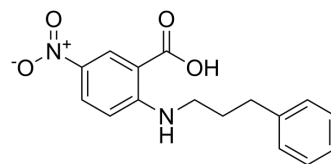


## NPPB

Cat. No.:	HY-101012		
CAS No.:	107254-86-4		
Molecular Formula:	C <sub>16</sub> H <sub>16</sub> N <sub>2</sub> O <sub>4</sub>		
Molecular Weight:	300.31		
Target:	Chloride Channel		
Pathway:	Membrane Transporter/Ion Channel		
Storage:	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	2 years
		-20°C	1 year



## SOLVENT & SOLUBILITY

In Vitro	DMSO : 100 mg/mL (332.99 mM; Need ultrasonic)			
		Solvent Concentration	Mass	
			1 mg	5 mg
	Preparing Stock Solutions		10 mg	
	1 mM	3.3299 mL	16.6495 mL	33.2989 mL
	5 mM	0.6660 mL	3.3299 mL	6.6598 mL
	10 mM	0.3330 mL	1.6649 mL	3.3299 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 (8.32 mM); Clear solution			

## BIOLOGICAL ACTIVITY

Description	NPPB is a blocker of the outwardly rectifying chloride channel (ORCC).
IC <sub>50</sub> & Target	ORCC <sup>[1]</sup>
In Vitro	0.1 mM NPPB in the bath solution reduces channel open probability from 0.89±0.06 to 0.11±0.04 (n=5, P<0.01) <sup>[1]</sup> . Dose-dependent inhibition of chloride currents is observed with a 50% inhibitory concentration (IC <sub>50</sub> ) of 125 μM NPPB. NPPB itself also shows cytotoxicity against glioma cells with a GI <sub>50</sub> of approximately 500 μM <sup>[2]</sup> . MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## PROTOCOL

### Cell Assay [2]

Cells are seeded in the 96-well microtiter plate at a density of  $5 \times 10^3$  cells per well and incubated at 37°C for 24 h in a humidified 5% CO<sub>2</sub> atmosphere. After removing the culture medium, fresh media containing various concentrations of NPPB is added, and incubated for 24 h. Next, 100 µL of Thiazolyl blue tetrazolium bromide at 0.5 mg/mL is added to each well and incubated at 37°C for 1 h. Cells are then dissolved in 100 µL of DMSO, and the absorbance is measured at 570 nm with a Microplate Reader. Concentration-response curves of NPPB are fitted to a Hill equation to obtain GI<sub>50</sub> and GI<sub>80</sub> (50% and 80% growth inhibition concentrations, respectively) values<sup>[2]</sup>.

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

### CUSTOMER VALIDATION

- Biochem J. 2023 May 2;BCJ20220614.

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### REFERENCES

[1]. Li J, et al. Enhancement of an outwardly rectifying chloride channel in hippocampal pyramidal neurons after cerebral ischemia. Brain Res. 2016 Aug 1;1644:107-17.

[2]. Park M, et al. Double Blockade of Glioma Cell Proliferation and Migration by Temozolomide Conjugated with NPPB, a Chloride Channel Blocker. ACS Chem Neurosci. 2016 Mar 16;7(3):275-85.

**Caution: Product has not been fully validated for medical applications. For research use only.**

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