PROTAC HK2 Degrader-1

Cat. No.: HY-155008

Molecular Formula: $C_{32}H_{28}Cl_2N_6O_5$

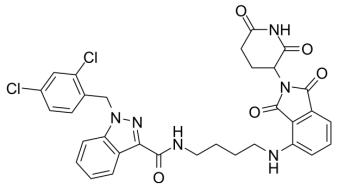
Molecular Weight: 647.51

Target: Hexokinase

Pathway: Metabolic Enzyme/Protease

Storage:

Powder	-20°C	3 years
	4°C	2 years
In solvent	-80°C	6 months
	-20°C	1 month



SOLVENT & SOLUBILITY

In Vitro

DMSO : 100 mg/mL (154.44 mM; Need ultrasonic)

Preparing Stock Solutions

Mass / Solvent / Concentration	1 mg	5 mg	10 mg
1 mM	1.5444 mL	7.7219 mL	15.4438 mL
5 mM	0.3089 mL	1.5444 mL	3.0888 mL
10 mM	0.1544 mL	0.7722 mL	1.5444 mL

Please refer to the solubility information to select the appropriate solvent.

BIOLOGICAL ACTIVITY

Description

PROTAC HK2 Degrader-1 is a PROTAC consisting of Lonidamine (HY-B0486) as a target protein Hexokinase 2 (HK2) inhibitor and Thalidomide (HY-14658) as a CRBN ligand-linked PROTAC. PROTAC HK2 Degrader-1 selectively inhibits the proliferation of breast cancer cells by forming a ternary complex through the ubiquitin-proteasome system to degrade Hexokinase 2 (HK2) protein leading to mitochondrial damage and cell death. PROTAC HK2 Degrader-1 effectively inhibits breast tumor growth and reduces the colonic side effects of cisplatin for breast cancer research[1].

IC_{50} & Target

DC50: 2.56 μM (Hexokinase 2, HK2 in 4T1); 0.79 μM ((Hexokinase 2, HK2 in MDA-MB-231)

In Vitro

PROTAC HK2 Degrader-1 inhibits the proliferation of 786-O, 4T1, PANC-1, HGC-27, and MCF-1 with IC_{50}s of 34.07 μM, 5.08 μM, 31.53 μM, 6.11 μM, and 21.65 μM, respectively[1].

PROTAC HK2 Degrader-1 degrades HK2 with DC_{50} values of was 2.56 μM (4T1) and 0.79 μM (MDA-MB-231), respectively[1].

PROTAC HK2 Degrader-1 (0.01-200 μM, 36 h) selectively suppresses breast cancer cell proliferation and stimulates HK2 protein degradation via the ubiquitin mediated proteasome pathway in a time and concentration dependent manner[1].

PROTAC HK2 Degrader-1 (10 μM for 4T1, 0.5 μM for MDA-MB-231, 24 h) degraded HK2 protein via the ubiquitin−proteasome system by forming a ternary complex[1].

PROTAC HK2 Degrader-1 (20 μM, 36 h) mediates degradation of HK2 that causing mitochondrial damage, releasing

cytochrome C to activate caspase-3, then PROTAC HK2 Degrader-1 cleaves GSDME to trigger thermal coma and promotes cellular release of danger signals, such as ATP, HMGB1, CRT, etc., thus inducing cellular immune death[1].
PROTAC HK2 Degrader-1 (20 μM, 36 h) can induce PD-L1 protein to internalize from the cell membrane to the cytoplasm and reduce the total amount of PD-L1 protein[1].
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Cell Cytotoxicity Assay[1]

Cell Line:	4T1, MDA-MB-231, PUMC-HUVEC-T1
Concentration:	20 μM
Incubation Time:	72 h (MTT), 48 h (CCK-8)
Result:	Showed the greatest impact on 4T1 and HGC-27 cells, with IC_{50} dosages of 5.08 and 6.11 μM. Selectively suppressed breast cancer cell proliferation and stimulates HK2 protein degradation. Prevented 4T1 cells to form a colony and had little influence on HUVECT-1.

Western Blot Analysis[1]

Cell Line:	4T1, MDAMB-231
Concentration:	20 μM; 0.01, 0.05, 0.1, 0.5, 1.0, 2.0, 5.0, 10, 20, 50, 100, 150, 200 μM; 10 μM; 0.5 μM.
Incubation Time:	36 h; 24 h
Result:	Degraded 71.06% of HK2 at 20 μM in 4T1 and MDAMB-231 cells. DC_{50}=2.56 μM (4T1) and 0.79 μM (MDA-MB-231), respectivley. Promoted the degradation of HK2 protein within 12 h, with the greatest degradation impact at 36 h in 4T1 cells and MDA-MB-231 cells. Degradation capacity was reduced, because pretreatment with Tha and LND occupy the protein pocket and disrupt the formation of the ternary complex of HK2, CRBN and C-02. Increased the expression of VDAC and Bax and decreased the level of Bcl-2 protein. had lower levels of full-length caspase-3 and higher levels of cleaved caspase-3. Cleaved GSDME through cleaved caspase-3, increasing the N-terminus of GSDME protein and thus triggering pyroptosis in 4T1 cells.

Immunofluorescence[1]

Cell Line:	4T1 and MDA-MB-231
Concentration:	20 μM
Incubation Time:	36 h
Result:	Caused the degradation of HK2 protein in a concentration dependent manner. Significantly reduced the visual yellow fluorescence of HK2 protein.

In Vivo

PROTAC HK2 Degrader-1 (50 mg/kg, Intraperitoneal injection, bid, for nine times, into six-weekold female BALB/c mice) inhibits tumor growth in 4T1 tumor models[1].
PROTAC HK2 Degrader-1 (50 mg/kg, Intraperitoneal injection, bid, for nine times, six-weekold female BALB/c mice) can induce GSDME-dependent pyroptosis to realize tumor immune response and effectively inhibit breast tumor growth[1].
PROTAC HK2 Degrader-1 (Cisplatin (HY-17394) 10mg/kg, i.v., C-02 50mg/kg, i.p., 25 days, into six-weekold female BALB/c mice) can sensitize Cisplatin (HY-17394) while reducing the colon side effects of Cisplatin (HY-17394), which has potential clinical value[1].
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Animal Model:	xenograft models , into six-weekold female BALB/c mice[1]
Dosage:	50 mg/kg
Administration:	Intraperitoneal injection, bid, for nine times.
Result:	Reduced proliferation and damaged nuclei in mouse models. Increased the levels of Cytokines IL-1β, IFN-γ, and TNF-α significantly and decreased the level of TGF-β and IL-10. Elevated levels of cleaved-Casp-3 and GSDME-N in tumor tissues of mouse.

Animal Model:	breast tumor model in mice by injecting 4T1 cells subcutaneously into six-weekold female BALB/c mice[1]
Dosage:	Cisplatin (HY-17394) 10mg/kg, 50mg/kg
Administration:	Cisplatin (HY-17394) (10mg/kg, i.v.), 50mg/kg, i.p., 25 days
Result:	Inhibited tumor growth and tumor volume. Decreased HK2 protein level, while co- treated with Cisplatin (HY-17394). Could alleviate Cisplatin (HY-17394) aggravated colon damage.

REFERENCES

[1]. Sang R, et al. Degradation of Hexokinase 2 Blocks Glycolysis and Induces GSDME-Dependent Pyroptosis to Amplify Immunogenic Cell Death for Breast Cancer Therapy. J Med Chem. 2023 Jun 27.

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