tual screening of new compounds possessing antiviral activity towards coxsackievirus B3 97-927 with high selectivity.

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Studying of Anti-Epstein–Barr Virus Activity of Amizon and their Derivative

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During last decades more and more attention is given to creation of preparations for pathogenetic therapy with the polyvalent pharmacological action. One of successful elaborations of the Ukrainian pharmacologists is the new non-narcotic analgesic Amizon with expressed antiphlogistic, antipyretic, interferon gene and immunomodulatory properties. Amizon—the derivative of isonicotinic acid (*N*-metyl-4-benzyl urea-pyridinit iodidum). The objective of the present investigation was to study the activity Amizon, as well as derivative, in which structure there is no iodine, against Epstein–Barr virus. As a model of EBV-infection in vitro we used the line of lymphoblastoid B-cells Raji. To study the cytotoxicity of investigated drugs they were entered into the culture of not infected cells in concentration from 0.1 up to 3000 μg/ml. In 48 h there was conducted the MTT-analysis of the investigated samples. It was shown, that the concentration that oppressed proliferative activity of cells on 50% (CD50), for Amizon has compounded 840 μg/ml, and for its derivative—2100 μg/ml, accordingly. The anti-virus activity was determined by a PCR method, using "Amply Sens 100 R" system (Russia). Drugs were investigated in concentrations of 0.1, 0.5, 1, 5, and 10 μg/ml. The analysis of obtained data allowed to determine concentrations, which oppressed the replication of the virus on 50%, that was shown by reduction of the number of genomic equivalents of EBV DNA on a cell testified. ED50 for Amizon has compounded 0.1 μg/ml, for its derivative—5 μg/ml. Thus, the low toxicity of investigated drugs was shown and their effective doses were determined. Proceeding from the index of selectivity that is 8400 for Amizon, 400 for its derivative, it is possible to make a conclusion about their availability for the further researches as of drugs that are active against an Epstein–Barr virus. Furthermore obtained data testify to importance of presence of iodine in structure of drug, as, apparently from the received data, the activity of derivative, not containing iodine, is below more than in 20 times.

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Development of Resistance to Oxoglaucine in Poliovirus Type 1 (LSc-2ab) and the Six Coxsackie B Viruses

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Up to date there is no safe and effective enterovirus specific drug available for clinical use. Still there is a clear need for continued development of new inhibitors of enterovirus replication. Oxoglaucine has proven its promising broad-spectrum antienterovirus effect in a pilot study of ours. It exerts a strong antiviral effect against the replication of poliovirus type 1and enterovirus B species. The selectivity ratio in most cases is above 100. Here the development of resistance to oxoglaucine in the case of poliovirus type 1(LSc-2ab) and the six coxsackie B viruses is studied in vitro. The tested viruses develop rapidly phenotypic signs or resistance. A correlation is established between the sensitivity to oxoglaucine and the necessary number of serial passages for the development and selection of resistant virus mutants. Viruses that have revealed the greatest sensitivity to the antiviral effect of oxoglaucine develop most rapidly resistant mutants. The resistant virus reaches high infectious titers in the presence of the compound. Reversion to sensitivity occurs when the selective oxoglaucine pressure is diminished. The obtained results serve as a proof for the specific and selective antienterovirus activity of oxoglaucine and serve as a basis for further studies on its mode of action.

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Efficacy of Therapeutic Intervention with an Oral Ether Lipid Analogue of Cidofovir (CMX001) in a Lethal Mousepox Model

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In the 21st century we are faced with the potential use of natural or recombinant VARV and MPXV as biological weapons, and the emergence of human MPXV. Such occurrences would require therapeutic and prophylactic intervention with antivirals. Cidofovir, an antiviral approved for the treatment of cytomegalovirus retinitis in AIDS patients, has activity against poxviruses, but must be administered intravenously and is associated with nephrotoxicity. An ether lipid analogue of CDV, CMX001 (HDP-CDV), has excellent oral bioavailability, minimal nephrotoxicity, and potent in vitro and in vivo antiviral activity against poxviruses. Using the mousepox model, we have staged the course of disease with biomarkers that include viral DNA copies in the blood, core body-temperature, blood sera clinical chemistry, blood cytokine changes and blood CD45+ cell changes. These biomarkers have been used to optimize

CMX001 therapeutic dosing and the maximum delay in treatment that can afford protection from lethal disease.

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Synthesis of P–O–C-linked Foscarnet–Peptide Conjugates and Sensitive Methods to Detect the Released Drug in Biological Samples

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The trisodium salt of phosphonoformic acid (PFA), foscarnet, is an analogue of pyrophosphate that inhibits a broad spectrum of viruses. A long-recognized limitation of foscarnet has been its very low oral bioavailability, which is due to the ionization of PFA at physiological pH. As a result, in the clinic it can only be administered intravenously. Here, we report the synthesis of a series of novel PFA prodrugs, created by incorporation of toxicologically benign amino acids or small peptides to abate the anionic state of the drug. Previous work done in our laboratory demonstrated the synthesis of P–N linked PFA-amino acid conjugates, which cleanly release the parent drug at physiological pH. In this work, conjugates of PFA monosalts were esterified by the alcohol side-chain group of serine using Mitsunobu chemistry to create the P–O–C link. The detection and analysis of foscarnet is made difficult by its lack of a visible–UV chromophore, therefore we have also sought improved methods to determine the parent drug released from the conjugates. Two new approaches (UV detection via formation of a Yb^{3+}/pyrocatechol violet complex, and fluorescence detection by formation of a 9,10-bis[(2,2′-dipicolylamino)methyl] anthracene zinc complex) will be compared with detection using LC–MS/MS.

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In Vivo Efficacy of CMX001 Against Herpes Simplex Virus Types 1 and 2

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CMX001, or HDP-cidofovir, has been previously reported to have excellent activity both in vitro and in vivo against vaccinia virus (VV), cowpox virus (CV) and human cytomegalovirus (HCMV). In the current studies, CMX001 was synthesized as a free acid form instead of the salt forms used previously and evaluated in murine models of herpes encephalitis and neonatal herpes. Compound was suspended in 0.4% carboxymethylcellulose to yield desired dosages in a 0.2 ml volume. Mice were lethally infected intranasally with herpes simplex virus (HSV), type I, E-377, MB-1 or HSV-2, strain MS and treatments were delayed until 24 h post viral infection. CMX001 was administered orally once daily at 2.5, 5 or 10 mg/kg beginning 24 h post HSV infection and continued for 7 days. CMX001 exhibited some toxicity at the 10 mg/kg dosages in uninfected and infected mice. Treatment with CMX001 significantly reduced mortality of HSV-1 infected mice at 5 and 2.5 mg/kg doses ($P < 0.001$). Also, CMX001 significantly reduced mortality in HSV-2 infected mice at 5 and 2.5 mg/kg doses ($P < 0.001$). Acyclovir (ACV) was administered twice daily beginning 24 h post HSV infection as a positive control at 30, 60, or 120 mg/kg. ACV was effective in reducing or eliminating mortality at all doses evaluated ($P < 0.001$). In these studies, CMX001 was as efficacious as ACV at non-toxic doses of 5 and 2.5 mg/kg. Additional evaluation of CMX001 will be required in order to assess its potential for the treatment of serious HSV types 1 and 2 infections in humans.

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A Synthetic Strategy to Different Cyclopentenyl-Nucleosides

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Carbocyclic nucleosides are compounds in which the furan ring has been replaced by a cyclopentane system. They possess increased metabolic stability against nucleoside phosphorylases as well a higher conformational flexibility. In the past, carbocyclic nucleoside analogues like abacavir showed very interesting antiviral properties *in vitro* and *in vivo*. Abacavir was approved as a HIV-drug for clinical application. Therefore, we were interested in a short and efficient stereoselective access to this class of compounds. As starting material 3-benzyloxymethylcyclopent-3-enol was chosen, that can be prepared from cyclopentadiene after deprotonation and alkylation using benzylchloromethylether to give symmetric benzyloxymethylcyclopentadiene. This compound isomerizes into two thermodynamically more stable benzyloxymethylcyclopentadienes. This mixture of compounds material can be used as precursor for the synthesis of different 3′,4′-cyclopentenyl-nucleosides. This material can be oxided to 3-benzyloxymethylcyclopent-3-enone. The β,γ-unsaturated ketone undergoes isomerization into 3-benzyloxymethylcyclopent-2-enone. After reduction, the resulting 3-benzyloxymethylcyclopent-2-enol can be used as precursor for the synthesis of different 4′,6′-cyclopentenyl-nucleosides. Moreover, this strategy offers the possibility for the synthesis of new carbocyclic nucleosides because the double bond can be functionalized before or after introduction of the nucleobase. The synthesized carbocyclic nucleosides were converted into their monophosphates by