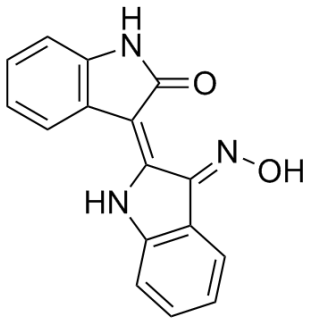
Indirubin-3'-monoxime

Cat. No.:	HY-19807
CAS No.:	160807-49-8
Molecular Formula:	$C_{16}H_{11}N_3O_2$
Molecular Weight:	277.28
Target:	GSK-3; Lipoxygenase
Pathway:	PI3K/Akt/mTOR; Stem Cell/Wnt; Metabolic Enzyme/Protease
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 6 months -20°C 1 month



SOLVENT & SOLUBILITY

In Vitro

DMSO : ≥ 37 mg/mL (133.44 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	3.6065 mL	18.0323 mL	36.0646 mL
5 mM	0.7213 mL	3.6065 mL	7.2129 mL
10 mM	0.3606 mL	1.8032 mL	3.6065 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (9.02 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

Indirubin-3'-monoxime is a potent GSK-3β inhibitor, and weakly inhibits 5-Lipoxygenase, with IC_{50}s of 22 nM and 7.8-10 μM, respectively; Indirubin-3'-monoxime also shows inhibitory activities against CDK5/p25 and CDK1/cyclin B, with IC_{50}s of 100 and 180 nM.

IC_{50} & Target

GSK-3β	CDK5/p25	CDK1/cyclin B	5-LOX
22 nM (IC_{50})	100 nM (IC_{50})	180 nM (IC_{50})	7.8-10 μM (IC_{50})

In Vitro

Indirubin-3'-monoxime inhibits GSK-3β by competing with ATP, with K_i of 0.85 μM, and K_m of 110 μM. Indirubin-3'-monoxime also inhibits tau phosphorylation by GSK-3β, with an IC_{50} value of around 100 nM. Indirubin-3'-monoxime completely inhibits the phosphorylation of the AT100 epitope[1]. Indirubin-3'-monoxime inhibits vascular smooth muscle cell (VSMC) proliferation with IC_{50} of ~2 μM. Indirubin-3'-monoxime blunts migration of VSMC stimulated with the PDGF. Indirubin-3'-

	monoxime interferes with the migratory response in VSMC, and also suppresses the production of pro-migratory LT in monocytes. Moreover, Indirubin-3'-monoxime inhibits 5-lipoxygenase (5-LO) product synthesis in monocytes and neutrophils, with the same potency (IC_{50}=5.0±1.1 and 3.7±1.2 μM, respectively). Indirubin-3'-monoxime is an inhibitor of 5-LO, with IC_{50} of 7.8-10 μM in cell-free assay[3]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	Indirubin-3'-monoxime (0.1, 0.2 and 0.4 mg/kg, i.p) dose dependently reverses the cognitive impairment and combats the elevated oxidative stress markers in HFD fed mice. Indirubin-3'-monoxime also dose dependently lowers the serum glucose, TGs, TC and insulin levels, and improves the β-cell functioning in HFD fed mice. Moreover, Indirubin-3'-monoxime treatment significantly decreases HOMA-IR levels compared to HFD group. Indirubin-3'-monoxime (0.4 mg/kg) significantly attenuates the increased EL in the HFD group[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	GSK-3β is expressed in and purified from insect Sf9 cells. It is assayed, following a 1/100 dilution in 1 mg/mL BSA, 10 mM DTT, with 5 μL of 40 μM GS-1 peptide as a substrate, in buffer A, in the presence of 15 μM[γ-^{32}P]ATP (3000 Ci/mmol; 1 mCi/mL) in a final volume of 30 μL. After 30-min incubation at 30°C, 25-μL aliquots of supernatant are spotted onto 2.5×3-cm pieces of Whatman P81 phosphocellulose paper, and, 20 s later, the filters are washed five times (for at least 5 min each time) in a solution of 10 mL of phosphoric acid/liter of water. The wet filters are counted in the presence of 1 mL of ACS scintillation fluid[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [3]	Cytotoxicity of Indirubin-3'-monoxime in monocytes is analysed by MTT assay in a 96-well format using a multi-well scanning spectrophotometer. Neutrophils (5×10^6 cells/mL) or monocytes (2×10^6 cells/mL) are incubated for 30 min with Indirubin-3'-monoxime, and the viability of the cells is analysed by MTT assay. Compared with vehicle (0.3% DMSO), no significant acute cytotoxicity is observed (neutrophils: 103.9±4.4%; monocytes: 129.4±5.4%; n=3, each)[3]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [2]	Male mice (5-6 weeks old) are randomLy assigned into five groups (n=10). Group 1: receive normal pellet diet (NPD); Group 2: receive a HFD; Group 3-5 receive HFD for 8 weeks followed by Indirubin-3'-monoxime treatment (0.1, 0.2 and 0.4 mg/kg i.p, respectively) once daily for 1 week. Indirubin-3'-monoxime is dissolved in (2.5% v/v) DMSO in saline. The mice in NPD and HFD groups receive an equivalent volume of vehicle (2.5% v/v DMSO in saline). Doses of Indirubin-3'-monoxime are selected. Mice are kept under standard husbandry conditions (22±1°C and 60% humidity) and maintained on a 12/12-h light/dark schedule with free access to food and water for 8 weeks. Body weight is recorded weekly throughout the experimental period[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Leclerc S, et al. Indirubins inhibit glycogen synthase kinase-3 beta and CDK5/p25, two protein kinases involved in abnormal tau phosphorylation in Alzheimer's disease. A property common to most cyclin-dependent kinase inhibitors? J Biol Chem. 2001 Jan 5;276(1):251-60.

[2]. Sharma S, et al. Neuroprotective role of Indirubin-3'-monoxime, a GSKβ inhibitor in high fat diet induced cognitive impairment in mice. Biochem Biophys Res Commun. 2014 Oct 3;452(4):1009-15.

[3]. Blazevic T, et al. Indirubin-3'-monoxime exerts a dual mode of inhibition towards leukotriene-mediated vascular smooth muscle cell migration. Cardiovasc Res. 2014 Mar 1;101(3):522-32.

Caution: Product has not been fully validated for medical applications. For research use only.

Tel: 609-228-6898 Fax: 609-228-5909 E-mail: tech@MedChemExpress.com
Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA