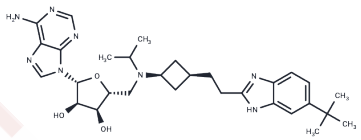
Data Sheet (Cat.No.T3099)

TargetMol

Pinometostat

Chemical Properties



CAS No. : 1380288-87-8

Formula: C30H42N8O3

Molecular Weight: 562.71

Appearance: no data available

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

Biological Description

Description	Pinometostat (EPZ-5676) is a potent DOT1L histone methyltransferase inhibitor (Ki=80 pM). Pinometostat has antitumor activity and can be used in experiments to study a variety of leukemia treatments.
Targets(IC50)	Histone Methyltransferase
In vitro	**METHODS**: MLL-r and non-MLL-r AML cells were treated with Pinometostat (0.1-10 μM) for 4-16 days and cell proliferation was detected by flow cytometry. **RESULTS**: The MV4-11, MOLM-13 and NOMO-1 cell lines carrying MLL-AF4 or MLL-AF9 fusions showed a dramatic decrease in cell numbers after 8 days of treatment, with IC50 values below 1 μM. In contrast, the proliferation of U-937 or HL-60 cells, which are all lacking in MLL-r, was not affected by Pinometostat. Surprisingly, DOT1L inhibition had no effect on MLL-AF9-positive THP-1 cells, whereas it significantly reduced the proliferation of the non-MLL-r OCI-AML3 cell line. [1] **METHODS**: Human leukemia cells MV4-11 were treated with Pinometostat (0.06-1000 nM) for 4 days and target protein expression levels were detected by Western Blot. **RESULTS**: A concentration-dependent decrease in overall cellular methylated H3K79 levels was observed in MV4-11 expressing MLL-AF4 at increasing concentrations of Pinometostat. [2]
In vivo	**METHODS**: To detect anti-tumor activity in vivo, Pinometostat (50 mg/kg, 2% DMSO+30% PEG 300+5% Tween80+63% PBS) was injected intraperitoneally into NSG mice bearing MDA-MB-468 tumors every two days and administered six times. **RESULTS**: Pinometostat significantly reduced tumor volume and primary tumor metastasis within 10 weeks. [3]
Cell Research	EPZ-5676 is dissolved in DMSO. To analyse inhibition of histone methylation in MV4-11 cells following EPZ-5676 treatment, extracted histones (400 ng) are fractionated on a 10-20% Tris HCl gels with Tris-Glycine SDS running buffer under denaturing conditions and transferred to nitrocellulose filters. Filters are cut into strips and incubated for 1 hour in blocking buffer at room temperature (RT) and then incubated overnight at 4°C in blocking buffer. Filters are washed 3 times for 5 minutes with wash buffer (Phosphate buffered saline (PBS) including 0.01% Tween 20 (PBST)) and incubated with infrared tagged secondary antibody at RT for 1 hour. Filters are washed in PBST and reprobed for 1 hour at RT with the appropriate total histone antibody control (mouse anti-histone H3 (1:20,000), CST 3638, or mouse anti-histone H4 (1:10,000), CST 2935). Filters are washed again in PBST and incubated with infrared tagged secondary antibody (IRDye 800Cw

donkey-anti-mouse IgG (1:20,000), Li-Cor 926-32212) at RT for 1 hour. After a final ish in PBST, filters are scanned using the Odyssey infared imager (Li-cor). To analyse inhibition of H3K79 methylation in peripheral blood mononuclear cells (PBMCs) from rats dosed with EPZ-5676, 20 μL of PBMC whole cell lysate is fractionated on denaturing gels and analysed by immunoblotting with antibodies to H3K79me2 or total H3. Signal intensities specific for the H3K79me2 antibody and total histone H3 control antibody are quantified using Odyssey software. The H3K79me2 signal intensity is normalized by dividing it by the total histone H3 control signal intensity in the same lane.

Solubility Information

Solubility	Ethanol: 85 mg/mL (151.05 mM),Sonication is recommended. DMSO: 60 mg/mL (106.63 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	1.7771 mL	8.8856 mL	17.7711 mL
5 mM	0.3554 mL	1.7771 mL	3.5542 mL
10 mM	0.1777 mL	0.8886 mL	1.7771 mL
50 mM	0.0355 mL	0.1777 mL	0.3554 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

Lonetti A, et al. Inhibition of Methyltransferase DOT1L Sensitizes to Sorafenib Treatment AML Cells Irrespective of MLL-Rearrangements: A Novel Therapeutic Strategy for Pediatric AML. Cancers (Basel). 2020 Jul 20;12(7):1972.

Yu S, Zhou C, He J, et al. BMP4 drives primed to naïve transition through PGC-like state. Nature Communications. 2022, 13(1): 1-15

Chen R, Xie W, Cai B, et al. Establishment and Identification of a CiPSC Lineage Reprogrammed from FSP-tdTomato Mouse Embryonic Fibroblasts (MEFs). Stem Cells International. 2018 Dec 25;2018:5965727

Daigle SR, et al. Potent inhibition of DOT1L as treatment of MLL-fusion leukemia. Blood. 2013 Aug 8;122(6):1017-25.

Kurani H, et al. DOT1L Is a Novel Cancer Stem Cell Target for Triple-Negative Breast Cancer. Clin Cancer Res. 2022 May 2;28(9):1948-1965.

Zhao X, Li X, Sun H, et al.Dot1l cooperates with Npm1 to repress endogenous retrovirus MERVL in embryonic stem cells.Nucleic Acids Research.2023: gkad640.

Ma Z, Huang X, Kuang J, et al.Cpt1a Drives primed-to-naïve pluripotency transition through lipid remodeling. Communications Biology.2024, 7(1): 1223.