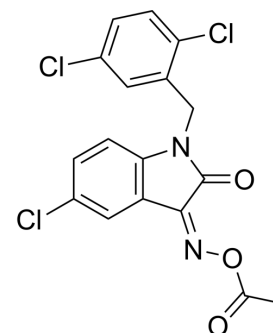


LDN-57444

Cat. No.:	HY-18637		
CAS No.:	668467-91-2		
Molecular Formula:	C ₁₇ H ₁₁ Cl ₃ N ₂ O ₃		
Molecular Weight:	397.64		
Target:	Deubiquitinase; Apoptosis		
Pathway:	Cell Cycle/DNA Damage; Apoptosis		
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 : 25 mg/mL (62.87 mM; Need ultrasonic)				
		Solvent Concentration	Mass 1 mg	5 mg	10 mg
	Preparing Stock Solutions	1 mM	2.5148 mL	12.5742 mL	25.1484 mL
		5 mM	0.5030 mL	2.5148 mL	5.0297 mL
10 mM		0.2515 mL	1.2574 mL	2.5148 mL	
Please refer to the solubility information to select the appropriate solvent.					
In Vivo	<ol style="list-style-type: none"> Add each solvent one by one: 50% PEG300 >> 50% saline Solubility: 10 mg/mL (25.15 mM); Suspended solution; Need ultrasonic Add each solvent one by one: 10% DMSO >> 90% corn oil Solubility: ≥ 2.5 mg/mL (6.29 mM); Clear solution 				

BIOLOGICAL ACTIVITY

Description	LDN-57444 is a reversible, competitive and site-directed inhibitor of ubiquitin C-terminal hydrolase L1 (UCH-L1), with an IC ₅₀ of 0.88 μM and a K _i of 0.40 μM; LDN-57444 also suppresses UCH-L3 activity, with an IC ₅₀ of 25 μM.
IC₅₀ & Target	IC ₅₀ : 0.88 μM (UCH-L1), 25 μM (UCH-L3) ^[1] K _i : 0.40 μM (UCH-L1) ^[1]
In Vitro	LDN-57444 is a reversible, competitive inhibitor of UCH-L1, with an IC ₅₀ of 0.88 μM, and also suppresses UCH-L3 activity, with an IC ₅₀ of 25 μM ^[1] . LDN-57444 (LDN, 5 μM for 1 hr) inhibits 70% of Uch activity in hippocampal slices of the mouse brain. LDN-57444 (5 μM for 2 hr) does not reduce potentiation further in APP/PS1 slices or in wt slices exposed to 200 nM Aβ ^[2] . LDN-57444 (25-100 μM) inhibits ubiquitin-proteasome activity dose-dependently in SK-N-SH cells. LDN-57444 (50 μM) also

induces apoptotic cell death, causes the endoplasmic reticulum stress and results in expression of spliced XBP-1(XBP-1s, 48KD) in SK-N-SH cells^[3].

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

In Vivo

LDN-57444 (0.4 mg/kg, i.p.) blocks the beneficial effect of V-Uch-L1, and worsens contextual conditioning performance as the mice are exposed to the context at 1, 7, 14, and 21 days after training^[2].

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

PROTOCOL

Kinase Assay ^[1]

To start an assay, 0.5 μ L of 5 mg/mL test compound (including LDN-57444, about 50 μ M final reaction concentration) or DMSO control is aliquoted into each well. Both enzyme and substrate are prepared in UCH reaction buffer (50 mM Tris-HCl [pH 7.6], 0.5 mM EDTA, 5 mM DTT, and 0.5 mg/mL ovalbumin). 25 μ L of 0.6 nM UCH-L1 is then added to each well except substrate control wells, followed by plate shaking for 45-60 s on an automatic shaker. The enzyme/compound mixture is incubated at room temperature for 30 min before 25 μ L of 200 nM Ub-AMC is added to initiate the enzyme reaction. The reaction mixture (300 pM UCH-L1, 100 nM Ubiquitin-AMC with 2.5 μ g test compound) is incubated at room temperature for 30 additional minutes prior to quenching the reaction by the addition of 10 μ L 500 mM acetic acid per well. The fluorescence emission intensity is measured on a LJI Analyst using a coumarin filter set (ex = 365 nm, em = 450 nm) and is subtracted by the intrinsic compound fluorescence to reveal the enzyme activity. A DMSO control (0.5 μ L of DMSO, 25 μ L of UCH-L1, 25 μ L of ubiquitin-AMC, 10 μ L of acetic acid), enzyme control (25 μ L of UCH-L1, 25 μ L of buffer, 10 μ L of acetic acid), substrate control (25 μ L of buffer, 25 μ L of ubiquitin-AMC, 10 μ L of acetic acid), and inhibitor control (0.5 μ L of ubiquitin aldehyde [100 nM stock], 25 μ L of UCH-L1, 25 μ L of ubiquitin-AMC, 10 μ L of acetic acid) are also performed in each assay plate to ensure quality and reproducibility. The UCH-L1 enzymatic reactions are manually repeated twice using the same protocol to confirm the results for the hit compounds from the primary robot-assisted screen^[1].

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

Cell Assay ^[3]

Cell viability is measured by a quantitative colorimetric assay with MTT. After drug treatment SK-N-SH cells are incubated for 4 h with 5 g/L MTT and then DMSO is added for 15 min. The absorption is quantified at 570 nm using a micro-plate reader ^[3].

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

Animal Administration ^[2]

Each animal is placed individually into the conditioning chamber. The electric current is gradually increased (0.1 mA for 1 sec. at 30 sec. intervals increasing the shock intensity by 0.1 mA to 0.7 mA). Animal behavior is evaluated for the first visible response to the shock (flinch), the first extreme motor response (run/jump), and the first vocalized distress (scream). Threshold to flinching, jumping, and screaming is quantified for each animal by averaging of the shock intensity at which each animal manifests a behavioral response of that type to the foot shock. Visual, motor, and motivation skills are also tested with visible platform training by measuring the time and the speed to reach a visible platform placed within a pool filled with water. Both time to reach the platform and swimming speed are recorded and analyzed with a video tracking system. No difference is observed among different groups of mice in the experiments in which fear conditioning is tested both in the presence of LDN-57444 (LDN) and TAT fusion proteins. To decide the time of administration of LDN-57444, a series of preliminary experiments are performed in which the inhibitor is injected intra-peritoneally at different intervals (4 hrs before, 1 hr before, 1 hr after and 4 hrs after) from the electric shock. During the training phase, there is no difference in the freezing of LDN-57444- or vehicle-injected mice^[2].

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

CUSTOMER VALIDATION

- Nat Commun. 2022 Mar 31;13(1):1700.
- EMBO J. 2022 Jul 11;e108791.

- Cell Chem Biol. 2021 Apr 27;S2451-9456(21)00213-0.
- J Med Chem. 2022 Oct 11.
- Cancer Sci. 2020 Sep;111(9):3174-3183.

See more customer validations on www.MedChemExpress.com

REFERENCES

- [1]. Liu Y, et al. Discovery of inhibitors that elucidate the role of UCH-L1 activity in the H1299 lung cancer cell line. Chem Biol. 2003 Sep;10(9):837-46.
- [2]. Gong B, et al. Ubiquitin hydrolase Uch-L1 rescues beta-amyloid-induced decreases in synaptic function and contextual memory. Cell. 2006 Aug 25;126(4):775-88.
- [3]. Tan YY, et al. Endoplasmic reticulum stress contributes to the cell death induced by UCH-L1 inhibitor. Mol Cell Biochem. 2008 Nov;318(1-2):109-15.
-

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

Tel: 609-228-6898

Fax: 609-228-5909

E-mail: tech@MedChemExpress.com

Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA