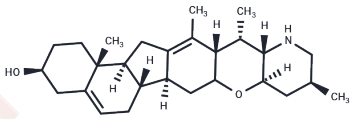
Data Sheet (Cat.No.T2825)

TargetMol

Cyclopamine

Chemical Properties



CAS No. : 4449-51-8

Formula: C27H41NO2

Molecular Weight: 411.62

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	Cyclopamine (11-Deoxojervine) is a natural small molecule steroidal alkaloid extracted from plants of the genus Aconitum. Cyclopamine acts as an antagonist of the Hedgehog pathway with an IC50 of 46 nM in cellular assays. Additionally, Cyclopamine functions as a selective Smo inhibitor. Cyclopamine is applicable for research in embryonic development, tumorigenesis, and targeted therapies.
Targets(IC50)	Hedgehog/Smoothened,Endogenous Metabolite,Smo
In vitro	**Methods**: Primary hepatocytes isolated from C57BL/6N mice were treated with Cyclopamine (10 μM) and Rapamycin (50 nM) for 24 hours. Mitochondrial oxygen consumption rate (OCR) was measured using the Seahorse XF analyzer to calculate parameters including basal respiration, maximal respiration, and ATP yield. **Results**: The combination of Cyclopamine and Rapamycin significantly reduced maximal respiration and ATP yield. [1] **Methods**: HeLa cells were treated with Cyclopamine (5 μM) for 24 hours. Western blot analysis measured the ratio of mature (31 kDa) to immature (53 kDa) forms of cathepsin D. **Results**: Cyclopamine treatment significantly reduced the mature/immature cathepsin D ratio, indicating impaired lysosomal maturation. [2]
In vivo	**Methods**: Transgenic Drosophila expressing APP-C99-Gal4 and UAS-GRIM (γ-secretase cleavage activates GRIM, causing eye roughness) were fed food containing Cyclopamine (100 nM) from the larval to adult stage. Observations were made within 24 hours post-adult emergence. **Results**: The severity of the rough eye phenotype was significantly reduced in the cyclopamine-treated group, and in vivo reduction of γ-secretase-mediated APP cleavage was also observed. [2]
Kinase Assay	This assay measures the end stage of the Hh signaling pathway, that is, the transcriptional modulation of Gli, using Luciferase as readout (Gli-Luc assay). Cyclopamine is prepared for assay by serial dilution in DMSO and then added to empty assay plates. TM3Hh12 cells (TM3 cells containing Hh-responsive reporter gene construct pTA-8xGli-Luc) are resuspended in F12 Ham's/DMEM (1:1) containing 5% FBS and 15 mM Hepes pH 7.3, added to assay plates and incubated with Cyclopamine for approximately 30 minutes at 37 °C in 5% CO2. 1 nM Hh-Ag 1.5 is then added to assay plates and incubated at 37 °C in the presence of 5% CO2. After 48 hours, either Bright-Glo or MTS reagent is added to the assay plates and luminescence or absorbance at 492

Kinase Assay	nm is determined. IC50 value, defined as the inflection point of the logistic curve, is determined by non-linear regression of the Gli-driven luciferase luminescence or absorbance signal from MTS assay vs log10 (concentration) of Cyclopamine using the R statistical software pack [1].
Cell Research	Cells were cultured in triplicate in 96-well plates in assay media to which 5E1 monoclonal antibody, ShhNp and/or cyclopamine were added at 0 h at concentrations indicated in the main text. Viable cell mass was determined by optical density measurements at 490 nm (OD490) at 2 and 4 days using the CellTiter96 colorimetric assay. Relative growth was calculated as OD (day 4) 2 OD (day 2)/OD (day 2) [3].
Animal Research	A total of 0.1 ml Hanks' balanced salt solution and matrigel (1:1) containing 2 × 10^6 cells were injected subcutaneously into CD-1 nude mice. Tumours were grown for 4 days to a minimum volume of 125 mm3; treatment was initiated simultaneously for all subjects. Mice were injected subcutaneously with vector alone (triolein:ethanol 4:1 v/v) or a cyclopamine suspension (1.2 mg per mouse in triolein: ethanol 4:1 v/v) daily for 7 days. At the end of the treatment period, tumours were excised from mice, weighed and then fixed for 3 h at 4 °C with 4% paraformaldehyde, embedded in paraffin wax and sectioned (6 μm). Apoptotic cells were identified by TUNEL using recombinant Tdt as previously described29. Sections were then counterstained with eosin. Eight ×20-magnified fields from regions corresponding to the exterior, middle and interior of two control and two cyclopamine-treated tumours were chosen at random [5].

Solubility Information

Solubility	DMSO: 4.12 mg/mL (10.01 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.4294 mL	12.1471 mL	24.2943 mL
5 mM	0.4859 mL	2.4294 mL	4.8589 mL
10 mM	0.2429 mL	1.2147 mL	2.4294 mL
50 mM	0.0486 mL	0.2429 mL	0.4859 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

Spormann L, et al. Cyclopamine and Rapamycin Synergistically Inhibit mTOR Signalling in Mouse Hepatocytes, Revealing an Interaction of Hedgehog and mTor Signalling in the Liver. Cells. 2020 Jul 31;9(8):1817.
Zheng H T, Fu T, Zhang H Y, et al. Progesterone-regulated Hsd11b2 as a barrier to balance mouse uterine corticosterone. Journal of Endocrinology. 2020, 244(1): 177-187
Vorobyeva AG, et al. Cyclopamine modulates γ-secretase-mediated cleavage of amyloid precursor protein by altering its subcellular trafficking and lysosomal degradation. J Biol Chem. 2014 Nov 28;289(48):33258-74.
Li B, Yan Y P, He Y Y, et al.IHH, SHH, and primary cilia mediate epithelial-stromal cross-talk during decidualization in mice.Science Signaling.2023, 16(774): eadd0645.
Berman DM, et al. Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours. Nature. 2003 Oct 23;425(6960):846-51.
Zhang Y, Wu T, Wang Y, et al.Reciprocal FGF19-GLI2 signaling induces epithelial-to-mesenchymal transition to promote lung squamous cell carcinoma metastasis.Cellular Oncology.2023: 1-14.
Feldmann G, et al. Blockade of hedgehog signaling inhibits pancreatic cancer invasion and metastases: a new paradigm for combination therapy in solid cancers. Cancer Res. 2007 Mar 1;67(5):2187-96.
Sex-biased gene expression during neural differentiation of human embryonic stem cells
Thayer SP, et al. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. Nature. 2003 Oct 23; 425(6960):851-6.
Ma W, et al. Reduced Smoothened level rescued Aβ-induced memory deficits and neuronal inflammation in animal models of Alzheimer's disease. J Genet Genomics. 2018 May 20;45(5):237-246.
Zheng H T, Fu T, Zhang H Y, et al. Progesterone-regulated Hsd11b2 as a barrier to balance mouse uterine corticosterone[J]. Journal of Endocrinology. 2020, 244(1): 177-187.