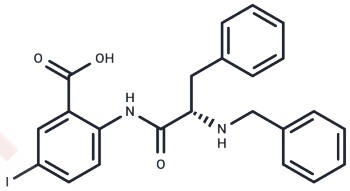
Data Sheet (Cat.No.T6209)

TargetMol

CW-069

Chemical Properties



CAS No. : 1594094-64-0

Formula: C23H21IN2O3

Molecular Weight: 500.33

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	CW-069 (IC50=75 μM), an allosteric selective inhibitor of microtubule motor protein HSET, exhibits remarkable specificity over KSP.
Targets(IC50)	Microtubule Associated,Kinesin
In vitro	CW069 increases multipolar spindles in N1E-115 cells with supernumerary centrosomes without altering bipolar spindle morphology in normal human dermal fibroblast cells. CW069 inhibits growth in cancer N1E-115 cells with IC50 of 10 μM, but not in NHDF or primary human bone marrow cells. [1]
Kinase Assay	In Vitro Enzymatic ATPase Assay: The protocol is optimized for use with full-length, N-terminal, 6His-tagged HSET and KSP, and measured the MT-stimulated activity of the proteins. Inhibition of the Gsp synthetase activity of HSET/KSP is observed spectrophotometrically by coupling the hydrolysis of ATP to oxidation of NADH via pyruvate kinase/lactate dehydrogenase reactions. The assay is initiated by adding purified Gsp synthetase/amidase (12.8 nM) to an assay mixture containing the following components (final concentration): 6 nM protein, 0.07 mg/ml MTs (University Biologicals), 1.56 mM glutathione, 10 mM spermidine, 2 mM ATP, 2.7 mM MgCl2, 1 mM phospho(enol) -pyruvate, 0.2 mM NADH, 50 μg/ml lactate dehydrogenase, 100 μg/ml pyruvate kinase, and various concentrations of inhibitor all in 50 mM Na PIPES (pH 6.8) at 37°C. The ADP-Glo detection assay (Promega) is performed as described in the manufacturer's instructions. All compound additions were performed using a multidrop BioMek Nxp. Plates were read using a Pherastar microplate reader.
Cell Research	Cells are cultured in DMEM supplemented with 10% fetal calf serum (FCS) at 37°C and 5% CO2. All compounds used in the Sulforhodamine B colorimetric (SRB) assay are dissolved in DMSO and diluted in culture medium to a final concentration of 0.2% DMSO. For the SRB assay and live-cell imaging, cells are seeded in 96-well plates at a density of 2,500 cells per well. After 24 hr, the cells are treated with compound for 72 hr, with triplicate wells for each concentration. For the SRB assay, the cells are then fixed with trichloroacetic acid (TCA) and stained with SRB. Fluorescence is quantified using an Infinite 200 PRO plate-reader at a wavelength of 545 nm. Compound-treated wells are compared with solvent control wells and the concentration of compound that results in 50% of the solvent-control cell growth is designated as the IC50 concentration, calculated using Graphpad PRISM 6. At least three biological replicates are performed for each assay.(Only for Reference)

Solubility Information

Solubility	Ethanol: 4 mg/mL (7.99 mM),Sonication is recommended. DMSO: 93 mg/mL (185.88 mM),Sonication is recommended. H2O: < 1 mg/mL (insoluble or slightly soluble), (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 3.3 mg/mL (6.6 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	1.9987 mL	9.9934 mL	19.9868 mL
5 mM	0.3997 mL	1.9987 mL	3.9974 mL
10 mM	0.1999 mL	0.9993 mL	1.9987 mL
50 mM	0.040 mL	0.1999 mL	0.3997 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

Watts CA, et al. Chem Biol. 2013, 20(11), 1399-1410.