conditions needed for *de novo* RNA-polymerase activity. Finally, oligomerization experiments in the presence of the non-nucleoside inhibitor (NNI) PF-254027 gave a statistically significant reduction in the FRET signal, suggesting a new connection between NS5B oligomerization and NNI binding. These results together with data reported by other groups have been used to *in silico* simulation docking studies that have allowed us to infer the fingers–thumb geometry of the interaction.

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In vitro Selection of HCV Replicons with Reduced Sensitivity to PSI-352938, A Cyclicphosphate Prodrug of β-D-2′-α-F-2′-β-*C*-Methylguanosine

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PSI-352938 is a cyclicphosphate prodrug of β-D-2′-deoxy-2′-α-fluoro-2′- β-*C*-methylguanosine monophosphate. PSI-352938 inhibited HCV replication in genotype (GT) 1a, 1b and 2a replicon cells with EC_{50} values of 0.20 μM, 0.13 μM and 0.14 μM, respectively. Cross resistance studies showed replicon cells containing the NS5B S282T variant remained fully susceptible to PSI-352938. In this study, we describe the selection of HCV replicons with lowered susceptibility to PSI-352938. Selection studies were performed using GT 1a (H77), 1b (Con1), or 2a (J6/JFH-1) replicon cell lines. Selected HCV replicons with lowered susceptibility were analyzed for mutations within NS5B. Replicon mutants were constructed by site directed mutagenesis and examined for resistance to PSI-352938. After 158 days, HCV GT 2a replicon cells with a 19.2-fold decrease in sensitivity to PSI-352938 were selected Sequence analysis identified mutations within the HCV NS5B, including S15G, R222Q, C223Y/H, V321I, and L320I. Evaluation of these mutations indicated that combinations of at least three amino acid changes that included C223H was required for a significant loss of sensitivity to PSI-352938. With the exception of S15G, these mutations were located in close proximity to the conserved D220 and D318 residues at the active site. We were unable to select for resistant GT 1a or 1b replicons and subsequently determined that the C223Y/H mutation was lethal for the GT 1b replicon. Similar results have been obtained with a related guanosine analog monophosphate prodrug PSI-353661. The difficulty in selecting resistant variants to PSI-352938 compared to other inhibitors such as non-nucleoside analog inhibitors of NS5B, HCV NS3 protease inhibitors and NS5A inhibitors, which select for mutations rapidly both *in vitro* and *in vivo*, suggests that PSI-352938 has a very high barrier to resistance. The unique resistance profile of PSI-352938 makes it a promising compound for mono- and/or combination therapy with other HCV inhibitors, including other nucleoside/tide analogs.

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Human subtilase Site-1 Protease (S1P): An Emerging Host Cell Target for Hepatitis C Virus (HCV) Infection and HCV-associated Steatogenesis

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In this study, we hypothesize that targeting cellular enzymes acting as master regulators of lipid homeostasis could represent a powerful approach to developing a novel class of broad-spectrum anti-virals against infection associated with human viruses such as HCV, Dengue virus, and rotaviruses, whose replication and pathogenesis depend on the interaction with lipid droplets (LDs). In the case of HCV, overstimulation of host lipid metabolism in the liver during viral infection promotes cholesterol intracellular storage in host LDs, a critical cellular event for HCV replication, assembly, and budding. One such master regulator of cholesterol metabolic pathways is the host subtilase S1P. Also known as SKI-1, S1P plays a critical role in the proteolytic activation of SREBPs, which control the expression of key enzymes of cholesterol and fatty-acid biosynthesis. Here, we report that strategic manipulation of cellular S1P activity levels by engineered serine protease inhibitors (serpins), Spn4A variants (Richer et al., 2004), provides a means of effectively inhibiting the S1P-dependent proteolytic cleavage of SREBPs in hepatoma cells, a critical step in the hijacking of the host cholesterol pathways by HCV. First, we describe the bioengineering, serpin functionality, and specificity studies of our novel S1P-directed Spn4A variant using a recombinant adenovirus (Ad) system. We demonstrated the anti-proteolytic and anti-HCV activities of our new Ad-expressing variant directed at S1P [reactive site loop: –RRKR– → –RRLL–]. Expression of the Ad_Spn4A_RRLL in Huh-7.5.1 cells results in inhibition of the S1P-mediated activation of SREBP-2 and down-regulation of the SREBP-2 target gene products as revealed by Western blotting. Using fluorescence microscopy, we found that specific inhibition of S1P reduces the abundance of LDs in Huh-7.5.1 cells. As hypothesized, inhibiting S1P activity blocked HCV infection (JFH-1 strain) of Huh-7.5.1 cells in a dose-dependent manner. The results of our studies contribute to our understanding of the HCV lifecycle and associated steatogenesis and to efforts in developing novel host-directed broad-spectrum anti-virals.

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Reference

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DMPK and Metabolism Studies of Nucleoside Phosphoramidates Including INX-08189, A Novel Double Pro-drug and Clinical Candidate for Hepatitis C Virus Therapy

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Hepatitis C Virus (HCV) infection is a serious health problem that leads to chronic liver disease, such as cirrhosis and hepatocellular carcinoma, in an estimated 2–15% of the world's population. A collaboration between Inhibitex and the University of Caridiff in Wales has produced a novel double pro-drug approach to the