产品名称：NMS-873
产品别名：NMS-873

生物活性：

Description	NMS-873 is a potent, selective allosteric **VCP/p97** inhibitor with **IC_{50}** value of 30 nM.
IC_{50} & Target	IC50: 30 nM[1]
In Vitro	NMS-873 has antiproliferative effect on a panel of tumor cell lines with IC50 values in the range of 0.08 μM to 2 μM. For HCT116 and HeLa cells, the IC50 values are 0.4 μM and 0.7 μM, respectively. NMS-873 reduces VCP sensitivity to trypsin digestion, preventing degradation of the linker-D2 domain. NMS-873 induces clear, dose-dependent accumulation of poly-Ub proteins and stabilization of cyclin E and Mcl-1 at doses consistent with its antiproliferative IC50 value[1].

Solvent&Solubility

In Vitro:

DMSO : 20.5 mg/mL (39.37 mM; Need ultrasonic and warming)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.9206 mL	9.6030 mL	19.2060 mL
	5 mM	0.3841 mL	1.9206 mL	3.8412 mL
	10 mM	0.1921 mL	0.9603 mL	1.9206 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 (4.80 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (4.80 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 (4.80 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (4.80 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 (4.80 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (4.80 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]. Paola Magnaghi, et al. Covalent and allosteric inhibitors of the ATPATP ase VCP/p97 induce cancer cell death. Nat Chem Biol. 2013 Jul 28. doi: 10.1038/nchembio.1313.

实验参考：

Cell Assay	Cells are seeded at 1,600 cells per well in 384-well white clear-bottom plates. Twenty-four hours after seeding, cells are treated with the compounds (eight dilution points, in duplicate, for each compound) and incubated for an additional 72 h at 37°C under a 5% CO_2 atmosphere. Cells are then lysed, and the ATP content in each well is determined using a thermostable firefly luciferase-based assay from Promega as a measure of cell viability. IC_{50}values are calculated using the percentage of growth of treated cells versus the untreated control. [1]
Kinase Assay	The ATPase activity and the kinetic parameters of recombinant wild-type VCP and its mutants are evaluated by monitoring ADP formation in the reaction, using a modified NADH-coupled assay46. As ADP and NADH are ATP-competitive inhibitors of VCP ATPase activity, the standard protocol for the NADH-coupled assay is modified into a two-step procedure. In the first part, an ATP-regenerating system (40 U/mL pyruvate kinase and 3 mM phosphoenolpyruvate) recycles the ADP produced by VCP activity, keeps the substrate concentration constant (thus preventing product inhibition) and accumulates a stoichiometric amount of pyruvate. In the second part, the VCP enzymatic reaction is quenched with 30 mM EDTA and 250 μM NADH and stoichiometrically oxidized by 40 U/mL lactic dehydrogenase to reduce accumulated pyruvate. The decrease of NADH concentration is measured at 340 nm using a Tecan Safire 2 reader plate. The assay is performed in 96- or 384-well UV platesin a reaction buffer with 50 mM Hepes, pH 7.5, 0.2 mg/mL BSA, 10 mM $MgCl_2$ and 2 mM DTT. [1]
References	[1]. Paola Magnaghi, et al. Covalent and allosteric inhibitors of the ATPATP ase VCP/p97 induce cancer cell death. Nat Chem Biol. 2013 Jul 28. doi: 10.1038/nchembio.1313.