产品名称：**IOX2**
产品别名：**IOX2**

生物活性：

Description	IOX2 is a specific prolyl hydroxylase-2 (PHD2) inhibitor with IC_{50} of 22 nM.
IC_{50} & Target	IC50: 22 nM (PHD2)[1]
In Vitro	IOX2 is at least 2-5000 fold selective, as judged by IC_{50} values, for PHD2 over the KDMs and Factor Inhibiting HIF(FIH)[1]. IOX2 significantly upregulates the transcription of VEGF-A and BNIP3 in normal human epidermal keratinocytes (NHEK) and normal human dermal fibroblasts (NHDF) when grown under normoxia and hypoxia. IOX2 efficiently promotes HIF-1α stability, nuclear translocation, and target gene expression in keratinocytes and fibroblasts. In addition, IOX2 significantly upregulates biosynthesis and transcription of VEGF-A and BNIP3 in sulfur mustard (SM)-exposed NHEK and NHDF grown under hypoxia. These results suggest that application of IOX2 is useful for restoring of SM-affected HIF-1α stability and signaling activity in keratinocytes and fibroblasts[2].
In Vivo	To investigate the utility of IOX2 as in vivo functional probes, IOX2 is tested to upregulate HIF signaling in a whole organism, that is, transgenic zebrafish (Danio rerio). Because the expression of the PHD3 encoding gene is regulated by HIF in humans and zebrafish, PHD3 levels are a readout of HIF activity. A zebrafish hypoxia reporter line is generated expressing GFP with the phd3 promoter elements. Transgenic wild-type embryos at 3 days postfertilization treated with compounds (10 μM) for 2 days displayed clear increase in phd3:EGFP expression in the liver, relative to controls. Significant increases in GFP levels are observed with IOX2[1].
Solvent&Solubility	***In Vitro:*** **DMSO : 25 mg/mL (70.95 mM;** Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.8382 mL	14.1908 mL	28.3817 mL
	5 mM	0.5676 mL	2.8382 mL	5.6763 mL
	10 mM	0.2838 mL	1.4191 mL	2.8382 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 (7.10 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (7.10 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。

References	[1]. Chowdhury R, et al. Selective small molecule probes for the hypoxia inducible factor (HIF) prolyl hydroxylases. ACS Chem Biol. 2013 Jul 19;8(7):1488-96. [2]. Deppe J, et al. Impairment of hypoxia-induced HIF-1α signaling in keratinocytes and fibroblasts by sulfur mustard is counteracted by a selective PHD-2 inhibitor. Arch Toxicol. 2016 May;90(5):1141-50.

实验参考：

Cell Assay	Both VHL-defective (renal carcinoma cells with an empty vector, RCC4) and VHL-competent cells human embryonic kidney HEK293T, osteosarcoma U2OS and RCC4/VHLHA (RCC4 stably transfected with C-terminal HA-tagged wt VHL) are used. Cells are treated with DMSO (control) and tested compounds (e.g., IOX2) (dissolved in DMSO except for DMOG which is dissolved in PBS and added directly to culture medium) for 4-5 h. Cell extracts are probed with antibodies to hydroxy-Pro564 (CODD-OH) and hydroxy-Asn803 (CAD-OH). HIF-1α band intensities are used to normalize hydroxylation signals[1]
Animal Administration	Zebrafish[1] Phd3:gfp$^{sh144/sh144}$ fish (Danio rerio) are incrossed to produce phd3:gfp$^{sh144/sh144}$ embryos, these are raised at 28°C in E3 medium. The phd3:gfp$^{sh144/sh144}$ line is a hypoxia reporter line created by BAC recombination of the phd3 reporter GFP construct. At 3 days post fertilization, potential inhibitors (e.g., IOX2) are added in fresh medium at 10 μM in 1% DMSO. The embryos are incubated with the compounds for a further 48 h. Embryos are anesthetized at 5 dpf by immersion in tricaine. Lateral view images of the embryos are taken using a fluorescent dissecting stereomicroscope (both bright-field and fluorescent). Fluorescent images are analyzed using Image J.
Kinase Assay	Inhibition assays are carried out in 384-well white ProxiPlates in 10 μL of reaction volume. Standard reaction mixtures consisted of the compound (in 2% DMSO final concentration), enzyme mix (0.001 μM of PHD2, 10 μM of Fe(II), 100 μM of ascorbate) and peptide mix (0.06 μM of biotinylated C-terminal oxygen dependent degradation domain (CODD) peptide, 2 μM of 2OG) in 50 mM HEPES pH 7.5, 0.01% Tween-20 and 0.1% BSA buffer. Compounds (e.g., IOX2) are preincubated with the enzyme mix for 15 min before being incubated with peptide mix for 10 min at 22°C. Each reaction is quenched with 5 μL of 30 mM EDTA. The bead mix containing AlphaScreen beads is preincubated for 1h with a rabbit monoclonal antibody selective for hydroxy-HIF1α (Pro564) and are added to the wells for a further 1 h at 22°C. The plates are then analyzed with an Envision plate reader. The IC_{50} values are calculated using nonlinear regression with normalized dose-response fit using Prism GraphPad (n≥3) [1]
References	[1]. Chowdhury R, et al. Selective small molecule probes for the hypoxia inducible factor (HIF) prolyl hydroxylases. ACS Chem Biol. 2013 Jul 19;8(7):1488-96. [2]. Deppe J, et al. Impairment of hypoxia-induced HIF-1α signaling in keratinocytes and fibroblasts by sulfur mustard is counteracted by a selective PHD-2 inhibitor. Arch Toxicol. 2016 May;90(5):1141-50.