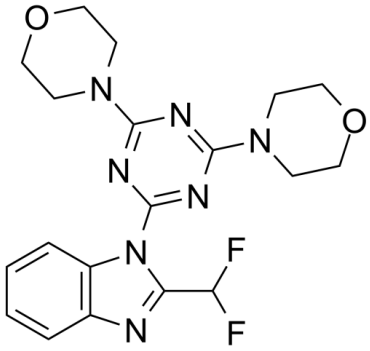
Data Sheet

Product Name:	ZSTK474
Cat. No.:	CS-0083
CAS No.:	475110-96-4
Molecular Formula:	C19H21F2N7O2
Molecular Weight:	417.41
Target:	Autophagy; PI3K
Pathway:	Autophagy; PI3K/Akt/mTOR
Solubility:	DMSO : 2 mg/mL (4.79 mM; Need ultrasonic)



BIOLOGICAL ACTIVITY:

ZSTK474 is an ATP-competitive pan-class I **PI3K** inhibitor with **IC_{50}**s of16 nM, 44 nM, 4.6 nM and 49 nM for PI3Kα, PI3Kβ, PI3Kδ and PI3Kγ, respectively. IC50 & Target: IC50: 16 nM (PI3Kα), 44 nM (PI3Kβ), 4.6 nM (PI3Kδ), 49 nM (PI3Kγ)[1] **In Vitro:** Lineweaver-Burk plot analysis revealed that ZSTK474 inhibits all four PI3K isoforms in an ATP-competitive manner. The K_i values determined for the four PI3K isoforms showed that ZSTK474 inhibited the PI3Kδ isoform most effectively with a K_i of 1.8 nM, whereas the other isoforms are inhibited with 4-10-fold higher K_i values. Therefore, ZSTK474 should be regarded as a pan-PI3K inhibitor. We also determined the IC_{50} values for inhibiting the four PI3K isoforms with ZSTK474 and LY294002. The IC_{50} values of ZSTK474 (16, 44, 4.6 and 49 nM for PI3Kα, PI3Kβ, PI3Kδ and PI3Kγ, respectively) are shown to be consistent with the K_i values (6.7, 10.4, 1.8 and 11.7 nM for PI3Kα, PI3Kβ, PI3Kδ and PI3Kγ, respectively), which further supported the idea that ZSTK474 inhibits PI3Kδ most potently. Even at a concentration of 100 μM, ZSTK474 inhibits mTOR activity rather weakly[1]. **In Vivo:** In mice subjected to MCAO, treatment with ZSTK474 is tested at dosages of 50, 100, 200, and 300 mg/kg. Since the 200 mg/kg dose produces significant improvement and no obvious toxic effects ($P<0.01$), mice are treated with ZSTK474 at a dose of 200 mg/kg/day daily for three post-MCAO days during the remaining experiments of this study. Neurological function is examined in mice suffered from MCAO followed by 24, 48, and 72 h of reperfusion. In the ZSTK474 group, neurological function scores are significantly better than the control group except the corner test[2].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay: [1]The linear phase of each kinetic reaction is defined at the respective enzyme amount (0.05, 0.1, 0.12 and 1 μg/mL for PI3Kα, PI3β, PI3δ and PI3γ, respectively) and reaction time (20 min). PI3K activity is assayed at various concentrations of ATP (5, 10, 25, 50, 100 μM) in the presence of increasing concentrations of ZSTK474. A Lineweaver-Burk plot is developed by plotting 1/v (the inverse of v, where v is obtained by subtracting the HTRF signal of the kinase test sample from the HTRF signal of the minus-enzyme control) versus 1/[ATP] (the inverse of the ATP concentration). For the minus-enzyme control, PIP2 is incubated with ATP in the absence of kinase. To determine the K_i value (inhibition constant) of ZSTK474 for each PI3K isoform, the slope of the respective Lineweaver-Burk plot is replotted against the ZSTK474 concentration. The K_i values are calculated by analysis using GraphPad Prism 4[1]. **Animal Administration:** ZSTK474 is suspended in 5 % hydroxypropylcellulose in water as a solid dispersion (Mice)[2].[2]Mice[2]
Mice are randomly assigned to receive different doses of ZSTK474 (50, 100, 200, and 300 mg/kg) to determine the optimum dose; in our experiment, the optimum dose is 200 mg/kg. Then mice are randomly assigned to one of three groups: a sham-operated group (phosphate-buffered saline, PBS); a control group (MCAO+PBS); a ZSTK474-treated group (MCAO+ZSTK474). In the ZSTK474-treated group, the mice are given the optimum dose of 200 mg/kg ZSTK474. In the sham-operated group and control group, mice are given an equivalent volume of PBS. All mice receive that same dose daily via oral gavage beginning at 6 h after the onset of focal ischemia and continuing for two more days, i.e., for a total of 3 days.

References:

[1]. Kong D, et al. ZSTK474 is an ATP-competitive inhibitor of class I phosphatidylinositol 3 kinase isoforms. Cancer Sci, 2007, 98(10), 1638-1642.

[2]. 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.

[3]. 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.

CAIndexNames:

1H-Benzimidazole, 2-(difluoromethyl)-1-(4,6-di-4-morpholinyl-1,3,5-triazin-2-yl)-

SMILES:

FC(C1=NC2=C(N1C3=NC(N4CCOCC4)=NC(N5CCOCC5)=N3)C=CC=C2)F

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