产品名称：LDN 57444
产品别名：LDN-57444

生物活性：

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.
IC_{50} & Target	IC50: 0.88 μM (UCH-L1), 25 μM (UCH-L3)[1] Ki: 0.40 μM (UCH-L1)[1]
In Vitro	LDN-57444 is a reversible, competitive inhibitor of UCH-L1, with an IC50 of 0.88 μM, and also suppresses UCH-L3 activity, with an IC50 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].
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].
Solvent&Solubility	***In Vitro:*** **DMSO : 25 mg/mL** (**62.87 mM;** Need ultrasonic) **H_2O : < 0.1 mg/mL** (insoluble)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
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

*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。

储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

In Vivo:

请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 **In Vitro** 方式配制澄清的储备液，再依次添加助溶剂：

——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶

1.请依序添加每种溶剂： 10% DMSO →90% corn oil

Solubility: ≥ 2.5 mg/mL (6.29 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (6.29 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的实验。

以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。

[1]. Liu Y, et al. Discovery of inhibitors that elucidate the role of UCH-L1 activity in the H1299 lung cancer

References	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.

实验参考：

Cell Assay	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].
Animal Administration	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].
Kinase Assay	To start an assay, 0.5 μL of 5 mg/mL test compound (including LDN-57444, about 50 μM 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 μL 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 μL 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 μg test compound) is incubated at room temperature for 30 additional minutes prior to quenching the reaction by the addition of 10 μL 500 mM acetic acid per well. The fluorescence emission intensity is measured on a LJL 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 μL of DMSO, 25 μL of UCH-L1, 25 μL of ubiquitin-AMC, 10 μL of acetic acid), enzyme control (25 μL of UCH-L1, 25 μL of buffer, 10 μL of acetic acid), substrate control (25 μL of buffer, 25 μL of ubiquitin-AMC, 10 μL of acetic acid), and inhibitor control (0.5 μL of ubiquitin aldehyde [100 nM stock], 25 μL of UCH-L1, 25 μL of ubiquitin-AMC, 10 μL 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].
	[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.

References	[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.