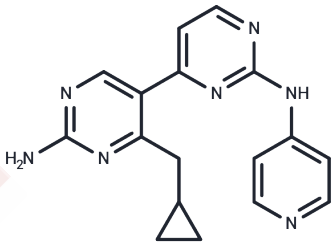
Data Sheet (Cat.No.T6945)

TargetMol

Vps34-PIK-III

Chemical Properties



CAS No. : 1383716-40-2

Formula: C17H17N7

Molecular Weight: 319.36

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	Vps34-PIK-III (VPS34-IN2), a selective inhibitor of VPS34 enzymatic activity, inhibits autophagy and results in the stabilization of autophagy substrates.
Targets(IC50)	Autophagy,PI3K
In vitro	VPS34 enzymatic function is essential for LC3 lipidation in mammalian cells and PIK-III is a robust inhibitor of autophagy and LC3 lipidation in mammalian cells. In H4 cells, PIK-III inhibits the formation of autolysosomes and increases the cytosolic signal of LC3 under basal conditions and when autophagy is induced with the mTOR inhibitor AZD8055. In a CCCP-induced mitophagy model, PIK-III inhibits the clearance of mitochondria.PIK-III treatment leads to an increase in the levels of LC3-I in H4 and PSN1 cells. In Panc10.05 cells, PIK-III increases the levels of LC3-II in parallel with LC3-I suggesting a cell type-specific response[1].
In vivo	The DFX-induced NCOA4-dependent turnover of FTH1 and FTL is blocked with PIK-III which suggests an autophagy-dependent process[2].
Kinase Assay	In vitro tyrosine kinase assays.: Assay of IGF-1R-catalyzed substrate phosphorylation of pTG, using a 96-well plate tyrosine kinase assay kit, is performed. We use recombinant epidermal growth factor receptor, immunoprecipitated IR from HEPG2, immunoprecipitated IGF-1R from P6 cells, and IGF-1R immunodepleted supernatant from P6 (representing "non-IGF-1R tyrosine kinases"). After 30-min treatment of the receptors with the desired compounds in the kinase buffer [50 mM HEPES buffer (pH 7.4), 20 mM MgCl2, 0.1 MnCl2, and 0.2 Na3VO4], the kinase reaction is activated by addition of ATP. The phosphorylated polymer substrate is probed with a phosphotyrosine-specific monoclonal antibody conjugated to horseradish peroxidase, clone PT-66. Color is developed with horseradish peroxidase chromogenic substrate O-phenylenediamine dihydrochloride and quantitated by spectrophotometry (ELISA reader). IGF-1R tyrosine autophosphorylation is analyzed by a sandwich ELISA assay. Briefly, 96-well plates are coated overnight at 4°C with 1 μg/well of an antibody to IGF-1R β-subunit. The plates are blocked with 1% BSA in PBS Tween for 1 h, and then 80 μg/well of total protein lysate from the P6 cell line is added. As a negative control we use total protein lysate from the R- cell line. The investigated compounds are added in tyrosine kinase buffer without ATP at room temperature for 30 min before kinase activation with ATP. Kinase assay is performed using the Sigma kit (see above). After spectrophotometry the IC50 values of inhibitors are determined using the REGRESSION

Kinase Assay	function of Statistica program.
Cell Research	To determine whether inhibition of VPS34 function impacts autophagy,LC3 and known autophagy substrates such as damaged mitochondria or the autophagy cargo receptor p62 are monitored. H4 cells expressing mCherry-GFP-LC3 are treated overnight with the indicated compounds, fixed, stained with Hoechst 33342 and imaged by automated acquisition. HeLa cells expressing GFP-Parkin are treated with PIK-III for 12 h followed by the addition of CCCP for 12 h, fixed, stained for endogenous Tom20 and imaged. (Only for Reference)

Solubility Information

Solubility	H2O: < 1 mg/mL (insoluble or slightly soluble), DMSO: 125 mg/mL (391.41 mM),Sonication is recommended. Ethanol: 59 mg/mL (184.74 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+90% Saline: 10 mg/mL (31.31 mM),Suspension. 10% DMSO+40% PEG300+5% Tween 80+45% Saline: 2 mg/mL (6.26 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	3.1313 mL	15.6563 mL	31.3126 mL
5 mM	0.6263 mL	3.1313 mL	6.2625 mL
10 mM	0.3131 mL	1.5656 mL	3.1313 mL
50 mM	0.0626 mL	0.3131 mL	0.6263 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

Honda A, et al. Potent, Selective, and Orally Bioavailable Inhibitors of VPS34 Provide Chemical Tools to Modulate Autophagy in Vivo. ACS Med Chem Lett. 2015 Nov 13;7(1):72-6.
Dowdle WE, et al. Selective VPS34 inhibitor blocks autophagy and uncovers a role for NCOA4 in ferritin degradation and iron homeostasis in vivo. Nat Cell Biol. 2014 Nov;16(11):1069-79.

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