Product Datasheet

Technical Data

Formula	$C_{35}H_{46}FN_5O_9S$					
Molecular Weight	731.83	**CAS No.**	850876-88-9			
Solubility (25°C)*	In vitro	DMSO	144 mg/mL (196.76 mM)			
		Ethanol	144 mg/mL (196.76 mM)			
		Water	Insoluble			
	In vivo (Add solvents to the product individually and in order)	Oral Administration	Homogeneous suspension	CMC-NA	≥5mg/ml	Taking the 1 mL working solution as an example, add 5 mg of this product to 1 ml of CMC-Na solution, mix evenly to obtain a homogeneous suspension with a final concentration of 5 mg/ml.
		Injection	Clear solution	5%DMSO [1]→ 40%PEG300 [2]→ 5%Tween80 [3]→ 50%ddH2O Validated by Capsid Bio labs. Should you need adjustments to this formulation, contact our sales team for custom testing.	3.600mg/ml (4.92mM)	Taking the 1 mL working solution as an example, add 50 μL of 72 mg/ml clarified DMSO stock solution to 400 μL of PEG300, mix evenly to clarify it; add 50 μL of Tween80 to the above system, mix evenly to clarify; then continue to add 500 μL of ddH2O to adjust the volume to 1 mL. The mixed solution should be used immediately for optimal results.
			Clear solution	5% DMSO [1]→ 95% Corn oil Validated by Capsid Bio labs. Should you need adjustments to this formulation, contact our sales team for custom testing.	1.210mg/ml (1.65mM)	Taking the 1 mL working solution as an example, add 50 μL of 24.2 mg/ml clear DMSO stock solution to 950 μL of corn oil and mix evenly. The mixed solution should be used immediately for optimal results.

* <1 mg/ml means slightly soluble or insoluble.
* Please note that Capsid Bio tests the solubility of all compounds in-house, and the actual solubility may differ slightly from published values. This is normal and is due to slight batch-to-batch variations.
* Room temperature shipping (Stability testing shows this product can be shipped at 2-8°C for storage purposes.)

Preparing Stock Solutions

Biological Activity

Description	Danoprevir 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.
Targets	HCV NS3/4A protease [1] 0.2 nM-3.5 nM
In vitro	Danoprevir (0.29 nM) inhibits the reference genotype 1 NS3/4A protease half-maximally, but a high dose of this compound (10 μM) shows no appreciably inhibition in a panel of 79 proteases, ion channels, transporters, and cell surface receptors. It remains bound to and inhibits NS3/4A for more than 5 hours after its initial association. This chemical (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 it, but the R155K substitution confers a high level (62-fold increase) of resistance to this compound. [2] In Huh7.5 cells transfected with chimeric recombinant virus, it 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 concentration of this compound required to eliminate replicon RNA from cells. [1]
Features	A peptidomimetic inhibitor of the NS3/4A protease of hepatitis C virus (HCV).

Protocol (from reference)

Kinase Assay:[1]

- **Continuous fluorescent resonance energy transfer (FRET) assay**

  The assay buffer contains 25 μM NS4A peptide, 50 mM Tris-HCl, pH 7.5, 15% vol/vol, 10 mM dithiothreitol, and 0.5 μM 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 this compound-NS3/4A complex is assessed by preincubating 10 nM NS3/4A with a two-fold excess of this chemical 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 this compound is initiated by the addition of enzyme to an otherwise-complete reaction mixture. Additional control reactions lack either this chemical 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 this compound 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

https://pubmed.ncbi.nlm.nih.gov/18824605/

https://pubmed.ncbi.nlm.nih.gov/18637752/

https://pubmed.ncbi.nlm.nih.gov/21480315/

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SHIPPING & STORAGE

Capsid Bio products are transported at room temperature. If you receive the product at room temperature, please rest assured, the Capsid Bio Quality Inspection Department has conducted experiments to verify that the normal temperature placement of one month will not affect the biological activity of powder products. After collecting, please store the product according to the requirements described in the datasheet. Most Capsid Bio products are stable under the recommended conditions.

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EU Headquarters
Gajeva ulica 28, HR-10000 Zagreb, Croatia

US Headquarters
12222 Merit Dr STE 130 Dallas, Texas, 75251

UK Headquarters
121 ABILITY HOUSE, BROOKER ROAD, EN9 1JH, ESSEX

Orders: Sales@capsidbio.com
Enquiries: info@capsidbio.com
www.capsidbio.com