

Product Data Sheet

Product Name: ABC99
Cat. No.: GC42669

Chemical Properties

Cas. No. 2331255-53-7

Chemical Name 7-(4-chlorobenzyl)-1,3-dioxohexahydroimidazo[1,5-a]pyrazin-2(3H)-yl-2,3-dihydro-4H-benzoxo[b][1,4]oxazine-4-carboxylate

SMILES C1C=CC=C(CN2CC(C(N(OC(N3C(C=CC=C4)=C4OCC3)=O)C5=O)=O)N5CC2)C=C1

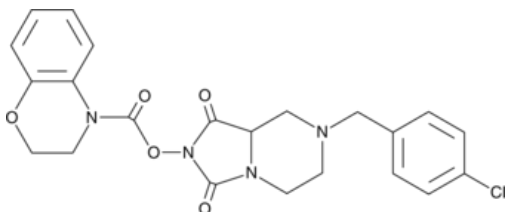
Formula C₂₂H₂₁ClN₄O₅ M.Wt 456.9

Solubility 1mg/mL in Chloroform, 1mg/mL in acetonitrile, 1mg/mL in methyl acetate 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 Human stem cells from the apical papilla (hSCAPs)

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Preparation Method Human stem cells from the apical papilla (hSCAPs) were recovered and cultured in a cell culture incubator under the conditions of 37°C with 5% CO₂ (v/v). The culture medium was refreshed every 3 days. The normal proliferation medium (PM) contained α-MEM, 10% fetal bovine serum, and 1% penicillin. When cells reached 80% confluency, odontoblastic medium (OM) contained α-MEM, 10% fetal bovine serum, 50mg/L L-Ascorbic acid, 5mmol/L sodium β-phosphoglycerate, and 10nmol/L dexamethasone, was used to induce odontoblastic differentiation. To down-regulate the extracellular NOTUM level, ABC99, a small molecule inhibitor of NOTUM, was added to the OM medium at 2ug/ml. Cells were collected on odontoblastic induction day 3, 7, and 14. Alkaline phosphatase (ALP) staining, Alizarin red (ARS) staining and qRT-PCR were performed according to the manufacturer's Instructions.

Reaction Conditions 2μg/mL; 7–14 days

Applications ABC99 suppressed odontoblastic differentiation of human stem cells from apical papilla (hSCAPs), reduced ALP activity, blocked mineralized nodule formation, and decreased DSPP, DMP1 and ALP mRNA levels.

Animal experiment [2]:

Animal models C57BL/6J mice

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8-week-old, specific-pathogen-free C57BL/6J mice were kept in an air-conditioned room at a temperature of $23 \pm 1^\circ\text{C}$, a relative humidity of 50–70%, and a 12h light/dark cycle. APKS tumor organoids were orthostatic implanted into the colonic mucosa of C57BL/6J mice under endoscopic guidance to establish an in situ colorectal cancer model. Mice were treated with

Preparation Method ABC99(10mg/kg/day; p.o.) or vehicle (corn oil) control after 4 weeks of engraftment and tumour growth. Mice are treated for indicated number of weeks (week 0-4 weeks post-implantation) (n=7 mice). Tumour weight, number of macrometastases in the liver and number of lymph node metastases in mice were qualified. Bioluminescent imaging (BLI) and Kaplan–Meier survival analysis were performed to monitor tumor burden and compare survival.

Dosage form 10mg/kg/day; p.o.; 4 weeks

Applications ABC99 almost completely prevented liver metastasis and lymph-node metastasis, reduced primary tumor weight in orthotopic colorectal cancer mice.

References:

[1] Zhao Q, Ren H, Wang N, Yuan X, Zhao Y, Wen Q. NOTUM plays a bidirectionally modulatory role in the odontoblastic differentiation of human stem cells from the apical papilla through the WNT/ β -catenin signaling pathway. *Arch Oral Biol.* 2024;160:105896.

[2] Tian Y, Wang X, Cramer Z, et al. APC and P53 mutations synergise to create a therapeutic vulnerability to NOTUM inhibition in advanced colorectal cancer. *Gut.* 2023;72(12):2294-2306.

Background

ABC99 is an N-hydroxyhydantoin (NHH)-carbamate-based, irreversible and selective inhibitor of Wnt-deacylating enzyme NOTUM^[1]. NOTUM is a secreted serine hydrolase that negatively modulates Wnt/ β -catenin signaling by removing the essential palmitoleate moiety from Wnt proteins^[2]. ABC99 is usually used in models of osteoporosis, colorectal cancer and neurodegenerative diseases where

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Wnt signaling is insufficient^{[3][4]}.

In vitro, ABC99 (2µg/mL; 7–14 days) suppressed odontoblastic differentiation of human stem cells from apical papilla (hSCAPs), reduced ALP activity, blocked mineralized nodule formation, and decreased DSPP, DMP1 and ALP mRNA levels^[5]. ABC99 (50nM and 500nM; 14 days) significantly reversed the inhibition of osteogenic differentiation of hPDLSCs by recombinant human NOTUM, restored ALP activity and calcium nodule formation, and increased the protein/mRNA levels of Osterix, OCN and Runx2^[6].

In vivo, ABC99 (10mg/kg/day; p.o.; 4 weeks) almost completely prevented liver metastasis and lymph-node metastasis, reduced primary tumor weight, and significantly inhibited Ki67⁺ cell proliferation in orthotopic colorectal cancer mice^[7].

References:

- [1] Suciú RM, Cognetta AB 3rd, Potter ZE, Cravatt BF. Selective Irreversible Inhibitors of the Wnt-Deacylating Enzyme NOTUM Developed by Activity-Based Protein Profiling. *ACS Med Chem Lett.* 2018;9(6):563-568.
- [2] Bayle ED, Svensson F, Atkinson BN, et al. Carboxylesterase Notum Is a Druggable Target to Modulate Wnt Signaling. *J Med Chem.* 2021;64(8):4289-4311.
- [3] Zhao Y, Jolly S, Benvegna S, Jones EY, Fish PV. Small-molecule inhibitors of carboxylesterase Notum. *Future Med Chem.* 2021;13(11):1001-1015.
- [4] Larrick JW, Mendelsohn AR. Roads to the Fountain of Youth? Rejuvenating Intestinal Stem Cells. *Rejuvenation Res.* 2019;22(4):342-347.
- [5] Zhao Q, Ren H, Wang N, Yuan X, Zhao Y, Wen Q. NOTUM plays a bidirectionally modulatory role in the odontoblastic differentiation of human stem cells from the apical papilla through the WNT/β-catenin signaling pathway. *Arch Oral Biol.* 2024;160:105896.
- [6] Yang P, Li C, Kou Y, et al. Notum suppresses the osteogenic differentiation of periodontal ligament stem cells through the Wnt/Beta catenin signaling pathway. *Arch Oral Biol.* 2021;130:105211.
- [7] Tian Y, Wang X, Cramer Z, et al. APC and P53 mutations synergise to create a therapeutic vulnerability to NOTUM inhibition in advanced colorectal cancer. *Gut.* 2023;72(12):2294-2306.

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