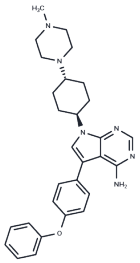
Data Sheet (Cat.No.T10206)

TargetMol

A-419259

Chemical Properties



CAS No. : 364042-47-7

Formula: C29H34N6O

Molecular Weight: 482.62

Storage: Keep away from direct sunlight,Store at low temperature
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	A-419259 (RK-20449) is an apoptosis inducer that selectively inhibits the Src family of kinases, including Src,LCK, and Lyn, with an IC50=3 to 9 nM.
Targets(IC50)	Apoptosis,Src
In vitro	A-419259 selectively inhibits Src kinases (IC_{50} < 0.003 μM), blocks proliferation, and induces apoptosis in Bcr-Abl$^+$ leukemia cells by suppressing Stat5 and Erk signaling, with minimal effect on normal cells[1].
Kinase Assay	In vitro kinase assays are performed on His(6)-tagged Lck (residues 62-509) and full-length c-Abl purified from Sf-9 cells, and commercial sources of Lyn, Src and PKC. Lck, Lyn, Src and Abl activities are measured with an ELISA-based assay. Flat bottom 96-well ELISA plates are incubated with a 200 μg/mL solution of Poly(Glu,Tyr) 4 : 1 substrate in phosphate buffered saline (PBS) for 1 h at 37°C and then washed with PBS containing 0.1% Tween-20 (PBS-T). Inhibitor dilutions are added to the washed plates already containing the appropriate enzyme in kinase assay buffer (250 mM Mopso, pH 6.75, 10 mM MgCl2, 2 mM MnCl2, 2.5 mM DTT, 0.02% BSA, 2 mM Na3VO4, 5% DMSO, 5 μM ATP). After incubation at room temperature for 20 min, plates are washed three times with PBS-T and plate-bound phosphotyrosine is detected with an anti-phosphotyrosine-HRP antibody conjugate and subsequent color development with K-Blue reagents. All assays are optimized to use the least amount of enzyme necessary for a reproducible signal-to-noise ratio [1].
Cell Research	K-562 cells are grown in RPMI 1640 supplemented with 10% fetal calf serum (FCS), and 50 g/mL Gentamycin. Meg-01 cells are cultured in Vitacell modified RPMI 1640 (ATCC), supplemented with 10% FCS and 50 μg/mL Gentamycin. The human GM-CSF-dependent myeloid leukemia cell line TF-1 is grown in RPMI 1640 supplemented with 10% FCS, 50 μg/mL Gentamycin, and 1 ng/mL of recombinant human GM-CSF. DAGM murine myeloid leukemia cells are cultured in RPMI 1640 supplemented with 10% FCS, 50 μg/mL Gentamycin, and 0.5 ng/mL recombinant IL-3. Concentrated stock solutions of PP2 (5 mM) and A-419259 (2 mM) are prepared in DMSO and stored at -20°C. Cellular proliferation is measured using the Live/Dead growth assay. This assay employs calcein-AM, a fluorogenic esterase substrate that is taken up by viable cells and hydrolyzed intracellularly, releasing a green fluorescent product. Briefly, 10^4 cells are

Cell Research	plated per well in 96-well plates for each day of a 4-day time course. Various concentrations of PP2, A-419259 or vehicle control are added to the wells (five wells per concentration per day) and the plates are incubated at 37°C. At each time point, one plate is centrifuged at 1500 g for 10 min to pellet the cells. Cells are washed with phosphate buffered saline (PBS), and calcein-AM is added to each well to a final concentration of 1 μM. Plates are incubated in the dark at room temperature for 1 h. The plates are then read at 485/530 nm (excitation/emission) using a fluorescent plate reader and data are analysed with SoftMax Pro software [1].

Solubility Information

Solubility	H2O: < 0.1 mg/mL (insoluble) DMSO: 10 mg/mL (20.72 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: 1 mg/mL (2.07 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	2.072 mL	10.3601 mL	20.7202 mL
5 mM	0.4144 mL	2.072 mL	4.144 mL
10 mM	0.2072 mL	1.036 mL	2.072 mL
50 mM	0.0414 mL	0.2072 mL	0.4144 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

Wilson MB, et al. Selective pyrrolo-pyrimidine inhibitors reveal a necessary role for Src family kinases in Bcr-Abl signal transduction and oncogenesis. Oncogene. 2002 Nov 21;21(53):8075-88.

Pene-Dumitrescu T, et al. An inhibitor-resistant mutant of Hck protects CML cells against the antiproliferative and apoptotic effects of the broad-spectrum Src family kinase inhibitor A-419259.Oncogene (2008) 27, 7055-7069