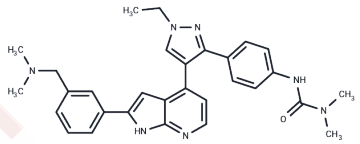
Data Sheet (Cat.No.T6129)

TargetMol

GSK-1070916

Chemical Properties



CAS No. : 942918-07-2

Formula: C30H33N7O

Molecular Weight: 507.63

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-1070916 (GSK-1070916A) is a reversible and ATP-competitive inhibitor of Aurora B/C with IC50 of 3.5 nM/6.5 nM. It displays >100-fold selectivity against the closely related Aurora A-TPX2 complex. Phase 1.
Targets(IC50)	Apoptosis,FLT,AMPK,Aurora Kinase,Tie-2
In vitro	GSK1070916 selectively inhibits Aurora B and Aurora C with Ki of 0.38 nM and 1.5 nM over Aurora A with Ki of 490 nM. Inhibition of Aurora B and Aurora C is time-dependent, with an enzyme-inhibitor dissociation half-life of >480 min and 270 min respectively. In addition, GSK1070916 is also a competitive inhibitor with respect to ATP. [1] Human tumor cells treated with GSK1070916 shows dose-dependent inhibition of phosphorylation on serine 10 of Histone H3, a substrate specific for Aurora B. Moreover, GSK1070916 inhibits the proliferation of tumor cells with EC50 values of <10 nM in over 100 cell lines spanning a broad range of tumor types, with a median EC50 of 8 nM. Although GSK1070916 has potent activity against proliferating cells, a dramatic shift in potency is observed in primary, nondividing, normal human vein endothelial cells. Furthermore, GSK1070916-treated cells do not arrest in mitosis but instead fails to divide and become polyploid, ultimately leading to apoptosis. [2] In another study, it is also reported high chromosome number associated with resistance to the inhibition of Aurora B and C suggests cells with a mechanism to bypass the high ploidy checkpoint are resistant to GSK1070916. [3]
In vivo	GSK1070916 (25, 50, or 100 mg/kg) shows dose-dependent inhibition of phosphorylation of an Aurora B-specific substrate in mice and consistent with its broad cellular activity, has antitumor effects in 10 human tumor xenograft models including breast, colon, lung, and two leukemia models. [2]
Kinase Assay	Kinase Assay: The ability of GSK1070916 to inhibit the Aurora enzymes is measured using in vivo kinase assays. The assays measure the ability of Aurora A, Aurora B and Aurora C to phosphorylate a synthetic peptide substrate. Biotin-Ahx-RARRRLSFFFFAKKK-NH2 is used for the Aurora A-TPX2 LEADseekerTM assay and 5FAM-PKAtide is used for the IMAPTM assay for all three Aurora kinases. To take into account time-dependent inhibition of Aurora enzymes, Aurora A-TPX2, Aurora B-INCENP and Aurora C-INCENP are incubated with GSK1070916 at various concentrations for 30 min before the reactions are initiated with the addition of substrates. For the Aurora A LEADseekerTM assay, final assay conditions are 0.5 nM Aurora A-TPX2, 1 μM peptide substrate, 6 mM MgCl2, 1.5 μM ATP, 0.003 μCi/μL [γ-33P] ATP in 50 mM Hepes, pH 7.2, 0.15 mg/mL BSA,

Kinase Assay	0.01% Tween-20, 5 mM DTT and 25 mM KCl. The reactions are incubated at room temperature (25 °C) for 120 min and terminated by the addition of LEADseekerTM beads in PBS containing EDTA (final concentration 2 mg/mL beads and 25 mM EDTA). The plates are then sealed, and the beads are allowed to settle overnight. Product formation is quantified using a Viewlux Imager. For the IMAPTM assays, Aurora A-TPX2 (final concentration 1 nM), Aurora B-INCENP (final concentration 2 nM) or Aurora C-INCENP (final concentration 2.5 nM) is added to the compound-containing plates in 5 µL of buffer (25 mM Hepes, pH 7.2, for Aurora A, 25 mM Hepes, pH 7.5, for Aurora B and 20 mM Hepes, pH 7.2, for Aurora C) containing 0.15 mg/mL BSA, 0.01% Tween 20 and 25 mM NaCl. This mixture is incubated at room temperature for 30 min. To start the reaction, 5 µL of a substrate solution is added containing the same Hepes buffer as used for the pre-incubation, 25 mM NaCl, MgCl2 (2, 4 and 4 mM for Aurora A, B and C respectively), DTT (4, 4 and 2 mM for Aurora A, B and C respectively), ATP (4, 4 and 10 µM for Aurora A, B and C respectively), 200 nM 5FAM-PKAtide, 0.01% Tween 20 and 0.15 mg/mL BSA. The reactions are incubated at room temperature for 120 min for Aurora A and B and 60 min for Aurora C. These reactions are then terminated by the addition of 10 µL of 1:500 (1:600 for Aurora C) Progressive Binding Reagent in 95% Progressive Binding Buffer A and 5% Progressive Binding Buffer B. Plates are incubated at room temperature for approx. 90-120 min (time allowed for equilibrium to be reached). Plates are read in a Molecular Devices Analyst plate reader in fluorescence polarization mode.
Cell Research	Cells are plated in 96-well plates in the recommended growth media and incubated at 37 °C in 5% CO2 overnight. The following day, the cells are treated with serial dilutions of GSK1070916. At this time, one set of cells is treated with CellTiter-Glo for a time equal to 0 (T = 0) measurement. Following a 6- to 7-d incubation with compound, cell proliferation is measured using the CellTiter-Glo reagent according to the manufacture's recommended protocol. As inhibition of Aurora B induces endomitosis, the degree of which differs depending on the cell type, an extended compound treatment time is required to accurately reflect the effects on cell viability across a large panel of cell lines. For analysis of cell viability, values from wells with no cells are subtracted for background correction and the data plotted as a percent of the DMSO-treated control samples using Microsoft Excel XLfit4 software. The EC50 values represent the concentration of GSK1070916 where 50% maximal effect is observed(Only for Reference)

Solubility Information

Solubility	DMSO: 72.5 mg/mL (142.82 mM),Sonication is recommended. Ethanol: 8 mg/mL (15.76 mM),Sonication is recommended. H2O: < 1 mg/mL (insoluble or slightly soluble), (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 3.3 mg/mL (6.5 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	1.9699 mL	9.8497 mL	19.6994 mL
5 mM	0.394 mL	1.9699 mL	3.9399 mL
10 mM	0.197 mL	0.985 mL	1.9699 mL
50 mM	0.0394 mL	0.197 mL	0.394 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

Anderson K, et al. Biochem J, 2009, 420(2), 259-265.
Hardwicke MA, et al. Mol Cancer Ther, 2009, 8(7),1808-1817.
Moy C, et al. J Transl Med, 2011, 9, 110.