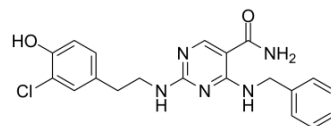


AS1517499

Cat. No.:	HY-100614		
CAS No.:	919486-40-1		
Molecular Formula:	C ₂₀ H ₂₀ ClN ₅ O 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	1 mg	5 mg	10 mg
	Concentration			
	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

- 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
- 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 IC₅₀ of 21 nM.

IC₅₀ & Target

STAT6
 21 nM (IC₅₀)

In Vitro

AS1517499 shows potent STAT6 inhibition with an IC₅₀ value of 21 nM, and also inhibits IL-4-induced Th2 differentiation of mouse spleen T cells with an IC₅₀ 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% CO₂), 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 recombinant 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 µL/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

- J Exp Med. 2018 Aug 6;215(8):2175-2195.
- Cancer Immunol Res. 2020 Nov;8(11):1426-1439.
- Theranostics. 2021 Jan 1;11(3):1192-1206.
- J Immunol. 2021 Mar 1;206(5):963-977.
- J Immunol. 2020 Nov 25;ji2000779.

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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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