methylenecyclopropane guanosine nucleoside analog, is active *in vitro* against HCMV and MCMV with EC_{50}'s of 0.27–0.49 μM (*J. Med. Chem.* 47: 566, 2004). *In vivo* it produces a 2–5 $\log_{10}$ reduction in virus titers in SCID mice (*AAC* 48: 4745, 2004). Recent studies in rats and dogs demonstrated good oral bioavailability with minimal toxic side effects at therapeutic concentrations thereby establishing a good therapeutic index for CPV (*Antiviral Res*. 82: A46, 2009). Other studies determined that the mechanism of action of CPV involves inhibition of viral DNA synthesis (*AAC* 49: 1039, 2005). We previously discovered that resistance of HCMV to CPV maps to a mutation in the *UL97* gene resulting in a truncated pUL97 devoid of both the ATP binding region and kinase activity domain (*Antiviral Res*. 78: A54, 2008). Taken together, we hypothesize that CPV must be phosphorylated to a triphosphate to inhibit HCMV DNA synthesis and viral replication. We now have examined if pUL97, a viral phosphotransferase, is the protein responsible for the initial phosphorylation of CPV to a monophosphate (CPVMP). Kinetic studies with CPV as the substrate for pUL97 established that CPVMP forms in a time-dependent manner and has an observed K_M of 1750 ± 210 μM. Ten μM maribavir, a pUL97 inhibitor, resulted in a complete loss of CPVMP formation. Lineweaver–Burk analysis demonstrated a K_i of 3.0 ± 0.3 nM. We previously determined that guanylate kinase (GMPK) preferentially phosphorylates the (+)-isomer of CPVMP to its triphosphate compared to the (−)-isomer (*Antiviral Res*. 82: A69, 2009). Incubation of CPV with both pUL97 and GMPK gave a phosphorylation profile similar to that of (+)-CPVMP incubated with GMPK alone leading us to conclude that pUL97 stereoselectively phosphorylates CPV to its (+)-enantiomer. We hypothesize that this results in the active form of the drug (+)-CPVTP that inhibits HCMV DNA polymerase.

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Screening and Rational Design of Low Molecular Weight HIV Fusion Inhibitors

Miriam Gochin[1,4,*], Guangyan Zhou[1], Dong Wu[1], Landon Whitby[2], Dale Boger[2], Thomas Chung[3]

[1] *Touro University, Vallejo, USA;* [2] *Scripps Research Institute, San Diego, USA;* [3] *Burnham Institute, San Diego, USA;* [4] *University of California, San Francisco, USA*

Screening and rational design of low molecular weight HIV fusion inhibitors Miriam Gochin Touro University – California; UCSF Department of Pharmaceutical Chemistry The peptide HIV-1 fusion inhibitor T-20 has been successful as a salvage therapy for patients who do not respond to other medications or who harbor drug-resistant strains of the virus. Lack of oral bioavailability of T-20 coupled with irritation at the injection site and high cost has limited its use. Small molecule inhibition of HIV fusion has been highly sought after for the past 10 years, but so far no potent low molecular weight inhibitors have been found. We will describe a high throughput screening project dedicated to discovery and development of low molecular weight HIV-1 fusion inhibitors. We have coupled HTS assays with structural, binding and biological assays, for detection and validation of hits. We will demonstrate applications to both random and focused libraries including: (1) the 300,000 compound NIH Small Molecule Repository, with the discovery of novel scaffolds for fusion inhibition; (2) rational design of an in-house set of fusion-inhibitory compounds; (3) investigation of a comprehensive peptidomimetic library to identify the side-chain composition required for activity. Our results show a strong correlation between affinity of the molecules for the gp41 hydrophobic pocket and activity in a cell–cell fusion assay, and provide extensive SAR data for compound optimization.

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A New Tacaribe Arenavirus Infection Model to Explore the Antiviral Activity of a Novel Aristeromycin Analog

Brian Gowen[1,*], Kie-Hoon Jung[1], Deanna Larson[1], Wei Ye[2], John Morrey[1], Donald Smee[1], Stewart Schneller[2]

[1] *Institute for Antiviral Research and Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, USA;* [2] *Chemistry & Biochemistry Department, Auburn University, Auburn, USA*

A growing number of arenaviruses can cause a devastating viral hemorrhagic fever (VHF) syndrome. They pose a public health threat as emerging infectious disease agents and because of their potential use as bioterror agents. Ribavirin, the only licensed antiviral that has been used to treat severe arenaviral infections lacks specificity and has had mixed success. All of the highly pathogenic New World arenaviruses (NWA) phylogenetically segregate into clade B and require maximum BSL-4 containment facilities for their study. Tacaribe virus (TCRV) is a nonpathogenic member of clade B that is closely related to the NWA VHF group at the amino acid level. Despite this relatedness, TCRV lacks the ability to antagonize the host interferon response, which likely contributes to its inability to cause disease in animals other than newborn mice. Due to the challenges of working with newborn mice, we have developed a new mouse model based on TCRV challenge of AG129 γ-, β-, and α-interferon (IFN) receptor deficient mice. Titration of the virus by intraperitoneal (i.p.) challenge of AG129 mice resulted in an LD_{50} of ~100 $CCID_{50}$ (50% cell culture infectious doses). Virus was readily detected in the spleen, lung, serum, liver, and brain 4–8 days after inoculation. MY-24, an aristeromycin derivative active against TCRV in cell culture at 0.9 μM, administered i.p. once daily for 7 days, offered highly significant ($p < 0.001$) protection against mortality in the AG129 TCRV infection model. It did not, however, appear to appreciably reduce tissue or serum viral titers, but a more comprehensive analysis is currently underway. The present data suggest that MY-24 may ameliorate disease by blunting the effects of the host response that play a role in disease pathogenesis. The new AG129 mouse TCRV infection model provides a means to evaluate compounds that do not require complete host IFN pathways to impart their antiviral activity in a BSL-2 setting.

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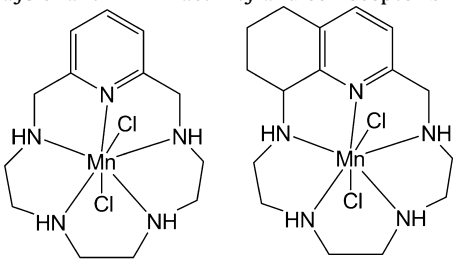
Metal Complexes of Macrocyclic Polyamines Targeting the Cellular HIV Co-receptors, CXCR4 and CCR5

Sunil Hamal[1,*], Dana Huskens[2], Dominique Schols[2], Thomas Bell[1]

[1] *Department of Chemistry, University of Nevada, Reno, USA;* [2] *Rega Institute for Medical Research, Department of Microbiology and Immunology, Katholieke Universiteit Leuven, Leuven, Belgium*

Some macrocyclic polyamines and their metal complexes possess anti-HIV activity. For example, 9-benzyl-3-methylene-

1,5-bis(*p*-toluenesulfonyl)-1,5,9-triazacyclododecane (CADA, or cyclotriazadisulfonamide) specifically down-modulates CD4, the principal cellular receptor for HIV. Bicyclams (e.g. AMD3100) and their metal complexes inhibit HIV by a different mechanism, specifically interacting with the cellular co-receptor, CXCR4. SH04 and SH06, the manganese complexes of 3,6,9,12,18-pentaazabicyclo[12,3,1]octadeca-1(18),14,16-triene and 3,4,5,6,7,8,9,10,11,-12,13,13a,14,15,16-pentdecahydro-2,17-etheno-1,4,7,10,13-benzopentaaza-cyclopentadecine, respectively, inhibit HIV replication and also interact with both of the cellular HIV co-receptors, CXCR4 and CCR5. Synthesis of several analogues of these compounds is in progress to decipher in more detail the mechanisms of chemokine receptor interaction and antiviral activity. For example, analogs of SH04 containing copper or zinc, instead of manganese, have been synthesized and tested for activity against HIV and for chemokine receptor interaction. Also, SH06 was previously tested as a racemic mixture, so efforts are also in progress to prepare the pure enantiomers for further assays of anti-HIV-1 activity and co-receptor binding.



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Changes in Human Cytomegalovirus Transcriptional Patterns Induced by Antiviral Drugs

Caroll Hartline[1,*], Dirk Dittmer[2], John Drach[3], Frances Chen[1], R. Curtis Hendrickson[1], Elliot Lefkowitz[1], Mark Prichard[1]

[1] *The University of Alabama School of Medicine, Birmingham, USA;* [2] *University of North Carolina at Chapel Hill, Chapel Hill, USA;* [3] *University of Michigan, Ann Arbor, USA*

The expression of herpesvirus genes is temporally regulated and the three kinetic classes of transcripts have been defined in part by transcriptional responses to antiviral drugs, such as phosphonoacetic acid. Since many compounds have been identified that inhibit the replication of human cytomegalovirus (HCMV) by novel mechanisms, we sought to characterize changes in transcriptional patterns that occurred in response to selected antiviral drugs. Compounds evaluated included ganciclovir and cidofovir (inhibitors of DNA polymerase), maribavir (inhibitor of the UL97 kinase), and 1H-β-D-ribofuranosyl-2-bromo-5,6-dichlorobenzimidazole (BDCRB), which inhibits the cleavage/packaging of the viral genome. A quantitative real time RT PCR array was developed that provided a global evaluation of viral transcripts levels. This approach measured levels of 136 viral transcripts at 72 h following infection. Data were normalized to cellular transcripts and ANOVA was used to identify changes that were significant relative to an untreated virus control. This analysis revealed that: (1) distinct kinetic classes of transcripts were less discernable in HCMV than in other herpesviruses, (2) inhibitors of DNA synthesis reduced many viral transcripts and was not restricted to early/late, or late messages, (3) maribavir reduced a subset of transcripts that were also reduced by the DNA synthesis inhibitors, and (4) BDCRB reduced levels of very few viral messages. These data will help to define the complex transcriptional regulation of this virus and will be a useful tool for characterizing the mechanism of action of new compounds that inhibit the virus.

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Antiviral Activity of Acyclic Nucleoside Phosphonates with Branched 2-(2-Phosphonoethoxy)ethyl Chain

Dana Hocková[1,*], Antonín Holý[1], Graciela Andrei[2], Robert Snoeck[2], Jan Balzarini[2]

[1] *Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic;* [2] *Rega Institute for Medical Research, KU Leuven, Leuven, Belgium*

Several drugs that are in current clinical use for the treatment of viral infections belong to the acyclic nucleoside phosphonates (ANPs) (De Clercq and Holý, 2005). Some of the recently prepared ANPs containing the 2-(2-phosphonoethoxy)ethyl function (PEE) were studied as antimalarial compounds (Hocková et al., 2009). When guanine or hypoxanthine is present as the nucleobase in their molecule, they inhibit Plasmodium falciparum hypoxanthine-guanine-xanthine phosphoribosyltransferase and can selectively discriminate between the human and the parasite enzyme (Hocková et al., 2009; Keough et al., 2009). Unexpectedly, some of these ANPs with the PEE-chain branched in the b-position to the phosphonate group exhibit antiviral activity against VZV, CMV, HIV, HSV-1 and HSV-2, although the parent unbranched PEE-ANPs are inactive. The influence of the heterocyclic base and substituent R will be discussed. The cytotoxic and cytostatic activities of the compounds were also evaluated.

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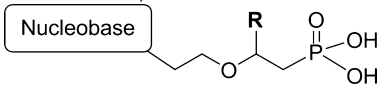

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