

## Product Data Sheet

Product Name: Cl-amidine

Cat. No.: GC35706

### Chemical Properties

Cas. No. 913723-61-2

SMILES O=C(N[C@H](C(N)=O)CCCNC(CCl)=N)C1=CC=CC=C1

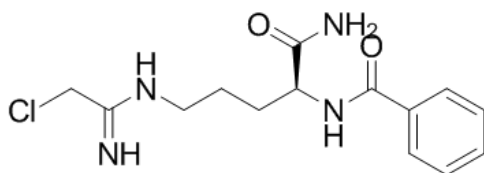
Formula  $C_{14}H_{19}ClN_4O_2$  M.Wt 310.78

Solubility Soluble in DMSO 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 HCT 116 wild-type (WT) colon cancer cells, HCT 116 p53<sup>-/-</sup> colon carcinoma cells, LS-180 colon cancer cells

**Caution: Product has not been fully validated for medical applications. For research use only.**

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HCT 116 WT and p53<sup>-/-</sup> cells were cultured in McCoy's medium supplemented with 10% Newborn Calf Serum, 2 mM glutamine, penicillin (10 U/ml) and streptomycin (10 µg/ml). LS-180 colon cancer cells were cultured in DMEM Medium supplemented with 10% Newborn Calf Serum, 2 mM glutamine, penicillin (10 U/mL) and streptomycin (10 µg/mL).

**Reaction Conditions** 1×10<sup>6</sup> were incubated in 6 well plates in McCoy's 5A medium 1 day before treatment and then treated with 50 µg/mL Cl-amidine. The cells were harvested and fixed with ice cold ethanol. After washing with 1x PBS containing 0.5% BSA twice, the cells were incubated with DNA staining solution consisting of Propidium Iodide (PI; 10 µg/mL) and RNase (0.1 mg/mL) for 30 min at room temperature in the dark.

**Applications** Cl-amidine could lead to a cell cycle arrest at the G1 phase through a p53-mediated mechanism. Cl-amidine could cause miRNA-16 induction in a p53-dependent manner.

### Animal experiment [2]:

**Animal models** Male C57BL/6J mice (18–26 g)

**Preparation Method** Mice were injected with intra-peritoneal Cl-amidine dissolved in DMSO, or vehicle DMSO 1 h after cecal ligation and puncture (CLP). DMSO was also injected into mice that were not subjected to CLP (n = 12/group). Mortality was monitored for 10 days after surgeries.

**Dosage form** 40 mg/kg

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**Applications** Cl-amidine protects mice from sepsis-induced lethality. The inhibition of PAD by Cl-amidine significantly suppresses the CitH3 production, while the CitH3 neutralization markedly improves the survival in septic mice.

### References:

- [1]. Cui X, et al. The induction of microRNA-16 in colon cancer cells by protein arginine deiminase inhibition causes a p53-dependent cell cycle arrest. PLoS One. 2013;8(1):e53791.
- [2]. Ting Z, et al. Protective effect of Cl-amidine against CLP-induced lethal septic shock in mice. Sci Rep. 2016; 6: 36696.

### Background

Cl-amidine (N- $\alpha$ -benzoyl-N5-(2-chloro-1-iminoethyl)-l-ornithine amide), is a PAD4 inactivator with enhanced potency. PAD Inhibitor was proved to exhibit enhanced cell killing in PAD-expressing osteosarcoma cells and block the formation of neutrophil extracellular traps. Cl-amidine, as a PAD inhibitor, could reduce the clinical signs and symptoms of colitis, and induced apoptosis of inflammatory cells. The inhibition of PAD by Cl-amidine markedly suppresses the production of CitH3, and neutralization of CitH3 improves survival markedly in septic mice.<sup>[1][2]</sup>

Cl-amidine is a more potent inhibitor of PAD which has the IC<sub>50</sub> of  $5.9 \pm 0.3 \mu\text{M}$ . In vitro experiment indicated that Cl-amidine caused a cell cycle arrest at the G1 phase and miRNA-16 induction through a p53-mediated mechanism. In vivo experiment demonstrated that the enhancement of the p300GBD–GRIP1 interaction mediated by PAD4 was antagonized by Cl-amidine in a dose-dependent manner, and that Cl-amidine treatment caused only a minimal reduction in the efficiency of the interaction, indicating that the inhibitory effect of this compound targeted at the active PAD4 enzyme.<sup>[1][3]</sup>

### References:

- [1]. Luo Y, et al. Inhibitors and inactivators of protein arginine deiminase 4: functional and structural characterization. Biochemistry. 2006 Oct 3;45(39):11727-36.
- [2]. Ting Z, et al. Protective effect of Cl-amidine against CLP-induced lethal septic shock in mice. Sci

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