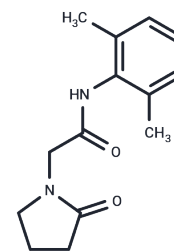


## Nefiracetam

## Chemical Properties

CAS No. :	77191-36-7
Formula:	C <sub>14</sub> H <sub>18</sub> N <sub>2</sub> O <sub>2</sub>
Molecular Weight:	246.3
Storage:	Powder: -20°C for 3 years   In solvent: -80°C for 1 year Actual storage temperature shall be subject to the COA.



## Biological Description

Description	Nefiracetam (DM9384) are underway in Phase 2 trials. Nefiracetam, a GABAergic, cholinergic, and monoaminergic neuronal system enhancer, is used for Ro 5-4864-induced convulsions.
Targets(IC50)	Calcium Channel, GABA Receptor, GluR, AChR, iGluR, PKC
In vitro	In DDY mice, Nefiracetam (>10 mg/kg) effectively inhibits convulsions induced by Ro 5-4864. Oral administration of Nefiracetam suppresses Ro 5-4864-induced seizures in EL mice. Administering Nefiracetam (1 time/day) prior to each training session facilitates the acquisition of avoidance responses.
In vivo	Nefiracetam, at low concentrations (0.01-0.1 μM), induces a transient inhibition of Ach-evoked currents, while at higher concentrations (1-10 μM), it provides a sustained enhancement of these currents. Specifically, 1 μM Nefiracetam doubles the long-lasting component of the calcium channel current without affecting the transient component. After a 10-minute treatment with Nefiracetam, Ach-induced current is reduced to 30% at 0.01 μM and 38% at 0.1 μM of the control levels. The compound interacts with the PKC pathway, augmenting the activity of nicotinic Ach receptors, thereby increasing the release of presynaptic glutamate, and leading to potentiation of synaptic transmission in the hippocampus. This mechanism may underlie cognitive enhancement elicited by Nefiracetam through its interaction with PKA and PKC pathways. In primary cultures of rat hippocampal neurons, Nefiracetam increases the ratio of nicotinic-sensitive excitatory postsynaptic currents. Moreover, in rat hippocampal slices in both the CA1 region and the dentate gyrus, Nefiracetam induces long-lasting synaptic potentiation, which can be inhibited by α-bungarotoxin and mecamylamine.
Kinase Assay	Assay of glutamate released: Hippocampal slices (400 μM) are prepared from the guinea pig brain using standard techniques. A slice is fixed on a pair of silver wire electrodes (10 Hz, 5 V, 0.1 ms in duration) at 1-minute intervals for 10 minutes and submerged in 1 mL standard artificial cerebrospinal fluid (ACSF) (in mM: 125 mM NaCl, 5 mM KCl, 1.24 mM KH <sub>2</sub> PO <sub>4</sub> , 1.3 mM MgSO <sub>4</sub> , 2 mM CaCl <sub>2</sub> , 26 mM NaHCO <sub>3</sub> , and 10 mM glucose) oxygenated with 95% O <sub>2</sub> and 5% CO <sub>2</sub> at 36 °C in the presence and absence of tetrodotoxin (TTX) (0.5 μM). In a different set of experiments, electrical stimulation is applied to slices treated with Nefiracetam (1 μM) in the presence and absence of α-bungarotoxin (50 nM) or mecamylamine (3 μM). A 100 μL aliquot of the medium filtered with millipore filters (0.45 μM) is injected onto the cation-exchanger column of the autoanalyser to separate amino acids and the amount of glutamate released is

## A DRUG SCREENING EXPERT

Kinase Assay	calculated using known amino acid standard concentrations.
Cell Research	The injected oocytes are transferred to the recording chamber 24 to 48 hours after incubation and continuously superfused at room temperature (20 to 22 °C) in a standard frog Ringer's solution (115 mM NaCl, 2 mM KCl, 1.8 mM CaCl <sub>2</sub> , and 5 mM HEPES, pH 7.0). Ca <sup>2+</sup> -free extracellular solution consisted of 115 mM NaCl, 2 mM KCl, 5 mM MgCl <sub>2</sub> , 5 mM HEPES, and 1 mM EGTA, pH 7.0. To remove the effect of the muscarinic ACh receptor, 1 μM atropine is added to the extracellular solution. ACh-activated currents are recorded using two-electrode, voltage-clamp techniques. The currents are analyzed on a microcomputer using pClamp software. ACh is bath-applied to oocytes. Nefiracetam is dissolved in distilled water at 1 mM for stock solution and diluted into concentrations required with the extracellular solution. (Only for Reference)

### Solubility Information

Solubility	DMSO: 70 mg/mL (284.21 mM),Sonication is recommended. Ethanol: 24.6 mg/mL (99.88 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 2 mg/mL (8.12 mM),Sonication is recommended. <i>Please add the solvents sequentially, clarifying the solution as much as possible before adding the next one. Dissolve by heating and/or sonication if necessary. Working solution is recommended to be prepared and used immediately. The formulation provided above is for reference purposes only. In vivo formulations may vary and should be modified based on specific experimental conditions.</i>

### Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	4.0601 mL	20.3004 mL	40.6009 mL
5 mM	0.812 mL	4.0601 mL	8.1202 mL
10 mM	0.406 mL	2.030 mL	4.0601 mL
50 mM	0.0812 mL	0.406 mL	0.812 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.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

### Reference

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- Nishizaki T, et al. Mol Pharmacol, 1998, 53(1), 1-5.
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