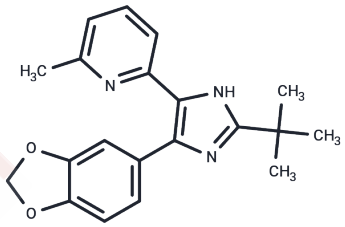
Data Sheet (Cat.No.T2462)

TargetMol

SB-505124

Chemical Properties



CAS No. : 694433-59-5

Formula: C20H21N3O2

Molecular Weight: 335.4

Storage: Powder: -20°C for 3 years | In solvent: -80°C for 1 year
Actual storage temperature shall be subject to the COA.

Biological Description

Description	SB505124 is a selective inhibitor of TGFβR for ALK4, ALK5.
Targets(IC50)	ALK,TGF-beta/Smad
In vitro	In the rabbit GFS model, SB505124 reduced intraocular pressure (IOP) at the GFS surgical site and diminished subconjunctival cellular infiltration and scarring. Additionally, in mice treated with tacrolimus (TAC) and FK12EC KO mice, SB505124 inhibited the activation of endothelial TGF-β receptors and the induction of hyalinosis in renal arterioles.
In vivo	SB505124 has been identified as a reversible ATP-competitive and selective inhibitor of ALK4 and ALK5. It inhibits ALK4 with an IC50 of 129 nM, exhibiting 2.5 times less selectivity towards ALK5, and does not inhibit ALK2 at concentrations up to 10 μM. SB505124 suppresses endogenous Smad2 phosphorylation in COS-1 cells. Both ALK4 and ALK5 activation lead to Smad1 and Smad2 phosphorylation, with SB505124 inhibiting this activation. This compound, in a concentration-dependent manner, inhibits Transforming Growth Factor-β (TGF-β)-induced Smad2 phosphorylation in HepG2 human liver cancer cells, C2C12 mouse myoblasts, and Mv1Lu mink lung cells. SB505124 effectively inhibits the activity of TGF-β and activin-induced CAGA12-luciferase and ARE-luciferase reporter constructs in a concentration-dependent fashion. In Human Umbilical Vein Endothelial Cells (HUVEC), SB505124 (500 nM) obstructs the effects of TGF-β1 on F-actin assembly and prevents TGF-β-induced ROS production.
Kinase Assay	In Vitro Protein Kinase Assay : Kinase assays are performed as described by Laping et al., 2002 using the kinase domain of ALK5 and full-length N-terminal fused GST-Smad3. Kinase assays are performed with 65 nM GST-ALK5 and 184 nM GST-Smad3 in 50 mM HEPES, 5 mM MgCl2, 1 mM CaCl2, 1 mM dithiothreitol, and 3 μM ATP. Reactions are incubated with 0.5 μCi of [33P]γATP for 3 hours at 30 °C. Phosphorylated protein is captured on P-81 paper , washed with 0.5% phosphoric acid, and counted by liquid scintillation. Alternatively, Smad3 or Smad1 protein is also coated onto FlashPlate Sterile Basic Microplates. Kinase assays are then performed in FlashPlates with same assay conditions using either the kinase domain of ALK5 with Smad3 as substrate or the kinase domain of ALK6 (BMP receptor) with Smad1 as substrate. Plates are washed three times with phosphate buffer and counted by TopCount.

Cell Research	Cell viability is measured as described by Laping et al., 2002 or by using the modified tetrazolium salt WST-1. XTT assay: The cells are serum-deprived for 24 hours and then treated with SB505124 for 48 hours to assess the cellular toxicity. Cell viability is determined by incubating cells for 4 hours with XTT labeling and electron coupling reagent according to the manufacturer's directions. Live cells with active mitochondria produce an orange-colored product, formazan, which is detected using a plate reader at between A450 nm and A500 nm with a reference wavelength greater than 600 nm. The absorbance values correlate with the number of viable cells. Modified tetrazolium salt WST-1: Approximately 2000 cells are seeded in 96-well dishes in 100 μL of 0.2% FBS phenol red-free media overnight. The cells are treated with 50 μL of SB505124 (to achieve the final concentrations indicated) for 30 minutes before being treated with or without TGF-β1 and TNF-α to a final volume of 200 μL. Cell growth is measured at the indicated time points by incubating each well with 10 μL of WST-1 for 3 hours at 37 °C. Metabolically active cells cleave WST-1 to water-soluble formazan, which is directly quantitated with an enzyme-linked immunosorbent assay plate reader. Each experiment is done at least twice, and treatment for each cell line is done in triplicate. (Only for Reference)

Solubility Information

Solubility	DMSO: 125 mg/mL (372.69 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 2 mg/mL (5.96 mM),Sonication is recommended. *Please add the solvents sequentially, clarifying the solution as much as possible before adding the next one. Dissolve by heating and/or sonication if necessary. Working solution is recommended to be prepared and used immediately. The formulation provided above is for reference purposes only. In vivo formulations may vary and should be modified based on specific experimental conditions.*

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.9815 mL	14.9076 mL	29.8151 mL
5 mM	0.5963 mL	2.9815 mL	5.963 mL
10 mM	0.2982 mL	1.4908 mL	2.9815 mL
50 mM	0.0596 mL	0.2982 mL	0.5963 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

Reference

DaCosta Byfield S, et al. Mol Pharmacol. 2004, 65(3), 744-752.
Kalli M, Mpekris F, Wong C K, et al. Activin A signaling regulates IL13Rα2 expression to promote breast cancer metastasis. Frontiers in Oncology. 2019 Feb 5;9:32
Liu S, Yu X, Guo Q, et al.Periodontal ligament-associated protein-1 promotes osteoclastogenesis in mice by modulating TGF-β1/Smad1 pathway.Journal of Periodontology.2023
Hu T, et al. Am J Physiol Renal Physiol. 2005, 289(4), F816-825.
Misumi S, et al. Eur J Neurosci. 2008, 28(6), 1049-1059.
Goto N, et al. Cancer Sci. 2011, 102(8), 1501-1508.
Sapitro J, et al. Mol Vis. 2010 ,16, 1880-1892.
Marini KD, et al. Inhibition of activin signaling in lung adenocarcinoma increases the therapeutic index of platinum chemotherapy. Sci Transl Med. 2018 Jul 25;10(451). pii: eaat3504.
Kalli M, Mpekris F, Wong C K, et al. Activin A signaling regulates IL13Rα2 expression to promote breast cancer metastasis[J]. Frontiers in oncology. 2019 Feb 5;9:32.