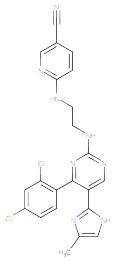
Data Sheet (Cat.No.T2310)

TargetMol
A DRUG SCREENING EXPERT

CHIR-99021

Chemical Properties



CAS No. : 252917-06-9

Formula: C22H18Cl2N8

Molecular Weight: 465.34

Appearance: no data available

Storage: Powder: -20°C for 3 years | In solvent: -80°C for 1 year

Biological Description

Description	CHIR-99021 (CT99021) is an activator of the Wnt/β-catenin signaling pathway and a GSK-3α/β inhibitor (IC50=10/6.7 nM) with selective and oral activity.CHIR-99021 induces cellular autophagy, which enhances self-renewal in mouse and human embryonic stem cells.
Targets(IC50)	GSK-3,Wnt/beta-catenin,Autophagy
In vitro	METHODS: Mouse stem cells ES-D3 were treated with CHIR-99021 (1-10 μM) for 72 h. Cell growth inhibition was detected using MTT. RESULTS: CHIR-99021 dose-dependently inhibited ES-D3 cell growth with an IC50 of 4.9 μM.[1] METHODS: Mouse embryonic stem cells J1 mESCs and mouse embryoma cells F9 mEC were treated with CHIR-99021 (3 μM) for 24 h. The expression levels of target proteins were detected by immunofluorescence. RESULTS: After CHIR-99021 treatment, β-linker proteins were increased in the cytoplasm and nucleus of J1-mESCs and F9-mEC cells. [2] METHODS: Human Tenon fibroblast HTFs were treated with CHIR-99021 (5 μM) for 48 h, and the expression levels of target proteins were detected by Western Blot. RESULTS: The production of the active form of GSK-3β (p-GSK-3β (Y216)) was significantly reduced by CHIR-99021 treatment. [3]
In vivo	METHODS: To test the antitumor activity in vivo, CHIR-99021 (37.5 mg/kg/twice daily on days 0-3, 6-10, 13-17, and 20) was orally administered and paclitaxel (10 mg/kg/one dose on day 0) was intraperitoneally injected into Balb/c nude mice harboring human non-small cell lung cancer tumor H1975. RESULTS: CHIR-99021 and paclitaxel synergistically inhibited NSCLC tumor growth in vivo. [4] METHODS: To investigate whether direct pharmacological inhibition of GSK-3 alters the positive potentiation of alcohol in mice, CHIR-99021 (1-10 mg/kg) was administered by single intraperitoneal injection to C57BL/6J mice with a history of alcohol or sucrose self-administration. RESULTS: CHIR-99021 dose-dependently increased alcohol-enhanced responses with no effect on sucrose self-administration or locomotor activity. ChIR-99021 significantly decreased pGSK-3β expression in all brain regions tested, decreased PICK1 and increased total GluA2 expression only in NAcb. [5]

Kinase Assay	Kinases were purified from SF9 cells through the use of their His or Glu tag. Glu-tagged proteins were purified as described, and His-tagged proteins were purified according to the manufacturer's instructions. Kinase assays were 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 MgCl2, 1 mM EGTA, 1 mM dithiothreitol, 25 mMβ-glycerophosphate, 1 mM NaF, and 0.01% bovine serum albumin). Peptides had Km values from 1 to 100 µM. CHIR 99021 or CHIR GSKIA was added in 3.5 µl of Me2SO, followed by ATP to a final concentration of 1 µM. After incubation, triplicate 100-µl aliquots were transferred to Combiplate 8 plates containing 100 µl/well of 50 µM ATP and 20 mM EDTA. After 1 hour, the wells were 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 were at room temperature. The percentage of inhibition was calculated as 100 × (inhibitor ? no enzyme control)/(Me2SO control ? no enzyme control) [4].
Cell Research	The Wnt/beta-catenin reporter assay was performed with the M50 Super 8× TOPFlash and M51 Super 8× FOPFlash vector containing the firefly luciferase gene under the control of TCF/LEF binding sites or mutated bindings sites. 12,500 cells were seeded overnight on gelatine-coated 96-well plates in LIF-containing ES cell medium. On the next day, the cells were transfected using Lipofectamine with one of the aforementioned vectors plus pGL4.75 [hRluc/CMV] encoding the renilla luciferase reporter gene hRluc as a transfection control. Six hours after transfection the medium was changed to medium devoid of LIF, with reduced serum, and supplemented with 5 µM CHIR-99021. The Dual-Luciferase? reporter assay system was employed 48 and 72 h after the medium change to follow the luminescence reaction using a GloMax?-multi detection system [4].
Animal Research	Blood was obtained by shallow tail snipping at lidocaine-anesthetized tips. Blood glucose was measured directly or heparinized plasma was collected for measurement of glucose or insulin. Animals were pre-bled and randomized to vehicle control or GSK-3 inhibitor treatment groups. For glucose tolerance tests (GTTs), animals fasted throughout the procedure with food removal early in the morning, 3 h before the 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 was measured, food was removed ~16 h before test agent administration. The glucose challenges in the GTT were 1.35 g/kg i.p. (ipGTT) or 2 g/kg via oral gavage (oGTT). CHIR-99021 were formulated as solutions in 20 mmol/l citrate-buffered 15% Captisol or as fine suspensions in 0.5% carboxymethylcellulose [1].

Solubility Information

Solubility	DMSO: 9.3 mg/mL (20 mM), (< 1 mg/ml refers to the product slightly soluble or insoluble)

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.149 mL	10.7448 mL	21.4897 mL
5 mM	0.4298 mL	2.149 mL	4.2979 mL
10 mM	0.2149 mL	1.0745 mL	2.149 mL
50 mM	0.043 mL	0.2149 mL	0.4298 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Reference

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.