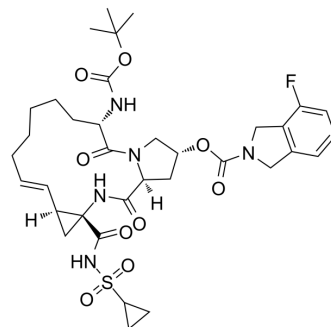


Danoprevir

Cat. No.:	HY-10238		
CAS No.:	850876-88-9		
Molecular Formula:	C ₃₅ H ₄₆ FN ₅ O ₉ S		
Molecular Weight:	731.83		
Target:	HCV Protease; HCV; SARS-CoV		
Pathway:	Anti-infection; Metabolic Enzyme/Protease		
Storage:	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	2 years
		-20°C	1 year



SOLVENT & SOLUBILITY

In Vitro

DMSO : ≥ 100 mg/mL (136.64 mM)
 * "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent 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

Please refer to the solubility information to select the appropriate solvent.

In Vivo

- Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
 Solubility: ≥ 2.5 mg/mL (3.42 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% corn oil
 Solubility: ≥ 2.5 mg/mL (3.42 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

Danoprevir (ITMN-191) is an orally active NS3/4A protease inhibitor for hepatitis C virus (HCV) with an IC₅₀ of 0.29 nM and is selective for NS3/4A over a panel of 53 proteases (IC₅₀ higher than 10 μM). Danoprevir (ITMN-191) inhibits HCV genotypes 1a, 1b, 4, 5, and 6 (IC₅₀s=0.2-0.4 nM) as well as 2b and 3a (IC₅₀s=1.6, 3.5 nM)^{[1][2]}. Danoprevir is also a SARS-CoV 3CL^{PRO} inhibitor with an IC₅₀ of 0.05 μM^[3].

IC₅₀ & Target

IC₅₀: 0.29 nM (NS3/4A protease), 0.2-3.5 nM (HCV genotypes 1a, 1b, 2b, 3a, 4, 5, 6)^[2]

In Vitro

In Huh7.5 cells transfected with chimeric recombinant virus, Danoprevir (ITMN-191) 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)^[1].

Danoprevir (ITMN-191) inhibits the reference genotype 1 NS3/4A protease half-maximally, but a high dose of Danoprevir (ITMN-191) (10 μ M) shows no appreciable inhibition in a panel of 79 proteases, ion channels, transporters, and cell surface receptors. Danoprevir (ITMN-191) remains bound to and inhibits NS3/4A for more than 5 hours after its initial association. Danoprevir (ITMN-191) (45 nM) eliminates a patient-derived HCV genotype 1b replicon from hepatocyte-derived Huh7 cells with an EC₅₀ of 1.8 nM^[2].

In HCV subgenomic replicon cell lines containing the individual mutations, V36M, R109K, and V170A substitutions confer little or no resistance to Danoprevir (ITMN-191), but the R155K substitution confers a high level (62-fold increase) of resistance to Danoprevir^[3].

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

In Vivo

Danoprevir (ITMN-191) (30 mg/kg, p.o.) 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^[2].

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

PROTOCOL

Kinase Assay ^[2]

The assay buffer contains 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 {Ac-DE-Dap(QXL520)-EE-Abu- ψ -[COO]-AS-Cys(5-FAMsp)-NH₂}. K2040 enzyme (50 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 \times 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.

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Animal Administration ^[2]

Pharmacokinetic properties are evaluated in rats and monkeys. Sprague-Dawley rats (three per time point) are administered a 30-mg/kg of body weight dose of ITMN-191 by oral gavage (a 6-mg/mL solution in water). Cynomolgus monkeys (two per time point) are administered a 30-mg/kg dose of ITMN-191 by oral gavage (a 3-mg/mL solution in water). For each species, terminal blood samples and the entire perfused liver are collected 1, 4, 8, 12, and 24 h after dose administration. Blood samples are collected in EDTA, processed for plasma by centrifugation at 5°C, and stored at -20°C until analysis is performed. Liver samples are snap-frozen and stored at -70°C until analysis is performed. Blank, standard, and unknown plasma samples and homogenized liver containing an internal standard (ITMN-191 analog) are treated with acidified acetonitrile and centrifuged to remove precipitated proteins. The density of liver tissue is taken into account to allow concentrations in both compartments to be expressed as weight per unit volume. The cleared supernatants are diluted 1:1 into high-performance liquid chromatography grade water and analyzed on a 4000 Q-trap liquid chromatography-tandem mass spectrometer fitted with the Turbo-Ionspray source operating in negative-ion mode. Analytes and internal standards are monitored using multiple-reaction-monitoring scans and calibrated with ABI Analyst software, version 1.4.2. The calibration standards ranges from 0.0169 ng/mL to 37.0 ng/mL and from 7.47 ng/mL to 5,440 ng/mL for the quantification of plasma samples and liver homogenates, respectively. Quadratic fitting with 1/x weighting is utilized where an R² value of > 0.999 is achieved in both matrices.

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CUSTOMER VALIDATION

- Nat Methods. 2018 Jul;15(7):519-522.
- Signal Transduct Target Ther. 2021 May 29;6(1):212.

- Nat Commun. 2014 Oct 30;5:5352.
- Int J Radiat Oncol Biol Phys. 2016 Nov 15;96(4):867-876.
- Virology. 2014 May;456-457:300-9.

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- [1]. Imhof I, et al. Genotype differences in susceptibility and resistance development of hepatitis C virus to protease inhibitors telaprevir (VX-950) and danoprevir (ITMN-191). *Hepatology*. 2011 Apr;53(4):1090-9.
- [2]. Seiwert, Scott D., et al. Preclinical characteristics of the hepatitis C virus NS3/4A protease inhibitor ITMN-191 (R7227). *Antimicrobial Agents and Chemotherapy* (2008), 52(12), 4432-4441.
- [3]. Bartels DJ, et al. Natural prevalence of hepatitis C virus variants with decreased sensitivity to NS3.4A protease inhibitors in treatment-naive subjects. *J Infect Dis*. 2008 Sep 15;198(6):800-7.
- [4]. Qi Sun, et al. Bardoxolone and bardoxolone methyl, two Nrf2 activators in clinical trials, inhibit SARS-CoV-2 replication and its 3C-like protease. *Signal Transduct Target Ther*. 2021 May 29;6(1):212.
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Caution: Product has not been fully validated for medical applications. For research use only.

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