产品名称：

2-Cyclohexyl-6-methoxy-N-[1-(1-methylethyl)-4-piperidinyl]-7-[3-(1-pyrrolidinyl)propoxy]-4-quinazoli

产品别名：**UNC0638**

生物活性：

Description	UNC0638 selectively inhibits **G9a** and **GLP histone methyltransferase** activity with **IC_{50}**s of less than 15 nM and 19 nM, respectively.
IC_{50} & Target	IC50: <15 nM (G9a), 19±1 nM (GLP)[1]
In Vitro	UNC0638, an inhibitor of G9a and GLP with excellent potency and selectivity over a wide range of epigenetic and non-epigenetic targets.The Ki of UNC0638 is determined to be 3.0±0.05 nM (n=2). Consistent with this, the Morrison Ki for UNC0638 is 3.7±0.2 nM (n=3). The selectivity of UNC0638 over a wide range of epigenetic targets is evaluated. Notably, UNC0638 is inactive against other H3K9 (SUV39H1 and SUV39H2), H3K27 (EZH2), H3K4 (SETD7, MLL and SMYD3), H3K79 (DOT1L) and H4K20 (SETD8) methyltransferases, as well as PRDM1, PRDM10 and PRDM12. In addition, UNC0638 is inactive against protein arginine methyltransferases PRMT1 and PRMT3, and HTATIP, a histone acetyltransferase. Of note, UNC0638 has weak but measurable activity against JMJD2E (IC50=4,500±1,100 nM), a Jumonji protein demethylase and DNA methyltransferase DNMT1 (IC50=107,000±6,000 nM). Nevertheless, the selectivity of UNC0638 for G9a and GLP over JMJD2E is >200-fold, and selectivity for G9a and GLP over DNMT1 is >5,000-fold[1]. UNC0638 is a type of small molecule that can specifically inhibit the enzyme activity of histone methyltransferase EHMT and reduce the H3K9 dimethylation (H3K9me2) levels in cells[2].
Solvent&Solubility	***In Vitro:*** **DMSO : ≥ 30 mg/mL (58.85 mM)** * "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	1.9618 mL	9.8091 mL	19.6182 mL
5 mM	0.3924 mL	1.9618 mL	3.9236 mL
10 mM	0.1962 mL	0.9809 mL	1.9618 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 (4.90 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (4.90 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 (4.90 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.90 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 (4.90 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.90 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]. Vedadi M, et al. A chemical probe selectively inhibits G9a and GLP methyltransferase activity in cells. Nat Chem Biol. 2011 Jul 10;7(8):566-74. [2]. Fu L, et al. Effects of the Histone Methyltransferase Inhibitor UNC0638 on Histone H3K9 Dimethylation of Cultured Ovine Somatic Cells and Development of Resulting Early Cloned Embryos. Reprod Domest Anim. 2014 Apr;49(2):e21-5.

实验参考：

Cell Assay	MDA-MB-231, PC3, HCT116 cells are cultured in RPMI with 10% FBS, 22RV1 cells in alphaMEM and 10% FBS, MCF7 and IMR90 cells in DMEM with 10% FBS. Cells are grown in the presence or absence of UNC0638 (10 nM, 100 nM, 1 μM, 10 μM, and 100 μM) for stated amount of time. The media is removed and replaced with DMEM 10% FBS without phenol red supplemented with 1mg/mL of MTT and incubated for 1-2 h. Live cells reduce yellow MTT to purple formazan. The resulting formazan is solubilized in acidified isopropanol and 1% Triton and absorbance measured at 570 nm, corrected for 650 nm background[1].
Animal Administration	Mice[1] Standard DMPK studies in male Swiss albino mice (3 animals per data point) are conducted, following intravenous (IV, 1 mg/kg), oral (PO, 3 mg/kg), and intraperitoneal (IP, 2.5 mg/kg) administration of UNC0638.
Kinase Assay	The enzymatic reactions are conducted in duplicate at room temperature for 1 hour in a 50 μL mixture containing PKMT assay buffer, substrate coated plate, 10 M SAM, a HMT enzyme (EZH2 (800 ng/reaction), MLL (300 ng/reaction), PRMT1 (0.5 ng/reaction), SUV39H1 (75 ng/reaction) and UNC0638 (0-1.25 μM). After enzymatic reactions, 100 μL of first antibody is added to each well and the plate is incubated at room temperature for an additional 1 h. 100 μL of secondary antibody is added to each well and the plate is incubated at room temperature for an additional 30 min. 100 μL of developer reagents are added to wells and luminescence is measured using a BioTek SynergyTM 2 microplate reader. Enzyme activity assays are performed in duplicates at each concentration. The luminescence data are analyzed using the computer software, Graphpad Prism[1].
	[1]. Vedadi M, et al. A chemical probe selectively inhibits G9a and GLP methyltransferase activity in cells. Nat Chem Biol. 2011 Jul 10;7(8):566-74.

References	[2]. Fu L, et al. Effects of the Histone Methyltransferase Inhibitor UNC0638 on Histone H3K9 Dimethylation of Cultured Ovine Somatic Cells and Development of Resulting Early Cloned Embryos. Reprod Domest Anim. 2014 Apr;49(2):e21-5.

源叶生物