Oral Session 3: Hepatitis Viruses

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HCV Culture System and Antiviral Development

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Identification of Halosalicylamide Derivatives as a Novel Class of Allosteric Inhibitors of HCV NS5B Polymerase

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Hepatitis C virus is the major etiological agent of non-A, non-B hepatitis. The disease is a major health problem with an estimated 170 million people infected worldwide. The current standard of care for treating HCV is a combination therapy of pegylated interferon-α (PEG-IFN-α) plus ribavirin. Patients infected with genotype 1 HCV do not respond as well and therefore demonstrated a need for HCV drug intervention. Because of its demonstrated vital role in viral replication, HCV NS5B polymerase has been the most studied viral protein target for small molecule HCV therapy. We developed an assay that is capable of identifying inhibitors targeting both initiation and elongation steps of the polymerization reaction. We undertook the hits evaluation by using the RNA binding assay to eliminate RNA intercalators and compounds interfering with the RNA substrate binding. We then evaluated the hits with respect to elongation NTP substrates. A gel-based assay was developed to differentiate initiation inhibitors from elongation inhibitors. Inhibitors of known binding sites were also used as probes in the inhibitor/screening hits competition studies. The structure and activity relationship revealed the absolute requirement of the salicylamide moiety for optimum activity. Methylation of either the hydroxyl group or the amide group of the salicylamide moiety abolished the activity while the substitutions on both phenyl rings are acceptable. The optimized compound demonstrated low micromolar biochemical activity and low micromolar replicon activity, and represented a novel class of allosteric inhibitors of HCV NS5B polymerase. Assay development work, screening hits evaluation strategies, mechanism of inhibition studies and detailed structure and activity relationship will be presented.

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Anti-Hepatitis C Virus Replicon Activity of Alkoxyalkyl Esters of (*S*)-HPMPA and Other Acyclic Nucleoside Phosphonates

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First generation hepatitis C virus (HCV) nucleoside inhibitors validated the viral RNA polymerase as an antiviral target. However, these initial nucleosides are limited by modest potency and poor tolerability. We have recently shown that hexadecyloxypropyl (*S*)-9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine (HDP-(*S*)-HPMPA) possesses substantial anti-HCV activity. Using an HCV replicon system utilizing HCV genotypes 1b and 2a, we evaluated the anti-HCV activity of additional alkoxyalkyl acyclic nucleoside phosphonates and compared their activity to HDP-(*S*)-HPMPA. We report preliminary activity for the HDP esters of cidofovir (CDV), tenofovir (TFV), 9-(5-phosphono-pent-2-en-1-yl)guanine (PPenG), and 9-(5-phosphono-pent-2-en-1-yl)adenine (PPenA). Compounds were tested in a 96-well plate format using cells stably expressing a hepatitis C genotype 1b or 2a luciferase replicon. Cells were incubated in triplicate with compounds (up to 50 μM) for 48 h. Luciferase activity was determined using BrightGlo (Promega). Cytotoxicity was measured using MultiTox-Fluor (Promega). EC_{50} and CC_{50} values were determined using Prism4 (GraphPad). In the hexadecyloxypropyl ester series, HDP-(*S*)-HPMPA was the most active compound with an EC_{50} of 2.0 μM. The order of activity of the compounds was HDP-(*S*)-HPMPA > HDP-PPenG > HDP-CDV > HDP-TFV ≫ HDP-PPenA. Octadecyloxyethyl-(*S*)-HPMPA was the most active acyclic nucleoside phosphonate with an EC_{50} of 1.3 μM in genotype 1b and 0.68 μM in genotype 2a replicons. Our results establish that certain lipid esters of acyclic nucleoside phosphonates, which have much different structures than the current 2′- or 4′-modified nucleosides, are active and selective inhibitors of HCV replication. Alkoxyalkyl esters of (*S*)-HPMPA, PPenG and CDV are active inhibitors of HCV replication. ODE-(*S*)-HPMPA is the most active alkoxyalkyl acyclic nucleoside phosphonate identified to date. Additional studies are underway to better define structure–activity relationships, mechanisms of action and potential resistance profiles.

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