Belnacasan

Cat. No.: HY-13205

CAS No.: 273404-37-8

Molecular Formula: $C_{24}H_{33}ClN_4O_6$

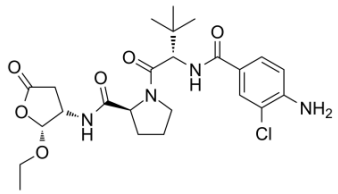
Molecular Weight: 509

Target: Caspase

Pathway: Apoptosis

Storage:

Powder	-20°C	3 years
	4°C	2 years
In solvent	-80°C	6 months
	-20°C	1 month



SOLVENT & SOLUBILITY

In Vitro

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

H_2O : < 0.1 mg/mL (insoluble)

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

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

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.08 mg/mL (4.09 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: 2.08 mg/mL (4.09 mM); Suspended solution; Need ultrasonic
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.08 mg/mL (4.09 mM); Clear solution

BIOLOGICAL ACTIVITY

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 K_is 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 IC_{50} 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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [2]	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, 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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [2][3]	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. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Cell Metab. 2019 Sep 3;30(3):477-492.e6.
- Brain Behav Immun. 2017 Oct;65:99-110.
- Brain Behav Immun. 2016 Aug;56:175-86.
- Cell Death Dis. 2020 Jun 18;11(6):470.
- Cell Death Dis. 2020 Apr 17;11(4):244.

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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.

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