

产品名称：**AZ20**
产品别名：**AZ20**

生物活性:					
Description	AZ20 is a potent and selective inhibitor of ATR with an IC50 of 5 nM, and has 8-fold selectivity against mTOR (IC50=38 nM).				
IC50 & Target	ATR	mTOR	PI3Kα		
	5 nM (IC50)	38 nM (IC50)	13000 nM (IC50)		
In Vitro	AZ20 inhibits ATR immunoprecipitated from HeLa nuclear extracts with an IC50 of 5 nM and ATR mediated phosphorylation of Chk1 in HT29 colorectal adenocarcinoma tumor cells with an IC50 of 50 nM[1].				
In Vivo	AZ20 (25, 50 mg/kg, p.o.) has high permeability combined with good stability to rat hepatocytes and, despite the lack of progress in achieving markedly higher solubility, has respectable bioavailability in a low dose rat PK study. AZ20 (25, 50 mg/kg, p.o.) leads to significant tumor growth inhibition in female nude mice bearing LoVo tumors[1].				
Solvent&Solubility	In Vitro: DMSO : ≥ 100 mg/mL (242.42 mM) * "≥" means soluble, but saturation unknown.				
	Preparing Stock Solutions	<div>Solvent / Mass / Concentration</div>	1 mg	5 mg	10 mg
		1 mM	2.4242 mL	12.1209 mL	24.2418 mL
		5 mM	0.4848 mL	2.4242 mL	4.8484 mL
		10 mM	0.2424 mL	1.2121 mL	2.4242 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℃, 6 months; -20℃, 1 month。 -80℃ 储存时，请在 6 个月内使用，-20℃ 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 (6.06 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.06 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 (6.06 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.06 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 生理盐水中，混合均匀。				

References	<p>[1]. Foote KM, et al. Discovery of 4-[[4-[(3R)-3-Methylmorpholin-4-yl]-6-[1-(methylsulfonyl)cyclopropyl]pyrimidin-2-yl]-1H-indole (AZ20): a potent and selective inhibitor of ATR protein kinase with monotherapy in vivo antitumor activity. J Med Chem. 2013 Mar 14</p>
实验参考:	
Cell Assay	<p>Compound dose ranges are created by diluting in 100% DMSO and then further into assay medium (EMEM, 10% FCS, 1% glutamine) using a Labcyte Echo acoustic dispensing instrument. Cells are plated in 384-well Costar plates at 9×10^4 cells per mL in 40 μL of EMEM, 10% FCS, 1% glutamine and grown for 24 h. Following addition of compound the cells are incubated for 60 min. A final concentration of 3 μM 4NQO (prepared in 100% DMSO) is then added using the Labcyte Echo, and the cells are incubated for a further 60 min. The cells are fixed by adding 40 μL of 3.7% v/v formaldehyde solution for 20 min. After removal of fix, cells are washed with PBS and permeabilized in 40 μL of PBS containing 0.1% Triton X-100. The cells are then washed, and 15 μL primary antibody solution (pChk1 Ser345) is added. The plates are incubated at 4°C overnight. The primary antibody is then washed off, and 20 μL of secondary antibody solution and 1 μM Hoechst 33258 added for 90 min at room temperature. The plates are washed and left in 40 μL of PBS. Plates are then read on an ArrayScan VTI instrument to determine staining intensities, and dose responses are obtained and used to determine the IC₅₀ values for the compounds. [1]</p>
Animal Administration	<p>Female Swiss nu/nu mice are housed in negative pressure isolators. LoVo tumor xenografts are established in 8- to 12-week-old mice by injecting 1×10^7 tumor cells subcutaneously (100 μL in serum free medium) on the left dorsal flank. Animals are randomized into treatment groups when tumors become palpable. AZ20 is prepared in 10% DMSO/40% propylene glycol/50% water and administered orally. Tumors are measured up to three times per week with calipers. Tumor volumes are calculated and the data plotted using the geometric mean for each group versus time. [1]</p>
Kinase Assay	<p>ATR for use in the in vitro enzyme assay is obtained from HeLa nuclear extract by immunoprecipitation with rabbit polyclonal antiserum raised to amino acids 400-480 of ATR contained in the following buffer: 25 mM HEPES (pH 7.4), 2 mM MgCl₂, 250 mM NaCl, 0.5 mM EDTA, 0.1 mM Na₃VO₄, 10% v/v glycerol, and 0.01% v/v Tween 20. ATR-antibody complexes are isolated from nuclear extract by incubating with protein A-Sepharose beads for 1 h and then through centrifugation to recover the beads. In the well of a 96-well plate, 10 μL ATR-containing Sepharose beads are incubated with 1 μg of substrate glutathione S-transferase-p53N66 (NH₂-terminal 66 amino acids of p53 fused to glutathione S-transferase are expressed in E. coli) in ATR assay buffer (50 mM HEPES (pH 7.4), 150 mM NaCl, 6 mM MgCl₂, 4 mM MnCl₂, 0.1 mM Na₃VO₄, 0.1 mM DTT, and 10% (v/v) glycerol) at 37°C in the presence or absence of inhibitor. After 10 min with gentle shaking, ATP is added to a final concentration of 3 μM and the reaction continued at 37°C for an additional 1 h. The reaction is stopped by addition of 100 μL of PBS, and the reaction is transferred to a white opaque glutathione coated 96-well plate and incubated overnight at 4°C. This plate is then washed with PBS/0.05% (v/v) Tween 20, blotted dry, and analyzed by a standard ELISA technique with a phosphoserine 15 p53 antibody. The detection of phosphorylated glutathione S-transferase-p53N66 substrate is performed in combination with a goat anti-mouse horseradish peroxidase-conjugated secondary antibody. Enhanced chemiluminescence solution is used to produce a signal, and chemiluminescent detection is carried out via a TopCount plate reader. The resulting calculated % enzyme activity is then used to determine the IC₅₀ values for the</p>

	compounds (IC_{50} taken as the concentration at which 50% of the enzyme activity is inhibited). [1]
References	<p>[1]. Foote KM, et al. Discovery of <u>4-{4-[(3R)-3-Methylmorpholin-4-yl]-6-[1-(methylsulfonyl)cyclopropyl]pyrimidin-2-yl}-1H-indole (AZ20): a potent and selective inhibitor of ATR protein kinase with monotherapy in vivo antitumor activity</u>. J Med Chem. 2013 Mar 14</p>



源叶生物