

Product Data Sheet

Product Name: Hippuric acid (2-Benzamidoacetic acid)

Cat. No.: GC33778

Chemical Properties

Cas. No. 495-69-2

SMILES O=C(O)CNC(C1=CC=CC=C1)=O

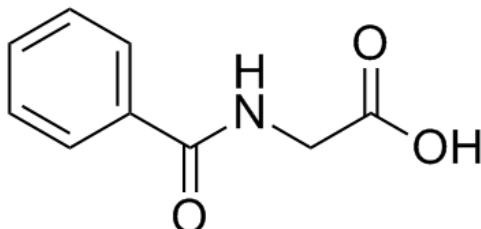
Formula C9H9NO3 M.Wt 179.17

Solubility DMSO : ≥ 100 mg/mL (558.13 mM) 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 RAW 264.7 cells and primary mouse bone marrow cells

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Preparation Method

Non adherent bone marrow cells from 4-week-old female C57BL/J mice were isolated. Bone marrow cells were flushed from femurs and then cultured in T175 Flasks for 2 days to let stromal cells attach. Two days later, non adherent cells were collected and cultured in appropriate plates with appropriate cell densities. These cells are considered as hematopoietic osteoclast progenitor cells. Conditional serum for treatment of cells was taken from female rats either fed 10% BB supplemental diet or standard rodent casein diet for 4 weeks. RAW 264.7 cells and primary mouse bone marrow cells were cultured in α -Minimum Essential Medium supplemented with 10% fetal bovine serum, penicillin (100units/ml), streptomycin (100 μ g/ml), and glutamine (4mM). Cells were seeded in 96-, 12-, or 6-well cell culture plates at appropriate density of cells per well for morphology, RNA, and protein expression experiments. At 85% confluence, cells in 96-well plates were treated with 2.5% rat serum (7.5% FBS) in the presence of 50ng/ml of soluble RANKL for osteoclastic cell morphologic study. RAW264.7 cells or nonadherent bone marrow cells were cultured in 96-well plates (2×10^4 cells/well) in the presence or absence of 50ng/ml of RANKL. Cells were treated with 60 μ g/dL Hippuric acid. After 4 days for RAW264.7 cell cultures and 5 days for bone marrow cell cultures, the cells were fixed with 4% paraformaldehyde and stained for tartrate-resistant acid phosphatase (TRAPase) activity using a TRAPase Staining Kit. TRAP-positive cells containing >3 nuclei in each well were counted as osteoclasts under an epifluorescent microscope. For osteoclast resorption activity assay, cells in the culture plates were fixed using 2.5% glutaraldehyde with or without Von Kossa staining. The areas of hydroxyapatite resorption were observed by light microscopy and analyzed using the Image J software. Cell viability was measured by MTT assay.

Reaction Conditions

60 μ g/dL; 4-5days

Applications

Treatment of RAW 264.7 cells and primary mouse bone marrow cells with Hippuric acid significantly inhibited osteoclastogenesis and osteoclast resorptive activity.

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Animal experiment [2]:

Animal models Male Sprague Dawley rats

Preparation Method Male Sprague Dawley rats at the age of 7 weeks were housed in a pathogen-free environment. The environment was that with a humidity of 50-70%, temperature of 22-24°C, and the day and night cycle was 12/12h. These rats were given a normal laboratory diet with ad libitum access to water. After 1 week of adaptation, fifty rats were subjected to either sham surgery (kidney exposure operation only) or 5/6NX surgery. The 5/6NX model commonly used to simulate typical CKD symptoms and study PGBT-promoted renal fibrosis. Two weeks after surgery (experimental week 1), excluding the rats that died during the operation, rats in the sham group ($n=9$) were treated with vehicle (0.5% DMSO), and 5/6NX rats were randomized into two groups ($n=9$ each) and treated as follows: (1) a 5/6NX group, 0.5% DMSO; (2) a 5/6NX + Hippuric acid group, 100mg/kg Hippuric acid. All treatments were administered intraperitoneally, five times per week for 10 weeks. Rats serum creatinine (SCr) and blood urea nitrogen (BUN) contents were evaluated using a BS-420 automated biochemical analyzer. Kidney samples were fixed for histological and immunohistochemical analyses. Total intracellular ROS and H₂O₂ concentrations were determined using commercial ROS and H₂O₂ assay kits.

Dosage form 100mg/kg; intraperitoneal injection; five times per week for 10 weeks

Applications Hippuric acid significantly increased serum creatinine and blood urea nitrogen levels, promoted renal fibrosis, and disrupted redox homeostasis in a 5/6 nephrectomy rat model of chronic kidney disease.

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References:

- [1] Zhao H, Lazarenko OP, Chen JR. Hippuric acid and 3-(3-hydroxyphenyl) propionic acid inhibit murine osteoclastogenesis through RANKL-RANK independent pathway. *J Cell Physiol.* 2020;235(1):599-610.
- [2] Sun B, Wang X, Liu X, et al. Hippuric Acid Promotes Renal Fibrosis by Disrupting Redox Homeostasis via Facilitation of NRF2-KEAP1-CUL3 Interactions in Chronic Kidney Disease. *Antioxidants (Basel).* 2020;9(9):783.

Background

Hippuric acid (2-Benzamidoacetic acid) is an orally active metabolite^[1]. Hippuric acid can be produced by the metabolism of polyphenols and benzoic acid by gut microbiota^[2]. Hippuric acid is usually used in the study of cardiovascular diseases, metabolic diseases, and inflammation/immunology^{[3][4]}.

In vitro, treatment of RAW 264.7 cells and primary mouse bone marrow cells with Hippuric acid (60µg/dL; 4-5days) significantly inhibited osteoclastogenesis and osteoclast resorptive activity^[5].

In vivo, Hippuric acid (100mg/kg; intraperitoneal injection; five times per week for 10 weeks) significantly increased serum creatinine and blood urea nitrogen levels, promoted renal fibrosis, and disrupted redox homeostasis in a 5/6 nephrectomy rat model of chronic kidney disease^[6]. Hippuric acid (150mg/kg/day; p.o.; 7 days) significantly reduced disease activity index (DAI) scores, colon shortening, and histological inflammation scores, increased colon length, and alleviated histopathological damage in the colon of DSS-induced colitis mice^[7].

References:

- [1] Nativelle C, Picard K, Valentin I, Lhuguenot JC, Chagnon MC. Metabolism of n-butyl benzyl phthalate in the female Wistar rat. Identification of new metabolites. *Food Chem Toxicol.* 1999;37(8):905-917.
- [2] Edwards SJ, Carter S, Nicholson T, et al. (-)-Epicatechin and its colonic metabolite hippuric acid protect against dexamethasone-induced atrophy in skeletal muscle cells. *J Nutr Biochem.* 2022;110:109150.

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- [3] Pero RW. Health consequences of catabolic synthesis of hippuric acid in humans. *Curr Clin Pharmacol.* 2010;5(1):67-73.
- [4] Santhakumar AB, Stanley R, Singh I. The ex vivo antiplatelet activation potential of fruit phenolic metabolite hippuric acid. *Food Funct.* 2015;6(8):2679-2683.
- [5] Zhao H, Lazarenko OP, Chen JR. Hippuric acid and 3-(3-hydroxyphenyl) propionic acid inhibit murine osteoclastogenesis through RANKL-RANK independent pathway. *J Cell Physiol.* 2020;235(1):599-610.
- [6] Sun B, Wang X, Liu X, et al. Hippuric Acid Promotes Renal Fibrosis by Disrupting Redox Homeostasis via Facilitation of NRF2-KEAP1-CUL3 Interactions in Chronic Kidney Disease. *Antioxidants (Basel).* 2020;9(9):783.
- [7] Yang Y, Huang S, Liao Y, et al. Hippuric acid alleviates dextran sulfate sodium-induced colitis via suppressing inflammatory activity and modulating gut microbiota. *Biochem Biophys Res Commun.* 2024;710:149879.

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