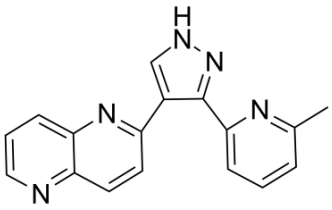
RepSox

Cat. No.:	HY-13012
CAS No.:	446859-33-2
Molecular Formula:	$C_{17}H_{13}N_5$
Molecular Weight:	287.32
Target:	TGF-β Receptor
Pathway:	TGF-beta/Smad
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 6 months -20°C 1 month



SOLVENT & SOLUBILITY

In Vitro

DMSO : 33.33 mg/mL (116.00 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	3.4804 mL	17.4022 mL	34.8044 mL
	5 mM	0.6961 mL	3.4804 mL	6.9609 mL
	10 mM	0.3480 mL	1.7402 mL	3.4804 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 7.5 mg/mL (26.10 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 7.5 mg/mL (26.10 mM); Clear solution
3. Add each solvent one by one: 5% DMSO >> 40% PEG300 >> 5% Tween-80 >> 50% saline
   Solubility: ≥ 1.67 mg/mL (5.81 mM); Clear solution
4. Add each solvent one by one: 5% DMSO >> 95% (20% SBE-β-CD in saline)
   Solubility: ≥ 1.67 mg/mL (5.81 mM); Clear solution
5. Add each solvent one by one: 1% DMSO >> 99% saline
   Solubility: ≥ 0.33 mg/mL (1.15 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

RepSox (E-616452) is a potent and selective of the TGFβR-1/ALK5 inhibitor which inhibits ALK5 autophosphorylation with an IC_{50} of 4 nM.

IC_{50} & 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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

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-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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Sci Adv. 2021 Apr 14;7(16):eabb2213.
- Biomaterials. 2018 Dec 6;193:30-46.
- Stem Cell Res Ther. 2020 Apr 16;11(1):157.
- Biomedicines. 2020 Nov 9;8(11):485.
- J Cell Mol Med. 2021 Apr;25(7):3498-3510.

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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.

Caution: Product has not been fully validated for medical applications. For research use only.

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