Table 1
Effect of peramivir treatment (IM) on weight loss and survival in mice infected with H1N1 virus (prophylaxis model).

Treatment	Dose level (mg/kg)	Percent survival	Mean weight change (±SEM) day 4
Vehicle, uninfected	0	100	0.32 ± 0.22
Vehicle, infected saline treated	0	20	−3.41 ± 0.17
Peramivir	1.0	60*	−2.11 ± 0.22**
Peramivir	3.0	60*	−1.51 ± 0.33**
Peramivir	10.0	90**	−1.84 ± 0.19**
Peramivir	30.0	100**	−1.83 ± 0.17**

* $p < 0.03$ vs. vehicle, infected control group.
** $p < 0.001$ vs. vehicle, infected control group.

Conclusion: These data demonstrate efficacy of parenterally administered peramivir against the recently isolated pandemic flu virus.

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52

In Vitro Dose Ranging Studies for Serine Protease Inhibitor, MK-4519, Against a Hepatitis C Virus (HCV) Replicon using the Bellocell System

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Background: Development of new anti-HCV agents has been limited due to the inability to effectively grow HCV in cell culture. This problem is obviated by use of a HCV replicon system. We have developed an in vitro system to examine the pharmacodynamically-linked parameters and monitor the exposure response of MK-4519 against HCV using a HCV replicon-bearing cell line.

Methods: The HCV replicon cell line, 2209–23, was obtained from Roche (Palo Alto, CA). The replicon contains a Renilla luciferase reporter gene that was used to monitor HCV replication kinetics. We inoculated 2209–23 cells into four BelloCell bottles at a concentration of 6×10^7 cells per bottle. One bottle served as a control and three bottles were treated with various concentrations of MK-4519, which was obtained from Merck. Medium, with or without drug, was infused into the system for 14 days. Replicon kinetics and cell growth were monitored daily by harvesting 6 carrier flakes in quadruplicate. Three sets of 6 flakes were trypsinized to remove 2209–23 cells from the flakes and viable cells were counted. RNA was later extracted from these trypsinized cells and used for sequencing of the NS3 gene. The remaining flakes were immersed in Renilla luciferase lysis buffer and frozen until the end of the study. Luciferase activity was quantified from all samples simultaneously to determine the effect of each drug on the HCV replicon.

Results: In the BelloCell system, MK-4519 significantly reduced luciferase activity in the 2209–23 cells in a dose dependent manner. Cell viability assays confirmed that the suppression of luciferase activity was not due to cytotoxicity. Sequencing analysis detected genotypic changes in the replicon as a result of drug exposure. Mutation A156T, which is associated with phenotypic drug resistance, was present only in replicons exposed to drug.

Conclusion: These studies indicate that the BelloCell system is an effective and relevant in vitro method to model in vivo pharmacodynamics for antiviral agents active against HCV. This system can be used to optimize dosing for anti-HCV compounds for inhibition of viral replication and suppression of resistance.

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53

Maporal Hantavirus ββ-Integrin Utilization and Sensitivity to Favipiravir

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Hantaviruses are members of the Bunyaviridae family of viruses. Pathogenic hantaviruses are the etiologic agents of hemorrhagic fever with renal syndrome (HFRS), a disease principally endemic in the Old World, and hantavirus pulmonary syndrome (HPS), a disease primarily restricted to the Americas. Maporal virus (MPRLV), a recently isolated hantavirus, has been found to cause disease in hamsters that resembles HPS in humans. However, the virus has not been linked to human cases of HPS. Considerable evidence suggests that ββ-integrin usage mediating infection may serve to distinguish pathogenic from non-pathogenic hantaviruses, but this receptor usage pattern information is not yet available for MPRLV. Although ribavirin has been shown to be effective in treating HFRS, it lacks specificity and has toxicity. Moreover, there are no effective antivirals for the treatment of HPS. Considering the above, we have investigated MPRLV (1) susceptibility to favipiriavir (T-705), (2) ββ-integrin-mediated mechanism of entry, and (3) genetic determinants of pathogenicity. Favipiravir, a pyrazine derivative reported to be active against related bunyaviruses, was found to be active against MPRLV, Dobrava virus (DOBV), and Prospect Hill virus (PHV) (EC_{50} = 65–93 μM) with therapeutic indexes of 77, 65, and 82, respectively. Using antibodies targeting specific integrin chains, we found infection of Vero E6 cells with MPRLV to be dependant on ββ3 integrins, similar to that reported for other pathogenic hantaviruses such as DOBV included in our studies. ββ1-Integrin chain-specific antibodies and fibronectin did not block MPRLV or DOBV infectivity as observed with the non-pathogenic PHV. Phylogenic analysis of characteristic degron sequences and ITAM motifs in the G1 cytoplasmic tails of MPRLV and other hantaviruses emphasizes the close genetic proximity of MPRLV to other HPS-causing hantaviruses. The data presented suggests that MPRLV may be pathogenic to humans and that it and other hantaviruses tested are sensitive to favipiravir in cell culture.

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54

Small Molecule Inhibitors of Dengue Virus Replication are Active *In Vivo*

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Flaviviruses, including dengue virus, West Nile virus, yellow fever virus, Japanese encephalitis virus, and tick-borne encephalitis virus, are a group of viruses transmitted by mosquitoes or ticks in the Flaviviridae family, that are significant pathogens responsible for emerging infectious disease in both humans and animals. Dengue virus is considered to be one of the most important viruses

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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55

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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56

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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57

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