**BIOLOGICAL ACTIVITY**

**Description**
PSI-352938 (PSI-938) is a hepatitis C virus (HCV) nucleotide inhibitor[^1].

**IC₅₀ & Target**
HCV[^1]

**In Vitro**
PSI-352938 (PSI-938) and PSI-353661 inhibit HCV genotype (GT) 1b replicon replication with 50% effective concentrations (EC₅₀) of 0.13±0.076 μM and 3.0±1.4 nM, respectively, and are similarly active against GT 1a and 2a replicons and infectious viruses. Metabolism of PSI-352938 and PSI-353661 generates the same 5′-triphosphate metabolite, PSI-352666, which is similarly active against NS5B polymerases from GT 1 to 4[^1]. PSI-352938 (PSI-938) is a novel cyclic phosphate prodrug of β-D-2′-deoxy-2′-α-fluoro-2′-β-C-methylguanosine 5′-monophosphate that has potent activity against HCV. PSI-352938 (PSI-938) has similar activity against genotype 1a, 1b, and 2a replicons, with EC₅₀ ranging from 0.13 to 0.20 μM and EC₉₀ values ranging from 0.35 to 0.74 μM. PSI-352938 (PSI-938) also effectively inhibits HCV replication in the infectious virus assays: the EC₅₀ and EC₉₀ values are 0.28±0.083 μM and 0.63±0.018 μM, respectively, against the H77 infectious virus and 0.39±0.31 μM and 1.16±0.64 μM, respectively, against the JFH-1 infectious virus. In contrast, PSI-352938 is not active against HBV or HIV up to the highest concentration tested (EC₅₀>100 μM[^2]).

**PROTOCOL**

**Cell Assay[^1]**
GT 1a, 1b, and 2a replicon cells are cultured in the presence of G418 (0.75 mg/mL for GT 1a, 0.25 mg/mL for GT 1b and 2a) and increasing concentrations of PSI-352938 (PSI-938) or PSI-353661 starting at their respective EC₅₀ or EC₉₀. As a no-compound control, replicon cells are maintained in parallel in the equivalent percent volume (0.2%) of DMSO. Cells are passaged whenever they reach ~80% confluence and replenished with G418 medium containing fresh compound. At various passages, cells are tested for sensitivity to PSI-352938 (PSI-938) and PSI-353661. For each assay, 3-fold dilutions of test compound are added to cells in duplicate and incubated at 37°C in a humidified 5% CO₂ atmosphere for 4 days. Inhibition of HCV replicon RNA replication is determined by real-time PCR (RT-PCR) using primers that anneal to the 5′ untranslated region or by measuring the levels of luminescence expressed via the firefly or Renilla luciferase reporter gene using the Bright-Glo or Renilla-Glo reagent, respectively. EC₅₀ and EC₉₀, the concentrations at which 50% and 90% inhibition are achieved, are determined using GraphPad Prism software. Aliquots of cells are also saved for RNA isolation, cDNA synthesis, and PCR amplification for sequencing analysis[^1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.
REFERENCES
