ferent HCV subgenomic (genotype 1b) replicon systems with a 50% effective concentration (EC_{50}) of 1–5 μg/ml. The concentration that reduced the growth of exponentially proliferating Huh 5-2 cells was >30 μg/ml thus resulting in a selectivity index ~25. The anti-HCV activity observed in Huh 5-2 was corroborated by means of RT-qPCR. LCTA-949 inhibited also efficiently the replication of the HCV_{cc} (JFH/J6 chimera) as assessed by RT-qPCR and by monitoring expression of viral antigen. Unlike various selective HCV inhibitors, LCTA-949 is very efficient in clearing cells from HCV replicons. The fact that the compound inhibits subgenomic replicon replication at concentrations that are similar to those that inhibit HCVcc replication, indicate that the compound inhibits intracellular RNA replication. At the ultrastructural level, treatment of either uninfected or infected cells with LCTA-949 results in the formation of multilamellar bodies (MLB). The potential effect of MLB formation on the HCV replication is currently being studied. Semisynthetic hydrophobic derivatives of glycopeptides antibiotics may thus be an interesting route to explore novel antiviral strategies against HCV.

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Eradication of Persistent Bovine Viral Diarrhea Virus Infection in Cell Culture by Antiviral Treatment: How to Get Ahead of the Viral Evasion Strategy

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The bovine viral diarrhea virus (BVDV) is a member of the family of Flaviviridae. BVDV exists as two biotypes, i.e. cytopathogenic (cp) and non-cytopathogenic (ncp). The ncp variant can establish a persistent infection in live stock as well as in cell culture by eluding the host innate immunity. Here we report on how such a persistent BVDV infection can be completely eradicated from mammalian cells. To this end the persistently infected cells were treated for a number of consecutive passages either with interferon alpha, the interferon inducer polyIC or the small molecule pestivirus inhibitor BPIP [Paeshuyse et al., 2006. J. Virol. 80, 149–160] or a combination thereof. Afterwards the cells were passaged two more times in cell culture medium without inhibitors. For each passage the presence of intracellular and extracellular viral RNA was monitored. An initial experiment resulted in a rapid decline of viral RNA for all inhibitors studied. Combined these data enabled the design of different drug regimes that resulted in the total eradication of BVDV ncp in cell cultures. The results obtained could help better understanding how pestiviruses establish persistent infections and how persistently infected cells can be cleared from such an infection. The latter can be of value for sanitation of precious cell lines that are contaminated by an ncp BVDV infection. Furthermore it is being investigated whether prolonged antiviral treatment of persistently infected cells can restore the innate immunity.

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N,N′-Bis(1,2,3-thiadiazol-5-yl)benzene-1,2-diamine Targets the HIV-1 Retroviral Nucleocapsid Zinc Fingers

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From an extensive structure-activity relationship study we have identified *N,N′*-bis(1,2,3-thiadiazol-5-yl)benzene-1,2-diamine (NV038) that efficiently blocks the replication of various strains of HIV-1, HIV-2 and SIV. NV038 inhibited the replication of HIV-1 at a 50% effective concentration of 17.3 μM and was not toxic for the host cells up to 300 μM tested, resulting in a selectivity index greater than 17. The compound was equipotent against several drug resistant virus strains. Time-of-addition experiments indicate that NV038 interferes with an event of the viral replication cycle following the viral entry but preceding or coinciding the early reverse transcription step, pointing towards an interaction with the viral nucleocapsid protein (NCp7). NCp7 is a small protein with two 'CCHC' zinc fingers flanked by basic residues, where both determinants are required for high affinity binding to RNA. The anti-HIV activity of NV038 decreased in the presence of Gag containing VLPs, suggesting its inhibitory effect is caused by an interaction with one of the Gag structural proteins. In fact, *in vitro*, NV038 efficiently chelates the zinc ions and depletes zinc from NCp7, which is paralleled by the inhibition of the NCp7-induced destabilization of cTAR. A chemical model suggests that the two carbonyl oxygens of both esters present in NV038 are involved in the chelation of the Zn^{2+}-ion. Besides the structural features required for zinc chelation other structural elements prove to be crucial for specific target recognition. This new lead and our mechanistic study provide insight into the design of further derivatives against this target with improved efficacy and selectivity.

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In Vitro Combination Studies of ANA598 with Anti-HCV Agents Demonstrate Enhanced Anti-viral Activity

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ANA598 is a potent direct-acting antiviral inhibitor of HCV NS5B polymerase that is currently in a Phase II clinical trial in combination with SOC. ANA598 exhibits subnanomolar potency against genotype 1 NS5B polymerase enzymes and dissociates slowly with a $t_{1/2}$ of ~2 h. Nanomolar *in vitro* potency was observed for clinical isolates tested with a mean EC_{50} for genotype 1b of 2.8 nM ($n = 10$) and 27 nM for genotype 1a ($n = 9$). Due to the potential for rapid emergence of resistance mutations to any single direct antiviral used as monotherapy in hepatitis C, future treatment of chronic HCV infection is expected to be in combination with SOC or with complementary direct antivirals. We describe here the *in vitro* assessment of ANA598 in combination with SOC, and several other classes of clinically advanced direct acting antiviral agents.