CHIR-98014

Cat. No.: HY-13076

CAS No.: 252935-94-7

Molecular Formula: $C_{20}H_{17}Cl_2N_9O_2$

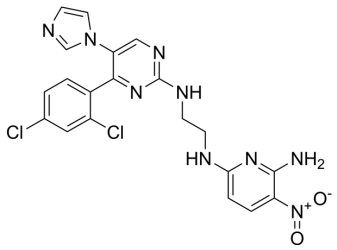
Molecular Weight: 486.31

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)

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

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 IC_{50}s 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

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 EC_{50}s of 106 nM and 107 nM, respectively[1]. CHIR-98014 (1 μM) reduces the viability of ES-CCE cells by 52%, with IC_{50} 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 EC_{50} 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 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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
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 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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

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

[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.

Caution: Product has not been fully validated for medical applications. For research use only.

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