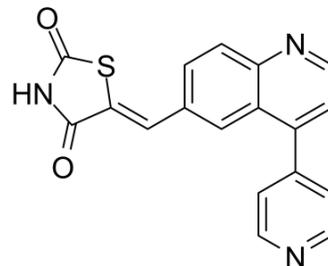


GSK1059615

Cat. No.:	HY-12036		
CAS No.:	958852-01-2		
Molecular Formula:	C ₁₈ H ₁₁ N ₃ O ₂ S		
Molecular Weight:	333.36		
Target:	PI3K; mTOR; Apoptosis		
Pathway:	PI3K/Akt/mTOR; Apoptosis		
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 : 5 mg/mL (15.00 mM; Need ultrasonic)			
		Solvent Concentration	Mass	
			1 mg	5 mg
			10 mg	
Preparing Stock Solutions	1 mM	2.9998 mL	14.9988 mL	29.9976 mL
	5 mM	0.6000 mL	2.9998 mL	5.9995 mL
	10 mM	0.3000 mL	1.4999 mL	2.9998 mL
Please refer to the solubility information to select the appropriate solvent.				
In Vivo	<ol style="list-style-type: none"> Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline Solubility: 0.5 mg/mL (1.50 mM); Suspended solution; Need ultrasonic Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: 0.5 mg/mL (1.50 mM); Suspended solution; Need ultrasonic Add each solvent one by one: 10% DMSO >> 90% corn oil Solubility: ≥ 0.5 mg/mL (1.50 mM); Clear solution 			

BIOLOGICAL ACTIVITY

Description	GSK1059615 is a dual inhibitor of PI3Kα/β/δ/γ (reversible) and mTOR with IC ₅₀ of 0.4 nM/0.6 nM/2 nM/5 nM and 12 nM, respectively.			
IC₅₀ & Target	PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ
	0.4 nM (IC ₅₀)	0.6 nM (IC ₅₀)	2 nM (IC ₅₀)	5 nM (IC ₅₀)
	mTOR 12 nM (IC ₅₀)			

In Vitro	GSK1059615 inhibits PI3K α , β , γ and δ , with K_i of 0.42 nM, 0.6 nM, 0.47 nM and 1.7 nM, respectively ^[1] . In T47D and BT474 cancer cells, GSK1059615 inhibits the phosphorylation of Akt at S473, with IC_{50} of 40 nM ^[2] . MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	GSK1059615 (25 mg/kg) effectively inhibits tumor growth in xenograft mice models of BT474 or HCC1954 breast cancer cells [1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay ^[2]

The measurement of the GSK1059615-dependent inhibition of the PI3Ks is accessed using a HTRF based PI3K profiling assay kit. 400 pM enzyme is used in PI3K α and δ assays, 200 pM in PI3K β assays, and 1 nM in PI3K γ assay. In addition, the PI3K α , β , and δ assays are run with 150 mM NaCl and 100 μ M ATP, while the PI3K γ assay is run with no NaCl and 15 μ M ATP. All reactions are run at 10 μ M PIP2. GSK1059615 is serially diluted (3-fold in DMSO), and 50 nL is transferred to a 384-well low-volume assay plate. PI3K Reaction Buffer is prepared by diluting the stock 1:4 with de-ionized water. Freshly prepared DTT is added at a final concentration of 5 mM on the day of use. Enzyme addition and GSK1059615 pre-incubation are initiated by the addition of 2.5 μ L of PI3K in reaction buffer. Plates are incubated at room temperature for 15 min. Reactions are initiated by addition of 2.5 μ L of 2 \times substrate solution (PIP2 and ATP in 1 \times reaction buffer). Plates are incubated at room temperature for one hour. Reactions are quenched by the addition of 2.5 μ L of stop solution. The quenched reactions are then processed to detect product formation by adding 2.5 μ L of Detection Solution. Following a 1-hour incubation in the dark, the HTRF signal is measured on the Envision plate reader set for 330 nm excitation and dual emission detection at 620 nm (Eu) and 665 nm (APC). The IC_{50} value is then obtained.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Cell Assay ^[2]

Cells are plate at a density of 1×10^4 cells per well in clear flat-bottomed 96-well plates and incubated overnight. Then, GSK1059615 is added and the plates are incubated for 30 min. At the end of incubation, media is aspirated from the plates, and the plate is wash once with cold PBS. 80 μ L MSD Lysis buffer is added into each well and the plates are incubated on a shaker at 4 $^{\circ}$ C for at least 30 min. For Akt duplex assay, plates are washed with 200 μ L/well wash buffer for 4 times and tapped on paper towel to blot. Then, 60 μ L lysates is added to each well and the plates are incubated on shaker at room temperature for 1 hour. After another 4 times washing, antibody is added (25 μ L per well) and the plates are incubated on shaker for 1 hour and washed again. Finally, Read Buffer is added (150 μ L per well) and the plates are read immediately. IC_{50} values are then obtained.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Sci Transl Med. 2018 Jul 18;10(450):eaaq1093.
- Front Pharmacol. 2020, 11:580407.
- Molecules. 2020 Apr 23;25(8):1980.
- Patent. US20200360350A1.
- Patent. US20200113874A1.

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REFERENCES

[1]. Carnero A. Novel inhibitors of the PI3K family. Expert Opin Investig Drugs. 2009 Sep;18(9):1265-77.

[2]. Maira SM, et al. From the bench to the bed side: PI3K pathway inhibitors in clinical development. Curr Top Microbiol Immunol, 2010, 347, 209-239.

[3]. Takashi Kei Kishimoto, et al. Methods and compositions for attenuating gene therapy anti-viral transfer vector immune responses. US 20160074531 A1.

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

Tel: 609-228-6898

Fax: 609-228-5909

E-mail: tech@MedChemExpress.com

Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA