

# Human Herpes Simplex Virus: Life Cycle and Development of Inhibitors

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**Abstract**—WHO reports that 90% of human population is infected by different types of herpesviruses, which develop latency or cause oral and genital herpes, conjunctivitis, eczema herpeticum, and other diseases. Herpesvirus almost always accompanies HIV-infection and complicates AIDS treatment. Herpes simplex virus type 1 is one of the most wide spread viruses from the Herpesviridae family. HSV virion, genome structure, replication mechanisms, antiherpes drug development strategies, including design of prodrugs, and mutations causing ACV-resistance in clinical HSV isolates are discussed in this review.

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*Key words:* HSV, herpes simplex, life cycle, replication, drugs, mutations, resistance

According to the World Health Organization, viruses of the Herpesviridae family infect 90% of the Earth's population. Humans are the hosts of at least nine unique herpes viruses. The most prevalent is herpes simplex virus type 1 (HSV-1), which establishes latent infection but reactivates causing cutaneous or genital herpes, conjunctivitis, keratitis, encephalitis, or eczema herpeticum. HSV often coinfects HIV-infected patients, complicating treatment of AIDS. HSV-1 might be also involved in the pathogenesis of multiple sclerosis [1] and result in male infertility [2].

This review describes the structure, mechanism of replication, and search for new inhibitors of HSV-1.

*Abbreviations:* ACV, acyclovir; AraA, adenine arabinoside; BVDU, (E)-5-(2-bromovinyl)-2'-deoxyuridine (brivudin); CMV, cytomegalovirus; DAI, DNA-dependent activator of interferon regulatory factor; GCV, ganciclovir; HFC-1, host cell factor 1; HHV-6A, 6B, 7, 8, human herpes virus; HIV, human immunodeficiency virus; HpACV, acyclovir *H*-phosphonate; HSV-1, herpes simplex virus-1; IFI16,  $\gamma$ -interferon-inducible protein; IRF-3, interferon regulatory factor 3; LAT, latency associated transcript; ND-10, nuclear domain 10; PCV, penciclovir; PFA, phosphonoformic acid; PMEAs, 9-(2-phosphonylmethoxyethyl)-adenine; RR, ribonucleotide reductase; VZV, varicella zoster virus.

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## HERPES SIMPLEX VIRUS TYPE 1: GENERAL DESCRIPTION, LIFE CYCLE, AND REPLICATION

**General description of Herpesviridae family.** The Herpesviridae family includes more than 200 species that infect mammals, birds, reptiles, amphibians, fish, and bivalves. It is assumed that herpes viruses and tailed bacteriophages descend from a common ancestor in spite of differences in their morphology and hosts [3]. Conventionally, the Herpesviridae family involves viruses that share a common virion structure. The viral particle consists of a double-stranded DNA core surrounded by an icosahedral capsid consisting of 162 capsomeres surrounded by protein unstructured matrix called tegument, which, in turn, is surrounded by a lipid bilayer envelope with embedded branched glycoproteins.

Referring to these morphological characteristics, various viruses infecting different hosts are classified as herpes viruses.

To date, nine types of human herpes viruses have been identified: herpes simplex viruses types 1 and 2, varicella zoster virus, Epstein–Barr virus, cytomegalovirus, roseoloviruses HHV-6 (A and B) and HHV-7, and Kaposi sarcoma-associated herpes virus (HHV-8). Epstein–Barr virus and HHV-8 are carcinogenic [4, 5].

At the end of the 1970s, Herpesviridae was subdivided into three subfamilies.

1. The Alphaherpesvirinae subfamily includes lytic viruses with relatively short life cycle that infect different cell types, replicate rapidly, and establish latency mainly in sensory ganglia. Simplex virus (HSV-1 and -2), varicella zoster virus (VZV), and some avian viruses are referred to Alphaherpesvirinae. VZV provokes chickenpox in children and herpes zoster in adults.

2. The Betaherpesvirinae subfamily includes viruses with long life cycle and slow progression of infection of a limited number of hosts. Infected cells thrive and increase in size (cytomegaly). Latent infection localizes in secretory glands, lymphoreticular cells, kidneys, and other tissues. Human cytomegalovirus (CMV) and roseoloviruses (HHV-6) are Betaherpesvirinae.

3. The Gammaherpesvirinae subfamily includes Kaposi sarcoma-associated herpes virus (HHV-8), Epstein–Barr virus (EBV) that causes Burkitt's lymphoma mainly in Central Africa residents, and infectious mononucleosis in USA and other countries [4].

Herpes viruses are highly complex. Their genomes encode many enzymes essential for nucleotide metabolism (thymidine kinase, thymidylate synthase, deoxyuridine triphosphatase, ribonucleotide reductase), DNA replication (DNA polymerase, helicase, primase), DNA repair (uracil N-glycosylase, UL2), and posttranslational modifications (protein kinases). DNA synthesis and nucleocapsid assembly take place in the nucleus, and virion processing and maturation proceeds in the cytoplasm. The production of a new viral generation always results in host cell death.

Under latent infection, cells bear a circular form of the viral genome, and only a small amount of RNA is transcribed. In a latent infection, the viral genome retains its ability to replicate and to provoke disease on reactivation. The mechanism of reactivation is not studied completely and can vary in different organisms. Different cell types maintain latency of distinct herpes viruses. For example, HSV-1 is detected only in neurons and ganglia that innervate liable to infection epithelium [6], while latent EBV is observed mainly in B-cells [7].

**HSV-1 virion structure.** HSV-1 virion structure is shown in Fig. 1A. Cryoelectron tomography has provided the most detailed data on the virion structure at resolution of 7 nm [9]. The virions are spherical particles 186 nm in diameter with glycoprotein spikes protruded from each virion, making their full diameter about 225 nm. The nucleocapsid occupies an eccentric position: on one virion side (the proximal pole), it is close to the envelope; on the other side (the distal pole), it is 30–35 nm apart from it. The tegument is an amorphous layer with some structured regions containing 7-nm width filaments apposed to the membrane.

The virion consists of 40 proteins of viral and cellular origin, 10 of which are glycosylated. Eleven proteins are located on the virion surface.

The *core* contains the linear double-stranded DNA genome wrapped as a toroid. A small fraction of the viral DNA appears to be circular. Host polyamines spermine and spermidine are found in the viral core, neutralizing the negative charges on the viral DNA and providing its proper packing. The virion contains 70,000 and 40,000 molecules of spermine and spermidine per virion, respectively. The polyamines are strongly bound to the DNA and cannot be exchanged with added radioactively labeled polyamines. By the degradation of the outer envelope using detergents and urea, spermidine, but not spermine, can be removed from the virion. Recently, polyamines and modified polyamines have been considered as possible regulators or inhibitors of some viral infections. Dextran-conjugated polyamines, in particular dextran-propan-1,3-diamine, inhibited HSV-1 growth in BS-C-1 cell line [10].

The *tegument* is comprised of 26 proteins, some of them participating in capsid transport to the nucleus and other organelles (UL36, UL37, ICP0) [11], viral DNA entry into the nucleus (VP1-2, UL36) [12], activation of early genes transcription (VP16, encoded by *UL48* gene) [13], suppression of cellular protein biosynthesis, and mRNA degradation (VHS, UL41) [14].

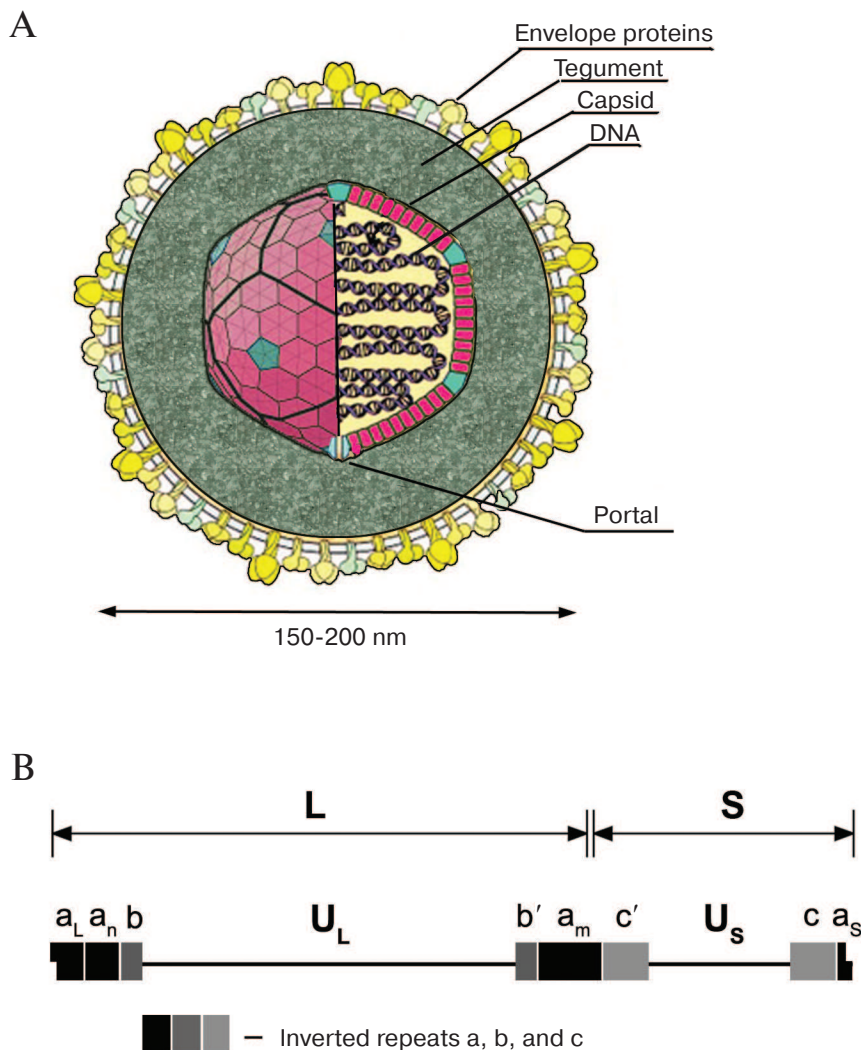
The tegument contains RNA-binding proteins US11, UL47, and UL49 presumably bound to viral and cellular transcripts packaged in the virion.

The *capsid* has icosahedral configuration and is composed of 162 capsomeres (Fig. 1A) – 150 hexons and 12 pentons.

Three types of capsids can be isolated from infected cells: A-capsids (procapsids) lack both scaffold proteins and viral DNA; B-capsids do not contain viral DNA but contain the protein scaffold for it; C-capsids contain the viral genome [15, 16].

Capsids of any type consist of four principal proteins: the major capsid protein UL19 (VP5), VP26 accessory protein (UL35), and also UL18 (VP23) and UL38 (VP19C) proteins, whose functions are not well studied. Six copies of the major capsid protein, VP5, form the hexons, and five copies form the pentons. Six copies of VP26 occupy the outer surfaces of the hexons formed by VP5. A single molecule of VP19C and two copies of VP23 form a triplex that binds surrounding capsomeres to form connections between them. In the center of every capsomere, there is a channel joining the virion outer surface and core. The channels in hexamers are 4 nm in diameter, and in pentamers they are slightly narrower, and in B-capsids these channels are completely closed. The capsid contains UL6 protein, which forms the portal on the vertex of one of the 12 capsid axes, through which the viral genome is presumably packed into the capsid [17], and VP24 (UL26) protease, breaking the scaffold during DNA packaging.

The *outer envelope* of the virion consists of lipid bilayer and 11 glycoproteins (gB, gC, gD, gE, gG, gH, gI, gJ,



**Fig. 1.** A) HSV-1 virion structure; B) HSV-1 genome structure. The long component of the genome ( $U_L$ ) is flanked by inverted repeats designated as **ab** and **ab'**, the short one ( $U_S$ ) is flanked by **ac** and **ac'** sequences. The number of **a** sequence repeats at the  $U_L$ – $U_S$  junction and at the  $U_L$  terminus is variable. The terminal  $a_L$  and  $a_S$  sequences are unique and asymmetric, and  $a_n$  and  $a_m$  are terminal **a** sequence repeats at  $n \geq 0$  and  $m \geq 1$ . The structure of the **a** sequence (400–500 bp) is highly conserved, but it consists of a variable number of repeat elements. The terminal sequence of  $U_L$  component ( $a_L$ ) is truncated and contains one 5'-overhanging nucleotide, and the  $a_S$  sequence contains one 3'-overhanging nucleotide. Upon genome circularization,  $a_L$  and  $a_S$  sequences join. The figure is based on data presented in article [8].

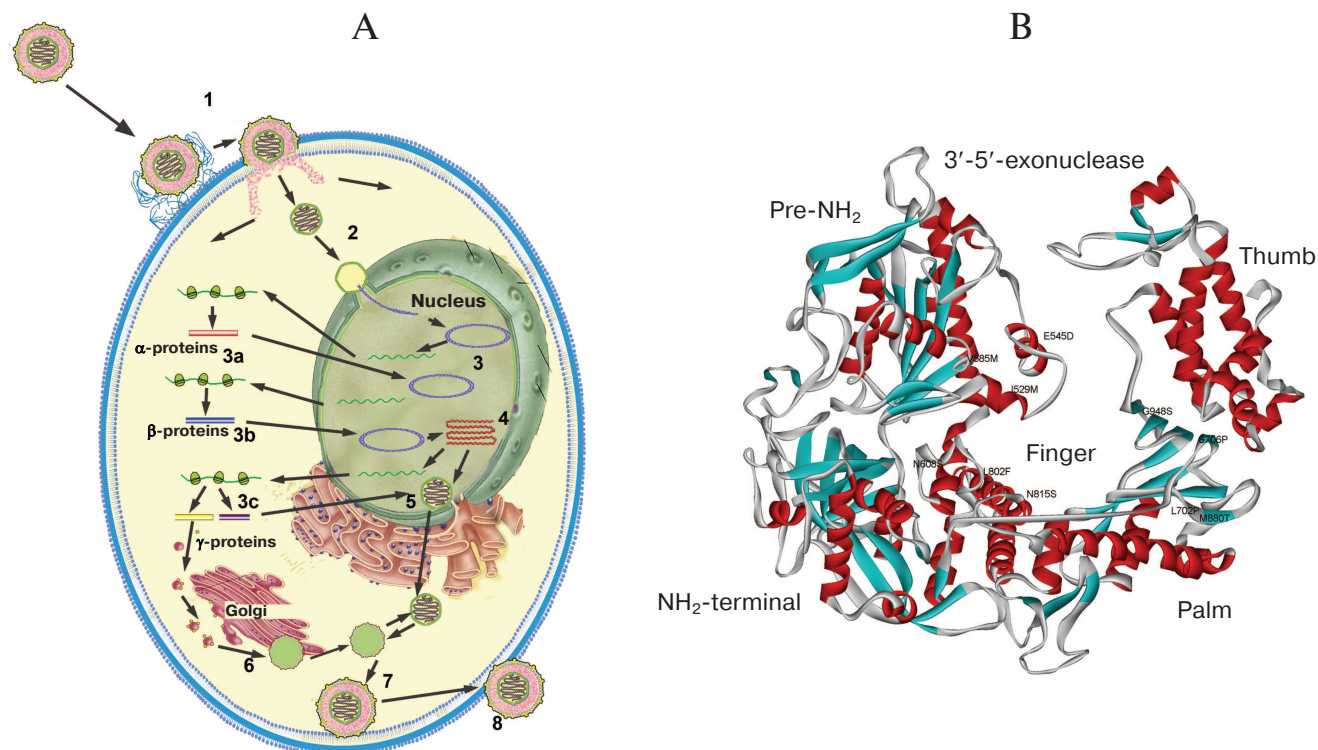
gK), membrane gL, and gM (Fig. 1A) [18], and at least two unglycosylated membrane proteins (UL20 and US9). The lipid bilayer is formed by cell membrane during virus egress by exocytosis. The function of glycoproteins in virus entry into the cell is now studied extensively.

**HSV-1 genome structure.** The HSV-1 genome (GenBank accession number X14112) (Fig. 1B) was estimated to be a 152,261-bp linear double-stranded GC-rich (G+C, 68%) DNA sequence [19]. The ends of the DNA are possibly held together or in close proximity inasmuch as a small fraction of the packaged DNA appears to be circular. The linear DNA circularizes in the absence of protein biosynthesis after entering from the nucleus of infected cells.

The HSV-1 genome can be considered as consisting of two unique units, long ( $U_L$ ) and short ( $U_S$ ), separated by a set of inverted repeats. The repeats bracketing the  $U_L$  component are designated **ab** and **ab'**, whereas those of the  $U_S$  component are **ac** and **ac'** (Fig. 1B).

Due to the presence of inverted repeats, the  $U_L$  and  $U_S$  units of the genome can be inverted relative to one another to yield four linear isomers. However, it was shown that neither the presence of internal repeats nor orientation of the genome components affect viral viability in *Vero* cells [20].

The HSV-1 genome encodes around 90 transcriptional units, and at least 84 encode proteins. With some minor exceptions, each viral transcript encodes a single



**Fig. 2.** A) HSV-1 life cycle: 1) virion attachment and entry into the cell; 2) transport to the nucleus; 3) viral gene expression: immediate early (a), early (b), and late (c); 4) viral DNA replication; 5) nucleocapsid assembly; 6) capsid maturation; 7) primary envelope formation; 8) egress. B) Overall structure of HSV-1 DNA polymerase in ribbon diagram. The figure was composed by the PDB Viewer program and is based on data represented in article [53].

protein and does not contain any introns. Several transcripts appear not to encode open reading frames (ORFs). Those best known are the latency-associated transcripts (LATs) [21] and *oriS* encoded regulatory microRNAs [22].

During the course of the HSV-1 infection, different genes are expressed, one gradually turning on and regulating another. According to this cascade fashion, the viral genes are classified into at least three general classes:  $\alpha$  or immediate early,  $\beta$  or early, and  $\gamma$  or late genes [23]. Immediate early genes are mapped near the termini of  $U_L$  and  $U_S$ . The  $\alpha_0$  and  $\alpha_4$  genes map within the inverted repeats of  $U_L$  and  $U_S$ , respectively [24].

#### Life cycle, gene expression, and replication of HSV-1.

The viral life cycle can be divided into the following major steps: entry into the host cell, expression of viral genes, replication, virion assembly, and egress of the new generation of viral particles (Fig. 2A). In permissive cell lines, this cycle takes about 18–20 h.

To date, two HSV-1 entry pathways have been proposed (Fig. 2A). The main mechanism assumes the fusion of the viral envelope with the plasma membrane and further transport of the viral capsid to the nucleus. The essential stage of this process is interaction of surface glycoproteins of the virus with specific cell surface receptors. The additional pathway by which the virus enters the cell

is endocytosis of the enveloped virion followed by fusion of the envelope with intracellular vesicles [25].

Attachment of the virion to the cell surface is mediated by viral glycoproteins C (gC) and B (gB), which interact with cell surface glycosaminoglycans, in particular heparan sulfate [26].

The interaction between four glycoproteins, gD, gB, and the heterodimer gH/gL, is required for viral entry into the host cell by fusion of the viral outer envelope with the plasma membrane [27, 28]. Glycoprotein gD can bind to the receptors of three types: nectin-1 and nectin-2, herpes virus entry mediator (HVEM), and 3-*O*-sulfated heparan sulfate (3-*O*-S-HS). The last is produced by 3-*O*-sulfotransferases 2–7 (3-OST) [25] making them attractive therapeutic targets for the development of anti-herpetic drugs [29].

In addition to binding of gD to cellular receptors, it triggers membrane fusion by interaction with the gB and gH/gL complex. The exact mechanism and participants of this process are poorly understood, but it is known that the N-terminal region of gD interacts with cellular receptors causing the release of its C-terminal domain, which activates gB and gH/gL complex thereby triggering membrane fusion. When gD is not bound to the ligand, the C-terminal domain is blocked [27]. An interesting addition-

al function of gD is suppression of apoptosis in the HSV-infected cell [30]. Interaction between gB and paired immunoglobulin-like type 2 receptor  $\alpha$  (PILR $\alpha$ ) is necessary for viral entry into the cell. Upon addition of antibodies to these receptors, infection in cell culture is suppressed [31].

After entering of the viral particle, which is the capsid coated with the tegument, it is transported to the nuclear pores with subsequent transfer into the nucleus (Fig. 2A). HSV-1 travels through the cells for rather long distances, especially in neurons. Using indirect immunofluorescence microscopy, it was shown that the viral capsid is transported to the nucleus along a network of microtubules. The transport is powered by the cytoplasmic motor protein dynein [32]. In a cell-free system, capsids coated with the inner tegument exposing US3, UL36, UL37, ICP0, UL14, UL16, and UL21 proteins recruited motor proteins associated with microtubules (dynein, dynactin, kinesin-1, and kinesin-2). The most likely candidates to play a role of a linking chain between the motor proteins and the capsids are UL36 and UL37 proteins. The capsids, which are not coated with the tegument or are coated with tegument that contains other proteins, do not bind the motor proteins. Presumably, when the outer envelope of the virion is fused with the cell membrane, the outer tegument proteins remain bound to the membrane. So, the proteins of the inner tegument are exposed to the capsid surface and bind to the motor proteins [11].

On the surface of the nuclear membrane, the capsid is associated with nuclear pore complex [33]. The inner tegument protein UL36 (VP1/2) bearing a nuclear localization signal [34] and nucleoporins Nup358 and Nup214, which bind the capsid indirectly or directly, appeared to be key participants in this process. The capsid binds to the nuclear pore complex in such a way that its unique “portal”-containing vertex sits just above the nuclear pore. Presumably, all these interactions are necessary for transport of the viral DNA by the nuclear import pathway mediated by importin  $\beta$  [35].

Transcription and replication of the viral genome (Fig. 2A) as well as the assembly of progeny capsids take place within the nucleus. The infection comes with reorganization of the nucleus causing an increase of its size, disruption of nucleolus [36] and nuclear domain-10 (ND-10) [37], and chromatin condensation and subsequent destruction of the latter and the nuclear lamina [38] in the late steps of infection. Key cellular processes – transcription [39], splicing of the cellular RNA [40], protein biosynthesis [41], and cellular response to infection [42] – are also blocked. All these steps increase the efficiency of viral replication and transcription.

The viral mRNA is synthesized by the host cell RNA-polymerase II with the participation of viral factors in all steps in infection. Viral proteins regulate sequential transcriptional cascades ( $\alpha$ ,  $\beta$ , and  $\gamma$  genes; Fig. 2A) and a series of posttranslational modifications.

For the transcription of immediate early  $\alpha$  genes, the presence of the tegument protein VP16 is important [43]. Unlike other viral genes, all  $\alpha$  genes contain several copies of the consensus sequence: 5'-GyATGnTAA-TGArATTCTTnGGG-3', where y is a pyrimidine base, r is a purine base, n is any base [43]. The cellular transcription factor Oct-1 binds to this sequence. VP16 protein interacts with this transcription factor and together with HCFC1 protein forms a complex that activates transcription of  $\alpha$  genes.

An intriguing feature of VP16 is its ability to regulate methylation and demethylation of histone H3 that binds with non-nucleosomal viral DNA at the  $\alpha$ ,  $\beta$  and  $\gamma$  gene promoters during infection. During infection, VP16 triggers a cascade of viral gene expression by directly or indirectly activating the viral  $\alpha$  gene promoters and removal of histone H3. H3 histone binding to the  $\alpha$  gene promoters is most likely the result of the cellular response to foreign DNA detected by the cell in the nucleus in order to inactivate it [44].

Six genes (*ICP0*, *ICP4*, *ICP22*, *ICP27*, *ICP47*, and *US1.5*) are ascribed to the group of immediate early genes; five of them (*ICP0*, *ICP4*, *ICP22*, *ICP27*, and *US1.5*) activate transcription of  $\beta$  genes at least in several types of cells. Immediate early proteins accomplish multiple functions and perform dramatic reorganization of cellular processes in the interests of the virus. For instance, ICP0 protein contains the E3-domain possessing ubiquitin ligase activity towards a wide range of substrates. Through direct or indirect interaction or by substrate phosphorylation, the triggering of proteasomal degradation of some of the proteins participating in cellular defense against the viral infection can occur. So, in a primary culture of fibroblasts the target of ICP0-mediated ubiquitination resulting in proteasomal degradation is interferon-inducible protein 16 (IFI16) localized in the nucleus. This DNA sensor triggers the cascade of the innate immune response signaling IRF-3 activation [45]. ICP0 similar to VP16 protein mentioned above can activate viral chromatin condensation and decondensation [46].

At the same time, in a confrontation between the virus and the cell the latter also has some tools for suppression of infection. For example, a DNA-dependent activator of interferon-regulatory factor (DAI) – the cytosolic DNA sensor, in addition to membrane-associated Toll-like receptor 9, recognizes the pathogen's DNA [47] and inhibits HSV-1 early gene expression via repression of *ICP0* promoter activation [48]. ICP22 protein functions as a repressor in a number of cellular and viral promoters. Using immunoprecipitation, this protein was shown to form a complex with transcription elongation factor b (P-TEFb), like viral transcriptional activator VP16, and to block its binding to viral promoters [49].

The main function of the  $\alpha$  gene-encoded proteins is activation of  $\beta$  gene expression. Proteins and enzymes

encoded by the  $\beta$  genes are involved in viral genome replication (e.g. HSV DNA polymerase, UL30), regulation of nucleotide metabolism (e.g. thymidine kinase, UL23), suppression of early  $\alpha$  genes, and activation of late  $\gamma$  genes. Regulation of  $\beta$  and  $\gamma$  gene expression is more diverse; that is why the start of initiation, duration, and level of expression of these genes do not coincide, in contrast to  $\alpha$  gene expression regulation.

Being a result of low translation initiation efficiency, the level of expression of the key replication protein DNA polymerase is below as compared to other  $\beta$  genes, for instance, thymidine kinase. Upstream (+55) and downstream from the translation initiation site, the transcript of this gene contains sequences forming stable hairpins that might prevent access of cellular initiation factors. The level of expression of DNA polymerase reaches its maximum only 4 h after infection [50].

After initiation, viral DNA synthesis switches from a  $\Theta$  replication mechanism to a rolling-circle mechanism [51], the latter producing concatemeric molecules that are cleaved during the process of nucleocapsid assembly.

The first step in replication of HSV DNA is the unwinding of the double helix by UL9 and/or ICP8 (UL29) proteins in the AT-rich regions of the *oriL* or *oriS* origins of replication. The latter are present in one copy in  $U_L$  of the genome, and in two copies in  $U_S$  of the genome, respectively. ICP8 binds ssDNA fragments, and UL9 binds specifically to *oriS* and unwinds it. Then helicase–primase complex composed of UL5, UL8, and UL52 proteins is loaded. Its helicase activity efficiently catalyzes unwinding of dsDNA only if single-stranded overhang of greater than six nucleotides is available. The primase function can also be accomplished by the primase subunit of the cellular DNA polymerase  $\alpha$ .

The leading and lagging DNA strands are synthesized by viral DNA polymerase (UL30) complexed with processivity factor UL42. The latter protein differs from proliferating cell nuclear antigen (PCNA) – it binds DNA as a monomer and thus does not form a toroidal structure [52].

In addition to seven viral proteins, a few cellular proteins appeared to participate in the replication. These are DNA ligase, topoisomerase II, and various components of the DNA repair and homologous recombination systems [54]. Moreover, the cellular chaperone Hsp90 was found to be essential for the viral replication; its inhibition impairs the latter and results in viral DNA polymerase mislocalization to the cytoplasm and its proteasome-dependent degradation [55].

Some viral proteins participate in nucleotide metabolism, e.g. thymidine kinase (UL23), ribonucleotide reductase (UL39, UL40), deoxyuridine triphosphatase (UL50), uracil N-glycosylase (UL2), and alkaline nuclease (UL12). These enzymes are essential for viral DNA synthesis and repair because the production of the corresponding host cell enzymes is suppressed.

After the viral DNA replication initiation, the levels of expression of late  $\gamma$  genes, especially encoding capsid proteins, increase providing the assembly of progeny virions. The capsid assembly and viral genome packaging occur in the nucleus (Fig. 2A) followed by nucleocapsid egress from the nucleus via nuclear pore or by budding through the nuclear membrane. With the participation of UL36 and UL37 proteins, the capsid is transported from the nucleus to the cytoplasm [56], where the virion maturation and outer shell formation occurs. The release of the virion from the cell by exocytosis accomplishes the envelope formation (Fig. 2A). As well as during virus entry into the cell, the egress of virions is associated with microtubule-based transport and with the UL37 interaction with molecular motor dyx1c1. Using live-cell imaging, dyx1c1 depletion was shown to result in striking reduction in capsid movement in the cytoplasm during egress [57].

HSV-infected cells produce not only infective virions but also non-infectious light particles (L-particles), which are devoid of viral capsids and genomes. They presumably facilitate the infection by delivering additional tegument proteins to the host cell. Clathrin-like coats are probably associated with virion and L-particle envelopment in virion assembly sites [58].

A functional screening assay using small interfering RNAs (siRNA) has shown that at least 15 host proteins are implicated in pathways that are most likely relevant for HSV-1 viability and the viral propagation. Among them intracellular transport (ARF1, HSPA8, RAB2A, RAB5A, RAB6A, RAB10, RAB11A) and cytoskeleton (KRT10) components as well as proteins involved in gene expression (DDX3X, HSPA8, EIF4H), signal transduction (CD59, MIF, YWHAG, YWHAZ), and apoptosis (MIF, YWHAZ) have been identified. The incorporation of most of these proteins within mature viral particles seems to be necessary for the optimal course of the next round of infection, and the depletion of one of the above proteins results in poorer viral replication without any significant effect on the cell viability [59]. One more cellular protein, the small GTPase Rab27a, colocalizes with viral glycoproteins gH and gD in the *trans*-Golgi network (TGN) and probably takes part in viral egress from oligodendroglial cells. The viral titer of Rab27a-silenced infected cells is significantly decreased [1].

An interesting property of HSV-1 is its ability to establish a latent infection. After primary infection, HSV-1 either replicates productively in epithelial cells or enters sensory neuron axons and moves to the neuronal cell nucleus. There, the viral DNA remains circular and does not possess any lytic gene expression; however, latency associated transcripts (LATs) are expressed and then spliced to give some mRNAs. Both the transcriptionally active and silent regions of latent HSV DNA have a nucleosomal structure similar to that of cellular chromatin [60]. Recent views on the functions of LATs are

conflicting, but their suggested major function is generation of miRNAs and siRNAs that downregulate ICP0 and other lytic gene expression. HSV-1 latency, reactivation, and recurrent diseases are studied in rabbit and mouse eye models [6].

**HSV-1 DNA polymerase.** HSV-1 DNA polymerase is the key enzyme in viral DNA replication. It belongs to the family B polymerases that includes human polymerases  $\alpha$ ,  $\delta$  and  $\epsilon$ . HSV-1 DNA polymerase is a 136-kDa protein being at least 300 amino acid residues longer than other B polymerases and exhibiting 16-50% sequence homology.

DNA polymerase associates with the DNA-binding accessory protein UL42 (65 kDa), which binds to the C-terminal region of the enzyme and acts as a processivity factor [61, 62]. The DNA polymerase region, which binds UL42, contains a C-terminal nuclear localization signal (NLS) corresponding to the RRMLHR motif (amino acid residues 1224-1229) [63]. In addition to polymerase activity, HSV polymerase has 3'-5'-exonuclease activity [64], presumably 5'-3'-exonuclease activity, and RNase H activity [65] required for removing RNA primers during synthesis of Okazaki fragments. Unexpectedly, HSV-1 DNA polymerase exhibits apurinic/aprimidinic (AP) and 5'-deoxyribose phosphate (dRP) lyase activities typical for repair polymerase [66]. AP activity of HSV-1 DNA polymerase in conjunction with the viral uracil N-glycosylase (UL2) is involved in a viral DNA repair system similar to cellular base excision repair (BER). The functional consequence of interaction between UL30 and UL2 is replication block of uracil-containing templates upstream from template uracil due to an AP-site [67].

HSV-1 polymerase is comprised of six structure domains. In addition to domains that usually perform the DNA polymerase activity, i.e. palm, fingers, and thumb domains, it has a pre-NH<sub>2</sub> domain, an NH<sub>2</sub>-domain, and a 3'-5'-exonuclease domain containing regions exo I, exo II (region IV), and exo III ( $\delta$ -C region) [53] (Fig. 2B). Regions III and VI belong to the fingers, regions I, II, and VII are located in the palm subdomain, and the thumb subdomain contains the conservative region V. Located in palm subdomain residues D717, D888, and F718 are involved in metal ion coordination required for polymerase catalysis. The ribose of the incoming nucleotide interacts with the strictly conservative Y722 residue, providing a "steric gating" effect against incorporation of ribonucleotides into the growing DNA chain. The side chain of N815 stacks against the base of the incoming nucleotide and stabilizes it. The R785, R789, and K811 residues from the fingers domain interact with the phosphate groups of the incoming nucleotide and are important for the positioning of the phosphate moiety to the 3'-OH of the primer. The KKKY (938-941) motif and residues Y818, Y884, and D886 serve for sensing mismatches in newly synthesized DNA duplex [53].

It was found that the pre-NH<sub>2</sub> domain, namely the conservative motif FYNPYL (amino acid residues 44-49) in the herpes virus, is required for efficient HSV-1 replication. Mutant viruses containing DNA polymerases without the extreme N-terminal 52 residues exhibited 5-7-fold decreases in viral yield, and virus with DNA polymerase without 141 residue failed to replicate. Mutant enzymes lacking the N-terminal 52 residues and containing six alanine residues instead of the FYNPYL motif displayed basal polymerase activity similar to that of wild-type HSV-1 polymerase *in vitro*. According to a suggested scenario, this HSV-1 polymerase region interacts with an unknown factor that recruits the polymerase to the replication fork. The possible candidates are helicase-primase complex component UL8, single-stranded DNA binding protein ICP8, alkaline nuclease UL12, and chaperone Asf1b [68].

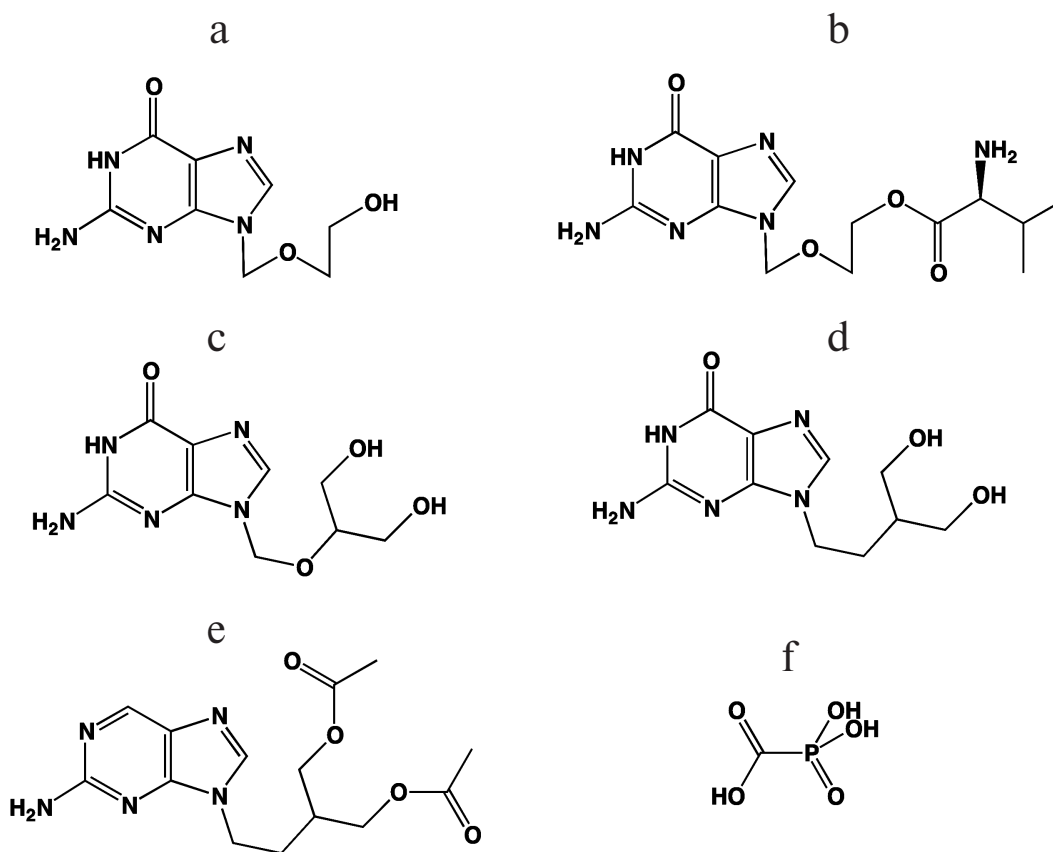
#### INHIBITORS OF HERPES VIRUS REPLICATION: CLINICAL DRUGS AND LABORATORY DEVELOPMENTS

As mentioned in the first section of this review, human herpetic infection is widespread and is a central problem in both Russia and abroad [69]. Among HIV-infected patients who are coinfecting by the herpes virus, about 6-10% have virus strains resistant to available antiherpetic drugs.

Most modern drugs for the treatment of herpetic infections are based on the use of modified nucleosides or their prodrugs [70]<sup>1</sup>. The action of drugs is directed mostly to the suppression of activity of the main replication enzyme of the virus – the DNA polymerase. It should be noted that the drugs do not save the patient from the recurrent character of the disease, and their prolonged administration can cause the emergence of resistant virus strains. These circumstances make the search for new antiherpetic drugs and their new targets a high priority. Below we present data on the application of clinically approved antiherpetic drugs as well as the search for new substances with high efficiency and low toxicity suppressing the replication of human HSV-1.

**Clinically approved antiherpetic drugs.** The first nucleoside antiherpetic drug, 5-iodo-2'-deoxyuridine, was created in the end of 1960s by W. Prusoff [71] and was widely used in clinical practice to treat herpetic keratitis. This was the first antiviral drug based on a nucleoside analog, and this became the basis for the development of antiviral therapy. In the next two decades, trifluorothymidine, vidarabine, and brivudin, which are also nucleoside analogs, were approved to therapy. However, these drugs showed low selectivity and rather high toxicity and were

<sup>1</sup> A prodrug is a medication that does not manifest any antiviral action itself, but forms an inhibitor of the virus after penetration into the infected cell due to chemical or enzymatic activation.



**Fig. 3.** Chemical structures of antiherpetic drugs used in clinics: a) acyclovir; b) valacyclovir; c) ganciclovir; d) penciclovir; e) famciclovir; f) foscarnet.

used only for topical administration. The second generation of antiherpetic drugs was created based on acyclic nucleosides (acyclovir, valacyclovir, ganciclovir, penciclovir, and famciclovir) that suppress infections caused by HSV-1, HSV-2, VZV, and CMV [69, 70]. The chemical structures of these compounds are presented in Fig. 3.

*Acyclovir and valacyclovir.* Acyclovir (ACV, earlier referred to as acycloguanosine; Fig. 3a) was first proposed as an antiherpetic drug in 1977 [72]. A new period in the development of antiherpetic chemotherapy started with its discovery. To date, acyclovir remains the gold standard in the search for antiherpetic drugs [73], and the author of its development, Gertrude Elion, was awarded the Nobel Prize in Physiology and Medicine in 1988 [74].

Acyclovir turned out to be an effective and low-toxicity drug. The mechanism of action of ACV is based on its phosphorylation by viral thymidine kinase with the formation of corresponding monophosphate (ACVMP). The next two phosphorylation steps are catalyzed by cellular kinases with the formation of triphosphate (ACVTP), which acts as a substrate of viral DNA polymerase, is incorporated in the viral DNA chain, and terminates its synthesis [72].

Disadvantages of acyclovir are poor oral bioavailability (10–30%), limited solubility in water, and short half-life of the drug in the bloodstream. Therefore, rather high doses and frequent drug administration are necessary to support the proper concentration of acyclovir in the patients' blood that, in turn, increases toxicity. To improve solubility and increase bioavailability, several prodrugs of acyclovir were prepared, and glycine and L-alanine esters were the first among the acyclovir prodrugs [70]. However, the drugs had rather high toxicity during clinic investigations.

The L-valine ester of acyclovir – valacyclovir – turned out to be an effective and safe drug (Fig. 3b). The increased oral bioavailability of valacyclovir can be attributed to rapid intestinal absorption via the human intestinal peptide transporter hPEPT1, followed by rapid conversion to ACV by ester hydrolysis in the small intestine [70].

*Ganciclovir.* Ganciclovir (GCV) – 9-(1,3-dihydroxy-2-propoxymethyl)guanine – is an acyclic guanosine analog (Fig. 3c) that is structurally related to ACV [75]. The drug appeared to be active towards CMV, HSV-1, HSV-2, VZV, and EBV; however, it is used in clinic only for the treatment of CMV infections. As with ACV, the oral



bioavailability of ganciclovir is limited and, to overcome this problem the prodrug of ganciclovir, the valine ester of ganciclovir, was synthesized [76].

**Penciclovir and famciclovir.** Penciclovir (PCV, Fig. 3d) is an acyclic guanosine analog with a structure that is similar to that of ACV and GCV but without the oxygen atom in the acyclic “sugar” moiety and with an OH group in the position equivalent to that of the 3'-OH group in the natural deoxynucleoside. PCV was synthesized for the first time at Beecham Pharmaceuticals Laboratories. The drug was less active than acyclovir against HSV-1 in experiments with infected cells; however, it efficiently suppressed VZV replication in animals and is widely used for treatment of HSV-1 induced skin lesions. In contrast to acyclovir, the drug also inhibited the replication of an HSV-1 strain encoding a mutant DNA polymerase [77]. Like ACV, PCV is converted to the monophosphate by the viral thymidine kinase. The initial step of PCV phosphorylation to PCVMP is more efficient than the phosphorylation of ACV, but the PCVTP formed in infected cells is less active than ACVTP as a substrate of HSV DNA polymerase. Its oral bioavailability was even lower than that of ACV. To improve these limitations, the PCV prodrug famciclovir, the diacetyl derivative of penciclovir, was synthesized (Fig. 3e). Famciclovir is converted to penciclovir in two steps *in vivo*: removal of the two acetyl groups by esterase and oxidation of the purine by aldehyde oxidase. It should be noted that famciclovir is inactive in cell cultures since oxidation of penciclovir does not occur there; however, being administered orally, it was found to be even more effective than acyclovir in eliminating the virus from its target sites. The more important advantage of famciclovir is its ability to prevent latent infection of HSV-1. When treating with famciclovir, significantly less latent virus was detected in mice ganglia as compared to valacyclovir [78]. The reason of preventing recurrences of infectious virus remains to be elucidated.

**Foscarnet.** Foscarnet (PFA) (Fig. 3f) is a pyrophosphate analog and a nonnucleoside inhibitor of HSV DNA polymerase. Foscarnet is a noncompetitive inhibitor with respect to nucleotide substrates; it binds to the enzyme active site and prevents the binding of incoming nucleotide [79]. PFA is not widely used in clinic because of high toxicity compared to acyclovir and is used only if the treatment with acyclovir and other nucleoside drugs is ineffective, e.g. in patients that have acquired resistance to them [80].

**Search for new antiherpetic drugs.** A series of interesting new nucleoside analogs having antiherpetic activity in cell cultures and laboratory animals has been described. Among such compounds worth mentioning are guanine derivatives: acyclic H2G (Fig. 4a), carbocyclic cyclobutane (lobucavir) (Fig. 4b) and cyclopropane (A-5021) (Fig. 4c) analogs. These compounds did not pass clinical trials and are not approved as drugs because of increased toxicity [81]; however, they can be used as the basis for the development of new drugs.

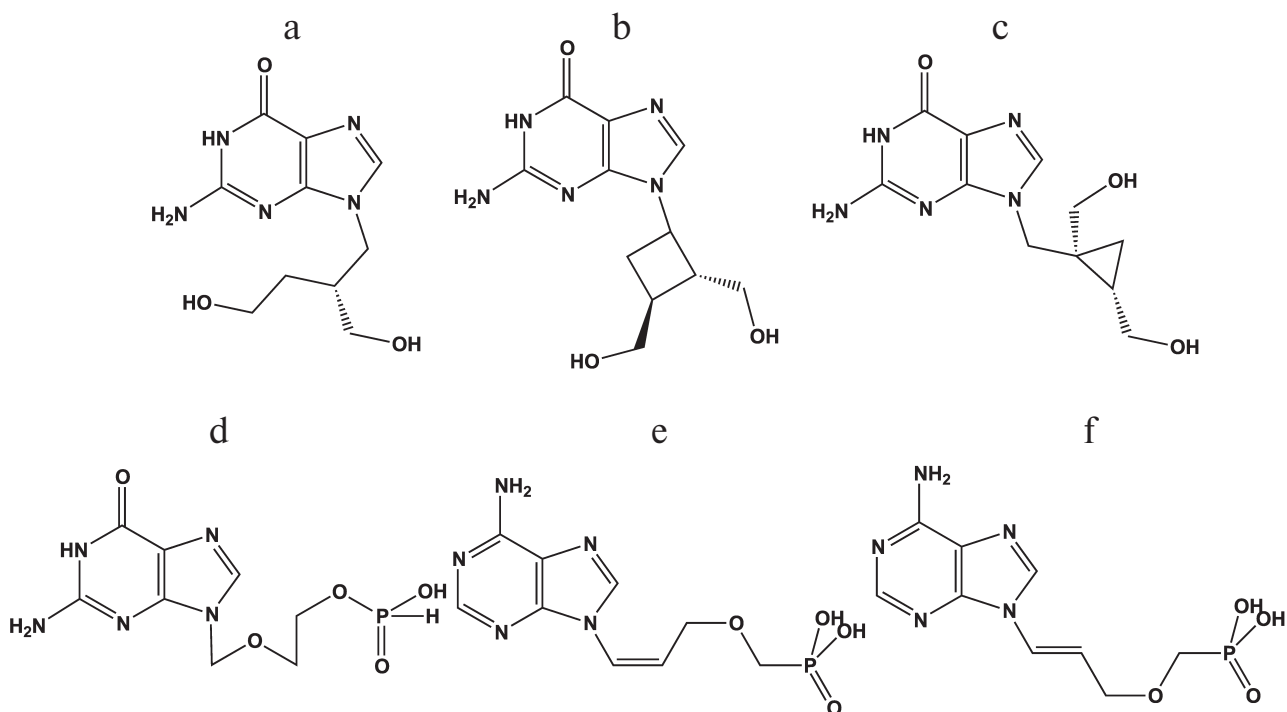
**Nucleoside phosphonate derivatives.** To date, three phosphonate derivatives of acyclic nucleosides acting directly without the primary phosphorylation stage for activation in the cell are used in clinics. These are cidofovir, a cytidine analog applied for treatment of CMV infection, and adefovir and tenofovir, adenine analogs that are employed in therapy of hepatitis B and HIV, respectively. Acyclic unsaturated phosphonate analogs, (Z)- and (E)-isomers of 9-[3-(phosphonometoxyprop)-1-en-1-yl]adenine [75], and a phosphonate analog of acyclovir (HpACV) (Fig. 4d) [82] were synthesized and tested as HSV-1 inhibitors.

An interesting property of the acyclic unsaturated phosphonate analogs is their ability to inhibit both replication of HSV-1 and human immunodeficiency virus in cells. In this case, the concentration of Z-isomer (Fig. 4e) that suppresses the development of viruses by 50% ( $IC_{50}$ ) was substantially lower than that of E-isomer (Fig. 4f). Toxicity of both compounds was lower than that of the known anti-HIV drug [2-(6-amino-9H-purin-9-yl)ethoxymethyl]phosphonic acid (PMEA). Both isomers also inhibit thymidine kinase-deficient HSV-1 strains resistant to acyclovir, since no first phosphorylation stage is required for their activation. The synthesized diphosphates acted as substrates of both HIV reverse transcriptase and HSV-1 DNA polymerase, were incorporated into 3'-end of the primer-template, and terminated further elongation [83]. These compounds were not substrates of the cellular DNA polymerase  $\alpha$ , consistent with data on their low toxicity found in cell culture experiments. These compounds simultaneously suppress both HIV and HSV, a rare achievement.

Properties of *H*-phosphonate acyclovir (HpACV, Fig. 4d) are interesting. This drug suppressed HSV replication in cell culture and lowered the probability of lethal outcome of HSV-infected laboratory animals [84]. It was noted that like acyclovir, HpACV and interferon  $\alpha$  act synergistically [85]. An unusual feature of HpACV is the suppression of acyclovir-resistant virus strains deficient in thymidine kinase; its concentration was only twice higher than that in the case of acyclovir-sensitive strains. However, the inhibiting concentration of acyclovir increased 500-fold in similar experiments. The resistance of strains to HpACV appears more slowly than to ACV and at higher concentrations (100–800  $\mu\text{g/ml}$  against 2.5–100  $\mu\text{g/ml}$ , respectively). Thus, we assume that ACV metabolism differs substantially from that of HpACV. Indeed, in contrast to ACV, which should be phosphorylated by thymidine kinase upon uptake into the cell, HpACV in *Vero* cells is mainly converted into acyclovir monophosphate (ACVMP), and only the small fraction is hydrolyzed to ACV [84].

**Derivatives of triazolopyrimidines.** Derivatives of 1,2,3-triazolo[1,5- $\alpha$ ]pyrimidine are the base for the synthesis of many physiologically active compounds.

Compound (a) in Fig. 5 was described as an inhibitor of a PTEN-deficient cancer cell line [86], while com-



**Fig. 4.** Chemical structures of antiherpetic drugs: a) H2G; b) lobucavir; c) A-5021; d) HpACV; e) (Z)-9-[3-(phosphonomethoxyprop)-1-en-1-yl]adenine; f) (E)-9-[3-(phosphonomethoxyprop)-1-en-1-yl]adenine.

compound (c) manifested antiherpetic activity [87], and ribosylated compound (b) was active against rhinoviruses [88]. Derivatives of triazolopyrimidines were studied as HSV-1 inhibitors in *Vero* cells [87]. The chemical structures of these compounds are shown in Figs. 5c-5g.

It was shown that acyclic derivatives of triazolopyrimidines display antiherpetic activity in cell culture, while their triphosphates inhibit DNA synthesis catalyzed by the HSV-1 DNA polymerase. The strongest inhibitor was triphosphates of compound (e) with R = SMe [87]. Consequently, one of the targets of these compounds might be the herpes virus DNA polymerase.

Along with the study of herpes replication inhibitors targeting DNA synthesis, intensive search for other viral targets have been performed.

**Helicase–primase inhibitors.** In the last 10 years, several new classes of compounds manifesting antiherpetic activity due to suppression of the virus helicase–primase complex (UL5, UL52, UL8) were developed. In 2002, the Bayer Company investigated a series of thiazole derivatives as antiherpetic compounds in cellular and animal models with higher efficiency than acyclovir and its derivatives. Leader compound BAY 57-1293 (N-[5-(aminosulfonyl)-4-methyl-1,3-thiazol-2-yl]-N-methyl-2-[4-(2-pyridinyl)phenyl]acetamide) (Fig. 6a) manifested significant antiherpetic properties both in cell lines and in animal models without cross-resistance relative to acyclovir.

The mechanism of BAY 57-1293 action is the complete blocking of the viral DNA synthesis after the transcription of immediate early genes. Analysis of 10 virus strains resistant to the compound showed that the resistance appears due to mutations in genes coding UL5 and/or UL52 – components of the helicase–primase complex of the virus, which was confirmed by the inhibition of ATPase activity of the complex *in vitro*. It should be noted that the frequency of appearance of viruses resistant to this compound was lower by an order of magnitude compared with the appearance of resistance to acyclovir.

The only disadvantage of this compound is dose-dependent hyperplasia of the urinary bladder of the rat model after peroral intake of the compound. However, no toxicological effects were observed for dogs under the same conditions. It should be mentioned that primary sulfonamides suppressing dehydratase cause hyperplasia of the epithelium of the urinary bladder in rodents but not in other animals including humans [89]. In addition, BAY 57-1293 considerably more efficiently reduced HSV-2 reactivation in a guinea pig model than valacyclovir during therapy at the early infection stage [90].

In parallel, a series of related compounds that suppresses HSV replication *in vitro* and *in vivo* was developed at Boehringer Ingelheim Pharmaceuticals.

The BILS 179 BS compound (Fig. 6b) is approximately 10-fold more effective than ACV in cell culture

and suppresses the development of herpetic infection in animals [91].

In contrast with the compounds described above, the oxadiazolylphenyl derivative (ASP2151, Fig. 6c) actively inhibited HSV-1 and HSV-2 as well as VZV [92]. The efficiency of ASP2151 considerably exceeded that of acyclovir, and this compound successfully passed through phase II clinical trials in 2011.

**Ribonucleotide reductase inhibitors.** Ribonucleotide reductase (RR) is an important enzyme in virus replication in herpetic infection of eyes and for virus reactivation from latent to active state in skin infections. To suppress herpes RR, a class of thiocarbonyl hydrazones was initially proposed. However, it turned out that they inhibit not only the viral enzyme, but also the cellular homolog; therefore, they can be considered only for local application.

Hydroxyurea, which is also a nonselective inhibitor of RR, enhanced the suppression of the HSV replication in *Vero* cells by ACV and other nucleoside drugs by a factor of 3-4-fold. The effect also manifested for strains resistant to ACV with mutations in both TK and DNA polymerase genes [93].

The BILD 1633 SE compound (Fig. 6e), a peptidomimetic of the C-end of the small subunit of herpes RR, inhibited virus enzyme at the concentration of 3 nM

and was active both in wild-type virus and strains resistant to ACV at the concentration of about 0.4 mM with depression of infection for a nude mouse line.

Synergistic action is mentioned for compounds BILD 1633 SE and ACV, since the suppression of viral RR activity led to decrease in the dGTP pool and increase in the ratio of the ACV concentration to dGTP in the cell [94].

**Inhibitors of viral attachment and entry into the cell.** Heparan sulfate, a herpes virus entry mediator (HVEM), nectines-1 and -2 on the cell surface, and virus glycoproteins gB, gC, gD, gH, and gL are involved in HSV attachment and entry into the cell (see section "Herpes Simplex Virus Type 1: General Description, Life Cycle, and Replication"). It was many times demonstrated that polyanions have antiviral properties *in vitro* since they resemble heparan sulfate chains and competitively inhibit binding of gB and gC to the cell. For example, a mixture of highly sulfated oligosaccharides of mannose (PI-88) was proposed as an antiherpetic drug; however, it manifested no virucidal properties [95]. Analysis of mutations in resistant viruses, which were cultured in the presence of PI-88, showed that glycoproteins gC, gB, and gD are responsible for the virus sensitivity to the drug [95]. The same authors [96] created a series of compounds based on various oligosaccharides and PI-88 conjugated

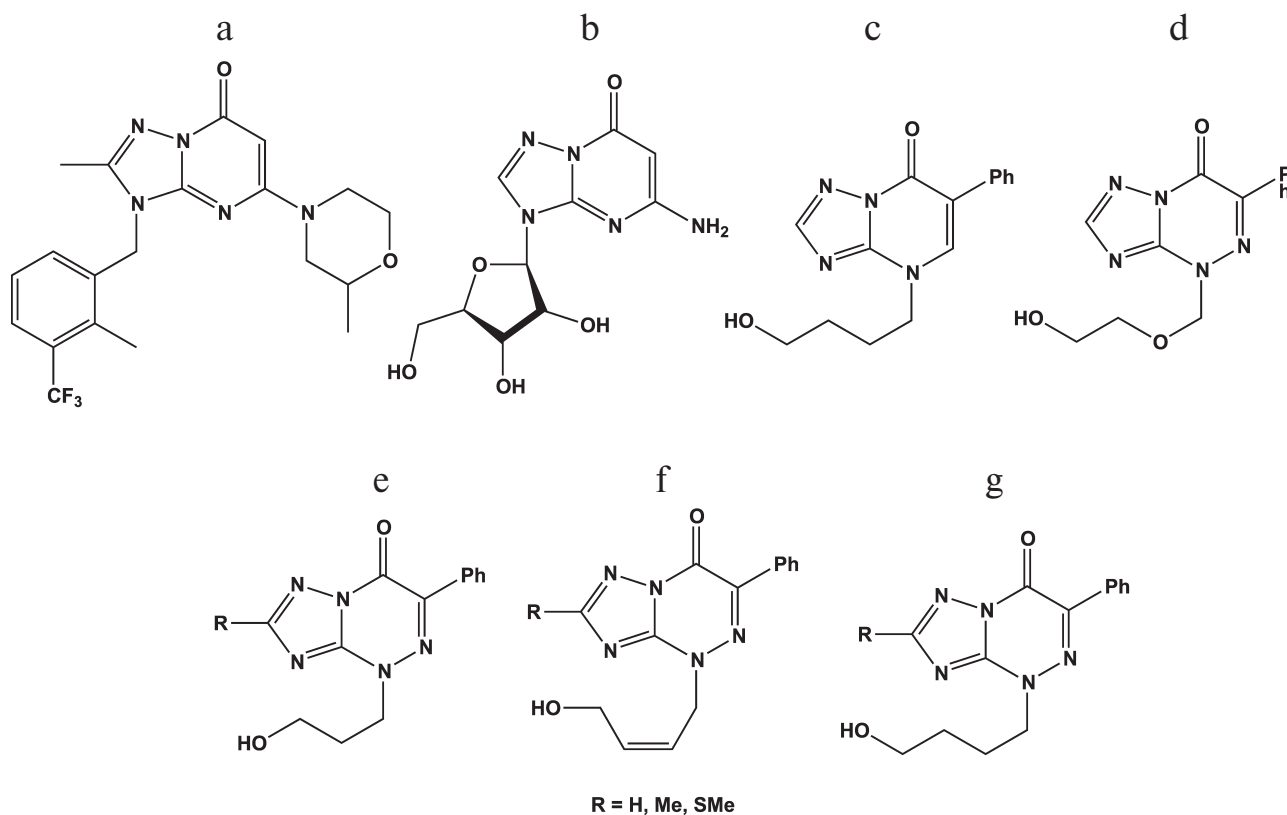
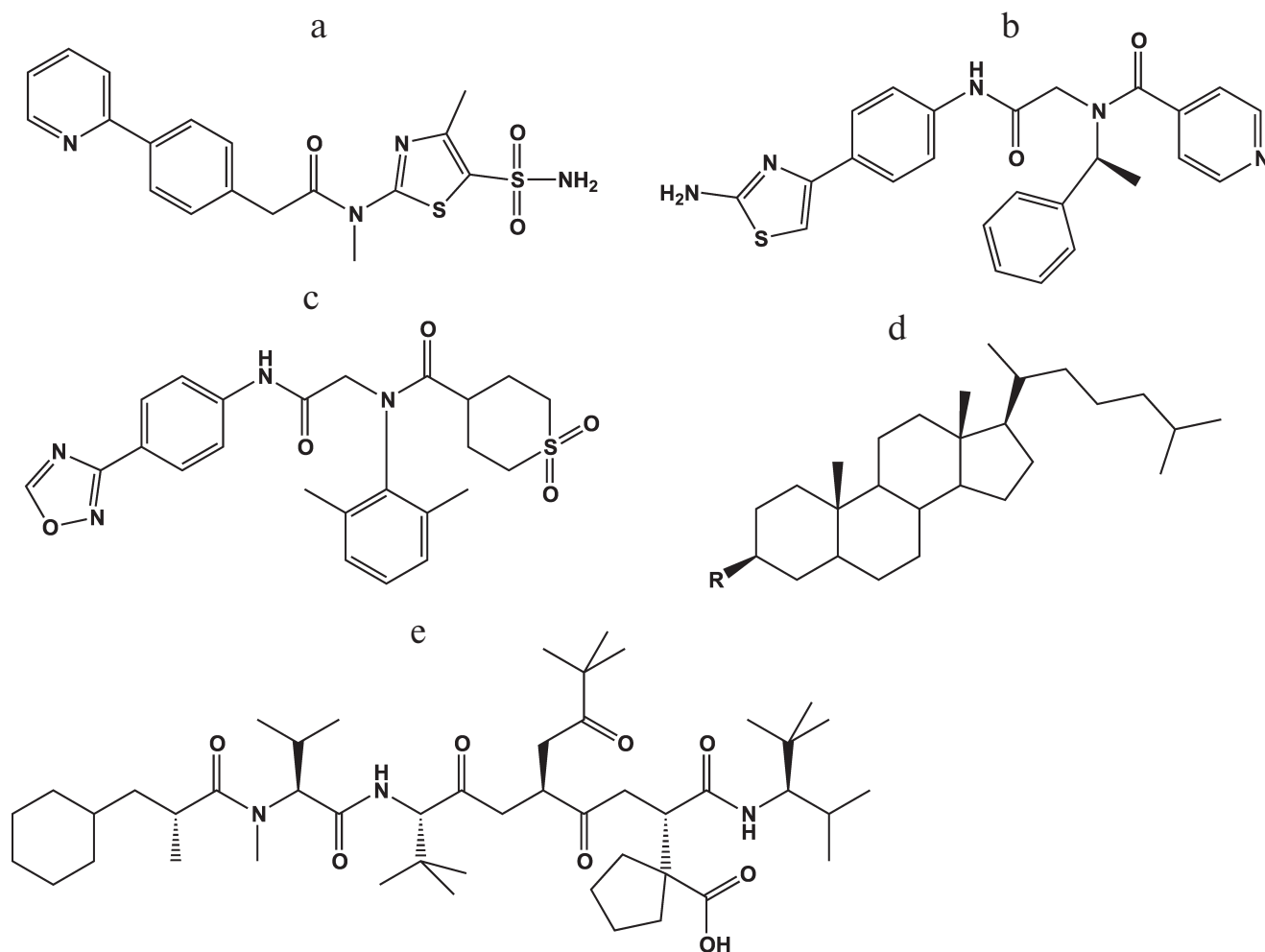


Fig. 5. Chemical structures of derivatives of triazolopyrimidines.



**Fig. 6.** Chemical structures of inhibitors of viral helicase–primase (a-c), adhesion and entry of the virus into the cells (d), and viral ribonucleotide reductase (e).

with hydrophobic aglycone groups, one of which, cholestanyl glycoside of sulfated tetrasaccharide [Man $\alpha$ (1,3)-Man $\alpha$ (1,3)-Man $\alpha$ (1,2)-Man] (Fig. 6d). This compound suppressed not only the virus replication in the cell culture GMK AH-1 ( $IC_{50} = 2.1 \mu\text{g/ml}$ ) but also competed with heparan sulfate for binding with viral glycoproteins, which prevented the penetration of the virus into the cell and its further transfer from cell to cell. The compound also inactivated the virus particles, which is apparently the result of destabilization of the viral envelope by the lipophilic cholestanyl group.

A common disadvantage of sulfated oligosaccharides and polysaccharides is the partial destruction of the usually impermeable for pathogens intestine mucus layer [97].

Retrocyclin 2, a short cyclic peptide from the group of  $\Theta$ -defensins [98], and lactoferrin [99] also protect cells against HSV-1 entry due to binding with virus glycoproteins. In contrast to oligosaccharides, which are suitable for local application only, lactoferrin suppresses the

development of skin symptoms of herpetic infection in mice with oral administration [99].

Thus, the number of promising viral targets and classes of compounds with substantial antiherpetic properties considerably increased during the last decade. However, no new effective and low-toxicity clinical drugs against both wild-type viruses and drug-resistant strains have appeared.

#### GENOTYPIC CHARACTERIZATION OF DRUG-RESISTANT HSV CLINICAL ISOLATES

As mentioned above, long drug use leads to the emergence of resistant HSV strains, making the disease course uncontrollable. In 95% of cases, ACV resistance is caused by mutations in viral thymidine kinase, which performs the initial phosphorylation step of ACV, followed by two subsequent steps carried out by cellular kinases. The resulting

ACVTP acts as a chain terminator of DNA synthesis. Mutations in the DNA polymerase gene, which determine the antiviral drug resistance, are found only in 5%. Resistance can also be induced by simultaneous mutations in both enzymes [100]. The situation is also complicated by the fact that the most clinical isolates appear to be heterogeneous, causing simultaneous coinfection by several variants of ACV-stable variants of the virus.

A large number of mutations are revealed in the thymidine kinase and DNA polymerase genes of HSV clinic isolates and laboratory clones resistant to acyclovir and other drugs [101]; some of them lead to the loss or alteration in enzyme activity or specificity and, consequently, to increased resistance (Fig. 7).

Mutation R220H in thymidine kinase leads to the loss of the virus sensitivity to ACV, PCV, and GCV [102], whose antiviral activity is directly associated with the enzyme function. The authors of paper [103] described similar mutation R220K in the HSV-2 gene of thymidine kinase, which leads (along with other substitutions) to lowering sensitivity to GCV and BVDU by 1-2 orders of magnitude. At the same time, thymidine kinase-independent adenine arabinoside (AraA) suppresses the replication of the mutant virus almost at the same extent as the wild-type HSV-1 [85]. This mutation is also present in the thymidine kinase gene of a laboratory strain resistant to HpACV; however, it does not affect the sensitivity of the virus to HpACV since its mechanism of action is inde-

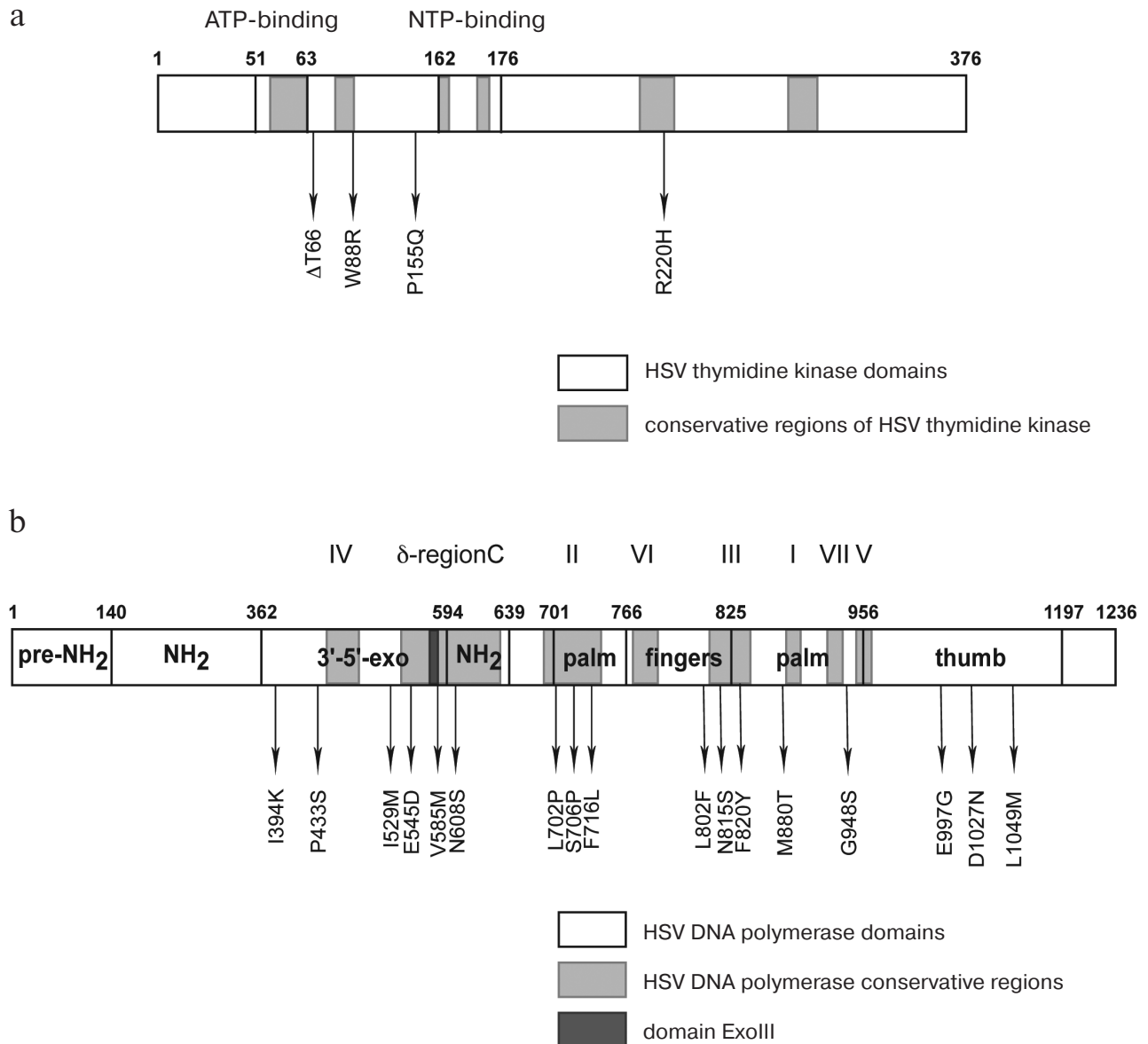


Fig. 7. Arrangement of amino acid substitutions from various clinic isolates and laboratory clones relative to conservative regions and domains: a) of thymidine kinase; b) of HSV-1 DNA polymerase [102, 105].

pendent of thymidine kinase. Mutation of G59R in the ATP-binding site of the enzyme plays a significant role in lowering its activity (Fig. 7a).

We identified more than 20 mutations in HSV-1 DNA polymerase [102]; some of them are substantial for enzyme functioning (Fig. 7b).

The substitutions 1394K, P433S, and V585M are localized in the 3'-5'-exonuclease domain of the enzyme, and mutation V585M is located in an ExoIII (572-585) conservative region. The authors [104] showed that mutations in this region cause resistance. Mutation D581A, which is located in the immediate proximity to V585M, leads to almost complete loss of 3'-5'-exonuclease activity of the enzyme, but along with partial retention of polymerase activity [64]. New amino acid substitutions I159M and E545D affecting the enzyme activity were found in the 3'-5'-exonuclease domain of DNA polymerase from an HSV-1 clinical isolate [102]. Mutations in the exonuclease site of the DNA polymerase typically impair the proofreading activity, lower the accuracy of the DNA synthesis, and, consequently, increase the mutation rate.

Mutation N608S is in the conserved  $\delta$ -region C and leads to resistance of an HSV-1 laboratory strain to ACV and HpACV [105]. A clone with the L702H mutation in the conserved region II of HSV-1 DNA polymerase showed resistance toward acyclovir but remained sensitive or insignificantly resistant towards penciclovir and ganciclovir. The substitution of hydrophobic leucine 702 by proline can promote a conformational change in the  $\beta$ -sheet in the "palm domain" coordinating magnesium ions and triphosphate in the enzyme active site [106]. A similar effect is caused by mutation F761L since it is located near amino acid residue D717 coordinating magnesium ions. When substituting hydrophobic valine 715 by more polar methionine [107] and neighboring hydrophobic phenylalanine 716 by polar lysine [102], sensitivity of the virus to ACV is lost.

According to crystallographic data [53], mutation M880T (Figs. 2B and 7b), which is located rather close to the catalytic triad (D717, D886, and D888) and to the binding site for the phosphate residue of the nucleotide and for magnesium ions, can form steric obstacles for binding phosphate residues and lead to lowering the sensitivity of the virus both to nucleoside analogs and PFA.

Authors of paper [108] showed that the N815S mutant is also resistant to acyclovir and its analogs. According to the crystallographic data [53], side chain N815 is opposed to the base of the nucleotide "entering" the active site of the enzyme. Computer modeling suggested that the side chain of mutant residue S815 has spatial orientation differing from that of the N815 residue of the native strain; the polymerase mutant at this residue does not incorporate ACVMP into the growing polynucleotide chain [109].

Thus, resistance of both clinic isolates and laboratory clones is explained by mutations in both DNA poly-

merase and thymidine kinase of the herpes virus, and substitutions of the same amino acid residue differently affect the sensitivity of enzymes to various antiherpetic drugs.

## CONCLUSION

This review does not pretend to represent all the data on herpes viruses, their interactions with the host cell, and development of antiherpetic drugs overall. From the instant of the creation of the gold standard of antiherpetic therapy, acyclovir, many new effective compounds appeared and another understanding of strategies in the search for drugs has come, one of which is the creation of prodrugs with lowered toxicity, and a multitude of investigations elucidating the details of the interaction of the virus with a cell was implemented [110]. The main purpose of this review was to show that the investigation of the herpes virus and search for inhibitors of its replication still remain a topical problem, which requires further efforts of the chemical, biological, pharmaceutical, and medical communities.

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