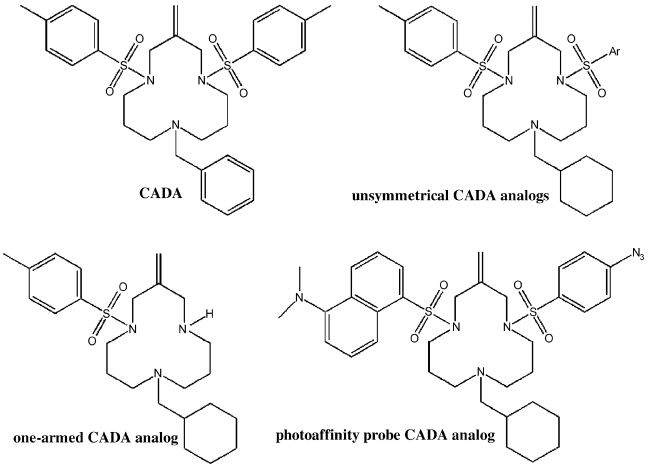
in preparing unsymmetrical analogs with two different arenesulfonyl side-arms is based on initial molecular modeling studies and on the potencies of the two unsymmetrical CADA analogs KKD015 and KKD016 (IC_{50} values for CD4 down-modulation are 1.72 ± 0.25 μM and 0.97 ± 0.13 μM, respectively). These results suggested that decreased symmetry may likely lead to sustained activity of the compounds. Using a new synthetic route, seven new unsymmetrical CADA analogs have been successfully prepared. All of these compounds exhibited CD4 down-modulating activity in the lower micromolar range. In fact, one of the new analogs showed a ca. 50-Fold increase in potency relative to CADA (IC_{50} values for CD4 down-modulation are 0.012 ± 0.010 μM for the new analog and 0.65 ± 0.21 μM for CADA). Thus, qualitative structure-activity relationships observed for these compounds suggest additional structures for unsymmetrical analogs that may be explored. New CADA compounds that are currently being prepared include a one-armed analog, and a photoaffinity probe bearing one side-arm consisting of a dansyl fluorophore and a second side-arm consisting of a photoactive aryl azide unit.



doi:10.1016/j.antiviral.2010.02.432

123

The Discovery and Efficacy of a Small Molecule Inhibitor of Ebola Capsid Assembly in an Animal Model

Jean Francis[1,*], Warren Kalina[3], Travis Warren[3], Katie Edwards[2], I-ting Jaing[1], A. Pia Abola[1], Abhar Nissar[1], Anatoliy Kitaygorodskyy[1], Clarence R. Hurt[1], Sina Bavari[3], William Hansen[1], Vishwanath R. Lingappa[1]

[1] *Prosetta Bioconformatics, San Francisco, USA;* [2] *CUBRC, Inc, Buffalo, USA;* [3] *Integrated Toxicology Division, USAMRIID, Ft. Detrick, USA*

We have taken a novel approach for identifying viral capsid assembly inhibitors by targeting cellular host proteins rather than viral gene products. A cell free protein synthesis (CFPS)-based system was used to express and assemble the Ebola nucleocapsid (NP), VP35 and VP24 proteins. The system was adapted to an assay platform to screen this assembly pathway by an ELISA and the hits were identified from a small molecule library. When synthesized in the presence of the active compounds, the assembled structures show differential protease sensitivity compared to control, consistent with altered assembly architecture. Hits were validated by plaque reduction assay against live virus in cell culture. The impressive therapeutic profile of one of the early hits, justified moving the compound forward into preliminary *in vivo* efficacy studies. This compound provided complete protection of mice challenged with 1000 LD_{50} Ebola virus at a compound dose of 5 mg/kg (IP) daily for 5 days. Preliminary optimization of the potency and safety profiles for this pre-lead series resulted in a promising structure activity relationship (SAR) demonstrating very impressive improvements over the initial hits in this series. Together, the *in vitro* and *in vivo* experiments have demonstrated the potential of this approach for discovering anti-Ebola therapeutics.

doi:10.1016/j.antiviral.2010.02.433

124

Structure–Activity Relationships of D- and L-analogs of Maribavir and 1-Beta-D-ribofuranosyl-2-bromo-5,6-dichlorobenzimidazole (bdcrb) Against Human Herpesvirus 6

Samuel Frederick[1,*], Shannon Daily[1], Kathy Borysko[2], Leroy Townsend[2], John Drach[2], Mark Prichard[1]

[1] *The University of Alabama at Birmingham School of Medicine, Birmingham, USA;* [2] *School of Dentistry and College of Pharmacy, University of Michigan, Ann Arbor, USA*

Human cytomegalovirus (HCMV) has been shown to be susceptible to a host of benzimidazole nucleoside analogs including, 1H-β-D-ribofuranosyl-2-bromo-5,6-dichlorobenzimidazole (BDCRB) and 1H-β-L-ribofuranosyl-2-isopropylamino-5,6-dichlorobenzimidazole (maribavir, MBV). Neither of these analogs exhibits good antiviral activity against either variant of human herpesvirus 6 (HHV-6A, HHV-6B), notwithstanding the relative conservation of their molecular targets. We evaluated nine analogs of both MBV and five analogs BDCRB against both variants of HHV-6. Neither the L- nor D-analogs of MBV exhibited detectable antiviral activity against these viruses. However, two L-analogs of BDCRB (L-ribosyl BDCRB and (−)-carbocyclic BDCRB) were identified that had good antiviral activity against HHV-6A (EC_{50} = 2.8 and 5.5 μM, respectively). Both molecules also exhibited more modest inhibition against HHV-6B (EC_{50} = 9.7 and 15 μM, respectively). Both molecules retained antiviral activity against HCMV (EC_{50} = 1.3–3.8 μM). This contrasts with results for D-ribosyl analogs of BDCRB, which were active against HCMV, but not either variant of HHV-6. These data taken together suggest that the substituent in the 2-position of the heterocycle, as well as the configuration of the ribose were essential for antiviral activity. The compounds that were active against HHV-6 did not appear to inhibit viral DNA synthesis, and failed to inhibit the enzymatic activity of the U69 protein kinase, suggesting that their mechanism of action was similar to that of BDCRB. Additional studies will be required to determine the effect of the analogs on the cleavage/packaging of the HHV-6 genome.

Acknowledgements: Supported by contract N01-AI-30049 from the NIAID, NIH and a grant from Burroughs Wellcome Co.

doi:10.1016/j.antiviral.2010.02.434

125

Stereospecific Phosphorylation of Cyclopropavir by pUL97 and Inhibition by Maribavir

Brian G. Gentry[1,*], Jeremy P. Kamil[2], Donald M. Coen[2], Jiri Zemlicka[3], John C. Drach[1]

[1] *University of Michigan, Ann Arbor, USA;* [2] *Harvard Medical School, Boston, USA;* [3] *Wayne State University, Detroit, USA*

Human cytomegalovirus (HCMV) is a widespread pathogen that can cause severe disease in immunologically immature and immunocompromised individuals. Cyclopropavir (CPV), a

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.

Acknowledgements: Supported by grants from NIH (CA32779, AI26077) and Microbiotix, Inc., plus funds from the University of Michigan.

doi:10.1016/j.antiviral.2010.02.435

126

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.

doi:10.1016/j.antiviral.2010.02.436

127

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.

Acknowledgements: Supported by N01-AI-30048, N01-AI-30063 (awarded to Southern Research Institute), and U19-AI-56540, Virology Branch, NIAID, NIH.

doi:10.1016/j.antiviral.2010.02.437

128

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-