LY2857785

Cat. No.: HY-12293

CAS No.: 1619903-54-6

Molecular Formula: $C_{26}H_{36}N_6O$

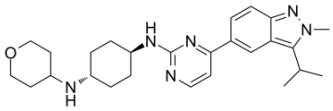
Molecular Weight: 448.6

Target: CDK; Apoptosis

Pathway: Cell Cycle/DNA Damage; Apoptosis

Storage:

Powder	-20°C	3 years
	4°C	2 years
In solvent	-80°C	6 months
	-20°C	1 month



SOLVENT & SOLUBILITY

In Vitro

DMSO : 10 mg/mL (22.29 mM; Need ultrasonic)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.2292 mL	11.1458 mL	22.2916 mL
5 mM	0.4458 mL	2.2292 mL	4.4583 mL
10 mM	0.2229 mL	1.1146 mL	2.2292 mL

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

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 1 mg/mL (2.23 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 1 mg/mL (2.23 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 1 mg/mL (2.23 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

LY2857785 is a type I reversible and competitive ATP kinase inhibitor against CDK9 (IC_{50} 11 nM) and other transcription kinases CDK8 (IC_{50} 16 nM), and CDK7 (IC_{50} 246 nM).

IC_{50} & Target

CDK9	CDK8	CDK7
0.011 μM (IC_{50})	0.016 μM (IC_{50})	0.246 μM (IC_{50})

In Vitro

LY2857785 shows good selectivity against a panel of 114 protein kinases, with only 5 other protein kinases inhibited with

potency (IC_{50}) less than 0.1 μM, and a total of 14 kinases less than 1 μM. At the cellular level, LY2857785 inhibits CTD P-Ser2 and CTD P-Ser5 in U2OS cells at IC_{50}s 0.089 (n=13) and 0.042 (n=1) μM, respectively. However, LY2857785 only induces a moderate G_2-M DNA content increase, from 35% to 55%, with EC_{50} 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 with IC_{50}s 0.04, 0.2, and 0.5 μM for MV-4-11, RPMI8226, and L363 cells, respectively. LY2857785-induced cancer cell apoptosis is also time dependent, reaching maximal potency at 8 hours with IC_{50} 0.5 μM in L363 cells[1].

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

In Vivo

In HCT116 xenograft tumor-bearing mice, LY2857785 demonstrates dose-dependent RNAP II CTD P-Ser2 inhibition potently with TED50 of 4.4 mg/kg and TEC50 of 0.36 μM. LY2857785 also shows 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). LY2857785 demonstrates the most dramatic tumor regression in the AML MV-4-11 xenograft tumor model either by i.v. bolus in mice or i.v. infusion in rats[1].

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PROTOCOL

Kinase Assay [1]

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 $MnCl_2$, 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, $MgCl_2$ 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 λ_{ex}=610 nm, λ_{em}=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/[^{33}P] and the ATP mix is incubated at room temperature for 40 minutes. Reactions are quenched with the addition 5 μL of 3% phosphoric acid, 10 μL of the reaction are spotted onto a filtermat, 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[1].

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Cell Assay [1]

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 compounds (e.g, 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 antiactivated 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[1].

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Animal Administration [1]

Mice and Rats[1]

For xenograft models, human cancer cells U87MG, MV-4-11, A375, 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 Dunnett method, one-way ANOVA.

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CUSTOMER VALIDATION

- Cell Chem Biol. 2018 Feb 15;25(2):135-142.e5.
- Sci Rep. 2018 Jun 21;8(1):9472.
- Biochem Biophys Res Commun. 2019 Jun 11;513(4):967-973.
- Oncotarget. 2017 Nov 3;8(63):107206-107222.

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REFERENCES

[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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