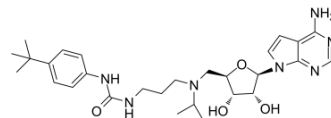


EPZ004777

Cat. No.:	HY-15227		
CAS No.:	1338466-77-5		
Molecular Formula:	C ₂₈ H ₄₁ N ₇ O ₄		
Molecular Weight:	539.67		
Target:	Histone Methyltransferase; Apoptosis		
Pathway:	Epigenetics; Apoptosis		
Storage:	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	6 months
		-20°C	1 month



SOLVENT & SOLUBILITY

In Vitro

DMSO : ≥ 100 mg/mL (185.30 mM)
 * "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent Concentration	Mass		
		1 mg	5 mg	10 mg
	1 mM	1.8530 mL	9.2649 mL	18.5298 mL
	5 mM	0.3706 mL	1.8530 mL	3.7060 mL
	10 mM	0.1853 mL	0.9265 mL	1.8530 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

- Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
 Solubility: ≥ 3 mg/mL (5.56 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
 Solubility: ≥ 3 mg/mL (5.56 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% corn oil
 Solubility: ≥ 3 mg/mL (5.56 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

EPZ004777 is a potent, selective DOT1L inhibitor with an IC₅₀ of 0.4 nM.

IC₅₀ & Target

IC₅₀: 0.4 nM (DOT1L)^[1]

In Vitro

EPZ004777 demonstrates potent, concentration-dependent inhibition of DOT1L enzyme activity with an IC₅₀ of 400±100 pM. EPZ004777 displays remarkable selectivity for inhibition of DOT1L over other HMTs (PRMT5, 521±137 nM; others, >50 μM).

The effect of extended EPZ004777 treatment is remarkably specific for the MLL-rearranged cell lines. The number of viable MV4-11 and MOLM-13 cells is dramatically reduced by EPZ004777, whereas the growth of Jurkat cells is unaffected. A small population of MV4-11 cells remain viable in the presence of EPZ004777, but their number remain constant when growth curves are tracked over longer periods indicating that they have ceased to divide. The proliferation of MLL-AF9-transformed cells is strongly inhibited by EPZ004777 at concentrations of 3 μ M or greater^[1]. EPZ004777 selectively inhibits proliferation of MLL-AF10 and CALM-AF10 transformed murine bone marrow cells^[2].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

In Vivo

EPZ004777 is well tolerated and no overt toxicity is observed. Complete blood count analysis after 14 days of continuous exposure to EPZ004777 revealed a statistically significant increase in the total white blood cell count, which resulted from an increase in neutrophils, monocytes, and lymphocytes. EPZ004777 (50, 100, or 150 mg/mL) administration is well tolerated, and no significant weight loss is observed^[1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay ^[1]

Avian (chicken) erythrocyte oligonucleosomes are purified. EPZ004777 is serially diluted 3-fold in DMSO for a total of ten concentrations, beginning at 1 μ M. A 1 μ L aliquot of each inhibitor dilution is plated in a 384-well microtiter plate. The 100% inhibition control consisted of 2.5 μ M final concentration of the product inhibitor S-adenosyl-L-homocysteine, (SAH). Compound is incubated for 30 min with 40 μ L 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 μ L per well of substrate mix comprising assay buffer with 200 nM 3H-SAM (80 Ci/mmol), 600 nM unlabeled SAM, and 20 nM nucleosomes are added to initiate the reaction (both substrates are present in the final reaction mixture at their respective K_M values, an assay format referred to as "balanced conditions"). Reactions are incubated for 120 min and quenched with 10 μ L per well of 800 μ M SAM. Incorporation of radioactivity into nucleosome substrate is measured in a flashplate. 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 K_M values^[1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Cell Assay ^[1]

For assessment of cell proliferation and viability in human cell lines, exponentially growing cells are plated, in triplicate, in 96-well plates at a density of 3×10^4 cells/well in a final volume of 150 μ L. Cells are incubated in the presence of 3 μ M (proliferation curve), or increasing concentrations (IC₅₀ determination) of EPZ004777 up to 50 μ M. 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. On days of cell counts, growth media and EPZ004777 are replaced and cells split back to a density of 5×10^4 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 continued until IC₅₀ values stabilized (day 18 for THP-1 cells, day 14 for all other cell lines)^[1].

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Animal Administration ^[1]

Mice^[1]

Nine-week-old female nude mice (nu/nu) are injected subcutaneously with MV4-11 cells in the right flank (200 μ L of a 5×10^7 cells/mL suspension in a 1:1 mixture of PBS and Matrigel). Mice are randomized to treatment groups when tumor sizes reached 300-400 mm³. Six mice received subcutaneous implant of osmotic pumps, containing 50 mg/mL EPZ004777 in 10% ethanol, 90% water, and five control mice received no pump implant. Six days after pump implant, animals are sacrificed and tumor samples from treated and control animals are collected for immunoblot analysis. For the disseminated leukemia model, MV4-11 cells are transduced with the pMMP-LucNeo retrovirus. Eight-week-old female NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ (NSG) mice are purchased from Jackson Laboratories. A total of 1×10^7 MV4-11-LucNeo cells are injected intravenously via the lateral tail vein. Engraftment of disseminate leukemia is determined by bioluminescence imaging after injection of 75 mg/kg of D-luciferin. Animals with documented leukemia are divided into treatment groups consisting of vehicle (15% ethanol, 50% PEG300, 35% water) loaded osmotic pumps, or EPZ004777 at 50, 100, or 150 mg/mL. Osmotic pumps are replaced after one week. Irritation caused by compound precipitation is observed in the 100 and 150 mg/mL dose groups, precluding additional pump replacements. Animals are monitored daily for clinical symptoms, and are euthanized when

they displayed signs of distress consistent with terminal leukemic disease. Log-rank analysis is used to determine statistical significance of the survival curves.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Cell Syst. 2018 Apr 25;6(4):424-443.e7.
- Hematol Oncol. 2019 Dec;37(5):617-625.
- Patent. US20180263995A1.

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REFERENCES

[1]. Daigle SR, et al. Selective killing of mixed lineage leukemia cells by a potent small-molecule DOT1L inhibitor. Cancer Cell. 2011 Jul 12;20(1):53-65.

[2]. Chen L, et al. Abrogation of MLL-AF10 and CALM-AF10-mediated transformation through genetic inactivation or pharmacological inhibition of the H3K79 methyltransferase Dot1l. Leukemia. 2013 Apr;27(4):813-22.

Caution: Product has not been fully validated for medical applications. For research use only.

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