产品名称：GSK343
产品别名：GSK343

生物活性：

Description	GSK343 is a highly potent and selective EZH2 inhibitor with an IC50 of 4 nM.
IC_{50} & Target	IC50: 4 nM (EZH2), 240 nM (EZH1)[1]
In Vitro	GSK343, which contains an n-propyl group at the 4-position of the pyridone, has EZH2 K_i^{app}=1.2±0.2 nM. In this 6-day proliferation assay, among the cell lines evaluated in this study, the prostate cancer cell line LNCaP is the most sensitive to EZH2 inhibition, with growth IC_{50} value of 2.9 μM for GSK343[1]. GSK343 is found to have half maximal inhibitory concentration values of 13 μM in HeLa cells and 15 μM in SiHa cells[2].
In Vivo	Compare with the controls, GSK343 (5 mg/kg)-treated mice exhibits significantly inhibited tumor growth. The average tumor volume and weight of the GSK343-treated cohort is remarkably reduced. As early as 20 days post-implantation, a significant reduction in tumor growth is observed in the GSK343-treated cohort relative to the control cohort; this difference persisted through the remainder of the study. In addition, compare with the control cohort, the GSK343-treated animals in the xenograft model show a remarkable increase in messenger RNA levels of E-cadherin but a significant decrease in vimentin messenger RNA levels[2].
Solvent&Solubility	***In Vitro:*** **DMSO : 15.62 mg/mL (28.84 mM;** Need ultrasonic)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	1.8461 mL	9.2304 mL	18.4607 mL
5 mM	0.3692 mL	1.8461 mL	3.6921 mL
10 mM	0.1846 mL	0.9230 mL	1.8461 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: ≥ 1.56 mg/mL (2.88 mM); Clear solution

此方案可获得 ≥ 1.56 mg/mL (2.88 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 15.599999 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: ≥ 1.56 mg/mL (2.88 mM); Clear solution

此方案可获得 ≥ 1.56 mg/mL (2.88 mM，饱和度未知) 的澄清溶液。

	以 1 mL 工作液为例，取 100 μL 15.599999 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: ≥ 1.56 mg/mL (2.88 mM); Clear solution 此方案可获得 ≥ 1.56 mg/mL (2.88 mM，饱和度未知) 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 15.599999 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Sharad K, et al. Identification of Potent, Selective, Cell-Active Inhibitors of the Histone Lysine Methyltransferase EZH2. ACS Med Chem Lett. 2012 Oct 19;3(12):1091-6. [2]. Ding M, et al. The polycomb group protein enhancer of zeste 2 is a novel therapeutic target for cervical cancer. Clin Exp Pharmacol Physiol. 2015 May;42(5):458-64.

实验参考：

Cell Assay	To account for varying doubling rates among cancer cell lines, the optimal cell seeding is determined empirically for all cell lines by examining their growth in a 384-well plate over 6 days with a wide range of seeding densities. Cells are then plated at the optimal seeding density and allowed to adhere overnight. Cells are treated in duplicate with a 20-point 2-fold dilution series of GSK343 or 0.147% DMSO (vehicle control) and incubated for 6 days at 37°C in 5% CO_2. Cells are then lysed with 25 μL CellTiter-Glo per well and chemiluminescence is quantified with a TECAN Safire2 microplate reader. In addition, an untreated plate of cells is harvested at the time of GSK343 addition (T_0) to quantify the starting number of cells. CTG values after 6 days of treatment are expressed as a percent of the T_0value and plotted against GSK343 concentration. Data are fit with a 4-parameter equation to generate a concentration response curve and the concentration of GSK343 required to inhibit 50% of growth (gIC_{50}) is determined[1].
Animal Administration	Mice[2] Six-week-old female nude BALB/c mice are used. To study the effect of the EZH2 inhibitor GSK343, 5 mg/kg in 100-μL phosphate-buffered saline is injected intraperitoneally every other day into BALB/c nude mice (n=6) after the tumor volume reaches 100 mm^3. In this analysis, the negative control group (n=6) received saline. After 40 days, the mice are killed, and the subcutaneous tumors are surgically excised, weighed, photographed, sectioned, and fixed in 10% formalin. The expression levels of E-cadherin, N-cadherin, and vimentin in the tumours are measured by real-time reverse transcription polymerase chain reaction.
Kinase Assay	Activity against EZH2 is assessed using 5 member PRC2 complex (Flag-EZH2, EED, SUZ12, AEBP2, RbAp48). The assay protocol may be summarized as follows: 10 mM stocks of GSK343 are prepared from solid in 100% DMSO. An 11 point serial dilution master plate is prepared in 384 well format (1:3 dilution, columns 6 and 18 are equal volume DMSO controls) and dispensed to assay ready plates using acoustic dispensing technology to create a 100 nL stamp of GSK343 and DMSO controls. The assay additions consisted of equal volume additions of 10 nM EZH2 and the substrate solution (5 μg/mL HeLa nucleosomes and 0.25 μM [^{3}H]-SAM) dispensed into assay plates using a multi-drop combi dispense. Reaction plates are incubated for 1 hr and quenched with an equal volume addition of 0.5 mg/mL PS-PEI Imaging Beads (RPNQ0098) containing 0.1 mM unlabeled SAM. The plates are sealed, dark adapted for 30 minutes, and a 5 minute endpoint luminescence

	image is acquired using a Viewlux imager. Plate statistics such as Z' and signal to background as well as dose response curves are analyzed using ActivityBaseXE. The in vitro biochemical activity of EZH1 is assessed as part of a 5 member PRC2 complex using a 384 well SPA assay identical to EZH2. Buffer components, reagent dispensing, GSK343 plate preparation, quench conditions and data analysis are identical for EZH1 and EZH2 with final assay concentrations of 20 nM EZH1, 5 μg/mL HeLa nucleosomes and 0.25 μM [^{3}H]-SAM. Further data analysis, pIC_{50} pivots and visualizations are enabled by TIBCO Spotfire[1].
References	[1]. Sharad K, et al. Identification of Potent, Selective, Cell-Active Inhibitors of the Histone Lysine Methyltransferase EZH2. ACS Med Chem Lett. 2012 Oct 19;3(12):1091-6. [2]. Ding M, et al. The polycomb group protein enhancer of zeste 2 is a novel therapeutic target for cervical cancer. Clin Exp Pharmacol Physiol. 2015 May;42(5):458-64.