

Product Data Sheet

Product Name: Danoprevir (RG7227)

Cat. No.: GC12879

Chemical Properties

Cas. No. 850876-88-9

Chemical Name (2R,6S,13aS,14aR,16aS,Z)-6-((tert-butoxycarbonyl)amino)-14a-((cyclopropylsulfonyl)carbamoyl)-5,16-dioxo-1,2,3,5,6,7,8,9,10,11,13a,14,14a,15,16,16a-hexadecahydrocyclopro[1,2-a]pyrrolo[1,4]diazacyclopentadecin-2-yl 4-fluoroisoindoline-2-carboxylate

SMILES FC1=CC=CC2=C1CN(C2)C(O[C@@H]3C[C@](C(N[C@@]4(C(NS(=O)(C5CC5)=O)=O)[C@@](/C=C\CCCC[C@@H]6N([H])C(OC(C)(C)C)=O)([H])C4=O)([H])N(C6=O)C3)=O

Formula C₃₅H₄₆FN₅O₉S

M.Wt 731.83

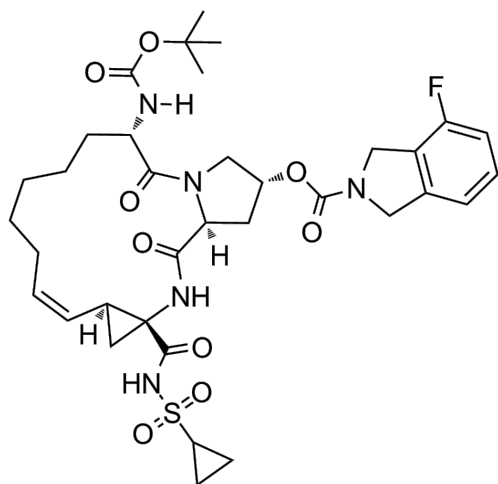
Solubility ≥ 32.6 mg/mL in DMSO, ≥ 46.4 mg/mL in EtOH with ultrasonic

Storage Store at -20°C

General tips For obtaining a higher solubility, please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution: ship with blue ice. All other available size: ship with RT, or blue ice upon request.

Structure



Protocol

Caution: Product has not been fully validated for medical applications. For research use only.

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

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Kinase experiment [1]:

Binding assays

Protease activity for K2040 and genotype 1 to 6 NS3 proteins was followed in a continuous fluorescent resonance energy transfer (FRET)-based assay. The assay buffer contained 25 μ M NS4A peptide, 50 mM Tris-HCl, pH 7.5, 15% (vol/vol) glycerol, 0.6 mM lauryldimethylamine N-oxide, 10 mM dithiothreitol, and 0.5 μ M fluorescein/QXL520-labeled FRET substrate. Typically, 50 pM K2040 enzyme was added to initiate the reaction. Reactions were set up in black 96-well plates, and fluorescence data were collected using a SpectraMax M5 plate reader. Recovery of activity from preformed ITMN-191-NS3/4A complex was assessed by preincubating 10 nM NS3/4A with a twofold excess of ITMN-191 in 1 $\times$ assay buffer for 15 min, followed by a rapid 200-fold dilution of the preformed complex into assay buffer containing substrate.

Cell experiment [1]:

Cell lines

Huh7 cells harboring HCV replicon

Preparation method

The solubility of this compound in DMSO is >32.6mg/mL. General tips for obtaining a higher concentration: Please warm the tube at 37 °C for 10 minutes and/or shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Reacting condition antiviral assays: 100 nM to 5 pM cytotoxicity assays: 1 mM to 5.6 nM

Applications

ITMN-191 displayed a high degree of specificity for its intended target. In replicon-bearing cells, ITMN-191 (3.7 nM-15 nM) promoted a 3.7 log₁₀ reduction in replicon levels upon 14 days of in vitro treatment but did not clear HCV replicon from every cell. Treatment with ITMN-191 (45 nM) reduced HCV replicon RNA levels and completely cleared replicon RNA.

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Animal experiment [1]:

Animal models Rats and monkeys

Dosage form Oral gavage, 30 mg/kg

Application Danoprevir (30 mg/kg) administered to rats or monkeys shows that its concentrations in liver 12 hours after dosing exceed the Danoprevir concentration required to eliminate replicon RNA from cells.

Other notes Please test the solubility of all compounds indoor, and the actual solubility may slightly differ with the theoretical value. This is caused by an experimental system error and it is normal.

References:

[1]. Seiwert S D, Andrews S W, Jiang Y, et al. Preclinical characteristics of the hepatitis C virus NS3/4A protease inhibitor ITMN-191 (R7227)[J]. Antimicrobial agents and chemotherapy, 2008, 52(12): 4432-4441.

Background

Danoprevir (R7227) is a potent and selective inhibitor of Hepatitis C Virus (HCV) NS3/4A protease, a chymotrypsin-like serine protease playing an essential role in the viral replication process of HCV, that non-covalently binds to and hence inhibits HCV NS3 protease with 50% inhibition concentration IC₅₀ values ranging from 0.2 to 3.5 nM. X-ray crystallographic analysis has revealed that the cyclopropyl acylsulfonamide of danoprevir occupies the SI/SI' pocket of HCV NS3 protease with the acyl carbonyl oxygen forming hydrogen bonds to Gly137 and Ser138 in the oxyanion hole of the protease active site and the acyl sulfonamide nitrogen forming a hydrogen bond with His57.

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[1].Jiang Y, Andrews SW, Condroski KR, Buckman B, Serebryany V, Wenglow sky S, Kennedy AL, Madduru MR, Wang B, Lyon M, Doherty GA, Woodard BT, Lemieux C, Do MG, Zhang H, Ballard J, Vigers G, Brandhuber BJ, Stengel P, Josey JA, Beigelman L, Blatt L, Seiwert SD. Discovery of Danoprevir (ITMN-191/R7227), a Highly Selective and Potent Inhibitor of Hepatitis C Virus (HCV) NS3/4A Protease. *J Med Chem.* 2013 May 28. [Epub ahead of print]

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