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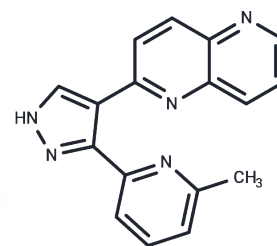
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RepSox

Chemical Properties

CAS No. :	446859-33-2
Formula:	C ₁₇ H ₁₃ N ₅
Molecular Weight:	287.32
Appearance:	no data available
Storage:	store at low temperature Powder: -20°C for 3 years In solvent: -80°C for 1 year



Biological Description

Description	RepSox (ALK5 Inhibitor II) is a TGFβR-1/ALK5 inhibitor that selectively inhibits the binding of ATP to ALK5 and the autophosphorylation of ALK5 (IC ₅₀ =23/4 nM). RepSox induces adipogenesis in MEFs cells.
Targets(IC ₅₀)	ALK,TGF-beta/Smad
In vitro	<p>METHODS: Mouse embryonic fibroblast MEFs were treated with RepSox (10 μM) for 10 days and cellular lipids were detected using oil red O staining.</p> <p>RESULTS: Many adipocyte-like cells appeared in RepSox-treated MEFs, and oil red O staining of lipid droplets confirmed adipocyte differentiation. [1]</p> <p>METHODS: Sheep fibroblasts were treated with RepSox (10-25 nM) for 3 days, and cell morphology was observed using a microscope.</p> <p>RESULTS: After three days of RepSox treatment, sheep fibroblasts acquired a significantly different morphology and changed from a spindle shape to an elongated shape with more clusters. [2]</p>
In vivo	<p>METHODS: To test the effect on the differentiation of EGCs from intestinal glial cells, RepSox (3-10 mg/kg in 0.5% CMC-Na) was injected intraperitoneally to GFAP-Cre: Rosa26-tdTomato mice once daily for two weeks.</p> <p>RESULTS: RepSox promotes the conversion of EGCs into neurons and improves gastrointestinal motility in vivo. [3]</p> <p>METHODS: To detect anti-tumor activity in vivo, RepSox (5-20 mg/kg) was injected intraperitoneally into a nude mouse model bearing osteosarcoma 143B every two days for three weeks.</p> <p>RESULTS: RepSox effectively inhibited the growth of osteosarcoma and had low toxicity in nude mice. [4]</p>
Kinase Assay	The kinase domain of ALK5 is cloned by PCR and expressed in a baculovirus/Sf9 cells system. The protein is 6-His tagged in the C terminus and purified by affinity chromatography using a Ni ²⁺ -column, and the obtained material is used to assess compound activity in an autophosphorylation assay. Purified enzyme (10 nM) is incubated in 50 μL of Tris buffer (Tris 50 mM, pH 7.4; NaCl, 100 mM; MgCl ₂ , 5 mM; MnCl ₂ , 5 mM; and DTT, 10 mM). The enzyme is preincubated with different concentrations of RepSox (0.1% DMSO final concentration in the test) for 10 min at 37°C. The reaction is then initiated by the addition of 3 μM ATP (0.5 μCi γ- ³³ P-ATP). After 15 min at 37°C, phosphorylation is stopped by the addition of SDS-PAGE sample buffer (50 mM Tris-HCl, pH 6.9, 2.5% glycerol, 1% SDS, and 5% β-mercaptoethanol). The samples are boiled for 5

min at 95°C and run on a 12% SDS-PAGE. Dried gels are exposed to a phosphor screen overnight. ALK5 autophosphorylation is quantified using a Storm imaging system[1].

Cell Research

RepSox is dissolved in DMSO and stored, and then diluted with appropriate media (DMSO 1%) before use[1]. To test anti-TGF-β activity of compounds, HepG2 cells are seeded in 96 well microplates at a concentration of 35000 cells per well in 200 μL of serum-containing medium. The microplates are then placed for 24 h in a cell incubator at 37°C, 5% CO2 atm. RepSox dissolved in DMSO are then added at concentrations of 50 nM to 10 μM (final concentration of DMSO 1%) for 30 min prior to the addition of recombinant TGF-β (1 ng/mL). After an overnight incubation, the cells are washed with PBS and lysed by addition of 10 μL of passive lysis buffer. Inhibition of luciferase activity relative to control groups is used as a measure of compound activity. A concentration response curve is constructed from which an IC50 value is determined graphically[1].

Solubility Information

Solubility

Ethanol: < 1 mg/mL (insoluble or slightly soluble),
H2O: < 1 mg/mL (insoluble or slightly soluble),
DMSO: 15 mg/mL (52.21 mM),Sonication is recommended.
(< 1 mg/ml refers to the product slightly soluble or insoluble)

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	3.4804 mL	17.4022 mL	34.8044 mL
5 mM	0.6961 mL	3.4804 mL	6.9609 mL
10 mM	0.348 mL	1.7402 mL	3.4804 mL
50 mM	0.0696 mL	0.348 mL	0.6961 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.

Reference

Tu WZ, et al. RepSox, a small molecule inhibitor of the TGFβ receptor, induces brown adipogenesis and browning of white adipocytes. Acta Pharmacol Sin. 2019 Dec;40(12):1523-1531.

Inhibitor · Natural Compounds · Compound Libraries · Recombinant Proteins

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