CHIR-99021 trihydrochloride

Cat. No.: HY-10182B

CAS No.: 1782235-14-6

Molecular Formula: $C_{22}H_{21}Cl_5N_8$

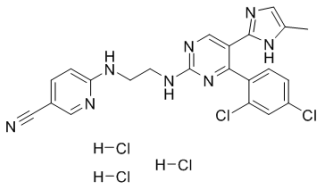
Molecular Weight: 574.72

Target: GSK-3; Wnt; β-catenin; Autophagy

Pathway: PI3K/Akt/mTOR; Stem Cell/Wnt; Autophagy

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 : ≥ 32 mg/mL (55.68 mM)

H_2O : 19 mg/mL (33.06 mM; Need ultrasonic and warming)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Mass / Solvent / Concentration	1 mg	5 mg	10 mg
1 mM	1.7400 mL	8.6999 mL	17.3998 mL
5 mM	0.3480 mL	1.7400 mL	3.4800 mL
10 mM	0.1740 mL	0.8700 mL	1.7400 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: ≥ 2.58 mg/mL (4.49 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.58 mg/mL (4.49 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.58 mg/mL (4.49 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

CHIR-99021 trihydrochloride (CT99021 trihydrochloride) is a potent and selective GSK-3α/β inhibitor with IC_{50}s of 10 nM and 6.7 nM. CHIR-99021 trihydrochloride shows >500-fold selectivity for GSK-3 over CDC2, ERK2 and other protein kinases. CHIR-99021 trihydrochloride is also a potent Wnt/β-catenin signaling pathway activator. CHIR-99021 trihydrochloride enhances mouse and human embryonic stem cells self-renewal. CHIR-99021 trihydrochloride induces autophagy[1][2][3].

IC_{50} & Target	GSK-3β 6.7 nM (IC_{50})	GSK-3α 10 nM (IC_{50})	cdc2 8800 nM (IC_{50})

In Vitro

CHIR-99021 inhibits human GSK-3β with K_i values of 9.8 nM[1]. CHIR-99021 is a small organic molecule that inhibits GSK3α and GSK3β by competing for their ATP-binding sites.In vitro kinase assays reveal that CHIR-99021 specifically inhibits GSK3β (IC_{50}=~5 nM) and GSK3α (IC_{50}=~10 nM), with little effect on other kinases[4]. In the presence of CHIR-99021 the viability of the ES-D3 cells is reduced by 24.7% at 2.5 μM, 56.3% at 5 μM, 61.9% at 7.5 μM and 69.2% at 10 μM CHIR-99021 with an IC_{50} of 4.9 μM[2].

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

In Vivo

In ZDF rats, a single oral dose of CHIR-99021 (16 mg/kg or 48 mg/kg) rapidly lowers plasma glucose, with a maximal reduction of nearly 150 mg/dl 3-4 h after administration[1]. CHIR99021 (2 mg/kg) given once, 4 h before irradiation, significantly improves survival after 14.5 Gy abdominal irradiation (ABI). CHIR99021 treatment significantly blocks crypt apoptosis and accumulation of p-H2AX$^+$ cells, and improves crypt regeneration and villus height. CHIR99021 treatment increases Lgr5$^+$ cell survival by blocking apoptosis, and effectively prevents the reduction of Olfm4, Lgr5 and CD44 as early as 4 h[5].

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

PROTOCOL

Cell Assay [3]

The viability of the mouse ES cells is determined after exposure to different concentrations of GSK3 inhibitors for three days using the MTT assay. The decrease of MTT activity is a reliable metabolism-based test for quantifying cell viability; this decrease correlates with the loss of cell viability. 2,000 cells are seeded overnight on gelatine-coated 96-well plates in LIF-containing ES cell medium. On the next day the medium is changed to medium devoid of LIF and with reduced serum and supplemented with 0.1-1 μM BIO, or 1-10 μM SB-216763, CHIR-99021 or CHIR-98014. Basal medium without GSK3 inhibitors or DMSO is used as control. All tested conditions are analyzed in triplicates[3].

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Animal Administration [1][4]

Rats[1]

Primary hepatocytes from male Sprague Dawley rats that weighed <140 g are prepared and used 1-3 h after isolation. Aliquots of 1×10^{6}cells in 1 mL of DMEM/F12 medium plus 0.2% BSA and CHIR-99021(orally at 16 or 48 mg/kg) or controls are incubated in 12-well plates on a low-speed shaker for 30 min at 37°C in a CO_2-enriched atmosphere, collected by centrifugation and lysed by freeze/thaw in buffer A plus 0.01% NP40; the GS assay is again performed.

Mice[4]

Mice 6-10 weeks old are used. The PUMA$^{+/+}$ and PUMA$^{-/-}$ littermates on C57BL/6 background (F10) and Lgr5-EGFP (Lgr5-EGFP-IRES-creERT2) mice are subjected to whole body irradiation (TBI), or abdominal irradiation (ABI). Mice are injected intraperitoneally (i.p.) with 2 mg/kg of CHIR99021 4 h before radiation or 1 mg/kg of SB415286 28 h and 4 h before radiation. Mice are sacrificed to collect small intestines for histology analysis and western blotting. All mice are injected i.p. with 100 mg/kg of BrdU before sacrifice.

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

CUSTOMER VALIDATION

- Nat Med. 2016 May;22(5):547-56.
- Mol Cell. 2017 Mar 2;65(5):873-884.e8.
- Nat Chem Biol. 2020 Aug 10.
- Small. 2020 Apr 27:e2001371.
- Cell Death Differ. 2020 Jul;27(7):2158-2175.

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REFERENCES

[1]. Ring DB, et al. Selective glycogen synthase kinase 3 inhibitors potentiate insulin activation of glucose transport and utilization in vitro and in vivo. Diabetes. 2003 Mar;52(3):588-95.

[2]. Bennett CN, et al. Regulation of Wnt signaling during adipogenesis. J Biol Chem. 2002 Aug 23;277(34):30998-1004.

[3]. Naujok O, et al. Cytotoxicity and activation of the Wnt/beta-catenin pathway in mouse embryonic stem cells treated with four GSK3 inhibitors.BMC Res Notes. 2014 Apr 29;7:273.

[4]. Wang X, et al. Pharmacologically blocking p53-dependent apoptosis protects intestinal stem cells and mice from radiation. Sci Rep. 2015 Apr 10;5:8566.

[5]. Ye S, et al. Pleiotropy of glycogen synthase kinase-3 inhibition by CHIR99021 promotes self-renewal of embryonic stem cells from refractory mouse strains. PLoS One. 2012;7(4):e35892.

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