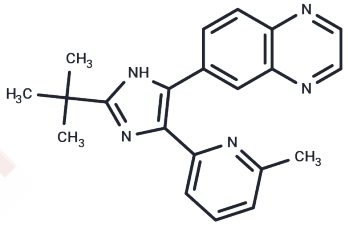
Data Sheet (Cat.No.T1763)

TargetMol

SB 525334

Chemical Properties



CAS No. : 356559-20-1

Formula: C21H21N5

Molecular Weight: 343.42

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	SB-525334 is a potent and selective inhibitor of the TGF-β1R and ALK5 (IC50: 14.3 nM).
Targets(IC50)	ALK,TGF-beta/Smad
In vitro	SB-525334 (1 μM) blocked TGF-beta1-induced phosphorylation and nuclear translocation of Smad2/3 in renal proximal tubule cells and inhibited TGF-beta1-induced increases in plasminogen activator inhibitor-1 (PAI-1) and procollagen alpha1 (I) mRNA expression in A498 renal epithelial carcinoma cells [1]. The combination with SB525334 significantly augmented the cytotoxicity of gemcitabine in both parental and gemcitabine-resistant pancreatic cancer cells. SB525334 significantly increased apoptotic cell death in gemcitabine-resistant cells [2].
In vivo	Orally administered doses of 1, 3, or 10 mg/kg/day SB-525334 for 11 days produced statistically significant reductions in renal PAI-1 mRNA [1]. SB-525334 (10 mg/kg or 30 mg/kg) was orally administered at twice a day. Lungs were isolated 5, 7, 9 and 14 days after Bleomycin (BLM) treatment. BLM treatment led to significant pulmonary fibrotic changes accompanied by significant upregulation of ECM mRNA expressions, Smad2/3 nuclear translocation, CTGF expression, myofibroblast proliferation and type I collagen deposition. SB-525334 treatment attenuated the histopathological alterations in the lung, and significantly decreased the type I and III procollagen and fibronectin mRNA expression [3].
Kinase Assay	To determine the potency of the ALK5 inhibitor SB-525334 at the enzyme level, purified GST-tagged kinase domain of ALK5 was incubated with purified GST-tagged full-length Smad3 in the presence of 33P-γATP and different concentrations of SB525334. The readout is radioactively labeled Smad3. To determine the selectivity of SB-525334, purified GST-tagged kinase domain of ALK2 and ALK4 were incubated with GST-tagged full-length Smad1 and Smad3, respectively, in the presence of different concentrations of SB-525334 (n=3). IC50 value determinations were calculated with GraphPad software using a sigmoidal dose-response curve [1].
Cell Research	RPTE cells were seeded on microscope slides. The following day, the cells were starved by removal of epidermal growth factor and serum for 24 h prior to dosing. Cells were dosed with 10 ng/ml TGF- 1 or 1 M SB-525334 or a combination of both. Slides were pretreated with SB-525334 or starve media for 3 h prior to a 1-h incubation at 37°C with TGF- 1 or starve media. The cells were then fixed for 15 min in 4% ice-cold

Cell Research	paraformaldehyde. The cells were permeabilized for 10 min in 0.3% Triton X-100/PBS at room temperature. The slides were incubated for 30 min in a blocking solution containing 0.3% bovine serum albumin, 10% FBS, 0.3% Triton X-100/PBS, and 5% milk in PBS. A 1:200 dilution of primary mouse anti-Smad2/3 antibody was applied to each slide for overnight incubation. A 1:200 dilution of anti-mouse IgG fluorescein secondary antibody was applied to each slide for 30 min at room temperature. The slides were then viewed using an argon blue 488 nM laser in a confocal microscope. Nuclear signal intensity was analyzed using 1D Image Analysis software. The relative intensity was determined by the mean intensity of the nucleus and expressed as percent control [1].
Animal Research	To identify the optimal treatment length for puromycin aminonucleoside's effect on extracellular matrix in the kidney, 18 Sprague-Dawley (SD) rats (200 -250 g) were injected with 15 mg/100 g of puromycin aminonucleoside in 0.9% saline or sham 0.9% saline only intraperitoneally. Animals were sacrificed at 24 h (n = 3+2 control), day 4 (n=3), day 8 (n = 3), day 10 (n = 3), day 15 (n = 2), and day 20 (n = 2). A 24-h urine collection and plasma sample were taken at 9:00 AM everyday. Urine and plasma chemistry were measured at GlaxoSmithKline Laboratories Animal Science using an Olympus clinical analyzer. Proteinuria was measured as a concentration (mg/deciliter) and then converted to total protein excreted over a 24-h period using urine flow (mL/24 h). The creatinine clearance was calculated by multiplying urine creatinine levels (mg/mL) by urine flow (mg/mL/100 g b.wt.) and then dividing that product by plasma creatinine (mg/mL). To determine the effect of SB-525334 on renal disease in the PAN model, SD rats were pretreated by oral gavage with 1, 3, or 10 mg/kg/day of SB-525334 once a day. The following day, PAN was injected at 15 mg/100 g to the appropriate rats. Treatment groups continued to receive SB-525334. Ten days after PAN injection the rats were sacrificed, and blood, urine, and kidneys were collected at the termination point for analysis [1].

Solubility Information

Solubility	H2O: < 1 mg/mL (insoluble or slightly soluble) DMSO: 50 mg/mL (145.59 mM),Sonication is recommended. Ethanol: 50 mg/mL (145.59 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 (5.82 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	2.9119 mL	14.5594 mL	29.1189 mL
5 mM	0.5824 mL	2.9119 mL	5.8238 mL
10 mM	0.2912 mL	1.4559 mL	2.9119 mL
50 mM	0.0582 mL	0.2912 mL	0.5824 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

Grygielko ET, et al. Inhibition of gene markers of fibrosis with a novel inhibitor of transforming growth factor-beta type I receptor kinase in puromycin-induced nephritis. J Pharmacol Exp Ther. 2005 Jun;313(3):943-51.
Peng H, Shen J, Long X, et al. Local Release of TGF-β Inhibitor Modulates Tumor-Associated Neutrophils and Enhances Pancreatic Cancer Response to Combined Irreversible Electroporation and Immunotherapy, Local Release of TGF-β Inhibitor Modulates Tumor-Associated Neutrophils and Enhances Pancreatic Cancer Response to Combined Irreversible Electroporation and Immunotherapy. Advanced Science. 2022: 2105240
Kim YJ, et al. Transforming growth factor beta receptor I inhibitor sensitizes drug-resistant pancreatic cancer cells to gemcitabine. Anticancer Res. 2012 Mar;32(3):799-806.
Higashiyama H, et al. Inhibition of activin receptor-like kinase 5 attenuates bleomycin-induced pulmonary fibrosis. Exp Mol Pathol. 2007 Aug;83(1):39-46.