KN-62

Cat. No.: HY-13290

CAS No.: 127191-97-3

Molecular Formula: $C_{38}H_{35}N_5O_6S_2$

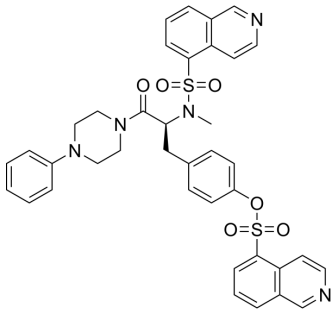
Molecular Weight: 721.84

Target: CaMK; P2X Receptor

Pathway: Neuronal Signaling; Membrane Transporter/Ion Channel

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 (138.53 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	1.3853 mL	6.9267 mL	13.8535 mL
5 mM	0.2771 mL	1.3853 mL	2.7707 mL
10 mM	0.1385 mL	0.6927 mL	1.3853 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 (3.46 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (3.46 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

KN-62 is a selective and reversible inhibitor of calmodulin-dependent protein kinase II (CaMK-II) with a K_i of 0.9 μM for rat brain CaMK-II. KN-62 directly binds to the calmodulin binding site of CaMK-II. KN-62 displays noncompetitive antagonism at $P2X_7$ receptors in HEK293 cells, with an IC_{50} value of approximately 15 nM.

IC_{50} & Target

P2X7 Receptor	CaMK II
	0.9 μM (Ki)

In Vitro

KN-62 potently antagonizes ATP-stimulated Ba^{2+} influx into fura-2 loaded human lymphocytes with an IC_{50} of 12.7 nM and complete inhibition of the flux at a concentration of 500 nM[1].

	?KN-62 does not inhibit the activity of autophosphorylated Ca2+/CaM kinase II. KN-62 inhibits the Ca2+/calmodulin-dependent autophosphorylation of both alpha (50 kDa) and beta (60 kDa) subunits of Ca2+/CaM kinase II dose dependently in the presence or absence of exogenous substrate[2]. ?In human leukemic B lymphocytes, KN-62 reduces the rate of permeability increase to larger permeant cations, like ethidium, induced by Bz-ATP with an IC_{50} of 13.1 nM[4]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	KN62 (5?mg/kg/day; ip; three times a week for 6 weeks) significantly reduces the liver metastatic tumor burden in five weeks old BALB/c athymic nude mice inoculated with TAMR-MCF-7 cells[3]. ?KN-62 (1 μg/site, i.c.v.) prevents the antidepressant-like behavior and antidepressant-like behaviors of $ZnCl_2$ (10 mg/kg, p.o.)[5]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	Lymphocytes (1×10^7/mL) are cultured with [^{3}H]-oleic acid (2-5 μCi/mL, specific activity 10 Ci/mmol) for 20-24 h in RPMI-1640 medium supplemented with Gentamicin (40 μg/mL), 10% heat inactivated foetal calf serum (FCS) at 37°C to label membrane phospholipids. Labelled cells are washed twice in HEPES buffered saline followed by a final wash in either HEPES buffered saline or 150 mM KCl medium containing HEPES 10 mM, pH 7.4, bovine serum albumin (BSA) 1 g/L and D-glucose 5 mM and $CaCl_2$ 1 mM. Three mL aliquots containing 1.1×10< sup>7/mL lymphocytes are warmed to 37°C and incubated with or without KN-62 or KN-04 (1 nM-500 nM) for 5 min, then 900 mL aliquots are added to 100 uL butanol (final concentration 30 mM) for a further 5 min, and stimulated with 1 mM ATP for 15 min with gentle mixing in the continued presence of inhibitor or diluent. The phospholipase D reaction is terminated by addition of 1 mL of 20 mM $MgCl_2$ followed by centrifugation and addition of 1 mL ice cold methanol. Membrane lipids are extracted into chloroform/HCl at 4°C under N_2, and separated by silica gel thin layer chromatography (t.l.c.) with the solvent system, ethyl acetate/iso-octane/acetic acid/water (13:2:3:10, v/v) under saturating conditions. Sample spots are located by autoradiography and [^{3}H]-phosphatidylbutanol ([^{3}H]-PBut) spots identified by an authentic standard. [^{3}H]-PBut and [^{3}H]-phospholipid spots are scraped into scintillant fluid (PPO in toluene, 4 g/L) and counted in a liquid scintillation counter. The quantity of [^{3}H]-PBut is presented as a percentage of total ^{3}H labelled-cellular phospholipids. Phospholipase D assays are performed in triplicate[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [4]	All experiments are performed using adherent HEK293 cells stably transfected with cDNA encoding the human $P2X_7$ receptor. Adherent cells on 12-well polylysine-coated plates are incubated at 37°C in 1 mL physiological salt solution (125 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1.5 mM $CaCl_2$, 25 mM NaHEPES (pH 7.5), 10 mM D-glucose, 1 mg/mL BSA). Antagonists(e.g., KN-62) are added from 1,000× stock solutions dissolved in DMSO. Cells are preincubated with antagonists (e.g., KN-62) for 15 min prior to stimulation for 10 min with 3 mM ATP (final concentration). Reactions are terminated by rapid aspiration of the extracellular medium in each well. The adherent cells in each well are then extracted overnight with 1 mL 10% HNO_3. K^+ content in these nitric acid extracts is assayed by atomic absorbance spectrophotometry. Duplicate or triplicate wells are run for all test conditions in each separate experiment[4]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [5]	Mice[3] Female Swiss mice (45-55 days old, weighing 30-45 g) are used. The following drugs are used: $ZnCl_2$ (1 or 10 mg/kg), H-89 (1 μg/site, PKA inhibitor), KN-62 (1 μg/site, CAMKII inhibitor), chelerythrine (1 μg/site, PKC inhibitor), PD98059 (5 μg/site, MAPKK/MEK 1/2 inhibitor), U0126 (5 μg/site, MEK1/2 inhibitor), LY294002 (10 nmol/site, PI3K inhibitor), AR-A014418 (0.001 μg/site, selective GSK-3β inhibitor). $ZnCl_2$ is dissolved in distilled water and administered orally (p.o.). H-89, KN-62, chelerythrine, PD98059, U0126, LY294002, AR-A014418 are dissolved in saline (0.9% NaCl) at a final concentration of 1% dimethyl sulfoxide (DMSO) and administered by intracerebroventricular (i.c.v.) route. The drugs are freshly prepared before treatment and administered in a volume of 10 mL/kg body weight (p.o. route) or 5 μL/site (i.c.v. route). Control animals receive the appropriate vehicle. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Clin Transl Med. 2022 May;12(5):e849.
- Cell Syst. 2018 Apr 25;6(4):424-443.e7.
- Cell Commun Signal. 2021 Oct 11;19(1):103.
- Cell Biol Toxicol. 2021 Jul 20.
- Mol Neurobiol. 2023 Jan 30.

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REFERENCES

[1]. Gargett CE, et al. The isoquinoline derivative KN-62 a potent antagonist of the P2Z-receptor of human lymphocytes. Br J Pharmacol. 1997 Apr;120(8):1483-90.

[2]. Ravi RG, et al. Potent P2X7 Receptor Antagonists: Tyrosyl Derivatives Synthesized Using a Sequential Parallel Synthetic Approach. Drug Dev Res. 2001 Oct;54(2):75-87.

[3]. Manosso LM, et al. Antidepressant-like effect of zinc is dependent on signaling pathways implicated in BDNF modulation. Prog Neuropsychopharmacol Biol Psychiatry. 2015 Jun 3;59:59-67.

[4]. H Hidaka, et al. Pharmacology of protein kinase inhibitors. Annu Rev Pharmacol Toxicol. 1992;32:377-97.

[5]. Miso Park, et al. Involvement of the P2X7 receptor in the migration and metastasis of tamoxifen-resistant breast cancer: effects on small extracellular vesicles production. Sci Rep. 2019 Aug 12;9(1):11587.

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