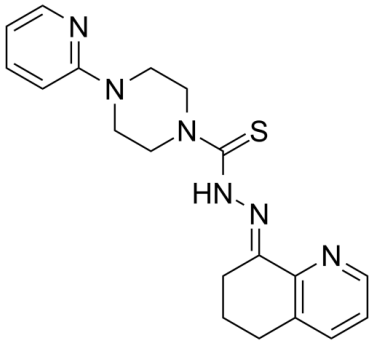
Data Sheet

Product Name:	COTI-2
Cat. No.:	CS-8156
CAS No.:	1039455-84-9
Molecular Formula:	C19H22N6S
Molecular Weight:	366.48
Target:	Apoptosis; MDM-2/p53
Pathway:	Apoptosis
Solubility:	H2O : < 0.1 mg/mL (insoluble); DMSO : 6.67 mg/mL (18.20 mM; Need ultrasonic)



BIOLOGICAL ACTIVITY:

COTI-2, an anti-cancer drug with low toxicity, is an orally available third generation activator of **p53 mutant** forms. COTI-2 acts both by reactivating mutant p53 and inhibiting the **PI3K/AKT/mTOR** pathway. COTI-2 induces **apoptosis** in multiple human tumor cell lines. COTI-2 exhibits antitumor activity in HNSCC through p53-dependent and -independent mechanisms. COTI-2 converts mutant p53 to wild-type conformation[1][2][3]. IC50 & Target: p53[1] **In Vitro:** COTI-2 efficiently inhibits the proliferation rate of all the tested cell lines following 72 h of treatment. COTI-2 is significantly effective at inhibiting tumor cell proliferation in all three cell lines (COLO-205, HCT-15, and SW620). Relatively low concentrations of COTI-2 are active against all human glioblastoma cell lines tested (U87-MG, SNB-19, SF-268, and SF-295). COTI-2 treatment of SHP-77 cells with approximate IC_{50} concentrations results in the induction of early apoptosis among 40 to 47% of total cells[2]. **In Vivo:** COTI-2 significantly inhibits tumor growth in the HT-29 human colorectal tumor xenografts at a dose of 10 mg/kg. In addition to reducing tumor volumes at specific times post-treatment, COTI-2 also delays the time required for tumors to reach specified volumes. COTI-2 also significantly inhibits tumor growth in the SHP-77 SCLC xenograft model at a dose as low as 3 mg/kg. COTI-2 treatment both reduces U87-MG tumor volumes at specific times post-treatment and lengthens 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 mm^3 while tumors in animals treated with COTI-2 take double that time (10 days) to reach a similar mean volume (857 mm^3). COTI-2 treatment effectively inhibits OVCAR-3 xenograft growth regardless of the route of administration[2].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay: [2]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[2]. **Cell Assay:** 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.[2]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 manufacturer's instructions. Briefly, 5 μL of Annexin V and 7AAD are added to 1×10^5 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 $CaCl_2$) is added to the cells. Finally, cells are analyzed using a flow cytometer[2]. **Animal Administration:** [2]SHP-77 and HT-29 cells are injected in 50% matrigel into flanks of NCr-nu mice (2×10^6 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 growth of established tumors is assessed. HT-29 xenografts are allowed to grow to 200 mm^3 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[2].

References:

[1]. Duffy MJ, et al. Mutant p53 as a target for cancer treatment. Eur J Cancer. 2017 Sep;83:258-265.

[2]. Salim KY, et al. 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.

[3]. Lindemann A, et al. COTI-2, A Novel Thiosemicarbazone Derivative, Exhibits Antitumor Activity in HNSCC through p53-dependent and -independent Mechanisms. Clin Cancer Res. 2019 Sep 15;25(18):5650-5662.

CAIndexNames:

1-Piperazinecarbothioic acid, 4-(2-pyridinyl)-, 2-(6,7-dihydro-8(5H)-quinolinylidene)hydrazide

SMILES:

S=C(N1CCN(C2=NC=CC=C2)CC1)N/N=C3CCCC4=C\3N=CC=C4

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