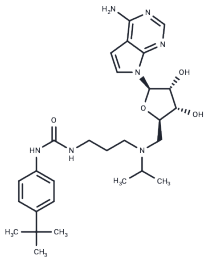
Data Sheet (Cat.No.T3081)

TargetMol

EPZ004777

Chemical Properties



CAS No. : 1338466-77-5

Formula: C28H41N7O4

Molecular Weight: 539.67

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	EPZ004777 is a potent and selective DOT1L inhibitor with an IC50 of 0.4 nM.
Targets(IC50)	Apoptosis,Histone Methyltransferase
In vitro	After inhibition of DOT1L, EPZ004777 selectively inhibited the proliferation of MLL-rearranged cell lines and MLL-AF9-transformed murine hematopoietic cells.EPZ004777 also induced differentiation and apoptosis in MLL-rearranged cell lines. EPZ004777 selectively inhibited cellular H3K79 methylation and suppressed the expression of key MLL fusion target genes.EPZ004777 selectively inhibited the proliferation of hematopoietic cells in mice transformed with MLL-AF10 and CalM-AF10.EPZ004777 inhibition of DOT1L resulted in a significant decrease in the proliferation of hematopoietic cells in mice transformed with MLL-AF6. MLL-AF6 target gene expression was reduced and cell cycle arrest was observed.
In vivo	After inhibition of DOT1L, EPZ004777 selectively inhibited the proliferation of MLL-rearranged cell lines and MLL-AF9-transformed murine hematopoietic cells.EPZ004777 also induced differentiation and apoptosis in MLL-rearranged cell lines. EPZ004777 selectively inhibited cellular H3K79 methylation and suppressed the expression of key MLL fusion target genes.EPZ004777 selectively inhibited the proliferation of hematopoietic cells in mice transformed with MLL-AF10 and CalM-AF10.EPZ004777 inhibition of DOT1L resulted in a significant decrease in the proliferation of hematopoietic cells in mice transformed with MLL-AF6. MLL-AF6 target gene expression was reduced and cell cycle arrest was observed.
Kinase Assay	Determination of Inhibitor IC50 Values: EPZ004777 is serially diluted 3-fold in DMSO for a total of ten concentrations, beginning at 1 mM. A 1 µL aliquot of each inhibitor dilution is plated in a 384-well microtiter plate. The 100% inhibition control consisted of 2.5 mM ? nal concentration of the product inhibitor S-adenosyl-L-homocysteine, (SAH). Compound is incubated for 30 min with 40 ml per well of 0.25 nM DOT1L(1-416) in assay buffer (20 mM TRIS [pH 8.0] 10 mM NaCl, 0.002% Tween 20, 0.005% Bovine Skin Gelatin, 100 mM KCl, and 0.5 mM DTT). 10 ml per well of substrate mix comprising assay buffer with 200 nM 3H-SAM (American Radiolabeled Chemicals: 80 Ci/mmol), 600 nM unlabeled SAM, and 20 nM nucleosomes are added to initiate the reaction (both substrates are present in the ?nal reaction mixture at their respective KM values). Reactions are incubated for 120 min and quenched with 10 ml per well of 800 mM SAM.

Kinase Assay	Incorporation of radioactivity into nucleosome substrate is measured in a ?ashplate. IC50 values for enzymes in the histone methyltransferase panel are determined under similar balanced assay conditions with both SAM and protein/peptide substrate present at concentrations equal to their respective KM values.
Cell Research	For assessment of cell proliferation and viability in human cell lines, exponentially growing cells are plated, in triplicate, in 96-well plates in a ?nal volume of 150 ml. Cells are incubated in the presence of 3 μM (proliferation curve), or increasing concentrations (IC50 determination) of EPZ004777 up to 50 μM. Viable cell number is determined every 3–4 days for up to 18 days using the Guava Viacount assay and analyzed on a Guava EasyCyte Plus instrument according to the manufacturer's protocol. On days of cell counts, growth media and EPZ004777 are replaced and cells split back to a density of 5×104 cells/well. Total cell number is expressed as split-adjusted viable cells per well. For each cell line, IC50 values are determined from concentration-dependence curves at each time point using Graphpad Prism software. Experiments to determine IC50 values continues until IC50 values stabilized (day 18 for THP-1 cells, day 14 for all other cell lines). For assessment of the effect of EPZ004777 treatment on transformed murine hematopoietic progenitors, cells from two independent transductions for each virus are plated in 24-well plates at a density of 0.5–1×105 cell/well in 1 ml media in 24-well plates and exposed to increasing concentrations of EPZ004777 up to 30 mM. Cells are counted and replated at equal cell numbers in fresh media with fresh compound every 3–4 days. For MTT assays, cells from serial replatings are harvested on day 10 and plated, in triplicate at 2×104 cells/well in 100 ml media with the appropriate concentration of EPZ004777. Cells are incubated for 2.5 days, the exposed to 10 ml MTT reagent for 3 hr, and lysed over night in 100 ml MTT- solubilization buffer (both from Cell Proliferation Kit I [MTT]). (Only for Reference)

Solubility Information

Solubility	Ethanol: 54 mg/mL (100.06 mM),Sonication is recommended. DMSO: 54 mg/mL (100.06 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 (3.71 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	1.853 mL	9.2649 mL	18.5298 mL
5 mM	0.3706 mL	1.853 mL	3.706 mL
10 mM	0.1853 mL	0.9265 mL	1.853 mL
50 mM	0.0371 mL	0.1853 mL	0.3706 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

Daigle SR, et al. Cancer Cell. 2011, 20(1), 53-65.
Zhou Z, Chen H, Xie R, et al. Epigenetically modulated FOXM 1 suppresses dendritic cell maturation in pancreatic cancer and colon cancer. Molecular Oncology. 2019, 13(4): 873-893
Chen L, et al. Leukemia. 2013, 27(4), 813-822.
Deshpande AJ, et al. Blood. 2013, 121(13), 2533-2541.
Zhou Z, Chen H, Xie R, et al. Epigenetically modulated FOXM 1 suppresses dendritic cell maturation in pancreatic cancer and colon cancer[J]. Molecular oncology. 2019 Apr;13(4):873-893.