Data Sheet (Cat.No.T2414)

TargetMol

Torkinib

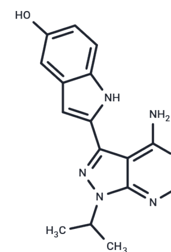
Chemical Properties

CAS No. : 1092351-67-1

Formula: C16H16N6O

Molecular Weight: 308.34

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	Torkinib (PP 242) (PP 242) is a selective and ATP-competitive mTOR inhibitor (IC50: 8 nM). It also inhibits mTORC1/2 (IC50s: 30/58 nM).
Targets(IC50)	Apoptosis,Mitophagy,Autophagy,mTOR,PI3K
In vitro	Torkinib (PP242) potently inhibited mTOR (IC50: 8 nM) but was much less active against other PI3-K family members. Testing of this compound against 219 protein kinases revealed remarkable selectivity relative to the protein kinome. In BT549 cells, PP242 inhibited the phosphorylation of Akt, the mTOR substrate p70S6K, and its downstream target S6 [1]. PP242 suppressed growth by > 90%, with low nanomolar potency (GI50: 12 nM). PP242 had greater anti-proliferative potency relative to rapamycin in a panel of solid tumor cell lines carrying either PI3K gain-of-function or PTEN loss-of-function [2].
In vivo	In mouse p190 model, short-term oral dosing with Torkinib in a dose-dependent manner significantly reduced leukemic burden in the spleen and bone marrow. In a long-term survival study, oral dosing of Torkinib (30 and 60 mg/kg) significantly delayed the onset of leukemia [2]. In fat and liver, Torkinib was able to completely inhibit the phosphorylation of Akt at S473 and T308. Surprisingly, Torkinib was only partially able to inhibit the phosphorylation of Akt in skeletal muscle and was more effective at inhibiting the phosphorylation of T308 than S473, despite it's ability to fully inhibit the phosphorylation of 4EBP1 and S6 [3].
Kinase Assay	Purified kinase domains were incubated with inhibitors at 2- or 4-fold dilutions over a concentration range of 50 - 0.001 μM or with vehicle (0.1% DMSO) in the presence of 10 μM ATP, 2.5 μCi of γ-32P-ATP and substrate. Reactions were terminated by spotting onto nitrocellulose or phosphocellulose membranes, depending on the substrate; this membrane was then washed 5-6 times to remove unbound radioactivity and dried. Transferred radioactivity was quantitated by phosphorimaging and IC50 values were calculated by fitting the data to a sigmoidal doseresponse using Prism software [1].
Cell Research	Cells were seeded in triplicate wells of 96-well flat bottom culture plates for 48 hr in the presence of increasing concentrations of indicated inhibitors. Cell viability and median-effect dose affecting growth (GIC50) was determined using the MTS assay. Absorbance values (490 nm) were normalized to controls and expressed as %MTS conversion. Wells lacking cells but with MTS added was used as the zero value when normalizing. For drug combination experiments, a range of fixed ratios of inhibitors was used to assess synergy using the combination index (CI) with CalcuSyn software according to the

Cell Research	median-effect method as previously described. For proliferation experiments with PC-3, SKOV3, 786-O, and U87 cells, the CellTiter-Glo Luminescent reagent was used following the manufacturer's instructions. Quantitation was performed as mentioned above [2].
Animal Research	Drugs were prepared in 100 μl of vehicle containing 20% DMSO, 40% PEG-400, and 40% saline. Six-wk-old male C57BL/6 mice were fasted overnight prior to drug treatment. PP242 (0.4 mg), rapamycin (0.1 mg), or vehicle alone was injected IP. After 30 min for the rapamycin-treated mouse or 10 min for the PP242 and vehicle-treated mice, 250 mU of insulin in 100 μl of saline was injected IP. 15 min after the insulin injection, the mice were killed by CO2 asphyxiation followed by cervical dislocation. Tissues were harvested and frozen on liquid nitrogen in 200 μl of cap lysis buffer. The frozen tissue was thawed on ice, manually disrupted with a mortar and pestle, and then further processed with a micro tissue-homogenizer. The protein concentration of the cleared lysate was measured by Bradford assay and 5-10 μg of protein was analyzed by Western blot as described above [3].

Solubility Information

Solubility	DMSO: 55 mg/mL (178.37 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: 2 mg/mL (6.49 mM),Sonication is recommended. *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	3.2432 mL	16.2159 mL	32.4317 mL
5 mM	0.6486 mL	3.2432 mL	6.4863 mL
10 mM	0.3243 mL	1.6216 mL	3.2432 mL
50 mM	0.0649 mL	0.3243 mL	0.6486 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

Apsel B, et al. Targeted polypharmacology: discovery of dual inhibitors of tyrosine and phosphoinositide kinases. Nat Chem Biol. 2008 Nov;4(11):691-9.
Janes MR, et al. Effective and selective targeting of leukemia cells using a TORC1/2 kinase inhibitor. Nat Med. 2010 Feb;16(2):205-13.
Feldman ME, et al. Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. PLoS Biol. 2009 Feb 10;7(2):e38.