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产品名称：WWL70
产品别名：WWL70

生物活性：

Description	WWL70 is a selective alpha/beta hydrolase domain 6 (ABHD6) inhibitor with an IC_{50} of 70 nM.
IC_{50} & Target	IC50: 70 nM (ABHD6)[1]
In Vitro	At 1 h after WWL70 (10 μM) treatment, 2-Arachidonoylglycerol (2-AG) is increased by 20% compare to untreated cells. At either 1 or 10 μM, WWL70 completely blocks the lipopolysaccharide (LPS)-induced increase of PGE_2. The enhanced mRNA expression of mPGES-1 and mPGES-2 by LPS is also reduced by WWL70. The IC_{50} of WWL70 to inhibit the PGE_2 biosynthesis is about 100 nM[2].
In Vivo	Although post-treatment with WWL70 at 5 mg/kg does not have any effect, treatment with WWL70 at 10 mg/kg improves the performance significantly. WWL70 treatment improves motor coordination of traumatic brain injury (TBI) mice in a concentration dependent manner. The latency to fall in animals treated with WWL70 at 5 mg/kg increases from 74.92±4.8 to 99.57±5.21 on day 3 ($p<0.01$) and from 87.32±4.42 to 100.14±3.56 on day 7 ($p<0.05$) post-injury when compare with the vehicle-TBI groups. At 10 mg/kg, WWL70 treatment improves motor coordination starting on day 1 post-injury. WWL70 treatment completely restores the ability of TBI mice to continuously alternate arms during Y maze exploration (69.67±4.98 %)[3].
Solvent&Solubility	***In Vitro:*** **DMSO : 17.33 mg/mL (39.61 mM;** Need ultrasonic) **H_2O : < 0.1 mg/mL** (insoluble)

	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
Preparing Stock Solutions	1 mM	2.2858 mL	11.4288 mL	22.8577 mL
	5 mM	0.4572 mL	2.2858 mL	4.5715 mL
	10 mM	0.2286 mL	1.1429 mL	2.2858 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→40% PEG300 →5% Tween-80 → 45% saline

Solubility: 2.5 mg/mL (5.71 mM); Suspended solution; Need ultrasonic

此方案可获得 2.5 mg/mL (5.71 mM)的均匀悬浊液，悬浊液可用于口服和腹腔注射。

以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀；向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。

References	[1]. Li W, et al. A functional proteomic strategy to discover inhibitors for uncharacterized hydrolases. J Am Chem Soc. 2007 Aug 8;129(31):9594-5. [2]. Tanaka M, et al. WWL70 attenuates PGE2 production derived from 2-arachidonoylglycerol in microglia by ABHD6-independent mechanism. J Neuroinflammation. 2017 Jan 10;14(1):7. [3]. Tchantchou F, et al. Selective inhibition of alpha/beta-hydrolase domain 6 attenuates neurodegeneration, alleviates blood brain barrier breakdown, and improves functional recovery in a mouse model of traumatic brain injury. J Neurotrauma. 2013 Apr 1;30(7):565-79.

实验参考：

Cell Assay	BV2 cells (90% confluence) in 10-cm dishes are treated with WWL70 (10 μM) for 1 h. After rinsing with PBS once, the cells are collected by centrifugation at 5000 g for 2 min. The pellet is suspended with 0.1 mL of 0.02% trifluoroacetic acid (TFA) and 1 nmol of 2-AG-d_8 by pipetting and dispersed in 4 mL of acetonitrile in a silanized glass tube to precipitate the debris overnight at -20°C. The supernatant after centrifuged at 5000 g for 5 min is transferred to a new glass tube and evaporated under a nitrogen gas stream in a mild hot water bath (approximately 35°C). 2-AG is resuspended with 0.1 mL of acetonitrile and stored at -80°C until mass analysis[2].
Animal Administration	Seven-week-old, male C57BL/6 mice weighing 25 to 30 g are used in this study. Animals are maintained under a controlled environment with a temperature of 23±2°C, a 12 h light/dark cycle, and access to food and water ad libitum. WWL70 (5 mg/kg or 10 mg/kg) in physiologic saline or an equal volume of 1% DMSO in saline (10 mL/kg) is injected intraperitoneally, and then once a day for 3, 7, or 21 days depending on the experimental design. During the 21-day treatment regimen, animals are subjected to a battery of behavioral tests at different time points. Two hours after the last injection on day 21 post-injury, animals are sacrificed and brain tissues are collected for histological analysis[3].
Kinase Assay	One hundred micrograms per milliliter of BV2 microsomes are pre-incubated with WWL70 for 5 min at 23°C, then mixed with 10 μM of Arachidonic acid (AA) for 1 min at 23°C. 500 μg/mL brain microsomes are incubated with 10 μM of AA for 2 min at 23°C. The reaction is stopped by mixing with stannous acid (5 mg/mL in 0.1 N HCl) to deactivate the enzyme and convert intermediate PGH_2 to $PGF_{2\alpha}$, followed by the measurement of PGE_2 concentration by Enzyme-linked immunoassay (EIA). The activity is determined after subtraction with the amount of PGE_2 in the microsome fraction incubated without substrate[2].
References	[1]. Li W, et al. A functional proteomic strategy to discover inhibitors for uncharacterized hydrolases. J Am Chem Soc. 2007 Aug 8;129(31):9594-5. [2]. Tanaka M, et al. WWL70 attenuates PGE2 production derived from 2-arachidonoylglycerol in microglia by ABHD6-independent mechanism. J Neuroinflammation. 2017 Jan 10;14(1):7. [3]. Tchantchou F, et al. Selective inhibition of alpha/beta-hydrolase domain 6 attenuates neurodegeneration, alleviates blood brain barrier breakdown, and improves functional recovery in a mouse model of traumatic brain injury. J Neurotrauma. 2013 Apr 1;30(7):565-79.