

# **Product** Data Sheet

# Salinomycin

Cat. No.: HY-15597

CAS No.: 53003-10-4

Molecular Formula:  $C_{42}H_{70}O_{11}$ Molecular Weight: 751

Target: Wnt; β-catenin; Bacterial; Autophagy; Mitophagy; Apoptosis; Antibiotic; Parasite

Pathway: Stem Cell/Wnt; Anti-infection; Autophagy; Apoptosis

Storage: Powder -20°C 3 years

 $\begin{array}{ccc} & 4^{\circ}\text{C} & 2 \text{ years} \\ \text{In solvent} & -80^{\circ}\text{C} & 6 \text{ months} \\ & -20^{\circ}\text{C} & 1 \text{ month} \end{array}$ 

## **SOLVENT & SOLUBILITY**

In Vitro DMSO : ≥ 36.7 mg/mL (48.87 mM)

\* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent Mass Concentration	1 mg	5 mg	10 mg
	1 mM	1.3316 mL	6.6578 mL	13.3156 mL
	5 mM	0.2663 mL	1.3316 mL	2.6631 mL
	10 mM	0.1332 mL	0.6658 mL	1.3316 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 (3.33 mM); Clear solution
- 2. Add each solvent one by one: 10% DMSO >> 90% corn oil Solubility: ≥ 2.5 mg/mL (3.33 mM); Clear solution
- 3. Add each solvent one by one: 5% DMSO >> 40% PEG300 >> 5% Tween-80 >> 50% saline Solubility: 2.5 mg/mL (3.33 mM); Suspended solution; Need ultrasonic

# **BIOLOGICAL ACTIVITY**

Description

Salinomycin (Procoxacin), a polyether potassium ionophore antibiotic, selectively inhibits the growth of gram-positive bacteria. Salinomycin is a potent inhibitor of Wnt/β-catenin signaling, blocks Wnt-induced LRP6 phosphorylation.

Salinomycin (Procoxacin) shows selective activity against human cancer stem cells<sup>[1][2][3]</sup>.

IC<sub>50</sub> & Target Coccidia

#### In Vitro

Salinomycin is a potent inhibitor of the Wnt signaling cascade. Incubation of the malignant lymphocytes with Salinomycin induces apoptosis within 48 h, with a mean  $IC_{50}$  of 230 nM. Salinomycin is also an antibiotic potassium ionophore, has been reported recently to act as a selective breast cancer stem cell inhibitor<sup>[1]</sup>.

Salinomycin is a novel and an effective anticancer drug, inhibits SW620 cells and Cisp-resistant SW620 cells with IC $_{50}$  of 1.54±0.23 µM and 0.32±0.05 µM, respectively. Salinomycin is found to have the ability to kill both cancer stem cells (CSCs) and therapy-resistant cancer cells. After continuous Salinomycin treatment for 48 h, the apoptotic cells are observed under the microscope and counted randomly at least 100 cells in one field. The number of apoptotic cells which are stained by Hoechst33342 is significantly increased in Cisp-resistant SW620 cells (20.20±3.72) than that of SW620 cells (9.40±2.07) per 100 cells (p<0.05). After treatment with Salinomycin for 48 h, flow cytometric analysis is used to detect the cell apoptosis both in SW620 cells and Cisp-resistant SW620 cells. The cell apoptotic rate in Cisp-resistant SW620 cells (37.82±3.63%) is significantly higher than that of SW620 cells (16.78±2.56%) (p<0.05)<sup>[2]</sup>.

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

#### In Vivo

After administration of 4 mg/kg Salinomycin (Sal), 8 mg/kg Salinomycin and 10 uL/g saline water for 6 weeks, the mice are sacrificed. The size of the liver tumors in the Salinomycin treatment groups diminishes compare with the control group. The mean diameter of the tumors decreases from 12.17 mm to 3.67 mm (p<0.05) and the mean volume (V=length×width²×0.5) of the tumors decreases from 819 mm³ to 25.25 mm³ (p<0.05). Next, the tumors are harvested, followed by HE staining, immunohistochemistry, and TUNEL assays, to assess the anti-tumor activity of Salinomycin. HE staining shows that the structure of the liver cancer tissue:nuclei of different sizes, hepatic cord structure is destroyed. Immunohistochemistry shows that PCNA expression is lower after Salinomycin treatment. HE staining and TUNEL assays indicates the Salinomycin-treated groups has higher apoptosis rates than control. Furthermore, immunohistochemistry shows an increased Bax/Bcl-2 ratio after Salinomycin treatment. The protein expression of  $\beta$ -catenin decreases in the Salinomycin treatment groups compared with control [4].

Salinomycin is a kind of monocarboxylic acid polyether type antibiotics, produced by the fermentation of Streptomyces albus, possesses a specific cyclic structure, and can form a complex compound with the pathogenic microorganisms and the extracellular cations of coccidian, especially K<sup>+</sup>, Na<sup>+</sup>, Rb<sup>+</sup>, to alter the intracellular and extracellular ion concentrations<sup>[5]</sup>. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## **PROTOCOL**

#### Cell Assay [2]

For cisplatin or Salinomycin IC $_{50}$  analysis in SW620 cells or Cisp-resistant SW620 cells, cells (1×10<sup>4</sup>/well) are cultured in 96-well plates and treated with different chemotherapeutics (cisplatin, Salinomycin) in different concentrations for 48 h. Then 20  $\mu$ L of cell counting kit-8 (CCK-8) is added into each of the 96-wells. After 4 h incubation at 37°C, the optical density (OD) values are detected at 450 nm using the scan reader. Cell growth inhibiting rates are described as cell inhibiting curves and the IC $_{50}$  parameters (inhibiting concentration of 50% cells) are evaluated by Xlfit 5.2 software. For cell proliferation analysis, SW620 cells or Cisp-resistant SW620 cells (5×10<sup>3</sup>/well) are also seeded in 96-well plates in serum-containing medium and treated with cisplatin (5  $\mu$ M, according to the calculated IC50 values of cisplatin in SW620 cells) for 0, 12, 24, 48, 72 and 96 h. Then 20  $\mu$ L cell counting kit-8 is added into each of the 96-wells. After 4-h incubation at 37°C, the coloring reactions are also quantified at 450 nm<sup>[2]</sup>.

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# Animal Administration [3][4]

#### Mice<sup>[3]</sup>

Nude mice (nu/nu; 4-6 weeks of age) are used. HepG2 cells are suspended in 100 mL 1:1 serum-free DMEM and Matrigel. Mice are anesthetized with ketamine/xylazine and after surgically opening the abdomen, HepG2 cells are inoculated into the liver parenchyma and mice are monitored every 3 days for 35 days. Finally, 18 nude mice are divided into three groups that are intraperitoneally injected daily for 6 weeks: two Salinomycin-treated groups (4 mg/kg Salinomycin group, 8 mg/kg Salinomycin group) and the control group (saline water group).

Rats<sup>[4]</sup>

A total of 10 male rats are used in the experiment. After a routine anesthesia, the abdomen is opened. After a resuspension of high glucose medium not containing serum DMEM, and matrigel, the bladder transitional cancer cell line T24 is inoculated in the parenchyma of bladder in rats, and then the abdomen is sutured. After operation, the rats are randomized into the experiment group and the control group with five in each group. After operation, the rats in the experiment group

are immediately given intraperitoneal injection of Salinomycin with a dosage of 8 mg/kg, while the rats in the control group are given intraperitoneal injection of normal saline. A close observation is paid during the drug administration period. After 15 d, the rats are sacrificed by cervical dislocation, and the complete tumor tissues are stripped to observe the tumor growth and metastasis.

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## **CUSTOMER VALIDATION**

- EMBO Mol Med. 2019 Oct;11(10):e9930.
- J Control Release. 2020 Oct 10;326:387-395.
- Acta Biomater. 2022 Aug 23;S1742-7061(22)00501-3.
- Pharmacol Res. 2020 May;155:104751.
- Cell Death Dis. 2023 Mar 11;14(3):193.

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#### **REFERENCES**

[1]. Lu D, et al. Salinomycin inhibits Wnt signaling and selectively induces apoptosis in chronic lymphocytic leukemia cells. Proc Natl Acad Sci U S A. 2011 Aug 9;108(32):13253-7.

[2]. Zhou J, et al. Salinomycin induces apoptosis in cisplatin-resistant colorectal cancer cells by accumulation of reactiveoxygen species. Toxicol Lett. 2013 Oct 24;222(2):139-45.

- [3]. Wang F, et al. Salinomycin Inhibits Proliferation and Induces Apoptosis of Human Hepatocellular Carcinoma Cells In Vitro and In Vivo. PLoS One. 2012; 7(12): e50638.
- [4]. Qu H, et al. Effect of salinomycin on metastasis and invasion of bladder cancer cell line T24. Asian Pac J Trop Med. 2015 Jul;8(7):578-82.
- $[5]. \ Klose\ J, et\ al.\ Salinomycin: Anti-tumor\ activity\ in\ a\ pre-clinical\ colorectal\ cancer\ model.\ PLoS\ One.\ 2019\ Feb\ 14;14(2):e0211916.$
- [6]. Naujokat C, et al. Salinomycin as a drug for targeting human cancer stem cells. J Biomed Biotechnol. 2012;2012:950658.

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

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