developed, optimized and validated a cytopathic effect (CPE) based, high-throughput screening (HTS) assay using the viability endpoint CellTiter Glo (Promega, Madison, WI) to identify novel anti-viral drugs against bluetongue virus infection in BSR cells. The 72 h assay against Bluetongue-10 virus was validated in 384-well plates with Z values >0.70. The signal-to-background at different multiplicity of infection (MOI) was >15 for MOI of 0.05 and >7 for MOI of 0.01, respectively. The small molecule compound library from the NIH molecular libraries screening center program has been screened using this assay. In addition, a secondary assay using Caspase-3/7 Glo (Promega, Madison, WI) to measure apoptosis was also developed. The apoptotic inducer Staurosporine served as a positive control and the apoptosis inhibitor Ac-DEVD-CHO served as negative control for the development of the apoptosis assay. This secondary assay was used to confirm hits and exclude false positives, including apoptosis inhibitors. Mechanism of action studies will be under taken on the hits to help prioritize them for drug development and for additional studies in other flaviviruses including DNV, WNV, and Yellow fever virus infection.

doi:10.1016/j.antiviral.2008.01.127

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Antioxidant Properties of Oseltamivir—A Specific Na Inhibitor of Influenza Virus Infection Type A in Influenza Virus Infected Mice and in some Model Systems

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The present study was designed to investigate some aspects of the effect of oseltamivir on the "oxidative stress" in alveolocytes, isolated from influenza virus infected mice. It was established that supplementation of mice with oseltamivir has protection against oxidative damages in lung of mice experimentally infected with influenza virus A/Aichi/2/68(H3N2) (1.5 LD50). Two products of lipid peroxidation in cell suspension were determined: malondialdehyde, and lipofuscine-like products. The results showed that influenza virus infection A/Aichi/2/68 (H3N2)was accompanied with a significant increase of the endogenous lipid peroxidation products and development of oxidative stress. We find that oseltamivir treatment led to a decrease of the products of lipid peroxidation on the 5th and on the 7th day after the inoculation. In order to elucidate the mechanism of the oseltamivir influence over the oxidative damages, experiments were carried out with some model systems. The capability of oseltamivir to scavenge superoxide radicals (scavenging properties) was studied in a system of xanthine–xanthine oxidase to generate superoxide. The amount of superoxide was measured spectrophotometrically by the NBT-test. Data is shown as a spectrophotometric scavenging index (SpSI). We concluded that oseltamivir does not show superoxide radical scavenging properties and its antioxidant-like effect observed in vivo is not a result of its direct action on the processes of lipid peroxidation and/or interaction with antioxidant enzymes. Our findings with model systems do not prove an antioxidant effect of the drug on the processes of lipid peroxidation in applied models of concentration range 10–0.01 mM. The mechanism of oseltamivir action on lipid peroxidation in influenza virus infection most probably is based on its antiviral activity.

Acknowledgements: This work is supported by Society of Medical Science, Medical University-Sofia, Research grant no. 57/2007, leader Dr. M. Mileva, PhD.

doi:10.1016/j.antiviral.2008.01.128

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Hit QSAR Analysis of Anti-Coxsackievirus B3 Activity of [(Biphenyloxy)Propyl]Isoxazole Derivatives

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Keywords: Coxsackievirus B3; Selectivity index; QSAR; Drug design

Diseases caused by coxsackieviruses (CVB) are widely distributed. Prophylaxis and treatment of these infections are important health care tasks. Drug design simply based on results of empirical screening is not very effective and can be substantially improved by usage of computer-based technologies. The objective of the present work is quantitative structure–activity relationship (QSAR) analysis of antiviral activity of various [(biphenyloxy)propyl]isoxazole derivatives and consequent drug design by means of HiT QSAR. Hierarchic QSAR technology (HiT QSAR) was used as a main tool of investigation. Simplex descriptors used and possibility of the statistical inverse task solution allow development of directed molecular design of new effective antiviral drugs. Thorough investigation of the relationship between antiviral activity against the clinical CVB3 isolate 97-927 ($\log_{10}$ IC_{50}, μM), and selectivity index (ratio of cytotoxicity to antiviral activity) and the structure of 25 [(biphenyloxy)propyl]isoxazole derivatives were carried out. Cytotoxicity on HeLa cells values ($\log_{10}$ CC_{50}, μM) were taken from results of virtual screening by HiT QSAR model developed by us. Obtained PLS QSAR models are quite satisfactory ($R^2 = 0.91$–0.97, $Q^2 = 0.78$–0.94, $R^2_{test} = 0.75$–0.91). It was found that compounds with high antiviral activity and selectivity have to contain oxadiazole or *p*-fluorophenyl fragments. Vice versa, the insertion of *p*-carboxymethyl-benzene, *p*-1,2,3-trifluoro-benzene and, especially, biphenyl fragments as a terminal substituents into investigated compounds substantially decrease both their antiviral activity and selectivity. High impact of atoms individuality and electrostatic factors was found for both properties, plus additionally lipophilicity is important for antiviral activity and H-bonding for selectivity. Obtained models have been used for drug design and consensus vir-

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

doi:10.1016/j.antiviral.2008.01.129

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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.

doi:10.1016/j.antiviral.2008.01.130

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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.

doi:10.1016/j.antiviral.2008.01.131

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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