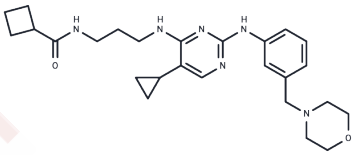
Data Sheet (Cat.No.T0097)

TargetMol

MRT67307

Chemical Properties



CAS No. : 1190378-57-4

Formula: C26H36N6O2

Molecular Weight: 464.6

Storage: Keep away from direct sunlight,Keep away from moisture
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	Through its effects on ULK1 and ULK2, MRT67307 blocks autophagy. MRT67307 prevents the phosphorylation of IRF3 and the production of IFN-β and increases toll-like receptor-induced IL-10 and IL-1ra secretion in macrophages. MRT67307 is a kinase inhibitor that has been shown to inhibit TBK1, MARK1-4, IKKε, and NUAK1 (IC50 values are 19, 27-52, 160, and 230 nM, respectively), the salt-inducible kinases (SIKs; IC50s =250, 67, and 430 nM for SIK1, SIK2, and SIK3, respectively) and ULK1 and ULK2 (IC50s = 45 and 38 nM, respectively).
Targets(IC50)	Autophagy,IκB/IKK
In vitro	In macrophages, MRT67307 prevents the phosphorylation of IRF3 and the production of IFNβ. In wild-type MEFs, MRT67307 enhances the IL-1-stimulated phosphorylation of p105 at Ser933 and RelA at both Ser468 and Ser536, and also enhances IL-1-stimulated activation of NF-κB-dependent gene transcription. [1] MRT67307 increases IL-10 production and suppresses proinflammatory cytokine production via a cAMP response element-Binding protein (CREB)-regulated transcriptional coactivator (CRTC) 3 Dependent Mechanism. [2] In addition, MRT67307 inhibit ULK and block autophagy in MEF cells. [3]
Kinase Assay	Substrates and kinases are diluted in 50?mM Tris/HCl (pH?7.5), 0.1% 2-mercaptoethanol, 0.1?mM EGTA and 10?mM magnesium acetate. Reactions are initiated with [γ-32P]ATP (2500 c.p.m./pmol) to a final concentration of 0.1?mM and terminated after 15?min at 30°C by the addition of SDS and EDTA (pH?7.0) to final concentrations of 1.0% (w/v) and 20?mM respectively. After heating for 5?min at 100°C and separation by SDS/PAGE, the phosphorylated proteins are detected by autoradiography.
Cell Research	Cells are rinsed in ice-cold PBS and extracted in lysis buffer (50 mM Tris·HCl at pH 7.4, 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 5 mM sodium pyrophosphate, 10 mM sodium β-glycerol 1-phosphate, 1 mM DTT, 1 mM sodium orthovanadate, 0.27mol/Lsucrose, 1% (vol/vol) Triton X-100, 1 μg/mL aprotinin, 1 μg/ mL leupeptin, and 1 mM phenylmethylsulphonyl fluoride). Cell extracts are clarified by centrifugation at 14,000 × g for 10 min at 4°C, and protein concentrations are determined by using the Bradford assay. FLAG-CRTC3 is purified on anti-FLAG M2 agarose, whereas endogenous CRTC3 is immunoprecipitated from cell extracts by using anti-CRTC3 raised against the peptide CWKEEKHPGFR (S277D bleed 2) and coupled to Protein GSepharose. To detect proteins in

Cell Research	cell lysates, 20 μg of protein extract is separated by SDS/PAGE. After transfer to PVDF membranes, proteins are detected by immunoblotting and visualized by treating the blots with ECL followed by autoradiography. The following antibodies are used for immunoblotting: pSer133 CREB, pSer171 CRTC2, total CRTC2, GAPDH, total STAT3, pTyr705 STAT3, FLAG (M2 clone), CRTC3, HA (3F10), and 14-3-3; and antibodies against pSer329 (S256D bleed 2) and pSer370 (S253D bleed 2) of CRTC3 are raised against the phosphopeptides GLQSSRpSNPSIQ and RLFSLpSNPSLST.

Solubility Information

Solubility	DMSO: 120 mg/mL (258.29 mM),Sonication is recommended. Ethanol: 83 mg/mL (178.65 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 3.3 mg/mL (7.1 mM),Sonication is recommended. *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.*

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.1524 mL	10.7619 mL	21.5239 mL
5 mM	0.4305 mL	2.1524 mL	4.3048 mL
10 mM	0.2152 mL	1.0762 mL	2.1524 mL
50 mM	0.043 mL	0.2152 mL	0.4305 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

Clark K, et al. Biochem J. 2011, 434(1), 93-104.
Clark K, et al. Proc Natl Acad Sci U S A. 2012, 109(42), 161986-161991.
Petherick KJ, et al. J Biol Chem. 2015, 290(18), 11376-11383.