

methylenecyclopropane guanosine nucleoside analog, is active *in vitro* against HCMV and MCMV with EC<sub>50</sub>'s of 0.27–0.49  $\mu$ M (*J. Med. Chem.* 47: 566, 2004). *In vivo* it produces a 2–5 log<sub>10</sub> 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  $1750 \pm 210 \mu$ M. Ten  $\mu$ M maribavir, a pUL97 inhibitor, resulted in a complete loss of CPVMP formation. Lineweaver–Burk analysis demonstrated a  $K_i$  of  $3.0 \pm 0.3$  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

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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

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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  $\gamma$ -,  $\beta$ -, and  $\alpha$ -interferon (IFN) receptor deficient mice. Titration of the virus by intraperitoneal (i.p.) challenge of AG129 mice resulted in an LD<sub>50</sub> of  $\sim 100$  CCID<sub>50</sub> (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  $\mu$ M, 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

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