产品名称：**AT7867**
产品别名：**AT7867**

生物活性：

Description	AT7867 is a potent ATP-competitive inhibitor of Akt1/Akt2/Akt3 and p70S6K/PKA with IC_{50}s of 32 nM/17 nM/47 nM and 85 nM/20 nM, respectively.			
IC_{50} & Target	Akt2	Akt1	Akt3	PKA
	17 nM (IC_{50})	32 nM (IC_{50})	47 nM (IC_{50})	20 nM (IC_{50})
In Vitro	The inhibition of AKT2 by AT7867 is shown to be ATP-competitive with a K_i of 18nM. AT7867 also displays potent activity against the structurally related AGC kinases p70S6K and PKA, but shows a clear window of selectivity against kinases from other kinase sub-families. In vitro growth inhibition studies show that AT7867 blocks proliferation in a number of human cancer cell lines. AT7867 appears to be most potent at inhibiting proliferation in MES-SA uterine, MDA-MB-468 and MCF-7 breast, and HCT116 and HT29 colon lines (IC_{50} values range from 0.9-3 μM), and least effective in the two prostate lines tested (IC_{50} values range from 10-12 μM) [1].			
In Vivo	In vivo: Following oral administration at 20 mg/kg, the elimination of AT7867 from plasma appears to be similar to that observed after i.v. administration. Plasma levels of AT7867 remain above 0.5 μM for at least 6 hours following an oral dose of 20 mg/kg. Assuming linear pharmacokinetics following i.v. administration, the bioavailability by the oral route is calculated to be 44%. In vivo pharmacodynamic (PD) biomarker studies are therefore performed with this model. Following pharmacokinetic and tolerability studies, doses of AT7867 (90 mg/kg p.o. or 20 mg/kg i.p.) are administered to athymic mice bearing MES-SA tumors and the phosphorylation status of GSK3β and S6RP in tumors is monitored over time. Clear inhibition of phosphorylation of the two markers of pathway activity is seen at 2 and 6 hours following treatment with AT7867. By 24 hours, total levels of both GSK3β and S6RP are greatly reduced[1].			
Solvent&Solubility	***In Vitro:*** **DMSO : 10 mg/mL (29.60 mM;** Need ultrasonic)			

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.9599 mL	14.7995 mL	29.5989 mL
	5 mM	0.5920 mL	2.9599 mL	5.9198 mL
	10 mM	0.2960 mL	1.4799 mL	2.9599 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: ≥ 1 mg/mL (2.96 mM); Clear solution

	此方案可获得 ≥ 1 mg/mL (2.96 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 10.0 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: ≥ 1 mg/mL (2.96 mM); Clear solution 此方案可获得 ≥ 1 mg/mL (2.96 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 10.0 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: ≥ 1 mg/mL (2.96 mM); Clear solution 此方案可获得 ≥ 1 mg/mL (2.96 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 10.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Grimshaw KM, et al. AT7867 is a potent and oral inhibitor of AKT and p70 S6 kinase that induces pharmacodynamic changes and inhibits human tumor xenograft growth. Mol Cancer Ther, 2010, 9(5), 1100-1110.

实验参考：

Cell Assay	Cells are plated in 96-well microplates at 16,000 cells per well in medium supplemented with 10% FBS, and grown for 24 hours before treatment with AT7867. AT7867 or vehicle control are added to the cells for 1 hour. Following this, cells are fixed with 3% paraformaldehyde, 0.25% glutaraldehyde, 0.25% Triton-X100, washed and blocked with 5% milk in tris-buffered saline with 0.1% Tween-20 (TBST) prior to overnight incubation with a phospho-GSK3β (serine 9) antibody. The plates are then washed, secondary antibody added, and enhancement of the signal performed using DELFIA reagents. Europium counts are normalized to the protein concentration, and the IC_{50} value for each inhibitor is calculated in GraphPad Prism using non-linear regression analysis and a sigmoidal dose-response (variable slope) equation[1]
Animal Administration	Mice[1] Male athymic BALB/c mice (nu/nu) are used. A single dose of AT7867 is administered to BALB/c mice at 5 mg/kg intravenously (i.v.) and 20 mg/kg per os (p.o.). Plasma samples are collected from duplicate animals at each of the following time points; 0.083, 0.167, 0.33, 0.67, 1, 2, 4, 6, 16 and 24 hours following i.v. dosing and at 0.25, 0.5, 1, 2, 4, 6 and 24 hours following p.o. dosing. Mice are bled by cardiac puncture and all blood samples are centrifuged to obtain plasma, which is then frozen at -20°C until analysis. For bioanalysis, all plasma samples are prepared by protein precipitation with acetonitrile containing internal standard. Quantification of sample extracts is by comparison with a standard calibration line constructed with AT7867 and using an inhibitor specific liquid chromatography tandem mass spectrometry (LC-MS/MS) method. Pharmacokinetic parameters are determined.
	Kinase assays for AKT2, PKA, p70S6K and CDK2/cyclinA are all carried out in a radiometric filter binding format. Assay reactions are set up in the presence of compound. For AKT2, the AKT2 enzyme and 25 μM AKTide-2T peptide (HARKRERTYSFGHHA) are incubated in 20 mM MOPS, pH 7.2, 25 mM β-glycerophosphate, 5 mM EDTA, 15 mM $MgCl_2$, 1 mM sodium orthovanadate, 1 mM

Kinase Assay	DTT, 10 μg/mL BSA and 30 μM ATP (1.16 Ci/mmol) for 4 hours. For PKA, the PKA enzyme and 50 μM peptide (GRTGRRNSI) are incubated in 2 mM MOPS, pH 7.2, 25 mM β-glycerophosphate, 5 mM EDTA, 15 mM $MgCl_2$, 1 mM orthovanadate, 1 mM DTT and 40 μM ATP (0.88 Ci/mmol) for 20 minutes. For p70S6K, the p70S6K enzyme and 25 μM peptide substrate (AKRRRLSSLRA) are incubated in 10 mM MOPS, pH 7, 0.2 mM EDTA, 1 mM $MgCl_2$, 0.01% β-mercaptoethanol, 0.1 mg/mL BSA, 0.001% Brij-35, 0.5% glycerol and 15μM ATP (2.3 Ci/mmol) for 60 minutes. For CDK2, the CDK2/cyclinA enzyme and 0.12 μg/ml Histone H1 are incubated in 20 mM MOPS, pH 7.2, 25 mM β-glycerophosphate, 5 mM EDTA, 15 mM $MgCl_2$, 1 mM sodium orthovanadate, 1 mM DTT, 0.1 mg/ml BSA and 45 μM ATP (0.78 Ci/mmol) for 4 hours. Assay reactions are stopped by adding an excess of orthophosphoric acid and the stopped reaction mixture is then transferred to Millipore MAPH filter plates and filtered. The plates are then washed, scintillant added and radioactivity measured by scintillation counting on a Packard TopCount. IC_{50} values are calculated from replicate curves using GraphPad Prism software. AKT1 and 3 enzyme assays are carried out, while all other enzyme assays are performed[1]
References	[1]. Grimshaw KM, et al. AT7867 is a potent and oral inhibitor of AKT and p70 S6 kinase that induces pharmacodynamic changes and inhibits human tumor xenograft growth. Mol Cancer Ther, 2010, 9(5), 1100-1110.