上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话：021-61312973　　传真：021-55068248
网址：www.shyuanye.com
邮箱：shyysw@sina.com

# 产品名称：CHIR-99021 (trihydrochloride)
# 产品别名：CT99021 trihydrochloride

## 生物活性：

**Description**

CHIR-99021 trihydrochloride (CT99021 trihydrochloride) is a GSK-3α/β inhibitor with IC50 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.

**$IC_{50}$ & Target**

| GSK-3β | GSK-3α | cdc2 |
|---|---|---|
| 6.7 nM ($IC_{50}$) | 10 nM ($IC_{50}$) | 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[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].

**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**

| Solvent / Mass / 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 |

*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。

储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

***In Vivo:***

请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 In Vitro 方式配制澄清的储备液，再依次添加助溶剂：

——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶

1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline

Solubility: ≥ 2.58 mg/mL (4.49 mM); Clear solution

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| | 此方案可获得 ≥ 2.58 mg/mL (4.49 mM，饱和度未知) 的澄清溶液。<br>以 1 mL 工作液为例，取 100 μL 25.8 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。<br><br>2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline)<br>Solubility: ≥ 2.58 mg/mL (4.49 mM); Clear solution<br>此方案可获得 ≥ 2.58 mg/mL (4.49 mM，饱和度未知) 的澄清溶液。<br>以 1 mL 工作液为例，取 100 μL 25.8 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀。<br><br>3.请依序添加每种溶剂： 10% DMSO →90% corn oil<br>Solubility: ≥ 2.58 mg/mL (4.49 mM); Clear solution<br>此方案可获得 ≥ 2.58 mg/mL (4.49 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的实验。<br>以 1 mL 工作液为例，取 100 μL 25.8 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。 |
| **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.<br>[2]. Bennett CN, et al. Regulation of Wnt signaling during adipogenesis. J Biol Chem. 2002 Aug 23;277(34):30998-1004.<br>[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.<br>[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. |

**实验参考：**

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| **Cell Assay** | 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** | Rats[1]<br>Primary hepatocytes from male Sprague Dawley rats that weighed <140 g are prepared and used 1-3 h after isolation. Aliquots of $1\times10^6$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.<br>Mice[4]<br>Mice 6-10 weeks old are used. The *PUMA*$^{+/+}$ and *PUMA*$^{-/-}$ littermates on C57BL/6 background (F10) |

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