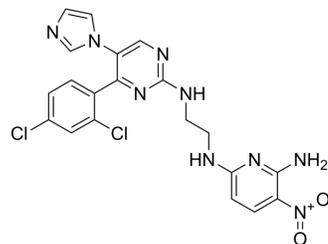


CHIR-98014

Cat. No.:	HY-13076		
CAS No.:	252935-94-7		
Molecular Formula:	C ₂₀ H ₁₇ Cl ₂ N ₉ O ₂		
Molecular Weight:	486.31		
Target:	GSK-3		
Pathway:	PI3K/Akt/mTOR; Stem Cell/Wnt		
Storage:	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	2 years
		-20°C	1 year



SOLVENT & SOLUBILITY

In Vitro

DMSO : 12.5 mg/mL (25.70 mM); ultrasonic and warming and heat to 60°C)

Concentration	Solvent	Mass		
		1 mg	5 mg	10 mg
Preparing Stock Solutions	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

Please refer to the solubility information to select the appropriate solvent.

BIOLOGICAL ACTIVITY

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.

IC₅₀ & Target

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 K_i 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].

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

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

PROTOCOL**Kinase Assay**^[1]

Polypropylene 96-well plates are filled with 300 μ L/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-3 α (27 nM, and 0.5 μ M biotin-CREB peptide); GSK-3 β (29 nM, and 0.5 μ M biotin-CREB peptide); cdc2 (0.8 nM, and 0.5 μ M biotin histone H1 peptide); erk2 (400 units/mL, and myelin basic protein-coated Flash Plate); PKC- α (1.6 nM, 0.5 μ M biotin-histone H1 peptide, and 0.1 mg/mL phosphatidylserine + 0.01 mg/mL diglycerides); PKC- ζ (0.1 nM, 0.5 μ M biotin-PKC-86 peptide, and 50 μ g/mL phosphatidylserine + 5 μ g/mL diacylglycerol); akt1 (5.55 nM, and 0.5 μ M biotin phospho-AKT peptide); p70 S6 kinase (1.5 nM, and 0.5 μ M biotin-GGGKRRRLASLRA); p90 RSK2 (0.049 units/mL, and 0.5 μ M biotin-GGGKRRRLASLRA); c-src (4.1 units/mL, and 0.5 μ M biotin-KVEKIGEGTYGVVYK); Tie2 (1 μ g/mL, and 200 nM biotin-GGGGAPEDLYKDFLT); flt1 (1.8 nM, and 0.25 μ M KDRY1175 [B91616] biotin-GGGGQDGKDYIVLPI-NH2); KDR (0.95 nM, and 0.25 μ M KDRY1175 [B91616] biotin-GGGGQDGKDYIVLPI-NH2); bFGF receptor tyrosine kinase (RTK; 2 nM, and 0.25 μ M KDRY1175 [B91616] biotin-GGGGQDGKDYIVLPI-NH2); IGF1 RTK (1.91 nM, and 1 μ M biotin-GGGGKKKSPGEYVNIIEFG-amide); insulin RTK (using DG44 IR cells); AMP kinase (470 units/mL, 50 μ M SAMS peptide, and 300 μ M AMP); pdk1 (0.25 nM, 2.9 nM unactivated Akt, and 20 μ M each of DOPC and DOPS + 2 μ M PIP3); CHK1 (1.4 nM, and 0.5 μ M biotin-cdc25 peptide); CK1- ϵ (3 nM, and 0.2 μ M biotin-peptide); DNA PK (see 31); and phosphatidylinositol (PI) 3-kinase (5 nM, and 2 μ g/mL PI). Test compounds or controls are added in 3.5 μ L of DMSO, followed by 50 μ L of ATP stock to yield a final concentration of 1 μ M ATP in all cell-free assays. After incubation, triplicate 100- μ L aliquots are transferred to Combiplate eight plates containing 100 μ L/well 50 μ M ATP and 20 mM EDTA. After 1 h, the wells are rinsed five times with PBS, filled with 200 μ L 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% \times (inhibited - no enzyme control)/(DMSO control - no enzyme control)^[1].

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Cell Assay^[2]

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 μ M BIO, or 1-10 μ M 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].

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Animal Administration^[1]

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].

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CUSTOMER VALIDATION

- Cell Res. 2022 Jun;32(6):513-529.

- Cancer Res. 2019 Feb 1;79(3):534-545.
- SSRN. 2023 Jun 20.

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REFERENCES

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- [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.
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