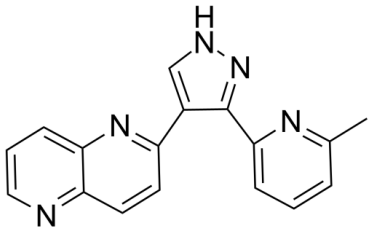
Data Sheet

Product Name:	RepSox
Cat. No.:	CS-1321
CAS No.:	446859-33-2
Molecular Formula:	C17H13N5
Molecular Weight:	287.32
Target:	TGF-β Receptor
Pathway:	TGF-beta/Smad
Solubility:	DMSO : ≥ 52 mg/mL (180.98 mM)



BIOLOGICAL ACTIVITY:

RepSox is a potent and selective of the **TGFβR-1/ALK5** inhibitor which inhibits ALK5 autophosphorylation with **IC_{50}** of 4 nM. IC50 & Target: IC50: 4 nM (ALK5 autophosphorylation)[1] **In Vitro:** RepSox also inhibits ATP binding to ALK5 with IC_{50} of 23 nM. RepSox shows potent activity in both binding and cellular assays and exhibits selectivity over p38 mitogen-activated protein kinase. with IC_{50} of >16 μM[1]. RepSox act s as an inhibitor of the Tgfβ1 kinase. Treatment with 25 μM RepSox almost completely eliminates Smad3 phosphorylation, indicating that RepSox strongly inhibits Tgfβ signaling in somatic cells. RepSox is most effective at replacing Sox2 during days 10-11 after transduction and that therefore cultures of Oct4, Klf4, and cMyc-transduced MEFs give rise to intermediates capable of responding to RepSox treatment. These intermediates appear at day 4 post-transduction and peak at days 10-11. Treatment with RepSox decreased the proportion of cells in G_2/M phase of the cell cycle, indicating it does not increase the proliferation rate of these partially reprogrammed cells[2].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay: [1]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 Assay:** RepSox is dissolved in DMSO and stored, and then diluted with appropriate media (DMSO 1%) before use[1].[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% CO_2 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 IC_{50} value is determined graphically[1].

References:

[1]. Gellibert F, et al. Identification of 1,5-naphthyridine derivatives as a novel series of potent and selective TGF-beta type I receptor inhibitors. J Med Chem.

2004 Aug 26;47(18):4494-506.

[2]. Ichida JK, et al. A small-molecule inhibitor of tgf-Beta signaling replaces sox2 in reprogramming by inducing nanog. Cell Stem Cell. 2009 Nov 6;5(5):491-503.

CAIndexNames:

2-(3-(6-methylpyridin-2-yl)-1H-pyrazol-4-yl)-1,5-naphthyridine

SMILES:

CC1=NC(C2=NNC=C2C3=NC4=CC=CN=C4C=C3)=CC=C1

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