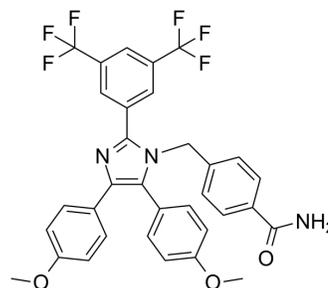


Apoptozole

Cat. No.:	HY-15098		
CAS No.:	1054543-47-3		
Molecular Formula:	C ₃₃ H ₂₅ F ₆ N ₃ O ₃		
Molecular Weight:	625.56		
Target:	HSP; Apoptosis		
Pathway:	Cell Cycle/DNA Damage; Metabolic Enzyme/Protease; Apoptosis		
Storage:	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	2 years
		-20°C	1 year



SOLVENT & SOLUBILITY

In Vitro

DMSO : ≥ 100 mg/mL (159.86 mM)
 * "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent Concentration	Mass		
		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

Please refer to the solubility information to select the appropriate solvent.

In Vivo

- Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
 Solubility: ≥ 2.5 mg/mL (4.00 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
 Solubility: 2.5 mg/mL (4.00 mM); Suspended solution; Need ultrasonic
- Add each solvent one by one: 10% DMSO >> 90% corn oil
 Solubility: ≥ 2.5 mg/mL (4.00 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

Apoptozole (Apoptosis Activator VII) 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.

IC₅₀ & Target

HSP70	HSC70
0.14 μM (Kd)	0.21 μM (Kd)

In Vitro	<p>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].</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>
In Vivo	<p>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].</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>

PROTOCOL

Kinase Assay ^[1]	<p>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 MgCl₂, pH 7.4) as the final 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].</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>
Cell Assay ^[3]	<p>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].</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>
Animal Administration ^[3]	<p>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 $volume = w \times l^2/2$, 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].</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>

CUSTOMER VALIDATION

- Theranostics. 2021 Feb 20;11(9):4187-4206.
- Redox Biol. 2024 Jan 24, 103035.
- CNS Neurosci Ther. 2019 Sep;25(9):1030-1041.
- mSphere. 2023 Feb 28;e0067922.
- Exp Cell Res. 2023 Mar 22;426(2):113565.

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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.
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Caution: Product has not been fully validated for medical applications. For research use only.

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