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

Jessen, H.J., Schulz, T., Balzarini, J., Meier, C., 2008. Bioreversible protection of nucleoside diphosphates. Angew. Chem. Int. Ed. 47, 8719–8722.

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

Periyasamy Selvam[1,*], Narayanan Murugesh[2], Markandavel Chandramohan[3], Christophe Panpannecouque[4], Erick De Clercq[4]

[1] *Devaki Amma Memorial College of Pharmacy, Malapuram, India;* [2] *Institute of Pharmacology, Madurai Medical College, Madurai, India;* [3] *Bharat Ratna Kamarajar Liver Hospital and Research Centre, Madurai, India;* [4] *Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium*

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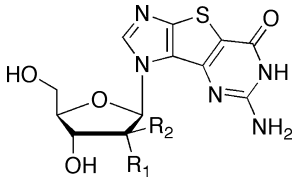
Synthesis of a Series of 2′-Modified Tricyclic Nucleosides as Potential HCV Agents

Matthew Tomney[1,*], Brent Korba[2], Sarah Zimmermann[1], Katherine Seley-Radtke[1]

[1] *University of Maryland Baltimore County, Baltimore, USA;* [2] *Georgetown University Medical Center, Washington, USA*

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

Julissa Trahan[1,*], Nadejda Valiaeva[1,2], James Beadle[1,2], Karl Hostetler[1,2]

[1] *Veterans Medical Research Foundation, San Deigo, USA;* [2] *University of California San Diego Department of Medicine, La Jolla, USA*

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

and weight was stable throughout the 7 day dosing period and the 7 day drug washout even in the 30 mg/kg group. Of the three new compounds tested, ODE-HPMPDAP appears to have no acute oral toxicity and should be evaluated further in view of its 20 nM EC50 against vaccinia virus in vitro.

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Cidofovir: Intratympanic Delivery and Hearing Loss

Jonette Ward [1,*], Alisa Reece [1], Kevin Li [2], Daniel Choo [1]

[1] *Center for Hearing and Deafness Research, CCHMC, Cincinnati, USA;* [2] *College of Pharmacy, UC, Cincinnati, USA*

Congenital Cytomegalovirus (CMV) is the leading cause of infectious-related sensioneural hearing loss (SNHL) worldwide. Approximately 90% of newborns infected with CMV are asymptomatic at birth, of these 20% exhibit SNHL. Clinicians are developing novel ways to treat SNHL caused by CMV infection. Our lab is exploring the intratympanic route (IT) for delivery of established antivirals to treat CMV related SNHL with promising preliminary results. IT injections provide an advantage over systemic delivery because IT delivery shields the patient from serious side effects. Moreover, the similarities in the anatomy and physiology of the guinea pig (GP) and human ear allows this to be a relevant model to study. Accordingly, viral kinetics studies and auditory brainstem responses (ABR) have shown that direct inoculation of guinea pig CMV (GPCMV) into the bulla of a GP is a consistent and reliable model for CMV infection. Studies are ongoing for IT injections of cidofovir (CDV) for the treatment of GPCMV related hearing loss. Administering CDV at different time points post viral inoculation is proving significant. The viral kinetics show replication begins at day 6 post-surgical inoculation. IT injection of CDV administered at day 7 shows the most impact in hearing improvement. ABR, *realtime* PCR, and histological data confirms that CDV given IT inhibits viral replication and improves hearing without manifesting any side effects. This data demonstrates that CDV given IT prevents the virus from replicating and shows an improvement in hearing loss by day 21. The lab is also exploring a unique application of drug-delivery, a temperature sensitive copolymer used as a transporter of antivirals. *In vitro* data shows that CDV in a temperature sensitive copolymer extends the effective life of the drug. To document the location and migration of these gels *in vivo*, MRI images of guinea pigs ears were taken at different time points post-IT injection of copolymer gels. The MRI data has shown that the gel can be injected IT and is located at the round window. Ongoing *in vivo* studies that include hearing and kinetic data will determine if the temperature sensitive copolymer gel can be used as a transporter for time controlled drug release into the inner ear.

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Toward A More Complete Anti-Herpesvirus SAR for 2nd Generation Methylenecyclopropane Nucleosides

John D. Williams [1,*], Atiyya R. Khan [1], Mark N. Prichard [2], Sam L. Frederick [2], Geraldine M. Jefferson [2], Jiri Zemlicka [3], Norton P. Peet [1], Terry L. Bowlin [1]

[1] *Microbiotix, Inc., Worcester, USA;* [2] *University of Alabama at Birmingham, Birmingham, USA;* [3] *Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, USA*

The discovery of methylenecyclopropane nucleosides (MCPNs) has lead to a large number of promising analogs with potent antiherpesvirus activity. One factor potentially limiting the further development of the MCPNs, however, is a stereocenter included in the first generation compounds (1). In an attempt to synthesize compounds without the problematic stereocenter, Zemlicka developed a second generation of methylenecyclopropane nucleosides that included an additional hydroxymethyl substituent (2). Unlike the thoroughly explored first generation MCPNs, only a few representatives of the second generation MCPNs were synthesized, including the guanine analog ZSM-I-62 (cyclopropavir, CPV), which is now in preclinical development for HCMV. The potent antiherpes activity of CPV and the other second generation MCPNs that were synthesized prompted us to further explore the structure, and elaborate the SAR for this series. Herein, we report the results of our investigations toward expanding the structure–activity relationship within the second generation of MCPNs. Several of the new analogs demonstrated low micromolar activity against HCMV and EBV, and some of the compounds also have moderate activity against HHV-8. We will discuss the relationships between amine, ether, and thioether analogs and the anti-herpes activities thereof.

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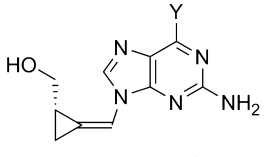
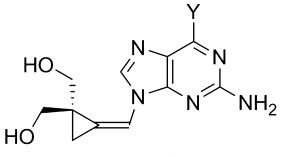


Y = NHR, OR, SR

1, first generation MCPN



Y = NHR, OR, SR

2, second generation MCPN

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Stereoselective Synthetic Strategy to Potentially Antiviral Active Carbocyclic L-Nucleosides and L-Nucleotides

Claudia Worthmann [1,*], Sönke Jessel [1], Jan Balzarini [2], Chris Meier [1]

[1] *Organic Chemistry, Department of Chemistry, Faculty of Sciences, University of Hamburg, Hamburg, Germany;* [2] *Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium*

Since L-nucleosides like 3TC **1** and L-FMAU **2** show potent activity against HBV-replication it would be of interest if this also applies to their carbocyclic analogues. In addition to a higher stability towards phosphorylases the carbocyclic moiety prefers a specific conformation depending on the substituents on the cyclopentane system. This leads to a different structure–activity–relationship (SAR) for carbocyclic compounds which may effect their biological properties in comparison with natural nucleosides.