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Synthesis of Novel CADA Analog Prodrugs Designed as Down-Modulators of the CD4 Receptor

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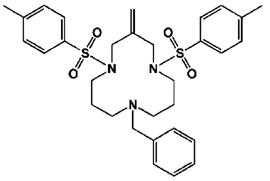
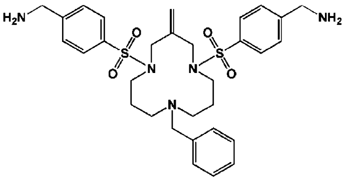
Cyclotriazadisulfonamide (CADA) inhibits HIV replication by specifically down-modulating expression of the of the CD4 receptor protein on host cells. Many analogs of CADA have been synthesized in order to enhance potency, reduce toxicity, and improve physical properties, especially solubility and cell permeability (Bell et al., 2006, J. Med. Chem., 49, 1291). These analogs have also been used to develop a three-dimensional quantitative structure-activity relationship (3D-QSAR) computer model. Current studies are aimed at developing a pro-drug approach involving novel CADA analog ES02. This compound is expected to have a CD4 down-modulation potency that is similar to that of CADA, according to our 3D-QSAR model. ES02 is the parent compound for prodrugs bearing dipeptide chains that are covalently bonded to the two amino groups of the aminomethylbenzenesulfonyl side arms. Cleavage of these chains by dipeptidyl-peptidase IV (Garcia-Aparicio et al., 2006, J. Med. Chem., 49, 5339) is expected to convert the prodrugs into ES02. The synthesis of ES02 uses a new palladium-catalyzed macrocyclization method. Two synthetic routes have been explored, one involving the bis(bromobenzenesulfonyl) analog of CADA, and a new route using the corresponding bis(cyanobenzenesulfonyl) analog as an intermediate. The anti-HIV and CD4 down modulation activities of the novel CADA compounds will be presented.



CADA



ES02

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Rational Drug Design—Screening and Synthesis of Potential Deoxyhypusine Synthase Inhibitors Targeting HIV-1 Replication

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The introduction of HAART was a key improvement in combating HIV that prolonged patients expectancy of life. However, in order to reduce side-effects and upcoming multidrug resistance in long-term HAART it is mandatory to address new targets and to identify new potential drugs.

Within the HIV replication cycle various host cell-factors play an important role, e.g. the eukaryotic initiation factor 5A (eIF-5A). Activation of eIF-5A involves a unique post-translational modification of a specific lysine residue to the unusual amino acid hypusine. This modification is catalyzed by subsequent action of human deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH). Recently, it was shown that DHS inhibition efficiently prohibits the activation of eIF-5A leading to suppression of HIV replication (Hauber et al., 2005).

Based on X-ray crystal data of DHS and known inhibitors such as GC7 and CNI-1493, structure-based drug design approaches were applied in order to discover novel DHS inhibitors. Using the molecular docking software FlexX in combination with the HYDE scoring function (Reulecke et al., 2008), several suggestions for potential DHS inhibitors were proposed.

Here, we present the synthesis and the biological evaluation of several selected compounds from this set of potential inhibitors. These compounds were tested for inhibition of DHS in an enzymatic assay, inhibition of HIV-1 replication *in vitro* and for potential cytotoxic effects.

References

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Lipophilic Nucleoside Diphosphate Prodrugs—Synthesis and Properties

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Nucleoside analogs are commonly used as antiviral and antitumor agents. The antiviral effect of the majority of nucleoside analogs such as 3′-deoxy-3′-azidothymidine (AZT) or 2′,3′-dideoxy-2′,3′-didehydrothymidine (d4T) depends on their conversion into the ultimately bioactive nucleoside triphosphate (NTP). However, cellular kinases often catalyze the biotransformation via the mono- and diphosphate insufficiently with the result of a loss in antiviral activity.

AZT is very slowly phosphorylated by thymidylate kinase to AZTDP which leads to severe side-effects during AZT treatment. Considering this, it is remarkable that there were only a few attempts made to synthesize lipophilic nucleoside diphosphate prodrugs (NDP prodrugs) in the past. Recently, we reported on the first successful bioreversible protection of nucleoside diphosphates as bis-(acyloxybenzyl) phosphate diesters (Jessen et al., 2008). The pyrophosphate protecting groups is cleaved by esterases/lipases inside cells resulting in the formation of the NDP.

To investigate the structure–activity relationship a series of more lipophilic bis-(4-acyloxy-benzyl)-d4T diphosphates (BAB-d4TDPs) was synthesized with yields up to 65%. We used longer alkyl chains as acyl moieties. The aim of our studies was to identify a correlation between the antiviral activity of the compounds and its lipophilicity.

Very interesting antiviral data found in CEM/TK$^-$ cells will be shown. These data point to a dependence between the alkyl chain length and the antiviral activity. Additionally, we will present the synthesis and the biological properties (pH- and cell extract stability, cytotoxicity) to give further insights into the behaviour of this novel series of BAB-d4TDPs.

Reference

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Synthesis, Antiviral and Cytotoxicactivities of 2-Phenyl, 3-Substituted Quinazolin-4(3H)-Ones

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Quinazolin-4-(3H)-one is a versatile lead molecule for the design of potential bioactive agents and its derivatives were reported to possess broad spectrum activities. 2-Phenyl-3-substituted quinazolin-4-(3H)-ones were reported to have anti-HIV activity and anti-cancer activity. Quinazolinones were screened for their wide spectrum anti-viral activity and they yield potential for further studies. A series of novel 2,3-disubstitutedquinazolin-4(3H)-ones have been synthesized by condensation of 2-substituted benzo[1,3]oxazine-4-ones and anthranilic acid. Their chemical structures were assigned by means of spectral analysis (FT-IR, ^{1}H NMR, MS). Synthesized compounds were evaluated for *in vitro* antiviral activity against HIV, HSV, vaccinia virus and other viruses. The compounds displayed cytotoxicity in MT-4 cells and were inactive against HIV-1 and -2 replication at non-cytotoxic concentrations. 2-(o-Phenyl carboxylic acid)-5,7-bromo-3-phenyl quinazolin-4(3H)-one and 2-(4-dibromo-2-phenyl carboxylic acid)-3-phenyl quinazolin-4(3H)-one showed activity against HSV and vaccinia virus. 2-(o-Phenyl carboxylic acid)-5,7-bromo-3-phenyl quinazolin-4(3H)-one inhibited the replication of HSV-1, -2 and vaccinia virus at an IC_{50} of 12 μg/ml. It was cytotoxic at 100 μg/ml. These compounds may be suitable as leads for designing newer derivatives and further molecular modification in this series may help optimizing antiviral activity.

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Synthesis of a Series of 2′-Modified Tricyclic Nucleosides as Potential HCV Agents

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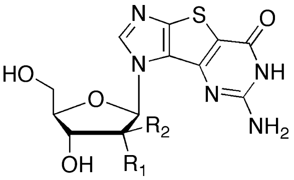
The hepatitis C virus (HCV) is a blood borne virus currently infecting 4 million people in the United States and over 170 million people worldwide. HCV is one of the leading causes of long term liver cirrhosis, which can result in liver failure and death. Compounding the impact of this disease is the rapid progression of liver degradation in patients that are co-infected with human immunodeficiency virus (HIV). Current therapies have exhibited low response rates and significant toxic side effects, thus there is an urgent need to develop more effective treatments.

HCV requires an RNA-dependent RNA-polymerase (RdRp) to replicate, more specifically the NS5B protein. NS5B has been shown to be essential in the HCV replication complex, thus is considered an ideal target. Multiple studies have shown that potent inhibitory activity against NS5B has resulted from structural modifications to the 2′-position including 2′-OMe, 2′-Me and 2′-F substitutions. Moreover, an analogue of 2′-C-methylguanosine has recently progressed to Phase I clinical trials. Related to this, a hetero-expanded purine tricyclic guanosine nucleoside synthesized in our laboratories exhibited moderate activity against HCV. Thus, combining these leads has focused our efforts on the development of 2′-modified analogues of the expanded tricyclic guanosine. The synthesis and preliminary studies are described herein.



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Acute Toxicity of Oral Octadecyloxyethyl Esters of 3-Hydroxy-2-(Phosphonomethoxy) Propyl Nucleosides in Balb/c Mice

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Octadecyloxyethyl esters of 3-hydroxy-2-(phosphonomethoxy) propyl adenine, guanine and diaminopurine (ODE-HPMPA, ODE-HPMPG, ODE-HPMP-DAP) have been synthesized and their antiviral activity evaluated against HCMV, vaccinia, and cowpox (Valiaeva, et al., 2009. Antiviral Res. 84, 254–259). These compounds have EC50 values ranging from 3 to 77 nM against these viruses in vitro. ODE-cidofovir (ODE-CDV) has previously been reported to have good oral bioavailability and in vivo antiviral activity in lethal poxvirus models of infection but shows significant oral toxicity at 30 mg/kg and above. To assess the acute toxicity of the new compounds, we gave 3, 10 and 30 mg/kg to mice daily for 7 days by oral gavage. Female Balb/c mice weighing approximately 18 g were dosed orally at the indicated doses for 7 days. Mice were observed and body weights were taken daily during dosing. After dosing was stopped, observations and body weight measurements were continued three times a week for one week. A control group received only the vehicle (0.9% saline). ODE-HPMPA at 30 mg/kg showed acute oral toxicity. Although there was no mortality in this group, dosing was stopped on day 4 because of a 20% loss of body weight from baseline. However, there were no signs of acute toxicity or weight loss in the 3 and 10 mg/kg groups. ODE-HPMPG at 30 mg/kg produced marked weight loss in all mice. Dosing was stopped in two mice at day 5 because of greater than 20% weight loss. Nevertheless 75% of the animals in this group died. Mice in the 10 mg/kg group showed moderate weight loss but tolerated dosing for the full 7 days. No mortality occurred in this group. In the 3 mg/kg group, no ill effects were noted. Mice treated orally with ODE-HPMP-DAP showed no signs of acute toxicity at all doses