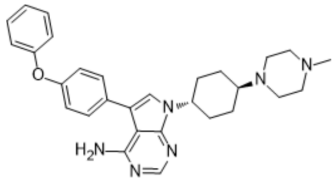
A 419259

Cat. No.:	HY-15764
CAS No.:	364042-47-7
Molecular Formula:	$C_{29}H_{34}N_6O$
Molecular Weight:	482.62
Target:	Src; Apoptosis
Pathway:	Protein Tyrosine Kinase/RTK; 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 : 100 mg/mL (207.20 mM; ultrasonic and warming and heat to 60°C)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.0720 mL	10.3601 mL	20.7202 mL
	5 mM	0.4144 mL	2.0720 mL	4.1440 mL
	10 mM	0.2072 mL	1.0360 mL	2.0720 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (5.18 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (5.18 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

A 419259 is a broad-spectrum pyrrolo-pyrimidine inhibitor, has high selectivity towards the Src family. A 419259 shows inhibitory effect for Src, Lck and Lyn with IC_{50} of 9 nM, <3 nM and <3 nM, respectively[1][2].

IC_{50} & Target

IC50: 9 nM (Src), <3 nM (Lck), <3 nM (Lyn), 3 μM (Abl)[1]

In Vitro

"A419259 is a second-generation pyrrolopyrimidine that blocks proliferation and induces apoptosis in CML cell lines. It induces apoptosis in K-562 cells and also inhibits Meg-01 proliferation (IC_{50}=0.1 μM)[1].
In the absence of IL-3, A-419259 strongly inhibits DAGM/Bcr-Abl cell proliferation (IC_{50}=0.1-0.3 μM)[1].
A-419259 also inhibits overall SFK activity in CML cell lines and blocks Src kinase activation (IC_{50}=0.1-0.3 μM)[1].
A 419259 (1 μM; 16 h) inhibits endogenous SFK (c-Src and Lck) activity, thereby inhibiting Src-driven differentiation of mES

cells toward primitive ectoderm-like cells[2].
A 419259 (0.3, 1 μM; 5 days) has no effect on undifferentiated colony morphology of hES cells grown in mTeSR medium[2].
"

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

PROTOCOL

Kinase Assay [1]

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 $MgCl_2$, 2 mM $MnCl_2$, 2.5 mM DTT, 0.02% BSA, 2 mM Na_3VO_4, 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].

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Cell Assay [1]

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 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 SpectraMax Gemini XS fluorescent plate reader and data are analysed with SoftMax Pro software[1].

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CUSTOMER VALIDATION

- Blood. 2016 Jun 23;127(25):3237-52.
- Patent. US20170333436A1.

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REFERENCES

[1]. 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.

[2]. Zhang X, et al. Src-family tyrosine kinase activities are essential for differentiation of human embryonic stem cells. Stem Cell Res. 2014 Nov;13(3 Pt A):379-89.

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

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