

Product Data Sheet

Product Name: Indirubin
Cat. No.: GC12975

Chemical Properties

Cas. No. 479-41-4

SMILES O=C1/C(NC2=CC=CC=C21)=C3C4=CC=CC=C4NC\3=O

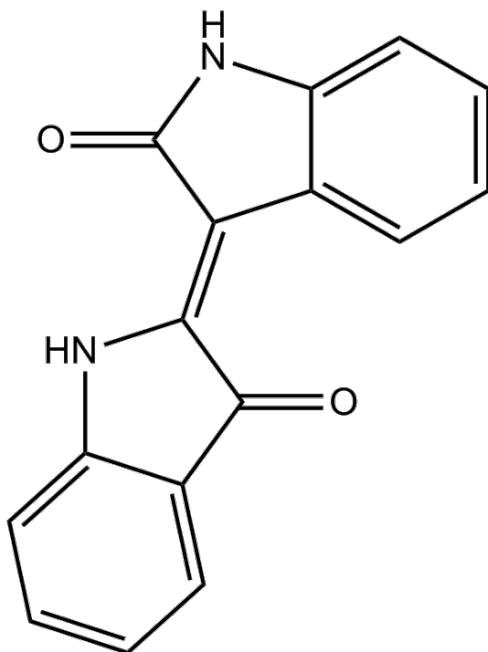
Formula $C_{16}H_{10}N_2O_2$ M.Wt 262.26

Solubility DMF>1 mg/ml, DMSO>10 mg/ml Storage Store at -20°C

General tips For obtaining a higher solubility, please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution : ship with blue ice All other available size: ship with RT, or blue ice upon request.

Structure



Protocol

Cell experiment [1]:

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Cell lines HL-60 cells

Over a period of 6 days, HL-60 cells transformed into neutrophil-like cells under the influence of 25% DMSO and 25ng/ml G-CSF while being treated with Indirubin (1μmol/L). The generation of Oonline images from differentiated cells stimulated by opsonized zymosan occurred through the reduction process of cytochrome c as detailed in previous research. Fluo-3 fluorescence changes provided measurements for the calcium response of HL-60 cells differentiated into neutrophil-like cells under specified experimental conditions. The 5×10^4

Preparation Method cells that received Indirubin treatment and subsequent washing were placed into black clear-bottomed 96-well plates and preincubated with Fluo-3 reagent from the Calcium Assay Kit and 2×5mmol/L probenecid to block dye release at 37°C for 1 hour. The calcium response experiments utilized an integrated fluid transfer workstation operating at 37°C with an excitation wavelength of 485nm and an emission wavelength of 525nm. Record data as relative fluorescence units (RFU) at 2-second intervals. A dye-exclusion test with trypan blue examined cell viability.

Reaction
Conditions 1μmol/L; 6 days

Applications Indirubin enhanced superoxide production by terminally differentiated HL-60 cells in the presence of opsonized zymosan, and enhanced the neutrophilic differentiation of HL-60 cells.

Animal experiment [2]:

Animal models Kunming pregnant mice

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Preparation Method	Thirty-six Kunming pregnant mice were randomly divided into six groups: the experimental groups included a control group and subsequent groups treated with dimethyl sulfoxide (DMSO), LPS alone, and LPS with Indirubin at concentrations of 25µmol/L, 50µmol/L, and 100µmol/L, respectively, for 24 hours. The mice underwent separation from their offspring two hours before receiving an anesthetic injection of pentobarbital sodium salt which consisted of 0.25g dissolved in 50mL phosphate-buffered saline (PBS) and administered at 10 mg per 20g intraperitoneally (i.p.). LPS groups were used as positive controls. The research group administered various Indirubin doses (25-100µmol/L) and 0.1% DMSO via intraperitoneal injection one hour before and twelve hours after LPS administration. Both the control group and LPS group received the same PBS volume. The mice received CO₂ euthanasia after 24 hours of LPS challenge before collecting mammary gland tissue to store at -80°C using a cell-freezer storage tube freezer. Measure the concentrations of IL-1β, IL-6 and TNF-α in mammary gland tissue through ELISA according to the kit provider's guidelines. Collect mammary gland tissues for histopathological analysis 24 hours after the LPS challenge. Research required tissue samples to undergo fixation in 4% paraformaldehyde for 48-72 hours followed by dehydration with graded alcohol and embedding in paraffin before staining with hematoxylin and eosin.
Dosage form	25µmol/L, 50µmol/L, and 100µmol/L for 24h; i.p.
Applications	Indirubin (100µmol/L) attenuated histopathological changes in the mammary gland, local tissue necrosis, and neutrophil infiltration in the LPS-induced mastitis mouse model.

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References:

- [1] Suzuki K, Adachi R, Hirayama A, et al. Indirubin, a Chinese anti-leukaemia drug, promotes neutrophilic differentiation of human myelocytic leukaemia HL-60 cells[J]. British journal of haematology, 2005, 130(5): 681-690.
- [2] Lai J, Liu Y, Liu C, et al. Indirubin inhibits LPS-induced inflammation via TLR4 abrogation mediated by the NF- κ B and MAPK signaling pathways[J]. Inflammation, 2017, 40: 1-12.

Background

Indirubin is a potent inhibitor of cyclin-dependent kinases (CDKs) (IC_{50} : 50-100nmol/L) and glycogen synthase kinase-3 β (GSK-3 β) (IC_{50} : 5-50nmol/L), with oral administration characteristics^[1]. Indirubin serves as the primary active ingredient within the traditional Chinese medicine Indigo naturalis and belongs to the bisindole alkaloid category^[2]. Indirubin shows promise as a medical treatment against tumors along with inflammatory conditions and neuropathic disorders and is effective against bacterial infections^[3].

In vitro, Indirubin showed an IC_{50} of 12.5 μ mol/L in glioma U87 and U118 cells for cell proliferation inhibition, and the IC_{50} in normal astrocytes was 100 μ mol/L^[2]. Indirubin (10 μ mol/L) activated AhR signaling and inhibited tumor cell proliferation and invasion in clear renal cell carcinoma and osteoma for 24h^[4]. Indirubin binds to ATP sites through competitive interactions and suppresses the functions of CDK1/cyclin B, CDK2/cyclin A and CDK2/cyclin E in starfish oocytes at 100 μ mol/L after 30h treatment^[5]. Indirubin increased opsonized zymosan-induced superoxide production in human myelocytic leukaemia HL-60 cells at low concentrations ranging from 10nmol/L to 1 μ mol/L^[6]. Indirubin (1 μ mol/L, 37 °C for 6 days) suppresses CDK2 expression and Rb protein phosphorylation in HL-60 myeloid leukemia cells while triggering interleukin-8 (IL-8) activity to promote PU.1 activation and neutrophil differentiation^[6]. Indirubin (1 μ mol/L) suppressed KBM-5 human chronic myeloid leukemia cell proliferation by reducing expression levels of NF- κ B-dependent protein cyclin D1^[6]. Indirubin (1 μ mol/L, 24h) significantly inhibited the phosphorylation of STAT3 in ovarian cancer cells, and cell apoptosis occurred after treatment of 1 μ mol/L Indirubin for 72h^[7].

In vivo, Indirubin treatment (25-100 μ mol/L) inhibited the expression of IL-1 β , IL-6, and TNF- α in the

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lipopolysaccharide (LPS)-stimulated mastitis mouse models in a dose-dependent manner^[8]. Indirubin (100µmol/L) ameliorated the pathological changes induced by LPS and reduced neutrophils and macrophages in the alveolar space, resulting in a reduction in the thickness of the mammary bleb^[8]. Inhibition of intersegmental blood vessel (ISV) formation in zebrafish embryos by treatment with indirubin at 100µmol/L for 24h^[9]. Indirubin (100µmol/L) showed mild toxicity in zebrafish embryos^[9]. Oral Indirubin (5-10mg/kg, 24h) significantly reduced the anxiety behavior of the male Swiss albino mice and exerted a sedative effect^[10].

References:

- [1] Leclerc S, Garnier M, Hoessel R, et al. Indirubins Inhibit Glycogen Synthase Kinase-3 β and CDK5/P25, Two Protein Kinases Involved in Abnormal Tau Phosphorylation in Alzheimer's Disease: A PROPERTY COMMON TO MOST CYCLIN-DEPENDENT KINASE INHIBITORS? 210[J]. Journal of Biological Chemistry, 2001, 276(1): 251-260.
- [2] Yang L, Li X, Huang W, et al. Pharmacological properties of indirubin and its derivatives[J]. Biomedicine & Pharmacotherapy, 2022, 151: 113112.
- [3] Ju Z, Sun J, Liu Y. Molecular structures and spectral properties of natural indigo and indirubin: Experimental and DFT studies[J]. Molecules, 2019, 24(21): 3831.
- [4] Ishida M, Mikami S, Shinojima T, et al. Activation of aryl hydrocarbon receptor promotes invasion of clear cell renal cell carcinoma and is associated with poor prognosis and cigarette smoke[J]. International journal of cancer, 2015, 137(2): 299-310.
- [5] Hoessel R, Leclerc S, Endicott J A, et al. Indirubin, the active constituent of a Chinese antileukaemia medicine, inhibits cyclin-dependent kinases[J]. Nature cell biology, 1999, 1(1): 60-67.
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- [8] Lai J, Liu Y, Liu C, et al. Indirubin inhibits LPS-induced inflammation via TLR4 abrogation mediated by the NF-kB and MAPK signaling pathways[J]. Inflammation, 2017, 40: 1-12.
- [9] Alex D, Lam I K, Lin Z X, et al. Indirubin shows anti-angiogenic activity in an in vivo zebrafish model and an in vitro HUVEC model[J]. Journal of ethnopharmacology, 2010, 131(2): 242-247.
- [10] Disha I J, Hasan R, Bhuia S, et al. Anxiolytic efficacy of Indirubin: in vivo approach along with receptor binding profiling and molecular interaction with GABAergic pathways[J]. ChemistryOpen,

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