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Ouabain Octahydrate

Cat. No.: HY-B0542

CAS No.: 11018-89-6

Molecular Formula: $C_{29}H_{60}O_{20}$

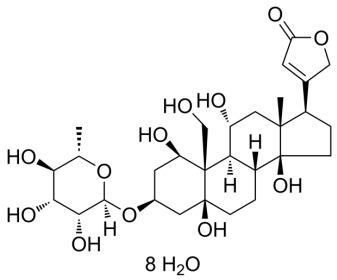
Molecular Weight: 728.77

Target: Na+/K+ ATPase; Autophagy

Pathway: Membrane Transporter/Ion Channel; Autophagy

Storage:

Powder	-20°C	3 years
	4°C	2 years
In solvent	-80°C	1 year
	-20°C	6 months



SOLVENT & SOLUBILITY

In Vitro

DMSO : ≥ 125 mg/mL (171.52 mM)

H_2O : 10 mg/mL (13.72 mM; ultrasonic and warming and heat to 60°C)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	1.3722 mL	6.8609 mL	13.7218 mL
5 mM	0.2744 mL	1.3722 mL	2.7443 mL
10 mM	0.1372 mL	0.6861 mL	1.3722 mL

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

In Vivo

1. Add each solvent one by one: PBS
   Solubility: 12.5 mg/mL (17.15 mM); Clear solution; Need ultrasonic and warming and heat to 60°C
2. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.08 mg/mL (2.85 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.08 mg/mL (2.85 mM); Clear solution
4. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.08 mg/mL (2.85 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

Ouabain Octahydrate is an inhibitor of Na^+/K^+-ATPase, used for the treatment of congestive heart failure.

In Vitro

Ouabain (100 μM) induces NLRP3 inflammasome activation and IL-1β release in macrophages. Ouabain-induced NLRP3

	inflammasome activation is mediated through K^+ efflux[1]. Ouabain (3 nM) alters the expression of EMT markers in NHK and ADPKD cells, and modifies cell-cell adhesion properties in ADPKD. Moreover, ouabain enhances migration of ADPKD cells, selectively modulates tight junctions, and modulates adherens junctions in ADPKD cells in a selective manner. Ouabain also activates TGFβ-Smad3 signaling, alters TER in ADPKD cells[2]. Ouabain (25, 50 or 100 nM) treatment significantly reduces cell proliferation and viability in Raji cells in a dose-dependent manner, with IC_{50} of 76.48±4.03 nM. Ouabain increases the number of apoptotic cells, induces autophagy, and upregulates Beclin-1 in Raji cells[4]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	Ouabain (3 mg/kg) significantly decreases cardiac contractile force with an enlarged LVESD when mice are primed with LPS. IL-1β deficiency attenuates ouabain-induced cardiac dysfunction and injury. IL-1β secreted by infiltrated macrophages contributes to ouabain-induced cardiac inflammation. Deficiency of NLRP3 and Casp1 attenuates ouabain-induced cardiac dysfunction and macrophage infiltration[1]. Ouabain (30 μg/kg, i.p.) modulates ABCB1 activity in thymocytes of Wistar rats and it has the same effect on Swiss mice at 300 μg/kg. After 14 days of ouabain treatment, the MAP of rats is significantly elevated[3]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Cell Assay [4]	Cell viability is determined using a Cell Counting Kit-8 assay. Briefly, 100 μL Raji cells (5×10^4/mL) are seeded in triplicate in a 96-well plate and treated with various concentrations of ouabain (400, 200, 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78 and 0.39 nM) for 48 h. Following the 48-h treatment, 10 μL CCK-8 reagent is added to each well, and the cells are incubated for an additional 3 h at 37°C. Optical density (OD) values at 450 nm are subsequently measured, and each ouabain concentration is assessed in triplicate. Raji cells cultured in medium without drug served as controls. Cell viability is calculated according to the following formula: Inhibition rate (%)=[1 − ($OD_{450(sample)}$ − $OD_{450(blank)}$)/($OD_{450(control)}$ − $OD_{450(blank)}$)] × 100. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [3]	Mice[3] A total of 8 mice are used in the present study, 4 in the control group and 4 in the ouabain-treated group. Animals are maintained under standard laboratory conditions, with room temperature controlled (22°C), and subjected to 12 h light-dark cycles with ad libitum access to food and water. At 24 h subsequent to the intraperitoneal injection with 300 μg/kg of ouabain or PBS, the Swiss mice are sacrificed by barbiturate overdose (86 mg/kg intraperitoneal injection of pentobarbital). The mesenteric lymph nodes and thymi are immediately removed and softly dissociated. The remaining cells are washed in PBS and centrifuged at 200 × g. The pellet is suspended in ice-cold RPMI-1640 culture medium supplemented with 10% FBS until required for the activity assays. Rat[3] Male Wistar rats are treated with daily intraperitoneal injections of 30 μg/kg of ouabain or its vehicle, phosphate-buffered saline (PBS). A total of 20 rats are used, 12 for acute treatment (n=6 rats/group in ouabain and control groups) and 8 for chronic treatment (n=4 rats/group in ouabain and control groups). Animals are maintained under standard laboratory conditions, with room temperature controlled (22°C), and subjected to 12 h light-dark cycles with ad libitum access to food and water. Prior to the first injection at 24 h and 7 and 14 days subsequent to the injection, the rats have their blood pressure measured by a computerized tail-cuff method. The animals are sacrificed by barbiturate overdose (86 mg/kg intraperitoneal injection of pentobarbital) after 24 h (acute treatment) or 14 days (chronic treatment) of ouabain injections, and the mesenteric lymph nodes, thymi and blood are collected. Full excisions of thymi and partial excisions of mesentheric lymph nodes are performed, while blood samples are collected by caudal venous puncture prior to animals sacrifice. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Emerg Microbes Infect. 2022 Dec;11(1):483-497.

- Br J Pharmacol. 2021 Jul 7.
- Life Sci. 2023 Dec 4, 122326.
- Biochem Pharmacol. 2022 Jul 21;115184.
- Int J Mol Sci. 2023 Feb 16;24(4):4000.

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REFERENCES

[1]. Kobayashi M, et al. The cardiac glycoside ouabain activates NLRP3 inflammasomes and promotes cardiac inflammation and dysfunction. PLoS One. 2017 May 11;12(5):e0176676.

[2]. Venugopal J, et al. Ouabain promotes partial epithelial to mesenchymal transition (EMT) changes in human autosomal dominant polycystic kidney disease (ADPKD) cells. Exp Cell Res. 2017 Jun 15;355(2):142-152.

[3]. Lima DB, et al. Ouabain-induced alterations in ABCB1 of mesenteric lymph nodes and thymocytes of rats and mice. Oncol Lett. 2016 Dec;12(6):5275-5280.

[4]. Meng L, et al. Ouabain induces apoptosis and autophagy in Burkitt's lymphoma Raji cells. Biomed Pharmacother. 2016 Dec;84:1841-1848.

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