产品名称：1-(2,4-二氯苯基)-5-(4-碘苯基)-4-甲基-N-1-哌啶基-1H-吡唑-3-甲酰胺

产品别名：AM251

生物活性：

Description	AM251 is a selective cannabinoid 1 (CB1) receptor antagonist with an IC_{50} of 8 nM, also acts as a potent GPR55agonist with an EC_{50} of 39 nM.
IC_{50} & Target	IC50: 8 nM (CB1 receptor)[1]
In Vitro	AM251 is a CB1 receptor antagonist/inverse agonist. AM251 produces an agonist response in HEK293 cells, similar to that found in the yeast expression system[2]. AM-251 reduces cholesteryl ester synthesis in unstimulated and acetylated LDL-stimulated Raw 264.7 macrophages, $CB2^{+/+}$ and $CB2^{-/-}$ peritoneal macrophages[3].
In Vivo	The CB1 antagonist AM251 (3 mg/kg, i.p.) decreases capsaicin-evoked nocifensive behavior ($F_{1,18}$=28.45, p<0.0001). This suppressive effect is genotype dependent ($F_{1,18}$=14.83, p<0.01), and the interaction between the effects of genotype and AM251 approached significance ($F_{1,18}$=4.704, p=0.0587). Planned comparisons reveal that AM251 reduces nocifensive behaviors in fatty-acid amide hydrolase (FAAH) KO mice (p<0.01) but fails to alter nocifensive behavior in WT mice (p>0.2) relative to their respective vehicle controls. AM251 (3 mg/kg, i.p.) reduces the duration of heat hypersensitivity in FAAH KO ($F_{1,9}$=21.43, p<0.01) but not WT mice (p>0.3). AM251 suppresses capsaicin-evoked heat hypersensitivity in a time-dependent manner in FAAH KO ($F_{5,9}$=4.349, p<0.01) but not in WT mice (p>0.3). Post-hoc analysis reveals that FAAH KO mice receiving vehicle (i.p.) display heightened thermal hypersensitivity at 30 (p<0.05), 60 (p<0.05), and 90 (p<0.001) minutes post-capsaicin in comparison to FAAH KO animals receiving AM251)[4]. One-way ANOVA shows that AM251 (AM-251) injected into the rats significantly decreases both of the percentage of entries in the open arms and time spent in the open arms, compare to controls. The Tukey-Kramer test analysis reveals a significant reduction for the doses of 1 mg/kg (P<0.05) and 5 mg/kg (P<0.01) compare to control rats in the time spent in the open arms. Also, AM251 significantly decreases percentage of entries in the open arms for the doses of 1 and 5 mg/kg (P<0.05)[5].
Solvent&Solubility	***In Vitro:*** **DMSO : 25 mg/mL (45.03 mM;** Need ultrasonic) **H_2O : < 0.1 mg/mL** (insoluble)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.8010 mL	9.0051 mL	18.0102 mL
	5 mM	0.3602 mL	1.8010 mL	3.6020 mL
	10 mM	0.1801 mL	0.9005 mL	1.8010 mL

*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 (4.50 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.50 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 400 μL PEG300 中，混合均匀向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。 2.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.5 mg/mL (4.50 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.50 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。
References	[1]. Bruno A, et al. Beyond radio-displacement techniques for identification of CB1 ligands: the first application of afluorescence-quenching assay. Sci Rep. 2014 Jan 20;4:3757. [2]. Sharir H, et al. Pharmacological characterization of GPR55, a putative cannabinoid receptor. Pharmacol Ther. 2010 Jun;126(3):301-13. [3]. Thewke D, et al. AM-251 and SR144528 are acyl CoA:cholesterol acyltransferase inhibitors. Biochem Biophys Res Commun. 2009 Apr 3;381(2):181-6. [4]. Carey LM, et al. A pro-nociceptive phenotype unmasked in mice lacking fatty-acid amide hydrolase. Mol Pain. 2016 May 13;12. pii: 1744806916649192. [5]. Komaki A, et al. Study the Effect of Endocannabinoid System on Rat Behavior in Elevated Plus-Maze. Basic Clin Neurosci. 2015 Jul;6(3):147-53. [6]. Jiang X, et al. Role of cannabinoid receptor type 1 in tibial and pudendal neuromodulation of bladder overactivity in cats. Am J Physiol Renal Physiol. 2017 Mar 1;312(3):F482-F488. [7]. Sun L, et al. Endocannabinoid activation of CB1 receptors contributes to long-lasting reversal of neuropathic pain by repetitive spinal cord stimulation. Eur J Pain. 2017 May;21(5):804-814.

实验参考：

Animal Administration	Mice[4] A total of 246 mice weighing 17-48 g are used in these experiments. Following determination of baseline responding, mice receive a single i.p injection (5 mL/kg) of AMG9810 (3 mg/kg, n=5 per group), AM251 (3 mg/kg, n=5 per group), or vehicle (n=6 per group). I.p. injections are performed 30 min prior to i.pl. capsaicin or vehicle administration. Paw withdrawal latencies are assessed before and 10, 30, 60, 90 and 120 min after intradermal injection of capsaicin or vehicle. Paw withdrawal latencies are measured in duplicate in each paw at each time point, and are reported as the mean of the two duplicate determinations from each animal, averaged across subjects. Rats[5] Male Wistar rats weighting 250-350 are used.The following agents are used: CB1 receptor agonist, Win-55212 (0.3, 1 and 5 mg/kg, i.p.); CB1 receptor antagonist, AM251 (0.3, 1 and 5 mg/kg, i.p.); endocannabinoid breakdown inhibitor, URB-597 (0.03, 0.1 and 0.3 mg/kg, i.p.) in the study. Physiological saline (0.9% sodium chloride) is used as the vehicle. All drugs are prepared freshly and administered intraperitoneally (i.p.) in a volume of 0.1 mL per 10 g of body weight of the rats. All substances are dissolved in physiological saline and are administrated 30 min before elevated

	plus-maze test.
Kinase Assay	Macrophages are seeded (2×10^6/well) in 12-well culture plates. AM-251 or SR144528 are added from 4 mM stock solutions prepared in DMSO, 1h prior to the addition of 7-ketocholesterol (7KC) from a 2 mg/mL ethanol stock solution. Controls are adjusted to receive equivalent volumes of DMSO and ethanol. After 16 h, caspase-3 activity is determined. All treatments are done in triplicate and the data presented as the mean RFLU/mg protein±SD[3].
References	[1]. Bruno A, et al. Beyond radio-displacement techniques for identification of CB1 ligands: the first application of afluorescence-quenching assay. Sci Rep. 2014 Jan 20;4:3757. [2]. Sharir H, et al. Pharmacological characterization of GPR55, a putative cannabinoid receptor. Pharmacol Ther. 2010 Jun;126(3):301-13. [3]. Thewke D, et al. AM-251 and SR144528 are acyl CoA:cholesterol acyltransferase inhibitors. Biochem Biophys Res Commun. 2009 Apr 3;381(2):181-6. [4]. Carey LM, et al. A pro-nociceptive phenotype unmasked in mice lacking fatty-acid amide hydrolase. Mol Pain. 2016 May 13;12. pii: 1744806916649192. [5]. Komaki A, et al. Study the Effect of Endocannabinoid System on Rat Behavior in Elevated Plus-Maze. Basic Clin Neurosci. 2015 Jul;6(3):147-53. [6]. Jiang X, et al. Role of cannabinoid receptor type 1 in tibial and pudendal neuromodulation of bladder overactivity in cats. Am J Physiol Renal Physiol. 2017 Mar 1;312(3):F482-F488. [7]. Sun L, et al. Endocannabinoid activation of CB1 receptors contributes to long-lasting reversal of neuropathic pain by repetitive spinal cord stimulation. Eur J Pain. 2017 May;21(5):804-814.