
Product Data Sheet

Product Name: Pitolisant

Cat. No.: GC36931

Chemical Properties

Cas. No. 362665-56-3

SMILES C1C=CC=C(CCCOCCCN2CCCCC2)C=C1Formula C₁₇H₂₆ClNO

M.Wt 295.85

Solubility DMF: 20 mg/ml, DMSO: 30 mg/ml, Ethanol: 30 mg/ml, PBS (pH 7.2): 10 mg/ml

Storage Store at -20°C

General tips For obtaining a higher solubility, please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C 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 **Protocol**

[35S]GTPγS binding assays are performed. CHO-K1 cells stably expressing the human H3 receptor (~400 fmol/mg protein) are homogenized in ice-cold buffer (50 mM Tris/HCl, pH 7.4). Homogenates are centrifuged twice (20,000g for 10 min at 4°C), and the final pellet is resuspended in 50 volumes of buffer. Membranes (550 μg of protein) are pretreated with adenosine deaminase (1 U/mL) and incubated for 60 min at 25°C with 0.1 nM [35S]GTPγS and the drugs to be tested in a final volume of 1 mL of assay buffer (50 mM Tris/HCl, 50 mM NaCl, 5 mM MgCl₂, 10 μM GDP, and 0.02% bovine serum albumin, pH 7.4). The nonspecific binding is

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Kinase experiment:

determined using 10 μ M nonradioactive GTP γ S. Incubations are stopped by rapid filtration under vacuum through GF/B glass fiber filters. After washing with ice-cold water, the radioactivity trapped on filters is counted by liquid scintillation spectrometry. A similar assay is used to assess competitive antagonism. In brief, membranes (10 μ g of protein) of HEK-293 cells stably expressing the human H3 receptor (\sim 600 fmol/mg protein) are preincubated in presence of Pitolisant in the buffer (50 mM Tris/HCl, pH 7.4, 10 mM MgCl₂, 100 mM NaCl, and 10 μ M GDP) in a 96-well microplate under gentle agitation at room temperature (19-20°C) for 30 min before the addition of 0.1 nM [³⁵S]GTP γ S (final volume 200 μ L). The nonspecific binding is determined using a 10 μ M concentration of nonradioactive GTP γ S. After 30 min, incubations performed in triplicate are stopped by rapid filtration under vacuum on a Multiscreen MAFCOB50 microplate. Radioactivity trapped on filters is counted by liquid scintillation spectrometry[1].

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Mice[2] Adult female Albino Swiss mice weighing 20-22 g are used in the study. Olanzapine or Pitolisant are suspended in 1 % Tween 80. The compounds or vehicle are administered intraperitoneally (i.p.) 30 min prior to the acute experiment. In the Pitolisant+Olanzapine group, Pitolisant is administered 15 min before Olanzapine. Subchronic treatment is done at about 9:00 am (0.2 mL Tween to control group, Pitolisant-10 mg/kg b.w. to Pitolisant group, Olanzapine-2 mg/kg b.w. to Olanzapine group, Pitolisant-10 mg/kg b.w. and Olanzapine after 15 min-2 mg/kg b.w. to Pitolisant+Olanzapine group) and at about 1:00 pm (Olanzapine group and Pitolisant+Olanzapine group). Rats[3] Male Wistar rats (220-300 g) receive vehicle (methylcellulose 1%, p.o.), Pitolisant (10 mg/kg, p.o.) or D-amphetamine (2.5 mg/kg, i.p. in saline). Ninety minutes later, they are killed by decapitation and nucleus accumbens are dissected out, weighed, frozen in liquid nitrogen and stored at -80°C. Tissues are homogenized in 1 mL of a 0.4 N perchloric acid/2.7 mM EDTA solution. After centrifugation (8000 rpm, 20 min, 4°C), supernatants are analysed by HPLC coupled to electrochemical detection. Tissue concentrations of dopamine (DA), dihydroxyphenyl acetic acid (DOPAC) and homovanillic acid (HVA) are determined and the corresponding ratios (DOPAC/DA, HVA/DA) are calculated.

Animal experiment:

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References:

- [1]. Ligneau X, et al. BF2.649 [1-{3-[3-(4-Chlorophenyl)propoxy]propyl}piperidine, hydrochloride], a nonimidazole inverse agonist/antagonist at the human histamine H₃ receptor: Preclinical pharmacology. *J Pharmacol Exp Ther.* 2007 Jan;320(1):365-75.
- [2]. Dudek M, et al. H₃ histamine receptor antagonist pitolisant reverses some subchronic disturbances induced by olanzapine in mice. *Metab Brain Dis.* 2016 Oct;31(5):1023-9.
- [3]. Uguen M, et al. Preclinical evaluation of the abuse potential of Pitolisant, a histamine H₃ receptor inverse agonist/antagonist compared with Modafinil. *Br J Pharmacol.* 2013 Jun;169(3):632-44.

Background

Pitolisant is a nonimidazole histamine H₃ receptor antagonist ($K_i = 0.16$ nM) and inverse agonist ($EC_{50} = 1.5$ nM).¹ It increases the levels of *te/e*-methylhistamine in mouse brain, indicating histaminergic neuron activity, with an ED_{50} value of 1.6 mg/kg. Pitolisant also increases dopamine and acetylcholine levels in the rat prefrontal cortex when administered at a dose of 10 mg/kg. It decreases the time spent in slow wave sleep and increases the time spent awake in cats. Pitolisant (2.5 and 5 mg/kg), when administered post-training, facilitates contextual fear memory consolidation and reverses dizocilpine-induced amnesia in mice.² When administered following reactivation, it reverses dizocilpine-induced reconsolidation deficits.

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1. Ligneau, X., Perrin, D., Landais, L., et al. BF2.649 [1-{3-[3-(4-chlorophenyl)propoxy]propyl}piperidine, hydrochloride], a nonimidazole inverse agonist/antagonist at the human histamine H₃ receptor: Preclinical pharmacology]. *Pharmacol. Exp. Ther.* 320(1)365-375(2007)

2. Brabant, C., Charlier, Y., and Tirelli, E. The histamine H₃-receptor inverse agonist pitolisant improves fear memory in mice. *Behav. Brain Res.* 243199-204(2013)

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