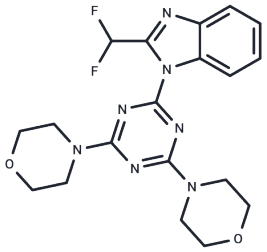
Data Sheet (Cat.No.T6168)

TargetMol

ZSTK474

Chemical Properties



CAS No. : 475110-96-4

Formula: C19H21F2N7O2

Molecular Weight: 417.41

Appearance: no data available

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

Biological Description

Description	PI3K Inhibitor ZSTK474 is an orally available, s-triazine derivative, ATP-competitive phosphatidylinositol 3-kinase (PI3K) inhibitor with potential antineoplastic activity.
Targets(IC50)	Autophagy,PI3K
In vitro	ZSTK474 at 1 μM potently reduces PI3K activity to 4.7% of the control level, whereas LY2194002 only reduces the activity to 44.6% of the control. ZSTK474 inhibits the activities of recombinant p110β, -γ, and -δ with IC50 of 17 nM, 53 nM, and 6 nM, respectively. ZSTK474 shows potent antiproliferative activity against a panel of 39 human cancer cell lines with mean GI50 of 0.32 μM, more effectively than that of LY294002 or wortmannin with mean GI50 of 7.4 μM or 10 μM, respectively. ZSTK474 treatment at 1 μM blocks membrane ruffling and generation of PIP3 induced by platelet-derived growth factor in murine embryonic fibroblasts (MEFs). ZSTK474 at 10 μM induces apoptosis in OVCAR3 cells, and induces complete G1-phase arrest but not apoptosis in A549 cells. ZSTK474 treatment at 0.5 μM significantly decreases the level of phosphorylated Akt and GSK-3β, as well as the cyclin D1 protein expression. ZSTK474 also inhibits the phosphorylation of other downstream signaling components that are involved in regulating cell proliferation including FKHRL1, FKHR, TSC-2, mTOR, and p70S6K in a dose-dependent manner. [1] ZSTK474 does not inhibit mTOR at 0.1 μM, and even at a concentration of 100 μM, ZSTK474 inhibits mTOR activity less than 40%. [2] ZSTK474 blocks VEGF-induced cell migration and the tube formation in human umbilical vein endothelial cells (HUVECs), and inhibits the expression of HIF-1α and secretion of VEGF in RXF-631L cells, exhibiting potent in vitro antiangiogenic activity. [3] ZSTK474 treatment inhibits the production of IFNγ and IL-17 in concanavalin A-activated T cells, and inhibits the proliferation and PGE(2) production by fibroblast-like synovial cells (FLS). [6]
In vivo	Oral administration of ZSTK474 inhibits the growth of subcutaneously implanted mouse B16F10 melanoma tumors in a dose-dependent manner, producing tumor regression of 28.5%, 7.1%, or 4.9% on day 14 at 100, 200, or 400 mg/kg, respectively, which is superior to that of the four major anticancer drugs irinotecan, cisplatin, doxorubicin, and 5-fluorouracil at their respective maximum tolerable doses with tumor regression of 96%, 35.7%, 24%, or 68.3%, respectively. ZSTK474 treatment at 400 mg/kg completely inhibits the growth of A549, PC-3, and WiDr xenografts in mice, and induces the regression of A549 xenograft tumors. [1] ZSTK474 significantly inhibits tumor growth in the RXF-631L xenograft model, correlated with a significantly reduced number of microvessels in the

	ZSTK474-treated mice. [3] Oral administration of ZSTK474 ameliorates the progression of adjuvant-induced arthritis (AIA) in rats. [6]
Kinase Assay	Inhibition of PI3K activity: A549 cells are lysed in a buffer containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM EDTA, and 1% Igepal CA-630, the lysates are centrifuged at 20,000 g and 4 °C for 10 minutes, and the supernatants are used as cell lysate (protein = 2-4 mg/mL). To immunoprecipitate PI3K, 200 μL of cell lysate are incubated with anti-p85 polyclonal antibody and protein G-agarose (5 μL). PI3Kα, PI3Kβ, and PI3Kδ can be immunoprecipitated by the anti-p85 polyclonal antibody. Agarose beads containing immunoprecipitates are washed twice with buffer A (20 mM Tris-HCl at pH 7.5, 150 mM NaCl, 5 mM EDTA, and 1% Igepal CA-630), once with buffer B (500 mM LiCl and 100 mM Tris-HCl at pH 7.5), once with distilled water, and once with buffer C (100 mM NaCl and 20 mM Tris-HCl at pH 7.5). Immunoprecipitates are suspended in 20 μL of buffer C containing phosphatidylinositol of 200 μg/mL. The mixture is preincubated with increasing concentrations of ZSTK474 at 25 °C for 5 minutes. [γ-32P]ATP (2 μCi per assay mixture; final concentration, 20 μM) and MgCl2 (final concentration, 20 mM) are added to start the reaction. The reaction mixture is incubated at 25 °C for 20 minutes. Phosphorylated products of phosphatidylinositol are separated by thin-layer chromatography and visualized by autoradiography. The phosphatidylinositol-3-phosphate region is scraped from the plate, and radioactivity is also measured with liquid scintillation spectroscopy. The level of inhibition for ZSTK474 is determined as the percentage of 32P counts per minute obtained without ZSTK474.
Cell Research	Cells are exposed to increasing concentrations of ZSTK474 for 48 hours. The inhibition of cell proliferation is assessed by measuring changes in total cellular protein by use of a sulforhodamine B assay. Apoptosis is assessed by chromatin condensation or by flow cytometry. For chromatin condensation assay, cells are stained with Hoechst 33342 and examined by fluorescence microscopy. Morphologic changes induced by ZSTK474, such as the condensation of chromatin, are indicative of apoptosis. For flow cytometry analysis, cells are harvested, washed with ice-cold PBS, and fixed in 70% ethanol. Cells are then washed twice with ice-cold PBS again, treated with RNase A (500 μg/mL) at 37 °C for 1 hour, and stained with propidium iodide (25 μg/mL). The DNA content of the cells is analyzed with a flow cytometer. (Only for Reference)

Solubility Information

Solubility	DMSO: 8.75 mg/mL (20.96 mM),Sonication is recommended. Ethanol: < 1 mg/mL (insoluble or slightly soluble), H2O: < 1 mg/mL (insoluble or slightly soluble), (< 1 mg/ml refers to the product slightly soluble or insoluble)

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.3957 mL	11.9786 mL	23.9573 mL
5 mM	0.4791 mL	2.3957 mL	4.7915 mL
10 mM	0.2396 mL	1.1979 mL	2.3957 mL
50 mM	0.0479 mL	0.2396 mL	0.4791 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

Yaguchi S, et al. J Natl Cancer Inst, 2006, 98(8), 545-556.
Han H W, Hahn S, Jeong H Y, et al. LINCS L1000 dataset-based repositioning of CGP-60474 as a highly potent anti-endotoxemic agent. Scientific Reports. 2018 Oct 8;8(1):14969
Kong D, et al. Cancer Sci, 2007, 98(10), 1638-1642.
Kong D, et al. Eur J Cancer, 2009, 45(5), 857-865.
Marone R, et al. Mol Cancer Res, 2009, 7(4), 601-613.
Yang S, et al. PLoS One, 2011, 6(10), e26343.
Wang P, et al. Class I PI3K inhibitor ZSTK474 mediates a shift in microglial/macrophage phenotype and inhibits inflammatory response in mice with cerebral ischemia/reperfusion injury. J Neuroinflammation. 2016 Aug 22;13 (1):192.
Liu F, et al. Prolonged inhibition of class I PI3K promotes liver cancer stem cell expansion by augmenting SGK3/GSK-3β/β-catenin signalling. J Exp Clin Cancer Res. 2018 Jun 25;37(1):122.
Han H W, Hahn S, Jeong H Y, et al. LINCS L1000 dataset-based repositioning of CGP-60474 as a highly potent anti-endotoxemic agent[J]. Scientific reports. 2018 Oct 8;8(1):14969.