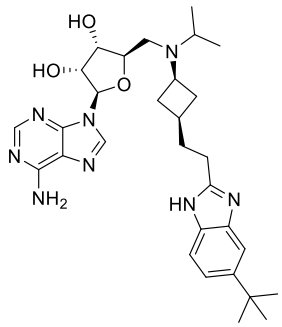
Product data sheet

MedKoo Cat#: 205892 Name: Pinometostat CAS#: 1380288-87-8 Chemical Formula: $C_{30}H_{42}N_8O_3$ Exact Mass: 562.33799 Molecular Weight: 562.71	
Product supplied as:	Powder
Purity (by HPLC):	≥ 98%
Shipping conditions	Ambient temperature
Storage conditions:	Powder: -20°C 3 years; 4°C 2 years.
	In solvent: -80°C 3 months; -20°C 2 weeks.

1. Product description:

Pinometostat, also known as EPZ-5676, is a small molecule inhibitor of histone methyltransferase with potential antineoplastic activity. Upon intravenous administration, EPZ-5676 specifically blocks the activity of the histone lysine-methyltransferase DOT1L, thereby inhibiting the methylation of nucleosomal histone H3 on lysine 79 (H3K79) that is bound to the mixed lineage leukemia (MLL) fusion protein which targets genes and blocks the expression of leukemogenic genes.

2. CoA, QC data, SDS, and handling instruction

SDS and handling instruction, CoA with copies of QC data (NMR, HPLC and MS analytical spectra) can be downloaded from the product web page under "QC And Documents" section. Note: copies of analytical spectra may not be available if the product is being supplied by MedKoo partners. Whether the product was made by MedKoo or provided by its partners, the quality is 100% guaranteed.

3. Solubility data

Solvent	Max Conc. mg/mL	Max Conc. mM
DMSO	57.6	102.36
DMF	30.0	53.31
Ethanol	65.0	115.51
Ethanol:PBS (pH 7.2) (1:8)	0.1	0.18

4. Stock solution preparation table:

Concentration / Solvent Volume / Mass	**1 mg**	**5 mg**	**10 mg**
1 mM	1.78 mL	8.89 mL	17.77 mL
5 mM	0.36 mL	1.78 mL	3.55 mL
10 mM	0.18 mL	0.89 mL	1.78 mL
50 mM	0.04 mL	0.18 mL	0.36 mL

5. Molarity Calculator, Reconstitution Calculator, Dilution Calculator

Please refer the product web page under section of "Calculator"

6. Recommended literature which reported protocols for in vitro and in vivo study

In vitro study

1. Godfrey L, Crump NT, Thorne R, Lau IJ, Repapi E, Dimou D, Smith AL, Harman JR, Telenius JM, Oudelaar AM, Downes DJ, Vyas P, Hughes JR, Milne TA. DOT1L inhibition reveals a distinct subset of enhancers dependent on H3K79 methylation. Nat Commun. 2019 Jun 26;10(1):2803. doi: 10.1038/s41467-019-10844-3. PMID: 31243293; PMCID: PMC6594956.
2. Zhang W, Zhao C, Zhao J, Zhu Y, Weng X, Chen Q, Sun H, Mi JQ, Li J, Zhu J, Chen Z, Pandolfi PP, Chen S, Yan X, Xu J. Inactivation of PBX3 and HOXA9 by down-regulating H3K79 methylation represses NPM1-mutated leukemic cell survival. Theranostics. 2018 Jul 30;8(16):4359-4371. doi: 10.7150/thno.26900. PMID: 30214626; PMCID: PMC6134928.

In vivo study

1. Yang W, Yu H, Huang J, Miao X, Wang Q, Wang Y, Cheng Y, He S, Zhao F, Meng L, Wang B, Qian F, Ren X, Jin M, Gu Y, Zhang Y, Cai W. Inhibition of Dot1L Alleviates Fulminant Hepatitis Through Myeloid-Derived Suppressor Cells. Cell Mol

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Gastroenterol Hepatol. 2021 Jan 23;12(1):81-98. doi: 10.1016/j.jcmgh.2021.01.013. Epub ahead of print. PMID: 33497867; PMCID: PMC8081916.
2. Song Z, Wei Z, Wang Q, Zhang X, Tao X, Wu N, Liu X, Qian J. The role of DOT1L in the proliferation and prognosis of gastric cancer. Biosci Rep. 2020 Jan 31;40(1):BSR20193515. doi: 10.1042/BSR20193515. PMID: 31939604; PMCID: PMC6997103.

7. Bioactivity

Biological target:

Pinometostat (EPZ-5676) is a DOT1L histone methyltransferase inhibitor with a Ki of 80 pM.

In vitro activity

EPZ5676, a DOT1L inhibitor approved for use in clinical trials, is less toxic and more effective than EPZ004777. Here, this study found that EPZ5676 was more efficient than EPZ004777 in down-regulating HOXA9, PBX3 and H3K79m2 expression in human NPMc+ cells (**Figure S5A**), and in promoting cell apoptosis (Figure S5B). EPZ5676 gradually reduced HOXA9 and PBX3 expression in OCI-AML3 cells as the treatment time increased. The levels of cleaved caspase 3 and PARP were also increased by EPZ5676 treatment in a time-dependent manner (Figure 5A). These changes were not observed in OCI-AML2 cells (Figure 5A). The two cell lines were treated with EPZ5676 for 3 days or 7 days and then were stained with Annexin V/PI for apoptosis detection. EPZ5676 induced a higher percentage of apoptotic cells in the OCI-AML3 line than in the OCI-AML2 line on both day 3 and day 7 (Figure 5B). Additionally, a decrease in HOXA9 and PBX3 expression and an increase in the expression of apoptosis-related proteins and the number of apoptotic cells were detected only in KG-1 cells stably transduced with mutant NPM1 but not in those transduced with the vector or WT NPM1 after 7 days of treatment with EPZ5676 (Figure 5C-D).

Reference: Theranostics. 2018 Jul 30;8(16):4359-4371. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6134928/

In vivo activity

Mononuclear cells (MNCs) accumulated significantly in the liver and spleen of *P. acnes*-primed control mice (Figure 2*A*), and both the percentage and absolute number of $CD4^+$ T cells increased dramatically in the liver and spleen (Figure 2*B* and *C*). By contrast, infiltration of MNCs and $CD4^+$ T cells decreased significantly both in the liver and spleen of EPZ-5676–treated mice (Figure 2*A*–*C*). To detect the proliferation of $CD4^+$ T cells, the study injected BrdU (5-bromo-2-deoxyuridine), a synthetic nucleoside that could be incorporated into newly synthesized DNA to monitor the cell proliferation, into EPZ-5676–treated or *P. acnes*-primed control mice. The flow cytometric analysis revealed that EPZ-5676 treatment decreased the frequencies of $BrdU^+$ $CD4^+$ T cells in the liver and spleen after *P. acnes* priming (Figure 2*D*), suggesting that Dot1L inhibition may regulate the proliferation of $CD4^+$ T cells in vivo. In addition, EPZ-5676–treated mice showed decreased $CD44^{hi}CD62L^{lo}$ $CD4^+$ T cells, and increased $CD62L^{hi}CD44^{lo}$ $CD4^+$ T cells (Figure 2*E*), suggesting that Dot1L inhibition suppressed $CD4^+$ T cell activation in vivo. The study also found reduced expression of chemokine receptors, such as CXCR3 and CCR7 on $CD4^+$ T cells (Figure 3*A* and *B*) and their respective chemokines CXCL9, CXCL10, and CCL21 in the liver of EPZ-5676–treated mice (Figure 3*C*). These results indicated that Dot1L inhibition also suppressed the chemotaxis of pathogenic $CD4^+$ T cells into the liver.

Reference: Cell Mol Gastroenterol Hepatol. 2021 Jan 23;12(1):81-98. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8081916/