

Product Data Sheet

Product Name: Gliotoxin
Cat. No.: GC17255

Chemical Properties

Cas. No. 67-99-2

Chemical Name (3R,5aS,6S,10aR)-6-hydroxy-3-(hydroxymethyl)-2-methyl-2,3,5a,6-tetrahydro-1H-3,10a-epidithiopyrazino[1,2-a]indole-1,4(10H)-dione

SMILES O[C@H]1C=CC=C2[C@@H]1N(C([C@@]3(N4C)CO)=O)[C@@](C4=O)(SS3)C2

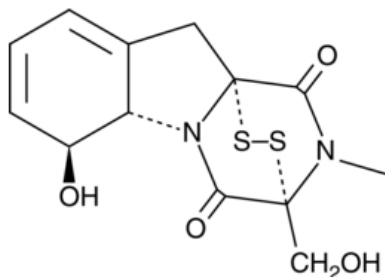
Formula $C_{13}H_{14}N_2O_4S_2$ M.Wt 326.38

Solubility 5mg/mL in DMSO, or in DMF Storage Store at -20°C, protect from light

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 A549 and L132 cells

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Preparation Method

A549 and L132 cells were seeded in 96 well plate at 5×10^3 cells/well and treated with different concentrations of Gliotoxin, FUM and combination of the two, dissolved in dimethyl sulfoxide (DMSO) for 24h, at 37°C. DMSO was used as the solvent control. After 24h incubation, 20µL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution (5mg/mL in PBS) was added to each well, and incubated for 3h at 37°C. The medium was then removed and the purple formazan product was dissolved in 100µL of DMSO. The absorbance was measured at 570nm (measurement) and 630nm (reference) using a 96-well plate EnSpire multimode reader. Data were collected for triplicates and used to calculate the respective means and the standard deviations.

Reaction Conditions

0, 2, 4, 6, 8, 10µM; 24h

Applications

Gliotoxin caused dose-dependent increase in cytotoxicity.

Animal experiment [2]:

Animal models

C57BL/6 mice

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Ten- to twelve-week-old male C57BL/6 mice were treated with hydrocortisone acetate (5mg/mouse, administered subcutaneously) every other day, beginning on day -4 relative to infection and ending on day +4, for a total of 5 doses. For inoculation, the mice were anesthetized with isoflurane, after which 5×10^6 conidia in 25 μ l of PBS containing 0.1% Tween-20 was instilled intranasally.

Preparation Method The mice were infected with *A. fumigatus* alone or with *A. fumigatus* mixed with exogenous 50ng/ml Gliotoxin. After 20h, the number of colonies in lung tissues (number of CFU/g) was also counted and calculated. For histopathological examination, the lung tissue sections obtained from mice from each group at 72h post-infection were dissected, fixed in 10% (vol/vol) formaldehyde, and stained with hematoxylin and eosin (HE) and periodic acid-Schiff (PAS).

Dosage form 1.25ng; 20, 72h; i.n.

Applications Exogenous Gliotoxin helped Gliotoxin synthesis deficient strain *gliP1* invade into the lung tissue and the lung fungal burden increased markedly in immunosuppressed mice.

References:

- [1] Gayathri, L., Akbarsha, M. A., & Ruckmani, K. (2020). In vitro study on aspects of molecular mechanisms underlying invasive aspergillosis caused by gliotoxin and fumagillin, alone and in combination. *Scientific reports*, 10(1), 14473.
- [2] Zhang, C., Chen, F., Liu, X., Han, X., Hu, Y., Su, X., Chen, Y., Sun, Y., & Han, L. (2019). Gliotoxin Induces Cofilin Phosphorylation to Promote Actin Cytoskeleton Dynamics and Internalization of *Aspergillus fumigatus* Into Type II Human Pneumocyte Cells. *Frontiers in microbiology*, 10, 1345.

Background

Gliotoxin, an immunosuppressive mycotoxin produced by pathogenic strains of *Aspergillus* and other

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fungi, is a specific inhibitor of 20S proteasomal chymotrypsin activity with an IC_{50} of $10\mu M$ ^[1]. Gliotoxin blocks the degradation of $I\kappa B\alpha$, thereby preventing the activation of NF- κB by inhibiting proteasome activity^[2]. Additionally, Gliotoxin inhibits the activity of farnesyltransferase (FTase) and geranylgeranyltransferase I (GGTase I), with IC_{50} values of $80\mu M$ and $17\mu M$, respectively^[3]. Gliotoxin can also inhibit phagocytosis by macrophages and the immune functions of other immune cells, and is commonly used in research related to immunology, inflammation, and tumors^{[4][5]}.

In vitro, A549 and L132 cells were treated with different concentrations of Gliotoxin (0, 2, 4, 6, 8, $10\mu M$; 24h) caused dose-dependent increase in cytotoxicity and the measured IC_{50} was 2.7 and $4.25\mu M$. In addition, treatment of A549 and L132 cells with IC_{50} concentrations of Gliotoxin for 6, 12, and 24 hours resulted a time-dependent increase in reactive oxygen species (ROS) levels^[6].

In vivo, exogenous Gliotoxin ($1.25ng$; 72h; intranasal administration) helped Gliotoxin synthesis deficient strain *gliP1* invade into the lung tissue and the lung fungal burden increased markedly in immunosuppressed mice^[7].

References:

- [1] Kroll, M., Arenzana-Seisdedos, F., Bachelierie, F., Thomas, D., Friguet, B., & Conconi, M. (1999). The secondary fungal metabolite gliotoxin targets proteolytic activities of the proteasome. *Chemistry & biology*, 6(10), 689–698.
- [2] Pahl, H. L., Krauss, B., Schulze-Osthoff, K., Decker, T., Traenckner, E. B., Vogt, M., Myers, C., Parks, T., Warring, P., Mühlbacher, A., Czernilofsky, A. P., & Baeuerle, P. A. (1996). The immunosuppressive fungal metabolite gliotoxin specifically inhibits transcription factor NF-kappaB. *The Journal of experimental medicine*, 183(4), 1829–1840.
- [3] Vigushin, D. M., Mirsaidi, N., Brooke, G., Sun, C., Pace, P., Inman, L., Moody, C. J., & Coombes, R. C. (2004). Gliotoxin is a dual inhibitor of farnesyltransferase and geranylgeranyltransferase I with antitumor activity against breast cancer in vivo. *Medical oncology (Northwood, London, England)*, 21(1), 21–30.
- [4] Schlam, D., Canton, J., Carreño, M., Kopinski, H., Freeman, S. A., Grinstein, S., & Fairn, G. D. (2016). Gliotoxin Suppresses Macrophage Immune Function by Subverting Phosphatidylinositol 3,4,5-Trisphosphate Homeostasis. *mBio*, 7(2), e02242.
- [5] Jia, X., Chen, F., Pan, W., Yu, R., Tian, S., Han, G., Fang, H., Wang, S., Zhao, J., Li, X., Zheng,

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D., Tao, S., Liao, W., Han, X., & Han, L. (2014). Gliotoxin promotes *Aspergillus fumigatus* internalization into type II human pneumocyte A549 cells by inducing host phospholipase D activation. *Microbes and infection*, 16(6), 491–501.

[6] Gayathri, L., Akbarsha, M. A., & Ruckmani, K. (2020). In vitro study on aspects of molecular mechanisms underlying invasive aspergillosis caused by gliotoxin and fumagillin, alone and in combination. *Scientific reports*, 10(1), 14473.

[7] Zhang, C., Chen, F., Liu, X., Han, X., Hu, Y., Su, X., Chen, Y., Sun, Y., & Han, L. (2019). Gliotoxin Induces Cofilin Phosphorylation to Promote Actin Cytoskeleton Dynamics and Internalization of *Aspergillus fumigatus* Into Type II Human Pneumocyte Cells. *Frontiers in microbiology*, 10, 1345.

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