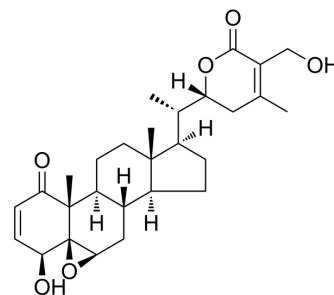


## Withaferin A

<b>Cat. No.:</b>	HY-N2065
<b>CAS No.:</b>	5119-48-2
<b>Molecular Formula:</b>	C <sub>28</sub> H <sub>38</sub> O <sub>6</sub>
<b>Molecular Weight:</b>	470.6
<b>Target:</b>	NF-κB; Ferroptosis
<b>Pathway:</b>	NF-κB; Apoptosis
<b>Storage:</b>	4°C, protect from light * In solvent : -80°C, 2 years; -20°C, 1 year (protect from light)



### SOLVENT & SOLUBILITY

<b>In Vitro</b>	DMSO : 50 mg/mL (106.25 mM; Need ultrasonic)				
		Solvent Concentration	Mass		
	<b>Preparing Stock Solutions</b>		1 mg	5 mg	10 mg
		1 mM	2.1249 mL	10.6247 mL	21.2495 mL
		5 mM	0.4250 mL	2.1249 mL	4.2499 mL
	10 mM	0.2125 mL	1.0625 mL	2.1249 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: ≥ 0.83 mg/mL (1.76 mM); Clear solution				
	2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: ≥ 0.83 mg/mL (1.76 mM); Clear solution				
	3. Add each solvent one by one: 10% DMSO >> 90% corn oil Solubility: ≥ 0.83 mg/mL (1.76 mM); Clear solution				

### BIOLOGICAL ACTIVITY

<b>Description</b>	Withaferin A is a steroidal lactone isolated from <i>Withania somnifera</i> , inhibits NF-κB activation and targets vimentin, with potent antiinflammatory and anticancer activities. Withaferin A is an inhibitor of endothelial protein C receptor (EPCR) shedding.
<b>IC<sub>50</sub> &amp; Target</b>	NFκB
<b>In Vitro</b>	Withaferin A has antiinflammatory activity, and potently inhibits NF-κB activation by preventing the TNF-induced activation of IκB kinase beta via a thioalkylation-sensitive redox mechanism <sup>[1]</sup> . Withaferin A also has anticancer activity. Withaferin A targets the IF protein vimentin, causes aggregation of vimentin

filaments in bovine aortic endothelial cells (BAECs) at 3  $\mu$ M, and induces vimentin fragmentation in endothelial cells at 10  $\mu$ M<sup>[2]</sup>.

Withaferin A (0.5, 1.5  $\mu$ M) alone or in combination with cisplatin (CIS) dose-dependently reduces tumorigenic potential of ALDH1 positive cancer stem cells (CSCs)<sup>[3]</sup>.

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

#### In Vivo

Withaferin A (2 mg/kg, i.p.) shows potent angiogenesis inhibitory activity via vimentin in mice<sup>[2]</sup>. Withaferin A (2 mg/kg) combined with cisplatin (CIS) regulates the expression of ALDH1 marker, and downregulates the expression of securin in tumors collected from mice<sup>[3]</sup>.

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

## PROTOCOL

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

Ovarian epithelial cancer cell line A2780 is maintained in RPMI1640 medium supplemented with insulin (5  $\mu$ g/mL), penicillin/streptomycin (100 IU/mL and 100  $\mu$ g/mL respectively) and 10% fetal bovine serum (FBS) from Hyclone. Withaferin A, cisplatin (CIS) and other reagents are prepared in DMSO. Cisplatin is prepared fresh each time<sup>[3]</sup>.

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

### Animal Administration <sup>[2]</sup>

Mice<sup>[2]</sup>

Vimentin homozygous-deficient mice (Vim<sup>-/-</sup>) and mice that are vimentin-heterozygous deficient (Vim<sup>+/-</sup>) in the 129/Svev background are used in the assay. In brief, mice between 4 and 6 wk of age are anesthetized by intraperitoneal (i.p.) injection of ketamine and xylazine. Corneas are topically anesthetized by application of proparacain eye drop, and 1  $\mu$ L drop of dilute 0.15 M sodium hydroxide is applied for 1 min. The cornea is immediately washed extensively in saline solution, and corneal and limbal epithelium gently removed by scraping. The cornea is topically treated with atropine eye drop and covered with tobramycin and erythromycin antibiotic eye ointment. Withaferin A or 12-D WS (2 mg/kg solubilized in DMSO) or vehicle (DMSO) is injected i.p. in respective drug or control groups of mice after their recovery from corneal injury, and subsequently every day thereafter for a period of 10 days. Mice are humanely killed and eyes enucleated. The anterior segment half of eyes are dissected and corneal buttons are prepared. Corneal tissues are fixed in 100% acetone for 20 min, washed in PBS for 1 hr, and blocked for 18 hr in 1% BSA-PBS at 4°C. Cornea whole-mount staining is performed by incubating tissues in FITC-conjugated rat anti-mouse CD31 antibody (1:333 dilution in 1% BSA-PBS) for 12 hr, washed away for 24 hr at 4°C in 1% BSA-PBS, and affixed to glass slides with a coverslip. Fluorescent staining is visualized on microscope, and quantified by importing digital images to NIH ImageJ<sup>[2]</sup>.

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

## CUSTOMER VALIDATION

- J Exp Clin Cancer Res. 2024 Feb 21;43(1):52.
- Cancer Cell Int. 2020 May 20;20:179.
- Aging. 2020 Dec 3;13(1):957-972.
- Vet Res. 2023 Jan 30;54(1):7.
- The Hong Kong Polytechnic University. 2023 Aug.

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## REFERENCES

[1]. Kaileh M, et al. Withaferin a strongly elicits I $\kappa$ B kinase beta hyperphosphorylation concomitant with potent inhibition of its kinase activity. J Biol Chem. 2007 Feb

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16;282(7);4253-64. Epub 2006 Dec 6.

[2]. Bargagna-Mohan P, et al. The tumor inhibitor and antiangiogenic agent withaferin A targets the intermediate filament protein vimentin. Chem Biol. 2007 Jun;14(6):623-34.

[3]. Kakar SS, et al. Withaferin A (WFA) inhibits tumor growth and metastasis by targeting ovarian cancer stem cells. Oncotarget. 2017 Aug 10;8(43):74494-74505.

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

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