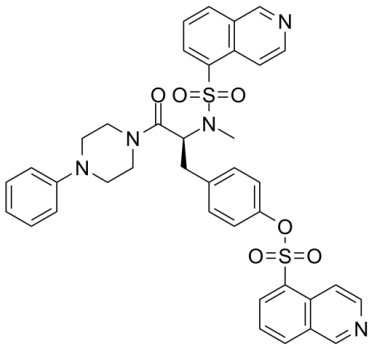
Data Sheet

Product Name:	KN-62
Cat. No.:	CS-0516
CAS No.:	127191-97-3
Molecular Formula:	C38H35N5O6S2
Molecular Weight:	721.84
Target:	Autophagy; CaMK; P2X Receptor
Pathway:	Autophagy; Membrane Transporter/Ion Channel; Neuronal Signaling
Solubility:	DMSO : ≥ 100 mg/mL (138.53 mM); H2O : < 0.1 mg/mL (insoluble)



BIOLOGICAL ACTIVITY:

KN-62 is a selective and potent inhibitor of calmodulin-dependent protein kinase II (**CaMK-II**) with **IC_{50}** of 0.9 μM, KN-62 also displays noncompetitive antagonism at **$P2X_7$** receptors in HEK293 cells, with an **IC_{50}** value of approximately 15 nM. IC50 & Target: IC50: 0.9 μM (CaMK II)[1], 15 nM ($P2X_7$ receptor, in HEK293 cells)[2] **In Vitro:** KN-62 is a selective antagonist of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). KN-62 potently antagonizes ATP-stimulated Ba^{2+} influx into fura-2 loaded human lymphocytes with an IC_{50} of 12.7±1.5 nM (n=3) and complete inhibition of the flux at a concentration of 500 nM. Similarly, KN-62 inhibits ATP-stimulated ethidium$^+$ uptake, measured by time resolved flow cytometry, with an IC_{50} of 13.1±2.6 nM (n=4) and complete inhibition of the flux at 500 nM[1]. KN-62 is found to be a potent antagonist in a functional assay, inhibition of ATP-induced K^+efflux in HEK293 cells expressing recombinant human $P2X_7$ receptors. 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. KN-62 at a concentration of 3 μM has no effect on ATP-induced ethidium influx through the rat $P2X_7$ receptor, while the IC_{50} at the human $P2X_7$ receptor is 0.1 μM. KN-62 has considerable selectivity for $P2X_7$ receptors within the P2 family[2]. **In Vivo:** The antidepressant-like behavior of $ZnCl_2$ (10 mg/kg, p.o.) ($p<0.01$) is prevented by CAMKII inhibitor KN-62 (1 μg/site, i.c.v.). The two-way ANOVA reveals a significantly main effect of KN-62 treatment [$F(1,28)=27.47$, $p<0.01$], no main effect of $ZnCl_2$ treatment [$F(1,28)=0.84$, $p>0.05$] and a significant effect of KN-62×$ZnCl_2$ treatment interaction [$F(1,28)=22.57$, $p<0.01$] to immobility time. As revealed by the post-hoc analysis, the anti-immobility effect of $ZnCl_2$ is completely prevented by treatment of animals with KN-62. No effect in locomotor activity in the open-field test is observed: (KN-62 treatment [$F(1,24)=1.97$, $p>0.05$], $ZnCl_2$ treatment [$F(1,24)=3.99$, $p>0.05$] and KN-62×$ZnCl_2$ treatment interaction [$F(1,24)=0.61$, $p>0.05$])[3].

PROTOCOL (Extracted from published papers and Only for reference)

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]. **Cell Assay:** KN-62 is dissolved in DMSO and stored, and then diluted with appropriate media before use[2].[2]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[2]. **Animal Administration:** KN-62 is dissolved in saline (0.9% NaCl) at a final concentration of 1% DMSO (Mice)[3].[3]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.

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.

CAIndexNames:

5-Isoquinolinesulfonic acid, 4-[(2S)-2-[(5-isoquinolinylsulfonyl)methylamino]-3-oxo-3-(4-phenyl-1-piperazinyl)propyl]phenyl ester

SMILES:

O=C(N1CCN(C2=CC=CC=C2)CC1)[C@H](CC3=CC=C(C=C3)OS(=O)(C4=CC=CC5=C4C=CN=C5)=O)N(C)S(=O)(C6=CC=CC7=C6C=CN=C7)=O

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