# Aloxistatin

**Cat. No.:** HY-100229

**CAS No.:** 88321-09-9

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

**Molecular Weight:** 342.43

**Storage:**

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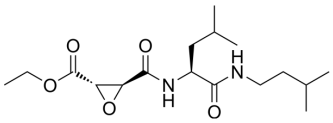
| Powder | -20°C | 3 years |
| | 4°C | 2 years |
| In solvent | -80°C | 2 years |
| | -20°C | 1 year |



## SOLVENT & SOLUBILITY

**In Vitro**

DMSO : 125 mg/mL (365.04 mM; Need ultrasonic)

Ethanol : ≥ 33.33 mg/mL (97.33 mM)

* "≥" means soluble, but saturation unknown.

**Preparing Stock Solutions**

| Concentration \ Solvent \ Mass | 1 mg | 5 mg | 10 mg |
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| 1 mM | 2.9203 mL | 14.6015 mL | 29.2030 mL |
| 5 mM | 0.5841 mL | 2.9203 mL | 5.8406 mL |
| 10 mM | 0.2920 mL | 1.4602 mL | 2.9203 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 (7.30 mM); Clear solution
2. Add each solvent one by one: 10% EtOH >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (7.30 mM); Clear solution
3. Add each solvent one by one: 10% EtOH >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (7.30 mM); Clear solution
4. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: 2.08 mg/mL (6.07 mM); Suspended solution; Need ultrasonic
5. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.08 mg/mL (6.07 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.

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| **$IC_{50}$ & 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].<br>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].<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |

## PROTOCOL

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| **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].<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |
| **Animal Administration** [4][5] | Mice and Pigs[4]<br>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.<br>Rats[5]<br>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 |

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| | 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.<br>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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