Apoptozole

Cat. No.: HY-15098

CAS No.: 1054543-47-3

Molecular Formula: $C_{33}H_{25}F_6N_3O_3$

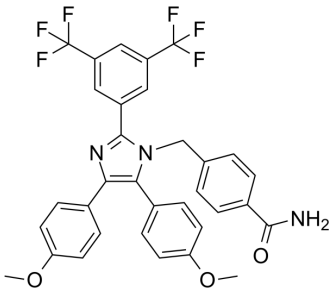
Molecular Weight: 625.56

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 / 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

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

In Vivo

1. 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
2. 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
3. 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.

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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	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 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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [3]	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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [3]	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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

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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