产品名称：ML-323
产品别名：ML-323

生物活性：

Description	ML-323 is a reversible, potent **USP1-UAF1** inhibitor with **IC_{50}** of 76 nM in a Ub-Rho assay. The measured inhibition constants of ML-323 for the free enzyme (**K_i**) is 68 nM.
IC_{50} & Target	IC50: 76 nM (USP1-UAF1, in Ub-Rho assay)[1] Ki: 68 nM (USP1-UAF1)[1]
In Vitro	ML-323 (ML323) is a highly potent inhibitor of the USP1-UAF1 deubiquitinase complex with excellent selectivity against human DUBs, deSUMOylase, deneddylase and unrelated proteases. ML-323 is a potent USP1-UAF1 inhibitor with IC50 values of 76 nM in a ubiquitin-rhodamine (Ub-Rho) assay and 174 nM and 820 nM in orthogonal gel-based assays using K63-linked diubiquitin (di-Ub) and monoubiquitinated PCNA (Ub-PCNA) as substrates, respectively. ML-323 probably exerts its inhibitory effect through an allosteric mechanism. The measured inhibition constants of ML-323 for the free enzyme (Ki) and the enzyme-substrate complex (K'i) are 68 nM and 183 nM. Besides, ML-323 potentiates Cisplatin cytotoxicity in non-small cell lung cancer and osteosarcoma cells[1]. ML-323 (ML323), a probe molecule that displays reversible, nanomolar inhibitory activity and excellent selectivity toward USP1/UAF1. In addition, ML-323 potentiates the cytotoxicity of Cisplatin and increases endogenous monoubiquitination levels of both PCNA and FANCD2, two known cellular targets of USP1/UAF1[2].
Solvent&Solubility	***In Vitro:*** **DMSO : ≥ 49 mg/mL (127.44 mM)** * "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.6009 mL	13.0046 mL	26.0092 mL
	5 mM	0.5202 mL	2.6009 mL	5.2018 mL
	10 mM	0.2601 mL	1.3005 mL	2.6009 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 (6.50 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (6.50 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 (6.50 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.50 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 (6.50 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.50 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]. Liang Q, et al. A selective USP1-UAF1 inhibitor links deubiquitination to DNA damage responses. Nat Chem Biol. 2014 Apr;10(4):298-304. [2]. Dexheimer TS, et al. Discovery of ML323 as a Novel Inhibitor of the USP1/UAF1 Deubiquitinase Complex. National Center for Biotechnology Information; 2010-2012 Oct 23.

实验参考：

Cell Assay	Cell viability is measured by a cell counting kit (CCK) assay using CCK-8 solution. For the colony-forming assay, cells are seeded at a density of 300-500 cells per well in six-well plates and grown overnight. Cells are then treated with ML-323 alone, Cisplatin alone or a combination of Cisplatin and ML-323 (1:1 or 1:4) at the indicated concentrations. Cells treated with an equal volume of DMSO and saline are used as a control. After 48 h of treatment, fresh growth medium is added, and cells are incubated for an additional 5-10 d to allow for colony formation. For UV combination treatment, the cells are treated with ML-323 at the indicated concentrations or an equal volume of DMSO. After 48 h, the medium is removed, and cells are irradiated at 254 nm at the indicated dosage. Fresh growth medium is added, and the cells are incubated for an additional 5-10 d to allow for colony formation. The cells without UV irradiation but treated with ML-323 or an equal volume of DMSO are used as controls and designated as 100%. After the formation of the colonies, cells are fixed with methanol and stained with 0.5% crystal violet. Colonies consisting of >50 cells are scored. The number of colonies is determined from triplicate plates. The dose-response curves are generated using GraphPad Prism and analyzed by using CalcuSyn to calculate the combination index, which is determined for the fraction of cells affected after the addition of fixed ratios of cisplatin and the USP1-UAF1 inhibitor[1].
Kinase Assay	For DUB profiling, ML-323 is tested at a single-dose of 10 μM in duplicate. The DUB activities are monitored using Ub-7-amido-4-methylcoumarin (AMC) as a substrate. The increase in fluorescent signal from free AMC is monitored over time, although only the initial linear portion of slope (signal/min) is used for analysis. The activity of enzyme with no compound is treated as 100%. For protease profiling, ML-323 is tested using threefold serial dilutions starting at 20 μM against 70 proteases. Proteases are pre-incubated with the compound for 5-15 min before the addition of the appropriate enzyme substrates. The enzyme activities are measured by reading the fluorescent signal from fluorescently labeled peptides[1].
References	[1]. Liang Q, et al. A selective USP1-UAF1 inhibitor links deubiquitination to DNA damage responses. Nat Chem Biol. 2014 Apr;10(4):298-304. [2]. Dexheimer TS, et al. Discovery of ML323 as a Novel Inhibitor of the USP1/UAF1 Deubiquitinase

	Complex. National Center for Biotechnology Information; 2010-2012 Oct 23.