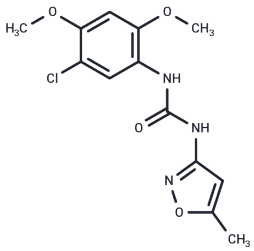
Data Sheet (Cat.No.T6950)

TargetMol

PNU-120596

Chemical Properties



CAS No. : 501925-31-1

Formula: C13H14ClN3O4

Molecular Weight: 311.72

Appearance: no data available

Storage: Powder: -20°C for 3 years | In solvent: -80°C for 1 year

Biological Description

Description	PNU-120596 (NSC-216666) is a positive allosteric modulator of α7 nAChR with EC50 of 216 nM.
Targets(IC50)	AChR
In vitro	PNU-120596 increases agonist (Ach)-evoked calcium flux mediated by an engineered variant of the human α7 nAChR. PNU-120596 increases agonists (choline and ACh)-evoked currents mediated by wild-type receptors and also demonstrates a pronounced prolongation of the evoked response in the continued presence of agonist in Xenopus oocytes. PNU-120596 increases the channel mean open time ofα7 nAChRs but has no effect on ion selectivity and relatively little, if any, effect on unitary conductance. When applied to acute hippocampal slices, PNU-120596 increases the frequency of ACh-evoked GABAergic postsynaptic currents measured in pyramidal neurons; this effect is suppressed by TTX, suggesting that PNU-120596 modulates the function of α7 nAChRs located on the somatodendritic membrane of hippocampal interneurons. [1] Besides the positive modulation to α7 nAChR, PNU-120596 induces a profound retardation of the kinetics of desensitization, raising the potential of Ca2+-induced toxicity through excessive stimulation of α7 nAChR. [2] PNU-120596 causes changes in cysteine accessibility at the inner beta sheet, transition zone and agonist binding site while binding to α7 nAChR. Binding sites for PNU-120596 are not in the agonist-binding sites and PNU-120596 enhances agonist-evoked gating of nicotinic receptors by eliciting conformational effects that are similar but nonidentical to the gating conformations promoted by Ach. [3]
In vivo	Systemic administration of PNU-120596 (1 mg/kg) to rats improves the auditory gating deficit caused by amphetamine, a model proposed to reflect a circuit level disturbance associated with schizophrenia. [1] When administered prior to Carrageenan, 30 mg/kg PNU-1230596 significantly blunts mechanical hyperalgesia and weight bearing deficits for up to 4 hours. PNU-120596 attenuates the carrageenan-induced increase in levels of TNF-α and IL-6 within the hindpaw oedema, diclofenac only attenuated IL-6 levels. Established mechanical hyperalgesia induced by Carrageenan or CFA is also partially reversed by PNU-120596. [4]
Kinase Assay	Ca2+ Fluorescence Assay: SH-EP1 human epithelial cells expressing a variant of theα7 nAChR (α7*) are grown in minimal essential medium (MEM) containing nonessential amino acids supplemented with 10% fetal bovine serum, L-glutamine, 100 U/ml penicillin/streptomycin, 250 ng/mL fungizone, 400 μg/mL hygromycin B, and 800

	μg/mL geneticin. α7* is a variant of the human α7 nAChR, with two point mutations in the first transmembrane domain (T230P and C241S) that allow for high functional expression in SH-EP1 cells [Groppi VE, Wolfe ML, Berkenpas MB (2003) U.S. Patent 6,693,172 B1]. Cells are grown in a 37 °C incubator with 6% CO2. Cells are trypsinized and plated in 96-well plates with dark side walls and clear bottoms at a density of 2 × 104 cells/well 2 days before analysis. Cells are loaded with a mixture of Calcium reen-1AM in anhydrous dimethylsulfoxide and 20% pluronic F-127. This reagent is added directly to the growth medium of each well to achieve a final concentration of 2 μM Calcium Green-1 AM. Cells are then incubated in the dye for 1 hour at 37 °C and then washed four times with Mark's modified Earle's balanced salt solution (MMEBSS) composed of the following (inmM): 4 CaCl2, 0.8 MgSO4, 20 NaCl, 5.3 KCl, 5.6 D-glucose, 20 Tris-HEPES, and 120 N-methyl-D-glucamine, pH 7.4. After the fourth cycle, the cells are allowed to incubate at 37 °C for at least 10 minutes. The final volume of MMEBSS in each well is 100 μL and atropine is added to all wells for a final concentration of 1 μM. A fluorometric imaging plate reader (FLIPR; Molecular Devices, Union City, CA) is set up to excite Calcium Green at 488 nm using 500 mW of power and reading fluorescence emission of >525 nm. A 0.5 seconds exposure is used to illuminate each well. Fluorescence is detected using an F-stop set of either 2.0 or 1.2. After 30 seconds of baseline recording, test compounds are added to each well of a 96-well plate in 50 μL of a 3 × stock.In each experiment, four wells are used as vehicle (0.2% DMSO) controls.
Cell Research	SH-SY5Y-α7 cells are plated on 96-well plates at a density of 15,000 cells per well (100 μL of 1.5 × 105 cells per mL) in complete growth medium and placed into a 37 °C incubator for 20 to 24 hours. Complete growth medium then is replaced with experimental medium alone ("PNU-120596 free") or containing appropriate concentrations of PNU-120596 and returns to the 37 °C ncubator for 20 to 24 hours. The medium is then replaced with fresh experimental medium and 20 μL per well MTS solution and returned to the 37 °C incubator for 3 hours, after which the plate is read on a microplate spectrophotometer at an absorbance of 490 nm. For all data analysis, data are normalized to untreated compound-free wells (100% cell viability) and a background absorbance taken from wells containing experimental medium and MTS solution.(Only for Reference)
Animal Research	Animal Models: male Sprague Dawley rats (weighing 250-300 g)Formulation: PNU-120596 is dissolved in 5% DMSO and 5% Solutol in PBS.Dosages: 1 mg/kgAdministration: PNU-120596 is intravenously administrated 5 minutes before auditory gating measurements.

Solubility Information

Solubility	DMSO: 31.2 mg/mL (100.09 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	3.208 mL	16.040 mL	32.0801 mL
5 mM	0.6416 mL	3.208 mL	6.416 mL
10 mM	0.3208 mL	1.604 mL	3.208 mL
50 mM	0.0642 mL	0.3208 mL	0.6416 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Reference

Hurst RS, et al, J Neurosci, 2005, 25(17), 4396-4405.
Ng HJ, et al, Proc Natl Acad Sci USA, 2007, 104(19), 8059-8064.
Barron SC, et al, Mol Pharmacol, 2009 76(2), 253-263.
Munro G, et al, Br J Pharmacol, 2012, doi: 10.1111/j.1476-5381.2012.02003.x