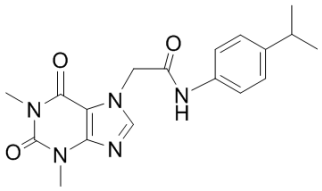
HC-030031

Cat. No.:	HY-15064
CAS No.:	349085-38-7
Molecular Formula:	$C_{18}H_{21}N_5O_3$
Molecular Weight:	355.39
Target:	TRP Channel
Pathway:	Membrane Transporter/Ion Channel; Neuronal Signaling
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 6 months -20°C 1 month



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

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (7.03 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (7.03 mM); Clear solution

BIOLOGICAL ACTIVITY

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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Cell Assay [2]	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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [2][3]	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. 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. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Nat Commun. 2016 Sep 15;7:12840.

- Sci Rep. 2016 Mar 17;6:23261.
- J Neuroendocrinol. 2020 Jun;32(6):e12876.
- Mol Pain. 2019 Jan-Dec;15:1744806919849201.
- Kyoto University. 2017 Mar.

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[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.

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