产品名称：EPZ004777
产品别名：EPZ004777

生物活性：

Description	EPZ004777 is a potent, selective DOT1L inhibitor with an IC_{50} of 0.4 nM.
IC_{50} & Target	IC50: 0.4 nM (DOT1L)[1]
In Vitro	EPZ004777 demonstrates potent, concentration-dependent inhibition of DOT1L enzyme activity with an IC_{50} 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 μM or greater[1]. EPZ004777 selectively inhibits proliferation of MLL-AF10 and CALM-AF10 transformed murine bone marrow cells[2].
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].
Solvent&Solubility	***In Vitro:*** **DMSO : ≥ 100 mg/mL (185.30 mM)** * "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	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

*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。

储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

In Vivo:

请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 **In Vitro** 方式配制澄清的储备液，再依次添加助溶剂：

——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶

1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline

Solubility: ≥ 3 mg/mL (5.56 mM); Clear solution

此方案可获得 ≥ 3 mg/mL (5.56 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 30.0 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀；向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。

	2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline) Solubility: ≥ 3 mg/mL (5.56 mM); Clear solution 此方案可获得 ≥ 3 mg/mL (5.56 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 30.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀。 3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 3 mg/mL (5.56 mM); Clear solution 此方案可获得 ≥ 3 mg/mL (5.56 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的实验。 以 1 mL 工作液为例，取 100 μL 30.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
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.

实验参考：

Cell Assay	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 μL. Cells are incubated in the presence of 3 μM (proliferation curve), or increasing concentrations (IC_{50} 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. 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, IC_{50} values are determined from concentration-dependence curves at each time point using Graphpad Prism software. Experiments to determine IC_{50} values continued until IC_{50} values stabilized (day 18 for THP-1 cells, day 14 for all other cell lines)[1].
Animal Administration	Mice[1] Nine-week-old female nude mice (*nu/nu*) are injected subcutaneously with MV4-11 cells in the right flank (200 μL 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 mm^3. 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.
Kinase Assay	Avian (chicken) erythrocyte oligonucleosomes are purified. EPZ004777 is serially diluted 3-fold in DMSO for a total of ten concentrations, beginning at 1 μM. A 1 μL aliquot of each inhibitor dilution is plated in a 384-well microtiter plate. The 100% inhibition control consisted of 2.5 μM final concentration of the product inhibitor S-adenosyl-L-homocysteine, (SAH). Compound is incubated for 30 min with 40 μL 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 μL 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 μL per well of 800 μM SAM. Incorporation of radioactivity into nucleosome substrate is measured in a flashplate. IC_{50} 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].
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.