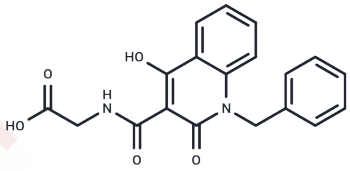
Data Sheet (Cat.No.T1823)

TargetMol

IOX2

Chemical Properties



CAS No. : 931398-72-0

Formula: C19H16N2O5

Molecular Weight: 352.34

Appearance: no data available

Storage: Powder: -20°C for 3 years | In solvent: -80°C for 1 year

Biological Description

Description	IOX2 (IOX 2) is a selective inhibitor of the Hypoxia Inducible Factor (HIF) Prolyl-Hydroxylases (PHD); active in cells with the IC50 value of 21 nM for PHD2/ELGN-1 and no inhibition at FIH (20uM).
Targets(IC50)	HIF/HIF Prolyl-Hydroxylase
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). Owing to the expression of the PHD3 encoding gene being 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[3].
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 IC50 values are calculated using nonlinear regression with normalized dose-response fit using Prism GraphPad (n≥3)[1].
Cell Research	IOX2 is prepared in DMSO and stored, and then diluted with appropriate medium before use[1]. 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].

Solubility Information

Solubility	DMSO: 7 mg/mL (19.87 mM),Sonication is recommended. Ethanol: < 1 mg/mL (insoluble or slightly soluble), (< 1 mg/ml refers to the product slightly soluble or insoluble)

Preparing Stock Solutions

	1mg	5mg	10mg
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
50 mM	0.0568 mL	0.2838 mL	0.5676 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Reference

Murray JK, et al. J Comb Chem, 2010, 12(5), 676-686.
Tian YM, et al. J Biol Chem, 2011, 286(15), 13041-13051.
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.