产品名称：HC-030031
产品别名：HC-030031

生物活性：

Description	HC-030031 is a potent and selective TRPA1 inhibitor, which antagonizes AITC- and formalin-evoked calcium influx with IC_{50}s of 6.2±0.2 and 5.3±0.2 μM, respectively.
IC_{50} & Target	TRPA1[1]
In Vitro	HC-030031 reversibly blocks TRPA1 currents with a similar potency, regardless of the agonist used; this includes blockade of currents elicited by reversible agonists, such as AITC, or irreversible agonists, such as N-methyl maleimide. HC-030031 blocks activation of TRPA1 by N-methyl maleimide, which opens the channel irreversibly through cysteine modification. HC-030031 does not block currents mediated by TRPV1, TRPV3, TRPV4, hERG, or NaV1.2 channels[1]. The potencies of HC-030031 versus cinnamaldehyde or allyl isothiocyanate (AITC or Mustard oil)-induced TRPA1 activation are 4.9±0.1 and 7.5±0.2 μM respectively (IC_{50}). These findings are similar to the previously reported IC_{50} of 6.2 μM against AITC activation of TRPA1. The ability of HC-030031 to block TRPA1 activation is tested in a FLIPR calcium-influx assay using HEK-293 cells stably expressing human TRPA1. Concentrations of HC-030031 from 0.3 to 60 μM are incubated with cells for 10 minutes prior to addition of an EC_{60} concentration of either cinnamaldehyde or AITC. HC-030031 dose-dependently blocks cinnamaldehyde- and AITC-induced calcium influx with IC_{50} values of 4.9 and 7.5 μM, respectively[2].
In Vivo	After injection of AITC (50 μL of 10%) into the rat hind paw, HC-030031 (300 mg/kg) significantly reduces flinching during the first 5 min. Over the remainder of the hour, HC-030031 decreases flinch frequency, a result that mirrors the effects observed on formalin-induced flinching[1]. In the rat, oral administration of HC-030031 reduces AITC-induced nocifensive behaviors at a dose of 100 mg/kg. Moreover, oral HC-030031 (100 mg/kg) significantly reverses mechanical hypersensitivity in the more chronic models of Complete Freunds Adjuvant (CFA)-induced inflammatory pain and the spinal nerve ligation model of neuropathic pain. One hour post-oral administration, HC-030031 significantly reduces the lifting duration following 1% AITC injection ($p<0.001$)[2]. HC-030031 completely reverses the enhanced mechanical firing in inflamed mice ($p<0.001$)[3].
Solvent&Solubility	***In Vitro:*** **DMSO : 25 mg/mL (70.35 mM;** Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.8138 mL	14.0691 mL	28.1381 mL
	5 mM	0.5628 mL	2.8138 mL	5.6276 mL
	10 mM	0.2814 mL	1.4069 mL	2.8138 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→ 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.5 mg/mL (7.03 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (7.03 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 (7.03 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (7.03 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]. McNamara CR, et al. TRPA1 mediates formalin-induced pain. Proc Natl Acad Sci U S A. 2007 Aug 14;104(33):13525-30. [2]. Eid SR, et al. HC-030031, a TRPA1 selective antagonist, attenuates inflammatory- and neuropathy-induced mechanical hypersensitivity. Mol Pain. 2008 Oct 27;4:48. [3]. Lennertz RC, et al. TRPA1 mediates mechanical sensitization in nociceptors during inflammation. PLoS One. 2012;7(8):e43597. [4]. Miyake T, et al. Cold sensitivity of TRPA1 is unveiled by the prolyl hydroxylation blockade-induced sensitization to ROS. Nat Commun. 2016 Sep 15;7:12840. [5]. So K, et al. Hypoxia-induced sensitisation of TRPA1 in painful dysesthesia evoked by transient hindlimb ischemia/reperfusion in mice. Sci Rep. 2016 Mar 17;6:23261.

实验参考：

Cell Assay	HEK-293 cells stably expressing human TRPA1 are plated into 384-well plates at a density of 20,000 cells/well 24 hours prior to assaying. On the day of assay, cells are loaded with 4 μM Fluo-4 dye and 0.08% pluronic acid for 1 hour at room temperature in assay buffer consisting of Hank's balanced salt solution supplemented with 20 mM HEPES, 2.5 mM probenecid, and 4% TR-40. Calcium influx assays are performed using the Fluorometric Imaging Plate Reader (FLIPR) TETRA. Concentration-response curves are generated for the TRPA1 agonists cinnamaldehyde and AITC prior to antagonist testing so EC_{60} concentrations could be determined. Titrations of HC-030031 are made from a DMSO stock solution and DMSO is kept to a constant of 0.4% in the assay. The antagonist is incubated with the cells for 10 minutes before the addition of an EC_{60} concentration of either cinnamaldehyde (18 μM) or AITC (6 μM) and calcium influx is monitored for an additional 10 minutes[2].
	Rats[2] Male Sprague-Dawley rats (200-500 g) are used in all experiments. HC-030031 (100, 300 mg/kg) is used. For all experiments, HC-030031 is suspended in 0.5% Methylcellulose and the drug is dosed p.o. at a volume of 10 mL/kg. Naproxen (20 mg/kg) is dissolved in sterile water and dosed p.o. to serve as a positive comparator for the CFA experiment. Pregabalin (20 mg/kg) is dissolved in sterile water and dosed p.o. to serve as a positive comparator for the neuropathic pain experiment.

Animal Administration	Mice[3] Adult male C57BL/6 mice (8-12 weeks old) are used. Mice are injected with a 30 μL emulsion of undiluted CFA into the medial left plantar hind paw. The vehicle control group is injected with 30 μL of sterile 0.9% saline solution. Two days after injection, at the peak of hypersensitivity, the magnitude of inflammation is measured at the midpoint of the hind paw using digital calipers (VWR). For one experiment, the membrane-impermeable sodium channel inhibitor lidocaine N-ethyl-bromide, also known as QX-314, (0.2% in saline; 30 μL) is injected with or without the TRPA1 agonist cinnamaldehyde (30 μM) into the left plantar hind paw 2 days post CFA injection. For another experiment, the TRPA1 antagonist HC-030031 (100 μg in 30 μL of 0.5% DMSO and 0.25% Tween-80 in PBS) is injected into the left plantar hind paw 2 days post CFA injection. Vehicle controls are injected with 30 μL 0.5% DMSO and 0.25% Tween-80 in PBS. All behavioral assays are completed between 1 and 4 hours following the QX-314, HC-030031 or vehicle injections.
References	[1]. McNamara CR, et al. TRPA1 mediates formalin-induced pain. Proc Natl Acad Sci U S A. 2007 Aug 14;104(33):13525-30. [2]. Eid SR, et al. HC-030031, a TRPA1 selective antagonist, attenuates inflammatory- and neuropathy-induced mechanical hypersensitivity. Mol Pain. 2008 Oct 27;4:48. [3]. Lennertz RC, et al. TRPA1 mediates mechanical sensitization in nociceptors during inflammation. PLoS One. 2012;7(8):e43597. [4]. Miyake T, et al. Cold sensitivity of TRPA1 is unveiled by the prolyl hydroxylation blockade-induced sensitization to ROS. Nat Commun. 2016 Sep 15;7:12840. [5]. So K, et al. Hypoxia-induced sensitisation of TRPA1 in painful dysesthesia evoked by transient hindlimb ischemia/reperfusion in mice. Sci Rep. 2016 Mar 17;6:23261.