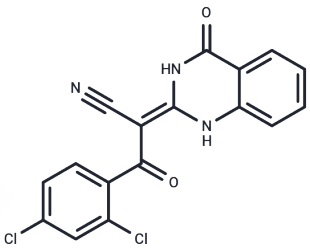
Data Sheet (Cat.No.T3156)

TargetMol

Ciliobrevin A

Chemical Properties



CAS No. : 302803-72-1

Formula: C17H9Cl2N3O2

Molecular Weight: 358.18

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	Ciliobrevin A (HPI-4) is an inhibitor of the hedgehog signaling pathway with an IC50 of less than 10 μM.
Targets(IC50)	Hedgehog/Smoothened
In vitro	Shh-EGFPFLAG-Gli2 cells cultured with Ciliobrevin A have truncated primary cilia, and this cellular organelle is absent in a significant fraction of Ciliobrevin A-treated cells. Ciliobrevin A perturbs primary cilia formation in the Shh-LIGHT2FLAG-Gli1 cells and promotes accumulation of FLAG-Gli1 at the distal tip of this organelle. Ciliobrevin A significantly inhibits the proliferation of these neuronal progenitors, as measured by histone H3 phosphorylation (pH3) levels, and reduces cellular levels of cyclin D1 protein and Gli1, Gli2, and N-Myc transcripts in the CGNPs. Ciliobrevin A can block the proliferation of SmoM2-expressing CGNPs and should be equally potent against CGNPs lacking Su(fu) function, whereas the Smo inhibitor Cyclopamine is ineffective against either oncogenic lesion. Ciliobrevin A prevents an increase in the FLAG-Gli2 full-length/repressor ratio upon Shh stimulation, but HPI-2 and HPI-3 have no significant effect. Ciliobrevin A increases ciliary levels of FLAG-Gli2 in a manner disproportionate to their effects on total FLAG-Gli2 levels[1].
Kinase Assay	Smo-binding assays are conducted with BODIPY-cyclopamine and Smo-overexpressing HEK 293T cells, using a CMVpromoter-based SV40 origin-containing expression construct for Smo-Myc3 (murine Smo containing three consecutive Myc epitopes at the C terminus). HEK 293T cells are seeded into eight-well chambered coverslips (80,000 cells/well) and cultured in DMEM containing 10% FBS, 100 U/mL penicillin, and 0.1 mg/mL streptomycin. The cells are cultured until they reached 55 to 65% confluency (14-18 h), after which they are transfected with the Smo-Myc3 expression construct and Transit-LT1. Twenty-four hours after transfection, the cells are washed with PBS and cultured in DMEM containing 0.5% FBS, 5 nM BODIPY-cyclopamine, and various concentrations of either cyclopamine or individual HPIs. After 30 min, 10 μM Hoescht 33342 is added to each well, and the HPIs are incubated with the cells for an additional 30 min. The cells are then washed two times with PBS buffer, once with phenol red-free DMEM containing 0.5% FBS, and immediately imaged using a DMI6000B compound microscope. Images are background-substracted using ImageJ software with a rolling ball size of 75 pixels, and BODIPY-cyclopamine intensity is then determined using Metamorph software. Circular regions with a diameter of 300 pixels are placed over regions containing uniformly confluent cells, and the pixel intensities of approximately

Kinase Assay	20 regions from four independent images is used to determine the average BODIPY-cyclopamine levels for each experimental condition[1].
Cell Research	Using MTS-8 assay to measure cell proliferation and the toxicity of this drug. 2000 cells were plated in 96-well plates per well. HPI-4 was added to cells at concentrations of 0, 5 and 10 μM in 100 μl DMEM/F12 with 10% FBS and incubated for 0, 1, 3, 6 and 9 days. Then, 10 μl MST-8 was added to the media in each well and incubated in an environment without light for 90 min. The absorbance value was measured using an enzyme microplate reader at 450 nm wavelength. The relative viability of cells was expressed by OD value.(Only for Reference)

Solubility Information

Solubility	DMSO: 65 mg/mL (181.47 mM),Sonication is recommended. Ethanol: 1 mg/mL (2.79 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: 2 mg/mL (5.58 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	2.7919 mL	13.9595 mL	27.9189 mL
5 mM	0.5584 mL	2.7919 mL	5.5838 mL
10 mM	0.2792 mL	1.3959 mL	2.7919 mL
50 mM	0.0558 mL	0.2792 mL	0.5584 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

Hyman JM, et al. Proc Natl Acad Sci U S A. 2009, 106(33):14132-7.
Xiang W, et al. Oncol Rep. 2014, 32(4):1622-30.
Jung IH, et al. PLoS One. 2011, 6(12):e27941.