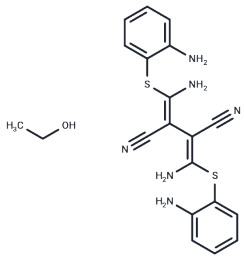
Data Sheet (Cat.No.T6223)

TargetMol

U0126-EtOH

Chemical Properties



CAS No. : 1173097-76-1

Formula: C18H16N6S2·C2H6O

Molecular Weight: 426.6

Storage: Powder: -20°C for 3 years | In solvent: -80°C for 1 year
Actual storage temperature shall be subject to the COA.

Biological Description

Description	U0126-etoh (U0126 Ethanol) is a non-ATP competitive inhibitor of MEK1 (IC50=72 nM) and MEK2 (IC50=58 nM) with selectivity. U0126-EtOH inhibited autophagy and mitophagy.
Targets(IC50)	Mitophagy,MEK,Autophagy,Influenza Virus
In vitro	**METHODS**: The transcriptional activity of AP-1 in COS-7 cells was detected after treatment with U0126-EtOH. **RESULTS**: U0126-EtOH inhibits AP-1 transcriptional activity (IC50=1 μM). [1] **METHODS**: HCT116 cells were treated with U0126-EtOH and colony formation was detected by soft AGAR growth assay. HeLa cells were treated with U0126-EtOH and the ELK1-luciferase reporter gene was detected. **RESULTS**: U0126-EtOH inhibited adheron-independent colony formation (IC50=19.4 μM), and U0126-EtOH inhibited EGF-stimulated Elk1 luciferase reporter gene (IC50=0.29 μM). [2] **METHODS**: Mouse RAS-3T3 cells were treated with U0126-EtOH, and the phosphorylation level of ERK1/2 was detected by ELISA. **RESULTS**: U0126-EtOH at a concentration of 10-40 μM inhibits MEK-mediated phosphorylation of ERK1/2. [3]
In vivo	**METHODS**: To study the anti-tumor activity of U0126-EtOH, U0126-EtOH (10.5 mg/kg) was intraperitoneally injected into mice every day for treatment. **RESULTS**: U0126-EtOH led to a significant reduction in tumor implantation and early growth. The tumor volume decreased by 60-70% 9 days after injection and remained in this state thereafter. [4] **METHODS**: To study the effect of U0126-EtOH on vascular constriction, rats received 120 minutes of temporary midcerebral artery occlusion (tMCAO), and then U0126-EtOH (30 mg/kg) was intraperitoneally injected into the rats. **RESULTS**: After treatment with U0126-EtOH, the vasoconstriction of S6c was significantly reduced. [5]
Kinase Assay	The amount of immunoprecipitated wild type MEK used in these assays was adjusted to give a similar amount of activity units as obtained with 10 nM recombinant MEK. All other assays were performed with a recombinant, constitutively activated mutant MEK-1 (ΔN3-S218E/S222D) or constitutively active MEK-2(S222E/S226D). Reaction velocities were measured using a 96-well nitrocellulose filter apparatus as described below. Unless otherwise noted, reactions were carried out at an enzyme concentration of 10

Kinase Assay	nM, in 20 mM Hepes, 10 mM MgCl2, 5 mM β-mercaptoethanol, 0.1 mg/ml BSA, pH 7.4, at room temperature. Reactions were initiated by the addition of [γ-33P]ATP into the premixed MEK/ERK/inhibitor reaction mixture, and an aliquot of 100 µl was taken every 6 min and transferred to the 96-well nitrocellulose membrane plate which had 50 mM EDTA to stop the reaction. The membrane plate was drawn and washed 4 times with buffer under vacuum. Wells were then filled with 30 µl of Microscint-20 scintillation fluid, and the radioactivity of33P-phosphorylated ERK was counted with a Top Count scintillation counter. Velocities were obtained from the slopes of radioactivity versus time plots. Concentrations of ERK and ATP were 400 nM and 40 µM, respectively, unless otherwise indicated [2].
Cell Research	HEK293 cells were maintained in Dulbecco's modification of Eagle's medium (low glucose) plus 10% foetal bovine serum. HeLa cells stably expressing wild type or kinase-dead LKB1 have been described. AMPK activity was determined by immunoprecipitate kinase assays using anti-AMPK-a1 and -a2 antibodies. Antibodies recognising AMPK phosphorylated on Thr-172 (anti-pT172), AMPK-α1 and -α2 and acetyl-CoA carboxylase-1 (ACC1) phosphorylated on Ser-80 [16] were described previously. Quantification of ratios of signals from phosphorylated and total protein using these antibodies was performed by dual labelling using the LI-COR Odyssey IR imager as described. Contents of ATP and ADP were determined for cells in 6 cm culture dishes by quickly pouring off the medium, adding 350 µl of ice-cold 5% perchloric acid, scraping the cells off with a plastic scraper, and centrifuging (14 000 · g; 3 min, 4 °C) to remove insoluble material. The perchloric acid was then extracted from the supernatant and nucleotides analysed by capillary electrophoresis of perchloric acid extracts as described previously. All incubations of cells were performed in triplicate and results are expressed as means ± S. E.M [3].
Animal Research	Prior to injection, FI cells were labeled with a stable fluorescent dye molecule, DiA at 10 µg/ml for 5 h at 37 1C. After washing to remove free DiA, cells were trypsinized for inoculation (U0126 experiments) or transfection (RNAi experiments). Biliary epithelial cells were injected subcutaneously, at the indicated times, into the tibia of nude mice. In the chemical experiments, 3h after inoculation, mice were treated with U0126 (10.5 mg/kg) daily by intraperitoneal injection. The length and width of each tumor were measured every day by using a caliper. The following formula was used to calculate tumor volumes ? width2 length/2. Mice were killed at the end of experiment. Tumors were immediately frozen in liquid nitrogen [5].

Solubility Information

Solubility	Ethanol: < 1 mg/mL (insoluble or slightly soluble) DMSO: 255 mg/mL (597.75 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 7.9 mg/mL (18.52 mM),Solution. *Please add the solvents sequentially, clarifying the solution as much as possible before adding the next one. Dissolve by heating and/or sonication if necessary. Working solution is recommended to be prepared and used immediately. The formulation provided above is for reference purposes only. In vivo formulations may vary and should be modified based on specific experimental conditions.*

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.3441 mL	11.7206 mL	23.4412 mL
5 mM	0.4688 mL	2.3441 mL	4.6882 mL
10 mM	0.2344 mL	1.1721 mL	2.3441 mL
50 mM	0.0469 mL	0.2344 mL	0.4688 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.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

Reference

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