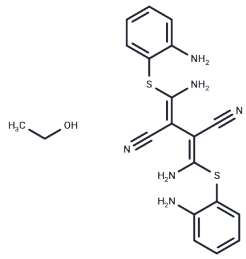
Data Sheet (Cat.No.T6223)

TargetMol

U0126-EtOH

Chemical Properties



CAS No. : 1173097-76-1

Formula: C18H16N6S2·C2H6O

Molecular Weight: 426.6

Appearance: no data available

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

Biological Description

Description	U0126-EtOH (U0126 Ethanol) is a non-ATP competitive specific inhibitor of MEK1/2 (IC50: 0.07/0.06 μM).
Targets(IC50)	Mitophagy,MEK,Autophagy,Influenza Virus
In vitro	U0126 antagonized AP-1 transcriptional activity via noncompetitive inhibition of the dual specificity kinase MEK with an IC50 of 0.07 microM for MEK 1 and 0.06 microM for MEK 2 [1]. In fibroblasts treated with TPA/serum, U0126 suppressed the up-regulation of c-Fos and c-Jun proteins by 50–80%. Treatment with 10 μM U0126 did not affect the protein levels of the constitutively expressed transcription factors SP-1 or JunD and Fra-1 [2]. U0126 caused phosphorylation and activation of AMPK) and increased phosphorylation of its downstream target acetyl-CoA carboxylase, in HEK293 cells. This effect only occurred in cells expressing the upstream kinase, LKB1 [3].
In vivo	Treatment of mice with U0126 via the aerosol route led to (i) inhibition of MEK activation in the lung (ii) reduction of progeny IAV titers compared to untreated controls (iii) protection of IAV infected mice against a 100× lethal viral challenge [4]. In all U0126 (10.5 mg/kg) experiments, engraftment and early tumor growth were significantly decreased. Furthermore, a 60–70% reduction in the volume of tumors treated with U0126 was obtained 9 days after injection and thereafter. Cdk1 expression was also strongly reduced in U0126-treated mice [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 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	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 7.9 mg/mL (18.52 mM),Solution. Ethanol: < 1 mg/mL (insoluble or slightly soluble) DMSO: 55 mg/mL (128.93 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)

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.

Reference

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