产品名称：CPI-1205
产品别名：CPI-1205

生物活性：

Description	CPI-1205, a highly potent and selective EZH2 inhibitor (biochemical IC50=2 nM, cellular EC50=32 nM).
IC_{50} & Target	IC50: 2 nM (EZH2)[1]
In Vitro	When tested within an in vitro hERG binding assay at concentration ranges of 45 nM to 100 μM, CPI-1205 shows an IC50 of 21.3 μM. CPI-1205 demonstrates modest selectivity (EZH1 IC50 of 52±11 nM) when tested against enhancer of zeste homologue 1 (EZH1), a methyltransferase highly related to EZH2. CPI-1205 achieves unbound exposures well above their respective cellular potencies; however, only the unbound exposure for CPI-1205 remains well above the cellular EC50 up to 4 h[1].
In Vivo	CPI-1205 is dosed at 160 mg/kg orally twice daily (po BID) for 25 days in tumor bearing female CB-17 SCID mice. Upon treatment of tumor-bearing CB-17 SCID mice with CPI-1205, tumor regression is observed within 2 weeks. By the end of day 25, significant tumor growth inhibition is recorded (>97% TGI relative to vehicle). CPI-1205 demonstrates robust antitumor effects in a Karpas-422 xenograft model when dosed at 160 mg/kg BID. CPI-1205 shows excellent oral bioavailability. CPI-1205 exhibits moderate clearance of 2.16 L/h/kg (40% liver blood flow), a half-life of ~1.6 h, similar volume of distribution (1.4 L/kg), and excellent bioavailability (100% F)[1].
Solvent&Solubility	***In Vitro:*** **DMSO： ≥ 50 mg/mL (96.42 mM)** * "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.9284 mL	9.6419 mL	19.2838 mL
	5 mM	0.3857 mL	1.9284 mL	3.8568 mL
	10 mM	0.1928 mL	0.9642 mL	1.9284 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: ≥ 2.5 mg/mL (4.82 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (4.82 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 25.0 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: ≥ 2.5 mg/mL (4.82 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.82 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 25.0 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: ≥ 2.5 mg/mL (4.82 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.82 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 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Vaswani RG, et al. Identification of (R)-N-((4-Methoxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-2-methyl-1-(1-(1-(2,2,2-trifluoroethyl)piperidin-4-yl)ethyl)-1H-indole-3-carboxamide (CPI-1205), a Potent and Selective Inhibitor of Histone Methyltransferase EZH2, Suitable for Phase I Clinical Trials for B-Cell Lymphomas. J Med Chem. 2016 Nov 10;59(21):9928-9941.

实验参考：

Cell Assay	Ten different doses of each test compound (in a series of 3-fold dilutions) are plated in duplicate 384-well tissue culture treated plates. HeLa cells grown in culture are trypsinized and counted. Cell are diluted to 67,000 cells per mL in10% DMEM and 15 μL (1,000 cells) are plated into each well using the Biotek MicroFloTM Select Dispenser. Plates are incubated at 37°C/5% CO_2 for 72 hrs. One of the duplicate plates is processed for AlphaLISA and the other for viability. Cell viability is assayed by adding 15 μL of Cell Titer Glo to each well with cells with media. The plates are incubated at RT for 15-20 minutes on a plate shaker at low speed. The plates are then read using an EnVision-Alpha Reader[1].
Animal Administration	Rats[1] CPI-1205 is orally administered in a GLP compliant toxicity study for 4 weeks to both Sprague-Dawley rats and beagle dogs followed by a 4-week recovery period. CPI-1205 is administered by oral gavage at single daily doses (QD) of 100, 300, and 600 mg/kg to rats for 28 days and at twice daily doses (BID) of 50, 150, and 500 mg/kg for 28 days to dogs. In general, CPI-1205 is well-tolerated in the 28-day GLP toxicology studies, and any findings are reversible over the recovery period.
Kinase Assay	PRC2 (wt or Y641N mutant), biotinylated nucleosome, H3K27me3 activator peptide and CPI-1205 (in DMSO) are incubated in 50 mM Tris, pH 8.5, 5 mM $MgCl_2$, 1 mM DTT, 70 uM Brij-35, and 0.1 mg/mL BSA for 30 minutes. Reaction is initiated by addition of [^{3}H]-SAM to final conditions of 5 nM PRC2, 200 nM nucleosome (concentration expressed as H3), activator peptide (3.6 μM) and 200 nM [^{3}H]-SAM in a total volume of 25 μL in 384 well Greiner plates. For CPI-1205 analysis assays are either single point or ten point dose-responses with final total DMSO of 0.8 or 1.6% (v/v). Typically assays are run for 60 minutes with <35% substrate turnover. After reaction assays are quenched by addition of 20 μL of 2 mM SAH and 200 mM EDTA in 50 mM Tris, pH 8.5. Reactions are transferred to streptavidin-coated FlashPlates, incubated for 2 h, aspirated, washed, and read on a TopCount[1].
References	[1]. Vaswani RG, et al. Identification of (R)-N-((4-Methoxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-2-methyl-1-(1-(1-(2,2,2-trifluoroethyl)piperidin-4-yl)ethyl)-1H-indole-3-carboxamide (CPI-1205), a Potent and Selective Inhibitor of

	Histone Methyltransferase EZH2, Suitable for Phase I Clinical Trials for B-Cell Lymphomas. J Med Chem. 2016 Nov 10;59(21):9928-9941.