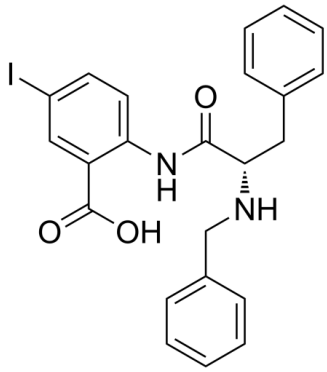
Data Sheet

Product Name:	CW-069
Cat. No.:	CS-2329
CAS No.:	1594094-64-0
Molecular Formula:	$C_{23}H_{21}IN_2O_3$
Molecular Weight:	500.33
Target:	Kinesin
Pathway:	Cell Cycle/DNA Damage; Cytoskeleton
Solubility:	DMSO : ≥ 52 mg/mL



BIOLOGICAL ACTIVITY:

CW-069 is an allosteric inhibitor of **microtubule motor protein HSET** with an **IC_{50}** of 75 μM. IC50 & Target: IC50: 75 μM (HSET)[1]
In Vitro: CW-069 is an allosteric inhibitor of HSET with an IC_{50} of 75 μM. CW-069 shows statistically significant selectivity over KSP. CW-069 potently suppresses N1E-115 cells, and less potently inhibits the NHDF cells, with IC_{50} of 86 ± 10 μM and 181 ± 7 μM, respectively. CW-069 (100 or 200 μM) causes increased multipolar spindles in N1E-115 cells with supernumerary centrosomes and shows no effect on altering bipolar spindle morphology in normal human dermal fibroblast cells. CW-069 (200 μM) cacuses multipolar anaphase and cell death induced in N1E-115 cells via transfection with HSET siRNA, and antagonizes inhibition of KSP by monastrol, but does not exert mitotic arrest in HeLa cells[1].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay: [1]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, 1.56 mM glutathione, 10 mM spermidine, 2 mM ATP, 2.7 mM $MgCl_2$, 1 mM phospho(enol)-pyruvate, 0.2 mM NADH, 50 μg/mL lactate dehydrogenase, 100 μg/mL pyruvate kinase, and **various concentrations of inhibitor (CW-069)** all in 50 mM Na PIPES (pH 6.8) at 37°C. The ADP-Glo detection assay is performed. All compound additions are performed using a multidrop BioMek Nxp. Plates are read using a Pherastar microplate reader[1]. **Cell Assay:** CW-069 is dissolved in DMSO and diluted in culture medium.[1]**NHDF cells, HeLa cells, BT549, MCF-7 and MDA-MB-231 cells** are verified by STR genotyping and all tested negative for mycoplasma. Cells are cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) at 37°C and 5% CO_2. All compounds (**CW-069**) 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 **(CW-069) 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 (CW-069)-treated wells are compared with solvent control wells and the concentration of compound that resulted in 50% of the solvent-control cell growth is designated as the IC_{50} concentration, calculated using Graphpad PRISM 6. At least three biological replicates are performed for each assay[1].

References:

[1]. Watts CA, et al. Design, synthesis, and biological evaluation of an allosteric inhibitor of HSET that targets cancer cells with supernumerary centrosomes. Chem Biol. 2013 Nov 21;20(11):1399-410.

CAIndexNames:

Benzoic acid, 5-iodo-2-[[(2S)-1-oxo-3-phenyl-2-[(phenylmethyl)amino]propyl]amino]-

SMILES:

O=C(NC1=CC=C(I)C=C1C(O)=O)[C@@H](NCC2=CC=CC=C2)CC3=CC=CC=C3

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