viruses. Compounds 05-103227, 05-102300 and 05-102691 yielded effective concentration (EC_{50}) values of 1–9 μM for VV and 6–58 μM for CV. Neutral red uptake and CellTiter-Glo cell viability assays were used to measure cellular cytotoxicity and it was determined that all of the compounds were relatively non-cytotoxic. Thus, these compounds are highly selective agents with 05-103227 and 05-102300 yielding selective indices of >167 and >346, respectively. The chemical structures of these small molecules shared characteristics with the potent antipoxvirus drug ST-246. We hypothesized that the compounds might act by a similar mechanism and tested them against an ST-246 resistant strain of VV. This mutant proved to be highly resistant to both 05-102300 and 05-102691, suggesting that these compounds also inhibited the F13L gene product, p37, which is the target for ST-246. The most effective compound of the three, 05-103227, retained activity against the F13L mutant suggesting that it does not target the same binding site on p37, or that it inhibits a different viral function. Additional experiments are underway to identify the molecular target of this compound and to determine the activity of each of these compounds in experimental animal infections.

doi:10.1016/j.antiviral.2008.01.124

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Synthesis and Antiviral Activity of Various N^4-Acyl Derivatives of Cidofovir and its 5-Azacytosine Counterpart, 1-(*S*)-[3-Hydroxy-2-(Phosphonomethoxy)Propyl]-5-Azacytosine

Marcela Krecmerova[1,*], Antonin Holy[1], Graciela Andrei[2], Lieve Naesens[2], Johan Neyts[2], Jan Balzarini[2], Erik De Clercq[2], Robert Snoeck[2]

[1] Gilead Sciences & IOCB Research Centre, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic; [2] Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

Investigation of new types of acyclic nucleoside phosphonates (ANPs) as antiviral agents resulted among others in discovery of 1-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine (HPMP-5-azaC), its cyclic form and several types of ester prodrugs, compounds active against DNA viruses with activity data similar or better compared to cidofovir and higher index of selectivity *in vitro*. In contrast to cidofovir, HPMP-5-azaC has more complicated metabolic profile due to its chemical and enzymatic instability. In aqueous solutions ring opening between C-6 and N-1 of the triazine moiety occurs and HPMP-5-azaC is successively degraded to 2-{[(2*S*)-3-hydroxy-2-(phosphonomethoxy)propyl]carbamoylguanidine via the intermediary *N*-formyl derivative. The final decomposition product has no cytotoxicity *in vitro* but it is antivirally inactive. Besides chemical decomposition, HPMP-5-azaC undergoes also extensive enzymatic deamination in cell cultures. To improve the stability towards deamination process we tried to transform HPMP-5azaC to diverse N^4-acyl prodrugs on the level of free phosphonic acids as well as on the level of some earlier already prepared ester prodrugs, e.g. hexadecyloxyethyl ester of cyclic HPMP-5-azaC. As acyl groups we selected even number fatty acid residues (e.g. behenoyl, stearoyl). Similar N^4-acyl compounds were prepared also from HPMPC (cidofovir) and some of its esters. Different reactivity of both systems towards acylation reactions and influence of introduction of N^4-acyl groups to stability and antiviral activity of compounds will be discussed.

doi:10.1016/j.antiviral.2008.01.125

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Antiviral Effects of Sulfated Exopolysaccharide from the Marine Microalga *Gyrodinium impudicum* Strain KG03

Chong-Kyo Lee[1,*], Hae Soo Kim[1], Ju Ryung Nam[1], Myung-Jin Lee[1], Joung-Han Yim[2], Hong Kum Lee[2]

[1] Pharmacology Res, Ctr, Korea Res. Inst. of Chemical Technology, Daejeon, Republic of Korea; [2] Polar Biocenter, Korea Polar Research Institute, Incheon, Republic of Korea

The sulfated exopolysaccharide p-KG03, which is produced by the marine microalga *Gyrodinium impudicum* strain KG03, exhibited impressive antiviral activity in vitro (EC_{50} = 26.9 mg/ml) against the encephalomyocarditis virus (EMCV). Depending on the p-KG03 concentration, the development of cytopathic effects in EMCV-infected HeLa cells was either inhibited completely or slowed. Moreover, p-KG03 did not show any cytotoxic effects on HeLa cells, even at concentrations up to 1000 mg/ml. The polysaccharide was purified by repeated precipitation in ethanol, followed by gel filtration. The p-KG03 polysaccharide had a molecular weight of 1.87×10^6, and was characterized as a homopolysaccharide of galactose with uronic acid (2.96%, w/w) and sulfate groups (10.32%, w/w). Antiviral activities of p-KG03 against various viruses – various picornaviruses, herpesviruses, influenza viruses and feline coronaviruses and HIV – will be reported. The biological activities of p-KG03 suggest that sulfated metabolites from marine organisms are a rich source of antiviral agents. The p-KG03 polysaccharide may be useful for the development of marine bioactive exopolysaccharides for use in biotechnological and pharmaceutical products.

doi:10.1016/j.antiviral.2008.01.126

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Developing a Novel High-throughput Screening Assay against Bluetongue Virus

Qianjun Li*, Lynn Rasmussen, Clinton Maddox, E. Lucile White

Southern Research Institute, Birmingham, USA

Arthropod borne viruses (arboviruses) are important human/animal pathogens that cause acute virus infections with severe diseases and/or death. Several recent human/animal epidemics are caused by arboviruses, including Dengue virus (DNV) in Asia, West Nile virus (WNV) in North America and Bluetongue virus (BTV) in Europe. There are no antiviral drugs available against these diseases. We have designed,

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

Milka Mileva[1,*], Angel S. Galabov[2], Lora Simeonova[2], Galina Gegova[2]

[1] Department of Medical Physics and Biophysics, Medical University, Sofia, Bulgaria; [2] Department of Virology, Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

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

E. Muratov[1,*], V. Kuz'min[1], A. Artemenko[1], E. Varlamova[1], V. Makarov[2], O. Riabova[2], P. Wutzler[3], M. Schmidtke[3]

[1] A.V. Bogatsky Physical-Chemical Institute, Odessa, Ukraine; [2] Research Center for Antibiotics, Moscow, Russia; [3] Institute of Virology and Antiviral Therapy, F. Schiller University, Jena, Germany

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-