

Product Data Sheet

Product Name: ZSTK474

Cat. No.: GC13617

Chemical Properties

Cas. No. 475110-96-4

Chemical Name 4-[4-[2-(difluoromethyl)benzimidazol-1-yl]-6-morpholin-4-yl]-1,3,5-triazin-2-yl]morpholine

SMILES C1COCCN1C2=NC(=NC(=N2)N3C4=CC=CC=C4N=C3C(F)F)N5CCOCC5

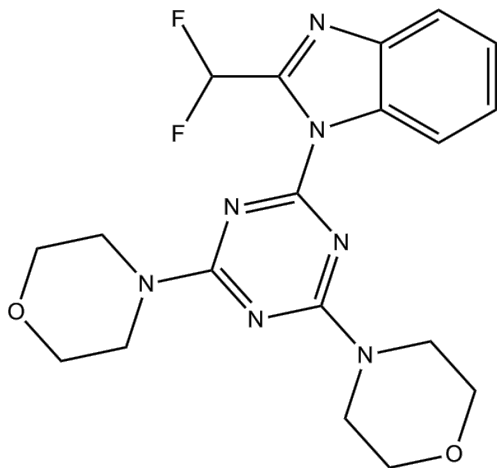
Formula $C_{19}H_{21}F_2N_7O_2$ M.Wt 417.41

Solubility $\geq 20.85\text{mg/mL}$ in DMSO Storage Store at -20°C

General tips For obtaining a higher solubility, please warm the tube at 37°C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution: ship with blue ice. All other available size: ship with RT, or blue ice upon request.

Structure



Protocol

Cell experiment [1]:

Cell lines Human glioblastoma cell lines (SF295, U87, U251)

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Preparation Method	Cells were cultured in DMEM or RPMI 1640 medium supplemented with 10% fetal bovine serum at 37°C, 5% CO ₂ . Cells were pre-treat with ZSTK474 at concentrations ranging from 0.01 to 2μM for 48 hours, followed by treatment with temozolomide (TMZ; 120–180μM) for an additional 48 hours.
Reaction Conditions	0.01 to 2μM; pre-treat 48h.
Applications	ZSTK474 synergistically enhanced temozolomide (TMZ)-induced cytotoxicity by suppressing homologous recombination repair. ZSTK474 significantly increased DNA double-strand breaks (DSBs), as evidenced by elevated γ-H2AX foci and comet assay results. ZSTK474 inhibited key HR repair proteins (BRCA1/2, Rad51, and p-ATM) and induced G0/G1 cell cycle arrest. Apoptosis was markedly enhanced in combination therapy, with cleaved PARP and caspase-3 levels upregulated. ZSTK474 also blocked PI3K/Akt pathway activation, further sensitizing glioblastoma cells to TMZ.
Animal experiment [2]:	
Animal models	BALB/C nude mice bearing WiT49 cell xenografts
Preparation Method	Mice were orally administered daily doses of ZSTK474 at 25, 50, and 100mg/kg for 14 days after tumor volume reached 90–100mm ³ . Tumor size and body weight were monitored every 2–3 days.
Dosage form	25–100mg/kg; p.o.; Daily for 14 days.

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Applications

ZSTK474 administration significantly inhibited subcutaneous tumor growth in a dose-dependent manner, reducing tumor volume and weight. Immunofluorescence analysis showed decreased expression of proliferation marker PCNA, invasion markers MMP2/MMP9, and angiogenesis marker VEGF in tumor tissues. ZSTK474 treatment also induced G0/G1 phase arrest in tumor cells by downregulating cyclin D and CDK4, and upregulating P21 expression, without causing significant toxicity or body weight changes.

References:

- [1] Namatame N, Tamaki N, Yoshizawa Y, et al. Antitumor profile of the PI3K inhibitor ZSTK474 in human sarcoma cell lines. *Oncotarget*. 2018 Oct 12;9(80):35141-35161.
- [2] Li M, Liu J, Jin L, et al. ZSTK474 targeting PIK3R3 inhibits the Wilms' tumor through G0 / G1 phase arrest. *PLoS One*. 2024 Oct 28;19(10):e0312178.

Background

ZSTK474 is an ATP-competitive pan-class I PI3K inhibitor that exhibits inhibitory activity against PI3K α (IC₅₀=16nM), PI3K β (IC₅₀=44nM), PI3K δ (IC₅₀=4.6nM), and PI3K γ (IC₅₀=49nM) subtypes^[1-2]. By inhibiting the PI3K/Akt signaling pathway, ZSTK474 downregulates phosphorylated Akt and GSK-3 β , thereby inducing tumor cell apoptosis and suppressing inflammatory responses^[3-4].

In vitro, treatment of sarcoma cells (RD-ES, A673), alveolar rhabdomyosarcoma cells (SJCRH30), or synovial sarcoma cells (SYO-1, Aska-SS, Yamato-SS) with ZSTK474 (0.0039–1 μ M) for 6–48 hours significantly inhibited the phosphorylation of PI3K downstream signaling proteins AKT (S473/T308) and ribosomal S6 protein (S235/236), while increasing the expression of cleaved PARP and inducing apoptosis^[5]. Pre-treatment of human glioblastoma cells (SF295, U87) with ZSTK474 (0.4–1.2 μ M) for 48 hours, followed by temozolomide (TMZ; 120–180 μ M) for an additional 48 hours, significantly suppressed cell proliferation, induced apoptosis, and increased DNA double-strand break damage^[6].

In vivo, daily oral administration of ZSTK474 (25–100mg/kg) for 14 days in BALB/C nude mice bearing subcutaneous Wilms tumor WiT49 cell xenografts. ZSTK474 significantly inhibited tumor growth and reduced tumor volume and weight^[7]. Intravenous administration of ZSTK474

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(2mg/kg/day) in combination with oncolytic vesicular stomatitis virus (VSV Δ 51; 2.5×10^7 pfu/kg/day) in BALB/c-nu/nu mice bearing 143B osteosarcoma cell xenografts. ZSTK474 significantly suppressed subcutaneous tumor growth and reduced tumor volume and weight after six treatments^[8].

References:

- [1] Kong DX, Yamori T. ZSTK474, a novel phosphatidylinositol 3-kinase inhibitor identified using the JFCR39 drug discovery system. *Acta Pharmacol Sin.* 2010 Sep;31(9):1189-97. doi: 10.1038/aps.2010.150.
- [2] Kong D, Yamori T. ZSTK474 is an ATP-competitive inhibitor of class I phosphatidylinositol 3 kinase isoforms. *Cancer Sci.* 2007 Oct;98(10):1638-42.
- [3] Dan S, Okamura M, Mukai Y, et al. ZSTK474, a specific phosphatidylinositol 3-kinase inhibitor, induces G1 arrest of the cell cycle in vivo. *Eur J Cancer.* 2012 Apr;48(6):936-43.
- [4] Yaguchi S, Fukui Y, Koshimizu I, et al. Antitumor activity of ZSTK474, a new phosphatidylinositol 3-kinase inhibitor. *J Natl Cancer Inst.* 2006 Apr 19;98(8):545-56.
- [5] Namatame N, Tamaki N, Yoshizawa Y, et al. Antitumor profile of the PI3K inhibitor ZSTK474 in human sarcoma cell lines. *Oncotarget.* 2018 Oct 12;9(80):35141-35161.
- [6] Jiao W, Zhu S, Shao J, et al. ZSTK474 Sensitizes Glioblastoma to Temozolomide by Blocking Homologous Recombination Repair. *Biomed Res Int.* 2022 Jul 13;2022:8568528.
- [7] Li M, Liu J, Jin L, et al. ZSTK474 targeting PIK3R3 inhibits the Wilms' tumor through G0 / G1 phase arrest. *PLoS One.* 2024 Oct 28;19(10):e0312178.
- [8] Jiang J, Wang W, Xiang W, et al. The phosphoinositide 3-kinase inhibitor ZSTK474 increases the susceptibility of osteosarcoma cells to oncolytic vesicular stomatitis virus VSV Δ 51 via aggravating endoplasmic reticulum stress. *Bioengineered.* 2021 Dec;12(2):11847-11857.

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