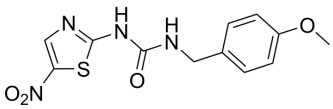
AR-A014418

Cat. No.:	HY-10512
CAS No.:	487021-52-3
Molecular Formula:	$C_{12}H_{12}N_4O_4S$
Molecular Weight:	308
Target:	GSK-3
Pathway:	PI3K/Akt/mTOR; Stem Cell/Wnt
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 1 year -20°C 6 months



SOLVENT & SOLUBILITY

In Vitro

DMSO : ≥ 100 mg/mL (324.68 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Mass / Solvent / Concentration	1 mg	5 mg	10 mg
	1 mM	3.2468 mL	16.2338 mL	32.4675 mL
	5 mM	0.6494 mL	3.2468 mL	6.4935 mL
	10 mM	0.3247 mL	1.6234 mL	3.2468 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 (8.12 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

AR-A014418 is a potent, selective and ATP-competitive GSK3β inhibitor (IC_{50}=104 nM; K_i=38 nM)[1].

IC_{50} & Target

GSK-3β
104 nM (IC_{50})

In Vitro

AR-A014418 blocks the phosphorylation of tau at a GSK3-specific site (Ser-396) in 3T3 fibroblasts expressing human four-repeat tau protein, with an IC_{50} of 2.7 μM, and protects cultured N2A cells from death cuased by PI3K/PKB pathway blockage. AR-A014418 also shows inhibitory effect on neurodegeneration mediated by beta-amyloid peptide in hippocampal slices[1].

AR-A014418 decreases neuroendocrine markers and suppresses neuroblastoma cell growth in NGP and SH-5Y-SY cells[2].

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

In Vivo	AR-A014418 (0-4 mg/kg, i.p.) delays the onset of symptoms, enhances motor activity, blocks disease progression, and postpons the endpoint of the disease in ALS mouse model with the G93A mutant human SOD1[3]. AR-A014418 suppresses acetic acid- and formalin-induced nociception in mice via modulating NMDA and metabotropic receptor signaling as well as TNF-α and IL-1β transmission in the spinal cord[4]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	The competition experiments are carried out in duplicate with 10 concentrations of the inhibitor in clear-bottomed microtiter plates. The biotinylated peptide substrate, biotin-AAEELDSRAGS(PO3H2)PQL, is added at a final concentration of 2 μM in an assay buffer containing 6 milliunits of recombinant human GSK3 (equal mix of both α and β), 12 mM MOPS, pH 7.0, 0.3 mM EDTA, 0.01% β-mercaptoethanol, 0.004% Brij 35, 0.5% glycerol, and 0.5 μg of bovine serum albumin/25 μL and preincubated for 10-15 min. The reaction is initiated by the addition of 0.04 μCi of [γ-^{33}P]ATP and unlabeled ATP in 50 mM $Mg(Ac)_2$ to a final concentration of 1 μM ATP and assay volume of 25 μL. Blank controls without peptide substrate are used. After incubation for 20 min at room temperature, each reaction is terminated by the addition of 25 μL of stop solution containing 5 mM EDTA, 50 μM ATP, 0.1% Triton X-100, and 0.25 mg of streptavidin-coated SPA beads corresponding to appr 35 pmol of binding capacity. After 6 h the radioactivity is determined in a liquid scintillation counter. Inhibition curves are analyzed by non-linear regression using GraphPad Prism. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	Cell viability is assessed by calcein/propidium iodide uptake. Calcein AM is taken up and cleaved by esterases present within living cells, yielding yellowish-green fluorescence, whereas PI is only taken up by dead cells, which become orange-red fluorescent. In brief, N2A cells are cultured for 2 days in vitro and then treated with 50 μM LY-294002 in the presence of AR-A014418 or vehicle (DMSO) for 24 h. Subsequently, N2A cells are incubated for 30 min with 2 μM PI and 1 μM calcein-AM. The cultures are then rinsed three times with Hanks' buffered saline solution containing 2 mM $CaCl_2$, and the cells are visualized by fluorescence microscopy using a Zeiss Axiovert 135 microscope. Three fields (selected at random) are analyzed per well (appr 300 cells/field) in at least three different experiments. Cell death is expressed as percentage of PI-positive cells from the total number of cells. In every experiment, specific cell death is obtained after subtracting the number of dead cells present in vehicle-treated cultures. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [3]	First, to examine the effects of GSK-3 inhibition on the clinical symptoms, life span, and motor behavior function of ALS, 56 Tg mice are divided into four groups. In each group, 0.5 mL of normal saline is mixed with either 0 μg (control group), 1 μg (group A), 2 μg (group B) or 4 μg (group C) of AR-A014418 per gram of mouse, and injected intraperitoneally into 14 animals per group 5 days a week beginning 60 days after birth. The mice are sacrificed at the endpoint described below. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- J Exp Clin Cancer Res. 2018 Jun 25;37(1):122.
- Cell Syst. 2018 Apr 25;6(4):424-443.e7.
- NPJ Biofilms Microbiomes. 2024 Jan 20;10(1):5.
- Oncogene. 2023 Jun 22.
- Phytomedicine. 2023 Sep 7, 155074.

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REFERENCES

[1]. Bhat R, Xue Y, Berg S, Structural insights and biological effects of glycogen synthase kinase 3-specific inhibitor AR-A014418. J Biol Chem. 2003 Nov 14;278(46):45937-45.

[2]. Carter YM, et al. Specific glycogen synthase kinase-3 inhibition reduces neuroendocrine markers and suppresses neuroblastoma cell growth. Cancer Biol Ther. 2014 May;15(5):510-5.

[3]. Koh SH, et al. Inhibition of glycogen synthase kinase-3 suppresses the onset of symptoms and disease progression of G93A-SOD1 mouse model of ALS. Exp Neurol. 2007 Jun;205(2):336-46

[4]. Martins DF, et al. The antinociceptive effects of AR-A014418, a selective inhibitor of glycogen synthase kinase-3 beta, in mice. J Pain. 2011 Mar;12(3):315-22.

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