Datasheet

Danoprevir (ITMN-191)

Product Name	Danoprevir (ITMN-191)
Catalogue Number	BSV-S1183
Chemical Formula	$C_{35}H_{46}FN_5O_9S$
Function	HCV protease inhibitor
CAS No.:	850876-88-9

Description:

Danoprevir(ITMN-191) is a peptidomimetic inhibitor of the **NS3/4A protease** of hepatitis C virus (HCV) with **IC50** of 0.2-3.5 nM, inhibition effect for HCV genotypes 1A/1B/4/5/6 is ~10-fold higher than 2B/3A. Phase 2.

Product Details:

Target: HCV NS3/4A protease [1] 0.2 nM-3.5 nM

Chemical name: 2H-Isoindole-2-carboxylic acid, 4-fluoro-1,3-dihydro-, (2R,6S,13aS,14aR,16aS)-14a-[[(cyclopropylsulfonyl)amino]carbonyl]-6-[[(1,1-dimethylethoxy)carbonyl]amino]-1,2,3,5,6,7,8,9,10,11,13a,14,14a,15,16,16a-hexadecahydro-5,16-dioxocyclopropa[e]pyrrolo[1,2-a][1,4]diazacyclopentadecin-2-yl ester

Formula: $C_{35}H_{46}FN_5O_9S$

Molecular weight: 731.83

Purity: 95.40 % (HPLC)

Solubility: 144 mg/mL (DMSO), 144 mg/mL (ethanol)

Storage: 3 years -20°C powder, 2 years -80°C in solvent

Regulatory/ Restrictions: For laboratory use only.

Preparing stock solutions:

Concentration/ Mass	1 mg	5 mg	10 mg
1 mM	1.3664 mL	6.8322 mL	13.6644 mL
5 mM	0.2733 mL	1.3664 mL	2.7329 mL
10 mM	0.1366 mL	0.6832 mL	1.3664 mL
50 mM	0.0273 mL	0.1366 mL	0.2733 mL

Biological activity:

In vitro:
Danoprevir (0.29 nM) inhibits the reference genotype 1 NS3/4A protease half-maximally, but a high dose of Danoprevir (10 μM) shows no appreciably inhibition in a panel of 79 proteases, ion channels, transporters, and cell surface receptors. Danoprevir remains bound to and inhibits NS3/4A for more than 5 hours after its initial association. Danoprevir (45 nM) eliminates a patient-derived HCV genotype 1b replicon from hepatocyte-derived Huh7 cells with an EC50 of 1.8 nM. [1] In HCV subgenomic replicon cell lines containing the individual mutations, V36M, R109K, and V170A substitutions confer little or no resistance to Danoprevir, but the R155K substitution confers a high level (62-fold increase) of resistance to Danoprevir. [2] In Huh7.5 cells transfected with chimeric recombinant virus, Danoprevir shows antiviral inhibition effects against HCV genotypes 1, 4 and 6 with IC50 of 2-3 nM, which are >100-fold lower than genotypes 2/3/5 (280-750 nM). [3]

In vivo:

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. [1]

A peptidomimetic inhibitor of the NS3/4A protease of hepatitis C virus (HCV).

Protocol *(Only for Reference)*

Kinase Assay: [1]	
Continuous fluorescent resonance energy	The assay buffer contains 25 μM 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 μM fluorescein/QXL520-labeled FRET substrate {Ac-DE-Dap(QXL520)-EE-Abu-ψ-[COO]-AS-Cys(5-FAMsp)-NH2}. K2040 enzyme (50

transfer (FRET) assay	pM) is added to initiate the reaction. Reactions are set up in black 96-well plates, and fluorescence data is collected. Control reactions lacking inhibitors and enzyme are included. Initial rates are calculated from the linear phase of the reaction (up to 1 hour) and are used to obtain IC50. Recovery of activity from preformed Danoprevir-NS3/4A complex is assessed by preincubating 10 nM NS3/4A with a two-fold excess of Danoprevir in 1× assay buffer for 15 min, followed by a rapid 200-fold dilution of the preformed complex into assay buffer containing substrate. A control reaction with the same final conditions without preincubation of NS3/4A and Danoprevir is initiated by the addition of enzyme to an otherwise-complete reaction mixture. Additional control reactions lack either Danoprevir or NS3. The progress of the reactions is followed over 5 hours.
Cell Assay: [1]	
Cell lines	Huh7 cells harboring HCV replicon
Concentrations	5 pM - 100 nM
Incubation Time	48 hours
Method	Serially diluted Danoprevir is added to Huh7 cells harboring the K2040 replicon 1 day after cell plating. For antiviral assays, after a 48-hour incubation, intracellular RNA is extracted, and the level of HCV replicon RNA is quantified by reverse transcription (RT)-PCR assay with the primers (5'-CACTCCCCTGTGAGGAACTACTG-3' and 5'-AGGCTGCACGACACTCATACT-3') and a probe (5'-6-FAM-CTTCACGCAGAAAGCGTCTAGCCATGG-MGBNFQ-3' using an ABI Prism 7900 sequence detection system. Here, FAM is 6-carboxyfluorescin and MGBNFQ is a molecular-groove binding non-fluorescence quencher specific to the HCV 5' untranslated region. Single-tube reactions are performed using the TaqMan Gold RT-PCR kit. Triplicate reactions for the RNA standards and samples are performed in 50 µL with 5 µL intracellular RNA (50 ng). RT is carried out at 48 °C for 30 min followed by 10 min at 95 °C. The PCR is run as follows: 15 seconds at 95 °C and 1 min at 60 °C for 40 cycles. Each RNA concentration is determined in triplicate. The absolute concentration of replicon RNA is calculated based on its signal relative to that of a standard curve generated by known concentrations of an in vitro-transcribed RNA corresponding to a genotype 1b 5' untranslated region. Replicon levels in the presence of Danoprevir are fitted to a four-parameter logistic function to obtain EC50.
Animal Study: [1]	
Animal Models	Sprague-Dawley rats, Cynomolgus monkeys
Dosages	30 mg/kg
Administration	Oral gavage

References:

[1] Seiwert SD, et al. Antimicrob Agents Chemother, 2008, 52(12), 4432-4441.
[2] Bartels DJ, et al. J Infect Dis, 2008, 198(6), 800-807.
[3] Imhof I, et al. Hepatology, 2011, 53(4), 1090-1099.