产品名称：UNC1999
产品别名：UNC1999

生物活性：

Description	UNC1999 is a SAM-competitive, potent and selective inhibitor of EZH2/1 with IC50s 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].
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].
Solvent&Solubility	***In Vitro:*** **DMSO : 125 mg/mL (219.40 mM;** Need ultrasonic) **H_2O : < 0.1 mg/mL** (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

*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: ≥ 2.25 mg/mL (3.95 mM); Clear solution

	此方案可获得 ≥ 2.25 mg/mL (3.95 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 22.5 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: 2.25 mg/mL (3.95 mM); Suspended solution; Need ultrasonic 此方案可获得 2.25 mg/mL (3.95 mM)的均匀悬浊液，悬浊液可用于口服和腹腔注射。 以 1 mL 工作液为例，取 100 μL 22.5 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: 2.25 mg/mL (3.95 mM); Clear solution; Need warming 此方案可获得 2.25 mg/mL (3.95 mM)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 22.5 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
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.

实验参考：

Cell Assay	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].
Animal Administration	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.
Kinase Assay	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].
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.