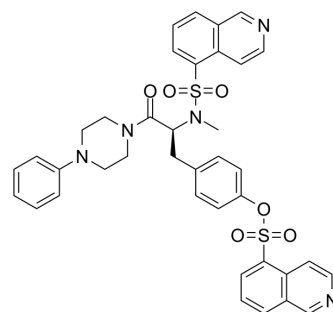


## KN-62

<b>Cat. No.:</b>	HY-13290		
<b>CAS No.:</b>	127191-97-3		
<b>Molecular Formula:</b>	C <sub>38</sub> H <sub>35</sub> N <sub>5</sub> O <sub>6</sub> S <sub>2</sub>		
<b>Molecular Weight:</b>	721.84		
<b>Target:</b>	CaMK; P2X Receptor		
<b>Pathway:</b>	Neuronal Signaling; Membrane Transporter/Ion Channel		
<b>Storage:</b>	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 (138.53 mM)  
 \* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent		1 mg	5 mg	10 mg
	Concentration	Mass			
	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

- 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
- 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<sub>i</sub> 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<sub>7</sub> receptors in HEK293 cells, with an IC<sub>50</sub> value of approximately 15 nM.

#### IC<sub>50</sub> & Target

CaMK II  
 0.9 μM (K<sub>i</sub>)

#### In Vitro

KN-62 potently antagonizes ATP-stimulated Ba<sup>2+</sup> influx into fura-2 loaded human lymphocytes with an IC<sub>50</sub> of 12.7 nM and complete inhibition of the flux at a concentration of 500 nM<sup>[1]</sup>.

KN-62 does not inhibit the activity of autophosphorylated Ca<sup>2+</sup>/CaM kinase II. KN-62 inhibits the Ca<sup>2+</sup>/calmodulin-dependent autophosphorylation of both alpha (50 kDa) and beta (60 kDa) subunits of Ca<sup>2+</sup>/CaM kinase II dose dependently in the presence or absence of exogenous substrate<sup>[2]</sup>.

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<sub>50</sub> of 13.1 nM<sup>[4]</sup>.

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<sup>[3]</sup>.

KN-62 (1 µg/site, i.c.v.) prevents the antidepressant-like behavior and antidepressant-like behaviors of ZnCl<sub>2</sub> (10 mg/kg, p.o.)<sup>[5]</sup>.

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## PROTOCOL

#### Kinase Assay <sup>[1]</sup>

Lymphocytes (1×10<sup>7</sup>/mL) are cultured with [<sup>3</sup>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<sub>2</sub> 1 mM. Three mL aliquots containing 1.1×10<sup>6</sup>/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 µL aliquots are added to 100 µL 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<sub>2</sub> followed by centrifugation and addition of 1 mL ice cold methanol. Membrane lipids are extracted into chloroform/HCl at 4°C under N<sub>2</sub>, 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 [<sup>3</sup>H]-phosphatidylbutanol ([<sup>3</sup>H]-PBut) spots identified by an authentic standard. [<sup>3</sup>H]-PBut and [<sup>3</sup>H]-phospholipid spots are scraped into scintillant fluid (PPO in toluene, 4 g/L) and counted in a liquid scintillation counter. The quantity of [<sup>3</sup>H]-PBut is presented as a percentage of total <sup>3</sup>H labelled-cellular phospholipids. Phospholipase D assays are performed in triplicate<sup>[1]</sup>.

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#### Cell Assay <sup>[4]</sup>

All experiments are performed using adherent HEK293 cells stably transfected with cDNA encoding the human P2X<sub>7</sub> 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<sub>2</sub>, 1.5 mM CaCl<sub>2</sub>, 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<sub>3</sub>. K<sup>+</sup> 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<sup>[4]</sup>.

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#### Animal Administration <sup>[5]</sup>

Mice<sup>[3]</sup>

Female Swiss mice (45-55 days old, weighing 30-45 g) are used. The following drugs are used: ZnCl<sub>2</sub> (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<sub>2</sub> 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.

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## CUSTOMER VALIDATION

- Cell Syst. 2018 Apr 25;6(4):424-443.e7.
- Clin Transl Med. 2022 May;12(5):e849.
- Cell Biol Toxicol. 2021 Jul 20.
- Cell Commun Signal. 2021 Oct 11;19(1):103.
- Mol Pharmacol. 2019 Mar;95(3):294-302.

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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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