产品名称：Apoptozole
产品别名：Apoptosis Activator VII

生物活性：

Description	Apoptozole is an inhibitor of the ATPase domain of Hsc70 and Hsp70, with K_ds of 0.21 and 0.14 μM, respectively, and can induce apoptosis.
IC_{50} & Target	HSP70 | HSC70 0.14 μM (Kd) | 0.21 μM (Kd)
In Vitro	Apoptozole is an inhibitor of Hsc70 and Hsp70, which binds to Hsc70 and Hsp70, with K_ds of 0.21 and 0.14 μM, respectively. Apoptozole (Apoptosis Activator VII; 1 μM) induces apoptosis in P19 cells. Apoptozole shows inhibitory activities against several cancer cell lines, such as SK-OV-3 (ovarian cancer cells), HCT-15 (colon cancer cells), and A549 (lung cancer cells), with IC_{50}s of 0.22, 0.25, and 0.13 μM, respectively[1]. Apoptozole binds to the ATPase domain of Hsc70 and Hsp70, but does not binds to other types of heat shock proteins such as Hsp60, Hsp90 or Hsp40[2]. Apoptozole (0-15 μM) suppresses the growth of A549 cells, HeLa cells, and MDA-MB-231 cells, with IC_{50}s ranging from 5 to 7 μM. Apoptozole (5 or 10 μM) shows no effect on associations of HSP70 with ASK1, JNK, or BAX, and does not induce AIF-mediated caspase-independent apoptosis in HeLa cells[3].
In Vivo	Apoptozole (Apoptosis Activator VII; 4 mg/kg, i.p.) exhibits antitumor activities in nude mice xenografted with A549, RKO (colorectal carcinoma), and HeLa cells[3].
Solvent&Solubility	***In Vitro:*** **DMSO : ≥ 100 mg/mL (159.86 mM)** **H_2O : < 0.1 mg/mL** (insoluble) * "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.5986 mL	7.9928 mL	15.9857 mL
	5 mM	0.3197 mL	1.5986 mL	3.1971 mL
	10 mM	0.1599 mL	0.7993 mL	1.5986 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 (4.00 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (4.00 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 (4.00 mM); Suspended solution; Need ultrasonic 此方案可获得 2.5 mg/mL (4.00 mM)的均匀悬浊液，悬浊液可用于口服和腹腔注射。 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。 3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.5 mg/mL (4.00 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.00 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 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Williams DR, et al. An apoptosis-inducing small molecule that binds to heat shock protein 70. Angew Chem Int Ed Engl. 2008;47(39):7466-9. [2]. Cho HJ, et al. Probing the effect of an inhibitor of an ATPase domain of Hsc70 on clathrin-mediated endocytosis. Mol Biosyst. 2015 Oct;11(10):2763-9. [3]. Ko SK, et al. A small molecule inhibitor of ATPase activity of HSP70 induces apoptosis and has antitumor activities. Chem Biol. 2015 Mar 19;22(3):391-403.

实验参考：

Cell Assay	Cells (5 × 10^5 per well) are plated in triplicate in 96-well plates in 0.1 mL of culture media with 10% FBS. After 24 hr, cells are treated with various concentrations of Apoptozole (0-15 μM) in culture media with 3% FBS (final volume: 0.2 mL per well) for 18, 48, and 72 hr before treatment with MTT. Absorbance at 570 nm is measured using a UV microplate reader[3].
Animal Administration	Male nude mice are housed in a pathogen-free room under controlled temperature and humidity. Mice aged 4 weeks are injected with tumor cells for the xenograft experiments. Viable A549 and RKO cells (5 × 10^6) and HeLa cells (5 × 10^6) are injected subcutaneously into the flank of mice. The A549 and RKO cell xenograft mice are immediately and randomly assigned to two groups. The first group (n = 10) is used as a control group and receives vehicle only. The second group (n = 10) receives intraperitoneal injections of Apoptozole (4 mg/kg/day) every other day for 2 weeks. The HeLa cell xenograft mice are immediately and randomly assigned to four groups. The first group (n = 10) is a control group receiving vehicle only. The second group (n = 10) receives intraperitoneal injections of Apoptozole (4 mg/kg/day) every other day for 2 weeks. The third group (n = 10) receives intraperitoneal injections of doxorubicin (15 mg/kg/day) every other day for 2 weeks. The fourth group (n = 10) receives intraperitoneal injections of Apoptozole (4 mg/kg/day) and doxorubicin (15 mg/kg/day) every other day for 2 weeks. Tumors in all mice are measured in two dimensions with calipers every 3 days and tumor volumes are calculated using the formula volume = w × l^2/2, where w is the width at the widest point of the tumor and l is the length perpendicular to w. The results from individual mice are plotted as average tumor volumes versus time [3]
	Stock solutions of malachite green (0.081% w/v), polyvinyl alcohol (2.3% w/v), and ammonium heptamolybdate tetrahydrate (5.7% w/v in 6 M HCl) are prepared and stored at 4°C. Three solutions are mixed with water in the ratio of 2 : 1 : 1 : 2 to prepare the malachite green reagent. For the determination of the ATPase activity of Hsc70, a master mixture of an ATPase domain of Hsc70 is prepared in assay buffer (100 mM Tris-HCl, 20 mM KCl, and 6 mM $MgCl_2$, pH 7.4) as the final

Kinase Assay	concentration of 1 mM. An aliquot (10 mL) of this mixture is added into each well of a 96-well plate. To this solution is added each compound (including Apoptozole) in assay buffer, and the plate is incubated for 30 min at room temperature. To start the reaction, 1 mL of 4 mM ATP is added to the solution. The final concentrations are 1 mM protein and 200 mM ATP in 20 mL of assay buffer. After 3 h incubation at 37°C, 80 mL of the malachite green reagent is added into each well. The samples are mixed thoroughly and incubated at 37°C for 15 min, and 10 mL of 34% sodium citrate is added to stop the nonenzymatic hydrolysis of ATP. The absorbance is determined at 620 nm on a SpectraMax 340 PC 384[1].
References	[1]. Williams DR, et al. An apoptosis-inducing small molecule that binds to heat shock protein 70. Angew Chem Int Ed Engl. 2008;47(39):7466-9. [2]. Cho HJ, et al. Probing the effect of an inhibitor of an ATPase domain of Hsc70 on clathrin-mediated endocytosis. Mol Biosyst. 2015 Oct;11(10):2763-9. [3]. Ko SK, et al. A small molecule inhibitor of ATPase activity of HSP70 induces apoptosis and has antitumor activities. Chem Biol. 2015 Mar 19;22(3):391-403.