Data Sheet (Cat.No.TQ0060)

LY2857785

Chemical Properties

CAS No.:	1619903-54-6
Formula:	C26H36N6O
Molecular Weight:	448.6
Appearance:	N/A
Storage:	0-4℃ for short term (days to weeks), or -20℃ for long term (months).

Biological Description

Description	LY2857785 is a type I competitive and reversible ATP kinase inhibitor against CDK7/CDK8/CDK9 (IC50s: 246 nM/16 nM/11 nM).
In vitro	At the cellular level, LY2857785 inhibits CTD P-Ser2 and CTD P-Ser5 in U2OS cells (IC50s: 0.089 and 0.042 μM). However, LY2857785 only induces a moderate G2-M DNA content increase, from 35% to 55%, with EC50 0.135 μM. LY2857785 shows potent compound exposure- and time-dependent cell proliferation inhibition in MV-4-11, RPMI8226, and L363 cells. When incubated between 4 to 24 hours, the cell growth inhibition potency reaches a maximal effect at 8 hours for MV-4-11, RPMI8226, and L363 cells (IC50s: 0.04, 0.2, and 0.5 μM). LY2857785-induced cancer cell apoptosis is also time-dependent, reaching maximal potency at 8 hours with IC50 0.5 μM in L363 cells.
In vivo	In HCT116 xenograft tumor-bearing mice, LY2857785 demonstrates dose-dependent RNAP II CTD P-Ser2 inhibition potently (TED50: 4.4 mg/kg; TEC50: 0.36 μM). LY2857785 also shows the significant duration of CTD P-Ser2 inhibition for 3 to 6 hours at TED70 (8 mg/kg) in HCT116 and MV-4-11 nude mice xenograft models. In the nude rat MV-4-11 xenograft model, LY2857785 similarly shows dose-dependent CTD P-Ser2 inhibition for 8 hours at TED70 (7 mg/kg) and TED90 (10 mg/kg).
Kinase Assay	CDK7 and CDK9 reaction mixtures contain 10 mM Tris-HCl (pH 7.4), 10 mM HEPES, 5 mM DTT, 10 μM ATP, 0.5 μCi 33p-ATP, 10 mM MnCl2, 150 mM NaCl, 0.01% Triton X-100, 2% DMSO, 0.05 mM CDK7/9ptide, and 2 nM CDK7/Mat1/cyclin H, or 2 nM CDK9/cyclin T1, respectively. CDK8/cyclin C reaction is performed in HEPES 30 mM, DTT 2 mM, MgCl2 5 mM, 0.015% Triton X-100, 5 μM ATP, and 400 nM of RBER-CHKStide containing 20 nM of enzyme. LY2857785 in DMSO is diluted serially 1:3 for dose-response. Reactions are carried out in 96-well polystyrene plates. The reactions are incubated at room temperature for 60 minutes and followed by termination with 10% H3PO4 or 10% trichloroacetic acid (TCA). For the filter binding assay, reactions are transferred to 96-well filter plates and measured by Microbeta scintillation counter. For ADP Transcreener Fluorescent Polarization Assays, reactions are quenched with ADP detection mix, incubated 2 hours at room temperature and then FP is measured at λex=610 nm, λem=670 nm on a Tecan Ultra 384 plate reader. The concentration of ADP product is calculated from millipolarization (μP) using a prepared ADP/ATP dilution series as a standard curve. Kinase profiling are carried out in 96-well polystyrene plates. Briefly, in a final volume of 25 μL the enzyme is incubated with the appropriate buffer, peptide substrate, and the diluted LY2857785. Reactions are initiated by the addition of ATP/[33P] and the ATP mix is incubated at room temperature for 40 minutes. Reactions are quenched with the addition of 5 μL of 3% phosphoric acid, 10 μL of the reaction are spotted onto a filter mat, washed 3 times for 5 minutes in 75 mM phosphoric acid and once in methanol. Once the filters are dry, they are submitted to scintillation counting.

Cell Research	Solid tumor cells are plated in poly-D-lysine coated and hematologic cell lines are seeded in noncoated 96-well plates overnight before being treated with LY2857785. Solid tumor cells are fixed with Prefer for 20 minutes at room temperature and permeated with 0.1% Triton X-100 in PBS for 15 minutes. Caspase-3 expression is measured by immunofluorescence with anti-activated caspase-3. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) activity is measured with In Situ Cell Death Detection Kit. Both assays are analyzed on Acumen Explorer laser-scanning fluorescence microplate cytometer. Hematologic tumor cells are assayed for cell viability with CellTiter-Glo Luminescent Cell Viability Assay.
Animal Research	For xenograft models, human cancer cells A375, U87MG, MV-4-11, and HCT116 are implanted into female nude rats or athymic nude female mice. The animals are dosed with saline, Rapamycin, or LY2857785, respectively. MV-4-11 xenografts in nude mice are treated by LY2857785 (4, 8, and 18 mg/kg) i.v. bolus. MV-4-11 xenografts in nude rats are treated with LY2857785 (3, 6, and 9 mg/kg) 4-hour i.v. infusion. An untreated vehicle control group is administered saline i.v. every 3 days. Flow cytometry analysis is conducted using Beckman Coulter's CXP software. Statistical significance of the effect of LY2857785 and/or control compounds is assessed by the Dunnett method, one-way ANOVA.

Solubility Information

Solubility	DMSO: 10 mg/mL (22.29 mM) (< 1 mg/ml refers to the product slightly soluble or insoluble)

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.229 mL	11.146 mL	22.292 mL
5 mM	0.446 mL	2.229 mL	4.458 mL
10 mM	0.223 mL	1.115 mL	2.229 mL
50 mM	0.045 mL	0.223 mL	0.446 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. The storage conditions and period of the stock solution: - 80 ℃ for 6 months; - 20 ℃ for 1 month. Please use it as soon as possible.

Reference

1. Yin T, et al. A novel CDK9 inhibitor shows potent antitumor efficacy in preclinical hematologic tumor models. Mol Cancer Ther. 2014 Jun;13(6):1442-56. Mol Cancer Ther. 2014 Jun;13(6):1442-56.

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