

产品名称: Ispinesib (SB-715992)

产品别名: 伊斯平斯

生物活性:					
Description	Ispinesib is a specific inhibitor of kinesin spindle protein (KSP), with a $K_{i\text{ app}}$ of 1.7 nM.				
IC <sub>50</sub> & Target	KSP				
	1.7 nM (K <sub>i app</sub> )				
In Vitro	Ispinesib is a potent, highly specific inhibitor of KSP, with a $K_{i\text{ app}}$ of 1.7 nM[1]. Ispinesib (150 nM) inhibits BT-474 and MDA-MB-468 cell lines, with GI <sub>50</sub> s of 45 and 19 nM, respectively[2]. Ispinesib (SB715992, 15 and 30 nM) suppresses the proliferation of PC-3 prostate cancer cell by 48.65% and 52.16%, and induces apoptosis of prostate cancer cell by 1094.88% and 1516.70%, respectively. Ispinesib up regulates genes responsible for apoptosis and cell cycle arrest, and down regulates genes responsible for cell proliferation and survival. The anti-proliferation and pro-apoptotic activities of Ispinesib can be enhanced by genistein[3].				
In Vivo	Ispinesib (SCID, 8 mg/kg; nude, 10 mg/kg, q4d × 3) reduces tumor volume in mice bearing tumor xenografts of ER-positive (MCF7), HER2-positive (KPL4, HCC1954, and BT-474), and triple-negative (MDA-MB-468) breast cancer cells via i.p. one dose every 4 days repeated three times[2].				
Solvent&Solubility	<b>In Vitro:</b> <b>DMSO : ≥ 125 mg/mL (241.75 mM)</b> <b>H<sub>2</sub>O : &lt; 0.1 mg/mL (insoluble)</b>  * "≥" means soluble, but saturation unknown.				
	<div>Preparing Stock Solutions</div>	<div><div>Solvent</div><div>Mass</div><div>Concentration</div></div>	1 mg	5 mg	10 mg
		1 mM	1.9340 mL	9.6701 mL	19.3401 mL
		5 mM	0.3868 mL	1.9340 mL	3.8680 mL
		10 mM	0.1934 mL	0.9670 mL	1.9340 mL
	<p>*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。</p> <p>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。</p> <p><b>In Vivo:</b></p> <p>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 <b>In Vitro</b> 方式配制澄清的储备液，再依次添加助溶剂：</p> <p>——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</p> <div><p>1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline</p><p>Solubility: ≥ 2.5 mg/mL (4.84 mM); Clear solution</p><p>此方案可获得 ≥ 2.5 mg/mL (4.84 mM，饱和度未知) 的澄清溶液。</p><p>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。</p></div> <div><p>2.请依序添加每种溶剂： 10% DMSO →90% corn oil</p><p>Solubility: ≥ 2.5 mg/mL (4.84 mM); Clear solution</p></div>				

	<p>此方案可获得 <math>\geq 2.5</math> mg/mL (4.84 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。</p> <p>以 1 mL 工作液为例, 取 100 <math>\mu</math>L 25.0 mg/mL 的澄清 DMSO 储备液加到 900 <math>\mu</math>L 玉米油中, 混合均匀。</p>
<b>References</b>	<p>[1]. <u>Lad L, et al. Mechanism of inhibition of human KSP by ispinesib. Biochemistry. 2008 Mar 18;47(11):3576-85.</u></p> <p>[2]. <u>Purcell JW, et al. Activity of the kinesin spindle protein inhibitor ispinesib (SB-715992) in models of breast cancer. Clin Cancer Res. 2010 Jan 15;16(2):566-76.</u></p> <p>[3]. <u>Davis DA, et al. Increased therapeutic potential of an experimental anti-mitotic inhibitor SB715992 by genistein in PC-3 human prostate cancer cell line. BMC Cancer. 2006 Jan 24;6:22.</u></p>
<b>实验参考:</b>	
<b>Cell Assay</b>	<p>PC-3 prostate cancer cells are seeded in 96 well plates at a density of <math>4 \times 10^3</math> cells/well. PC-3 cells are incubated for 24 hours to allow attachment to the surface of each well of the tissue culture plate. Then, the cells are treated with varying concentration of reagents and incubated for 1 to 3 days. First, PC-3 cells are treated with 15 and 30 nM of Ispinesib, respectively. Second, PC-3 cells are subjected to combinational treatments with 7.5 or 10 nM of Ispinesib plus 30 <math>\mu</math>M of genistein. Finally, PC-3 cells are pre-treated with 30 <math>\mu</math>M of genistein for 24 hours followed by treatment with 15 nM of Ispinesib. Control cells are treated with 0.3 mM <math>\text{Na}_2\text{CO}_3</math> (vehicle control). After treatment, PC3 cells are incubated at 37°C with MTT (0.5 mg/mL) for 2 hours and isopropyl alcohol at room temperature for 1 hour. The spectrophotometric absorbance of each sample is then determined by using ULTRA Multifunctional Micro Plate Reader at 595 nm [3].</p>
<b>Animal Administration</b>	<p>Mice with a tumor volume of <math>\sim 250</math> mm<sup>3</sup> receive a single dose of Ispinesib (10 mg/kg). Tumors are dissected, fixed in 10% buffered formalin, and embedded in paraffin, and 5-<math>\mu</math>m tissue sections are prepared. Antigen retrieval is done by boiling in 50 mM citrate buffer (pH 5.5), and sections are then incubated in 3% hydrogen peroxide, washed in PBS-0.1% Tween, and blocked in 10% goat serum. Phospho-histone H3 (PH3) antibody is detected using Alexa Fluor 488 secondary antibody. Images are taken with a microscope at <math>\times 10</math> magnification and captured using MetaMorph software to quantify PH3 expression by computing the area ratio of PH3-positive cells per total cells.</p> <p>Ki67/cleaved caspase-3 staining is done. Nonfluorescent images are taken on an Olympus BX41 microscope at <math>\times 20</math> magnification [2].</p>
<b>Kinase Assay</b>	<p>Kinesin specificity analysis is carried out using a pyruvate kinase-lactate dehydrogenase detection system that couples the production of ADP to oxidation of NADH. Absorbance changes are monitored at 340 nm. Steady-state studies using nanomolar concentrations of KSP are performed using a sensitive fluorescence-based assay utilizing a pyruvate kinase, pyruvate oxidase, and horseradish peroxidase coupled detection system that couples the generation of ADP to oxidation of Amplex Red to fluorescent resorufin. Generation of resorufin is monitored by fluorescence (<math>\lambda_{\text{excitation}} = 520</math> nm and <math>\lambda_{\text{emission}} = 580</math> nm). Steady-state biochemical experiments are performed in PEM25 buffer [25 mM Pipes-K<sup>+</sup> (pH 6.8), 2 mM <math>\text{MgCl}_2</math>, 1 mM EGTA] supplemented with 10 <math>\mu</math>M paclitaxel for experiments involving microtubules. The <math>\text{IC}_{50}</math> for steady-state inhibition is determined at 500 <math>\mu</math>M ATP, 5 <math>\mu</math>M MTs, and 1 nM KSP in PEM25 buffer. <math>K_{i \text{ app}}</math> (apparent inhibitor dissociation constant) estimates of Ispinesib are extracted from the concentration-response curves, with explicit correction for enzyme concentration [1].</p>
	<p>[1]. <u>Lad L, et al. Mechanism of inhibition of human KSP by ispinesib. Biochemistry. 2008 Mar</u></p>

<b>References</b>	<p><u>18;47(11):3576-85.</u></p> <p>[2]. <u>Purcell JW, et al. Activity of the kinesin spindle protein inhibitor ispinesib (SB-715992) in models of breast cancer. Clin Cancer Res. 2010 Jan 15;16(2):566-76.</u></p> <p>[3]. <u>Davis DA, et al. Increased therapeutic potential of an experimental anti-mitotic inhibitor SB715992 by genistein in PC-3 human prostate cancer cell line. BMC Cancer. 2006 Jan 24;6:22.</u></p>
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源叶生物