Preclinical Characterization of the Novel Hepatitis C Virus NS3 Protease Inhibitor GS-9451

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GS-9451 is a selective hepatitis C virus (HCV) NS3 protease inhibitor in development for the treatment of genotype 1 (GT1) HCV infection. Key preclinical properties of GS-9451, including in vitro antiviral activity, selectivity, cross-resistance, and combination activity, as well as pharmacokinetic properties, were determined. In multiple GT1a and GT1b replicon cell lines, GS-9451 had mean 50% effective concentrations (EC_{50}) of 13 and 5.4 nM, respectively, with minimal cytotoxicity; similar potency was observed in chimeric replicons encoding the NS3 protease gene of GT1 clinical isolates. GS-9451 was less active in GT2a replicon cells (EC_{50} = 316 nM). Additive to synergistic in vitro antiviral activity was observed when GS-9451 was combined with other agents, including alpha interferon, ribavirin, and the polymerase inhibitors GS-6620 and tegobuvir (GS-9190), as well as the NS5A inhibitor ledipasvir (GS-5885). GS-9451 retained wild-type activity against multiple classes of NS5B and NS5A inhibitor resistance mutations. GS-9451 was stable in hepatic microsomes and hepatocytes from human and three other tested species. Systemic clearance was low in dogs and monkeys but high in rats. GS-9451 showed good oral bioavailability in all three species tested. In rats, GS-9451 levels were ~40-fold higher in liver than plasma after intravenous dosing, and elimination of GS-9451 was primarily through biliary excretion. Together, these results are consistent with the antiviral activity observed in a recent phase 1b study. The results of in vitro cross-resistance and combination antiviral assays support the ongoing development of GS-9451 in combination with other agents for the treatment of chronic HCV infection.

MATERIALS AND METHODS

Compounds. The synthesis and structure-activity of GS-9451 has been described (14). GS-9451, GS-6620 (16), GS-9190 (17), GS-5885 (43), and BILN-2061 were synthesized by Gilead Sciences (Foster City, CA). VX-950 (telaprevir) was purchased from Acme Bioscience (Belmont, CA). RBV and Alpha IFN (IFN-α) were purchased from Sigma (St. Louis, MO) or R&D Systems (Minneapolis, MN).

Cell lines and replicon constructs. Huh-luc and Huh-Lunet cell lines were obtained from ReBlIkon GmbH (Mainz, Germany) (18). The S3 cell line was obtained from Christoph Seeger (Fox Chase Cancer Center, Philadelphia, PA) (19). HepG2 cells were obtained from the American Type Culture Collection (Manassas, VA). MT-4 cells were obtained from the National Institutes of Health AIDS Research and Reference Reagent Program (Germantown, MD). Lunet-CD81 cells were generated and described previously (20). Replicons 2aLucNeo−25 (JFH-1), HSG(1a, H77)-23, HSG-51, HSG-57, HSG-65, and GFP1b-7 (Con-1) have previously been described (21–23). Huh-Lunet and HepG2 cells were maintained in Dulbecco modified Eagle medium (DMEM) with GlutaMAX (Invitrogen,
Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, UT), 1 U of penicillin (Invitrogen)/ml, 1 g of streptomycin (Invitrogen)/ml, and 0.1 mM nonessential amino acids (Invitrogen). Replicon-containing cell lines were maintained in medium with addition of 0.5 mg of G418 (Invitrogen)/ml unless otherwise noted. MT-4 cells were maintained in RPMI 1640 medium ( Gibco) supplemented with 10% FBS. Replicons carrying the NS3 protease gene from patient isolates were generated previously by Qi et al. (24). Adapted GT2a J6/JFH viruses were generated previously (20).

**Transient transfection.** RNA was transcribed in vitro using a MEGAscript kit (Ambion, Austin, TX) and transfected into HuH-Lunet cells using the method of Lohmann et al. (25).

**Replicon assays.** Three-day replicon half-maximal effective concentration (EC_{50}) assays were conducted as described previously (22). Briefly, replicon cells were seeded into 96-well plates at a density of 5 × 10^3 cells per well. Compounds were serially diluted in 100% dimethyl sulfoxide (DMSO) in 3-fold steps and added to cells at a 1:200 dilution. Plates were incubated at 37°C with 5% CO_2 for 3 days. Luciferase expression was quantified in luciferase-encoding replicon cell lines using a commercial luciferase assay (Promega). Relative light units were converted into percentages relative to untreated controls (defined as 100%). EC_{50} values were calculated using the same equation as described above.

**Infectious virus antiviral activity assays.** Lunet-CD81 cells were seeded into 96-well plates and infected with a tissue culture-adapted GT2a virus for antiviral activity assays where NS3 activity was measured as a marker of viral replication as described previously (29).

**In vitro combination studies.** Replicon cells were seeded in 96-well plates at a density of 5 × 10^3 cells per well in 100 μl of DMEM culture medium, excluding G-418, and incubated overnight. Alternatively, replicon cells were seeded in 384-well plates at a density of 2,000 cells per well in 90 μl of DMEM. Compounds were serially diluted in DMSO and added to cells with one compound in the horizontal direction and with the other compound in the vertical direction. A defined set of drug concentrations to cells with one compound in the horizontal direction and with the other compound in the vertical direction. A defined set of drug concentrations was assessed in Sprague-Dawley rats, beagle dogs, cynomolgus monkeys, and humans, as well as cryopreserved Sprague-Dawley rat and human hepatocytes. Hepatic microsomal fractions and components for the NADPH-regenerating system were obtained from BD Biosciences (Woburn, MA). Cryopreserved hepatocytes, hepatocyte thawing medium, and Krebs-Henseleit buffer (KHB) medium were obtained from In Vitro Technologies (Baltimore, MD). All other chemicals were purchased from Sigma-Aldrich or VWR (West Chester, PA).

**Cross-resistance analysis.** Pi-Luc, a bicistronic replicon encoding the firefly luciferase gene (luc) downstream of the polio internal ribosome entry site (IRES) and the GT1b (Con-1) HCV nonstructural genes (NS3 to NS5B) controlled by the encephalomyocarditis virus IRES, was used for transient transfection studies (34). NS3 and NS5B mutations were introduced into the wild-type (WT) Pi-Luc replicon using a QuikChange II XL mutagenesis kit according to the manufacturer’s instructions (Stratagene, La Jolla, CA). Mutations were confirmed by DNA sequencing. Replicon RNAs were transcribed in vitro and transfected into HuH-Lunet cells for determination of susceptibility to GS-9451. GS-9451 was serially diluted in DMEM and tested at a high concentration of 50 μM, ranging down to a low concentration of 0.2 nM. The resistance fold change was calculated as a ratio of mutant EC_{50} versus wild-type EC_{50}.

**In vitro metabolic stability.** The metabolic stability of GS-9451 was assessed in pooled hepatic microsomes from Sprague-Dawley rats, beagle dogs, cynomolgus monkeys, and humans, as well as cryopreserved Sprague-Dawley rat and human hepatocytes. Hepatic microsomal fractions and components for the NADPH-regenerating system were obtained from BD Biosciences (Woburn, MA). Cryopreserved hepatocytes, hepatocyte thawing medium, and Krebs-Henseleit buffer (KHB) medium were obtained from In Vitro Technologies (Baltimore, MD). All other chemicals were purchased from Sigma-Aldrich or VWR (West Chester, PA).

**Pharmacokinetics and excretion in animals.** Animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources) using protocols approved by the Animal Care and Use Committee. Plasma pharmacokinetics was assessed in Sprague-Dawley rats, beagle dogs, and cynomolgus monkeys after intravenous infusion and oral administration. For intravenous dosing, fasted animals (n = 3) were infused over 30 min with GS-9451 at 1 mg/kg in a vehicle containing 1% ethanol, 1% propylene glycol, 4% Labrasol, 4% Solutol HS-15, and 90% phosphate buffer. For oral dosing, fasted animals (n = 3) were dosed with 10 mg/kg (rats), 4 mg/kg (dogs), or 5 mg/kg (monkeys) in a gavage vehicle containing 5% ethanol, 20% propylene glycol, 30% polyethylene glycol 400, and 45% phosphate buffer. Blood samples were collected at appropriate time points into tubes containing EDTA as anticoagulant. Plasma was separated via centrifugation and stored at −70°C.

The liver/plasma ratio was determined in Sprague-Dawley rats after an intravenous bolus dose of GS-9451 at 1 mg/kg. Liver and blood samples were obtained from 3 animals per time point. Blood samples were processed as described above. Liver was flash frozen and stored at −20°C. Biliary excretion was assessed in bile duct-cannulated Sprague-Dawley rats after a 30-min infusion of GS-9451 at 1 mg/kg or 10 mg/kg. Bile, urine, and plasma samples were collected at appropriate time points.
TABLE 1 Antiviral activity of GS-9451 against genotype 1 and 2a HCV replicons

<table>
<thead>
<tr>
<th>Compound</th>
<th>GT1a genotype, replicon cell type&lt;sup&gt;b&lt;/sup&gt;</th>
<th>GT1b genotype, replicon cell type</th>
<th>GT2a genotype, replicon cell type</th>
<th>Patient isolates&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HSG-23</td>
<td>Huh-luc</td>
<td>2aLucNeo-25</td>
<td>SL3</td>
</tr>
<tr>
<td>GS-9451</td>
<td>9.3 ± 0.9</td>
<td>1.8 ± 0.9</td>
<td>7.2 ± 0.7</td>
<td>7.2 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>1.8 ± 0.9</td>
<td>0.7 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>0.9 ± 0.8</td>
</tr>
<tr>
<td>BILN-2061*</td>
<td>19 ± 5.5</td>
<td>33 ± 0.3</td>
<td>62 ± 6.7</td>
<td>1346 ± 364</td>
</tr>
<tr>
<td>VX-950*</td>
<td>157 ± 6.0</td>
<td>322 ± 19</td>
<td>1346 ± 364</td>
<td>346 ± 110</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values represent the means from 10 patient isolates for each genotype.
<sup>b</sup> Values represent the means of at least two independent experiments.
<sup>c</sup> Values represent the means from 10 patient isolates for each genotype. n = 10 for both GT1a and GT1b.

RESULTS

Activity in cellular assays. In multiple GT1a (H77 strain) and GT1b (Con-1 strain) replicon cell lines, GS-9451 had mean EC<sub>50</sub> of 13 and 5.4 nM, respectively (Table 1), with minimal cytotoxicity (CC<sub>50</sub> of >50,000 nM in GT1a and >40,000 nM in GT1b). These data result in a selectivity index of >27,000 for both GT1a (HSG-51 cell line) and GT1b (Huh-luc cells) (Table 2). To assess the susceptibility of natural HCV variants to GS-9451, a panel of chimeric replicons carrying the NS3 protease gene from HCV-infected patient isolates was tested in a transient replicon antiviral assay. Of the 10 GT1a and 10 GT1b NS3 protease patient isolates tested, GS-9451 displayed potent activity against the GT1a and GT1b isolates with mean EC<sub>50</sub> of 8.7 and 11.8 nM, respectively (Table 1). These values are similar to the mean EC<sub>50</sub> of 9.1 nM obtained with the GT1b Con-1 laboratory strain of HCV in the transient replication assay (data not shown) and in stable GT1a and GT1b replicon cell lines (Table 1). In addition, the EC<sub>50</sub> of GS-9451 against the least susceptible clinical isolate was <30 nM (data not shown).

GS-9451 was less active when tested against GT2a HCV. Mean EC<sub>50</sub> were 316 and 251 nM when GS-9451 was tested against GT2a (JFH-1 strain) subgenomic replicon cells and cells infected with a GT2a (JFH-1 strain) virus, respectively.

Combination studies. To assess antiviral drug interactions, GS-9451 was tested in combination with approved or development-stage anti-HCV agents in a 3-day replicon assay. Antiviral drug interactions that deviated from additivity and reached statistical significance (at 95% confidence envelopes) were quantified using MacSynergy II software. GS-9451 had minor antiviral synergy when combined with IFN-α (32 nM<sup>2</sup>) and moderate antiviral synergy when combined with RBV (54 nM<sup>2</sup>) (Fig. 1). Combinations of GS-9451 with nucleoside (GS-6620) (16) or non-nucleoside (tegobuvir [GS-9190]) (17) NS5B polymerase inhibitors and an NSSA inhibitor (ledipasvir [GS-5885]) (43) showed additive activity toward minor synergy. No additive cytotoxicity was observed when GS-9451 was combined with ledipasvir, GS-6620, tegobuvir, IFN-α, or RBV at any of the tested concentrations (data not shown).

Cross-resistance. To investigate whether GS-9451 is active against reported HCV resistance mutations, transient transfection experiments were performed using a panel of site-directed mutants, including those resistant to NS3/4A protease inhibitors (35), as well as those resistant to nucleoside or non-nucleoside NS5B polymerase inhibitors and NSSA inhibitors (36, 37). The NS3 variants R155K, A156T, and D168V are cross-resistant to GS-9451 (1,480- to 8,321-fold, Fig. 2); however, a subset of VX-950-resistant variants (V36A/M, T54A, A156S) remained fully susceptible to GS-9451. GS-9451 retained full activity against vari-

TABLE 2 Cytotoxicity of GS-9451 after 3 days of exposure to HCV genotype 1 replicon cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>Genotype 1a replicon cell line (HSG-51)</th>
<th>Genotype 1b replicon cell line (Huh-luc)</th>
<th>Genotype 1b replicon cell line (GFP1b-7)</th>
<th>Genotype 1b replicon cell line (SL3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC&lt;sub&gt;50&lt;/sub&gt; (nM) SI</td>
<td>CC&lt;sub&gt;50&lt;/sub&gt; (nM) SI</td>
<td>CC&lt;sub&gt;50&lt;/sub&gt; (nM) SI</td>
<td>CC&lt;sub&gt;50&lt;/sub&gt; (nM) SI</td>
</tr>
<tr>
<td>GS-9451</td>
<td>&gt;50,000</td>
<td>&gt;27,778</td>
<td>&gt;50,000</td>
<td>&gt;27,778</td>
</tr>
<tr>
<td>BILN-2061*</td>
<td>27,221</td>
<td>4,462</td>
<td>50,000</td>
<td>71,428</td>
</tr>
<tr>
<td>VX-950*</td>
<td>44,000</td>
<td>807</td>
<td>50,000</td>
<td>129</td>
</tr>
</tbody>
</table>

<sup>a</sup> CC<sub>50</sub>, selectivity index, calculated as the CC<sub>50</sub> divided by the EC<sub>50</sub>. Values represent the means of two or more independent experiments.
<sup>b</sup> BILN-2061 and VX-950 were included as experimental controls to validate the assays.
ants resistant to various classes of NS5B polymerase and NS5A inhibitors (Fig. 2). D168V and R155K are resistant variants identified during GS-9451 in monotherapy (15). In the case of D168V, this variant was also selected in vitro by BILN-2061 (38). The R155K variant is resistant to all protease inhibitors described to date (36).

In vitro metabolic stability. GS-9451 was stable in the microsomal fractions of all tested species (t1/2 H11022 395 min) except the monkey, where moderate turnover was observed (t1/2 H11005 79 min, Table 3). In agreement with the observed microsomal stability, GS-9451 was also stable in human and rat cryopreserved hepatocytes (t1/2 H11022 395 min, Table 3).

Pharmacokinetics and excretion. GS-9451 showed good oral bioavailability in rats (62%), dogs (142%), and monkeys (49%). The systemic clearance of GS-9451 was low in dogs and monkeys but high in rats, with intravenous plasma elimination half-lives of 0.62, 4.2, and 3.9 h in rats, dogs, and monkeys, respectively (Table 4). To characterize the distribution of GS-9451, liver and plasma concentrations were quantified and compared at 2, 6, and 12 h after an intravenous bolus dose in rats. GS-9451 rapidly distributed to the liver with concentrations 40-fold higher than plasma at 2- and 6-h time points (Table 5); GS-9451 remained detectable in the liver at 12 h, whereas it was below the limit of quantification in the plasma at this time point. The elimination of GS-9451 was investigated in bile duct cannulated rats by quantification of GS-9451 concentrations in bile and urine, as well as by profiling and identifying the metabolites of GS-9451 in plasma and bile (data not shown). These studies indicated that GS-9451 is cleared primarily through biliary secretion of the unmodified GS-9451 and its acylglucuronide conjugates; very little GS-9451 or its metabolites were found in urine.

DISCUSSION

NS3 protease inhibitors (telaprevir and boceprevir) have become a key component of combination therapies for HCV in GT1 patients. Novel NS3 protease inhibitors are needed to improve treatment with better tolerability and lower drug-drug interactions.

<table>
<thead>
<tr>
<th>TABLE 3 Rate of metabolism of GS-9451 in hepatic microsomes and hepatocytes</th>
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<tbody>
<tr>
<td>Microsome or hepatocyte</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>Microsomal fractions</td>
</tr>
<tr>
<td>Rat</td>
</tr>
<tr>
<td>Dog</td>
</tr>
<tr>
<td>Monkeyb</td>
</tr>
<tr>
<td>Human</td>
</tr>
<tr>
<td>Hepatocytes</td>
</tr>
<tr>
<td>Rat</td>
</tr>
<tr>
<td>Human</td>
</tr>
</tbody>
</table>

a t1/2, half-life.

b Values for monkey represent the means of one experiment performed in duplicate.
GS-9451 is a novel acyclic, noncovalent NS3 inhibitor being developed for the treatment of GT1 HCV infection (14, 39).

Our preclinical studies demonstrate that GS-9451 is a selective inhibitor of GT1a and GT1b HCV using both laboratory strains, as well as a panel of NS3 protease gene isolates from 20 patients. Of note, in patient isolates, EC_{50}s for GS-9451 were similar against GT1a and GT1b, and we did not observe isolates with markedly reduced susceptibility compared to transient or stable subgenomic replicons. These findings suggest that GS-9451 will have similar efficacy across diverse viruses in GT1a and GT1b patients and are in agreement with recent phase 1b clinical results. The results in the clinic showed that at a dose of 200 mg once daily, median maximal HCV RNA reductions were ~3.2 log_{10} in GT1a patients and ~3.5 log_{10} in GT1b patients during 3 days of monotherapy (39). In contrast, GS-9451 has significantly less activity against GT2a (approximately 24- to 58-fold compared to GT1a and GT1b), suggesting that GS-9451 would be less efficacious in this patient population.

Based on the limited efficacy of PEG-IFN plus RBV and the rapid emergence of resistance to most DAAs, clinical research has focused on combination therapies for HCV. Ideally, drug combinations will result in more potent suppression of HCV and prevent the emergence of resistance which will ultimately translate into a rapid and complete eradication of HCV in high proportions of patients. With this in mind, critical preclinical studies used to evaluate new inhibitors include the cross-resistance profile and antiviral drug interactions in combination with other anti-HCV compounds.

The cross-resistance profile of GS-9451 was evaluated against known resistance mutations for several classes of HCV inhibitors. We observed that NS3 variants resistant to other cyclic or acyclic noncovalent NS3 inhibitors (R155K, A156T, and D168V) conferred significant cross-resistance to GS-9451 (1,480- to 8,321-fold); these findings were predictive of resistance mutations that emerged during phase 1b evaluation of GS-9451 (15). In contrast, a subset of VX-950 resistant variants (V36A/M, T54A, and A156S) remained susceptible to GS-9451, suggesting that it may be possible to re-treat some patients who fail VX-950 with combination regimens, including GS-9451. As expected, GS-9451 retained wild-type activity against all replicons encoding variants resistant to NS5A and NS5B inhibitors, including those that confer resistance to the development-stage inhibitors ledipasvir (NS5A inhibitor), sofosbuvir (nucleoside NS5B inhibitor), GS-9669 (site II non-nucleoside NS5B inhibitor) (40), and tegobuvir (site III/IV non-nucleoside NS5B inhibitor).

In vitro combination experiments indicate that GS-9451 has additive to synergistic antiviral activity when combined with all other drug classes tested, including standard-of-care agents (IFN-α and RBV) and the NS5B inhibitors (GS-6620 and tegobuvir in the present study), as well as sofosbuvir and GS-9669, as reported by coworkers (40, 41), and NS5A inhibitors (ledipasvir). Importantly, no significant antagonism or unexpected toxicities were observed during any of these in vitro drug combination studies. Overall, results of both cross-resistance and in vitro antiviral combination studies indicate that GS-9451 can be combined with PEG-IFN and RBV, as well as NS5A and NS5B inhibitors. Accordingly, several phase 2 studies are ongoing to investigate the safety and efficacy of GS-9451 in combination with other agents in both interferon-containing and interferon-free regimens.

GS-9451 also demonstrated favorable metabolic and pharmacokinetic properties in preclinical studies. The in vitro metabolic stability (t_{1/2} > 395 min) of GS-9451 in both hepatocytes and microsomal fractions indicates low potential for hepatic oxidative metabolism in humans. GS-9451 also has favorable pharmacokinetic properties in nonclinical species (a plasma t_{1/2} of ~4 h in dogs and monkeys). Additional profiling in rats demonstrated that GS-9451 was enriched in liver (the site of HCV replication) to ~40-fold above plasma concentrations. Studies in bile duct cannulated rats indicated that GS-9451 is cleared primarily through biliary secretion of the unmetabolized parent compound. The constitutive biliary secretion of rats (versus other nonrodent species) potentially explains the substantially shorter plasma t_{1/2} observed in rats (0.6 h).

Based on allometric scaling of these preclinical data, GS-9451 was predicted to have a plasma t_{1/2} of 6 to 10 h in humans, a finding supportive of once a day (QD) or twice-daily (BID) administration at low doses. During the phase 1b study in HCV-infected patients, GS-9451 was observed to have a median plasma t_{1/2} 14 to 17 h at doses of 60 to 400 mg administered once daily.

In summary, GS-9451 is a selective inhibitor of GT1 HCV NS3 with favorable preclinical pharmacokinetic properties. These results are consistent with the antiviral activity observed in a recent phase 1b monotherapy study (42). In addition, GS-9451 has an orthogonal resistance profile, as well as an additive to synergistic in vitro antiviral activity with respect to other classes of HCV in-

### TABLE 4 Mean plasma PK parameters for GS-9451 in preclinical species

<table>
<thead>
<tr>
<th>Species</th>
<th>Intravenous administration</th>
<th>Oral administration</th>
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<tbody>
<tr>
<td>Dose (mg/kg)</td>
<td>AUC_{0-∞} (nM·h)</td>
<td>CL (L/min/kg)</td>
</tr>
<tr>
<td>Rat</td>
<td>0.1</td>
<td>10</td>
</tr>
<tr>
<td>Dog</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Monkey</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

a AUC, area under the concentration-time curve; CL, clearance; C_{max}, maximum plasma concentration; %F, percent bioavailability; MRT, mean residence time; t_{1/2}, half-life; t_{max}, time to C_{max}; V_{ss}, volume of distribution at steady state. Values are expressed as means ± standard deviations for three males per species for each route of administration.

### TABLE 5 Mean GS-9451 concentrations in plasma and liver following a 1-mg/kg intravenous bolus dose in rats

<table>
<thead>
<tr>
<th>Time postinfusion (h)</th>
<th>Liver (nM)</th>
<th>Plasma (nM)</th>
<th>Liver/plasma ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1,830 ± 77</td>
<td>53.7 ± 20.3</td>
<td>40 ± 24</td>
</tr>
<tr>
<td>6</td>
<td>177 ± 78</td>
<td>4.2 ± 0.2</td>
<td>42 ± 19</td>
</tr>
<tr>
<td>12</td>
<td>20.6 ± 3.3</td>
<td>BLQ</td>
<td></td>
</tr>
</tbody>
</table>

a Values are means for three males per time point. The liver/plasma ratio at 12 h could not be calculated because the plasma levels at that time point were below the limit of quantitation (BLQ) of 1.0 nM.
hibitors. These results support the ongoing phase 2b investigation of GS-9451 in combination with other agents, including PEG-IFN, RBV, tegobuvir, and ledipasvir (NS5A inhibitor), as well as potential future studies in combination with sofosbuvir (nucleoside NS5B inhibitor) and GS-9669 (site II non-nucleoside NS5B inhibitor).

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REFERENCES


