# Data Sheet

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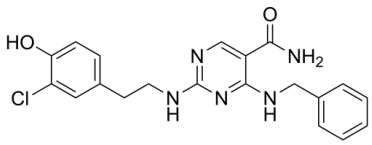
| **Product Name:** | AS1517499 |
| **Cat. No.:** | CS-6099 |
| **CAS No.:** | 919486-40-1 |
| **Molecular Formula:** | $C_{20}H_{20}ClN_5O_2$ |
| **Molecular Weight:** | 397.86 |
| **Target:** | STAT |
| **Pathway:** | JAK/STAT Signaling; Stem Cell/Wnt |
| **Solubility:** | DMSO : ≥ 35 mg/mL |




## BIOLOGICAL ACTIVITY:

AS1517499 is a potent and brain-permeable **STAT6** phosphorylation inhibitor with an **$IC_{50}$** of 21 nM. IC50 & Target: IC50: 21 nM (STAT6)[1] ***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]. ***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].

## PROTOCOL (Extracted from published papers and Only for reference)

**Cell Assay:** AS1517499 is dissolved in DMSO and stored, and then diluted with appropriate medium (0.3% DMSO) before use[2].[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 μL/well), and used for Western blot analyses[2]. **Animal Administration:** AS1517499 is dissolved in 20% DMSO in saline (Mice)[2].[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.

## 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.


## CAIndexNames:

5-Pyrimidinecarboxamide, 2-[[2-(3-chloro-4-hydroxyphenyl)ethyl]amino]-4-[(phenylmethyl)amino]-

## SMILES:

O=C(C1=CN=C(NCCC2=CC=C(O)C(Cl)=C2)N=C1NCC3=CC=CC=C3)N

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