in this family that cause human disease with an estimated 50–100 million infections per year worldwide. Although abundant research has been done, there are no approved vaccines or therapeutics available. An antiviral drug administered early during Dengue virus infection that inhibits viral replication and prevents the high viral load associated with the more severe forms of dengue would be an attractive strategy in the treatment and management of disease. The goal of the SIGA dengue program is to develop a small molecule therapeutic for the treatment and/or prevention of disease caused by dengue virus, with a final drug product that will be a safe, effective, and orally administered antiviral compound. Novel small molecule inhibitors have been identified that are potent and selective, with inhibitory activity against all four serotypes of dengue virus in vitro. These compounds have structures that are chemically tractable, in that they possess chemically stable functionalities and have potential drug-like qualities. Lead series have been identified and are being defined by spectrum of activity, mechanism of action, preliminary absorption, distribution, metabolism, and excretion (ADME) profiles, and pharmacokinetic (PK) evaluations. Two of these series have shown proof-of-concept efficacy in a murine model of disease. The identification and characterization of early stage dengue virus inhibitors with activity in a murine model of dengue virus infection represents a compelling start toward our goal.

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Biological Profiling of GS-9350, a Novel Pharmacoenhancer that Lacks Anti-HIV Activity and Exhibits Low Potential for Metabolic Adverse Effects *In Vitro*

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Background: Pharmacokinetic enhancement is frequently used to increase systemic exposure of HIV protease inhibitors (PIs). Ritonavir (RTV), a therapeutic PI, is also a potent mechanism-based inhibitor of CYP3A widely used at low dose to boost PIs. However, chronic use of RTV may cause various metabolic adverse effects, and its subtherapeutic dose could potentially induce PI resistance mutations. Here we describe the biological profile of GS-9350, a novel mechanism-based CYP3A inhibitor and pharmacoenhancer that is currently in advanced stage of clinical development.

Methods: Inhibition of proteases was evaluated using synthetic fluorescent substrates. Inhibition of HIV (including a panel primary isolates in PBMC), HBV and HCV were determined using standard assays. Effects on lipid accumulation and insulin-stimulated glucose uptake were assessed in human and mouse adipocytes, respectively.

Results: RTV inhibited HIV-1 protease and cathepsin D with IC_{50} values of 0.6 and 870 nM, respectively. In contrast, GS-9350 showed no inhibition of HIV-1 protease and cathepsin D at concentrations up to 30 mM. GS-9350 showed no anti-HIV activity at concentrations up to 30 and 90 mM in MT-2 cells, in the absence and presence of human serum, respectively. GS-9350 was also devoid of antiviral activity against a panel of HIV primary isolates and did not inhibit HBV or HCV. In addition, GS-9350 did not affect the *in vitro* anti-HIV activity of multiple approved antiretrovirals, including PIs. GS-9350 exhibited no effect on lipid accumulation in human adipocytes at concentrations up to 30 mM, while RTV showed significant inhibition (EC_{50} = 16 mM). GS-9350 and RTV also showed differential effects on the insulin-stimulated glucose uptake with <10% and 55% inhibition at 10 mM concentration, respectively.

Conclusions: In contrast to RTV, GS-9350 is devoid of antiretroviral activity due to the lack of HIV protease inhibition. In addition, GS-9350 does not affect adipocyte functions, suggesting a lower potential for metabolic adverse effects compared to RTV. Overall, GS-9350 exhibits an improved *in vitro* pharmacological profile relative to RTV, supporting its further clinical development.

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Production and Characterization of a Highly Infectious Genotype 1b/2a Chimeric Hepatitis C Virus in Cell Culture

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Genotype 1 hepatitis C virus (HCV) is the most prevalent HCV genotype in North America and Europe and the least responsive to the present standard of care. The recent development of cell culture systems based on intergenotypic recombinants of the genotype 2 JFH-1 strain, has made it possible to study infectious HCV encoding the structural genes of additional HCV genotypes including genotype 1b (con-1). Intergenotypic 1b/2a chimeric genomes replicate in transfected cells but produce very low viral titers, limiting the utility of this system. In this study, we generated cell culture adapted 1b/2a variants by serially passaging the virus in a novel Huh-7-Lunet cell clone. The adapted 1b/2a chimeric virus yielded significantly higher titers than the parental unadapted virus (4×10^4 vs. 3×10^1 $TCID_{50}$/ml, respectively). Furthermore, quantitative fluorescent microscopy indicated that the adapted virus formed larger foci and spread through cultures significantly faster than the parental virus. Sequence analyses revealed four potential adaptive mutations: A150V in core, V1056G and I1312V in NS3 and M2388I in NS5A. Experiments are ongoing to determine the impact of each mutation on enhancement of virus production and spread. We also validated our adapted 1b/2a virus for antiviral testing using a panel of known HCV inhibitors with distinct mechanisms of action. Overall, this novel adapted 1b/2a HCV chimera will facilitate the identification and characterization of novel HCV inhibitors including those that target steps in virus entry, assembly or release that involve genotype 1b structural genes.

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Excision of HIV-1 Proviral DNA using Tre-Recombinase: An Experimental Update

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HIV-1 integrates into the host chomosome and persists as a provirus flanked by long terminal repeats (LTR). To date, treatment regimens primarily target the virus enzymes, virus attachment or virus-cell fusion, but not the integrated provirus. Therefore, current antiretroviral therapies require lifelong treatment which, unfortunately, is frequently accompanied by the occurrence of