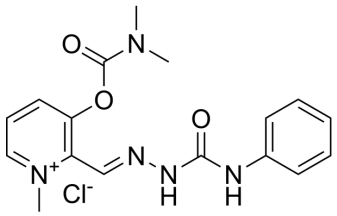
MHP 133

Cat. No.:	HY-101653
CAS No.:	147340-43-0
Molecular Formula:	$C_{17}H_{20}ClN_5O_3$
Molecular Weight:	377.83
Target:	Cholinesterase (ChE); mAChR; 5-HT Receptor
Pathway:	Neuronal Signaling; GPCR/G Protein
Storage:	Please store the product under the recommended conditions in the Certificate of Analysis.



BIOLOGICAL ACTIVITY

Description	MHP 133 is a agent with multiple CNS targets, and inhibits acetylcholinesterase (AChE) with K_i of 69 μM; also active against muscarinic M1 and M2 receptors, serotonin 5HT4 receptors, and imidazole I2 receptors.			
IC_{50} & Target	mAChR1	mAChR2	5-HT_4 Receptor	AChE 69 μM (Ki)
In Vitro	MHP-133 is be active (>50% displacement or activity) against muscarinic M1 and M2 receptors, serotonin 5HT4 receptors, and imidazole I2 receptors. MHP-133 exhibits this nicotinic-like activity in the cell line. Although the ED_{50} for inducing TrkA expression is only about 1 μM, it does predicts the cytoprotective action of MHP-133 in differentiated PC-12 cells deprived of growth factor for 24 h. MHP-133 (10-100 μM) significantly increases the levels of sAPP from cultured astrocytes by 40-60%. MHP-133 produces a bi-phasic effect on slice survival, particularly in the dentate gyrus and the CA1 regions[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.			
In Vivo	In rats, MHP-133 (50, 100, or 200 μg/kg, i.p.) enhances acquisition of the task and increases task accuracy. MHP-133 elicits significant improvements in task accuracies during sessions initiated 10 min after dosing[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.			

PROTOCOL

Kinase Assay [1]	Rat cerebral cortex is homogenized in ice cold 50 mM Tris-HCl buffer containing 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 2 mM $CaCl_2$ (pH 7.0). The homogenate is centrifuged at 37,200 g for 20 min at 0°C. The pellet is washed twice and resuspended in fresh buffer. For nicotinic receptor binding, [^{3}H]cytisine is incubated with 0.5 mg protein and various concentrations of MHP-133 or other ligand in a final volume of 250 μL ta 4°C for 120 min. About 10 μM (-)-nicotine is used to determine nonspecific binding. Bound radioactivity is isolated by rapid filtration through polyethyleneimine-treated glass fiber filters and by washing several times with icecold buffer (Tris-HCl, 50 mM). Filters are soaked in scintillation fluor for 6 h prior to quantification or radioactivity in a scintillation counter. Data are presented in triplicate. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	PC-12 cells are maintained in 150-cm^2 tissue-culture flasks in Dulbecco's modified Eagles medium containing 7% horse serum, 7% fetal calf serum, 1% nonessential amino-acids and 1% streptomycin (DMEM). The cells are incubated at 37°C in a 5% CO_2-enriched, humidified atmosphere. For the actual experiments PC-12 cells are plated on poly-L-lysine coated 24-well

	plates at a density of 40,000 cells per well in DMEM medium containing 50 ng/mL nerve growth factor (NGF). To attain maximum differentiation, the cells are maintained in DMEM.NGF medium for 7 days with the medium being changed every 2 or 3 days. Next, the differentiated cells are incubated with vehicle or with a test drug (prepared in serum-free DMEM media with no exogenous NGF) for 24 h. A parallel set of control cells are maintained in DMEM.NGF medium in each experiment. Cell viability (cytotoxicity) is determined by using the Cell Titer 96 cell proliferation/cytotoxicity assay kit, which is based on the cellular conversion of a 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenltetrazolium bromide (MTT) into a formazan product that can be detected spectrophotometrically. At the completion of the incubation period, the culture medium is aspirated and 15 µL of dye solution in DMEM is added. After 4 h at 37°C, 100 µL of solubilization/stop solution is added and the absorbance of solubilized MTT formazan products is measured at 579 nm. All data are normalized to untreated control cells in each plate. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [1]	Male Sprague-Dawley, outbred Wistar rats weighing 250-300 g are housed separately in our animal care facility for 1 week prior to experimentation. At the time of the experiment the 40 animals are randomLy assigned to one of four treatment groups, a saline vehicle group, or a group to be administered 50, 100 or 200 µg/kg of MHP-133. Vehicle (1 mL/kg body weight) is administered i.p. 30 min prior to testing in the Morris Water Maze apparatus. The apparatus consists of a water-filled (room temperature) tub 1.2 m in diameter. A mounting platform is fixed in place and slightly submerged in the northwest quadrant of the tub. The platform is similar in color to the inner surface of the tub so as to make it difficult to visualize. The tub is always maintained in the same orientation with respect to visual cues placed on the walls, around the testing room. Rats are tested by placing the animal in the water facing away from the platform. Four consecutive trials are administered with 10 min between trials. In each successive trial the rats are placed first in the south quadrant of the tub, followed by the north, east and west quadrants. The time required for the rat to find (place at least 2 paws on) the platform is monitored to the nearest 0.1 s. All rats found the platform in less than 90 s. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Buccafusco JJ, et al. MHP-133, a drug with multiple CNS targets: potential for neuroprotection and enhanced cognition. Neurochem Res. 2007 Jul;32(7):1224-37. Epub 2007 Apr 3.

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