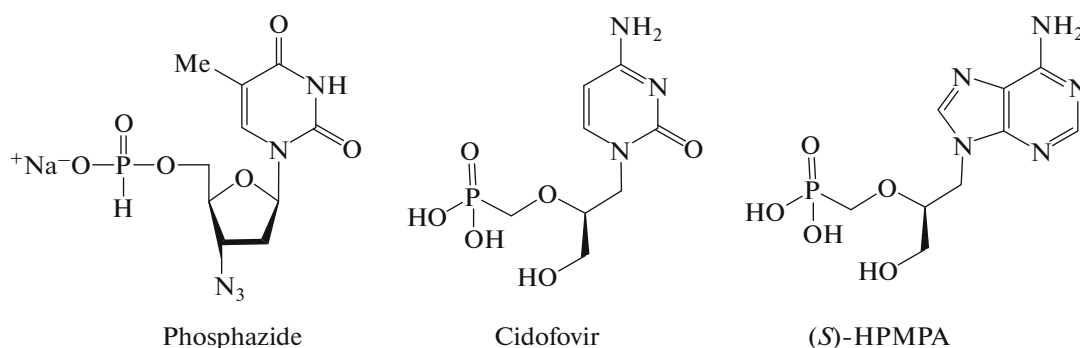


Fig. 4. Phosphonate prodrug forms of nucleotide analogs. (S)-HPMPA, (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine.

phorylated forms of modified nucleosides as medical preparations allows first rate-limiting step of nucleoside phosphorylation to be avoided and increases the efficiency of their action on cells infected with the virus [102, 103]. This strategy has a number of disadvantages, since the introduction of a polar phosphate group into the modified nucleosides significantly complicates the penetration of such compounds into the cell, and inside it they are exposed to the action of host phosphatases. In this regard, the use of 5'-phosphonate derivatives, which are not subject to dephosphorylation, instead of 5'-phosphate forms of nucleosides, increases the stability of the target compound and improves its penetration through the cell membrane. Based on phosphonate derivatives of nucleosides and their acyclic analogs, a number of drugs have been developed that can convert into triphosphorylated forms and suppress viral replication in the cell (Fig. 4, Table 2) [102, 104].

Phosphazide is a nucleoside reverse transcriptase inhibitor (NRTI) used in antiretroviral therapy for HIV [104]. A domestic drug based on it, Nikavir, has established itself as an effective non-toxic drug for antiretroviral therapy. Acyclic analogs of phosphazide, adefovir and tenofovir, used in antiretroviral therapy, suppress the reproduction of HIV and HBV [102]. Tenofovir disoproxil fumarate, marketed in 2001, has been shown to be more effective than tenofovir due to the presence of two biodegradable hydrophobic residues that improve cell penetration. Cidofovir (*S*-HPMPC) is a cytidine derivative modified at the ribofuranose fragment with preservation of 2'- and 5'-hydroxyl groups, active against most DNA viruses, including polyomaviruses, papillomaviruses, orthopoxviruses, HCV, VZV, Epstein-Barr virus, adenoviruses and CMV [102].

An alternative to the phosphonate method is the ProTide technology (PROdrug + nucleoTide), which was proposed in the early 1990s [105, 106]. This approach is based on the chemical modification of nucleoside 5'-phosphates by introducing two lipophilic biodegradable residues into the phosphate group (Fig. 5). The resulting derivatives easily penetrate the cell membrane, effectively accumulate in the

cell, and are gradually metabolized to biologically active nucleoside-5'-phosphates. The advantages of the ProTide technology in comparison to the phosphonate method include a decrease in the toxicity of modified nucleosides. The first experiments on the application of ProTide technology in antiviral therapy were associated with the use of aryl phosphate and phosphoramidate derivatives of zidovudine [106]. Using T lymphocytes (JM cell line), it was shown that zidovudine 5'-diaryl phosphate at a concentration of about 10 μM inhibited HIV-1 replication, while the original zidovudine was almost inactive in this cell line. Substitution of one aryl group for an alanine residue resulted in a significant increase in the inhibitory activity of zidovudine 5'-alanyl(aryl)phosphoramidate (EC_{50} 0.8 μM) compared to 5'-diaryl phosphate (EC_{50} 10 μM). The introduction of amino acid residues containing a hydrophobic side chain seems promising in view of their increased ability to penetrate into the cell and the possibility of hydrolytic cleavage by enzymes inside the cell.

The modified phosphonate nucleoside prodrug tenofovir alafenamide has a higher activity against HIV and HCV and is better distributed in tissues than tenofovir. In a range of purine and pyrimidine 2'-*C*-methyl-nucleosides, such derivatives as 7-deaza-2'-*C*-methyladenosine, 2'-methyl-2'-fluorocytidine, and 2'-methyl-2'-fluorouridine have been synthesized, which suppress the replication of a number of RNA and DNA viruses [103]. Modifications of the 5'-phosphate unit lead to an increase in the antiviral activity of 2'-*C*-methyl-nucleosides in the cell. The compound IDX-184, containing an *S*-pivaloyl-2-thioethyl fragment and an *N*-benzylphosphamide group, turned out to be a potent inhibitor of HCV replication (EC_{50} 0.03–0.2 μM). The IDX184 analogue, INX189, exhibited high antiviral activity against HCV (EC_{50} 0.01 μM). The presence of a modified phosphate residue contributes to the greater efficiency of INX189 due to the creation of a higher concentration of NTP in cells compared to the initial 2'-*C*-methylguanosine (the intracellular concentration of NTP from INX189 is 4 times higher than the concentration of NTP from

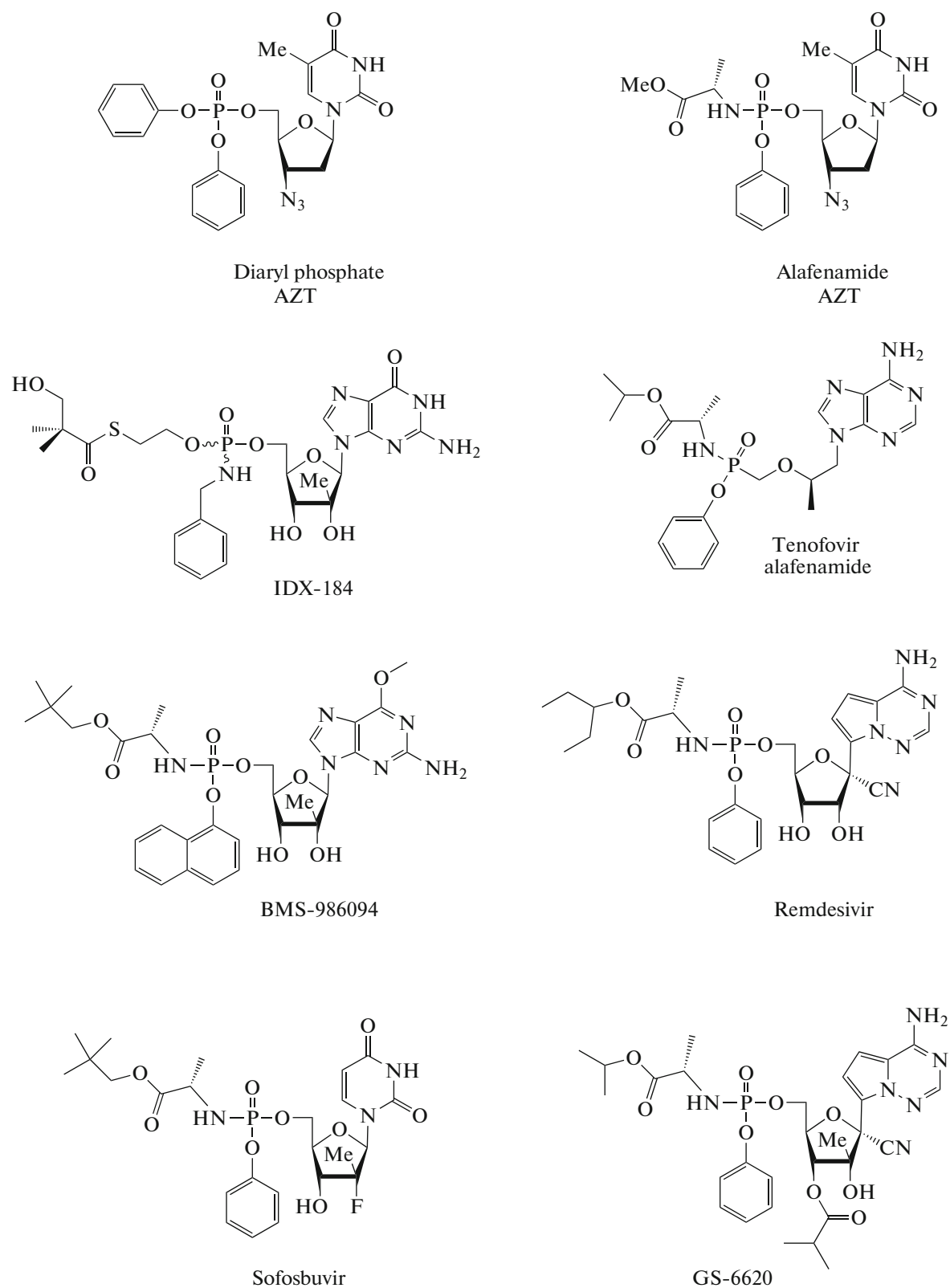


Fig. 5. Prodrug forms of nucleoside phosphate analogs obtained using the ProTide technology. AZT, azidothymidine.

2'-*C*-methylguanosine 24 h after cell culture treatment). The use of another 2'-*C*-methylguanosine derivative, BMS-986094 (INX-189), in combination with ribavirin produced a significant synergistic effect in inhibiting Dengue virus replication in vitro (EC_{90} 0.11 μ M for INX-189, EC_{90} 100 μ M for ribavirin; for combination of two drugs: EC_{90} 0.022 μ M for INX-189 and EC_{90} 10 μ M for ribavirin) [107].

During study of the structure–activity dependence of modified derivatives of pyrimidine nucleosides, it was shown that the ProTide derivative of 2'-methyl-2'-fluorouridine (Fig. 5) effectively inhibited HSV replication, while the initial nucleoside, 2'-methyl-2'-fluorouridine, showed very weak antiviral activity. The *Sp*-isomer (the position of substituents at the phosphorus atom corresponds to the *S*-configuration) of this ProTide derivative (the drug sofosbuvir) is much more active (EC_{90} 0.42 μ M) than the *Rp*-isomer (the position of the substituents at the phosphorus atom corresponds to the *R*-configuration, EC_{90} 7.5 μ M) and is used for the treatment of hepatitis C in combination with other drugs (Fig. 5).

Remdesivir, an analogue of adenosine-5'-monophosphate, is considered as a promising prodrug that can inhibit RNA-dependent RNA polymerase of coronaviruses. This drug was originally developed for the treatment of Ebola and Marburg hemorrhagic fever, and its antiviral activity against SARS-CoV (EC_{50} 0.069 μ M) and MERS-CoV (EC_{50} 0.032 μ M) was discovered. Remdesivir is metabolized to the triphosphate form much faster than its analogue, 1-cyano-4-aza-7,9-dideazaadenosine, which has significantly lower antiviral activity against SARS-CoV and MERS-CoV viruses. According to the recent studies, remdesivir effectively inhibits SARS-CoV-2 in Vero E6 cell culture (EC_{50} 0.77 μ M), while exhibiting low cytotoxicity (50% concentration of cell death (CC_{50} > 100 μ M) and a high selectivity index ($SI > 129.87$) [80].

By modifying the structure of remdesivir by introducing a 2'-*C*-methyl group and a 3'-*O*-isobutyryl group, a new prodrug nucleoside analogue (GS-6620, *Sp*-isomer) was obtained, which effectively accumulates in hepatocytes and suppresses the reproduction of HCV replicons at low concentrations (EC_{50} in range 68–427 nM) [108, 109].

In the cell, the phosphamide residue of the ProTide drug undergoes enzymatic cleavage in the presence of esterase (CES1) and cathepsin A (CTSA) to form the corresponding nucleoside 5'-monophosphates. That is why ProTide drugs are effective against viruses that infect cells with CES1 and CTSA expression (for example, HCV in hepatocytes) [110].

In some cases, the monophosphorylation step does not limit the overall rate of the triphosphorylation reaction, which may be due to the structural peculiarities of the nucleoside derivative. For example, for azidothymidine, the rate-limiting step is the formation of

the 5'-diphosphate form under the action of thymidylate kinase. Nucleoside diphosphate kinase works with low efficiency on non-optimal substrates: uridine-5'-diphosphate and 2',3'-didehydro-2',3'-dideoxyuridine-5'-diphosphate [111].

In connection with the above factors, the development of prodrug tri- and diphosphate forms of nucleosides is considered as a more universal approach that allows them to bypass potential rate-limiting steps and side metabolic processes occurring mainly at the level of nucleosides and their 5'-monophosphates (for example, deamination), and ultimately efficiently deliver biologically active NDP and NTP into the cell. In view of the low stability and high polarity of NDP and NTP, the main difficulties in the development of such drug forms are associated with the low stability of the pyrophosphate bond and the delivery of such compounds into the cell.

A new synthetic approach developed in the laboratory of prof. Chris Meier, suggests: (1) the use of partially charged forms of NDP and NTP, which are resistant to hydrolysis; (2) the introduction of biodegradable lipophilic residues at the terminal phosphate group. In this manner a number of biologically active transport forms of AZT and stavudine have already been obtained (Fig. 6) and their antiviral activity has been studied [111]. Chemical hydrolysis of the prodrug form of AZT in the form of δ -cycloSaligenyl diphosphate (Fig. 6a) leads to the formation of a mixture of 5'-mono- and 5'-diphosphate forms of AZT with a predominance of the monophosphate form in the mixture. Taking this into account, bis(acyloxybenzyl)nucleoside di- and triphosphates, DiP- and TriP-prodrugs, were proposed as more promising prodrug forms (Fig. 6b). Treatment of DiP- and TriP-derivatives of stavudine (d4T) with cell extracts, blood serum or carboxyesterase from pig liver led to the formation of predominantly corresponding di- and triphosphates; at the same time, asymmetric prodrug forms ($R_1 \neq R_2$, R_1 -Me or Bu, $R_2 > C_4H_9$) were more resistant to enzymatic hydrolysis of the pyrophosphate group compared to symmetric ones ($R_1 = R_2$).

In experiments on T cells, DiP and TriP derivatives of stavudine (Fig. 6b) inhibited HIV RT more effectively than the non-phosphorylated drug (d4T). Prodrugs of nucleosides containing acyl residues with $R_{1,2} = C_8H_{17}$ exhibited high anti-HIV activity in experiments on CEM cell culture, which proves their ability to penetrate cell membranes with subsequent release of phosphorylated d4T metabolites.

Thus, phosphate prodrug forms based on modified nucleosides are already a well-proven approach in the development of new antiviral agents. The introduction of hydrophobic residues in the 5'-phosphate group of the modified nucleoside (ProTide technology) makes it possible to increase the efficiency of its action as an inhibitor of viral replication. ProTide technology with the use of phosphamide derivatives of nucleosides is

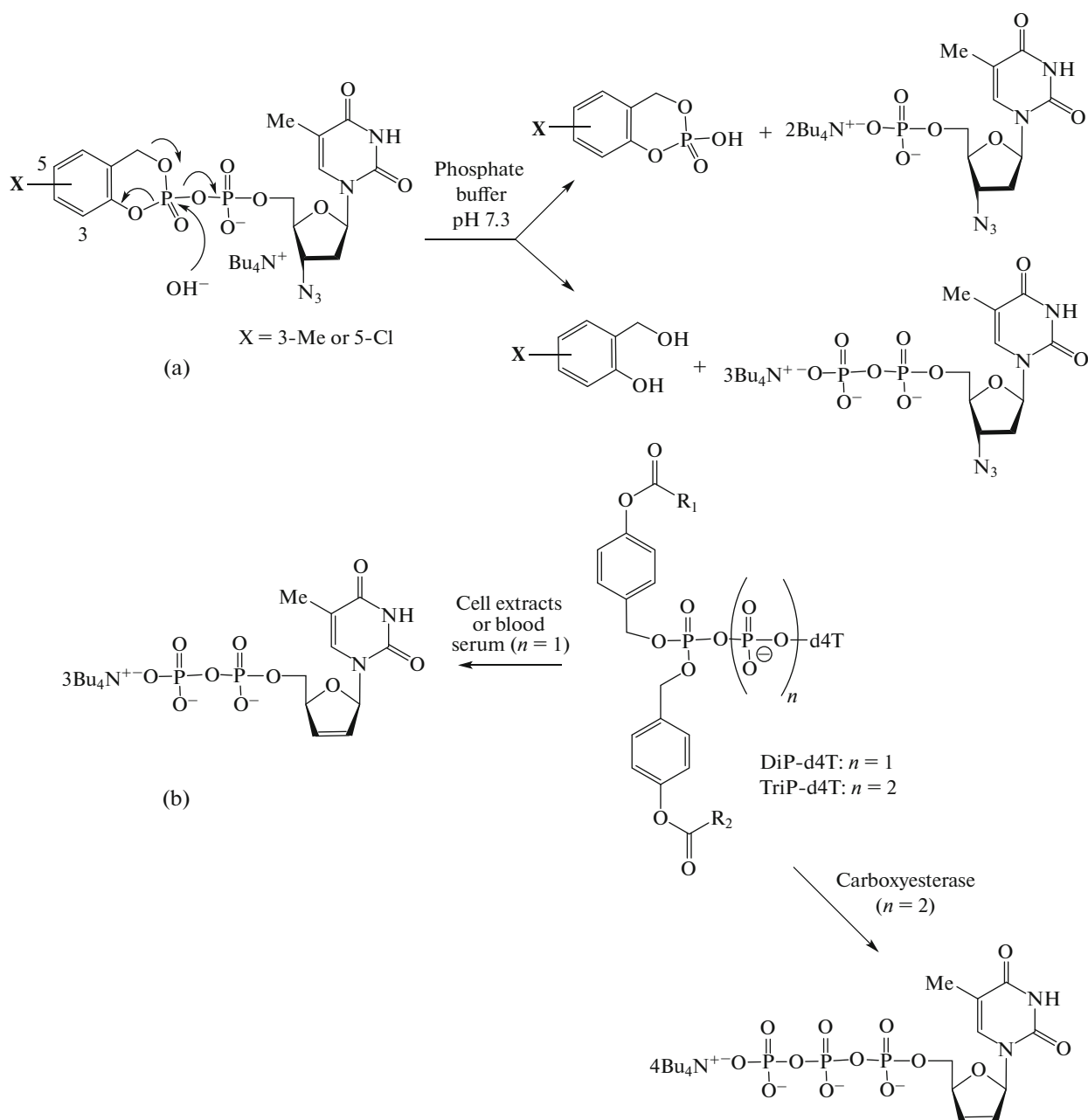


Fig. 6. Hydrolysis of diphosphate and triphosphate prodrugs of AZT (a) and stavudine d4T (b) (compiled from the data of [111]).

another modern approach for the creation of effective drugs for the treatment of viral and oncological diseases. The ProTide technology has served as the basis for the creation of 3 effective antiviral drugs: sofosbuvir for the treatment of hepatitis C, alafenamide tenofovir for the antiretroviral therapy of HIV and hepatitis C, and remdesivir for the treatment of COVID-19.

Nucleoside analogs containing nicotinic acid residues are considered as an alternative to the prodrug forms discussed above [112]. The introduction of nicotinic acid residues into the carbohydrate unit of the nucleoside makes it possible to reduce the toxicity of the drug, increase its stability in the cell, prolong its

life time in the bloodstream, and also provide permeability through the blood–brain barrier [113, 114].

HYDROPHOBIC COMPOUNDS WITH ANTI-VIRAL PROPERTIES

Non-Nucleoside Hydrophobic Compounds with Antiviral Properties

The mechanism of biological activity of a number of antiviral drugs with pronounced hydrophobic properties is based on the ability to block the work of viral enzymes by binding to a certain part of the enzyme. Unlike drugs created on nucleosides, the targets of

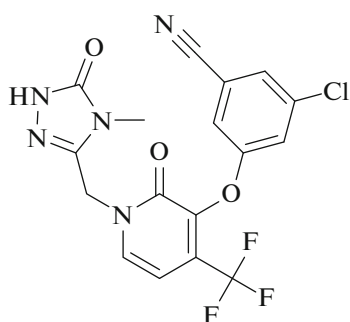


Fig. 7. The structure of Doravirine.

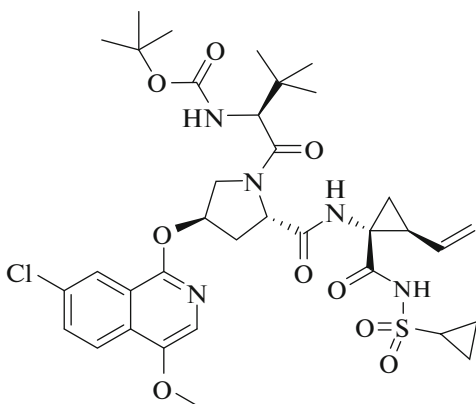


Fig. 8. The structure of Beclabuvir.

which are the nucleoside-binding active site of polymerases, these compounds do not undergo chemical transformations inside the cell. Their targets are the hydrophobic regions of viral enzymes.

The structure of HIV-1 RT contains a hydrophobic region, the so-called hydrophobic pocket, which is located at a distance of about 15 Å from the nucleotide-binding site [115]. When a hydrophobic compound enters this pocket and a strong bond is formed with it, the enzyme loses its conformational mobility, which leads to allosteric inhibition of its activity [116]. Inhibitors of a hydrophobic nature that are able to bind in the hydrophobic pocket of HIV-1 RT are non-nucleoside inhibitors (HIV-1 NNRTI). They are specific for the structure of the hydrophobic pocket of HIV-1 RT and have no activity against HIV-2 RT and other retroviruses. In clinical practice, these inhibitors are used in complex therapy of HIV-1 infection [45]. FDA-approved first-generation HIV-1 NNRTIs include nevirapine, delavirdine, and efavirenz; second-generation drugs include etravirine and rilpivirine. The class of diarylpyrimidines, the second generation drugs, turned out to be promising for the creation of inhibitors of this kind due to their conformational flexibility, as a result of which they can rearrange and adapt to various amino acid substitutions within the

hydrophobic pocket. In 2018, the FDA approved another drug of this class, doravirine (Fig. 7).

New variants of inhibitors of the diarylpyrimidine class are currently being actively investigated as potential antiviral agents [117–121]. Thus, HCV is an enveloped RNA virus that belongs to the *Flaviviridae* family. The structure of the RNA-dependent RNA polymerase of this virus, NS5B, contains a hydrophobic pocket located at a distance of 30 Å from the nucleotide-binding active site [122]. A large number of hydrophobic aromatic compounds capable of binding to this NS5B site have been investigated [123–129]. Currently, dasabuvir, an allosteric inhibitor of the enzymatic activity of NS5B from HCV genotypes 1a and 1b (IC₅₀ 2.8 and 10.7 nM, respectively), is used in the treatment of HCV. This drug has received FDA approval and is listed on the Essential Drugs List. Another drug of the piperazinecarboxamide class is beclabuvir (Fig. 8); it also acts as an allosteric inhibitor of RNA polymerase of HCV. The efficacy and safety of its use was studied in the following complexes: beclabuvir + pegylated interferon + ribavirin [130] and beclabuvir + asunaprevir (NS3 serine protease inhibitor) + daclatasvir (NS5A protein inhibitor) [131]. Based on clinical evaluation of the efficacy, safety and pharmacokinetic properties of these complexes, the Pharmaceutical and Food Safety Bureau of Japan (PFSB) approved the use of Ximency. This drug is the second of the above complexes: beclabuvir + asunaprevir + daclatasvir, with fixed doses of all components, for the treatment of patients infected with HCV genotype 1 [132].

Effect of Hydrophobic Substituents on the Antiviral Activity of Nucleoside Derivatives

Derivatives of pyrimidines, such as 2',5'-bis-O-(*tert*-butyldimethylsilyl)-pentafulanosyl-3'-spiro-5''-(4''-amino-1'',2''-oxathiolane-2'',2''-dioxide)pyrimidine (TSAO), are able to bind to HIV-1 RT in vitro and inhibit the activity of the enzyme by the allosteric mechanism [133–136]. However, when comparing the efficiency of inhibition of HIV RT by these nucleoside derivatives with the efficiency of NNRTI HIV, these derivatives of pyrimidines are significantly inferior. Their inhibitory effect is lost even with minor amino acid substitutions in the structure of HIV-1 RT, which do not affect the inhibitory properties of known HIV NNRTIs [137]. In addition, these nucleoside analogs are highly toxic, and attempts to reduce toxicity by varying the structure of substituents led to a decrease in their inhibitory activity [138].

Studies of the antiviral activity of various nucleoside derivatives were performed in cellular systems against a large group of flaviviruses, which are carried by arthropods (arboviruses) and belong to the causative agents of socially significant endemic diseases [139–144]. Among them are tick-borne encephalitis virus, Dengue virus, yellow fever virus, Japanese encephalitis virus, and

West Nile virus. It turned out that the highest antiviral activity *in vitro* is exhibited by nucleosides containing triphenylmethyl substituents at different positions of the pentafuranose ring. The mechanism of their action cannot yet be considered definitively established, although Eyer L. et al. [145], based on data on the inhibition of the Dengue virus in cellular systems, suggested that these nucleoside derivatives inhibit the process of viral RNA replication by interacting with the active center of RNA-dependent RNA polymerase.

In cases where bulky hydrophobic substituents are found in the nucleoside analog with aglycone, the antiviral effect is determined by the amphipathic nature of these compounds. They inhibit the initial stage of fusion of the outer membrane of enveloped viruses with the membranes of the infected cells. The antiviral efficacy of such nucleoside derivatives was demonstrated by the example of HSV-1 and HSV-2 (IC_{50} 48 nM) [146], as well as tick-borne encephalitis virus (IC_{50} 18–24 nM) [147]. In concentrations sufficient to create an antiviral effect, these nucleosides have neither a cytotoxic nor cytostatic effect on cells.

CONCLUSION

This review considers the structural and functional characteristics of known nucleoside analogues, which determine their antiviral or antiparasitic properties. Knowledge of the characteristics of cellular metabolism of natural nucleosides is necessary to identify those stages of metabolic processes that may be sensitive to drugs based on nucleosides. Nucleoside phosphorylases can be targets for antiparasitic agents, in cases where these enzymes are noticeably different in humans and the parasite. The antiviral effect of nucleoside analogs is mainly associated with their ability to undergo enzymatic phosphorylation inside the cell with the formation of modified nucleoside-5'-mono-, di- and triphosphates. These metabolites then compete with natural NTPs for binding to the active site of viral polymerases and either remain competitive inhibitors, or become terminators of viral DNA/RNA chain growth or triggers of viral genome mutagenesis. Nucleoside analogs bearing hydrophobic substituents can be both inhibitors of viral enzymes involved in the replication of viral nucleic acids and inhibitors of the process of viral penetration into the cell, and can also act on cellular enzymes indirectly involved in viral replication (ribavirin). The specific mechanism of antiviral action depends on the structure and position of the substituent in the nucleoside.

Specific inhibition of viral enzymes by nucleoside and non-nucleoside compounds containing a hydrophobic link can also occur by the allosteric mechanism if hydrophobic regions are present in the enzyme structure at a relatively short distance from the active site. Enzymes with such a structure include HIV-1 RT

and RNA-dependent RNA-polymerase of a number of viruses, which makes them promising targets in the search for new antiviral agents.

Almost all drugs, in addition to the desired effect, cause side effects associated with the toxic effect of the drug on healthy cells, and the toxic effect on the systems of inactivation and excretion of the drug by the liver and kidneys. Nucleoside-based drugs can be toxic as a result of inhibiting enzymes that metabolize natural nucleosides. Another serious problem limiting the use of nucleoside drugs is the development of drug resistance in an infectious agent. This is usually a consequence of long-term use of the drug in the treatment of latent and chronic infections. Resistance occurs when mutations leading to the formation of drug-resistant strains accumulate in the pathogen's genome [148]. To increase the effectiveness and reduce the dosage of the drug, the selectivity of its action on targets in the body is important.

Currently, when creating drugs based on nucleosides, one should adhere to a strategy that decreases the toxicity to human and increases their effectiveness of action on infected cells. This strategy is based on the use of prodrugs of modified nucleosides. The combination of hydrophobic residues at the 5'-phosphate group of the modified nucleoside (ProTide technology) enhances its effect on cells infected with the virus. The ProTide technology with the use of phosphoramidate nucleoside derivatives is a highly effective approach to the development of new drugs for the treatment of viral diseases. Using this technology, three effective antiviral drugs have been obtained: sofosbuvir for the treatment of hepatitis C, tenofovir alafenamide for antiretroviral therapy of HIV and hepatitis C, and remdesivir for the treatment of COVID-19. Currently, approaches are being developed related to the use of di- and triphosphate forms of nucleoside prodrugs capable of exerting a selective effect due to their accumulation in cells.

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COMPLIANCE WITH ETHICAL STANDARDS

In carrying out this work, all ethical standards were observed.

Conflict of interest. The authors declare that they have no conflicts of interest.

This article does not contain any studies involving humans and animals as objects.

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