Andrographolide

Cat. No.: HY-N0191

CAS No.: 5508-58-7

Molecular Formula: $C_{20}H_{30}O_5$

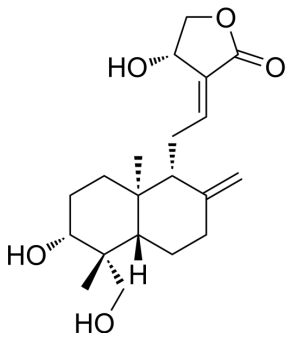
Molecular Weight: 350.45

Target: NF-κB; Autophagy; Influenza Virus; SARS-CoV; Parasite

Pathway: NF-κB; Autophagy; Anti-infection

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 : 100 mg/mL (285.35 mM; Need ultrasonic)

H_2O : 0.1 mg/mL (0.29 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.8535 mL	14.2674 mL	28.5347 mL
	5 mM	0.5707 mL	2.8535 mL	5.7069 mL
	10 mM	0.2853 mL	1.4267 mL	2.8535 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 (7.13 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (7.13 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (7.13 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

Andrographolide is a NF-κB inhibitor, which inhibits NF-κB activation through covalent modification of a cysteine residue on p50 in endothelial cells without affecting IκBα degradation or p50/p65 nuclear translocation. Andrographolide has antiviral effects.

IC_{50} & Target

p50

In Vitro	Andrographolide (AP) concentration-dependently suppresses receptor activator of nuclear factor kappa B ligand (RANKL)-mediated osteoclast differentiation and bone resorption in vitro and reduces the expression of osteoclast-specific markers. Andrographolide attenuates inflammation by inhibition of TNFα-induced NF-κB activation through covalent modification of reduced Cys^{62} of p50, without affecting IκBα degradation or p50/p65 nuclear translocation. Andrographolide also inhibits the ERK/MAPK signalling pathway without affecting p38 or JNK signalling. Andrographolide inhibits osteoclast differentiation of RAW 264.7 cells in a concentration-dependent manner. Andrographolide suppresses osteoclast formation in a concentration-dependent manner without any obvious cytotoxic effects, in both BMMs and RAW 264.7 cells. Andrographolide treatment substantially reduces the area of bone resorption. Only approximately 30% of the bone resorption observed in the control group is achieved after treatment with 2.5 μM Andrographolide. Osteoclastic bone resorption is almost completely inhibited after treatment with 10 μM Andrographolide[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	Treatment with Andrographolide (5 or 30 mg/kg) reduces the extent of bone loss induced by LPS. Moreover, Andrographolide slightly increases the BMD and cortex thickness compared to LPS treatment. Histological examination confirms the protective effects of Andrographolide on LPS-induced bone loss. LPS injection leads to inflammatory bone erosion and increased numbers of TRAP-positive osteoclasts[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	In vitro osteoclastogenesis assays are preformed to examine the effects of Andrographolide on osteoclast differentiation. Bone marrow macrophages (BMM) cells are prepared. Briefly, cells extracted from the femur and tibiae of a 6-week-old C57/BL6 mouse are incubated in complete cell culture media and 30 ng/mL M-CSF in a T-75 cm^2 flask for proliferation. When changing the medium, the cells are washed in order to deplete residual stromal cells. After reaching 90% confluence, cells are washed with PBS three times and trypsinized for 30 min to harvest BMMs. Cells adhering to the bottom of the dish are classified as BMMs; these BMMs are plated in 96-well plates at a density of 8×10^3 cells per well in triplicate and incubated in a humidified incubator containing 5% CO_2 at 37°C for 24 h. The cells are then treated with various concentrations of Andrographolide (0, 2.5, 5, or 10 μM) plus M-CSF (30 ng/mL) and RANKL (50 ng/mL). After 5 days, cells are fixed and stained for tartrate-resistant acid phosphatase (TRAP) activity. TRAP-positive multinucleated cells with more than five nuclei are counted as osteoclasts[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	Effects of Andrographolide on cell proliferation are determined with a CCK-8. BMMs are plated in 96-well plates at a density of 3×10^3 cells per well in triplicate. Twenty-four hours later, the cells are treated with increasing concentrations of Andrographolide (0, 2.5, 5, 10 or 20 μM) for 2 days. Next, 10 μL CCK-8 is added to each well, and the plates are then incubated at 37°C for an additional 2 h. The optical density (OD) is then measured with an ELX800 absorbance microplate reader at a wavelength of 450 nm (650 nm reference). The cell viability is calculated[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [1]	Mice[1] C57BL/6 mice (8 weeks old) are divided into four groups of seven mice each. Mice are injected i.p. with Andrographolide (5 or 30 mg/kg body weight) or PBS as a control 1 day before injection of LPS (5 μg/g body weight). Andrographolide or PBS is injected intraperitoneally every other day for 8 days. LPS is injected intraperitoneally on days one and four. All mice are killed 8 days after the initial LPS injection, and the left femurs of all animals are scanned with a high-resolution micro-CT at a resolution of 9 μm. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Nucleic Acids Res. 2021 Jan 8;49(D1):D1113-D1121.
- Int J Pharm. 2022 Nov 1;122361.
- Front Pharmacol. 2021 Mar 16;12:653035.
- J Cell Mol Med. 2019 Aug;23(8):5518-5531.
- Front Microbiol. 2018 Oct 8;9:2407.

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REFERENCES

[1]. Zhai ZJ, et al. Andrographolide suppresses RANKL-induced osteoclastogenesis in vitro and prevents inflammatory bone loss in vivo. Br J Pharmacol. 2014 Feb;171(3):663-75.

[2]. Gupta S, et al. Broad-spectrum antiviral properties of andrographolide. Arch Virol. 2017 Mar;162(3):611-623.

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