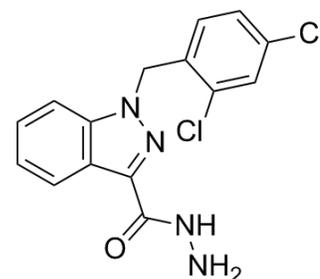


## Adjudin

<b>Cat. No.:</b>	HY-18996		
<b>CAS No.:</b>	252025-52-8		
<b>Molecular Formula:</b>	C <sub>15</sub> H <sub>12</sub> Cl <sub>2</sub> N <sub>4</sub> O		
<b>Molecular Weight:</b>	335.19		
<b>Target:</b>	Chloride Channel; Mitochondrial Metabolism		
<b>Pathway:</b>	Membrane Transporter/Ion Channel; Metabolic Enzyme/Protease		
<b>Storage:</b>	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	6 months
		-20°C	1 month



### SOLVENT & SOLUBILITY

<b>In Vitro</b>	DMSO : 16.67 mg/mL (49.73 mM; Need ultrasonic)				
		Solvent Concentration	Mass 1 mg	5 mg	10 mg
	<b>Preparing Stock Solutions</b>	1 mM	2.9834 mL	14.9169 mL	29.8338 mL
		5 mM	0.5967 mL	2.9834 mL	5.9668 mL
10 mM		0.2983 mL	1.4917 mL	2.9834 mL	
Please refer to the solubility information to select the appropriate solvent.					
<b>In Vivo</b>	1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline Solubility: ≥ 1.67 mg/mL (4.98 mM); Clear solution  2. Add each solvent one by one: 10% DMSO >> 90% corn oil Solubility: ≥ 1.67 mg/mL (4.98 mM); Clear solution				

### BIOLOGICAL ACTIVITY

<b>Description</b>	Adjudin is an extensively studied male contraceptive with a superior mitochondria-inhibitory effect. Adjudin is also a potent Cl <sup>-</sup> channel blocker.
<b>IC<sub>50</sub> &amp; Target</b>	Cl <sup>-</sup> channel <sup>[1]</sup> Mitochondria <sup>[2]</sup>
<b>In Vitro</b>	Adjudin is a potent blocker of Cl <sup>-</sup> channels: disrupting Cl <sup>-</sup> ion transport function results in a decline in sperm capacitation and fertilizing ability in humans in vitro <sup>[1]</sup> . Adjudin (ADD) is a mitochondria inhibitor <sup>[2]</sup> . Adjudin is a molecule that mediates adherens junction disruption at the Sertoli-germ cell interface. To investigate the effect of Adjudin on cancer cells, more than ten different types of human or mice cancer cell lines are treated with increasing concentrations of Adjudin and the cell

proliferation is measured by the modified MTT assay. Adjudin inhibits cell proliferation in a dose dependent manner in SGC-7901 (human gastric adenocarcinoma cell), MDA-MB-231 (human breast adenocarcinoma cell), Smmc-7721 (human hepatoma cell) and MIA Paca-2 (human pancreatic adenocarcinoma cell) cells. The IC<sub>50</sub> of Adjudin is determined to be 58.0 μM, 13.8 μM, 72.3 μM and 52.7 μM against SGC-7901, MDA-MB-231, Smmc-7721 and MIA Paca-2 cells, respectively, after treatment for 24 h. Similar results are obtained in other human and mice cancer cell lines. The IC<sub>50</sub> of Adjudin in A549 cells and PC3 cells is 63.1 μM and 93.0 μM, respectively. For WI-38 and BPH-1 cells, the IC<sub>50</sub> of Adjudin can be observed at more than 300 μM and 200 μM, respectively, which is about 5 times and 2 times more than that for the cancer cell lines A549 and PC3<sup>[3]</sup>.

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

#### In Vivo

To determine whether Adjudin can inhibit lung and prostate cancer growth in vivo, the effect of Adjudin is tested in a subcutaneous model of lung and prostate cancer. Human lung carcinoma cells A549 and prostate carcinoma cells PC3 are injected into athymic nude mice subdermally at the lower back site respectively. Mice are then randomized into two treatment groups with similar mean tumor sizes: Adjudin and vehicle (control). Approximately 2 weeks after tumor inoculation Adjudin is injected intraperitoneally once every three days in lung carcinoma cells and every other day in prostate carcinoma cells at 100 mg/kg. Adjudin treatment can be well tolerated in rodent. And Adjudin-treated mice show significant tumor growth inhibition compared with the control group (P<0.0001 in the human lung carcinoma cells A549 and P=0.006 in the prostate carcinoma cells PC3)<sup>[3]</sup>.

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## PROTOCOL

#### Cell Assay <sup>[3]</sup>

A549 cells, WI-38 cells, BPH-1 cells, PC-12 cells and other cell lines are seeded in 96-well plates at the density of 0.5×10<sup>4</sup>/well in the complete growth medium and incubated for 24 h. Then the growth medium is replaced with a serial dilution of Adjudin (300 μM, 100 μM, 30 μM, 10 μM, 3 μM and 0) in growth medium (without serum). The cells are incubated for another 24 h followed by the addition of 10 μl of Cell Counting Kit-8 solution to each well. After 4 h of incubation at 37 °C in the cell incubator, the absorbance at 450 nm is measured using a microplate reader<sup>[3]</sup>.

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#### Animal Administration <sup>[3]</sup>

Mice<sup>[3]</sup>

The male BALB/C nude mice weighing ~20g are equally implanted with A549 cells (0.5×10<sup>7</sup> cells) containing 3 mg/mL of matrigel and PC3 cells (1×10<sup>6</sup> cells) hypodermically. After 2 weeks, the mice with palpable tumors are divided into two groups (n=4 per group in each experiment and repeated with a total of three experiments): i.p. injection of Adjudin which is dissolved in corn oil from a DMSO stock solution with final administered quantity at 100 mg/kg (~300 μM used in vitro); the equivalent vehicle control group are administered with the same amount of corn oil and DMSO via i.p. injection. Adjudin or vehicles are administered every three day in A549 and every other day in PC3 up to 2 weeks. Tumor volumes are determined and calculated.

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

## REFERENCES

- [1]. Li K, et al. Inhibition of sperm capacitation and fertilizing capacity by adjudin is mediated by chloride and its channels in humans. Hum Reprod. 2013 Jan;28(1):47-59.
- [2]. Li X, et al. Combination delivery of Adjudin and Doxorubicin via integrating drug conjugation and nanocarrier approaches for the treatment of drug-resistant cancer cells. J Mater Chem B. 2015 Feb 28;3(8):1556-1564.
- [3]. Xie QR, et al. Male contraceptive Adjudin is a potential anti-cancer drug. Biochem Pharmacol. 2013 Feb 1;85(3):345-55.

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

Tel: 609-228-6898

Fax: 609-228-5909

E-mail: [tech@MedChemExpress.com](mailto:tech@MedChemExpress.com)

Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA