产品名称：氟甲基酮
产品别名：Z-VAD(OMe)-FMK；Z-Val-Ala-Asp(OMe)-FMK

生物活性：

Description	Z-VAD(OMe)-FMK (Z-Val-Ala-Asp(OMe)-FMK) is a cell-permeable and irreversible pan-caspase inhibitor[1]. Z-VAD(OMe)-FMK is an ubiquitin carboxy-terminal hydrolase L1 (UCHL1) inhibitor. Z-VAD(OMe)-FMK irreversibly modifies UCHL1 by targeting the active site of UCHL1[2].
IC_{50} & Target	Caspase
In Vitro	Z-VAD(OMe)-FMK (Z-Val-Ala-Asp(OMe)-FMK) is a broad-spectrum caspase inhibitor, has been shown to inhibit the intracellular activation of caspase-like proteases. The injection of Z-VAD(OMe)-FMK suppresses the caspase-3 activity in lung tissues, and significantly decreases the number of terminal dUTP nick-end labeling-positive cells[1]. Z-VAD(OMe)-FMK effectively inhibits UCHL1's reaction with hemagglutinin-tagged ubiquitin vinylmethyl ester (HA-UbVME) at the concentration of 100 μM[2]. Z-VAD(OMe)-FMK is administered intraperitoneally at 1 hour before and 6 hours after SAH. Expression of caspase-3 and positive TUNEL is examined as markers for apoptosis. Z-VAD(OMe)-FMK suppresses TUNEL and caspase-3 staining in endothelial cells, decreases caspase-3 activation, reduces BBB permeability, relieves vasospasm, abolishes brain edema, and improves neurological outcome[3]. Z-VAD(OMe)-FMK is a cell-permeable caspase inhibitor, efficiently blocks cell death induced by SMN deficiency[4].
In Vivo	The survival rate of mice is prolonged significantly by the injection of Z-VAD(OMe)-FMK (Z-Val-Ala-Asp(OMe)-FMK). All mice succumbed to LPS within 30 hours. By contrast, the mice treated with Z-VAD(OMe)-FMK survive significantly longer and 27% of the mice survived more than 7 days[1].

Solvent&Solubility

In Vitro:

DMSO : 100 mg/mL (213.91 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.1391 mL	10.6954 mL	21.3908 mL
	5 mM	0.4278 mL	2.1391 mL	4.2782 mL
	10 mM	0.2139 mL	1.0695 mL	2.1391 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 (5.35 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (5.35 mM，饱和度未知) 的澄清溶液。

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。

	2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.5 mg/mL (5.35 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (5.35 mM，饱和度未知) 的澄清溶液。 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 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀。 3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.5 mg/mL (5.35 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (5.35 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。
References	[1]. Kawasaki M, et al. Protection from lethal apoptosis in lipopolysaccharide-induced acute lung injury in mice by a caspaseinhibitor. Am J Pathol. 2000 Aug;157(2):597-603. [2]. Davies CW, et al. The co-crystal structure of ubiquitin carboxy-terminal hydrolase L1 (UCHL1) with a tripeptide fluoromethyl ketone (Z-VAE(OMe)-FMK). Bioorg Med Chem Lett. 2012 Jun 15;22(12):3900-4. [3]. Park S, et al. Neurovascular protection reduces early brain injury after subarachnoid hemorrhage. Stroke. 2004 Oct;35(10):2412-7. [4]. Ilangovan R, et al. Inhibition of apoptosis by Z-VAD-fmk in SMN-depleted S2 cells. J Biol Chem. 2003 Aug 15;278(33):30993-9.

实验参考：

Cell Assay	PCR products containing coding sequences for the dSMN (forward primer: 5′-TAA TAC GAC TCA CTA TAG GG AAG ACG TAC GAC GAG TCG-3′; and reverse primer: 5′-TAA TAC GAC TCA CTA TAG GG GTG GTG CTG GCT TCT TTC-3′; product length, 601bps; bold and italics letters represent T7 promoter sequences) and control *Drosophila Presenilin*(dPsn) gene (forward primer: 5′-TAA TAC GAC TCA CTA TAG GG TG GCT GCT GTC AAT CTC-3′; and reverse primer: 5′-TAA TAC GAC TCA CTA TAG GG CGA TAG CAA CGC TTC TTG-3′; product length: 543bps) are obtained and gel-purified. Double-stranded RNAs (dsRNA) are generated by transcription with Ribomax T7 Transcription kit and digested with Rnase-free DNase. The dsRNA products are ethanol precipitated and annealed by incubation at 65°C for 30 min and then slowly allowed to cool at room temperature. The annealed dsRNA products are analyzed on a 1% agaorse gel to ensure the majority of dsRNA existed as a single band. The dsRNA (2 μg) and/or plasmid DNAs (2 μg) are introduced into cells by using Cellfectin. Caspase inhibition is achieved by using 50 μM of Z-VAD(OMe)-FMK in the culture medium[4].
	Mice[1] Mice used in this study are 5- to 6-week-old (20 to 22 g) ICR males. Mice are injected with 30 mg/kg LPS from E. coli serotype O111:B4 through the tail vein. A single intravenous injection of Z-VAD(OMe)-FMK (0.25 mg) is made 15 minutes before LPS injection, followed by three intravenous injections of Z-VAD(OMe)-FMK (0.1 mg each) per hour. Control mice are injected with the same volume of 1% DMSO in sterile saline. Rats[3] Male Sprague-Dawley rats weighing 300 to 350 g are anesthetized with α-chloralose (40 mg/kg IP)

Animal Administration	and urethane (400 mg/kg IP). Animals are intubated, and respiration is maintained with a small animal respirator. Rectal temperature is maintained at 37±0.5°C with a heating pad. The left external carotid artery is isolated and a 4.0 monofilament nylon suture is inserted through the internal carotid artery to perforate the middle cerebral artery. SAH is confirmed at autopsy in each rat. Sham-operated rats underwent the same procedures except that the suture is withdrawn after resistance is felt. Z-VAD(OMe)-FMK (50 μM per 0.3 mL) is injected intraperitoneally at 1 hour before and 6 hours after SAH induction. In vehicle group, rats underwent SAH induction and are treated with the same volume of vehicle (DMSO diluted in physiological buffer solution). No treatment is applied in sham-operated animals.
References	[1]. Kawasaki M, et al. Protection from lethal apoptosis in lipopolysaccharide-induced acute lung injury in mice by a caspaseinhibitor. Am J Pathol. 2000 Aug;157(2):597-603. [2]. Davies CW, et al. The co-crystal structure of ubiquitin carboxy-terminal hydrolase L1 (UCHL1) with a tripeptide fluoromethyl ketone (Z-VAE(OMe)-FMK). Bioorg Med Chem Lett. 2012 Jun 15;22(12):3900-4. [3]. Park S, et al. Neurovascular protection reduces early brain injury after subarachnoid hemorrhage. Stroke. 2004 Oct;35(10):2412-7. [4]. Ilangovan R, et al. Inhibition of apoptosis by Z-VAD-fmk in SMN-depleted S2 cells. J Biol Chem. 2003 Aug 15;278(33):30993-9.

源叶生物