Product Data Sheet

Product Name: HS 014
Cat. No.: GC12840

Chemical Properties

Cas. No.	207678-81-7
Chemical Name	(R)-3-((Z)-((S)-2-((Z)-(((S)-1-((S)-1-((4S,5Z,8Z,10S,11Z,13S,14Z,16R,17E,19S,20Z,22S,23Z,25R)-19-((1H-imidazol-5-yl)methyl)-10-((1H-indol-3-yl)methyl)-22-(2-carboxyethyl)-13-(3-guanidinopropyl)-6,9,12,15,18,21,24-heptahydroxy-25-((Z)-(1-hydroxyethylidene)
SMILES	C/C(O)=N/[C@@](/C(O)=N/[C@@](/C(O)=N/[C@@](/C(O)=N\[C@](/C(O)=N/[C@@](/C(O)=N/[C@@](/C(O)=N/C/C(O)=N/1)([H])CC2=CNC3=CC=CC=C23)([H])CCCNC(N)=N)([H])CC4=CC5=CC=CC=C5C=C4)([H])CC6=CN=CN6)([H])CCC(O)=O)([H])CSSC[C@]1([H])C(N7CCC[C@@]7([H])C(N8CCC[C@@]8([H])/
Formula	$C_{71}H_{94}N_{20}O_{17}S_2$
M.Wt	1563.77
Solubility	Soluble to 1 mg/ml in Water
Storage	Desiccate at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 ℃ and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20℃ for several months.
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.
Structure	HS 014

Protocol

Cell experiment [1]:

Cell lines	Epidermal melanocytes and Keratinocytes

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Preparation Method	Epidermal melanocytes and keratinocytes (phototype III) were seeded in six-well plates and pre-incubated in medium lacking supplemented tetradecanoylphorbol acetate for 48 hours. Before stimulation, the specific MC4-R antagonist HS 014 (10nM-200nM) was added to the appropriate wells.
Reaction Conditions	10nM-200nM; 30min.
Applications	HS 014 significantly attenuated the induction of melanin formation in melanocytes stimulated by β-MSH or NDP-α-MSH over 72 hours. In both melanocytes and keratinocytes, HS 014 attenuated the cAMP response induced by β-MSH or NDP-α-MSH after 1 hour of stimulation.

Animal experiment [2]:

Animal models	Adult male Sprague-Dawley rats
Preparation Method	Rats were implanted with a radiotelemetry transmitter for core body temperature (Tc) monitoring and a guide cannula in the right lateral ventricle for intracerebroventricular (icv) injection. On the experiment day, rats received an intraperitoneal (i.p.) injection of bacterial endotoxin lipopolysaccharide (LPS; 30μg/kg) to induce fever, followed 30 minutes later by the icv injection of HS 014 or vehicle (artificial cerebrospinal fluid, aCSF). Tc and motor activity were recorded continuously, and tail skin temperature (Tsk) was measured hourly.

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Dosage form	1μg in 5μl; icv; single injection.
Applications	HS 014 had no significant effect on the LPS-induced febrile increase in core body temperature (Tc) or the associated decrease in tail skin temperature (Tsk) in rats. HS 014 also had no significant effect on Tc, Tsk, or motor activity in afebrile (saline-treated) rats. However, the same dose of HS 014 completely blocked the antipyretic and anti-vasoconstrictive effects of a co-administered selective MC4R agonist (MRLOB-0001; 150ng; icv) during the first phase of fever.

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References:
[1] Spencer JD, Schallreuter KU. Regulation of pigmentation in human epidermal melanocytes by functional high-affinity beta-melanocyte-stimulating hormone/melanocortin-4 receptor signaling. Endocrinology. 2009 Mar;150(3):1250-8.
[2] Sinha PS, Schiöth HB, Tatro JB. Activation of central melanocortin-4 receptor suppresses lipopolysaccharide-induced fever in rats. Am J Physiol Regul Integr Comp Physiol. 2003 Jun;284(6):R1595-603.

Background

HS 014 is a potent and selective melanocortin MC4 receptor antagonist (K_i=3.16nM). HS 014 regulates appetite and energy balance by blocking the interaction between the MC4 receptor and endogenous ligands, while also reducing inflammatory responses by inhibiting IL-1β-induced Fos expression[1-2]. HS 014 is utilized in research related to obesity, diabetes, and metabolic syndrome[3-4].

In vitro, epidermal melanocytes were pretreated with HS 014 (10nM–200nM) for 30 minutes, followed by stimulation with β-MSH (200nM) or the N-terminally modified α-MSH analog NDP-α-MSH (200nM) for 72 hours. HS 014 significantly inhibited melanin

formation and reduced cAMP production[5]. MC4R-overexpressing M1830 astrocytoma cells were treated with HS 014 (1nM) for 12 days. This treatment significantly downregulated the expression of p-JNK, ATF3, and c-Jun proteins, while also suppressing the activation of the JNK signaling pathway[6].

In vivo, HS 014 (1μg; 5μl) was administered via a single intracerebroventricular (icv) injection in rats that had been previously induced with fever by lipopolysaccharide (LPS; 30μg/kg; i.p.). HS 014 had no significant effect on the febrile response but completely blocked the antipyretic effect produced by the selective MC4R agonist MRLOB-0001 (150ng; icv)[7]. HS 014 (3.5ng or 100μg, intranasal infusion) was administered as a single dose 30 minutes before a single prolonged stress (SPS) exposure in rats. The 100μg dose significantly reduced the elevation in plasma corticosterone levels measured 30 minutes after the SPS stressor ended, while both doses prevented the stress-induced short-term increase in corticotropin-releasing hormone (CRH) mRNA levels in the basal medial hypothalamus, as well as the increase in tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH) mRNA levels in the locus coeruleus[8].

References:
[1] Schiöth HB, Mutulis F, Muceniece R, et al. Discovery of novel melanocortin4 receptor selective MSH analogues. Br J Pharmacol. 1998 May;124(1):75-82.
[2] Ercil NE, Galici R, Kesterson RA. HS014, a selective melanocortin-4 (MC4) receptor antagonist, modulates the behavioral effects of morphine in mice. Psychopharmacology (Berl). 2005 Jul;180(2):279-85.
[3] Kask A, Rägo L, Mutulis F, et al. Selective antagonist for the melanocortin 4 receptor (HS014) increases food intake in free-feeding rats. Biochem Biophys Res Commun. 1998 Apr 7;245(1):90-3.
[4] Gao Z, Lei D, Welch J, et al. Agonist-dependent internalization of the human melanocortin-4 receptors in human embryonic kidney 293 cells. J Pharmacol Exp Ther. 2003 Dec;307(3):870-7.
[5] Spencer JD, Schallreuter KU. Regulation of pigmentation in human epidermal melanocytes by functional high-affinity beta-melanocyte-stimulating hormone/melanocortin-4 receptor signaling. Endocrinology. 2009 Mar;150(3):1250-8.
[6] Zhao Y, Xin Y, Chu H. MC4R Is Involved in Neuropathic Pain by Regulating JNK Signaling Pathway After Chronic Constriction Injury. Front Neurosci. 2019 Sep 10;13:919.
[7] Loopuijt LD. Local application of L- threo-hydroxyaspartate and malonate in rats in

vivo induces rigidity and damages neurons of the substantia nigra, pars compacta. J Neural Transm (Vienna). 2002 Oct;109(10):1275-94.
[8] Serova LI, Laukova M, Alaluf LG, et al, Blockage of melanocortin-4 receptors by intranasal HS014 attenuates single prolonged stress-triggered changes in several brain regions. J Neurochem. 2014 Dec;131(6):825-35.