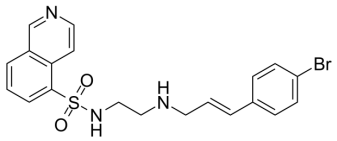
H-89

Cat. No.:	HY-15979
CAS No.:	127243-85-0
Molecular Formula:	$C_{20}H_{20}BrN_3O_2S$
Molecular Weight:	446.36
Target:	PKA; Autophagy
Pathway:	Stem Cell/Wnt; TGF-beta/Smad; Autophagy
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 : 100 mg/mL (224.03 mM; Need ultrasonic)

Preparing Stock Solutions	Mass / Solvent / Concentration	1 mg	5 mg	10 mg
	1 mM	2.2403 mL	11.2017 mL	22.4034 mL
	5 mM	0.4481 mL	2.2403 mL	4.4807 mL
	10 mM	0.2240 mL	1.1202 mL	2.2403 mL

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

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (5.60 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: 2.5 mg/mL (5.60 mM); Suspended solution; Need ultrasonic

BIOLOGICAL ACTIVITY

Description

H-89 is a potent and selective inhibitor of cyclic AMP-dependent protein kinase (protein kinase A) with IC_{50} of 48 nM and has weak inhibition on PKG, PKC, Casein Kinase, and others kinases.

IC_{50} & Target

IC50: 48 nM (protein kinase A)

In Vitro

H-89 inhibits protein kinase A, in competitive fashion against ATP. H-89 causes a dose-dependent inhibition of the forskolin-induced protein phosphorylation, with no decrease in intracellular cyclic AMP levels in PC12D cells. H-89 significantly inhibits the forskolin-induced neurite outgrowth from PC12D cells. H-89 (30 μM) inhibits significantly cAMP-dependent histone IIb phosphorylation activity in PC12D cell lysates[1]. H-89 (1-2 μM) significantly slows the repriming rate in rat skinned fibres, most likely due to it deleteriously affecting the T-system potential. H-89 (10-100 μM) inhibits net Ca^{2+} uptake

	by the SR and affectes the Ca^{32}-sensitivity of the contractile apparatus in rat skinned fibres[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	H-89 (0.2 mg/100g, i.p.) significantly increases seizure latency and threshold in PTZ-treated animals. H-89 (0.05, 0.2 mg/100 g, i.p.) prevents the epileptogenic activity of bucladesine (300 nM) with significant increase of seizure latency and seizure threshold[3]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	Kinase activities are assayed at 30°C for 2-5 min by measuring the transfer of ^{32}P from [γ-^{32}P]ATP to substrates. The reaction is terminated by adding 1 mL of 20% trichloroacetic acid, following the addition of 100 µg of bovine serum albumin as a carrier protein. The sample is centrifuged at 3000 rpm for 10 min, the pellet is resuspended in 5% trichloroacetic acid solution, the final pellet is dissolved in 1 mL of 1 N NaOH and the radioactivity is measured in a liquid scintillation counter. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	After 48 h in culture, PCl2D cells are cultured in test medium containing 30 µM H-89 for 1 h and then exposed to fresh medium that contained both 10 µM forskolin and 30 µM H-89. Cells are scraped off with a rubber policeman and sonicated in the presence of 0.5 mL of 6% trichloroacetic acid. To extract trichloroacetic acid, 2 mL of petroleum ether is added, the preparation mixed and centrifuged at 3000 rpm for 10 min. After aspiration of the upper layer, the residue sample solution is used for determination. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [1]	Male albino mice weighing 20-25 g are obtained. Pentoxifylline (25, 50, 100 mg/kg), bucladesine (50, 100, 300 nM/mouse) and H-89 (0.05, 0.1, 0.2 mg/100 g) are administered intraperitoneally (i.p.) 30 min before intravenous (i.v.) infusion of PTZ. In combination groups, the first and second components are injected 45 and 30 min before PTZ infusion. In all groups, the respective control animalsreceive an appropriate volume of vehicle. For the i.v. infusion, the needle is inserted into the lateral tail vein, fixed to the tail vein by a narrow piece of adhesive tape, and the animal is allowed to move freely. PTZ solution is infused at a concentration rate of 1 mL/min. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Signal Transduct Target Ther. 2023 Aug 9;8(1):290.
- Cell Metab. 2021 Sep 8;S1550-4131(21)00375-2.
- Cell Metab. 2021 Mar 2;33(3):565-580.e7.
- Cell Mol Immunol. 2023 Jan 5.
- Nat Metab. 2024 Mar 18.

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REFERENCES

[1]. Chijiwa T, et al. Inhibition of forskolin-induced neurite outgrowth and protein phosphorylation by a newly synthesized selective inhibitor of cyclic AMP-dependent protein kinase, N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide (H-89), of PC12D

[2]. Blazev R, et al. Effects of the PKA inhibitor H-89 on excitation-contraction coupling in skinned and intact skeletal muscle fibres. J Muscle Res Cell Motil. 2001;22(3):277-86.

[3]. Hosseini-Zare MS, et al. Effects of pentoxifylline and H-89 on epileptogenic activity of bucladesine in pentylenetetrazol-treated mice. Eur J Pharmacol. 2011 Nov 30;670(2-3):464-70.

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