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 ($4 \times 10^4$ vs. $3 \times 10^1$ 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 substantial toxicities and/or the development of drug-resistant viruses. Previously, we engineered a LTR-specific recombinase (Tre-recombinase) that can effectively excise integrated HIV-1 proviral DNA from infected human cell cultures, suggesting that customized enzymes might someday help to eradicate HIV-1 from the body. Here, we provide an update on our recent and further analyses of Tre-recombinase in various HIV-1 infection models. Moreover, we discuss potential future strategies to deliver Tre-recombinase into infected subjects.

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## Small Molecules Targeting Protein–Protein Interactions: A Promising Anti-HIV Strategy

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The development of multidrug-resistant viruses compromises the efficacy of anti-human immunodeficiency virus (HIV) therapy and limits treatment options. Therefore, new targets with a different mechanism of action with respect to anti-AIDS drugs so far in therapy need to be identified. In recent years, many examples of protein–protein interactions in the HIV life cycle and related inhibitors is growing rapidly (Busschots et al., 2009). Thus, protein–protein interactions (PPIs) provide an important new approach for the drug design against HIV infection. In our previous paper, a structure-based 3D pharmacophore model for potential inhibitors of the interaction between HIV-1-IN and its cellular cofactor LEDGF/p75 was developed and used for virtual screening of chemical databases, leading to the identification of interesting hits for further optimization (De Luca et al., 2009). Consequently, the rational design, synthesis and biological tests of some derivatives have been carried out. Our studies resulted in the discovery of compounds able to interfere with the IN-LEDGF/p75 interaction at micromolar concentration. Docking simulations were also performed with the aim to investigate the possible binding mode of our new compounds.

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## A Cell Protection Screen Reveals Potent Inhibitors of Multiple Stages of the Hepatitis C Virus Life Cycle

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The hepatitis C virus (HCV) life cycle involves multiple steps, but most current drug candidates target only viral replication. Inability to systematically discover inhibitors targeting multiple steps of the HCV life cycle has hampered antiviral development. We describe a new screen for HCV antivirals based on the alleviation of a HCV-mediated cytopathic effect experienced by an engineered cell line—n4mBid. This approach obviates the need for a secondary screen to avoid cytotoxic false positive hits. Application of our screen to 1280 compounds, many in clinical trials or approved for therapeutic use, yielded >200 hits. Of the 55 leading hits, 47 inhibited one or more aspects of the HCV life cycle by >40%. Six compounds blocked HCV entry to levels similar to an antibody (JS-81) targeting the HCV entry receptor CD81. Seven hits inhibited HCV replication and/or infectious virus production by >100-fold, with one (quinidine) inhibiting infectious virus production by 450-fold relative to HCV replication levels. The described approach is simple and inexpensive, and should enable the rapid discovery of new classes of HCV life cycle inhibitors.

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## Synthesis and Tissue Distribution Studies of Acyloxyalkyl Prodrug Derivative of an Anti-HBV Dinucleotide

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We have reported that phosphorothioate dinucleotides and trinucleotides are a new class of anti-HBV compounds with potent activity *in Vitro* and *in Vivo*. Recently, we had developed the acyloxyalkylester derivative as an oral prodrug of the model anti-HBV dinucleotide $[R_p,S_p]$-3′-dA-ps-$U_{2'OMe}$ (**1**). The current studies were undertaken to evaluate the distribution of the $^{35}$S-labeled prodrug in the liver and other organs in rats. The $^{35}$S-labeled prodrug was obtained as a solid in high specific activity (120 mCi/g; 84.9 mCi/mmol) by chemoselective *S*-alkylation of $^{35}$S-1. The requisite $^{35}$S-1 was synthesized using solid-phase phosphoramidite chemistry. Thus, controlled-pore-glass (CPG)-supported $dA^{NBz}$ was coupled to 5′DMT-2′-OMe-uridine-3′-phosphoramidite to generate the intermediate dinucleoside phosphite. The sulfurization of the phosphite using $^{35}$S-labeled 3H-1,2-benzodithiole-1,1-dioxide (independently synthesized) followed by deprotection of the CPG-bound dinucleoside phosphotriester, and HPLC purification gave $^{35}$S-1. $^{35}$S-1 was administered to rats, at a dose of 10 mg/kg, by intravenous (iv) and oral (po) routes. Radioactivity was readily detected in plasma at different time-points after both iv and po administration. Radioactivity concentrated in the liver and the ratio of liver to plasma concentration was as high as 2.9 (iv route) and 3.9 (po route) 1 h after dose administration. Other tissues – kidney, brain, spleen, and heart – contained minor amounts of radioactivity. The primary route of excretion of the radioactivity from the com-