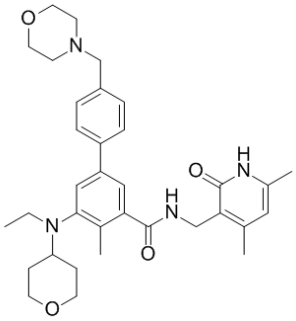
Data Sheet

Product Name:	EPZ–6438
Cat. No.:	HY-13803
CAS No.:	1403254-99-8
Molecular Formula:	C34H44N4O4
Molecular Weight:	572.74
Target:	Epigenetic Reader Domain; Histone Methyltransferase
Pathway:	Epigenetics
Solubility:	DMSO: 5.0 mg/mL (need ultrasonic)



BIOLOGICAL ACTIVITY:

EPZ–6438 inhibits the activity of human PRC2–containing wild–type **EZH2** with **Ki** of 2.5±0.5 nM.
IC50 & Target: Ki: 2.5 nM (EZH2)[1]
In Vitro: EPZ–6438 inhibits EZH2 in a manner competitive with the substrate S–adenosylmethionine (SAM). EPZ–6438 inhibits EZH1, EZH2(in peptide assay), EZH2 (in nucleosome assay) with $IC_{50}$of 392 nM, 11 nM and 16 nM, respectively. EPZ–6438 displays a 35–fold selectivity versus EZH1 and >4,500–fold selectivity relative to 14 other HMTs tested[1].
In Vivo: EPZ–6438 (125 mg/kg) induces tumor stasis during the administration period and produced a significant tumor growth delay compared with vehicle after the dosing period. Measuring EPZ–6438 plasma levels either 5 min before or 3 h after dosing on day 21 reveals a clear dose–dependent increase in systemic exposure[1]. Dose–dependent target inhibition is observed in PBMCs and bone marrow from rats dosed with EPZ–6438 (orally administered, 100, 300, or 1,000 mg/kg) as measured by ELISA[2].

PROTOCOL (Extracted from published papers and Only for reference)

Cell Assay: EPZ–6438 is dissolved in DMSO and stored, and then diluted with appropriate media before use[1]. [1]293T (CRL–11268), RD (CRL–136), SJCRH30 (CRL–2061), A204 (HTB–82), G401 (CRL–1441), G402 (CRL–1440), KYM–1 (JCRB0627), 293T, RD, SJCRH30, A204, G401, and G402 cells are used. On day 0, cells are either untreated, DMSO–treated, or treated with EPZ–6438 starting at 10 μM and decreasing in either threefold or fourfold dilutions. Plates are read on day 0, day 4, and day 7 using Cell Titer Glo, with compound/media being replenished on day 4. On day 7, the six–well plates are trypsinized, centrifuged, and resuspended in fresh media for counting by Vi–Cell. Cells from each treatment are replated at the original density in 96–well plates in triplicate. Cells are allowed to adhere to the plate overnight, and cells are treated as on day 0. On days 7, 11, and 14, plates are read using Cell Titer Glo, with compound/media being replenished on day 11. Averages of triplicates are used to plot proliferation over the time course, and calculate IC_{50} values. For cell cycle and apoptosis, G401 and RD cells are plated in 15–cm dishes in duplicate at a density of 1×10^6 cells per plate. Cells are incubated with EPZ–6438 at 1 μM, in a total of 25 mL, over a course of 14 d, with cells being split back to original plating density on day 4, 7, and 11. Cell cycle analysis and TUNEL assay are performed using a Guava flow cytometer[1].
Animal Administration: EPZ–6438 is prepared in 0.5% NaCMC plus 0.1% Tween 80 in water.[1][2]Mice[1]
SCID mice bearing s.c. G401 xenografts are inoculated s.c. at the right flank with G401 tumor cells (5×10^6 cells per mouse) in 0.2–mL mixture of base media and Matrigel for tumor development. The treatments are started when the tumor size reached 157 mm^3 for the tumor efficacy study (n=16 mice per group). EPZ–6438 (125 mg/kg, 250 mg/kg, and 500 mg/kg) or vehicle (0.5% NaCMC plus 0.1% Tween 80 in water) is administered orally BID at a dose volume of 10 μL/g for either 21 or 28 d. Animal body weights are measured every day during the first week, and then twice weekly for the remainder of the study. Tumor size is measured twice weekly in two dimensions using a caliper, and the volume is expressed in cubic millimeters.
Rats[2]

Male and female Sprague–Dawley rats (8 weeks old) are orally treated with EPZ–6438 (100, 300, or 1,000 mg/kg) or vehicle for 28 days once a day. On day 22, the females from the highest dose group received another dose and are subsequently euthanized on day 23 approximately 29 hours after the last dose. All other animals are euthanized on day 29 approximately 29 hours after the last dose administered on day 28. At euthanasia, the full blood volume is collected, peripheral blood mononuclear cells (PBMC) are isolated, and cell pellets are frozen and stored at -80°C before analysis. A 2–mm–thick slice of skin is formalin–fixed for 24 hours and transferred to 70% ethanol. The fixed tissues are paraffin embedded. Bone marrow samples are collected from femur, tibia, and hip bones, frozen and stored at -80°C before analysis.

References:

[1]. Knutson SK, et al. Durable tumor regression in genetically altered malignant rhabdoid tumors by inhibition of methyltransferaseEZH2. Proc Natl Acad Sci U S A. 2013 May 7;110(19):7922–7.

[2]. Knutson SK, et al. Selective inhibition of EZH2 by EPZ–6438 leads to potent antitumor activity in EZH2–mutant non–Hodgkin lymphoma. Mol Cancer Ther. 2014 Apr;13(4):842–54.

[3]. Majumder S, et al. Shifts in podocyte histone H3K27me3 regulate mouse and human glomerular disease. J Clin Invest. 2018 Jan 2;128(1):483–499.

Tel: 609-228-6898 Fax: 609-228-5909 E-mail: tech@MedChemExpress.com

Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA