

Product Data Sheet

Product Name: Indomethacin

Cat. No.: GC17556

Chemical Properties

Cas. No. 53-86-1

Chemical Name 2-[1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]acetic acid

SMILES CC1=C(C2=C(N1C(=O)C3=CC=C(C=C3)Cl)C=CC(=C2)OC)CC(=O)O

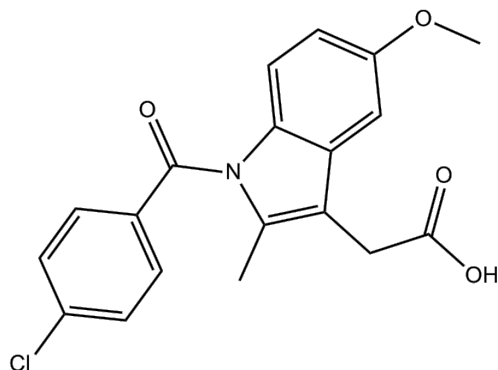
Formula $C_{19}H_{16}ClNO_4$ M.Wt 357.79

Solubility $\geq 100\text{mg/mL}$ in DMSO Storage 4°C , protect from light

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]:

Cell lines 3LL-D122 cells (Lewis lung carcinoma cells)

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Recombinant human COX-1 and COX-2 were expressed in Sf-9 cells and purified. Enzymatic activity was measured by use of a chromogenic assay based on the oxidation of N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD) during the reduction of PGG₂ to PGH₂. The assay mixture (180 µl) contains 100 mM sodium phosphate, pH 6.5, 1 µM hematin, 1 mg ml⁻¹ gelatin, 80-100 units of purified enzyme (one unit of enzyme is defined as the amount of enzyme required to produce an O.D. change of 0.001 min⁻¹ at 610 nm) and 4 µl of the test compound (indomethacin) in dimethylsulphoxide (DMSO). The mixture was preincubated at room temperature (22°C) for 15 min before initiation of the

Preparation Method enzymatic reaction by the addition of 20 µl of a solution of 1 mM arachidonic acid and 1 mM TMPD in assay buffer (without enzyme or hematin). The enzyme activity was measured by estimation of the initial velocity of TMPD oxidation over the first 36 s of the reaction. A non-specific rate of oxidation was observed in the absence of enzyme (0.007-0.01 O.D. min⁻¹) and was subtracted before the calculation of the % inhibition. IC₅₀ values were derived from the 4-parameter least squares non-linear regression analysis of the log-dose vs % inhibition plot. For measurement of COX-2 peroxidase activity, the reaction was initiated by the addition of 20 µl of a solution of 4 mM hydrogen peroxide and 0.5 mM TMPD in assay buffer.

Reaction Conditions 0, 20, 50, 100 and 150 µM ; 24 hours

Applications Indomethacin was a potent inhibitor of both COX-1 (IC₅₀=18±3 nM) and COX-2 (IC₅₀=26±6 nM).

Animal experiment [2]:

Animal models Male C57BL/6J mice (8-10 weeks) (carried LL (Lewis lung) carcinoma cells)

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Preparation Method	Indomethacin (prepared as a 100X stock of 1 mg/mL in 0.25 M Tris-HCl, pH = 8.5) was added in the drinking water at a final concentration of 10 µg/mL.
Dosage form	Mice were given drinking water containing 10 µg/mL(approx 2mg/kg/day) of indomethacin beginning one day before inoculation with LL carcinoma cells and continuing until the termination of the experiment.
Applications	Indomethacin given at a concentration of 10 µg/mL in the drinking water inhibited platelet COX-1 (thromboxane B2 formation) by >95% and significantly delayed the onset of tumor growth and the initial growth rate of the footpad tumors.

References:

- [1]. Riendeau D, Percival MD, et al. Biochemical and pharmacological profile of a tetrasubstituted furanone as a highly selective COX-2 inhibitor. Br J Pharmacol. 1997 May;121(1):105-17. doi: 10.1038/sj.bjp.0701076. PMID: 9146894; PMCID: PMC1564640.
- [2].Eli Y, Przedecki F, et al.Comparative effects of indomethacin on cell proliferation and cell cycle progression in tumor cells grown in vitro and in vivo. Biochem Pharmacol. 2001 Mar 1;61(5):565-71. doi: 10.1016/s0006-2952(00)00578-5. PMID: 11239499.

Background

Indomethacin (Indometacin) is a potent, orally active COX1/2 inhibitor with IC50 values of 18 nM and 26 nM for COX-1 and COX-2, respectively. Indomethacin has anticancer activity and anti-infective activity. Indomethacin can be used for cancer, inflammation and viral infection research[1-3].

Indomethacin(24 hours) significantly inhibited 3LL-D122 cell viability at 20 µM, with 50% inhibition at approx. 60 µM[2]. Indomethacin(1 or 10 uM of indomethacin)can suppress HT29 cancer cell migration through its influence on the focal complexes formation [4]. Indomethacin(1.54 µg/ml)during mitosis did not interfere with the M/G1 progression in synchronized BY-2 cells but it inhibited cAMP production at the beginning of the G1 phase and arrested the cell cycle progression at G1/S in tobacco bright yellow 2 (TBV-2) cell [5]. Inhibition of PGE2 production by indomethacin (50

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mM;3days) eliminated the macrophage suppression factor from the supernatant, and sensitized the resistant tumor cells (NIH3T3 or M109 cells) to macrophage cytotoxicity [6].

Indomethacin given at a concentration of 10 µg/mL in the drinking water (calculated to be approx. 2 mg/kg/day) inhibited platelet COX-1 (thromboxane B2 formation) by >95% and significantly delayed the onset of tumor growth and the initial growth rate of the footpad tumors[2]. Indomethacin(30 mg/kg ; p.o. ; 3-12h)can induce time-dependent apoptotic and autophagic cell death of gastric parietal cells (PCs) in mice[7]. Indomethacin(1 mg/kg for 28 days) by itself had no effect on tumor growth kinetics, nor did it improve the transient antitumor effect of Cyclophosphamide (CTX). However, the addition of indomethacin significantly enhanced the efficacy of mCD19CAR T therapy[8].

References:

- [1]. Riendeau D, Percival MD, et al. Biochemical and pharmacological profile of a tetrasubstituted furanone as a highly selective COX-2 inhibitor. Br J Pharmacol. 1997 May;121(1):105-17. doi: 10.1038/sj.bjp.0701076. PMID: 9146894; PMCID: PMC1564640.
- [2]. Eli Y, Przedecki F, et al. Comparative effects of indomethacin on cell proliferation and cell cycle progression in tumor cells grown in vitro and in vivo. Biochem Pharmacol. 2001 Mar 1;61(5):565-71. doi: 10.1016/s0006-2952(00)00578-5. PMID: 11239499.
- [3]. Amici C, La Frazia S, et al. Inhibition of viral protein translation by indomethacin in vesicular stomatitis virus infection: role of eIF2α kinase PKR. Cell Microbiol. 2015 Sep;17(9):1391-404. doi: 10.1111/cmi.12446. Epub 2015 May 13. PMID: 25856684; PMCID: PMC7162271.
- [4]. Guo YC, Chang CM, et al. Indomethacin inhibits cancer cell migration via attenuation of cellular calcium mobilization. Molecules. 2013 Jun 4;18(6):6584-96. doi: 10.3390/molecules18066584. PMID: 23736792; PMCID: PMC6269835.
- [5]. Ehsan H, Roef L, et al. Indomethacin-induced G1/S phase arrest of the plant cell cycle. FEBS Lett. 1999 Sep 24;458(3):349-53. doi: 10.1016/s0014-5793(99)01152-7. PMID: 10570938.
- [6]. Totary-Jain H, et al. Indomethacin sensitizes resistant transformed cells to macrophage cytotoxicity. Immunol Lett. 2016 Aug;176:1-7. doi: 10.1016/j.imlet.2016.05.011. Epub 2016 May 17. PMID: 27210423; PMCID: PMC6011832.
- [7]. Gebril SM, Ito Y, et al. Indomethacin can induce cell death in rat gastric parietal cells through alteration of some apoptosis- and autophagy-associated molecules. Int J Exp Pathol. 2020 Dec;101(6):230-247. doi: 10.1111/iep.12370. Epub 2020 Sep 28. PMID: 32985762; PMCID: PMC7691216.

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[8]. Aboeella NS, Brandle C, et al. Indomethacin-induced oxidative stress enhances death receptor 5 signaling and sensitizes tumor cells to adoptive T-cell therapy. J Immunother Cancer. 2022 Jul;10(7):e004938. doi: 10.1136/jitc-2022-004938. PMID: 35882449; PMCID: PMC9330341.

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