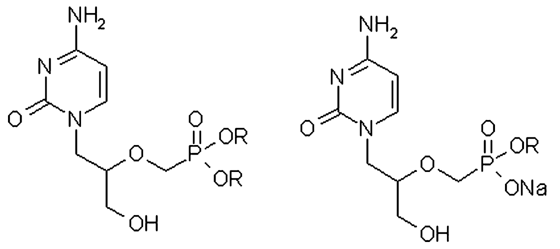

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The Activity of the New Adamantane Derivatives Against the Orthopoxviruses

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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 adamantyl 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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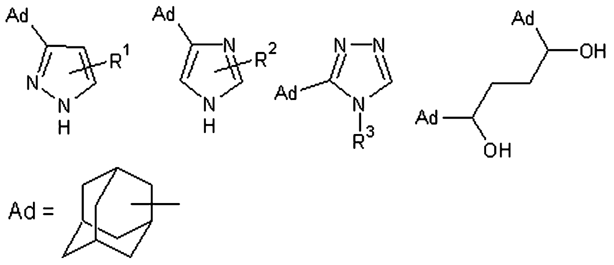

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The Influence of Combined Application of Interferon Inducers with Proteolysis Inhibitor on the Endogenic Interferon Level

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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 diverse types of prodrugs was carried out including alkoxyalkyl (hexadecyloxypropyl, octadecyloxyethyl, hexadecyloxyethyl), pivaloyloxymethyl (POM), 2,2,2-(trifluoro)ethyl,2-butylsalicylyl esters as well as peptide-conjugated phosphonates. New synthetic procedures including the utilization of hexafluorophosphate coupling agents for esterification of the phosphonate function were developed. All HPMPDAP and cHPMDAP prodrugs were synthesized as phosphonate monoesters. A detailed anti-poxvirus and other antiviral testing as well as comparison of properties of single types of prodrugs was carried out. Alkoxyalkyl esters emerged as the most potent anti-poxvirus prodrugs of HPMPDAP and its cyclic form.

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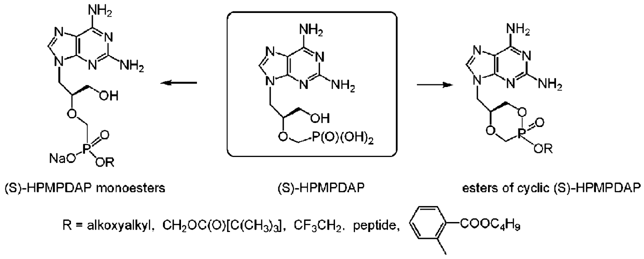

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New Nucleoside and *bis*-Nucleoside-Phosphonate Conjugates: Design, Stability, and Anti-HIV Evaluation

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Design of depot forms of anti-HIV drugs are widely used to reduce toxicity, improve drug pharmacokinetics, and overcome drug resistance. The obvious design rational for depot forms is that the conjugate will be converted by hydrolysis or enzyme action to active agents upon penetration into cells. Herein, we report the design, stability, and anti-HIV properties of phosphonate derivatives containing AZT, 3TC, and *bis*-nucleosides composed of mono- and heteronucleoside analogs with general structure Nu–O–P(O)(R)–O–Nu (Nu = AZT or 3TC). Among phosphonate depot forms of AZT or 3TC, the most perspective were their aminocarbonyl derivatives. They were stable in blood serum, displayed good anti-HIV activity and low toxicity in cell culture, improved pharmacokinetics, lower acute toxicity, and absence of cumulative effect if compared to that of AZT or 3TC, respectively. The stability in blood serum, anti-HIV activity and toxicity of phosphonate derivatives of *bis*-nucleosides were dependent on the structure of phosphonate moiety. Stability of morpholinecarbonyl-*bis*-AZT in blood serum was 30 min, at the same time for heterodimer ($R = ClCH_2$, Nu = AZT, 3TC) and homodimer ($R = CH_3{-}(CH_2)_5{-}NHC(O)$, Nu = AZT) was ≫6 h. The majority of compounds were less potent than parent nucleosides but their toxicity (CD_{50}) was considerably lower than those of the appropriate nucleoside. Therefore, a higher CD_{50} allowed better selectivity indexes (SI). Pharmacokinetic parameters of some phosphonate derivatives of nucleoside analogs and that of *bis*-nucleosides will be reported.

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RNA Polymerase Fidelity Variants of the Picornaviruses Uncover A Novel, Indirect RNA Mutagenic Activity for Amiloride Compounds

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Our laboratory studies the effects of RNA mutagens, and base analogs, ribavirin, 5-azacytidine and 5-fluorouracil on picornaviruses (poliovirus, Coxsackie B virus). In a screen to isolate RNA mutagen resistant variants of Coxsackie B3 virus, we identified a mutant presenting an A372V change in the viral RNA dependent RNA polymerase that conferred resistance to all 3 base analogs. This resistance was found to result from an increased polymerase fidelity, similar to our recently published work on ribavirin-resistant polioviruses that mapped to a different region of the polymerase (Vignuzzi et al., 2008). Interestingly, the A372V mutant had been previously isolated in a screen for resistance to amiloride compounds (inhibitors of Na^+ ion channels and the Na^+/H^+ exchanger), along with another polymerase mutant, S299T (Harrison et al., 2008). Since the same mutation would not expectedly confer resistance to two different antiviral mechanisms, we hypothesized that amiloride compounds had a previously unknown mutagenic activity. Indeed, we find that amiloride treatment of both Coxsackie virus and poliovirus increases their mutation frequencies. Furthermore, we show that higher fidelity variants of both viruses, presenting lower basal mutation frequencies, are more resistant to the RNA mutagenic effects of these compounds. Our results suggest that in addition to being replication inhibitors (as observed by other groups), amiloride compounds are the first described, non-nucleoside, indirect RNA mutagens. We are currently determining whether this mutagenic activity is the result of a direct interaction with the polymerase, or the result of intracellular alterations stemming from the inhibition of ion channels. Our data raises the question of whether this mutagenic activity is strong enough to act as an antiviral (through lethal mutagenesis) or whether it promotes viral evolution (through moderate mutagenesis). Implications for future drug development will be discussed.

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Overlap in Virus Specificity Leads to the Discovery of Small Molecules Active Against Rabies Virus, Cytomegalovirus, and Monkey Pox Virus

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The Prosetta Platform for cell-free protein synthesis (CFPS) and assembly of viral capsid-like structures has been used successfully to identify novel compounds with antiviral activity (see other