

## Product Data Sheet

Product Name: L-NMMA acetate

Cat. No.: GC16926

### Chemical Properties

Cas. No. 53308-83-1

Chemical Name (S,E)-2-amino-5-(2-methylguanidino)pentanoic acid compound with acetic acid (1:1)

SMILES OC([C@H](CCCN/C(N)=N/C)N)=O.O=C(C)O

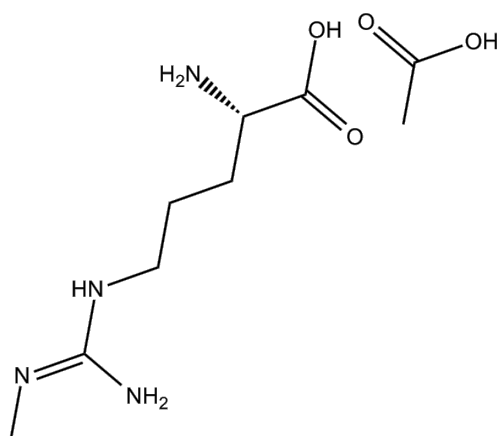
Formula  $C_7H_{16}N_4O_2 \cdot CH_3CO_2H$  M.Wt 248.28

Solubility DMSO: .25 mg/ml, Ethanol: 1 mg/ml, PBS (pH 7.2): 10 mg/ml Storage Store at -20°C

General tips For obtaining a higher solubility, please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution: ship with blue ice All other available size: ship with RT, or blue ice upon request.

Structure



### Protocol

#### Cell experiment [1]:

Cell lines MDA-MB-231 cells

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MDA-MB-231 cells were maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic. Cells were treated daily with N-[[3-(aminomethyl)phenyl]methyl]-ethanimidamide (1000, 2000, 4000 $\mu$ M), L-NMMA acetate (1000, 2000, 4000 $\mu$ M) for 96 hours. The proliferation of MDA-MB-231 cell was determined by adding premixed WST-1 reagent. Confluent cells were treated in starvation conditions (1% serum) for 72 hours. The medium was then changed by regular growth medium in the presence of inhibitors for 24 hours. A 'wound' was made in the cell monolayer using a 100- $\mu$ L pipette tip. Pictures were taken at 0 and 14 hours. Data were replicated in three independent experiments.

Preparation Method

Reaction Conditions      1000, 2000, 4000 $\mu$ M; 96h

Applications      L-NMMA acetate treatment significantly inhibited the proliferation and migration of the MDA-MB-231 cells.

### Animal experiment [2]:

Animal models      Three-colored guinea pigs of British species

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### Preparation Method

Clinical examinations before the experiment were performed to ensure that all guinea pigs did not have eye diseases, such as cataract, keratitis, and spontaneous myopia. The environment temperature of the laboratory was maintained at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  with a circadian rhythm of 12h/12h (day/night alteration). All the experimental guinea pigs were treated in accordance with the ethics for animal experiments and were guaranteed to have sufficient nutrient-rich feed every day. Fresh vegetables were supplemented to guinea pigs twice a day to provide vitamin C. The guinea pigs were divided into four groups (six animals in each group): a normal control (NC) group, a lens-induced myopia (LIM) group, a NOS inhibitor (L-NMMA acetate) injection group, and a NOS inhibitor solvent phosphate-buffered saline (PBS, pH7.2) group. The guinea pigs in the NC group were normally housed with no treatment, whereas the guinea pigs in the LIM group, L-NMMA group, and PBS group were fitted with  $-6.0\text{D}$  lenses on their right eyes to induce myopia. The pigs in the L-NMMA acetate group received  $5\mu\text{L}$  ( $0.02\mu\text{M}$ ) of L-NMMA acetate once every other day (3 times a week) through an intravitreal injection, and the pigs in the PBS group received only  $5\mu\text{L}$  of sterilized  $1 \times \text{PBS}$ . To ensure the success of the experiment, all the lenses were cleaned every morning and evening, and replaced immediately if scratches appeared. After 4 weeks of myopia induction, 3 guinea pigs in each group were randomly selected and anesthetized with  $10\text{g/L}$  of pentobarbital sodium by intraperitoneal injection. The blood and hair of guinea pig were rinsed with sterile  $0.9\%$  NaCl solution. Subsequently, the fascial tissues around the eye were carefully peeled off by sterile ophthalmic scissors and the eyeball was taken out immediately and put into the sampling tube containing fixative solution for 24h of routine dehydration. Next, paraffin embedding was processed and sectioned into  $3\text{-}\mu\text{m}$  sections for histopathological assessment and Masson Staining.

### Dosage form

$0.02\mu\text{M}/5\mu\text{L}$ , once every other day for 4 weeks; intravitreal injection

### Applications

L-NMMA acetate treatment significantly alleviated choroidal fibrosis in myopic guinea pigs.

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### References:

- [1] Granados-Principal S, Liu Y, Guevara M L, et al. Inhibition of iNOS as a novel effective targeted therapy against triple-negative breast cancer[J]. Breast Cancer Research, 2015, 17(1): 25.
- [2] Li T, Bao B, Hao Y, et al. Suppressive effect of nitric oxide synthase (NOS) inhibitor L-NMMA acetate on choroidal fibrosis in experimental myopic guinea pigs through the nitric oxide signaling pathway[J]. European Journal of Pharmacology, 2023, 960: 176111.

### Background

L-NMMA acetate is a potent inhibitor of Nitric-Oxide Synthase (NOS), with an IC<sub>50</sub> of 2μM<sup>[1]</sup>. L-NMMA acetate has been widely used to regulate the vascular tension and blood pressure of arterial rings in animals<sup>[2]</sup>.

In vitro, L-NMMA acetate treatment at 4000μM for 96 hours significantly reduced the proliferation and migration of the MDA-MB-231 and SUM159 cell lines<sup>[3]</sup>. L-NMMA acetate treatment (4000μM) for 72 hours inhibited the upregulation of inducible nitric oxide synthase (iNOS) induced by docetaxel in MDA-MB-468 cells and enhanced the Docetaxel-induced cell apoptosis<sup>[4]</sup>. L-NMMA acetate treatment (10000μM) for 42 hours significantly inhibited the expansion of porcine cumulus cells, without affecting nuclear or cytoplasmic maturation<sup>[5]</sup>.

In vivo, L-NMMA acetate treatment via intravitreal injection (0.02μM/5μL; once every other day) for 4 weeks inhibited the NO signaling pathway and slowed down the progression of choroidal fibrosis in myopic guinea pigs<sup>[6]</sup>. In endotoxemic dogs, 22-hours intravenous infusion of L-NMMA acetate (10mg/kg/h) increased systemic and pulmonary vascular resistance, marginally increased mean arterial pressure, and decreased oxygen delivery<sup>[7]</sup>.

### References:

- [1] Jung C S, Lange B, Zimmermann M, et al. Role of endogenous monomethylated L-arginine (L-NMMA) after subarachnoid hemorrhage[J]. Neurological Research, 2013, 35(7): 709-712.
- [2] Rees D D, Palmer R M, Schulz R, et al. Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo[J]. British journal of pharmacology, 1990, 101(3): 746.
- [3] Granados-Principal S, Liu Y, Guevara M L, et al. Inhibition of iNOS as a novel effective targeted

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- therapy against triple-negative breast cancer[J]. Breast Cancer Research, 2015, 17(1): 25.
- [4] Dávila-González D, Choi D S, Rosato R R, et al. Pharmacological inhibition of NOS activates ASK1/JNK pathway augmenting docetaxel-mediated apoptosis in triple-negative breast cancer[J]. Clinical Cancer Research, 2018, 24(5): 1152-1162.
- [5] Romero-Aguirregomez J, Santa Á P, García-Vázquez F A, et al. Nitric oxide synthase (NOS) inhibition during porcine in vitro maturation modifies oocyte protein S-nitrosylation and in vitro fertilization[J]. PLoS One, 2014, 9(12): e115044.
- [6] Li T, Bao B, Hao Y, et al. Suppressive effect of nitric oxide synthase (NOS) inhibitor L-NMMA acetate on choroidal fibrosis in experimental myopic guinea pigs through the nitric oxide signaling pathway[J]. European Journal of Pharmacology, 2023, 960: 176111.
- [7] Cobb J P, Natanson C, Quezado Z M, et al. Differential hemodynamic effects of L-NMMA in endotoxemic and normal dogs[J]. American Journal of Physiology-Heart and Circulatory Physiology, 1995, 268(4): H1634-H1642.

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