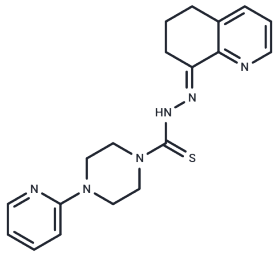
Data Sheet (Cat.No.T4325)

TargetMol

COTI-2

Chemical Properties



CAS No. : 1039455-84-9

Formula: C19H22N6S

Molecular Weight: 366.48

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	COTI-2, an orally available thiosemicarbazone, is an activator of mutant forms of the p53 protein with potential antineoplastic activity.
Targets(IC50)	Apoptosis,p53,MDM-2/p53
In vitro	COTI-2 (72 h) potently inhibits the proliferation rate of all the tested cell lines. In COLO-205, HCT-15, and SW620 cell lines, COTI-2 markedly inhibits tumor cell proliferation. Relatively low concentrations of COTI-2 are active against all human glioblastoma cell lines tested (U87-MG, SNB-19, SF-268, and SF-295). In SHP-77 cells, COTI-2 treatment with IC50 concentrations causes the induction of early apoptosis among 40 to 47% of total cells.
In vivo	In the HT-29 human colorectal tumor xenografts, COTI-2 (10 mg/kg) markedly inhibits tumor growth. Except for reducing tumor volumes at specific times post-treatment, COTI-2 also increases the time required for tumors to reach specified volumes. In the SHP-77 SCLC xenograft model, COTI-2 (3 mg/kg) also markedly inhibits tumor growth. COTI-2 can reduce U87-MG tumor volumes at specific times post-treatment and lengthen the time required for U87-MG xenografts to grow in nude mice. Control tumors in mice treated with vehicle alone take only 5 days to reach an average volume of 828 mm3 while tumors in animals treated with COTI-2 take double that time (10 days) to reach a similar mean volume (857 mm3). Regardless of the route of administration, COTI-2 potently inhibits OVCAR-3 xenograft growth.
Kinase Assay	The interaction of COTI-2 with 227 kinases is tested using the AMBIT BIOSCIENCES KINOMESCAN assay. In brief, streptavidin-coated magnetic beads are treated with biotinylated small molecule ligands for 30 min at 25°C to generate affinity resins for kinase assays. The liganded beads are blocked with excess biotin and washed with blocking buffer (1% BSA, 0.05% Tween 20, 1 mM DTT) to remove unbound ligand and to reduce non-specific binding. Binding reactions are assembled by combining phage lysates, liganded affinity beads, and COTI-2 in 1× binding buffer (20% SeaBlock, 0.17× PBS, 0.05% Tween 20, 6 mM DTT). All reactions are carried out in polystyrene 96-well plates that have been pre-treated with blocking buffer in a final volume of 0.1 mL.
Cell Research	COTI-2 is dissolved in 100% dimethyl sulfoxide stock solution and diluted in medium plus FBS such that final DMSO concentrations are 0.5 to 1.0% depending on the experiment. SHP-77 cells are cultured with various concentrations of COTI-2 for 48 h. Cells are then washed twice with 1× cold PBS and stained with Annexin V and 7AAD according to the

Cell Research	manufacturer's instructions. Briefly, 5 μL of Annexin V and 7AAD are added to 1×105 cells and incubated for 15 min at room temperature in the dark. Then 400 μL of the 1× binding buffer (100 mM HEPES, pH 7.4, 140 mM NaCl, 2.5 mM CaCl2) is added to the cells. Finally, cells are analyzed using a flow cytometer.
Animal Research	SHP-77 and HT-29 cells are injected in 50% matrigel into flanks of NCr-nu mice (2×106 cells per injection site) (n=5 mice per group). In the case of SHP-77 xenografts, treatment with COTI-2 begins prior to the appearance of palpable tumors. One day after injection of SHP-77 cells, animals receive 3 mg/kg of COTI-2 (once every two days, up to 38 days). Tumor sizes are estimated at 5, 10, 17, 24, and 38 days, by standard caliper measurements. In the case of HT-29 xenografts, the capacity of COTI-2 to suppress the growth of established tumors is assessed. HT-29 xenografts are allowed to grow to 200 mm3 before starting IP treatment with COTI-2 (10 mg/kg, 5 days a week for 7 weeks) or saline IP. Tumor growth is measured every 4 days by caliper measurement.

Solubility Information

Solubility	DMSO: 9.26 mg/mL (25.27 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+90% Saline: 0.93 mg/mL (2.54 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	2.7287 mL	13.6433 mL	27.2866 mL
5 mM	0.5457 mL	2.7287 mL	5.4573 mL
10 mM	0.2729 mL	1.3643 mL	2.7287 mL
50 mM	0.0546 mL	0.2729 mL	0.5457 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

Bykov VJN,etal.Targeting mutant p53 for efficient cancer therapy.Nat Rev Cancer. 2018 Feb;18(2):89-102.
Salim KY,etal.COTI-2, a novel small molecule that is active against multiple human cancer cell lines in vitro and in vivo.Oncotarget. 2016 Jul 5;7(27):41363-41379.
Duffy MJ, et al. Mutant p53 as a target for cancer treatment. Eur J Cancer. 2017 Sep;83:258-265.