

Product Data Sheet

Product Name: BAY 2416964

Cat. No.: GC60622

Chemical Properties

Cas. No. 2242464-44-2

SMILES O=C(C1=CC(C2=CC=C(Cl)C=C2)=NN(C3=CN(C)N=C3)C1=O)N[C@@H](C)CO

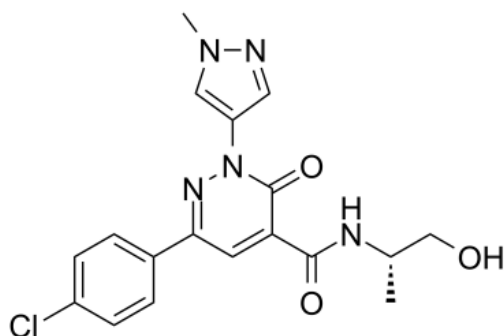
Formula $C_{18}H_{18}ClN_5O_3$ M.Wt 387.82

Solubility DMSO: 250 mg/mL (644.63 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 PyMT mouse mammary cancer cells

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

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

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Preparation Method

PyMT mouse mammary cancer cells grew in a DMEM/F12 medium mixture that included 10% v/v fetal bovine serum and 1% v/v l-glutamine along with 1µg/ml hydrocortisone and 5µg/ml insulin and 5ng/ml epidermal growth factor. The cells underwent incubation at 37°C and 5% CO₂ within a humidified environment until they reached 80% confluency before sub-culturing. PyMT cells were seeded into a 24-well plate at 5×10⁴ cells per well and received treatment with BAY 2416964 at concentrations of 0.1, 1 and 10µM for a period of 6 hours. The RNA isolation kit was used to extract RNA according to the manufacturer's instructions. The cell harvest and lysis process employed 10mM Tris-HCl buffer mixed with 1mM EDTA and 1% SDS to achieve pH 8.0. Samples underwent high intensity sonication for a total of two cycles each lasting thirty seconds with an on/off sequence. The NE-PER Nuclear and Cytoplasmic Extraction Reagents kit according to manufacturer's instructions was used to harvest cytoplasmic and nuclear fractions. The BCA Protein Assay Kit was used to determine the protein concentration. Protein samples moved through 4-20% SDS-PAGE gels before transfer to polyvinylidene fluoride membranes. The membranes underwent overnight incubation at 4°C with primary antibodies dissolved in 5% skim milk before being treated with secondary antibodies at room temperature for 1 hour. The antibodies used in the experiment included anti-AHR (bml-sa210-0100), anti-PARP1 (sc-7150), anti-CASPASE-3 (9662S), anti-Lamin A/C (2032S), anti-Tubulin (T5168) and anti-β-actin (AC-74). Commercial extended duration substrate was used to visualize the protein bands.

Reaction Conditions

0.1, 1 and 10µM; 6h

Applications

BAY 2416964 dose-dependently reduced the AHR signal transduction pathway, lowered the expression level of AHR protein, and decreased the expression level of Cyp1b1.

Animal experiment [2]:

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Animal models Male C57BL/Ly5.1 mice

Preparation Method The antitumor activity of BAY 2416964 was tested using the B16F10-OVA (clone 5) melanoma model in male C57BL/Ly5.1 mice. The mice were treated orally (p.o.) The mice received BAY 2416964 (30mg/kg/day) or vehicle treatment for a period of 14 days. Researchers collected tumor samples from all groups at the study's conclusion and used flow cytometry to measure different immune cell population percentages.

Dosage form 30mg/kg/day for 14 days; p.o.

Applications The BAY 2416964 treatment substantially reduced tumor expansion while raising immunostimulatory CD8⁺ T cells and natural killer (NK) cell levels within tumors in male C57BL/Ly5.1 mice.

References:

- [1] Olafsen N E, Das S, Gorrini C, et al. Long-term exposure to BAY2416964 reduces proliferation, migration and recapitulates transcriptional changes induced by AHR loss in PyMT-induced mammary tumor cells[J]. *Frontiers in Oncology*, 2024, 14: 1466658.
- [2] Kober C, Roewe J, Schmees N, et al. Targeting the aryl hydrocarbon receptor (AhR) with BAY 2416964: a selective small molecule inhibitor for cancer immunotherapy[J]. *Journal for Immunotherapy of Cancer*, 2023, 11(11): e007495.

Background

BAY 2416964 is a potent and orally active inhibitor targeting aryl hydrocarbon receptor (AhR) with IC₅₀ value of 341nM^[1]. BAY 24169649 has been widely used in anti-cancer research ^[2].

In vitro, BAY 2416964 strongly inhibited the expression of CYP1A1 induced by AhR in human monocyte U937 cells with IC₅₀ value of 21nM after treatment for 4h^[3]. After treatment with 10nM BAY 2416964 for 24 hours, the expression of IL-10 in Tet2-deficient B cells was significantly inhibited^[4]. Treatment with 100nM BAY 2416964 for 24 hours could significantly inhibit the

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expression of fibrotic factors (Fn, Col1a1 and α -SMA) induced by L-Kynurenine (100 μ M; 24h) and alleviate the fibrotic process in TCMK-1 cells^[5]. BAY 2416964 treatment (20 μ M; 48h) increased the expression of type I interferon and raised the levels of IFNB1, CXCL10 and CCL5 in MDA-MB-436 cells^[6].

In vivo, BAY 2416964 (20mg/kg/day; i.p.) treatment for one week can inhibit the expression of EZH2 caused by cisplatin (20mg/kg/day; i.p.) in the kidneys of C57BL/6 J mice, and alleviate cisplatin-induced renal dysfunction and tubular injury^[7]. Treatment with BAY 2416964 (p.o.) at a dose of 30mg/kg/day for half a month enhanced the production of IFN- γ and increased the frequency of immune-stimulated tumor-infiltrating CD8⁺ T cells and natural killer (NK) cells, inhibited tumor growth in the melanoma mouse model^[3].

References:

- [1] Gutcher I, Schmees N, Röse L, et al. 2-Heteroaryl-3-oxo-2, 3-dihydropyridazine-4-carboxamides for the treatment of cancer: U.S. Patent 11,795,164[P]. 2023-10-24.
- [2] Cohen Z, Petrenko E, Barisaac A S, et al. SLAYER: A Computational Framework for Identifying Synthetic Lethal Interactions through Integrated Analysis of Cancer Dependencies[J]. bioRxiv, 2024: 2024.05. 01.592073.
- [3] Kober C, Roewe J, Schmees N, et al. Targeting the aryl hydrocarbon receptor (AhR) with BAY 2416964: a selective small molecule inhibitor for cancer immunotherapy[J]. Journal for Immunotherapy of Cancer, 2023, 11(11): e007495.
- [4] Lu Z, Liu R, Wang Y, et al. Ten-eleven translocation-2 inactivation restrains IL-10-producing regulatory B cells to enable antitumor immunity in hepatocellular carcinoma[J]. Hepatology, 2023, 77(3): 745-759.
- [5] Ren Q, Cheng L, Guo F, et al. Fisetin improves hyperuricemia-induced chronic kidney disease via regulating gut microbiota-mediated tryptophan metabolism and aryl hydrocarbon receptor activation[J]. Journal of Agricultural and Food Chemistry, 2021, 69(37): 10932-10942.
- [6] Martin J C, da Silva Fernandes T, Chaudhry K A, et al. Aryl hydrocarbon receptor suppresses STING-mediated type I IFN expression in triple-negative breast cancer[J]. Scientific Reports, 2024, 14(1): 5731.
- [7] Wen L, Ren Q, Guo F, et al. Tubular aryl hydrocarbon receptor upregulates EZH2 to promote cellular senescence in cisplatin-induced acute kidney injury[J]. Cell Death & Disease, 2023, 14(1): 18.

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