产品名称： 2-烯丙基-1-(6-(2-羟基丙-2-基)吡啶-2-基)-6-(4-(4-甲基哌嗪-1-基)苯基氨基)-1H-吡唑并[3,4-D]嘧啶-3(2H)-酮
产品别名：Adavosertib；AZD1775; MK-1775

生物活性：

Description	Adavosertib (AZD-1775; MK-1775) is a potent Wee1 inhibitor with an IC50 of 5.2 nM.
IC_{50} & Target	IC50: 5.2 nM (Wee1)
In Vitro	Adavosertib (MK-1775) enhances the cytotoxic effects of 5-FU in p53-deficient human colon cancer cells. Adavosertib (MK-1775) inhibits CDC2 Y15 phosphorylation in cells, abrogates DNA damaged checkpoints induced by 5-FU treatment, and causes premature entry of mitosis determined by induction of Histone H3 phosphorylation[1]. Adavosertib (MK-1775) abrogates the radiation-induced G2 block in p53-defective cells but not in p53 wild-type lines[2]. The combination of NSC 613327 with Adavosertib (MK-1775) produces robust anti-tumor activity and remarkably enhances tumor regression response (4.01 fold) compared to NSC 613327 treatment in p53-deficient tumors[3].
In Vivo	In vivo, Adavosertib (MK-1775) potentiates the anti-tumor efficacy of 5-FU at tolerable doses[1]. Adavosertib (MK-1775) (60 mg/kg twice daily, p.o.) enhances H1299 xenograft tumor response to fractionated radiotherapy[2]. Adavosertib (MK-1775) (30 mg/kg. p.o.) regresses tumor growth in PANC198, PANC215 and PANC185 as compared to GEM treated mice[3].

Solvent&Solubility

In Vitro:

DMSO : 125 mg/mL (**249.70 mM**; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.9976 mL	9.9880 mL	19.9760 mL
	5 mM	0.3995 mL	1.9976 mL	3.9952 mL
	10 mM	0.1998 mL	0.9988 mL	1.9976 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: ≥ 2.08 mg/mL (4.16 mM); Clear solution

此方案可获得 ≥ 2.08 mg/mL (4.16 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 20.8 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: ≥ 2.08 mg/mL (4.16 mM); Clear solution

此方案可获得 ≥ 2.08 mg/mL (4.16 mM，饱和度未知) 的澄清溶液。

源叶生物

	以 1 mL 工作液为例，取 100 μL 20.8 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: 2.08 mg/mL (4.16 mM); Clear solution 此方案可获得 ≥ 2.08 mg/mL (4.16 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 20.8 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Hirai H, et al. MK-1775, a small molecule Wee1 inhibitor, enhances anti-tumor efficacy of various DNA-damaging agents, including 5-FU. Cancer Biol Ther. 2010 Apr;9(7):514-22. [2]. Bridges KA, et al. MK-1775, a novel Wee1 kinase inhibitor, radiosensitizes p53-defective human tumor cells. Clin Cancer Res. 2011 Sep 1;17(17):5638-48. Epub 2011 Jul 28. [3]. Rajeshkumar NV, et al. MK-1775, a potent Wee1 inhibitor, synergizes with NSC 613327 to achieve tumor regressions, selectively in p53-deficient pancreatic cancer xenografts.Clin Cancer Res. 2011 May 1;17(9):2799-806. Epub 2011 Mar 9.

实验参考：

Cell Assay	Total protein is extracted from the cell pellet using a lysis solution containing 50 mM HEPES (pH 7.9), 0.4 mol/L NaCl, and 1 mM EDTA and fortified with 10 μL/mL phosphatase inhibitor cocktail 1, 10 μL/mL phosphatase inhibitor cocktail 2, 10 μL/mL protease inhibitor, and 1% NP-40. Protein concentration of the lysates is determined by the Bio-Rad protein assay. Equal amounts of protein are separated by 12% SDS-PAGE and transferred to an Immobilon membrane. Nonspecific binding sites on the membrane are blocked in 5% nonfat dry milk in Tris (20 mM)-buffered saline (150 mM, pH 7.4) with 0.1% Tween (TBS-T). Protein signals are detected by incubating the membrane in primary antibody in 5% nonfat dry milk overnight at 4°C, followed by a 45-min incubation in the appropriate peroxidase-conjugated secondary antibody. The membrane is then developed by enhanced chemiluminescence with ECL plus Western Blotting Detection Reagents on a Typhoon 9400 scanner. [2]
Animal Administration	Tumor xenografts are produced in the leg by im inoculation of 1×10^6 Calu-6 cells in 10 μL. Irradiation and Adavosertib (MK-1775) treatment are started when tumors reach 8 mm diameter and continue for 5 days. Gamma-rays are delivered locally to the tumor-bearing legs of unanesthetized mice using a small-animal irradiator consisting of two parallel-opposed ^{137}Cs sources, at a dose rate of 5 Gy/min. Tumors are irradiated twice daily separated by 6 h. Adavosertib (MK-1775) is given by gavage in 0.1 mL volumes 1 h before and 2 h after the first daily radiation dose. [2]
References	[1]. Hirai H, et al. MK-1775, a small molecule Wee1 inhibitor, enhances anti-tumor efficacy of various DNA-damaging agents, including 5-FU. Cancer Biol Ther. 2010 Apr;9(7):514-22. [2]. Bridges KA, et al. MK-1775, a novel Wee1 kinase inhibitor, radiosensitizes p53-defective human tumor cells. Clin Cancer Res. 2011 Sep 1;17(17):5638-48. Epub 2011 Jul 28. [3]. Rajeshkumar NV, et al. MK-1775, a potent Wee1 inhibitor, synergizes with NSC 613327 to achieve tumor regressions, selectively in p53-deficient pancreatic cancer xenografts.Clin Cancer Res. 2011 May 1;17(9):2799-806. Epub 2011 Mar 9.