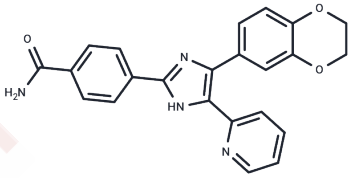
Data Sheet (Cat.No.T2449)

D4476

Chemical Properties



CAS No. : 301836-43-1

Formula: C23H18N4O3

Molecular Weight: 398.41

Appearance: no data available

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

Biological Description

Description	D4476 (Casein Kinase I Inhibitor) is an effective, selective, and cell-permeant CK1 (casein kinase 1) inhibitor(IC50=300 nM in a cell-free assay).
Targets(IC50)	Apoptosis,Casein Kinase,ALK,Autophagy
In vitro	D4476 (10 μM) effectively inhibits CK1 and ALK5 with the activities of about 7% and 22% versus control group, respectively. In H4IIE hepatoma cells, D4476 specifically inhibits the phosphorylation of endogenous forkhead box transcription factor O1a (FOXO1a) on Ser322 and Ser325, without affecting the phosphorylation of other sites.[1] D4476 induces cytotoxicity in ANBL6, INA6 and RPMI8226 lines; MM1S and U266 lines are less sensitive; and OPM1 line is totally resistant. High concentrations (50 μM) D4476 induced toxicity in all multiple myeloma (MM) lines. In MM cells, D4476 increases the protein levels of TP53, P27, and FADD, as well as the cell cycle progression and induction of apoptosis.[2] Treatment of leukemia stem cells (LSCs) with D4476 shows highly selective killing of LSCs over normal HSPCs.[3]
Kinase Assay	CK1 kinase assay: All protein kinase assays (25 μL) are carried out at room temperature (21℃). Assays are performed for 40 min using a Biomek 2000 Laboratory Automation Workstation in a 96-well format. The concentrations of magnesium acetate and [γ-33P] ATP (800 cpm/pmol) in the assays are 10 mM and 0.1 mM, respectively. Assays are initiated with MgATP and stopped by the addition of 5 μL of 0.5 M orthophosphoric acid. Aliquots are then spotted on to P30 ?ltermats, washed four times in 75 mM phosphoric acid to remove ATP, once in methanol, then dried and counted for radioactivity. CK1 δ (5-20 m-units), diluted in 20 mM Hepes, pH 7.5, 0.15 M NaCl, 0.1 mM EGTA, 0.1% (v/v) Triton X-100, 5 mM dithiothreitol, 50% (v/v) glycerol, is assayed against the peptide RRKDLHDDEEDEAMSITA in an incubation containing 20 mM Hepes, pH 7.5, 0.15 M NaCl, 0.1 mM EDTA, 5 mM DTT, 0.1% (v/v) Triton X-100 and 0.5 mM substrate peptide.
Cell Research	MM cells are seeded in triplicate into 96-well plates in 100 μL culture media. D4476 is added to each well at concentrations of 0, 5, 10, 20, 30, 40, and 50 μM in another 100 μL culture media. Cell viability is measured with MTT at the 72 h drug exposure. Absorbance is measured at 570 nm with spectrophotometer.(Only for Reference)

Solubility Information

Solubility	DMSO: 40 mg/mL (100.4 mM),Sonication is recommended. Ethanol: 19.9 mg/mL (49.95 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.510 mL	12.5499 mL	25.0998 mL
5 mM	0.502 mL	2.510 mL	5.020 mL
10 mM	0.251 mL	1.255 mL	2.510 mL
50 mM	0.0502 mL	0.251 mL	0.502 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

Rena G, et al. EMBO Rep. 2004, 5(1), 60-65.
Hu Y, et al. Leukemia. 2015, 29(2), 474-482.
Järås M, et al. J Exp Med. 2014,211(4), 605-612.
Bain J, et al. Biochem J. 2003, 371(Pt 1), 199-204.