polymerase, we analyzed by nuclear magnetic resonance spectroscopy the structure and dynamics of a DNA duplex containing cidofovir and an isosequential control DNA. The oligonucleotide was synthesized from the 5′-to-3′ end by adapting the solid-phase procedure described by Birkus et al. for (S)-HPMPA oligonucleotides [Birkus et al., 2004, Antivir. Chem. Chemother. 15, 23–33]. Reversed 5′ phosphoramidites were introduced into the oligonucleotide chain using phosphoramidite chemistry. The appropriately protected CDV monomer was prepared by a multistep synthesis starting from N^4-benzoylcytosine and (S)-glycidyl trityl ether and was added to the oligo using the phosphotriester condensation method. From the NMR it is clear that the cidofovir containing DNA is distorted in structure. No imino–imino contacts were found in the cidofovir containing DNA, implying that the base pairing dynamics are much higher than that of the control DNA. Further, the imino protons of the neighboring nucleobases near the cidofovir moiety exchange more easily than those in control DNA. These experiments suggest there is a bulge near to the location of cidofovir in the DNA core. These results should provide further details about the mechanisms by which CDV inhibits vaccinia polymerase activity.

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Inhibition of Calicivirus Replication in Mammalian Cells by RNAI

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Caliciviruses are non-enveloped viruses with a single stranded positive orientated RNA genome. They include human pathogenic (norovirus and sapovirus) and non-human pathogenic genera (vesivirus and lagovirus). Because of the increasing number of human infections and the absence of possibilities for vaccination as well as an antiviral therapy, it is of interest to develop antiviral strategies against human pathogenic caliciviruses. The objective of our study is to use the feline calicivirus in order to proof the concept of siRNA application as a new approach to control calicivirus infections. In contrast to the vast majority of caliciviruses that so far remain non-cultivable, the feline calicivirus (FCV, vesivirus) can be passed in CRFK-monolayers and is therefore used as a cell model for antiviral research. Our investigations include two different FCV strains (FCV-DD06 [GB Acc. No. DQ424892] and FCV-2024 [GB Acc. No. AF479590]). We have observed antiviral effects on several aspects of viral replication like reduction of infectious particles, the level of viral genomic RNA and inhibition of viral translation. siRNA-molecules targeting conserved genomic sequences lead either to a complete reduction of the viral titer or to a decrease of virions by two log scales (1.95E + 05 PfU/ml vs. a positive control of 4.08E + 07 PfU/ml). Similarly, viral genomic RNA level was decreased by about 30–50% in comparison to a positive control. Analysis by western blot reveals no detectable signal for structural and non-structural viral proteins after siRNA application. Quantification of the antiviral activity was performed with a cell proliferation assay resulting in an EC50 value of 0.69 μM. Our data underlines the potential of the siRNA approach as a novel strategy to control calicivirus infection.

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Developing Capsid Inhibitor V-073 for Poliovirus

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V-073, an enterovirus capsid inhibitor, has potent broad spectrum of anti-poliovirus activity *in vitro*. The compound has exhibited desirable attributes in nonclinical pharmacologic and toxicologic studies, which support the continued advancement of V-073 to first-in-human clinical studies. These attributes will be reviewed, as will the rationale for additional poliovirus antiviral development.

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Addition Antiflogistic to Viral Inhibitor

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Earlier we have been shown new viral inhibitors may be found among compounds with reliable anti-inflammatory properties. It may be supposed inflammatory and virus inhibitors influence at two independent parts of viral reproduction and therefore combine action of antiflogistic and antiviral remedies may be more potent in comparison with activity level of antiflogistic or virus inhibitor per se. These results present in table. Previous result seems to support this conclusion. Acyclovir is more potent than indometacin but a mixture is active as (I) ever in diminished dose. I + II overestimated as I and II if virus is added after I + II.

Comp	HSV-1, initial titer 4 IgID50, compounds are added in 24 h after virus infection			HSV-1, initial titer 5 IgID50, compounds are added in 24 h before virus infection		
	Acyclovir (I)	Indometacin (II)	I + II (mol:mol)	I	II	I + II (mol:mol)
Dose mkg/m	50	50	25	50	50	37.5
Titer $IgID_{50}$	1	2	1	1	3	<1.0

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Unsymmetrical CADA Analogs as Novel Down-modulators of the CD4 Receptor

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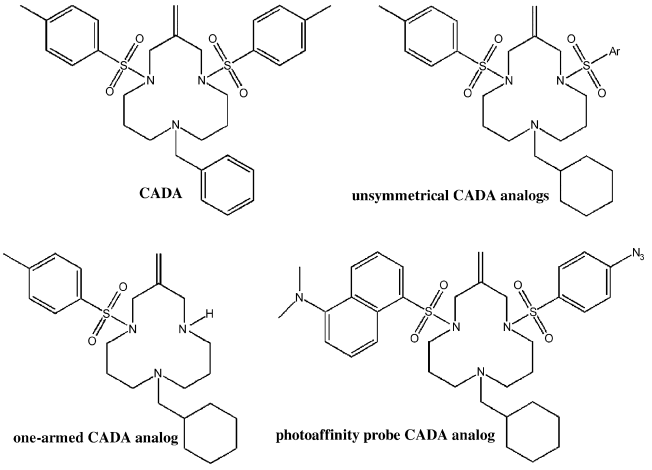
Cyclotriazadisulfonamide (CADA) inhibits the entry of HIV into $CD4^+$ target cells. This effect is due to the compound's ability to down-modulate the expression of the primary cellular receptor for HIV, CD4. Structural modifications of CADA have been made to increase potency, reduce toxicity, and improve physical properties. For 19 CADA analogs, a strict correlation between the CD4 down-modulating and antiviral activities has been observed. The interest

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.



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

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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.

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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

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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.

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Stereospecific Phosphorylation of Cyclopropavir by pUL97 and Inhibition by Maribavir

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