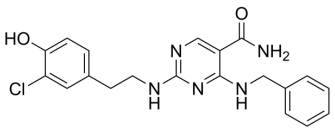
AS1517499

Cat. No.:	HY-100614
CAS No.:	919486-40-1
Molecular Formula:	$C_{20}H_{20}ClN_5O_2$
Molecular Weight:	397.86
Target:	STAT
Pathway:	JAK/STAT Signaling; Stem Cell/Wnt
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 6 months -20°C 1 month



SOLVENT & SOLUBILITY

In Vitro

DMSO : ≥ 35 mg/mL (87.97 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.5134 mL	12.5672 mL	25.1345 mL
5 mM	0.5027 mL	2.5134 mL	5.0269 mL
10 mM	0.2513 mL	1.2567 mL	2.5134 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (6.28 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (6.28 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

AS1517499 is a potent and brain-permeable STAT6 phosphorylation inhibitor with an IC_{50} of 21 nM.

IC_{50} & Target

STAT6
21 nM (IC_{50})

In Vitro

AS1517499 shows potent STAT6 inhibition with an IC_{50} value of 21 nM, and also inhibits IL-4-induced Th2 differentiation of mouse spleen T cells with an IC_{50} value of 2.3 nM and without influencing T-helper cell 1 (Th1) differentiation induced by IL-12. AS1517499 selectively inhibits Th2 differentiation without affecting Th1 differentiation[1].
In cultured human BSM cells, IL-13 (100 ng/mL) causes a phosphorylation of STAT6 and an up-regulation of RhoA, a

	monomeric GTPase responsible for Ca^{2+} sensitization of smooth muscle contraction: both events are inhibited by co-incubation with AS1517499 (100 nM)[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	In BALB/c mice that are actively sensitized and repeatedly challenged with ovalbumin antigen, an increased IL-13 level in bronchoalveolar lavage fluids and a phosphorylation of STAT6 in bronchial tissues are observed after the last antigen challenge. These mice have an augmented BSM contractility to acetylcholine together with an up-regulation of RhoA in bronchial tissues. Intraperitoneal injections of AS1517499 (10 mg/kg) 1 hour before each ovalbumin exposure inhibits both the antigen-induced up-regulation of RhoA and BSM hyperresponsiveness, almost completely[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Cell Assay [2]	Normal human BSM cells (hBSMCs) are maintained in SmBM medium supplemented with 5% fetal bovine serum, 0.5 ng/mL human epidermal growth factor (hEGF), 5 μg/mL insulin, 2 ng/mL human fibroblast growth factor-basic (hFGF-b), 50 μg/mL gentamicin, and 50 ng/mL amphotericin B. Cells are maintained at 37°C in a humidified atmosphere (5% CO_2), fed every 48 to 72 hours, and passaged when cells reached 90 to 95% confluence. Then the hBSMCs (passages 7-9) are seeded in 6-well plates and 8-well chamber slides at a density of 3,500 cells/cm2 and, when 80 to 85% confluence observed, cells are cultured without serum for 24 hours before addition of recombin is ant human IL-13. AS1517499 (100 nM) or its vehicle (0.3% DMSO) is treated 30 minutes before the addition of IL-13 (100 ng/mL). In some experiments, AS1517499 is treated 0 (co-incubation), 3, or 12 hours after the addition of IL-13. In another series of experiments, a selective Rho-kinase inhibitor Y-27632 (1 μM) or its vehicle (0.3% DMSO) is also applied 15 minutes before the IL-13 application. At the indicated time after the IL-13 treatment, cells are washed with PBS, immediately collected, and disrupted with 1× SDS sample buffer (250 μ L/well), and used for Western blot analyses[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [2]	Mice[2] Male BALB/c mice are used. Preparation of a murine model of allergic bronchial asthma, which has an in vivo AHR, is performed. In brief, BALB/c mice (8 wk of age) are actively sensitized by intraperitoneal injections of 8 μg ovalbumin (OVA) with 2 mg Imject Alum on Day 0 and Day 5. The sensitized mice are challenged with aerosolized OVA-saline solution (5 mg/mL) for 30 minutes on Days 12, 16, and 20. A control group of mice received the same immunization procedure but inhaled saline aerosol instead of OVA challenge. The aerosol is generated with an ultrasonic nebulizer and introduced to a Plexiglas chamber box (130×200 mm, 100 mm height) in which the mice are placed. Animals also received intraperitoneal injection with AS1517499 (1 or 10 mg/kg/d; dissolved in 20% DMSO in saline) or its vehicle 1 hour before each antigen inhalation (Days 12, 16, and 20). Twenty-four hours after the last OVA challenge, mice are killed by exsanguination from abdominal aorta under urethane (1.6 g/kg, intraperitoneally) anesthesia. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Cell Mol Immunol. 2022 Nov;19(11):1263-1278.
- J Exp Med. 2018 Aug 6;215(8):2175-2195.
- Cell Death Differ. 2021 Sep;28(9):2728-2744.
- Compos Part B-Eng. 2023 Jan.
- Cancer Immunol Res. 2020 Nov;8(11):1426-1439.

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REFERENCES

[1]. Nagashima S, et al. Synthesis and evaluation of 2-{[2-(4-hydroxyphenyl)-ethyl]amino}pyrimidine-5-carboxamide derivatives as novel STAT6 inhibitors. Bioorg Med Chem. 2007 Jan 15;15(2):1044-55.

[2]. Chiba Y, et al. A novel STAT6 inhibitor AS1517499 ameliorates antigen-induced bronchial hypercontractility in mice. Am J Respir Cell Mol Biol. 2009 Nov;41(5):516-24.

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