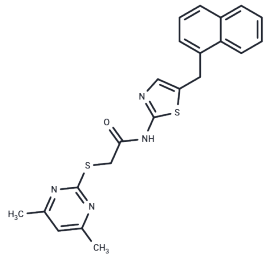
Data Sheet (Cat.No.T6984)

TargetMol

SirReal2

Chemical Properties



CAS No. : 709002-46-0

Formula: C22H20N4OS2

Molecular Weight: 420.55

Storage: Powder: -20°C for 3 years | In solvent: -80°C for 1 year
Actual storage temperature shall be subject to the COA.

Biological Description

Description	SirReal2 is a potent and selective Sirt2 inhibitor with IC50 of 140 nM.
Targets(IC50)	Sirtuin
In vitro	SirReal2 selectively inhibits Sirt2 via a Sirt2-specific amino acid network, and induces a tubulin hyperacetylation and a significant depletion of BubR1 in HeLa cells. [1]
In vivo	In vivo, SirReal2 inhibits Sirt2 activity without affecting the activity of the other Class-I sirtuins Sirt1 and Sirt3. [1]
Kinase Assay	Wee1 Mass Screening: Wee1 mass screening is performed using Amersham's p34cdc2 kinase SPA kit with some modifications. Briefly, 45–60 nM full-length Wee1 kinase is incubated with 25 μM compounds, 20 μM ATP, and 122–441 nM Cdc2/cyclin B in a final volume of 50 μl of enzyme dilution buffer [50 mM Tris (pH 8.0), 10 mM NaCl, 10 mM MgCl2, 1 mM DTT, and 0.1 mM Na3VO4]. After 30 min incubation at 30°C, 30 μl of [33P] ATP containing kinase buffer [67 mM Tris (pH 8.0), 40 mM NaCl, 13 mM MgCl2, 1 mM DTT, and 0.13 mM Na3VO4] containing 1 μM biotinylated peptide, and 0.25 μCi of [γ-33P]ATP is added to the reaction and incubated for another 30 min at 30°C. The reaction is stopped by adding 200 μl of stop buffer [50 μM ATP, 5 mM EDTA, 0.1% Triton X-100, and 1.25 mg/ml SPA beads in PBS]. After centrifugation at 2400 rpm for 15 min, the plate is counted with Wallac's Microbeta counter.
Cell Research	HeLa cells were plated in petri dishes (5?cm), incubated overnight to a confluency of 30–40% and then treated with SirReal2 dissolved in RPMI1640 medium supplemented with fresh 20% (v/v) FCS, 1% (v/v) penicillin, 1% (v/v) streptomycin, 1% (v/v), L-glutamine, 1% (v/v) DMSO for 5?h at various concentrations. Cells were then washed with prewarmed PBS (2?ml), lysed in SDS-PAGE sample buffer (70?μl, 50?mM Tris/HCl, 0.5?mM EDTA, 1 × Complete Protease Inhibitors, 2% (v/v) IGEPAL, 2% (w/v) SDS, 10% (v/v) glycerol, 50?mM NCA, 3.3?μM trichostatin A, 50?mM DTT, 0.01% (w/v) bromophenol blue, pH 6.8) and sonicated (5?min). Cell samples were then separated using SDS-PAGE (12.5% (w/v) polyacrylamide), transferred to an activated nitrocellulose membrane (Bio-Rad), blocked with non-fat dry milk (Roth, 5% (w/v), TBS, 0.1% (v/v) Tween 20) and probed with an anti-acetyl-α-tubulin antibody (1:1,000) and an anti-GAPDH antibody (1:2,000–1:5,000) as a loading control.

Solubility Information

Solubility	DMSO: 62.5 mg/mL (148.61 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 2 mg/mL (4.76 mM),Sonication is recommended. *Please add the solvents sequentially, clarifying the solution as much as possible before adding the next one. Dissolve by heating and/or sonication if necessary. Working solution is recommended to be prepared and used immediately. The formulation provided above is for reference purposes only. In vivo formulations may vary and should be modified based on specific experimental conditions.*

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.3778 mL	11.8892 mL	23.7784 mL
5 mM	0.4756 mL	2.3778 mL	4.7557 mL
10 mM	0.2378 mL	1.1889 mL	2.3778 mL
50 mM	0.0476 mL	0.2378 mL	0.4756 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.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

Reference

Rumpf T, et al. Nat Commun. 2015, 6, 6263.