产品名称：

9-cyclopentyl-2-(2-methoxy-4-(1-methylpiperidin-4-yloxy)phenylamino)-7-methyl-7H-purin-8(9H)-one

产品别名：**AZ3146**

生物活性：

Description	AZ3146 is a reasonably potent and selective Mps1 inhibitor with IC_{50} of 35 nM for $Mps1^{Cat}$.
IC_{50} & Target	$Mps1^{Cat}$
	35 nM (IC_{50})
In Vitro	In in vitro kinase assays, AZ3146 inhibits human Mps1Cat with IC50 of ~35 nM. AZ3146 also efficiently inhibits autophosphorylation of full-length Mps1 immunoprecipitated from human cells[1]. TTK specific kinase inhibitor AZ3146 can decrease HCC cell growth. In vitro cell cytotoxicity assays are performed on SMMC-7721 and BEL-7404 cells. IC50s are calculated as being 7.13 μM (BEL-7404) and 28.62 μM (SMMC-7721). Both cells are further treated under the concentration of IC50 for 4 days. Significant inhibitions of cell proliferation are observed[2]. HCT116 cells are cultured for 10 days in 0.8 μM (the GI50) of AZ3146 , then 2 μM AZ3146 for 3 weeks. Sixteen clones are isolated and cell lines generated, named AzR1-16, all of which are resistant to AZ3146-induced cell death in cell viability assays; AzR3 and 4 have a GI50 of approximately 3 μM (4-fold resistance), while the remaining clones have a GI50 of approximately 9 μM (11-fold resistance). When analyzing mitosis by time-lapse microscopy, while 2 μM AZ3146 causes the parental cell line to rapidly exited mitosis in 10 minutes[3].
Solvent&Solubility	***In Vitro:***
	DMSO : 100 mg/mL (**220.97 mM;** Need ultrasonic)

	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
Preparing Stock Solutions	1 mM	2.2097 mL	11.0485 mL	22.0970 mL
	5 mM	0.4419 mL	2.2097 mL	4.4194 mL
	10 mM	0.2210 mL	1.1049 mL	2.2097 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 (5.52 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (5.52 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 (5.52 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (5.52 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。
References	[1]. Hewitt L, et al. Sustained Mps1 activity is required in mitosis to recruit O-Mad2 to the Mad1-C-Mad2 core complex. J Cell Biol. 2010 Jul 12;190(1):25-34. [2]. Liu X, et al. TTK activates Akt and promotes proliferation and migration of hepatocellular carcinoma cells. Oncotarget. 2015 Oct 27;6(33):34309-20. [3]. Gurden MD, et al. Naturally Occurring Mutations in the MPS1 Gene Predispose Cells to Kinase Inhibitor Drug Resistance. Cancer Res. 2015 Aug 15;75(16):3340-54.

实验参考：

Cell Assay	The TTK inhibitor AZ3146 is disolved in DMSO at a concentration in 100 mM and diluted into 100 μM, 10 μM, 1 μM and 0.1 μM sequentially with DMEM containing 10% FBS before use. In vitrocytotoxicity assays are performed. HCC cells are plated into 96-well plates at the density of 3×103 per well. AZ3146 is added in the indicated concentrations the next day. The inhibitor treated cells are cultured and tested at a 24-hour intervals for 3-4 days using CCK-8[2].
Kinase Assay	His-tagged human Mps1Cat encoding amino acids 510-857 is generated. For kinase assays, 500 ng is added to buffer (25 mM Tris-HCl, pH 7.4, 100 mM NaCl, 50 μg/mL BSA, 0.1 mM EGTA, 0.1% β-mercaptoethanol, 10 mM MgCl2, and 0.5 μg/mL myelin basic protein), AZ3146, and 100 μM γ-[32P]ATP (2 μCi/assay). Reactions are incubated at 30°C for 20 min, spotted onto P81 paper, washed in 0.5% phosphoric acid, and immersed in acetone. Phosphate incorporation is determined by scintillation counting. For immunoprecipitation kinase assays, HeLa cells are treated with nocodazole for 14 h, mitotic cells isolated, washed in PBS, and lysed for 30 min in 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 0.5% NP-40, 5 mM EDTA, 5 mM EGTA, 40 mM β-glycerophosphate, 0.2 mM PMSF, 1 mM DTT, 1 mM sodium orthovanadate, 20 mM sodium fluoride, 1 μM okadaic acid, and complete EDTA-free protease inhibitor cocktail. Full-length Mps1 is immunoprecipitated. Purified complexes are washed with lysis buffer containing 100 mM NaCl and assayed as described for the recombinant protein. To quantify 32P incorporation, reactions are stopped with SDS sample buffer and separated by SDS-PAGE followed by phosphorimaging. The plate is analyzed using a phosphorimager using AIDA software. To assess the specificity of AZ3146, a single-point screen is carried using kinase profiling service. 50 kinases are selected and assayed with 1 μM AZ3146[1].
References	[1]. Hewitt L, et al. Sustained Mps1 activity is required in mitosis to recruit O-Mad2 to the Mad1-C-Mad2 core complex. J Cell Biol. 2010 Jul 12;190(1):25-34. [2]. Liu X, et al. TTK activates Akt and promotes proliferation and migration of hepatocellular carcinoma cells. Oncotarget. 2015 Oct 27;6(33):34309-20. [3]. Gurden MD, et al. Naturally Occurring Mutations in the MPS1 Gene Predispose Cells to Kinase Inhibitor Drug Resistance. Cancer Res. 2015 Aug 15;75(16):3340-54.