产品名称：CHIR-98014
产品别名：CHIR-98014

生物活性：

Description	CHIR-98014 is a potent, cell-permeable GSK-3 inhibitor with IC50s of 0.65 and 0.58 nM for GSK-3α and GSK-3β, respectively; it shows less potent activities against cdc2 and erk2.		
IC_{50} & Target [1]	GSK-3β	GSK-3α	cdc2
	0.58 nM (IC_{50})	0.65 nM (IC_{50})	3700 nM (IC_{50})
In Vitro	CHIR 98014 inhibits human GSK-3β with Ki value of 0.87 nM. CHIR 98014 causes GS stimulation in CHO-IR cells and rat hepatocytes, with EC50s of 106 nM and 107 nM, respectively[1]. CHIR-98014 (1 μM) reduces the viability of ES-CCE cells by 52%, with IC50 of 1.1 μM. Moreover, CHIR-98014 in combination with CHIR-99021 results in a significant activation of the Wnt/beta-catenin pathway in ES-D3 cells. In CHIR-98014 treated cells, the T gene expression is induced up to 2,500-fold. CHIR-98014 (1 μM) also yields around 50% Brachyury-positive cells, with EC50 of 0.32 μM[2]. CHIR98014 (10 μM) prevents loss of neurites caused by 20 μM PrP1-30 in cortical and hippocampal neurons, and substantially decreases the amount of dead cells[3].		
In Vivo	CHIR 98014 (30 mg/kg, i.p.) exhibits a significant reduction in fasting hyperglycemia within 4 h of treatment and shows improved glucose disposal during an ipGTT in markedly diabetic and insulin-resistant db/db mice[1].		
Solvent&Solubility	***In Vitro:*** **DMSO : 20 mg/mL (41.13 mM;** Need ultrasonic)		

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.0563 mL	10.2815 mL	20.5630 mL
	5 mM	0.4113 mL	2.0563 mL	4.1126 mL
	10 mM	0.2056 mL	1.0282 mL	2.0563 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 mg/mL (4.11 mM); Suspended solution; Need ultrasonic

此方案可获得 2 mg/mL (4.11 mM)的均匀悬浊液，悬浊液可用于口服和腹腔注射。

以 1 mL 工作液为例，取 100 μL 20.0 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。

[1]. Ring DB, et al. Selective glycogen synthase kinase 3 inhibitors potentiate insulin activation of glucose

References	transport and utilization in vitro and in vivo. Diabetes. 2003 Mar;52(3):588-95. [2]. 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. [3]. Zajkowski T, et al. Stabilization of microtubular cytoskeleton protects neurons from toxicity of N-terminal fragment of cytosolic prion protein. Biochim Biophys Acta. 2015 Oct;1853(10 Pt A):2228-39.

实验参考：

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[2].
Animal Administration	Blood is obtained by shallow tail snipping at lidocaine-anesthetized tips. Blood glucose is measured directly or heparinized plasma is collected for measurement of glucose or insulin. Animals are prebled and randomized to vehicle control or GSK-3 inhibitor treatment groups. For glucose tolerance tests (GTTs), animals are fasted throughout the procedure with food removal early in the morning, 3 h before first prebleed (db/db mice), or the previous night, 16 h before the bleed (ZDF rats). When the time course of plasma glucose and insulin changes in fasting ZDF rats is measured, food is removed ~16 h before test agent administration. The glucose challenges in the GTT are 1.35 g/kg i.p. (ipGTT) or 2 g/kg via oral gavage (oGTT). Test inhibitors are formulated as solutions in 20 mM citrate-buffered 15% Captisol or as fine suspensions in 0.5% carboxymethylcellulose[1].
Kinase Assay	Polypropylene 96-well plates are filled with 300 μL/well buffer (50 mM tris HCl, 10 mM MgCl2, 1 mM EGTA, 1 mM dithiothreitol, 25 mM β-glycerophosphate, 1 mM NaF, 0.01% BSA, pH 7.5) containing kinase, peptide substrate, and any activators. Information on the kinase concentration, peptide substrate, and activator for these assays is as follows: GSK-3α (27 nM, and 0.5 μM biotin-CREB peptide); GSK-3β (29 nM, and 0.5 μM biotin-CREB peptide); cdc2 (0.8 nM, and 0.5 μM biotin histone H1 peptide); erk2 (400 units/mL, and myelin basic protein-coated Flash Plate); PKC-α (1.6 nM, 0.5 μM biotin-histone H1 peptide, and 0.1 mg/mL phosphatidylserine + 0.01 mg/mL diglycerides); PKC-ζ (0.1 nM, 0.5 μM biotin-PKC-86 peptide, and 50 μg/mL phosphatidylserine + 5 μg/mL diacylglycerol); akt1 (5.55 nM, and 0.5 μM biotin phospho-AKT peptide); p70 S6 kinase (1.5 nM, and 0.5 μM biotin-GGGKRRRLASLRA); p90 RSK2 (0.049 units/mL, and 0.5 μM biotin-GGGKRRRLASLRA); c-src (4.1 units/mL, and 0.5 μM biotin-KVEKIGEGTYGVVYK); Tie2 (1 μg/mL, and 200 nM biotin-GGGGAPEDLYKDFLT); flt1 (1.8 nM, and 0.25 μM KDRY1175 [B91616] biotin-GGGGQDGKDYIVLPI-NH2); KDR (0.95 nM, and 0.25 μM KDRY1175 [B91616] biotin-GGGGQDGKDYIVLPI-NH2); bFGF receptor tyrosine kinase (RTK; 2 nM, and 0.25 μM KDRY1175 [B91616] biotin-GGGGQDGKDYIVLPI-NH2); IGF1 RTK (1.91 nM, and 1 μM biotin-GGGGKKKSPGEYVNIEFG-amide); insulin RTK (using DG44 IR cells); AMP kinase (470 units/mL, 50 μM SAMS peptide, and 300 μM AMP); pdk1 (0.25 nM, 2.9 nM unactivated Akt, and 20 μM each of DOPC and DOPS + 2 μM PIP3); CHK1 (1.4 nM, and 0.5 μM biotin-cdc25 peptide); CK1-ε (3 nM, and 0.2 μM biotin-peptide); DNA PK (see 31); and phosphatidylinositol (PI) 3-kinase (5 nM, and 2 μg/mL PI). Test compounds or controls are added in 3.5 μL of DMSO, followed by 50 μL of ATP stock to yield a final concentration of 1 μM ATP in all cell-free assays. After incubation,

	triplicate 100-μL aliquots are transferred to Combiplate eight plates containing 100 μL/well 50 μM ATP and 20 mM EDTA. After 1 h, the wells are rinsed five times with PBS, filled with 200 μL of scintillation fluid, sealed, left 30 min, and counted in a scintillation counter. All steps are performed at room temperature. Inhibition is calculated as 100% × (inhibited − no enzyme control)/(DMSO control − no enzyme control)[1].
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]. 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. [3]. Zajkowski T, et al. Stabilization of microtubular cytoskeleton protects neurons from toxicity of N-terminal fragment of cytosolic prion protein. Biochim Biophys Acta. 2015 Oct;1853(10 Pt A):2228-39.