产品名称：VX-765
产品别名：Belnacasan

生物活性：

Description	Belnacasan (VX-765) is an orally bioactive prodrug of VRT-043198, which is a potent and selective inhibitor of IL-converting enzyme (ICE)/caspase-1 with Kis of 0.8 nM and less than 0.6 nM for caspase-1 and caspase-4, respectively. Belnacasan (VX-765) inhibits the release of LPS-induced IL-1β and IL-18 by human PBMCs with an IC50 of ~0.7 μM[1][2].
IC_{50} & Target	Caspase-1
In Vivo	Belnacasan reduces inflammatory response in murine models of inflammatory disease[1]. Belnacasan (50-200 mg/kg) significantly reduces serum IL-1β levels by as much as 60%. It is noteworthy that the effect of Belnacasan on the release of IL-1β induced by LPS reached a plateau at 100 mg/kg. Belnacasan (25-100 mg/kg × 2) significantly reduces ear edema. Belnacasan also dose-dependently reduces the concentrations of cytokines, chemokines, and inflammatory mediators in the ear biopsy samples[2]. Belnacasan (25-200 mg/kg) significantly delays the time to seizure onset by 1.5- to twofold ($p<0.01$), reduces the number of seizures by 40% ($p<0.01$) and the total time spent in EEG seizure activity by 30 to 50% ($p<0.01$)[3].

Solvent&Solubility

In Vitro:

DMSO : 50 mg/mL (**98.23 mM;** Need ultrasonic)

H_2O : 1 mg/mL (**1.96 mM;** ultrasonic and warming and heat to 60°C)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.9646 mL	9.8232 mL	19.6464 mL
	5 mM	0.3929 mL	1.9646 mL	3.9293 mL
	10 mM	0.1965 mL	0.9823 mL	1.9646 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 (4.91 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (4.91 mM，饱和度未知) 的澄清溶液。

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。

2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline)

Solubility: ≥ 2.5 mg/mL (4.91 mM); Clear solution

	此方案可获得 ≥ 2.5 mg/mL (4.91 mM，饱和度未知) 的澄清溶液。 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 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀。 3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.5 mg/mL (4.91 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.91 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。 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]. Stack JH, et al. IL-converting enzyme/caspase-1 inhibitor VX-765 blocks the hypersensitive response to an inflammatory stimulus in monocytes from familial cold autoinflammatory syndrome patients. J Immunol. 2005 Aug 15;175(4):2630-4. [2]. Wannamaker W, et al. (S)-1-((S)-2-{[1-(4-amino-3-chloro-phenyl)-methanoyl]-amino}-3,3-dimethyl-butanoyl)-pyrrolidine-2-carboxylic acid ((2R,3S)-2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-amide (VX-765), an orally available selective interleukin (IL)-converting enzyme/caspase-1 inhibitor, exhibits potent anti-inflammatory activities by inhibiting the release of IL-1beta and IL-18. J Pharmacol Exp Ther. 2007 May;321(2):509-16. [3]. Ravizza T, et al. Inactivation of caspase-1 in rodent brain: a novel anticonvulsive strategy. Epilepsia. 2006 Jul;47(7):1160-8.

实验参考：

Cell Assay	A total of 2×10^5 cells/well (100 μL cell suspension) is distributed in triplicate in flat-bottom 96-well plates. Either 50 μL of Belnacasan (40 μM in RPMI 1640 complete medium containing 0.2% DMSO) or vehicle control is added to appropriate wells. Following a 30-min incubation at 37°C, 50 μL of LPS diluted in RPMI 1640 complete medium is added at final concentrations varying from 0.001 to 10 ng/mL. Cells are returned to a 37°C incubator. At 4 h after LPS addition, 75 μL of supernatant is removed from wells, cleared by centrifugation for 5 min at 1500 rpm, and stored at 4°C until assayed. Cells are returned to a 37°C incubator until 24 h after LPS addition, at which time 100 μL of supernatant is removed, cleared by centrifugation, and stored at 4°C. Supernatants are tested using ELISA kits for IL-1β, IL-6, IL-18, and IL-1α[1].
Animal Administration	Mice[2] Single doses of Belnacasan (10, 21, 43, and 84 mg/kg) in vehicle (25% Cremophor EL in water) are administered via oral gavage. Blood samples (approximately 0.25-0.3 mL) are collected before dose administration and 0.167, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 24 h after dosing via the retroorbital sinus and processed for plasma. A high-performance liquid chromatography/mass spectrometry methodology is used to determine the concentration of Belnacasan and VRT-043198 in plasma samples. Noncompartmental analysis is carried out using WinNonlin Pro, version 4.0.1. Rats[3] Male Sprague-Dawley rats (250-280 g) are used. Belnacasan (25, 50, 200 mg/kg) is dissolved in 20% Cremophor and injected ip in rats once a day for 3 consecutive days. On the fourth day, rats receive Belnacasan, 45 min and 10 min before intrahippocampal injection of kainic acid. Respective controls are similarly injected with vehicle before kainic acid.
	Enzyme inhibition is assayed by tracking of the rate of hydrolysis of an appropriate substrate labeled with either p-nitroaniline or aminomethyl coumarin (AMC) as follows: ICE/caspase-1,

Kinase Assay	suc-YVAD-p-nitroanilide; caspase-4, Ac-WEHD-AMC; caspase-6, Ac-VEID-AMC; caspase-3, -7, -8, and -9, Ac-DEVD-AMC; and granzyme B, Ac-IEPD-AMC. Enzymes and substrates are incubated in a reaction buffer [10 mM Tris, pH 7.5, 0.1% (w/v) CHAPS, 1 mM dithiothreitol, and 5% (v/v) DMSO] for 10 min at 37°C. Glycerol is added to the buffer at 8% (v/v) for caspase-3, -6, and -9 and granzyme B to improve stability of enzymes. The rate of substrate hydrolysis is monitored using a fluorometer. Assays for cathepsin B and trypsin are performed[2].
References	[1]. Stack JH, et al. IL-converting enzyme/caspase-1 inhibitor VX-765 blocks the hypersensitive response to an inflammatory stimulus in monocytes from familial cold autoinflammatory syndrome patients. J Immunol. 2005 Aug 15;175(4):2630-4. [2]. Wannamaker W, et al. (S)-1-((S)-2-{[1-(4-amino-3-chloro-phenyl)-methanoyl]-amino}-3,3-dimethyl-butanoyl)-pyrrolidine-2-carboxylic acid ((2R,3S)-2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-amide (VX-765), an orally available selective interleukin (IL)-converting enzyme/caspase-1 inhibitor, exhibits potent anti-inflammatory activities by inhibiting the release of IL-1beta and IL-18. J Pharmacol Exp Ther. 2007 May;321(2):509-16. [3]. Ravizza T, et al. Inactivation of caspase-1 in rodent brain: a novel anticonvulsive strategy. Epilepsia. 2006 Jul;47(7):1160-8.