上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话：021-61312973　　传真：021-55068248
网址：www.shyuanye.com
邮箱：shyysw@sina.com

产品名称：1-[3-[3-(4-氯丙基)丙氧基]丙基]-哌啶盐酸盐
产品别名：Pitolisant hydrochloride ；替洛利生盐酸盐 ；Ciproxidine; BF 2649

生物活性：	
Description	Pitolisant hydrochloride is a potent and selective nonimidazole inverse agonist at the recombinant human histamine H3 receptor (K_i=0.16 nM).
IC_{50} & Target	Ki: 0.16 nM (H3 receptor)[1] EC50: 1.5 nM (H3 receptor)[1]
In Vitro	On the stimulation of guanosine 5′-O-(3-[^{35}S]thio)triphosphate binding to this receptor, Pitolisant (BF2.649) behaves as a competitive antagonist with a K_i value of 0.16 nM and as an inverse agonist with an EC_{50} value of 1.5 nM and an intrinsic activity ~50% higher than that of ciproxifan. Pitolisant displaces [^{125}I]iodoproxyfan binding from mouse brain cortical membranes with an IC_{50} value of 26.4±4.5 nM. Taking into account the K_d value of the radioligand (161±9 pM), the deduced K_i value for Pitolisant is 14±1 nM. Pitolisant displaces [^{125}I]iodoproxyfan binding from membranes of rat glioma C6 cells stably expressing the human H_3 receptor with an IC_{50} value of 4.2±0.2 nM. Taking into account the K_d value of the radioligand (50±4 pM), the deduced K_i value for Pitolisant is 2.7±0.5 nM. Pitolisant progressively reverses this response with a Hill coefficient close to unity and an IC_{50} value of 330±68 nM, leading to a K_i value of 17±4 nM. Pitolisant elicits a dose-dependent decrease of the basal-specific [^{35}S]GTPγS binding to membranes with a maximal effect corresponding to 75±1% of the basal-specific binding and an EC_{50}value of 1.5±0.1 nM[1].
In Vivo	The administration of Pitolisantat a single dose of 10 mg/kg 30 min before a single dose of LY170053 (2 mg/kg b.w.) also significantly affects immobility time in the FST. Subsequent administration of the aforementioned drug sequence in mice statistically significantly increases the duration of immobility in comparison to the time determined in the control group in the FST. It decreased locomotor activity as well. In contrast, the results obtained in subchronic treatment after fifteen administrations of both drugs (Pitolisant 10 mg/kg b.w., and after 30 min LY170053 2 mg/kg b.w., and again after 4 h LY170053 2 mg/kg b.w.) show that the administration of Pitolisant followed by that of LY170053 equalized the locomotor activity in mice; in comparison to the level of motility in the control group, to which only Pitolisant is administered. More importantly, this combination of drugs significantly reduces immobility time to the level obtained in the control group in the forced swim test in mice [one-way ANOVA; $F_{(3,20)}$=4.226,P=0.0181][2]. Rats given Pitolisant (10 mg/kg) during the conditioning phase stayed 502±94 s on the paired texture, a value not statistically different from that of controls, indicating that Pitolisant did not support place preference[3].
	In Vitro: **H_2O : 100 mg/mL (300.92 mM;** Need ultrasonic) **DMSO : ≥ 43 mg/mL (129.40 mM)** * "≥" means soluble, but saturation unknown.

	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
Preparing	1 mM	3.0092 mL	15.0462 mL	30.0924 mL

<table>
<tr><td rowspan="3">Solvent&Solubility</td><td rowspan="2">Stock Solutions</td><td>5 mM</td><td>0.6018 mL</td><td>3.0092 mL</td><td>6.0185 mL</td></tr>
<tr><td>10 mM</td><td>0.3009 mL</td><td>1.5046 mL</td><td>3.0092 mL</td></tr>
<tr><td colspan="5">*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。
储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month。 -80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。
In Vivo:
请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 In Vitro 方式配制澄清的储备液，再依次添加助溶剂：
——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶
1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline
Solubility: ≥ 2.5 mg/mL (7.52 mM); Clear solution
此方案可获得 ≥ 2.5 mg/mL (7.52 mM，饱和度未知) 的澄清溶液。
以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀。

2.请依序添加每种溶剂： 10% DMSO →90% corn oil
Solubility: ≥ 2.5 mg/mL (7.52 mM); Clear solution
此方案可获得 ≥ 2.5 mg/mL (7.52 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的实验。
以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。</td></tr>
<tr><td>References</td><td colspan="5">[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 LY170053 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 H? receptor inverse agonist/antagonist compared with CRL 40476. Br J Pharmacol. 2013 Jun;169(3):632-44.</td></tr>
<tr><td colspan="6">实验参考：</td></tr>
<tr><td>Animal Administration</td><td colspan="5">Mice[2]
Adult female Albino Swiss mice weighing 20-22 g are used in the study. LY170053 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+LY170053 group, Pitolisant is administered 15 min before LY170053. 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, LY170053-2 mg/kg b.w. to LY170053 group, Pitolisant-10 mg/kg b.w. and LY170053 after 15 min-2 mg/kg b.w. to Pitolisant+LY170053 group) and at about 1:00 pm (LY170053 group and Pitolisant+LY170053 group).
Rats[3]</td></tr>
</table>

	Male Wistar rats (220-300 g) receive vehicle (methylcellulose 1%, p.o.), and Pitolisant (10 mg/kg, p.o.). 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.
Kinase Assay	[^{35}S]GTPγS binding assays are performed. CHO-K1 cells stably expressing the human H_3 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 [^{35}S]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 $MgCl_2$, 10 µM GDP, and 0.02% bovine serum albumin, pH 7.4). The nonspecific binding is determined using 10 µM nonradioactive GTPγS. 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 µg of protein) of HEK-293 cells stably expressing the human H_3 receptor (~600 fmol/mg protein) are preincubated in presence of Pitolisant in the buffer (50 mM Tris/HCl, pH 7.4, 10 mM $MgCl_2$, 100 mM NaCl, and 10 µM 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 [^{35}S]GTPγS (final volume 200 µL). The nonspecific binding is determined using a 10 µM concentration of nonradioactive GTPγS. 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].
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 LY170053 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 H? receptor inverse agonist/antagonist compared with CRL 40476. Br J Pharmacol. 2013 Jun;169(3):632-44.