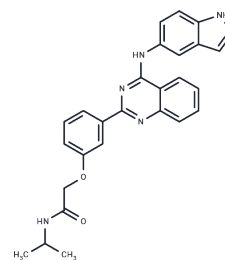


Belumosudil

Chemical Properties

CAS No. :	911417-87-3
Formula:	C ₂₆ H ₂₄ N ₆ O ₂
Molecular Weight:	452.51
Storage:	Powder: -20°C for 3 years In solvent: -80°C for 1 year Actual storage temperature shall be subject to the COA.



Biological Description

Description	Belumosudil (Rezurock) is an orally available, and specific ROCK2 inhibitor (IC ₅₀ /K _i : 60/41 nM).
Targets(IC ₅₀)	ROCK
In vitro	Belumosudil down-regulates the IL-17 and IL-21 secretion in human PBMCs, and leads to down-regulation of STAT3 phosphorylation, IRF4, and RORγt expression in CD4+ T cells. [2] Belumosudil inhibits the secretion of IL-21, IL-17, and IFNγ along with decreasing phosphorylated STAT3 and reduced protein expression of IRF4 and BCL6 in human peripheral blood mononuclear cells. [3]
In vivo	In mice, Belumosudil (200 mg/kg, p.o.) inhibits ROCK activity in brain and heart, as measured by the degree of MYPT1 phosphorylation. Belumosudil (200 mg/kg, p.o.) significantly reduces the area of perfusion defect, and reduces tissue loss in the ipsilateral hemisphere. [1] In a collagen-induced arthritis (CIA) mouse model, Belumosudil (200 mg/kg, i.p.) downregulates the progression of collagen-induced arthritis via targeting of the Th17-mediated pathway. [2] Belumosudil (150 mg/kg, i.p. or p.o.) effectively ameliorates cGVHD in a full MHC-mismatch model of multi-organ system cGVHD with bronchiolitis obliterans syndrome and a minor MHC-mismatch model of sclerodermatous GVHD. [3]
Kinase Assay	Cell-free enzyme assays are performed to determine the selective inhibition of ROCK1 and ROCK2 by SLX-2119. Reactions are performed on non-binding surface microplates. Four mU of human ROCK1 and ROCK2 are used to phosphorylate 30 μM of the synthetic ROCK peptide substrate S6 Long, prepared at American Peptide with the addition of 10 μM ATP, containing ³³ P-ATP in the presence of 10 mM Mg ²⁺ , 50 mM Tris, pH 7.5, 0.1 mM EGTA and 1 mM DTT at room temperature. One unit is the amount of kinase needed to catalyze the transfer of 1 nmol phosphate/min to the peptide. The reactions are allowed to proceed for 45 minutes and then stopped with 3% phosphoric acid to a final concentration of 1%. The reactions are captured on phospho cellulose filtration microplates and washed with 75 mM phosphoric acid and methanol using a vacuum manifold. Phosphorylation is measured on a Perkin-Elmer MicroBeta 1450.
Cell Research	SLX-2119 is dissolved in DMSO to obtain a stock solution of 20 mM. Western blots are used to determine whether HMVEC, NHDF and PASMIC express ROCK1 and ROCK2. The cells are incubated for 24 hours in 3 mL culture media containing SLX-2119. All cells are collected at passage 3 and lysed on ice in 25 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.5%

Cell Research	tritonX-100, 10% glycerol, 10 mM NaF and a protease inhibitor cocktail. Protein concentration is determined using a BCA protein assay reagent. Cell lysates (35 µg) are separated on 7.5% or 12.5% SDS-PAGE polyacrylamide gels and transferred to PVDF membrane filters. Membranes are blocked in 5% non-fat milk in TBS containing 0.1% Tween 20. Blots are probed with antibodies to ROCK1, ROCK2 or actin and washed well before incubation with HRP-conjugated secondary antibodies and visualization with an enhanced chemiluminescence (ECL) kit.
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Solubility Information

Solubility	H2O: < 1 mg/mL (insoluble or slightly soluble), DMSO: 260 mg/mL (574.57 mM),Sonication is recommended. Ethanol: < 1 mg/mL (insoluble or slightly soluble), (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 5 mg/mL (11.05 mM),Sonication is recommended. <i>Please add the solvents sequentially, clarifying the solution as much as possible before adding the next one. Dissolve by heating and/or sonication if necessary. Working solution is recommended to be prepared and used immediately. The formulation provided above is for reference purposes only. In vivo formulations may vary and should be modified based on specific experimental conditions.</i>

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.2099 mL	11.0495 mL	22.099 mL
5 mM	0.442 mL	2.2099 mL	4.4198 mL
10 mM	0.221 mL	1.1049 mL	2.2099 mL
50 mM	0.0442 mL	0.221 mL	0.442 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.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

Reference

- Lee JH, et al. Ann Clin Transl Neurol. 2014, 1(1), 2-14.
Zanin-Zhorov A, et al. Proc Natl Acad Sci U S A. 2014, 111(47), 16814-16819.
Flynn R, et al. Blood. 2016. pii: blood-2015-10-678706.

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