resistant variants were compared in one-step-growth experiments. The results did not reveal differences between pleconaril-sensitive and -resistant variants. Taken together, the results indicate that in addition to amino acid substitutions in position 1092 of the hydrophobic pocket, Ile1207 → Arg and Ile1207 → Lys correlate with a pleconaril-resistant phenotype. Possibly, substitution of amino acid 1207 of the GH-loop hinders conformational changes of this loop observed during drug binding and by this manner drug entry into the binding pocket or supports receptor binding and leads so to a reduced anti-CVB3 activity of pleconaril.

doi:10.1016/j.antiviral.2011.03.100

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Engineering Genetic Suppressor Elements against Hepatits C Virus

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Hepatitis C virus (HCV) infection is a major public health problem, putting infected individuals (~180 million worldwide) at risk of developing cirrhosis, hepatocellular carcinoma and liver failure. The current standard interferon-based therapy for HCV cures only ~50% of patients infected with the most common genotype. In this study, we describe the selection of a new class of antivirals against HCV – genetic suppressor elements (GSEs). GSEs are protein/nucleotide sequences derived from a gene or genome of interest that act as transdominant inhibitors of a particular biological function. Using an engineered hepatoma cell line – n4mBid – that undergoes significant HCV-induced cytopathic effect and cell culture-derived HCV (HCVcc), we developed an efficient selection system for isolating anti-HCV GSEs from a library comprising a fragmented HCV genome. Target cells expressing the 4th round enrichment library showed significant resistance to HCV cytopathic effect and reduced levels of permissivity to HCV infection. These studies represent the first report of successful selection of genetic anti-HCV elements. We are currently isolating individual GSEs from the selected cell population and characterizing their specific effects on the HCV life cycle.

doi:10.1016/j.antiviral.2011.03.101

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3′,5′di-*O*-Trityluridine Inhibits Flavivirus (Dengue and Yellow Fever Virus) Replication and Targets the Viral RNA Dependent RNA Polymerase

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The dengue fever virus (DENV) and the yellow fever virus (YFV) are members of the genus flavivirus in the family *Flaviviridae*. An estimated 50–100 million cases of DENV infections occur each year and approximately half a million patients require hospitalization. There is no vaccine or effective antiviral treatment available. There is an urgent need for potent and safe inhibitors of DENV replication; ideally such compounds should have broad-spectrum activity against flaviviruses.We report on the activity of 3′,5′di-*O*-trityluridine (DiTU) on flavivirus replication. The compound inhibits induction of DENV- and YFV-induced cytopathic effect (CPE) with the EC_{50} values in the low micromolar range. This was confirmed in virus yield reduction experiments, where dose-dependent inhibition of viral RNA synthesis was observed (DENV-2 EC_{50} = 1.2 μM; YFV-17D EC_{50} = 0.8 μM; Selectivity index > 125). Moreover, DiTU also efficiently inhibited viral protein synthesis in the same concentration range. Activity was demonstrated in DENV subgenomic replicons (which encodes only non-structural viral proteins) (EC_{50} = 3 μM) indicating that the compound inhibits intracellular events of the viral replication cycle. This observation was corroborated by the time-of-drug-addition studies, where DiTU was shown to inhibit flavivirus replication at a time point that coincides with the onset of intracellular viral RNA synthesis. DiTU efficiently inhibited highly purified DENV-2 polymerase (EC_{50} = 1.8 μM). Drug-resistant variants are currently being selected, but even following 10 passages, no such variants have so far been selected. In conclusion, our data indicate that the antiflavivirus activity of DiTU is the result of inhibition of the viral RNA dependent RNA polymerase, that this molecule has a high barrier to resistance and that it does not act as a nucleoside analogue.

doi:10.1016/j.antiviral.2011.03.102

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Identification of a Novel Antiviral Drug Targeting at Host Apoptotic Responses

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Bluetongue virus (BTV) is a multi-layered, double-stranded RNA and the prototype virus in the genus *Orbivirus* within the *Reoviridae* family. BTV is one of the most important diseases of domestic livestock, including sheep, goat, cattle, horse and other domestic animals, with $3 billion/year loss worldwide. Recently, the re-emerging of BTV has caused a major outbreak of disease in cattle and sheep in several countries across northern and western Europe. We present the identification and characterization of a novel antiviral against BTV by targeting at the host apoptotic response. This novel small molecule antiviral compound belongs to one of the six clusters of antivirals against BTV (Li et al., 2009), identified via a high throughput screening of a 200,000 compound library. This compound showed an IC_{50} at 0.69 ± 0.15 μM, with very low cytotoxicity (CC50 > 100 μM), demonstrated that it is high selective against BTV with a Selective Index (SI_{50}) over 100. This compound also reduced the BTV plaque formation by 2–3 logs in standard plaque assay. The Time-of-Addition assay showed that this compound inhibits the late event of the BTV viral life-cycle. Mechanism of action studies indicate that this compound might interact with the host apoptotic/autophagic mechinary. The identification and characterization of a novel antiviral against BTV could be further developed a new control and prevention measure against BTV.

doi:10.1016/j.antiviral.2011.03.103