USP7-IN-1

Cat. No.: HY-16709

CAS No.: 1381291-36-6

Molecular Formula: $C_{23}H_{24}ClN_3O_3$

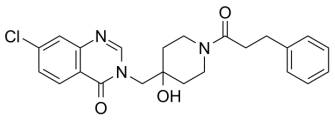
Molecular Weight: 425.91

Target: Deubiquitinase

Pathway: Cell Cycle/DNA Damage

Storage:

Powder	-20°C	3 years
	4°C	2 years
In solvent	-80°C	2 years
	-20°C	1 year



SOLVENT & SOLUBILITY

In Vitro

DMSO : 100 mg/mL (234.79 mM; Need ultrasonic)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.3479 mL	11.7396 mL	23.4791 mL
5 mM	0.4696 mL	2.3479 mL	4.6958 mL
10 mM	0.2348 mL	1.1740 mL	2.3479 mL

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

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (5.87 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (5.87 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (5.87 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

USP7-IN-1 is a selective and reversible inhibitor of ubiquitin-specific protease 7 (USP7), with an IC_{50} of 77 μM, and can be used for the research of cancer.

IC_{50} & Target

IC50: 77 μM (USP7)[1]

In Vitro

USP7-IN-1 (Example 2) is a selective and reversible inhibitor of USP7, with an IC_{50} of 77 μM, and shows no inhibition of USP8, USP5, Uch-L1, Uch-L3 or caspase 3. USP7-IN-1 inhibits the proliferation of HCT116 cells, with a GI_{50} of 67 μM[1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]

USP7 is diluted in USP buffer (50 mM Tris HCl; 0.5 mM EDTA (Ethylenediaminetetraacetic acid); 5 mM DTT; 0.01 % Triton X-100; Bovine Serum Albumin 0.05 mg/mL pH7.6). Compounds stocks (10 mM) are stored at -20°C in DMSO. Compounds (including USP7-IN-1) are tested at different concentrations: from 200 µM to 91 nM. Reactions are performed as duplicates in Black 384 well plates (10 µL final reaction volume). The substrate concentration for USP7 is 300 nM Ub-AMC. The concentrations of the enzyme (USP7) in specificity assays is 100 pM. The concentrations are determined in order to perform specificity assays under initial velocities at fixed substrate concentration. Compounds are pre-incubated with enzymes for 30 minutes at 25°C. Reactions are initiated by addition of substrate to the plates containing the enzymes (+/- compounds) diluted in assay buffer. Reactions are incubated for 60 minutes at 37°C. Reactions are stopped by adding acetic acid (100 mM final). Readings are performed on a Pherastar Fluorescent Reader. λ Emission 380 nm; λ Excitation = 460 nm. Data (mean values +/- standard deviation) are analyzed as % of control (no compound) and plotted as percentage versus the Log of the compound concentration using GraphPad. Data are fitted to a sigmoidal model (variable slope)[1].

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Cell Assay [1]

HCT116 colon cancer cells are maintained in Mc Coy's 5A medium containing 10% FBS, 3 mM glutamine and 1 % penicillin/streptomycin. Cells are incubated at 37°C in a humidified atmosphere containing 5% CO_2. Cell viability is assayed using the MTS technique in 96-well culture plates. MTS (3-(4,5-dimethyl-thiazol-2-yl)-5-(3-carboxy- methoxyphenyl)-2-(4-sulfophenyl)-2H-tetra-zolium) is a MTT-derived tetrazolium that is reduced in metabolically active cells into a soluble, cell-permeant formazan. The amount of formazan, detected by its absorbance at 492 nm is proportional to the number of living, metabolically active cells. 10^3 HCT116 cells are seeded per well. 24 hours later, the medium is changed and the cells treated in triplicate with the concentrations of each compound from 100 µM to 50 nM. The compounds (including USP7-IN-1) are diluted in 100% DMSO, whose final concentration on cells is kept at 0.5%. Cells are incubated with the compounds for 72 hours, and their viability then assayed by the addition of MTS for 2 hours. Absorbance at 492 nm is measured directly from the 96-well culture plates. GI_{50} (Growth Inhibition 50) concentrations for each compound are calculated using a sigmoidal variable slope fit. Values represent mean of three independent experiments[1].

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CUSTOMER VALIDATION

- Nat Commun. 2019 Jan 24;10(1):411.
- J Med Chem. 2022 Oct 11.
- Arch Pharm (Weinheim). 2023 May 17;e2200661.
- Harvard Medical School LINCS LIBRARY

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REFERENCES

[1]. SELECTIVE AND REVERSIBLE INHIBITORS OF UBIQUITIN SPECIFIC PROTEASE 7. WO 2013030218 A1

Caution: Product has not been fully validated for medical applications. For research use only.

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