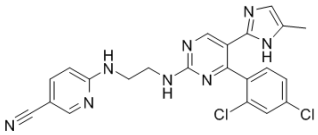
Data Sheet

Product Name:	CHIR-99021
Cat. No.:	CS-0181
CAS No.:	252917-06-9
Molecular Formula:	$C_{22}H_{18}Cl_2N_8$
Molecular Weight:	465.34
Target:	Autophagy; GSK-3
Pathway:	Autophagy; PI3K/Akt/mTOR; Stem Cell/Wnt
Solubility:	DMSO: ≥ 5.1 mg/mL



BIOLOGICAL ACTIVITY:

CHIR-99021 is a **GSK-3α/β** inhibitor with **IC_{50}** of 10 nM/6.7 nM; > 500-fold selectivity for GSK-3 versus its closest homologs CDC2 and ERK2, as well as other protein kinases.
IC50 & Target: IC50: 10 nM/6.7 nM (GSK-3α/β)[1]
In Vitro: CHIR 99021inhibits 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[2]. 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[3].
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[4].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay: [2]Kinases are purified from SF9 cells through use of their His or Glu tag. Glu-tagged proteins are purified, and His-tagged proteins are purified. Kinase assays are performed in 96-well plates with appropriate peptide substrates in a 300-μL reaction buffer (variations on 50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 1 mM EGTA, 1 mMdithiothreitol, 25 mMβ-glycerophosphate, 1 mM NaF, and 0.01% bovine serum albumin). Peptides has K_m values from 1 to 100 μM. CHIR 99021 or CHIR GSKIA is added in 3.5 μL of Me_2SO, followed by ATP to a final concentration of 1 μM. After incubation, triplicate 100-μL aliquots are transferred to Combiplate 8 plates containing 100 μL/well of 50 μM ATP and 20 mM EDTA. After 1 hour, the wells are rinsed five times with phosphate-buffered saline, filled with 200 μL of scintillation fluid, sealed, and counted in a scintillation counter 30 min later. All of the steps are at room temperature. The percentage of inhibition is calculated as 100×(inhibitor-no enzyme control)/(Me_2SO control-no enzyme control)[2]. **Cell Assay:** CHIR 99021 is dissolved in DMSO and stored, and then diluted with appropriate media before use[3].[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]. **Animal Administration:** CHIR 99021 is formulated as solutions in 20 mM citrate-buffered 15% Captisol or as fine

suspensions in 0.5% carboxymethylcellulose (Rat)[1].

CHIR 99021 is prepared in DMSO and diluted (Mice)[4].[1][4]Rat[1]

Primary hepatocytes from male Sprague Dawley rats that weighed <140 g are prepared and used 1-3 h after isolation. Aliquots of 1×10^6cells 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.

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.

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