产品名称：**VLX1570**
产品别名：**VLX1570**

生物活性：

Description	VLX1570 is a competitive inhibitor of proteasome deubiquitinases (DUBs) with an IC50 of approximate 10 μM.
IC_{50} & Target	IC50: appr 10 μM (Deubiquitinase)[2]
In Vitro	VLX1570 inhibits USP14 and UCHL5 activity of 19S regulatory particles, and the inhibition of USP14 is more pronounced. VLX1570 (1 μM) shows inhibitory activity against USP14 in KMS-11 myeloma cells. VLX1570 exhibits an IC_{50} of 0.58 μM on HCT116 cells[1]. VLX1570 binds to recombinant USP14 with K_d of 1.5-18 μM using two different sources of recombinant protein, and the K_d for recombinant UCHL5 is higher (14-18 μM) compared to that of USP14. VLX1570 has potent antiproliferative activities on multiple myeloma cells, with IC_{50}s of 43 ± 2 nM, 74 ± 2 nM, 126 ± 3 nM, and 191 ± 1 nM for KMS-11, RPMI8226, OPM-2, and OPM-2-BZ^R cells, respectively[2]. VLX1570 suppresses the viability of BCWM.1 cells, with an EC_{50} of 20.22 nM. VLX1570 (100, 250, 500 nM) induces significant apoptosis by 12 h in a dose-dependent manner in all Waldenstrom macroglobulinemia (WM) cell lines tested, including BCWM.1/IR (IR) and BCWM.1/BR (BR) subclones. VLX1570 (100, 250, 500 nM) also causes ER stress machinery and mitochondrial damage in WM cells. VLX1570 (250 nM) downregulates BCR-signalosome components and their end effectors, as well as CXCR4 expression in WM cells[3].
In Vivo	VLX1570 (3 mg/kg) significantly decreases tumor growth in mice bearing KMS-11 multiple myeloma cells[2]. VLX1570 (4.4 mg/kg, i.p.) markedly suppresses tumor growth, without obvious weight loss and other signs of systemic toxicity in the Waldenstrom macroglobulinemia (WM)-bearing mice[3].
Solvent&Solubility	***In Vitro:*** **DMSO : ≥ 32 mg/mL (68.17 mM)** * "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.1304 mL	10.6521 mL	21.3042 mL
5 mM	0.4261 mL	2.1304 mL	4.2608 mL
10 mM	0.2130 mL	1.0652 mL	2.1304 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。

References

[1]. Wang X, et al. Synthesis and evaluation of derivatives of the proteasome deubiquitinase inhibitor b-AP15. Chem Biol Drug Des. 2015 Nov;86(5):1036-48.

[2]. Wang X, et al. The proteasome deubiquitinase inhibitor VLX1570 shows selectivity for ubiquitin-specific protease-14 and induces apoptosis of multiple myeloma cells. Sci Rep. 2016 Jun 6;6:26979.

[3]. Paulus A, et al. Coinhibition of the deubiquitinating enzymes, USP14 and UCHL5, with VLX1570 is lethal to ibrutinib- or bortezomib-resistant Waldenstrom macroglobulinemia tumor cells. Blood Cancer J. 2016 Nov 4;6(11):e492.

实验参考：

Cell Assay	Cell viability is monitored by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. For the MTT assay, cells are suspended at 5 × 10^5 cells/mL, and 100 µL aliquots are dispended into 96-well microtiter plates and exposed to drugs using DMSO as control. At the end of incubations, 10 µL of a stock solution of 5 mg/mL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), is added into each well, and the plates are incubated 4 hours at 37°C. Formazan crystals are dissolved with 100 µL of 10% SDS/10 mM HCl solution overnight at 37°C. Since MTT assays are affected by mitochondrial activity, and since OXPHOS is affected by VLX1570, the acid phosphatase method49 are used to determine cell viability in some experiments. After washing twice with PBS, cells are lysed in 100 µL of 0.1 M sodium acetate, 0,1% Triton X-100, p-nitrophenylphosphate and incubated for 90 min at 37°C. At the end of the incubation, 10 µL NaOH is added to each well and A405 is determined[2].
Animal Administration	Animal experiment is performed with a sample size of 7 per group, 80% power at the 5% significance level to detect a difference in means of 1800 mm^3 between the 2 groups. For percentage change in IgM from baseline, with a sample size of 7 per group, 80% power at the 5% significance level is calculated to detect a difference in means of 450% between the 2 groups. Fourteen female NOD/SCID mice (6-8 weeks of age) are subcutaneously implanted with 1× 10^6 luciferase labeled RPCI-WM1 cells (Luc-RPCI-WM1), which are allowed to grow till a bioluminescent signal is observed by IVIS imaging (Day 20). On day 21, mice are randomized into 2 groups (n=7 each), with one group receiving vehicle (cremaphor+PEG+Tween) and the other receiving VLX1570 at 4.4 mg/kg via intraperitoneal injection. The investigator is not blinded to the group allocation. Both groups are respectively treated with either vehicle or VLX1570 every alternate day for 22 days. Sizes of the tumors is measured every 3- 4 days using direct caliper measurements, and volume of the tumorsis calculated using the formula $(width)^2$ × length/2. Bioluminescent tumor imaging is performed with the Xenogen imaging system on Days 0, 20, 30, 36 and 43 post-tumor implantation. Blood from mice is collected on the same days by submandibular venous puncture, with sera subsequently separated for quantification of human IgM levels' using ELISA. On Day 44, mice are sacrificed, and final tumor volume is measured in control and treatment arms. All images are obtained using a Canon D40 digital camera. No specific criteria for inclusion/exclusion are used as all mice formed tumors and are therefore included into the study[3].
Kinase Assay	Preparations of 26S proteasomes (1 nM) are pretreated with DMSO, VLX1570, or b-AP15 for 2 min in assay buffer (25 mM Tris, 5 mM $MgCl_2$, 10% glycerol, 0.05 mg/mL BSA, 2 mM ATP, and 1 mM DTT) before addition of Ubrhodamine. Fluorescence is monitored at 37°C using Ex/Em = 490 nm/520 nm to read data every 10 second for 30 min using a TECAN infinite 200 instrument. For UbVS labeling of KMS 11 cells, cell pellets are lysed from control or treated cells with buffer (50 mM HEPES pH 7.4, 250 mM sucrose, 10 mM $MgCl_2$, 2 mM ATP, 1 mM DTT) on ice for 30 min and removed debris by centrifugation. Twenty five µg of protein is labeled with 1 µM UbVS for 30 min at 37°C. Samples are resolved by SDS-PAGE and subjected to immunoblotting. For UbVS labeling of proteasomes, purified 19S proteasomes (50 nM) are pretreated with DMSO, VLX1570, or b-AP15 (50 µM) for 10 min at room temperature, followed by labeling with 1 µM HA-UbVS for 30 min at 37°C and by immunoblotting[1].
References	[1]. Wang X, et al. Synthesis and evaluation of derivatives of the proteasome deubiquitinase inhibitor b-AP15. Chem Biol Drug Des. 2015 Nov;86(5):1036-48. [2]. Wang X, et al. The proteasome deubiquitinase inhibitor VLX1570 shows selectivity for ubiquitin-specific protease-14 and induces apoptosis of multiple myeloma cells. Sci Rep. 2016 Jun

	6;6:26979. [3]. Paulus A, et al. Coinhibition of the deubiquitinating enzymes, USP14 and UCHL5, with VLX1570 is lethal to ibrutinib- or bortezomib-resistant Waldenstrom macroglobulinemia tumor cells. Blood Cancer J. 2016 Nov 4;6(11):e492.