Conclusion: These data demonstrate that all three drugs in the TCAD regimen contributed to prevent the emergence of resistance, as determined by virus breakthrough and/or the presence of resistance-associated mutations.

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Orally Bioavailable Anti-HBV Dinucleotide Acyloxyalkyl Prodrugs

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We have previously reported that phosphorothioate di-, and trinucleotides are a new class of anti-HBV compounds with potent activity in vitro and in vivo. We report here the evaluation of acyloxyalkylester prodrugs 2 and 3, derived from the anti-HBV dinucleotide [R_p,S_p]-3′-dA-ps-$U_{2'OMe}$ (**1**). The bioreversibility studies of 2 and 3 – using mouse, rabbit, and human serum – revealed that each isomer of **2** and **3** underwent stereospecific conversion to the active **1** at almost equal rates. The anti-HBV evaluation of **3** in the HepG2.2.15 cell lines revealed that the compound had antiviral potency similar to that of ADV, and antiviral activity against all tested Lamivudine and ADV-resistant mutants. The cytotoxicity evaluation using MDBK, Vero, and HFF cell lines showed that both prodrugs **2** and **3** had $CC_{50} > 1000$ μm indicating a high safety profile. The compounds **2** and **3** displayed high stability in simulated gastric fluid with $t_{1/2} > 1$ h. The pharmacological bioavailability studies of orally administered 2 and 3 in Swiss Webster mice revealed the presence of the dinucleotide 1 in liver. Biodistribution studies of ^{35}S-labeled-**3** in Sprague–Dawley rats revealed that the ratio of liver to plasma concentration of radioactivity was as high as 2.9 (iv route) and 3.9 (po route). The initial pharmacodynamic evaluations of **2**, and **3** at high doses of 300 and 400 mg/kg/day in the HBV transgenic mouse model showed that both compounds had strong anti-HBV activity. Dose-ranging studies of **3** at 1, 5, 10, and 100 mg/kg revealed a dose-dependent reduction of liver HBV DNA as determined by Southern blot analysis and RT-PCR. In summary, the dinucleotide prodrugs 2 and 3 represent the first-in-class orally bioavailable antiviral agents against HBV.

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Activation of Retinoic Acid Inducible Gene (RIG-I) by Nucleotide Analogs: A Potential Novel Mechanism for Antiviral Discovery

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Retinoic acid inducible gene (RIG-I) is a host cellular cytosolic protein, that acts as a viral sensor for recognition of double-stranded viral RNA, and stimulates type I interferon production thereby inhibiting viral replication and suppressing cellular permissiveness for virus infection. Using a novel cell-free assay, we have discovered that chemically modified short oligonucleotides induced rapid translocation (shuttling) of RIG-I on a double-stranded RNA (dsRNA) template. The shuttling of RIG-I on dsRNA may have two consequences in vivo: (a) the oligonucleotide-induced shuttling can cause prolonged occupancy of RIG-I on viral RNA and interfere with viral protein/nucleic acid interaction thereby inhibiting viral nucleic acid replication/translation; (b) rapid translocation of RIG-I can activate the downstream mitochondrial antiviral signaling pathway (MAVS) by efficiently exposing the caspase activation and recruitment domains (CARDs) of RIG-I for subsequent ubiquitination and interaction with MAVS to coordinate an immune or apoptotic response. Since RIG-I is a viral sensor that detects whole range of RNA viruses, it presents a unique host target for broad-spectrum antiviral intervention. We have discovered that the anti-HBV compound SB 40 and its oral prodrug SB 44 also induce rapid translocation of RIG-I on dsRNA. Although HBV is a DNA virus, it uses a pregenomic RNA (pgRNA) template for the initiation of DNA synthesis; therefore RIG-I may be a receptor for HBV pgRNA as well. Hence, the mechanism of antiviral action of SB 40 and SB 44 may also include the induction of shuttling of RIG-I on pgRNA of HBV that inhibit viral replication. Based upon studies with SB 40 and SB 44, we have identified certain structural and stereochemical attributes of short oligonucleotides that are important for rapid RIG-I translocation on dsRNA and established a strong rationale for the design and synthesis of focused libraries for lead optimization and discovery of potent antiviral compounds.

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Efficacy of 2′-C-Methylcytidine Against Yellow Fever Virus in a Hamster Model of Disease

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Yellow fever virus (YFV) causes periodic outbreaks of acute disease despite the availability of an effective vaccine. The National Institute of Allergy and Infectious Disease (NIAID) has listed YFV as a Category C priority pathogen, thus prioritizing the development of therapeutic intervention strategies for the treatment of disease caused by this flavivirus. Derivatives of the nucleoside analog 2′-C-methylcytidine (2′-C-MeC) are effective in improving disease in people infected with hepatitis C virus, a related flavivirus, but gastrointestinal side effects have inhibited clinical development. The compound 2′-C-MeC was found to have activity against YFV in Vero cells, which was confirmed by a virus yield reduction assay. The 90% effective concentration (EC_{90}) in Vero cells was 0.32 μg/ml and the 50% cytotoxic concentration (CC_{50}) was 32 μg/ml, yielding an