

## Product Data Sheet

Product Name: NS309  
Cat. No.: GC12528

### Chemical Properties

Cas. No. 18711-16-5

Chemical Name 6,7-dichloro-3-(hydroxyamino)-2H-indol-2-one

SMILES O=C1N=C2C(Cl)=C(Cl)C=CC2=C1NO

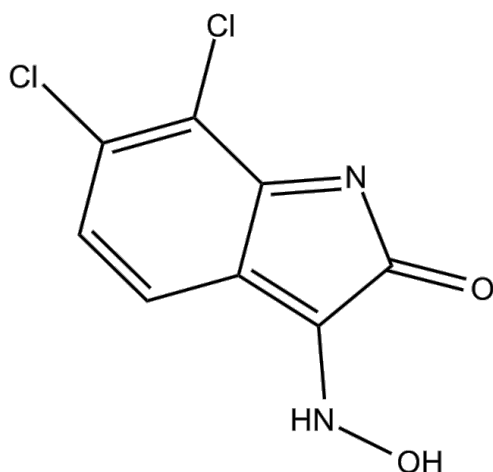
Formula  $C_8H_4Cl_2N_2O_2$  M.Wt 231.04

Solubility DMSO : 100 mg/mL (432.83 mM; Need ultrasonic) Storage Store at -20°C

General tips For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.

Structure



### Protocol

#### Cell experiment [1]:

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### Cell lines

Human coronary artery endothelial cells

The human coronary artery endothelial cells (HCAECs) at passage four received two washes of  $\text{Ca}^{2+}$ -free Dulbecco's Modified Eagle Medium (DMEM) before undergoing incubation with 0.05% trypsin and 0.02% EDTA for 1 to 2 minutes. Use a perforated whole-cell voltage-clamp method with an Axopatch-200B amplifier to record  $\text{K}^+$  currents. The bath solution contained 140mmol/L NaCl and 5mmol/L KCl along with 1mmol/L  $\text{CaCl}_2$  and 2mmol/L  $\text{MgCl}_2$  while 10mmol/L HEPES and 30mmol/L glucose were added to achieve pH 7.4 at a temperature of 22°C. The resistance measurement for the patch pipette ranged from 2 to 5 megaohms. The pipette solution contained 110mmol/L aspartate,

Preparation Method 20mmol/L KCl, 1mmol/L  $\text{MgCl}_2$ , 9mmol/L  $\text{CaCl}_2$ , 10mmol/L HEPES, 8mmol/L NaCl, 0.01mmol/L niflumic acid, and 10mmol/L BAPTA with a pH of 7.2 and free  $\text{Ca}^{2+}$  concentration of 1 $\mu\text{mol/L}$ . Cells received depolarization pulses every 5 seconds from -50mV holding potential reaching test potentials from -100 to +100mV in steps of +20mV for an overall 150mV duration. After treating cells with NS309 at 10 $\mu\text{mol/L}$  concentration tracings were collected across a potential range from -100 to +100mV while maintaining a holding potential of -50mV. The effect of the selective NS309 (10 $\mu\text{mol/L}$ ) on the whole cell  $\text{K}^+$  currents was examined. Both TRAM34 and apamin were also applied for testing the specificity of NS309.

### Reaction

#### Conditions

10 $\mu\text{mol/L}$ ; every 5 seconds

### Applications

NS309 treatment increased the total  $\text{K}^+$  currents of culture human coronary artery endothelial cells and induced the cell hyperpolarization.

### Animal experiment [2]:

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Animal models      Male Sprague-Dawley (SD) rats

Preparation Method      Male Sprague-Dawley (SD) rats (3 months old, 250-280g body weight) were randomly divided into three groups (n = 6 each group): the NS309 pretreated group received an intraperitoneal injection of NS309 at 2mg/kg concentration (100µl per 20g body weight) 30 minutes before the traumatic brain injury (TBI). The sham group rats went through surgical procedures without any traumatic injury while the remaining rats received traumatic injury treatments. A saline solution containing 0.9% NaCl mixed with 1% DMSO served as the vehicle. At 24 hours post-TBI the rats were sacrificed to measure brain water content and perform TUNEL staining as well as apoptosis factor measurement and immunohistochemistry, while at 14 and 28 days they were sacrificed to measure contusion volume and at 12, 24, and 72 hours they were used for inflammatory cytokine measurement and TSG-6 and NF-κB p65 expression analysis.

Dosage form      2mg/kg; i.p.

Applications      NS309 treatment significantly reduced brain edema and neurological deficits caused by TBI, decreased neuronal apoptosis and significantly reduced the levels of proinflammatory cytokines (IL-1β, IL-6, and TNF-α) and chemokines (MCP-1, MIP-2, and RANTES).

### References:

- [1]Liu Y, Xie A, Singh A K, et al. Inactivation of endothelial small/intermediate conductance of calcium-activated potassium channels contributes to coronary arteriolar dysfunction in diabetic patients[J]. Journal of the american heart association, 2015, 4(8): e002062.
- [2]Chen T, Zhu J, Hang C H, et al. The potassium SK channel activator NS309 protects against experimental traumatic brain injury through anti-inflammatory and immunomodulatory mechanisms[J]. Frontiers in pharmacology, 2019, 10: 1432.

### Background

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NS309 is the most potent positive gating modulator for intermediate-conductance  $\text{Ca}^{2+}$ -activated  $\text{K}^{+}$  channel  $\text{KCa3.1}$ , with an  $\text{EC}_{50}$  of  $75\text{nmol/L}$ <sup>[1]</sup>. NS309 was binding within the interface between calmodulin's N-lobe and the S4-S5 linker to exhibit the agonist activity, and NS309 forms hydrogen bonds between the NS309's carbonyl and residues N201 and R287<sup>[2]</sup>. NS309 functions as a pharmacological agent to enhance the activity of both small and intermediate conductance calcium-activated potassium channels<sup>[3]</sup>.

In vitro, NS309 can stimulate changes in the cell's ions<sup>[4]</sup>. The exposure of diabetes-derived human coronary endothelial cells to  $10\mu\text{mol/L}$  NS309 for 15 minutes led to a significant rise in total  $\text{K}^{+}$  current while simultaneously causing cell hyperpolarization<sup>[4]</sup>. Human red cells displayed near full dehydration after 2 hours when treated with NS309 at  $100\mu\text{mol/L}$  in a medium containing only  $4\mu\text{mol/L}$  external  $\text{Ca}^{2+}$ <sup>[5]</sup>. The administration of NS309 ( $10\mu\text{mol/L}$  for 2 minutes) led to a significant rise in whole cell small conductance  $\text{Ca}^{2+}$ -activated  $\text{K}^{+}$  currents while causing hyperpolarization of the resting membrane potential in detrusor smooth muscle cell of rats<sup>[6]</sup>.

In vivo, NS309 has neuroprotective effects<sup>[7]</sup>. Intraperitoneal injection of  $2\text{mg/kg}$  NS309 for 10 days significantly reduced brain edema and neuronal apoptosis in rats with traumatic brain injury, and decreased the number of neutrophils, lymphocytes and microglia<sup>[7]</sup>. The administration of NS309 through the intrathecal route at concentrations of  $100\mu\text{mol/L}$  for 30 minutes reduced complete Freund adjuvant (CFA)-induced mechanical hypersensitivity in the rat model of inflammatory pain<sup>[8]</sup>.

### References:

- [1]Nasburg J, Wulff H, Shim H, et al. Exploring the binding site and mechanism of action of NS309, a superagonistic positive gating modulator for the calcium-activated potassium channel  $\text{KCa3.1}$ [J]. Biophysical Journal, 2024, 123(3): 261a-262a.
- [2]Nasburg J A. Mapping the binding site of NS309, a superagonistic positive gating modulator for the calcium-activated potassium channel  $\text{KCa3.1}$ [J]. Biophysical Journal, 2023, 122(3): 252a.
- [3]Nasburg J A, Rouen K C, Dietrich C J, et al. NS309 Functions as a Superagonist for the Calcium-Activated Potassium Channel  $\text{KCa3.1}$ [J]. Molecular Pharmacology, 2025: 100018.
- [4]Liu Y, Xie A, Singh A K, et al. Inactivation of endothelial small/intermediate conductance of calcium-activated potassium channels contributes to coronary arteriolar dysfunction in diabetic patients[J]. Journal of the american heart association, 2015, 4(8): e002062.

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- [5]Seear R V, Lew V L. IKCa agonist (NS309)-elicited all-or-none dehydration response of human red blood cells is cell-age dependent[J]. Cell Calcium, 2011, 50(5): 444-448.
- [6]Parajuli S P, Hristov K L, Soder R P, et al. NS309 decreases rat detrusor smooth muscle membrane potential and phasic contractions by activating SK3 channels[J]. British journal of pharmacology, 2013, 168(7): 1611-1625.
- [7]Chen T, Zhu J, Hang C H, et al. The potassium SK channel activator NS309 protects against experimental traumatic brain injury through anti-inflammatory and immunomodulatory mechanisms[J]. Frontiers in pharmacology, 2019, 10: 1432.
- [8]Hipólito L, Fakira A K, Cabañero D, et al. In vivo activation of the SK channel in the spinal cord reduces the NMDA receptor antagonist dose needed to produce antinociception in an inflammatory pain model[J]. Pain, 2015, 156(5): 849-858.

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