SB-431542 is a potent and selective inhibitor of ALK5 with an IC50 value of 94 nM, and is also an inhibitor of TGF-β Receptor.

In Vitro: SB-431542 (1 μM) significantly reduces the TGF-β-induced nuclear accumulation of Smad proteins in A498 cells. SB-431542 inhibits TGF-β1-induced collagen Iα1 and PAI-1 mRNA with IC50 values of 60 and 50 nM, respectively. In addition, SB-431542 inhibits TGF-β1-induced fibronectin mRNA and protein with IC50 values of 62 and 22 nM, respectively. SB-431542 (10 μM) is a selective inhibitor of endogenous activin and TGF-β signaling but has no effect on BMP signaling in NIH 3T3 cells. TRKI, SB-431542, inhibits TGF-beta-induced transcription, gene expression, apoptosis, and growth suppression. SB-431542 attenuates the tumor-promoting effects of TGF-beta, including TGF-beta-induced EMT, cell motility, migration and invasion, and vascular endothelial growth factor secretion in human cancer cell lines. SB-431542 induces anchorage independent growth of cells that are growth-inhibited by TGF-beta, whereas it reduces colony formation by cells that are growth-promoted by TGF-beta. SB-431542 (0.3 μM) inhibits cell proliferation induced by TGF-β in MG63 cells.

In Vivo: SB-431542 (10 mg/kg, i.p.) decreases lung metastasis but does not significantly alter growth of the primary tumor 4T1 xenograft.

PROTOCOL (Extracted from published papers and Only for reference)

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**Kinase Assay:** A total of 100,000 cells from each pool of A549 and HT29 are seeded into each well of a 12-well plate. Cells are cultured in media containing 0.2% FBS for 18 hours, and then treated with 5 ng/mL TGF-β1 in the presence of SB-431542 (10 μM) in 0.5 mL of media for 24 hours. One hundred μLs of each supernatant media is used for VEGF assay according to the manufacturer’s instruction.

For TGF-β1 ELISA, 100,000 cells from each pool of A549, VMRC-LCD, and HT29 are seeded into each well of a 12-well plate and serum-starved for 20 hours. Cells are then treated with SB-431542 in 0.5 mL of serum-free RPMI media for 24 hours. One hundred μLs of each supernatant media is activated and used for TGF-β1 assay according to the manufacturer’s instruction.

Cell Assay: SB-431542 is dissolved at a concentration of 10 mM in DMSO. A498 cells are seeded at 5,000 to 10,000 cells/well in 96-well plates. The cells are serum-deprived for 24 h and then treated with SB-431542 for 48 h to assess the cellular toxicity. Cell viability is determined by incubating cells for 4 h 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 A 450 nm and A 500 nm with a reference wavelength greater than 600 nm. The absorbance values correlate with the number of viable cells.

Animal Administration: SB-431542 is formulated in 20% DMSO/80% corn oil. Ten thousand 4T1 cells are injected subcutaneously into the second mammary fat pad of 6-week-old Balb/c female mice. Tumors are measured twice weekly, and volume is calculated using the following formula: Volume = width²×length×0.52. Mice are randomly assigned to two treatment groups: control, n = 14 (20% DMSO/80% corn oil); SB-431542-treated, n = 15 (10 mg/kg body weight in 20% DMSO/80% corn oil, administered intraperitoneally three times per week starting one day after tumor cell inoculation. Primary tumors are resected when the volume at day 10 post-
injection of 4T1 cells. All mice are monitored daily and euthanized after 4 weeks. The metastases are dissected to snap-freeze for further analysis.

References:


