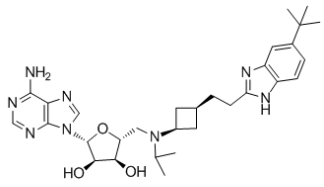
Data Sheet

Product Name:	EPZ–5676
Cat. No.:	HY-15593
CAS No.:	1380288-87-8
Molecular Formula:	C30H42N8O3
Molecular Weight:	562.71
Target:	Histone Methyltransferase
Pathway:	Epigenetics
Solubility:	DMSO: ≥ 47.8 mg/mL



BIOLOGICAL ACTIVITY:

EPZ–5676 is a potent and selective aminonucleoside inhibitor of **DOT1L histone methyltransferase** with **K_i** of < 80 pM, demonstrating > 37,000–fold selectivity against all other PMTs tested, and inhibits H3K79 methylation in tumor.

IC50 & Target: Ki: < 80 pM (DOT1L histone methyltransferase)

In Vitro: EPZ–5676 inhibits H3K79me2 with IC_{50} values of 3 nM and 5 nM in MV4–11 and HL60 cells, respectively. EPZ–5676 is a potent inhibitor of MV4–11 proliferation with an IC_{50} value of 3.5 nM[1]. EPZ–5676 induces a synergistic and durable antiproliferative effect, increases expression of differentiation markers and apoptosis as dingle agent, and demonstrates combination benefit in combination with AML standard of care drugs in MLL–r cells[2].

In Vivo: EPZ–5676 (70 mg/kg, i.p.) causes complete and sustained regression in a rat xenograft model of MLL–rearranged leukemia. 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].

PROTOCOL (Extracted from published papers and Only for reference)

Cell Assay: EPZ–5676 is dissolved in DMSO.[1]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.

Animal Administration: EPZ–5676 is dissolved in 10% ethanol in saline.[1]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 EPZ–5676 when tumor volumes reache approximately 100 mma^3. EPZ–5676 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.

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.

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