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Synthesis, properties and anti-EBV activity of a new series of 3'-modified *cyclo*Sal-BVDUMP pronucleotides

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A new series of lipophilic 5-bromovinyl-2'-deoxyuridine monophosphate (BVDUMP) derivatives will be presented as potential prodrugs of the antiviral agent BVDU. To these compounds the 5'-*cyclo*Sal-masking group technique has been applied in order to achieve the delivery of the monophosphate of BVDU inside the target cells and to increase membrane penetration. Furthermore, in addition to the free 3'-hydroxyl compound the 3'-hydroxyl function has been masked as a 3'-O-methyl ether as well as different 3'-O-ester of long aliphatic carboxylic esters and α-aminoacids having natural and non-natural C-α-configuration. It could be proven that the monophosphate BVDUMP was delivered from most of the compounds by means of chemical hydrolysis. In addition to the synthetic approach towards these compounds different physicochemical properties of the new derivatives have been studied and will be presented, i.e. lipophilicity and hydrolysis behaviour. The new compounds have been tested for their anti-EBV activity by means of an EBV DNA hybridization assay using a digoxigenine-labeled probe specific for the Bam H1-W-fragment of the EBV genome and by measuring viral capsid antigen expression in P3HR-1-cells by indirect immunofluorescence. Some of the new derivatives demonstrated in vitro anti-EBV activity.

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Antiviral Activity of the Non-nucleoside Analogue CMV423 in Lymphoblast Cells Infected with Human Herpesvirus 6

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The new antiviral compound CMV423 [2-chloro-3-pyridin-3-yl-5,6,7,8-tetrahydroindolizine-1-carboxamide] was previously shown to possess potent and selective activity against human cytomegalovirus *in vitro* [Snoeck et al., 1999, Antiviral Res., 41, p. A35, No. 12]. We now report on the antiviral efficacy of CMV423 in human T-cell lines infected with human herpesvirus 6 (HHV-6) (Variant A and B). Virus replication was measured by microscopic evaluation of the cytopathic effect, and by quantitation of viral DNA using a DNA hybridization assay. In HSB-2 cells infected with HHV-6A strain GS [multiplicity of infection (MOI): 0.001], CMV423 displayed an EC_{50} (concentration causing 50% inhibition of viral replication) of 0.2 μM, and a CC_{50} (concentration causing 50% inhibition of cell growth) of 200 μM, resulting in a selectivity index (ratio of CC_{50} to EC_{50}) of 1000. In comparison, foscarnet and cidofovir had EC_{50} values of 15 μM and 5 μM and selectivity indices of 32 and 3, respectively. The anti-HHV-6 activity of CMV423 was found to be markedly dependent on MOI, virus strain and cell line, with no considerable activity in MOLT-3 cells infected with HHV-6B (strain Z-29). This points to the role of a cellular factor in the antiviral response to CMV423. Studies are in progress to determine the inhibitory effect of CMV423 on the transcription of immediately early, early and late HHV-6 genes.

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A Flow Cytometric Assay System for Determining Antiviral Drug Efficacy against Lymphotropic Herpesviruses.

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Human herpesvirus 6 (HHV-6), human herpesvirus 7 (HHV-7), human herpesvirus 8 (HHV-8) and Epstein-Barr virus (EBV) belong to a group of lymphotropic herpesviruses that are responsible for a wide range of diseases in immunocompromised patients, including lymphoproliferative disorders, tumors, pneumonitis and graft versus host disease. Immunofluorescence assays (IFA) have been used previously in our studies to determine the efficacy of antiviral compounds against these viruses. While IFA offers sensitivity and reproducibility it is very labor intensive. Both IFA and flow cytometry utilize monoclonal antibodies to identify the production of specific viral antigens in cells, however flow cytometry offers advantages over IFA as it provides a means of rapid evaluation of antiviral activity with reduced technical time. The results obtained using flow cytometric assays correlated closely with those from IFA for drug efficacy. Assays using either EBV or HHV-8, members of the gammaherpesvirus subfamily have shown that while EBV responds well to acyclovir, HHV-8 is more sensitive to cidofovir (CDV). HHV-6 and cytomegalovirus, members of the betaherpesvirus family, were similar in their sensitivity to foscarnet (PFA) and ganciclovir (GCV). Since HHV-6 strains are divided into two groups, A and B, based on restriction endonuclease band patterns, we also compared the drug efficacy for strains from each group. The group A strain, HHV-6_{GS}, was inhibited by PFA and GCV in both Sup-T1 and HSB-2 cells while CDV was more active in Sup-T1 cells. A representative group B virus, HHV-6_{Z-29} in cord blood lymphocytes, was inhibited by GCV but not by PFA. Overall, PFA was more effective against the group A variant while GCV was more effective against the group B variant. These results indicate that flow cytometry assays can be utilized to evaluate large numbers of new antiviral agents against these lymphotropic herpesviruses. It provides results similar to those obtained by IFA and other published methods and is considerably less labor intensive.

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Development of a Moderate Throughput Assay Using TaqMan PCR Technology to Identify Inhibitors of Human Herpesvirus 8 (HHV-8).

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Human Herpesvirus 8 (HHV-8) is the etiologic agent of Kaposi's Sarcoma, a common malignancy in patients infected with HIV. We have developed a microtiter-based, moderate throughput assay to identify inhibitors of HHV-8 *in vitro*. This assay utilizes latently infected BCBL-1 cells treated with the phorbol ester TPA to induce virus expression. Real time detection is performed using TaqMan PCR technology, in which a fluorogenic probe hybridizes to the target of interest and results in the generation of a fluorescence signal proportional to the amount of product present. We have been able to screen up to 300 compounds per day for the identification of new active leads utilizing this assay system. IC50 values for known herpesvirus inhibitors were determined as follows: acyclovir, 27.9 uM; cidofovir, 0.45 uM; ganciclovir, 0.83 uM; and foscarnet, 61.8 uM. In addition, various classes of HIV inhibitors, including nucleoside and nonnucleoside reverse transcriptase inhibitors and protease inhibitors have been tested in this system. Finally, an internal drug discovery compound library consisting of a series of nucleoside analogs was evaluated for anti-HHV-8 activity in this assay system. Results obtained with these compounds will also be presented. A comparison of the effective concentrations of known herpesvirus inhibitors determined in our assay and those determined in previously published reports validate the use of this assay as a model system for identifying inhibitors of HHV-8.