Aloxistatin

Cat. No.: HY-100229

CAS No.: 88321-09-9

Molecular Formula: $C_{17}H_{30}N_2O_5$

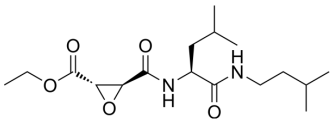
Molecular Weight: 342

Target: Cathepsin; SARS-CoV

Pathway: Metabolic Enzyme/Protease; Anti-infection

Storage:

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



SOLVENT & SOLUBILITY

In Vitro

DMSO : 125 mg/mL (365.50 mM; Need ultrasonic)

Ethanol : ≥ 33.33 mg/mL (97.46 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.9240 mL	14.6199 mL	29.2398 mL
5 mM	0.5848 mL	2.9240 mL	5.8480 mL
10 mM	0.2924 mL	1.4620 mL	2.9240 mL

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

In Vivo

1. Add each solvent one by one: 10% EtOH >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (7.31 mM); Clear solution
2. Add each solvent one by one: 10% EtOH >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (7.31 mM); Clear solution
3. Add each solvent one by one: 10% EtOH >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (7.31 mM); Clear solution
4. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: 2.08 mg/mL (6.08 mM); Suspended solution; Need ultrasonic
5. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.08 mg/mL (6.08 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

Aloxistatin (E64d) is a cell-permeable and irreversible broad-spectrum cysteine protease inhibitor. Aloxistatin (E64d) exhibits

	entry-blocking effect for MERS-CoV.
IC_{50} & Target	Cysteine protease[1]
In Vitro	Inhibition of protease-resistant prion protein (PrP-res) accumulation in ScNB cells by cysteine protease inhibitor Aloxistatin (E64d) with IC_{50} of 0.5±0.11 μM. For the cell surface PrP-sen detection, PrP-sen is immunoprecipitated from media treated with phosphatidylinositol-specific phospholipase C (PIPLC) to release pulse-^{35}S-labeled PrP-sen from the cell surface. Aloxistatin is maintained at 15 μM, respectively, in the labeling media of all but the control cells [1]. Aloxistatin (E64d) (which specifically blocks cysteine proteases, but not serine proteases such as granzymes) is able to completely block turnover of the CatL substrate Z-Phe-Arg-aminomethylcoumarin, when pre-incubated with NK-92 or YT 5 cells[2]. Aloxistatin (E64d) is a broad-spectrum cell-permeable inhibitor of cysteine proteases[3]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	Oral administration of Aloxistatin (E64d) to guinea pigs results in a dose-dependent reduction in brain, CSF and plasma A β(40) and Aβ(42). Aloxistatin also causes a biphasic dose-dependent reduction in brain CTFβ. Aloxistatin causes a dose-dependent increase in brain sAβPPα. The mean sAβPPα levels are significantly higher than the no dose group for Aloxistatin doses of 5 mg/kg/day or greater with the highest Aloxistatin dose resulting in the maximum increase in sAβPPα of about 54% more than the control group. Similar to the Aβ effect, oral Aloxistatin administration produces a biphasic dose-dependent reduction in brain cathepsin B activity. The minimum effective dose is about 1 mg/kg/day with the highest Aloxistatin dose causing the maximum reduction in brain cathepsin B activity of about 91% lower than that of the control group. Thus, Aloxistatin reduces guinea pig brain cathepsin B activity in a manner which is consistent with the compound inhibiting cathepsin B β-secretase activity[4]. Aloxistatin (E64d) inhibits the increases in the expression of $AT_{1A}R$ and ACE genes in rats. Administration of RNH-6270 or Aloxistatin reduces the increase in the superoxide production of the intramyocardial coronary arteries in HF rats[5]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Cell Assay [3]	Cell proliferation and apoptosis are assessed by staining for a proliferation marker, Ki67, or an apoptotic marker, cleaved caspase 3, following the protocol described above for the polarity markers. MCF10 variants are grown in 3D rBM overlay cultures for 4 days and are treated with 0.1 % DMSO, 5 μM CA074Me or 5 μM Aloxistatin. The percentage of structures that are positive for Ki67 or cleaved caspase 3 is determined by counting a total of 100 structures on two separate coverslips with a Zeiss Axiophot epifluorescent microscope. Structures are considered Ki67 positive if they contained at least one cell staining for Ki67. Structures are considered to be caspase 3 positive if they contained at least one cell that is positive for cleaved caspase 3 and the positive cell(s) is not localized in the center of a developing lumen[3]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [4][5]	Mice and Pigs[4] Guinea Pigs (male, Hartley strain, average weight 400 g corresponding to animals about 6 weeks old) are used. Male transgenic mice expressing human AβPP containing the wt β-secretase site and the London mutant β-secretase site sequences are used. Delivering a drug by gavage offers the advantage of accurate dosing but is traumatic and thus only suitable for relatively short dosing periods (up to about a week). Delivery by gavage is used for the guinea pig studies. Aloxistatin is suspended in Me2SO at the indicated concentrations (0.1, 1.0, 5, and 10 mg/kg) and administered by gavage daily using a feeding tube. Vehicle control animals are treated by gavage of Me2SO alone. Rats[5] Male inbred DS rats are used. Weaned rats are fed laboratory chow containing 0.3% NaCl until 7 weeks of age. DS rats fed an 8% NaCl diet after 7 weeks manifest compensated concentric left ventricular (LV) hypertrophy secondary to hypertension at 12 weeks and a distinct stage of fatal LV failure with lung congestion at 19 weeks. DS rats are therefore fed an 8% NaCl diet from 7 weeks of age and are randomized to an HF group, an Aloxistatin group (10 mg per kg of body mass per day, administered intraperitoneally every other day), or an RNH-6270 group (3 mg/kg per day in chow) from 12 to 19 weeks of age (n=10 for each group). The doses of RNH-6270 (an ARB) and Aloxistatin are determined in preliminary experiments and previous studies. DS rats maintained on the 0.3% NaCl diet served as age-matched controls (control group, n=10). At 19

weeks of age, all of the rats are euthanized by an intraperitoneal overdose of NSC 10816 (50 mg/kg), and the hearts are removed for biological and histological analyses. Arterial blood is collected from the abdominal aorta for the measurement of renin activity. Systolic blood pressure and heart rate are measured in conscious rats from 7 weeks of age, every week, using a noninvasive tail-cuff method. In separate experiments, 12-week-old DS rats, fed a low-salt diet from 7 weeks of age, are given vehicle, RNH-6270, or Aloxistatin in the same manner as in the above experiments (n=5 for each group), and the LV tissues for measuring targeting mRNAs and protein levels are immediately placed in liquid nitrogen and stored at -80°C.
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Cancer Cell. 2021 Mar 8;39(3):423-437.e7.
- Signal Transduct Target Ther. 2021 Mar 27;6(1):134.
- Cell Discov. 2021 Dec 14;7(1):119.
- Nat Commun. 2024 Jan 2;15(1):162.
- Nat Commun. 2021 Sep 17;12(1):5498.

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[1]. Doh-Ura K, et al. Lysosomotropic agents and cysteine protease inhibitors inhibit scrapie-associated prion protein accumulation. J Virol. 2000 May;74(10):4894-7.

[2]. Konjar S, et al. Human and mouse perforin are processed in part through cleavage by the lysosomal cysteine proteinase cathepsin L. Immunology. 2010 Oct;131(2):257-67.

[3]. Mullins SR, et al. Three-dimensional cultures modeling premalignant progression of human breast epithelial cells: role of cysteine cathepsins. Biol Chem. 2012 Dec;393(12):1405-16.

[4]. Hook G, et al. The cysteine protease inhibitor, E64d, reduces brain amyloid-β and improves memory deficits in Alzheimer's disease animal models by inhibiting cathepsin B, but not BACE1, β-secretase activity. J Alzheimers Dis. 2011;26(2):387-408.

[5]. Cheng XW, et al. Superoxide-dependent cathepsin activation is associated with hypertensive myocardial remodeling and represents a target for angiotensin II type 1 receptor blocker treatment. Am J Pathol. 2008 Aug;173(2):358-69.

[6]. Ji Yeun Kim, et al. Safe, High-Throughput Screening of Natural Compounds of MERS-CoV Entry Inhibitors Using a Pseudovirus Expressing MERS-CoV Spike Protein. Int J Antimicrob Agents. 2018 Nov;52(5):730-732.

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