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Study of Anti-Epstein–Barr Virus Activity of Novel Fluorinated Heterocyclic Nucleoside Analogues

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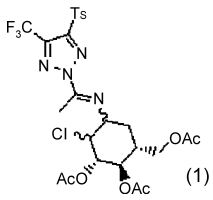
Although a number of the antiviral drugs can inhibit EBV replication, none of them has been licensed for the treatment of EBV infection in the clinic [1]. Search of new effective preparations capable to inhibit herpesviruses reproduction is conditioned by their certain resistance to different groups of chemical preparations. Synthesis of nucleoside analogues with triazole substituents which could be used as pharmaceuticals are of great interest [2]. We prepared new fluorinated 1,2,3-triazole derivatives (1–3) (Fig. 1) and studied their antiEBV activity. The activity of triazole substituents against EBV—lymphotropic and oncogenic virus was the object of present investigation. The line of lymphoblastoid B-cells Raji was used as a model of EBV-infection in vitro. The analysis of cytotoxicity of substances for cell line Raji was first stage of their investigation. They were studied in concentrations of 1000–1 μg/ml. In 48 h the MTT-analysis of investigated samples was conducted. The concentrations which inhibited the quantity of alive cells on 50% (CD_{50}) were for substance No. 1 – 600 μg/ml, No. 2 – 255 μg/ml and No. 3 – 800 μg/ml. An inhibition of reproduction of EBV in a cell culture was determined by reduction of a accumulation of the virus capsid antigen proteins on a cell. The anti-virus activity was determined by a cellular ELISA method, using Mab to VCA EBV (AbD Serotec, GB). Drugs were investigated in concentrations 100–0.1 μg/ml. The analysis of obtained data allowed to determine concentrations, which oppressed the accumulation of the virus proteins on 50 %. ED_{50} for No. 1 and No. 3 was 10 μg/ml, for No. 2 – 50 μg/ml. Thus, proceeding from the index of selectivity for the compound No. 1 – 60, No. 3 – 80, it is possible to make a conclusion about their availability for the further researches as of drugs that are active against an EBV.



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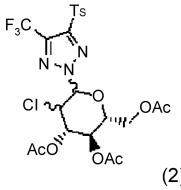

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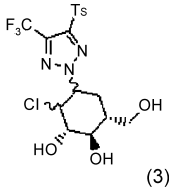

(3)

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Induction of Lytic Cytotoxicity by NF-(B Inhibitors in Epstein-Barr Virus-associated Gastric Carcinoma Cells

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Epstein-Barr virus (EBV) has been associated with epithelial malignancies like nasopharyngeal carcinoma (NPC), lymphoepitheliomas of several organs, and recently, it has been linked to gastric carcinoma (GC). Worldwide, EBV-associated GC represents about 10% of GC. EBV is found in every tumor cell in EBV-positive cancers, but not in normal cells, suggesting that EBV targeted strategies could be used to treat these tumors. EBV infection in tumor cells is generally restricted to the latent forms of viral infection. The antiviral nucleoside analogue ganciclovir (GCV) has been successful in eradicating virus-infected cells with the lytic, but not the latent, form of EBV. The switch from the latent to the lytic form can be induced by expression of either one of two immediate-early gene products, BZLF1 and BRLF1. Both genes are able to induce the entire program of lytic EBV gene expression. High levels of nuclear factor (NF)-(B can inhibit EBV lytic replication, suggesting that NF-(B inhibitors might reactivate the viral lytic cycle. In this study, we tested the effects of NF-(B inhibitors on inducing EBV lytic infection. We found that NF-(B inhibitors, including acetylsalicylic acid, induced the expression of the lytic genes BZLF1, BRLF1 and BMRF1, in an EBV-positive GC cell line. Cells exhibited decreased viability in a dose- and time-dependent manner when incubated with NF-(B inhibitors. In contrast, there was no significant effect on EBV-negative GC cells. The combination of GCV and NF-(B inhibitors enhanced the cytotoxic effect of GCV after lytic induction by NF-(B inhibitors. In conclusion, the combination of NF-(B inhibitors with anti-viral nucleoside analogues might be a useful therapeutic strategy for EBV-associated human gastric cancer.

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Anti-cytomegalovirus Activity of Membranotropic Polyacidic Agents Effects In Vitro

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The human cytomegalovirus (CMV) is the infectious agent lethally danger at HIV/AIDS and other immunodeficiency states. The carboxy-mimickers of polymeric backbone of nucleic acids, potential agonists (antiviral responses stimulators) or antagonists for viral genomes, were developed as promising candidates to multifunctional antiviral protecting counter-agents (AVA). The AVA membrane-tropic derivatives as have been shown [Antiviral Res. 1999–2007] are able efficiently prevent infecting the cells by various HIV-1 strains. Here we present and discuss the new data in focus of AVA modification by the cage-hydrocarbon and/or sulfoacidic pharmacophores and followed evaluation on the CMV infection experimental models in vitro (fig/tab). Within the tested AVA the active modifications were detected as the efficient inhibitors of CMV with high selectivity indexes up to 250, 4286 and 7500 at the prophylactic, therapeutic, and viricidal experimental schemes

correspondently. Modulating influences of the lipotropic (cage-hydrocarbon) pharmacophores on the anti-CMV activity were observed only under the viricidal and prophylactic experimental schemes, where the lipid membranes of cells and/or viral envelope are involved. But the dominant role in the AVA antiviral activity was played by the sulf-anionic modulation. The negative charge accumulation on AVA macromolecules, seems, amplifies a potential for electrostatic blocking virions/virus-cell adsorption, and agonistic stimulated cell resistance or antagonistic competition of this synthetic polyacids with the viral nucleic acids. The most promising compounds have been selected for the future explorations of mechanisms of the anti-CMV and anti-HIV activity.

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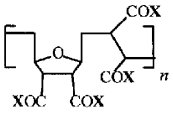

Where -X =
-OH/-O⁻ Na+, carboxyacid (CA), in part negative charged
-NH-Spacer$_1$-Adamantane (Ad), cage-tricyclic, mebranotropic
-NH-Spacer$_2$-exo-Norbornene (Nb), cage-bicyclic, mebranotropic
-O-Spacer$_3$-SO$_3^-$ Na+, sulfoacid (SA), full negative charged

AVA code	Various kind side groups (X), mol. ratio, CA : Ad : Nb : SA	Cytotoxicity [a], CC_{50}, μg/ml		Selectivity Index of Anti-CMV[b] activity SI = CC_{50}/EC_{50} (3 days)		
		1 day	3 days	Viricid.[c]	Prevent.[d]	Therap.[e]
ÀS. 470	1.00 : 0.00 : 0.00 : 0.00	3 750	3 500	< 10	< 10	< 10
ÀS. 473	0.94 : 0.06 : 0.00 : 0.00	3 200	2 500	< 10	< 10	< 10
ÀS. 632	0.93 : 0.07 : 0.00 : 0.00	3 600	2 400	41	< 10	< 10
ÀS. 504	0.92 : 0.00 : 0.08 : 0.00	3 200	1 700	< 10	< 10	< 10
ÀS. 677	0.86 : 0.00 : 0.08 : 0.06	-	1 440	66	22	< 10
ÀS. 678	0.79 : 0.00 : 0.08 : 0.13	1 800	1 420	355	189	< 10
ÀS. 679	0.67 : 0.00 : 0.08 : 0.25	1 000	500	**5 000**	91	< 10
ÀS. 688	0.60 : 0.00 : 0.00 : 0.40	4 000	3 000	**7 500**	**250**	**4 286**

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A Mass Spectrometry-based Method to Detect Antiviral Drug Resistance in Human Cytomegalovirus

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During antiviral therapy, the emergence of viral escape mutants that are resistant against the drug of choice is a major problem. Monitoring the introduction of resistance-associated mutations into the viral genome population can facilitate antiviral therapy management, including the selection of the most effective drug(s) in a given situation. We are currently assessing whether a mass spectrometry (MS)-based re-sequencing method (iSEQ by Sequenom, Inc., San Diego, USA) can improve the accuracy, speed, and sensitivity of the identification of resistance-associated mutations in the human cytomegalovirus genome (HCMV). Briefly, the assay employs four base-specific cleavage reactions of an amplicon of a relatively small region of the viral genome. The resulting four MS data sets are compared to *in silico* derived cleavage patterns from a database of reference sequences. Differences between the spectra derived from patient samples and those derived from viral reference sequences are indicators of sequence variations and can reveal potential resistance mutations. As resistance mutations against ganciclovir frequently occur in the phosphotransferase gene of HCMV (UL97), this gene was chosen to obtain proof of principle. A collection of patient samples was used to produce amplicons of 300–700 base pairs representing the 3′ half of the gene, which were analyzed using the Sequenom approach. The results were verified by traditional sequence analysis of the same samples. Our first data confirmed that detection of mutations or polymorphisms by SNP discovery is faster, but equally accurate compared to identification by regular sequencing. A more extensive comparison will be performed in the coming months.

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L-Analogs of 1-Beta-D-Ribofuranosyl-2-Bromo-5,6-Dichlorobenzimidazole (BDCRB) Inhibit Human Herpesvirus-6 Replication

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Benzimidazole nucleoside analogs have proven to be an abundant source of molecules with highly specific antiviral activity against human cytomegalovirus (HCMV). One analog, 1H-β-D-ribofuranosyl-2-bromo-5,6-dichlorobenzimidazole (BDCRB) inhibits the packaging of viral DNA, whereas a related L-ribosylnucleoside, 1H-β-l-ribofuranosyl-2-isopropylamino-5,6-dichlorobenzimidazole (maribavir) inhibits the HCMV UL97 protein kinase and is currently in Phase III clinical trials. Human herpesvirus-6 (HHV-6) is a related betaherpesvirus that is inhibited to a limited extent by maribavir but is insensitive to BDCRB. Therefore we hypothesized that other L-sugars in this series would be specific inhibitors of HHV-6. Of several compounds tested, two L-analogs of BDCRB (L-ribosyl BDCRB and (−)-carbocyclic BDCRB) have been identified that have good activity against the A variant of HHV-6 (EC_{50} = 2.6 and 2.4 μM, with selective indices of 11 and 5, respectively). Both compounds also inhibited HCMV in this concentration range (EC_{50} = 1.3–3.8 μM). These data differ with results for D-ribosyl analogs that were active against HCMV, but not HHV-6. Additional studies were conducted to examine their mechanism of action. Neither compounds inhibited viral DNA synthesis at high multiplicities of infection and no inhibition of HHV-6 U69 kinase activity was detected. Both results are consistent with a mechanism of action that is similar to that of BDCRB against HCMV and suggest that certain L-benzimidazole analogs have a mechanism of action similar to the D-benzimidazole analog BDCRB and differ from that of the L-analog maribavir. The results substantiate the prior observation that both the sugar moiety and the substituent in the 2-position of the heterocycle affect the mechanism of action and antiviral specificity of benzimidazole nucleosides.

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Comparative Efficacy of Treatment with CMX001 Versus Acyclovir in BALB/c Mice Infected with Herpes Simplex Virus

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Previous reports have shown excellent activity of CMX001 both in vitro and in vivo against vaccinia virus, cowpox virus, cytomegalovirus (CMV) and herpes simplex virus, Type 1 and 2 (HSV-1 and HSV-2). In cell culture, CMX001 has proven to be