viruses isolated in Yokohama during 2007–2008. As a result, it was appeared that **2** completely inhibited the infection of their drug-resistant viruses. Further, the virus inhibition activity of **2** was found to be 20-fold relative to **1**. This unique virus inhibitory action can be utilized to inhibit a broad spectrum of influenza viruses.

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Small Molecule Therapeutics of Viruses of Families *Bunyaviridae* and *Arenaviridae*

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The nucleoprotein of Rift Valley Fever Virus (RVFV NP), a member of family *Bunyaviridae*, and of Lassa Fever Virus (LASV NP), a member of family *Arenaviridae* both of which have helical capsids, have been expressed in a system for cell-free protein synthesis (CFPS). Assembly was assessed by velocity sedimentation on sucrose density gradients and occurs under conditions previously demonstrated to assemble icosahedral capsid-related structures for multiple virus families. The nucleoprotein assembly pathways of these helical capsid viruses appear distinct from each other as well as from those of the several families of icosahedral capsid viruses studied previously. Preliminary electron microscopic studies confirm an appearance of nucleoprotein assembled by CFPS that is similar to irradiation-inactivated, detergent treated, authentic RVFV and LASV, and markedly different from the structures formed for icosahedral capsid viruses, as would be expected. ELISA screens have been devised for identification of small molecules blocking these assembly pathways. Hits from these screens have been validated by plaque reduction assessment of live virus in cell culture. Partial overlap was observed between the compounds active against RVFV and those active against LASV, with some compounds active against one but not the other, and other compounds active against both. One possible explanation for these findings is that these viral families share a requirement for some host factors. Studies are proceeding on putative host target identification using drug column affinity chromatography of extracts prior to CFPS, on dissection of mechanism of drug action by analysis of the products of CFPS in the presence of compounds, and on structure activity relationship optimization to enhance potency and diminish toxicity.

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Efficacy of N-methanocarbathymidine Against Herpes Simplex Virus is Cell Cycle Dependent

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(North)-methanocarbathymidine (N-MCT) is a conformationally locked analog of thymidine that is a good inhibitor of herpes simplex virus (HSV) and orthopoxvirus replication in vitro and in vivo. This compound is phosphorylated by the thymidine kinase (TK) encoded by herpes simplex virus and also by the TK homologs encoded by the orthopoxviruses. However, the mechanism of action is complex and other cellular kinases also likely play a role in its metabolic activation in infected and uninfected cells. Isolates of HSV that are resistant to acyclovir are also comparatively resistant to N-MCT which was expected since mutations that reduce TK activity also reduce the activation of both compounds. However, the efficacy of the compound against acyclovir-resistant isolates varied widely depending on the state of the primary human foreskin fibroblast (HFF) cells used in these studies. When HFF cells were seeded 3 days prior to infection, the compound inhibited TK deficient strains of HSV-1 with EC_{50} values of 3–10 μM, but if cells were seeded two days prior to infection the compound was much less effective and had EC_{50} values of 48–66 μM. A similar effect was observed against TK deficient strains of HSV-2. Significant differences were not observed in the efficacy of cidofovir controls. The differential efficacy is likely related to the cell cycle since most of the cells are in the S phase of the cell cycle 2 days after the cells are seeded, and most are in G_1/G_0 by 3 days. It is unclear why the compound would be less effective in dividing cells; it is possible that increased levels of dTTP during S phase might compete with N-MCT triphosphate for incorporation by the viral DNA polymerase. Nonetheless, this observation is intriguing and could potentially be useful as the antiviral and the antitumor properties of this compound are of significant interest.

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Synthesis and Antiviral Activity of Adamantyl Modified Nucleoside Phosphonates: Analogs of Cidofovir

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Cidofovir (HPMPC, CDV, 1-(S)-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine) is a potent and selective anti-DNA virus agent. Cidofovir suppresses the in vitro growth of all human and animal DNA viruses thus for examined. In the ongoing search for new cidofovir analogues and derivatives, accruing attention is given to the development of neutral ester prodrugs to enhance oral absorption and improve pharmacological parameters. In the paper, we described the synthesis of novel nucleoside phosphonates modified by adamantyl moiety: R = $AdCH_2$, $AdCH_2CH_2$, $AdCH_2CH_2CH_2$, $AdOCH_2CH_2$, $AdOCH_2CH_2CH_2$, $AdOCH_2CH_2CH_2CH_2$, $AdOCH_2CH_2OCH_2CH_2$, 3-Et-$AdCH_2$, 3-Et-$AdCH_2CH_2$, 3-Et-$AdCH_2CH_2CH_2$, 3-Et-$AdOCH_2CH_2$, 3-Et-$AdOCH_2CH_2CH_2$, 3-Et-$AdOCH_2CH_2CH_2CH_2$, 3-Et-$AdOCH_2CH_2OCH_2CH_2$. This way of modification could allow developing new therapeutic agents having high level of bioavailability and can be able to act on two or more stages of reproductive cycle of DNA viruses.

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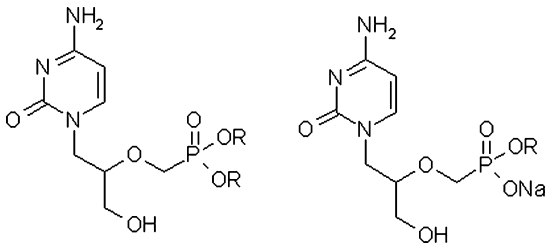

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The Activity of the New Adamantane Derivatives Against the Orthopoxviruses

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At present time the problem of development of drugs for prevention and treatment of orthopoxviral diseases becomes actual because the vaccination does not take place for a long time and there is a probability of arising of new centers of these infections, such as monkeypox in humans. Functional derivatives of cage compounds are as is known one of perspective substances for search of antiviral agents. During our investigation we have synthesized series of functional derivatives of adamantane: amides, hydrazones, hydroxy derivatives and wide range of adamantlyl substituted nitrogen containing heterocycles. Antiviral potency of synthesized compounds was evaluated against following orthopoxviruses: vaccinia, cowpox and mousepox in cell cultures (Vero, MK-2). More than 20 of synthesized compounds have showed very good antiviral action. Meanwhile, these substances have very low acute toxicity. Among them it is necessary to note adamantyl amides of p-bromobenzoic acid, which inhibits reproduction of orthopoxvirus in 2 mM concentration and adamantlyl disubstituted butanediol shows good potency against orthopoxvirus (IC_{50} = 2 mM). The presence of great number of high active compounds indicates some common principles of antiviral action of compounds, containing saturated cage moiety. Structures of compounds having activity against poxviruses allow supposing that their action occurs at the later stages of viral reproduction.

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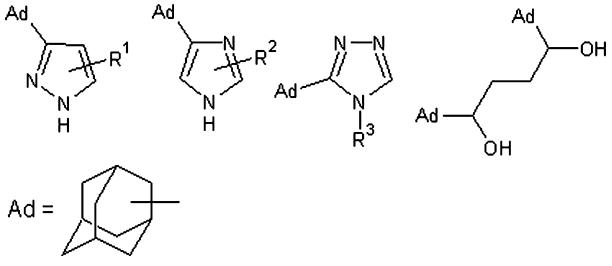

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The Influence of Combined Application of Interferon Inducers with Proteolysis Inhibitor on the Endogenic Interferon Level

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Increase of interferon formation intensity is the important problem for using its inducers as antivirals. We have supposed that one of the ways for the enhancement of antiviral efficacy of interferon inducers may be the prevention of hydrolysis of synthesized endogenic interferon by using proteolysis inhibitors. In our studies we used E-aminocaproic acid as a proteolysis inhibitor and amixin (tilorone) or SK-19 (which is a new phytoextract that we obtained) as interferon inducers. A noticeable increase of interferon level and prolongation of its circulation in the blood of experimental mice were established after the various schemes of combined application of interferon inducers and proteolysis inhibitors. E-aminocaproic acid has antiviral properties but it does not demonstrate interferon inducing activity. E-aminocaproic acid promoted the increase of interferon level in the blood of animals when used in 12 h after intraperitoneal injection of interferon inducer SK-19 in a dose of 40 mg/kg – in 16 times (from 80 to 1280 un/ml) and in 4 times (from 1280–2560 to 5120–10240 un/ml) when SK-19 was used in a dose of 60 mg/kg. A pique of interferon production in 24 h after per oral use of amixin in a dose of 200 mg/kg was from 640 to1280 un/ml. Combined application of this interferon inducer with E-aminocaproic acid (in 0.5–2 h after the use of amixin) stimulated interferon system more effectively and titers of serous interferon grew up to 2560–5120 un/ml. Also joint use of amixin and E-aminocaproic acid prolonged of interferon circulation: interferon was not detected in 48 h after amixin alone application and in case of its combination with E-ACA titers of serous interferon reached 20–40 un/ml. Higher antiviral efficacy of these schemes of combined application of SK-19 and amixin (tilorone) with proteolysis inhibitor E-aminocaproic acid than use of interferon inducers alone has been shown in the subsequent on experimental models of arboviral infections.

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Synthesis of Ester Prodrugs of 9-(S)-[3-Hydroxy-2-(phosphonomethoxy)propyl]-2,6-diaminopurine (HPMPDAP) as Anti-Poxvirus Agents

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Based on their in vitro activity and toxicity profile, (*S*)-9-[3-hydroxy-2-(phosphonomethoxy)propyl]-2,6-diaminopurine (HPMPDAP) and its cyclic form (cHPMPDAP) were selected for further evaluation as potential drug candidates against poxviruses. To optimize potency and bioavailability of these compounds for therapeutic applications, synthesis of structurally