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.

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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.

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

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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-