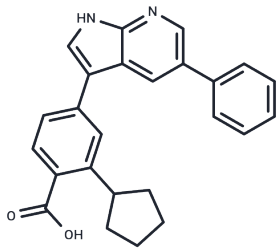
Data Sheet (Cat.No.T2622)

TargetMol

GSK 650394

Chemical Properties



CAS No. : 890842-28-1

Formula: C25H22N2O2

Molecular Weight: 382.45

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	GSK 650394 is an inhibitor of serum- and glucocorticoid-regulated kinases (SGK) that inhibits SGK1 and SGK2 (IC50=62/103 nM). GSK 650394 has antitumor activity and also inhibits osteoclast differentiation and prevents bone loss.
Targets(IC50)	Influenza Virus,SGK
In vitro	**METHODS**: HB1 cells were treated with Grazoprevir (6-30 nM) and G418 (0.5 mg/mL) for 3 weeks for in vitro resistance selection. **RESULTS**: Grazoprevir showed good mutational potency in all resistant variants and inhibited R155 and D168 mutations in particular. [1] **METHODS**: 315 compounds with electrophilic moieties were experimentally screened to determine if they would covalently bind to the active site cysteine of SARS-CoV-2 3CLpro. **RESULTS**: Among the compounds, Grazoprevir was able to inhibit the activity of SARS-CoV-2 3CLpro. [2]
In vivo	**METHODS**: To validate in vivo efficacy against chronic HCV, Grazoprevir (1 mg/kg) was administered orally to HCV-infected chimpanzees harboring chronic gt1a or gt1b infections twice daily for seven days. **RESULTS**: Grazoprevir was able to suppress viral load by 4-5 logs in vivo. [1] **METHODS**: To assess in vivo pharmacokinetics, Grazoprevir (5 mg/kg) was administered orally to rats. **RESULTS**: Plasma exposure to Grazoprevir was favorable, with an AUC of 0.7 μM h/mL. [3]
Kinase Assay	Scintillation proximity assay (SPA): SGK1 S422D (60–431 aa; 0.275 μg/mL final concentration) or SGK2 (0.875 μg/mL final concentration) are activated by PDK1 (1.1 μg/mL final concentration) in a buffer consisting of 50 mM Tris (pH 7.5), 0.1 mM EGTA, 0.1 mM EDTA, 10 mM MgCl2, 0.1% β-mercaptoethanol, 1 mg/mL BSA, and ATP (final concentration of 0.15 mM) and incubated for 30 min at 30°C. SGK2 is prepared exactly as described for SGK1, except it corresponded to the full-length protein. A solution containing biotinylated CROSStide peptide at a final concentration of 75 μM and γ32P-ATP corresponding to 2×106 cpm is prepared in the reaction buffer. In a 96-well plate, 5 μL of GSK650394 is added to 25 μL of the activated enzyme mixture. To this, 20 μL of the CROSStide mixture is added and incubated for 1 h at room temperature. Next, 50 μL of a 25 mg/mL slurry of streptavidin-coated SPA beads in PBS with 0.1 M EDTA, pH 8.0 is added. The plate is then sealed and centrifuged for 8 min at 2000 rpm, and the signal is detected by measuring for 30 sec/well in a Packard TopCount NXT Scintillation Counter.

Kinase Assay	The IC50 values of the inhibition of SGK1 and SGK2 activities by GSK650394 are calculated from these data using GraphPad Prism 3 Software.
Cell Research	LNCaP cells are plated at a density of 5,000 cells per well in 96-well plates in 100 µL PRF-RPMI 1640, supplemented with 8% CS-FBS, 0.1 mM NEAA, and 1 mM NaPyr. At day three, cells are treated with hormone with or without GSK650394 by removing 50 µL of the media and replacing this with 50 µL of PRF-RPMI 1640 with 8% CS-FBS, NEAA, NaPyr containing a 2X concentration of the appropriate hormone/inhibitor treatment. At days 5 and 7, the treatment is repeated. On the tenth day, the media is removed and the relative cell number is measured using the FluoReporter Blue assay according to the manufacturer's instructions.(Only for Reference)

Solubility Information

Solubility	DMSO: 50 mg/mL (130.74 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+90% Corn Oil: 2.5 mg/mL (6.54 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	2.6147 mL	13.0736 mL	26.1472 mL
5 mM	0.5229 mL	2.6147 mL	5.2294 mL
10 mM	0.2615 mL	1.3074 mL	2.6147 mL
50 mM	0.0523 mL	0.2615 mL	0.5229 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

Sherk AB, et al. Development of a small-molecule serum- and glucocorticoid-regulated kinase-1 antagonist and its evaluation as a prostate cancer therapeutic. Cancer Res. 2008 Sep 15;68(18):7475-83.
He Y, et al. Discovery of a novel Aurora B inhibitor GSK650394 with potent anticancer and anti-aspergillus fumigatus dual efficacies in vitro. J Enzyme Inhib Med Chem. 2022 Dec;37(1):109-117.
Jin LY, et al. GSK 650394 Inhibits Osteoclasts Differentiation and Prevents Bone Loss via Promoting the Activities of Antioxidant Enzymes In Vitro and In Vivo. Oxid Med Cell Longev. 2022 Sep 17;2022:3458560.
Peng HY, et al. Spinal SGK1/GRASP-1/Rab4 is involved in complete Freund's adjuvant-induced inflammatory pain via regulating dorsal horn GluR1-containing AMPA receptor trafficking in rats. Pain. 2012 Dec;153(12):2380-92.
Peng HY, et al. Spinal serum-inducible and glucocorticoid-inducible kinase 1 mediates neuropathic pain via kalirin and downstream PSD-95-dependent NR2B phosphorylation in rats. J Neurosci. 2013 Mar 20;33(12):5227-40.

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