产品名称：EPZ-5676
产品别名：Pinometostat

生物活性：

Description	Pinometostat (EPZ-5676) is a potent **DOT1L histone methyltransferase** inhibitor with a K_i of 80 pM.
IC_{50} & Target	Ki: < 80 pM (DOT1L histone methyltransferase)
In Vitro	Pinometostat (EPZ-5676) inhibits H3K79me2 with IC50 values of 3 nM and 5 nM in MV4-11 and HL60 cells, respectively. Pinometostat (EPZ-5676) is a potent inhibitor of MV4-11 proliferation with an IC50 value of 3.5 nM[1]. Pinometostat (EPZ-5676) induces a synergistic and durable antiproliferative effect, increases expression of differentiation markers and apoptosis as single agent, and demonstrates combination benefit in combination with AML standard of care drugs in MLL-r cells[2].
In Vivo	Pinometostat (EPZ-5676) (70 mg/kg, i.p.) causes complete and sustained regression in a rat xenograft model of MLL-rearranged leukemia. Pinometostat (EPZ-5676) (70, 35 mg/kg, i.v.) reduces HOXA9 and MEIS1 mRNA levels of tumors taken from rats, and reduces MLL-fusion target gene expression in vivo[1].

Solvent&Solubility

In Vitro:

DMSO : ≥ 47.8 mg/mL (84.95 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	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

*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: ≥ 1.67 mg/mL (2.97 mM); Clear solution

此方案可获得 ≥ 1.67 mg/mL (2.97 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 16.699999 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: ≥ 1.67 mg/mL (2.97 mM); Clear solution

此方案可获得 ≥ 1.67 mg/mL (2.97 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 16.699999 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: ≥ 1.67 mg/mL (2.97 mM); Clear solution 此方案可获得 ≥ 1.67 mg/mL (2.97 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 16.699999 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. Potent inhibition of DOT1L as treatment for MLL-fusion leukemia. Blood. 2013 Jun 25. [Epub ahead of print] [2]. Klaus CR, et al. DOT1L inhibitor EPZ-5676 displays synergistic antiproliferative activity in combination with standard of care drugs and hypomethylating agents in MLL-rearranged leukemia cells. J Pharmacol Exp Ther. 2014 Sep;350(3):646-56.

实验参考：

Cell Assay	To analyse inhibition of histone methylation in MV4-11 cells following Pinometostat 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 Pinometostat (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. [1]
Animal Administration	0.2 mL of a MV4-11 cell suspension (1×10^7 cells) in PBS is injected subcutaneously into female athymic nude mice (Crl:NU(Ncr)-Foxn1nu). Tumors are measured by calipers and mice are randomized according to tumor size into treatment groups (n=10) before the initiation of dosing with Pinometostat (EPZ-5676) when tumor volumes reache approximately 100 mma^3. Pinometostat is administered intraperitoneally three times daily for 28 days at 10 and 20 mg/kg in 10% ethanol in saline. Mice are weighed and tumors measured with calipers twice weekly until the end of the study[1]
References	[1]. Daigle SR, et al. Potent inhibition of DOT1L as treatment for MLL-fusion leukemia. Blood. 2013 Jun 25. [Epub ahead of print] [2]. Klaus CR, et al. DOT1L inhibitor EPZ-5676 displays synergistic antiproliferative activity in combination with standard of care drugs and hypomethylating agents in MLL-rearranged leukemia cells. J Pharmacol Exp Ther. 2014 Sep;350(3):646-56.

源叶生物