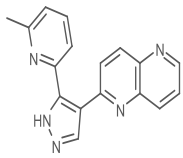
Data Sheet (Cat.No.T6337)

TargetMol
A DRUG SCREENING EXPERT

RepSox

Chemical Properties



CAS No.:	446859-33-2
Formula:	C17H13N5
Molecular Weight:	287.32
Appearance:	N/A
Storage:	0-4℃ for short term (days to weeks), or -20℃ for long term (months).

Biological Description

Description	RepSox is a potent and selective of the TGFβR-1/ALK5 inhibitor.
Targets(IC_{50})	ALK5: 4nM TGFβR1: 18nM
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 Ni2+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; MgCl2, 5 mM; MnCl2, 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-33P-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].
Animal Research	

Solubility Information

Solubility	DMSO: 53 mg/mL (184.5 mM) Ethanol: <1 mg/mL Water: <1 mg/mL (< 1 mg/ml refers to the product slightly soluble or insoluble)

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	3.48 mL	17.402 mL	34.804 mL
5 mM	0.696 mL	3.48 mL	6.961 mL
10 mM	0.348 mL	1.74 mL	3.48 mL
50 mM	0.07 mL	0.348 mL	0.696 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. The storage conditions and period of the stock solution: - 80 ℃ for 6 months; - 20 ℃ for 1 month. Please use it as soon as possible.

Reference

1. Gellibert F, et al. J Med Chem, 2004, 47(18), 4494-4506.
2. Ichida JK, et al. Cell Stem Cell, 2009, 5(5), 491-503.

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