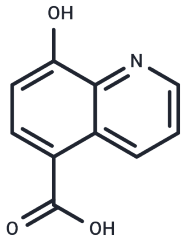
Data Sheet (Cat.No.T6545)

TargetMol

IOX1

Chemical Properties



CAS No. : 5852-78-8

Formula: C10H7NO3

Molecular Weight: 189.17

Storage: Powder: -20°C for 3 years | In solvent: -80°C for 1 year
Actual storage temperature shall be subject to the COA.

Biological Description

Description	IOX1 is the most effective broad-spectrum 2OG oxygenases(including the JmjC demethylases) inhibitor. The IC50 of IOX1 for KDM4A, KDM3A, is 0.6 and 0.1 uM, respectively.
Targets(IC50)	Histone Demethylase
In vitro	IOX1with an in vitro IC50 value in the micromolar range is the most effective against a representative panel of 2OG oxygenases, including non-JmjC 2OG oxygenases. In HeLa cells, however, its IC50 is 86uM, which efficacy is about a hundred-fold lower, possibly because of low cell permeability resulting from its polar C-5 carboxyl group.
Kinase Assay	AlphaScreen Assay: All reagents are diluted in 50 mM HEPES, 0.1% BSA, pH 7.5 supplemented with 0.01% Tween20 and allowed to equilibrate to room temperature prior to addition to plates. Catalytic turnover assays are run in 10 µL volumes in lowvolume 384-well plates at RT. The reaction consisted of enzyme (5 nM), biotinylated substrate peptide (30 nM), Fe(II) (1 µM), ascorbate (100 µM), 2OG (10 µM) and run at RT. For PHD2, the reaction consisted of enzyme (5 nM), biotinylated substrate peptide (60 nM), Fe(II) (20 µM), ascorbate (200 µM), 2OG (2 µM) and run at RT. EDTA is used to quench the reaction (5 µL), AlphaScreen donor (Streptavidin-conjugated) and acceptor (Protein A-conjugated) beads preincubated with peptide product antibodies are added (5 µL). Plates are foil-sealed to protect from light, incubated at room temperature for 60 minutes and read on a PHERAstar FS plate reader using an AlphaScreen 680 excitation/570 emission filter set. The final bead concentration in 20 µL reaction is 20 µg/mL. IC50 values are calculated in Prism 6 after normalisation against corresponding DMSO controls.
Cell Research	Antiproliferative activities of compounds are determined by the MTT assay. HeLa cells are seeded into 96-well plates and cultured at 37 °C for 24 h to achieve 70%. Subsequently, the medium is replaced with DMEM medium containing the tested compounds in different concentrations of 1-300 µM in 1% DMSO. Staurosporine in 0.03-10 µM final concentration is used as a control for cytotoxicity. After 24 h of treatment, the medium is replaced with CellTiter 96 Aqueous One Solution Reagent and incubated for 4 hours. CC50 values are calculated in Prism 6 software after normalisation against corresponding 1% DMSO treated cells and 1% DMSO in media (no cells) controls.(Only for Reference)

Solubility Information

Solubility	DMSO: 50 mg/mL (264.31 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 2 mg/mL (10.57 mM),Sonication is recommended. *Please add the solvents sequentially, clarifying the solution as much as possible before adding the next one. Dissolve by heating and/or sonication if necessary. Working solution is recommended to be prepared and used immediately. The formulation provided above is for reference purposes only. In vivo formulations may vary and should be modified based on specific experimental conditions.*

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	5.2863 mL	26.4313 mL	52.8625 mL
5 mM	1.0573 mL	5.2863 mL	10.5725 mL
10 mM	0.5286 mL	2.6431 mL	5.2863 mL
50 mM	0.1057 mL	0.5286 mL	1.0573 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.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

Reference

Schiller R, et al. ChemMedChem. 2014, 9(3), 566-571.
Huang Y, Su R, Sheng Y, et al. Small-molecule targeting of oncogenic FTO demethylase in acute myeloid leukemia. Cancer Cell. 2019, 35(4): 677-691. e10.
Huang Y, Su R, Sheng Y, et al. Small-molecule targeting of oncogenic FTO demethylase in acute myeloid leukemia [J]. Cancer Cell. 2019, 35(4): 677-691. e10.