产品名称：**HSP-990**
产品别名：**NVP-HSP990**

生物活性：

Description	NVP-HSP990 is a potent and selective Hsp90 inhibitor, with IC_{50} values of 0.6, 0.8, and 8.5 nM for Hsp90α, Hsp90β, and Grp94, respectively.			
IC_{50} & Target	HSP90α	HSP90β	GRP94	TRAP1 ATPase
	0.6 nM (IC_{50})	0.8 nM (IC_{50})	8.5 nM (IC_{50})	320 nM (IC_{50})
In Vitro	NVP-HSP990 is a potent and selective Hsp90 inhibitor, with IC50 values of 0.6, 0.8, and 8.5 nM for Hsp90α, Hsp90β, and Grp94, respectively. NVP-HSP990 (10 μM) shows no affect the ATPase activity of topoisomerase II, a GHKL (Gyrase, Hsp90, Histidine Kinase, MutL) family ATPase, closely related to Hsp90. NVP-HSP990 also exerts efficient effects on c-Met, Hsp70, p-ERK and p-AKT in CTL-16 cells, with EC50s of 37 ± 4, 20 ± 2, 11 ± 1, and 6 ± 1 nM, respectively. NVP-HSP990 suppresses the proliferation of BT474, A549, H1975 and MV4;11 cells, with GI50s of 7 ± 2, 28 ± 5, 35 ± 4, and 4 ± 1 nM, respectively[1]. NVP-HSP990 inhibits cellular proliferation of GTL-16, with an EC50 of 14 nM[2]. NVP-HSP990 (5-500 nM) inhibits the multiple myeloma cell lines, with IC50s of 27-49 nM after treatment for 72 h. NVP-HSP990 induces apoptosis in multiple myeloma cell lines (0-100 nM), leads to cell cycle arrest in the G2/M phase (25-200 nM), and induces apoptosis via caspase-8 followed by caspase-3 activation (100 nM). NVP-HSP990 increases HSP70 expression and interacts with Akt and ERK signaling. Moreover, NVP-HSP990 (100 nM) in combination with melphalan, causes synergistic effects on growth inhibition in multiple myeloma cells and increases cleavage of caspase-3, caspase-8, and caspase-9 and activates caspase-2[3].			
In Vivo	NVP-HSP990 (2.5 to 5 mg/kg twice weekly, or 5 to 15 mg/kg weekly, p.o.) causes dose proportional antitumor efficacy, without obvious loss or overt signs of toxicity in a GTL-16 tumor bearing mice. NVP-HSP990 (5 or 10 mg/kg weekly, p.o.) also results in significant inhibition of tumor growth in BT-474 breast cancer model. NVP-HSP990 (5 mg/kg twice weekly or 15 mg/kg weekly, p.o.) inhibits the growth of tumor in the MV4;11 xenograft model. Furthermore, NVP-HSP990 (0.5 mg/kg every day, 14, 5 mg/kg twice weekly, or 15 mg/kg weekly, p.o.) displays antitumor efficacy in H1975 and A549 tumor models[1]. NVP-HSP990 (5, 15 mg/kg, p.o.) shows prolonged suppression of c-Met levels with 30% and 50% reduction and exhibits antitumor activities in GTL-16 tumor xenograft[2].			

In Vitro:

DMSO： ≥ 33 mg/mL (86.98 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	**1 mg**	**5 mg**	**10 mg**
	1 mM	2.6358 mL	13.1791 mL	26.3581 mL
	5 mM	0.5272 mL	2.6358 mL	5.2716 mL
	10 mM	0.2636 mL	1.3179 mL	2.6358 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:

Solvent&Solubility	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 (6.59 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.59 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% corn oil Solubility: ≥ 2.5 mg/mL (6.59 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.59 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]. Menezes DL, et al. The novel oral Hsp90 inhibitor NVP-HSP990 exhibits potent and broad-spectrum antitumor activities in vitro and in vivo. Mol Cancer Ther. 2012 Mar;11(3):730-9. [2]. McBride CM, et al. Design, structure-activity relationship, and in vivo characterization of the development candidate NVP-HSP990. J Med Chem. 2014 Nov 13;57(21):9124-9. [3]. Lamottke B, et al. The novel, orally bioavailable HSP90 inhibitor NVP-HSP990 induces cell cycle arrest and apoptosis in multiple myeloma cells and acts synergistically with melphalan by increased cleavage of caspases. Eur J Haematol. 2012 May;88(5):406-15.

实验参考：

Cell Assay	GTL-16 Cells (1 × 10^3) are plated into 96 well tissue culture plates and cultured at 37°C, 5% CO_2. Serially diluted compounds (NVP-HSP990) are added to the cells and are incubated for 72 hrs. at 37°C, 5% CO_2. Cell proliferation is determined using Cell Viability assay [2]
Animal Administration	Human tumor xenograft models GTL-16, NCI-H1975, BT474, and MV4;11 are implanted subcutaneously with 50% Matrigel in nude or severe combined immunodeficient mice. Mice are randomized into cohorts (10 mice/group for efficacy) when tumors reach 200 to 500 mm^3. NVP-HSP990 is administered orally in a vehicle of 100% polyethylene glycol (PEG400). Tumor caliper measurements are converted into tumor volumes using the formula: [length × $(width)^2$]/2. Relative tumor inhibition is calculated as %T/C = 100 × dT/dC, where, dT or dC = difference of mean tumor volume of drug treatment (T) or vehicle (C) on the final day of the study and the randomization volume. Statistical comparisons are conducted using a one-way ANOVA, followed by the Dunn or Tukey post hoc test. Differences are statistically significant at $P < 0.05$[1]
Kinase Assay	His-tagged Hsp90α N-terminal domain protein (N-Hsp90α-His) is diluted to 2× the final concentration in the assay buffer consisting of 50 mM Hepes, pH 7, 6 mM $MgCl_2$, 20 mM KCl and 0.1% BSA. The Hsp90 is dispensed into 96 well polypropylene plates at 50 μL per well. In a separate polypropylene plate, test compounds (NVP-HSP990) are diluted to 40× their final concentration in 100% DMSO. Serial dilutions in DMSO are made in 3-fold increments. 2.5 μL of diluted compounds are transferred to the 50 μL of Hsp90 and mixed. Background wells receive 25 μM (final concentration) radicicol. Biotin-radicicol is diluted into assay buffer at 2× the final

	concentration and 50 µL are added to the Hsp90/compound plate. DMSO is at a final concentration of 2.5%. Samples are incubated at room temperature for 2 hours before 50 µL are transferred to NeutrAvidin-coated plates. Plates are incubated 1 hour, washed 3× with DELFIA wash buffer (5 mM Tris, pH 7.5, 0.1% Tween 20, 0.1% sodium azide, 0.9% NaCl), and then 50 µL per well of 3 nM Eu-anti-His diluted into DELFIA assay buffer are added. The plates are next incubated 2 hours at room temperature, washed 4×, and then 50 µL enhancement solution are added. Plates are gently shaken for 7-10 minutes before reading in a VICTOR2 instrument[2]
References	[1]. Menezes DL, et al. The novel oral Hsp90 inhibitor NVP-HSP990 exhibits potent and broad-spectrum antitumor activities in vitro and in vivo. Mol Cancer Ther. 2012 Mar;11(3):730-9. [2]. McBride CM, et al. Design, structure-activity relationship, and in vivo characterization of the development candidate NVP-HSP990. J Med Chem. 2014 Nov 13;57(21):9124-9. [3]. Lamottke B, et al. The novel, orally bioavailable HSP90 inhibitor NVP-HSP990 induces cell cycle arrest and apoptosis in multiple myeloma cells and acts synergistically with melphalan by increased cleavage of caspases. Eur J Haematol. 2012 May;88(5):406-15.