# 产品名称：Danoprevir
# 产品别名：丹诺普韦； ITMN-191; R7227; RO5190591; RG7227

## 生物活性：

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| **Description** | Danoprevir (ITMN-191) is an orally active NS3/4A protease inhibitor for hepatitis C virus (HCV) with an $IC_{50}$ of 0.29 nM and is selective for NS3/4A over a panel of 53 proteases ($IC_{50}$ higher than 10 μM). Danoprevir (ITMN-191) inhibits HCV genotypes 1a, 1b, 4, 5, and 6 ($IC_{50}$s=0.2-0.4 nM) as well as 2b and 3a ($IC_{50}$s=1.6, 3.5 nM) [1][2]. |
| **$IC_{50}$ & 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].<br>Danoprevir (ITMN-191) inhibits the reference genotype 1 NS3/4A protease half-maximally, but a high dose of Danoprevir (ITMN-191) (10 μM) shows no appreciably 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].<br>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]. |
| **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]. |
| **Solvent&Solubility** | ***In Vitro:***<br>**DMSO : ≥ 100 mg/mL (136.64 mM)**<br>* "≥" means soluble, but saturation unknown. |

**Preparing Stock Solutions**

| Solvent / Mass / Concentration | 1 mg | 5 mg | 10 mg |
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| 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 |

*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。

储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

***In Vivo:***

请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 **In Vitro** 方式配制澄清的储备液，再依次添加助溶剂：

——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶

1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline

Solubility: ≥ 2.5 mg/mL (3.42 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (3.42 mM，饱和度未知) 的澄清溶液。

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| | 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。<br><br>2.请依序添加每种溶剂： 10% DMSO →90% corn oil<br>Solubility: ≥ 2.5 mg/mL (3.42 mM); Clear solution<br>此方案可获得 ≥ 2.5 mg/mL (3.42 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的实验。<br>以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀 |
| **References** | [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.<br>[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.<br>[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. |

**实验参考：**

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| **Animal Administration** | 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. [2] |
| **Kinase Assay** | 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 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 $IC_{50}$. 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 |

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| | 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. [2] |
| **References** | [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.<br>[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.<br>[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. |