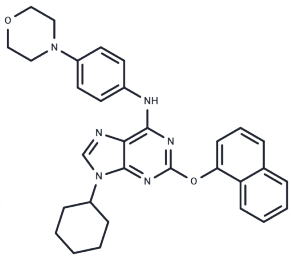
Data Sheet (Cat.No.T1810)

TargetMol

Purmorphamine

Chemical Properties



CAS No. : 483367-10-8

Formula: C31H32N6O2

Molecular Weight: 520.62

Storage: Store at low temperature
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	Purmorphamine (Shh Signaling Antagonist VI), which directly binds and activates Smoothened, blocks BODIPY-cyclopamine binding to Smo. It also is an inducer of osteoblast differentiation.
Targets(IC50)	Hedgehog/Smoothened,Autophagy,Smo
In vitro	In rat models, based on human mesenchymal stem cells, Purmorphamine upregulates the expression of ALP.
In vivo	In multipotent C3H10T1/2 cells, Purmorphamine can induce osteogenesis with an EC50 of 1 μM. It directly binds to Smoothened, thereby activating the Hedgehog pathway with an IC50 of 1.5 μM.
Kinase Assay	Binding assay: Smo binding assays are conducted with BODIPY-cyclopamine and Smo-overexpressing cells as previously described4,5, using CMV promoter-based, SV40 origin-containing expression constructs for Smo-Myc3, the deletion mutant Smo?CRD (deletion of amino acids 68 to 182), and Smo?CT (deletion of amino acids 556 to 793). HEK 293T cells are grown on poly-D-lysine-treated glass coverslips in 12-well plates until 70% confluency and then transfected with the appropriate expression construct (0.5 ?g/well) using FuGene 6 according the manufacturer's protocols. Two days after transfection, the HEK 293T cells are incubated with DMEM containing 0.5% bovine calf serum, 5 nM BODIPY-cyclopamine, and varying concentrations of Purmorphamine (0, 1.5, or 5 ?M) (1 mL/well) for 1 h at 37 ℃. The Smo-overexpressing cells are then washed with 1 × PBS buffer (1 mL/well), mounted with DAPI-containing medium, and visualized using a Leica DM4500B fluorescence microscope. For binding assays using fixed cells, the Smo-overexpressing HEK 293T cells are fixed with 3% paraformaldehyde in 1 × PBS buffer for 10 min at room temperature (1 mL/well), treated with 1 × PBS containing 10 mM glycine and 0.2% sodium azide for 5 min (1 mL/well), washed with 1 × PBS buffer (1 mL/well), and treated with the Purmorphamine-containing media described above for 4 h at room temperature.
Cell Research	C3H10T1/2 cells are expanded in T175 flasks; cells at 13th passage are detached by trypsin/EDTA and diluted in the growth media. The resulting cell suspension is then plated into black clear bottom 384-well plates with 2500 cells/well in 100 μL growth medium using a Multi-dropTM liquid delivery system. After overnight incubation, cells attached to the bottom of the wells. A stock solution of each Purmorphamine in DMSO

Cell Research	(500 nL) is delivered into corresponding well using a Mini TrakTM multiposition dispenser system to make a final concentration of 5 μM of Purmorphamine. Cells are then incubated at 37 ℃ with 5% CO2 in air atmosphere. After 4 days, the medium is removed and 10 μL of passive lysis buffer is added into each well. After 5 min, 10 μL of alkaline phosphatase substrate solution is added to each well. After incubating 15 min at room temperature, the plates are read on an Acquest high-throughput plate reader following the manufacturer's protocol.(Only for Reference)

Solubility Information

Solubility	DMSO: 31 mg/mL (59.54 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 (3.84 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	1.9208 mL	9.6039 mL	19.2079 mL
5 mM	0.3842 mL	1.9208 mL	3.8416 mL
10 mM	0.1921 mL	0.9604 mL	1.9208 mL
50 mM	0.0384 mL	0.1921 mL	0.3842 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

Sinha S, et al. Nat Chem Biol, 2006, 2(1), 29-30.
Singh K, Nawabjan S A, Zhang L, et al. Discovery of small-molecule modulators of the secretin receptor: Purmorphamine as novel anti-hypertensive agent. European Journal of Medicinal Chemistry. 2022: 114642.
Mendonça L S, Henriques D, Fernandes V, et al.Graft-derived neurons and bystander effects are maintained for six months after human iPSC-derived NESC transplantation in mice's cerebella.Scientific Reports.2024, 14(1): 3236.
Wu X, et al. J Am Chem Soc, 2002, 124(49), 14520-14521.
Wu X, et al. Chem Biol, 2004, 11(9), 1229-1238.
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