UNC1999

Cat. No.: HY-15646

CAS No.: 1431612-23-5

Molecular Formula: $C_{33}H_{43}N_7O_2$

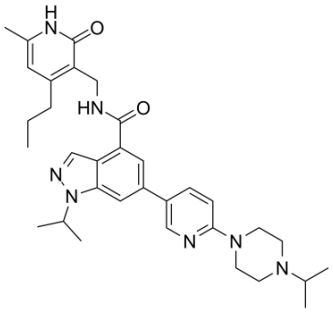
Molecular Weight: 569.74

Target: Histone Methyltransferase; Autophagy

Pathway: Epigenetics; Autophagy

Storage:

Powder	-20°C	3 years
	4°C	2 years
In solvent	-80°C	6 months
	-20°C	1 month



SOLVENT & SOLUBILITY

In Vitro

DMSO : 100 mg/mL (175.52 mM; Need ultrasonic)

H_2O : < 0.1 mg/mL (ultrasonic;warming;heat to 80°C) (insoluble)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	1.7552 mL	8.7759 mL	17.5519 mL
5 mM	0.3510 mL	1.7552 mL	3.5104 mL
10 mM	0.1755 mL	0.8776 mL	1.7552 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.25 mg/mL (3.95 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: 2.25 mg/mL (3.95 mM); Suspended solution; Need ultrasonic
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: 2.25 mg/mL (3.95 mM); Clear solution; Need warming

BIOLOGICAL ACTIVITY

Description

UNC1999 is a SAM-competitive, potent and selective inhibitor of EZH2/1 with IC_{50}s of <10 nM and 45 nM, repectively.

IC_{50} & Target

IC50: <10 nM (EZH2), 45 nM (EZH1)[1]

In Vitro

UNC1999, the first orally bioavailable inhibitor that has high in vitro potency for wild-type and mutant EZH2 as well as EZH1, a closely related H3K27 methyltransferase that shares 96% sequence identity with EZH2 in their respective catalytic

	domains. UNC1999 is highly selective for EZH2 and EZH1 over a broad range of epigenetic and non-epigenetic targets, competitive with the cofactor SAM, and non-competitive with the peptide substrate. UNC1999 has K_i values of 4,700 nM, 65 nM, 300 nM, and 1,500 nM for sigma1, sigma2, histamine H_3, and NET, respectively. NC1999 selectively kills DB cells, a DLBCL cell line with the EZH2 Y641N mutation. UNC1999 displays a concentration- and time-dependent inhibition of DB cell proliferation (EC_{50}=633±101 nM (n=3))[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	A single intraperitoneal (IP) injection of UNC1999 at 15, 50, or 150 mg/kg achieved high C_{max} (9,700-11,800 nM) and exhibited dose linearity in male Swiss albino mice. Both the 150 and 50 mg/kg IP doses resulted in the plasma concentrations of UNC1999 above its cellular IC_{50} over the entire 24 h period while the 15 mg/kg IP dose led to the plasma concentrations of UNC1999 above its cellular IC_{50} for approximately 12 h. We next examined whether UNC1999 is orally bioavailable and are pleased to find that a single 50 mg/kg oral dose of UNC1999 achieved high C_{max} (4,700 nM) and good exposure levels in male Swiss albino mice. The plasma concentrations of UNC1999 are maintained above its cellular IC_{50} for approximately 20 h following this single oral dose. It is worth noting that all doses including the 150 mg/kg IP dose are well tolerated by all test mice, and no adverse effects are observed[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	EZH2 Y641N mutant is generated and assayed by BPS Bioscience. A series of dilutions of UNC1999 are prepared with 10% DMSO in HMT assay buffer and 5 µL of the dilution is added to a 50 µL reaction so that the final concentration of DMSO is 1% in all of reactions. All of the enzymatic reactions are conducted in duplicate at room temperature for 60 minutes (EZH2) and 180 minutes (EZH2 Y641N) in a 50 µL mixture containing HMT assay buffer, S-adenoslymethionine, enzyme, and UNC1999. These 50 µL reactions are carried out in wells of a HMT substrate pre-coated plate. After enzymatic reactions, the reaction mixtures are discarded and each of the wells is washed three times with TBST buffer, and slowly shaken with Blocking Buffer for 10 minutes. Wells are emptied, and 100 µL of diluted 1°antibody is added. The plate is then slowly shaken for 60 minutes at room temperature. As before, the plate is emptied and washed three times, and shaken with Blocking Buffer for 10 minutes at room temperature. After discarding the Blocking Buffer, 100 µL of diluted 2°antibody is added. The plate is then slowly shaken for 30 minutes at room temperature. As before, the plate is emptied and washed three times, and shaken with Blocking Buffer for 10 minutes at room temperature. Blocking Buffer is discarded and a mixture of the HRP chemiluminescent substrates is freshly prepared. 100 µL of this mixture is added to each empty well. Immediately, the luminescence of the samples is measured in a BioTek SynergyTM 2 microplate reader[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	DB cells, a diffuse-large B-cell lymphoma cell line harboring the EZH2 Y641N mutation, are obtained from ATCC and cultured in RPMI 1640 supplemented with 10% fetal bovine serum, antibiotics, and various concentration of UNC1999 (0.1, 0.2, 0.3, 0.4, 0.5, 1, 2, 3, and 5 µM) or UNC2400 and DMSO control. The medium containing the test compound or control is refreshed every three days. The numbers of viable cells from at least three independent experiments are measured using TC20 automated cell counter system. Total histones are prepared from cell nuclei using an acidic extraction protocol. About 1 microgram of total histones is separated using 15% of SDS-PAGE, transferred to PVDF membranes, and probed with histone antibodies. Antibodies used in this study are those against EZH2, general H3, and H3K27me3[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [1]	Mice[1] Standard PK studies are conducted using male Swiss albino mice at Sai Life Sciences. Four doses (15, 50, and 150 mg/kg IP, and 50 mg/kg PO) of UNC1999 are evaluated. Each study lasted 24 h. Plasma concentrations of UNC1999 reported at each of the eight time points (0.08, 0.25, 0.5, 1, 2, 4, 8, and 24 h post dosing) are the average values from 3 test animals. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Proc Natl Acad Sci U S A. 2019 Feb 19;116(8):2961-2966.
- Transl Oncol. 2020 Oct;13(10):100819.
- Patent. US20180263995A1.

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REFERENCES

[1]. Konze KD, et al. An Orally Bioavailable Chemical Probe of the Lysine Methyltransferases EZH2 and EZH1. ACS Chem Biol. 2013;8(6):1324-34.

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