产品名称：(3E,5E)-1-Acryloyl-3,5-bis(4-nitrobenzylidene)piperidin-4-one
产品别名：b-AP15

实验参考：

Description	b-AP15 is a specific inhibitor of the deubiquitinating enzymes UCHL5 and Usp14.
IC_{50} & Target	UCHL5/Usp14[1]
In Vitro	Purified 19S proteasomes (5 nM) are treated with indicated concentrations of b-AP15 and DUB activity is determined by detectionof Ub-AMC cleavage. The IC50 value (2.1±0.411 μM) is determined from log concentration curves in Graph Pad Prism using non linear regression analysis. b-AP15 as a previously unidentified class of proteasome inhibitor that abrogates the deubiquitinating activity of the 19S regulatory particle. b-AP15 inhibited the activity of two 19S regulatory-particle-associated deubiquitinases, ubiquitin C-terminal hydrolase 5 (UCHL5) and ubiquitin-specific peptidase 14 (USP14), resulting in accumulation of polyubiquitin. b-AP15 induced tumor cell apoptosis that is insensitive to TP53 status and overexpression of the apoptosis inhibitor BCL2[1]. The ability of b-AP15 is determined to inhibit proteasome deubiquitinase activity using Ub-AMC as the substrate. An IC50 of 16.8±2.8 μM is observed[2]. b-AP15 is a specific USP14 and UCHL5 inhibitor, which blocks growth and induces apoptosis in MM cells[3].
In Vivo	b-AP15 (2.5 mg/kg) inhibits tumor growth in syngenic mice models with less frequent administration schedules. We administered b-AP15 to C57BL/6J mice with Lewis lung carcinomas (LLCs) using a 2-d-on, 2-d-off schedule and to BALB/c mice with orthotopic breast carcinoma (4T1) using a 1-d-on, 3-d-off schedule. b-AP15 significantly inhibited tumor growth in both models, with T/C=0.16 (P≤0.01) for the C57BL/6J mice and T/C=0.25 (P≤0.001) for the BALB/c mice. A reduction in the number of pulmonary metastases also is observed in the group of mice with 4T1 breast carcinomas treated with b-AP15[1].
Solvent&Solubility	***In Vitro:*** **DMSO : ≥ 44 mg/mL (104.91 mM)** * "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.3844 mL	11.9221 mL	23.8442 mL
	5 mM	0.4769 mL	2.3844 mL	4.7688 mL
	10 mM	0.2384 mL	1.1922 mL	2.3844 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 (5.96 mM); Suspended solution; Need ultrasonic

此方案可获得 2.5 mg/mL (5.96 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]. D'Arcy P, et al. Inhibition of proteasome deubiquitinating activity as a new cancer therapy. Nat Med. 2011 Nov 6;17(12):1636-40. [2]. Wang X, et al. The 19S Deubiquitinase Inhibitor b-AP15 is Enriched in Cells and Elicits Rapid Commitment to Cell Death. Mol Pharmacol. 2014 Jun;85(6):932-45. [3]. Tian Z, et al. A novel small molecule inhibitor of deubiquitylating enzyme USP14 and UCHL5 induces apoptosis in multiple myeloma and overcomes bortezomib resistance. Blood. 2014 Jan 30;123(5):706-16.

实验参考：

Cell Assay	Cell viability is monitored by either the fluorometric microculture cytotoxicity assay or the MTT assay. For the MTT assay, cells are seeded into 96-well flat-bottomed plates overnight and exposed to drugs, using DMSO as the control. At the end of incubations, 10 μl of a stock solution of 5 mg/mL MTT is added into each well, and the plates are incubated 4 hours at 37°C. Formazan crystals are dissolved with 100 μL 10% SDS/10 mM HCl solution overnight at 37°C. Absorbance is measured using an enzyme-linked immunosorbent assay (ELISA) plate reader at 590 nm[2].
Animal Administration	Mice [2]. For the squamous carcinoma model, 1×10^6 FaDu cells are subcutaneously injected into the right rear flank of female SCID mice. Tumor growth is measured by the formula length×width^2×0.44. When tumors have grown to a size of approximately 200 mm^3 (defined as day 0), mice are randomized to receive either vehicle (n=10) or b-AP15 (n=15) at 5 mg per kg of body weight by daily subcutaneous injection. For the colon carcinoma model, we subcutaneously injected 2.5×10^6 HCT-116 colon carcinoma cells overexpressing Bcl2 into the right flank of female nude mice. We treated mice with 5 mg of b-AP15 per kg of body weight by intraperitoneal injection. For the lung carcinoma model, we subcutaneously injected 2×10^5 LLC cells into the right rear flank of female C57/B6 mice. When tumors had grown to a size of approximately 50 mm^3 (defined as day 0), we randomized mice to receive either vehicle (n=4) or b-AP15 (n=4) at 5 mg per kg of body weight intraperitoneally, with a treatment cycle consisting of 2 d of treatment followed by 2 d of rest (2 d on, 2 d off) for 2 weeks.
Kinase Assay	For deubiquitinase inhibition assays, 19S regulatory particle (5 nM), 26S (5 nM) UCH-L1 (5 nM), UCH-L3 (0.3 nM), USP2CD (5 nM) USP7CD (5 nM) USP8CD (5 nM) or BAP1 (5 nM) is incubated with DMSO or b-AP15 and monitored the cleavage of ubiquitin-AMC (1,000 nM) using a Wallac VICTOR Multilabel counter or a Tecan Infinite M1000 equipped with 380 nm excitation and 460 nm emission filters[1].
References	[1]. D'Arcy P, et al. Inhibition of proteasome deubiquitinating activity as a new cancer therapy. Nat Med. 2011 Nov 6;17(12):1636-40. [2]. Wang X, et al. The 19S Deubiquitinase Inhibitor b-AP15 is Enriched in Cells and Elicits Rapid Commitment to Cell Death. Mol Pharmacol. 2014 Jun;85(6):932-45. [3]. Tian Z, et al. A novel small molecule inhibitor of deubiquitylating enzyme USP14 and UCHL5 induces apoptosis in multiple myeloma and overcomes bortezomib resistance. Blood. 2014 Jan 30;123(5):706-16.