Product Data Sheet

Product Name: M40
Cat. No.: GC13801

Chemical Properties

Cas. No.	143896-17-7
Chemical Name	(S,Z)-2-((Z)-((S)-2-((Z)-((2S,3R)-2-((Z)-((S)-2-((Z)-(2-amino-1-hydroxyethylidene)amino)-1-hydroxy-3-(1H-indol-3-yl)propylidene)amino)-1,3-dihydroxybutylidene)amino)-1-hydroxy-4-methylpentylidene)amino)-N'1-((3Z,5S,6Z,8S,9Z,11S,12Z,15Z,17S,18Z,20S)-1-((S)
SMILES	CC(C[C@@](/N=C(O)/[C@](/N=C(O)/[C@](/N=C(O)/C/N=C(O)/[C@](/N=C(O)/[C@](/N=C(O)/[C@](/N=C(O)/[C@](/N=C(O)/[C@](/N=C(O)/[C@](/N=C(O)/CN)([H])CC1=CNC2=CC=CC=C12)([H])[C@@](O)([H])C)([H])CC(C)C)([H])CC(O)=N)([H])CO)([H])C)([H])CC3=CC=C(O)C=C3)([H])CC(C)C)([H]
Formula	$C_{94}H_{145}N_{23}O_{24}$
M.Wt	1981.33
Solubility	Soluble to 1 mg/ml in sterile water
Storage	Desiccate at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 ℃ and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20℃ 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	M40

Protocol

Cell experiment [1]:

Cell lines	Nucleus tractus solitarius (NTS) neurons
	After the Sprague-Dawley neonatal rats was deeply anesthetized with

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Preparation Method	halothane, a craniotomy was performed, and the forebrain was ablated at the caudal border of the pons by transection. The caudal brainstem and cervical spinal cord were isolated by dissection inmodified Krebs' solution that contained 128.0mM NaCl, 3.0mM KCl, 0.5mM NaH_2PO_4, 1.5mM $CaCl_2$, 1.0mM $MgSO_4$, 21mM $NaHCO_3$, 1.0mM mannitol, 30.0mM glucose, and 10.0mM HEPES. The stomach, connected to the esophagus with the vagus nerves linking it to the brainstem, was kept, and all the other internal organs were removed. The preparation was then isolated and pinned, with the dorsal surface up, on a layer of Sylgard resin in a recording chamber. An incision was made on the lateral surface of the stomach wall to minimize possible gastric vagal fiber damage. The stomach was opened, and its contents were removed. The stomach was then pinned down, and both mucosa and serosa were exposed to Krebs' solution in the gastric compartment. The preparation was superfused with Krebs' solution at 23±1℃. The bathing solution was aerated continuously with a mixture of 95% O_2 and 5% CO_2 and adjusted to pH 7.35 to 7.45. Single tonic unitary discharges were recorded extracellularly in the medial subnucleus of the NTS by glass microelectrodes filled with 3M NaCl, with an impedance of 10 to 20 MΩ . A collision test was applied to identify orthodromic inputs to ensure that only second- or higher-order NTS neurons in the gastric vagal afferent system. Concentrations of galanin and M40 used in the experiment are 100nM. Each test compound was first dissolved in a small volume of Krebs' solution. The concentrated solution was then applied to the gastric compartment. The final drug concentration in the gastric compartment was calculated based on the amount of concentrated solution and the total Krebs volume in the gastric compartment. Drug solution was applied 5min prior to any pharmacological observation to provide sufficient time for drug delivery to reach a steady-state level. After each observation, drug was washed out from the compartment. The NTS neuronal responses observed during pretrial or pretreatment (control) were compared with post-trial (washout) to confirm that brainstem neuronal activity returned to the

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	control level after washout. .
Reaction Conditions	100nM; 5min
Applications	M40 significantly reversed the galanin induced inhibition effect in nucleus tractus solitarius (NTS) neurons.
Animal experiment [2]:	
Animal models	Male specific pathogen-free Sprague-Dawley rats
Preparation Method	The animals were anaesthetized with urethane (1.1g/kg) a and immediately tracheotomized. The femoral artery was cannulated with a plastic catheter containing heparin (50IU/ml, 0.9% NaCl w/v) to record blood pressure and heart rate. and the animal was placed in a stereotaxic frame. The head was flexed 45°, the neck muscles were dissected with an electric knife to avoid bleeding and the atlanto-occipital membrane was exposed. The surgical procedure took 8-10min and the animals were allowed to stabilize for at least 30min. During the whole experiment, the body temperature was maintained at 37.5±0.5℃ by means of a thermostatic blanket. To study if M40 alone exerts any effect on central 11 cardiovascular parameters, groups of rats: received intracisternal injections of M40 at doses of 1.0nmol.
Dosage form	1.0nmol; 50 min; intracisternal injection

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Applications	M40 injected alone produces a significant increase on mean arterial pressure of rat.

References:
[1] YUAN C S, DEY L, XIE J T, et al. Gastric effects of galanin and its interaction with leptin on brainstem neuronal activity [J]. The Journal of pharmacology and experimental therapeutics, 2002, 301(2): 488-93.
[2] NARVáEZ J A, DíAZ-CABIALE Z, HEDLUND P B, et al. The galanin receptor antagonist M40 blocks the central cardiovascular actions of the galanin N-

terminal fragment (1-15) [J]. Eur J Pharmacol, 2000, 399(2-3): 197-203.

Background

M40, a peptidergic galanin antagonist, is a 29 amino acid neuropeptide that is widely distributed in the central nervous system of both rats and humans[1]. M40 significantly blocked galanin-induced feeding in satiated rats[2]. M40, is high-affinity ligands at the spinal cord galanin receptors characterized with K_d values of 6.8±2.2nM[3].

M40 (10μM; 3h) increased insulin release from RIN5AH cells in the absence of galanin[4]. M40 (100nM; 5min) significantly reversed the galanin (100nM; 5min) induced inhibition effect in nucleus tractus solitarius (NTS) neurons[5].

M40 (1.0nmol; 50 min; intracisternal injection) can produce a significant increase on mean arterial pressure of rat[6]. M40 (0.02-2.0nmol; bilateral microinjections; 15min) in lateral septum (LS) dose dependently reduced defensive burying behavior in rats[7].

References:
[1] MCDONALD M P, CRAWLEY J N. Galanin receptor antagonist M40 blocks galanin-induced choice accuracy deficits on a delayed-nonmatching-to-position task [J]. Behavioral neuroscience, 1996, 110(5): 1025-32.
[2] CRAWLEY J N, ROBINSON J K, LANGEL U, et al. Galanin receptor antagonists M40 and C7 block galanin-induced feeding [J]. Brain research, 1993, 600(2): 268-72.
[3] XU X J, WIESENFELD-HALLIN Z, LANGEL U, et al. New high affinity peptide antagonists to the spinal galanin receptor [J]. Br J Pharmacol, 1995, 116(3): 2076-80.
[4] WANG Z L, KULKARNI R N, WANG R M, et al. Possible evidence for endogenous production of a novel galanin-like peptide [J]. J Clin Invest, 1997, 100(1): 189-96.
[5] YUAN C S, DEY L, XIE J T, et al. Gastric effects of galanin and its interaction with leptin on brainstem neuronal activity [J]. The Journal of pharmacology and experimental therapeutics, 2002, 301(2): 488-93.

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[6] KHOSHBOUEI H, CECCHI M, DOVE S, et al. Behavioral reactivity to stress: amplification of stress-induced noradrenergic activation elicits a galanin-mediated anxiolytic effect in central amygdala [J]. Pharmacol Biochem Behav, 2002, 71(3): 407-17.
[7] ECHEVARRIA D J, HERNANDEZ A, DIOGENES A, et al. Administration of the galanin antagonist M40 into lateral septum attenuates shock probe defensive burying behavior in rats [J]. Neuropeptides, 2005, 39(5): 445-51.