产品名称：P22077
产品别名：P 22077

生物活性：

Description	P 22077 is a cell-permeable **ubiquitin-specific protease 7 (USP7)** inhibitor with an **EC_{50}** of 8.01 μM. It also inhibits USP47 with an **EC_{50}** of 8.74 μM.
IC_{50} & Target	EC50: 8.01 μM (USP7), 8.74 μM (USP47)[1]
In Vitro	P 22077 is an inhibitor of USP7 and DUB USP47, with EC50s of 8.01 μM and 8.74 μM, respectively. P 22077 (15-45 μM) inhibits a much smaller subset of DUBs. P 22077 (25 μM) causes DUBs inhibition in HEK293T cells[1]. P 22077 (0-20 μM) greatly reduces the cell viability of Neuroblastoma (NB) cells including IMR-32, NGP, CHLA-255, and SH-SY5Y cells but without NB-19 and SK-N-AS cells. P 22077 (10 μM) increases p53 activity and induces apoptosis in p53 wild-type and HDM2-expressing NB cells. P 22077 (5 μM) enhances the cytotoxic effect of Dox and VP-16 on NB cells, and enhances Dox- and VP-16-induced p53-mediated apoptosis[2].
In Vivo	P 22077 (15 mg/kg, i.p. 21 days) shows potent antitumor activities in an xenograft mouse model bearing IMR-32-derived tumors; P 22077 also exhibits antitumor effects after treatment at 10 mg/kg for 14 days in mice bearing SH-SY5Y-derived tumors, and at 20 mg/kg for 12 days in mice bearing NGP-derived tumors[2].
Solvent&Solubility	***In Vitro:*** **DMSO : 50 mg/mL** (**158.57 mM;** Need ultrasonic)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	3.1714 mL	15.8569 mL	31.7138 mL
5 mM	0.6343 mL	3.1714 mL	6.3428 mL
10 mM	0.3171 mL	1.5857 mL	3.1714 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 (7.93 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (7.93 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]. Altun M, et al. Activity-based chemical proteomics accelerates inhibitor development for

References	deubiquitylating enzymes. Chem Biol. 2011 Nov 23;18(11):1401-12. [2]. Fan YH, et al. USP7 inhibitor P22077 inhibits neuroblastoma growth via inducing p53-mediated apoptosis. Cell Death Dis. 2013 Oct 17;4:e867.

实验参考：

Cell Assay	Cell viability assays are assessed using the Cell Counting Kit-8 (CCK-8, WST-8[2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2 H-tetrazolium, monosodium salt]). Cells are seeded in 96-well flat-bottomed plates at the density of 1×10^4 per well. After 24 h of incubation at 37°C, increasing concentrations of P 22077, Dox, VP-16, or their combinations are added to the wells. Twenty-four hours later, 10 μL of CCK-8 is added into each well and after 1 h of incubation, the absorbance is measure at 450 nm using the microplate reader. Each experiment is performed in replicates of six. Background reading of media only is used to normalize the results[2].
Animal Administration	The orthotopic Neuroblastoma (NB) mouse model is used in the assay. Briefly, 1.5×10^6 human IMR-32, SH-SY5Y, or NGP cells with luciferase expression are surgically injected into the left renal capsule of 5-week-old female NCR nude mice. IMR-32, SH-SY5Y, and NGP-derived xenografts are allowed to grow for ~2-3 weeks before randomizing the mice into a control group and a P 22077 treatment group. Each group consists of three or six mice. Animals are treated with DMSO or P 22077 by intraperitoneal (i.p.) injection every day for 12, 14, or 21 days. At the end of the experiments, all mice are killed. Tumors and the right side control kidneys are resected, weighed, and photographed[2].
Kinase Assay	Recombinant full length USP7, USP2 core, USP5, JOSD2, DEN1, PLpro core, and SENP2 catalytic core are generated. Amino terminal His6 tagged USP4, USP8, USP28, UCH-L1, UCH-L3, UCH-L5, and MMP13 are expressed in Escherichia coli. N-terminal His6 tagged USP15, USP20, and USP47 are expressed in Sf9 cells. All the recombinant proteins are purified by chromatography. Amino terminal tagged His6 Ub-PLA2 (Ub-CHOP), SUMO3-PLA2 (SUMO3-CHOP), ISG15-PLA2 (ISG15-CHOP), NEDD8-PLA2 (NEDD8-CHOP), Ub-EKL (Ub-CHOP2), and free catalytically active PLA2 are prepared[1].
References	[1]. Altun M, et al. Activity-based chemical proteomics accelerates inhibitor development for deubiquitylating enzymes. Chem Biol. 2011 Nov 23;18(11):1401-12. [2]. Fan YH, et al. USP7 inhibitor P22077 inhibits neuroblastoma growth via inducing p53-mediated apoptosis. Cell Death Dis. 2013 Oct 17;4:e867.