产品名称：**4-[[9-氯-7-(2-氟-6-甲氧基苯基)-5H-嘧啶并[5,4-D][2]苯并氮杂卓-2-基]氨基]-2-甲氧基苯甲酸**

产品别名：**Alisertib**

生物活性：

Description	Alisertib (MLN 8237) is an orally active and selective Aurora A kinase inhibitor (IC50=1.2 nM), which binds to Aurora A kinase resulting in mitotic spindle abnormalities, mitotic accumulation. Alisertib (MLN 8237) induces apoptosis and autophagy through targeting the AKT/mTOR/AMPK/p38 pathway in leukemic cells. Antitumor activity[1][2][3].

IC_{50} & Target	Aurora A	Aurora B
	1.2 nM (IC_{50})	396.5 nM (IC_{50})

In Vitro	Alisertib (MLN 8237) leads the MM cells to mitotic spindle abnormalities, mitotic accumulation, as well as inhibition of cell proliferation through apoptosis and senescence. Alisertib up-regulates p53 and tumor suppressor genes p21 and p27[1]. The decreased activity of Alisertib (MLN 8237) for the T217D/W277E Aurora A/TPX2 complex may reflect the increased affinity for ATP induced by cofactor binding to Aurora A[4]. Alisertib (MLN 8237) inhibits cell proliferation with IC50s ranging from 15 to 469 nM in different tumer cell lines[5].
In Vivo	Alisertib (MLN 8237) (30 mg/kg, p.o.) significantly reduces tumor burden and increases overall survival in xenograft-murine model of human-MM[1]. Alisertib (MLN 8237) (20, 30 mg/kg, p.o.) causes tumor growth inhibition in solid tumor xenograft models and regressions in in vivo models of lymphoma, and reduces FLT uptake in HCT-116 xenograft tumors[5]..

Solvent&Solubility

In Vitro:

DMSO： 9.33 mg/mL (17.98 mM; Need ultrasonic and warming)

H_2O : < 0.1 mg/mL (insoluble)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.9271 mL	9.6354 mL	19.2708 mL
	5 mM	0.3854 mL	1.9271 mL	3.8542 mL
	10 mM	0.1927 mL	0.9635 mL	1.9271 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 (4.82 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (4.82 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 (4.82 mM); Suspended solution; Need ultrasonic 此方案可获得 2.5 mg/mL (4.82 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 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 (4.82 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.82 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]. Güllü G, et al. A novel Aurora-A kinase inhibitor MLN8237 induces cytotoxicity and cell-cycle arrest in multiple myeloma Blood June 24, 2010 vol. 115 no. 25 5202-5213 [2]. Sloane DA, et al. Drug-Resistant Aurora A Mutants for Cellular Target Validation of the Small Molecule Kinase Inhibitors MLN8054 and MLN8237 ACS Chem. Biol., 2010, 5 (6), pp 563-576 [3]. Bavetsias V, et al. Aurora Kinase Inhibitors: Current Status and Outlook. Front Oncol. 2015 Dec 21;5:278. [4]. Sloane DA, et al. Drug-Resistant Aurora A Mutants for Cellular Target Validation of the Small Molecule Kinase Inhibitors MLN8054 and MLN8237 ACS Chem. Biol., 2010, 5 (6), pp 563-576 [5]. Manfredi MG, et al. Characterization of Alisertib (MLN8237), an investigational small-molecule inhibitor of aurora A kinase using novel in vivo pharmacodynamic assays.Clin Cancer Res. 2011 Dec 15;17(24):7614-7624.

实验参考：

Cell Assay	MM cell lines are incubated with DMSO or Alisertib (0.125-0.5 μM) in combination with conventional anti-MM agents for 72 hours. Cell viability is measured by MTT assay. The combination index (CI) is determined by isobologram analysis using CalcuSyn software, Version 2.0 (CI < 1 indicates synergistic effect; CI=1, additive effect; and CI > 1, no significant combination effect). [1]
Animal Administration	Mice are irradiated (200 cGy), and then 5×10^6 MM1.S cells are inoculated subcutaneously in the right flank. When tumor growth is measurable (2 weeks after the injection), mice are assigned into 4 groups (10 mice each) receiving vehicle orally (100 μL of 10% 2-hydroxypropyl-β-cyclodextrin/1% sodium bicarbonate) or Alisertib (7.5 mg/kg, 15 mg/kg, and 30 mg/kg in a final formulation in 10% 2-hydroxypropyl-β-cyclodextrin/1% sodium bicarbonate) for 21 consecutive days. The maximal tolerated dose of Alisertib in most mouse strains (continuous dosing for 21 days) is approximately 20 mg/kg twice a day (40 mg/kg per day). Tumor volumes are measured by a Vernier caliper every alternate day and calculated using the following formula: length×width^2×0.5. Mice are killed at the end of the treatment, 2 hours after the last treatment, or when tumor reaches 2 cm^3; tumors are immediately collected from mice and evaluated for induction of apoptosis and cell death by TdT-mediated dUTP nick end labeling (TUNEL) assay. [1]
	To measure Aurora A activity, 25 ng (12.5 mM final concentration) or 250 ng (125 nM final

Kinase Assay	concentration) of purified bacterially expressed Aurora A is assayed in the presence of the appropriate inhibitors (MLN8054, Alisertib), using Histone H3 as substrate for 20 min at 30°C in the presence of 100 μM [γ-^{32}P] ATP. For Aurora A/TPX2 assays, 50 ng of a TPX2 [1-43] peptide, representing a 2-fold molar excess over Aurora A, is included. The Aurora A/TPX2 complex is preformed in kinase reactions prior to subsequent addition of inhibitors and ATP[2]
References	[1]. Güllü G, et al. A novel Aurora-A kinase inhibitor MLN8237 induces cytotoxicity and cell-cycle arrest in multiple myeloma Blood June 24, 2010 vol. 115 no. 25 5202-5213 [2]. Sloane DA, et al. Drug-Resistant Aurora A Mutants for Cellular Target Validation of the Small Molecule Kinase Inhibitors MLN8054 and MLN8237 ACS Chem. Biol., 2010, 5 (6), pp 563-576 [3]. Bavetsias V, et al. Aurora Kinase Inhibitors: Current Status and Outlook. Front Oncol. 2015 Dec 21;5:278. [4]. Sloane DA, et al. Drug-Resistant Aurora A Mutants for Cellular Target Validation of the Small Molecule Kinase Inhibitors MLN8054 and MLN8237 ACS Chem. Biol., 2010, 5 (6), pp 563-576 [5]. Manfredi MG, et al. Characterization of Alisertib (MLN8237), an investigational small-molecule inhibitor of aurora A kinase using novel in vivo pharmacodynamic assays.Clin Cancer Res. 2011 Dec 15;17(24):7614-7624.