Indirubin-3'-monoxime

**Cat. No.:** HY-19807  
**CAS No.:** 160807-49-8  
**Molecular Formula:** C₁₆H₁₁N₃O₂  
**Molecular Weight:** 277.28  
**Target:** GSK-3; Lipoxygenase; CDK  
**Pathway:** PI3K/Akt/mTOR; Stem Cell/Wnt; Metabolic Enzyme/Protease; Cell Cycle/DNA Damage

**Storage:**  
Powder:  
-20°C, 3 years  
4°C, 2 years  
In solvent:  
-80°C, 2 years  
-20°C, 1 year

**SOLVENT & SOLUBILITY**

**In Vitro**  
DMSO: 125 mg/mL (450.81 mM; Need ultrasonic)

<table>
<thead>
<tr>
<th>Preparing Stock Solutions</th>
<th>Concentration</th>
<th>Mass</th>
<th>1 mg</th>
<th>5 mg</th>
<th>10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 mM</td>
<td></td>
<td>3.6065 mL</td>
<td>18.0323 mL</td>
<td>36.0646 mL</td>
</tr>
<tr>
<td></td>
<td>5 mM</td>
<td></td>
<td>0.7213 mL</td>
<td>3.6065 mL</td>
<td>7.2129 mL</td>
</tr>
<tr>
<td></td>
<td>10 mM</td>
<td></td>
<td>0.3606 mL</td>
<td>1.8032 mL</td>
<td>3.6065 mL</td>
</tr>
</tbody>
</table>

**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 IC₅₀s of 22 nM and 7.8-10 µM, respectively; Indirubin-3'-monoxime also shows inhibitory activities against CDK5/p25 and CDK1/cyclin B, with IC₅₀s of 100 and 180 nM.

**IC₅₀ & Target**

<table>
<thead>
<tr>
<th>Product</th>
<th>Data Sheet</th>
<th>Inhibitors</th>
<th>Screening Libraries</th>
<th>Proteins</th>
</tr>
</thead>
</table>

**In Vitro**  
Indirubin-3'-monoxime inhibits GSK-3β by competing with ATP, with Kᵢ of 0.85 µM, and Kₘ of 110 µM. Indirubin-3'-monoxime also inhibits tau phosphorylation by GSK-3β, with an IC₅₀ 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 IC₅₀ 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].

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### 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-32P]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].

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#### 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].

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#### 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].

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### CUSTOMER VALIDATION

- EBioMedicine. 2022 Apr;78:103950.

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### REFERENCES


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