Product Data Sheet

Product Name: KIN-59
Cat. No.: GC16460

Chemical Properties

Cas. No.	4152-77-6		
Chemical Name	5'-O-(triphenylmethyl)-inosine		
SMILES	O[C@H]1[C@@H](O)[C@H](N2C=NC3=C2N=CNC3=O)O[C@@H]1COC(C4=CC=CC=C4)(C5=CC=CC=C5)C6=CC=CC=C6		
Formula	$C_{29}H_{26}N_4O_5$	M.Wt	510.5
Solubility	≤0.3mg/ml in ethanol;16mg/ml in DMSO;5mg/ml in dimethyl formamide	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 ℃ and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20℃ for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		
Structure	KIN-59		

Protocol

Kinase experiment [1]:

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com
Address: 10292 Central Ave. #205, Montclair, CA, USA

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Preparation Method	500μL reaction solution containing 10mM Tris-HCl pH 7.6, 1mM EDTA, 150mM NaCl, 2mM phosphate and 100μM thymidine with 0.025U enzyme TPase was incubated for 60min at room temperature. The samples taken at 0, 20, 40, 60min, 100μL aliquots were boiled, cooled and analyzed by RP-8 HPLC (267nm) to quantify thymine formation. KIN-59 (1mM, 100μM, 10μM, and 0μM) were added to the reaction mixture to test for TPase inhibition.
Reaction Conditions	0–1000μM; 60min
Applications	KIN-59 inhibited TPase in a reversible, non-competitive manner with K_i value of about 39μM.

Cell experiment [2]:

Cell lines	Fetal bovine aortic endothelial GM7373 cells
Preparation Method	Serum-starved FGFR1-overexpressing GM7373 cells and VEGFR2-overexpressing GM7373 cells were incubated with 60μM KIN-59 or vehicle for 30min, after which 10ng/mL FGF2 or 30ng/mL VEGF was added.
Reaction Conditions	60μM; 30min
Applications	KIN-59 inhibited FGF2- but not VEGF-stimulated endothelial cell growth.

Animal experiment [2]:

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Animal models	Female, athymic, nude nu/nu mice
Preparation Method	Eight-week-old female, athymic, nude nu/nu mice, weighing about 25g, were inoculated subcutaneously with 200mL of serum-free DMEM containing 2×10^6 F2T-luc2.9 cells. KIN-59 treatment was started after 24 hours and continued till the end of the experiment. KIN-59 was administered subcutaneously at 15mg/kg, twice daily (once daily during the weekend) at a site distant from the tumor (inoculation) site.
Dosage form	15mg/kg; s.c; twice daily (once daily during the weekend)
Applications	KIN-59 inhibited the binding of fibroblast growth factor 2 (FGF2) to FGF receptor 1, preventing the growth and neovascularization of subcutaneous tumors induced by FGF2-transformed endothelial cells.

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References:
[1] Liekens, S., Hernández, A.I., Ribatti, D., et al. The nucleoside derivative 5'-O-trityl-inosine (KIN59) suppresses thymidine phosphorylase-triggered angiogenesis via a noncompetitive mechanism of action. The Journal of Biological Chemisty 279(28), 29598-29605 (2004).
[2] Liekens, S., Bronckaers, A., Belleri, M., et al. The thymidine phosphorylase inhibitor 5'-O-tritylinosine (KIN59) is an antiangiogenic multitarget fibroblast growth factor-2 antagonist. Mol. Cancer Ther. 11(4), 817-829 (2012).

Background

KIN-59 is a noncompetitive inhibitor against human and bacterial recombinant thymidine phosphorylase (TPase) with IC_{50} values of 67 and 44μM respectively[1]. KIN-59 is a purine riboside derivative that blocks the enzyme non-competitively with thymidine or phosphate, and requires both the intact 5′-O-trityl group and the ribose–hypoxanthine scaffold for activity[2].

In vitro, KIN-59 (0–100μM) incubated muse aortic endothelial cells (MAECs) for 5 days poorly inhibited cell proliferation with an IC_{50} value of 78 ± 2μM. KIN-59 also dose-dependently slowed endothelial cell migration in a 16-hour wound-healing assay[3]. KIN-59 (250nmol) incubated fertilized chicken eggs with or without 10μl of TPase for 4 days. KIN-59 not only annihilated the TPase-induced angiogenesis but also efficiently inhibited the formation of normal chick chorioallantoic membrane vessels in the absence of

exogenously added TPase[4]. KIN-59 (125–250μM) pretreatment on human or wild-type murine platelets for 5min before collagen, ADP, thrombin or collagen related peptide stimulation reversibly and dose-dependently suppressed induced aggregation[5].

In vivo, KIN-59 (15mg/kg) was injected subcutaneously in immunodeficient nude mice twice daily (once daily during the weekend) until day 20. KIN-59 inhibited the binding of fibroblast growth factor 2 (FGF2) to FGF receptor 1, preventing the growth and neovascularization of subcutaneous tumors induced by FGF2-transformed endothelial cells[6].

References:
[1] Liekens S, Balzarini J, Hernández A I, et al. Thymidine phosphorylase is noncompetitively inhibited by 5'-O-trityl-inosine (KIN59) and related compounds. Nucleosides Nucleotides Nucleic Acids. 2006;25(9-11):975-80.
[2] Casanova E, Hernandez A I, Priego E M, et al. 5'-O-tritylinosine and analogues as allosteric inhibitors of human thymidine phosphorylase. J Med Chem. 2006 Sep 7;49(18):5562-70.
[3] Liekens, S., Bronckaers, A., Hernández, A.I., et al. 5'-O-tritylated nucleoside derivatives: inhibition of thymidine phosphorylase and angiogenesis. Mol. Pharmacol. 70(2), 501-509 (2006).
[4] Liekens, S., Hernández, A.I., Ribatti, D., et al. The nucleoside derivative 5'-O-trityl-inosine (KIN59) suppresses thymidine phosphorylase-triggered angiogenesis via a noncompetitive mechanism of action. The Journal of Biological Chemisty 279(28), 29598-29605 (2004).
[5] Li W, Gigante A, Perez-Perez M J, et al. Thymidine phosphorylase participates in platelet signaling and promotes thrombosis. Circ Res. 2014 Dec 5;115(12):997-1006.
[6] Liekens, S., Bronckaers, A., Belleri, M., et al. The thymidine phosphorylase inhibitor 5'-O-tritylinosine (KIN-59) is an antiangiogenic multitarget fibroblast growth factor-2 antagonist. Mol. Cancer Ther. 11(4), 817-829 (2012).