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Novel 4′-Azido-2′-Deoxy-Nucleoside Analogs are Potent Inhibitors of NS5B-Dependent HCV Replication

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Two novel deoxynucleoside analogs have been identified as potent inhibitors of HCV replication targeting HCV polymerase NS5B. Despite lacking the 2′-a-hydroxy moieties of ribonucleosides, which are generally recognized by RNA polymerases through direct hydrogen bonding interaction, the deoxycytidine triphosphate analogs RO-0622-TP and RO-9187-TP were efficiently incorporated into nascent RNA by HCV polymerase NS5B in a template base specific manner, causing chain termination. Both nucleosides were also excellent substrates for deoxycytidine kinase and were phosphorylated with efficiencies similar to or higher than deoxycytidine. Consistent with these findings, high levels of triphosphate were formed in human hepatocytes and both compounds were potent inhibitors of HCV replication in the replicon system ($IC_{50} = 171 \pm 12$ nM and 24 ± 3 nM for RO-9187 and RO-0622, respectively; $CC_{50} > 1$ mM). Both compounds inhibited RNA synthesis by HCV polymerases from either genotypes 1a and 1b or containing the S282T point mutation with similar potencies, suggesting no cross-resistance with 2′-C-methyl nucleosides. Pharmacokinetic studies with RO-9187 in rats, dogs and monkeys showed that plasma concentrations exceeding HCV replicon IC_{50} values up to 150-fold could be achieved by low dose (10 mg/kg) oral administration. Therefore, 4′-azido-2′-deoxy nucleosides are a new class of antiviral nucleosides with promising preclinical properties as potential medicines for the treatment of HCV infection.

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Characterization of the Intracellular Metabolism of β-D-2′-Deoxy-2′-Fluoro-2′-C-Methyl-Cytidine and the Inhibition of HCV Polymerase NS5B by its 5′-Triphosphate Species

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β-D-2′-Deoxy-2′-fluoro-2′-C-methyl-cytidine (R1656, PSI-6130) is a potent inhibitor of hepatitis C virus (HCV) replication in the subgenomic HCV replicon system and its corresponding 5′-triphosphate is a potent inhibitor of the HCV RNA polymerase in vitro. In this study, the formation of R1656-triphosphate was characterized in primary human hepatocytes isolated from several independent donors. Intracellular concentrations of R1656 and its 5′-phosphorylated derivatives were determined, with R1656 parent compound being the major intracellular species. In addition, the deaminated derivative of R1656, β-D-2′-deoxy-2′-fluoro-2′-C-methyl-uridine (RO2433, PSI-6026) and its corresponding phosphorylated metabolites were identified in human hepatocytes after incubation with R1656. The formation of R1656-TP and RO2433-TP increased with time and reached steady state level at 48 h. R1656-TP and RO2433-TP were the major phosphorylated species at steady state. The formation of both R1656-TP and RO2433-TP demonstrated a linear relationship with the extracellular concentrations of R1656 up to 100 μM, suggesting a high capacity of human hepatocytes to generate the two triphosphates. The mean half lives of R1656-TP and RO2433-TP were 4.7 and 38 h, respectively. RO2433-TP also inhibited RNA synthesis by the native HCV replicase isolated from HCV replicon cells and the recombinant HCV polymerase NS5B with potencies comparable to those of R1656-TP. Incorporation of RO2433-MP into nascent RNA by NS5B led to chain termination, similar to that of R1656-MP. These results suggest that R1656 is metabolized to two pharmacologically active species in primary human hepatocytes. The long half life of RO2433-TP suggests the potential for investigating once daily dosing regimens of R1656 for the treatment of HCV infection.

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Sub Micromolar Inhibitors of HCV Generated from Inactive Nucleosides by Application of ProTide Technology

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We report the application of our phosphoramidate ProTide technology to various 4′-substituted ribonucleoside analogues, designed as potential inhibitors of hepatitis C virus (HCV) (Fig. 1). Thus, ProTides were prepared from 4′-azidouridine (AZU), -cytidine (AZC), -adenosine (AZA) and -5-methyluridine (AZMeU), besides other 4′-substituted uridines and cytidines. In each case, ProTide families included variations in the aryl, ester, and amino acid regions. A number of compounds showed potent inhibitory properties in cell culture without detectable cytotoxicity. These results confirm that phosphoramidate ProTides can deliver monophosphates of ribonucleoside analogues and suggest a potential path to the generation of novel antiviral agents against HCV infection. Of particular note was the sub-μM potency displayed by certain ProTides of AZU; a nucleoside analogue, which was itself inac-