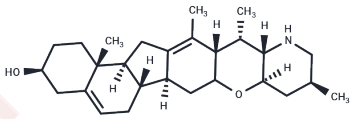
Data Sheet (Cat.No.T2825)

TargetMol

Cyclopamine

Chemical Properties



CAS No. : 4449-51-8

Formula: C27H41NO2

Molecular Weight: 411.62

Appearance: no data available

Storage: Powder: -20°C for 3 years | In solvent: -80°C for 1 year

Biological Description

Description	Cyclopamine (11-Deoxojervine), a Smoothened (Smo) antagonist (IC50: 46 nM in TM3Hh12 cells), belongs to the group of steroidal jerveratrum alkaloids.
Targets(IC50)	Hedgehog/Smoothened,Endogenous Metabolite,Smo
In vitro	The Chicken embryos Exposure to cyclopamine resulted in visible external defects, including cyclopia, microphthalmia, proboscis formation, amelia, thoracic lordosis, and decreased body size [2]. cyclopamine treatment reduced the growth of tumor cell lines from the oesophagus, stomach, biliary tract and pancreas by 75–95% compared with tomatidine controls [3]. In pancreatic cancer cell lines, Hh inhibition with cyclopamine resulted in down-regulation of snail and up-regulation of E-cadherin, consistent with inhibition of epithelial-to-mesenchymal transition, and was mirrored by a striking reduction of in vitro invasive capacity ($P < 0.0001$) [4].
In vivo	To examine the effects of cyclopamine treatment in vivo, subcutaneous xenografts from HUCCT1 cells, a metastatic cholangiocarcinoma cell line, were established in athymic mice. Tumours in cyclopamine-treated animals regressed completely by 12 days [3]. In the delayed treatment model, no difference in weight was noted between control and cyclopamine (1.2 mg) treated BxPC3-SMOlow tumours. By contrast, a 50–60% decrease in tumour mass was observed in Panc 05.04- and L3.6sl-derived tumours, respectively (Fig. 5b, c)—an even more marked effect was noted in the concurrent treatment model, which revealed an 84% reduction in tumour mass of L3.6sl-derived tumours [5].
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 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 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.

Reference

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