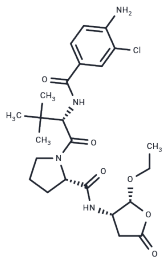
Data Sheet (Cat.No.T6090)

TargetMol

Belnacasan

Chemical Properties



CAS No. : 273404-37-8

Formula: C24H33ClN4O6

Molecular Weight: 508.99

Appearance: no data available

Storage: Powder: -20°C for 3 years | In solvent: -80°C for 1 year

Biological Description

Description	Belnacasan (VX-765) is an orally active inhibitor of IL-converting enzyme/caspase-1.
Targets(IC50)	Caspase
In vitro	VX-765 demonstrates antiepileptic properties by preventing the increase of IL-1β in the forebrain astrocytes of rats, thereby inhibiting the occurrence of epilepsy without significantly affecting the duration of post-discharge. In adult rats with genetic absence epilepsy, a 3-day administration of VX-765 significantly reduces the cumulative duration and decreases the average spike-wave discharges by 55% through the selective blockade of IL-1β biosynthesis. In acute epileptic mouse models, doses ranging from 50 mg/kg to 200 mg/kg of VX-765 delay the onset of the first epileptic episode and reduce the average number of seizures by 50% and the total duration by 64%. Additionally, a 200 mg/kg dose in a collagen-induced arthritis mouse model suppresses 60% of lipopolysaccharide-induced IL-1β production and leads to a dose-dependent significant reduction in inflammation scores, effectively protecting against joint lesions.
In vivo	VRT-043198 inhibits the release of IL-1β from peripheral blood mononuclear cells (PBMCs) and whole blood, with IC50 values of 0.67 μM and 1.9 μM, respectively. VX-765, an orally bioavailable prodrug of VRT-043198, demonstrates potent inhibition of ICE/caspase-1 and caspase-4, with Ki values of 0.8 nM and <0.6 nM, respectively.
Kinase Assay	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].
Cell Research	VX-765 is solubilized in DMSO and stored, and then diluted with RPMI 1640 complete medium (DMSO 0.2%) before use[1]. A total of 2×105 cells/well (100 μL cell suspension) is distributed in triplicate in flat-bottom 96-well plates. Either 50 μL of VX-765 (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].

Solubility Information

Solubility	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 9.3 mg/mL (18.27 mM),Solution. Ethanol: 93 mg/mL (182.71 mM),Sonication is recommended. DMSO: 45 mg/mL (88.41 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	1.9647 mL	9.8234 mL	19.6468 mL
5 mM	0.3929 mL	1.9647 mL	3.9294 mL
10 mM	0.1965 mL	0.9823 mL	1.9647 mL
50 mM	0.0393 mL	0.1965 mL	0.3929 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Reference

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