Indirubin-3'-monoxime

**Cat. No.:** HY-19807  
**CAS No.:** 160807-49-8  
**Molecular Formula:** C₁₆H₁₁N₃O₂  
**Molecular Weight:** 277.28  
**Target:** GSK-3; 5-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

<table>
<thead>
<tr>
<th>Solvent &amp; Solubility</th>
<th>In Vitro</th>
<th>DMSO : (\geq 37 \text{ mg/mL} (133.44 \text{ mM}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preparing Stock Solutions</strong></td>
<td></td>
<td><strong>1 mg</strong></td>
</tr>
<tr>
<td><strong>Concentration</strong></td>
<td><strong>Solvent</strong></td>
<td><strong>Mass</strong></td>
</tr>
<tr>
<td>1 mM</td>
<td></td>
<td>3.6065 mL</td>
</tr>
<tr>
<td>5 mM</td>
<td></td>
<td>0.7213 mL</td>
</tr>
<tr>
<td>10 mM</td>
<td></td>
<td>0.3606 mL</td>
</tr>
</tbody>
</table>

*“\(\geq\)” means soluble, but saturation unknown.*

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

### 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**  
**IC₅₀:** 22 nM (GSK-3β), 100 nM (CDK5/p25), 180 nM (CDK1/cyclin B)[¹], 7.8-10 µM (5-Lipoxygenase)[³]

**In Vitro**  
Indirubin-3'-monoxime inhibits GSK-3β by competing with ATP, with **Kᵢ** of 0.85 µM, and **Kᵣ** 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 (**IC₅₀**=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$^3$.

**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$^2$.

**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[y-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$^1$.

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)$^3$.

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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$^2$.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

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**REFERENCES**


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