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产品名称：3-氨基-6-氯-5-[(3S)-4-[1-[(4-氯苯基)甲基]-4-哌啶基]-3-乙基-1-哌嗪基]-2-哌嗪甲酰胺
产品别名：SCH 546738

生物活性：

Description	SCH 546738 is a potent, orally active and non-competitive CXCR3 antagonist, the affinity constant (Ki) of SCH 546738 binding to human CXCR3 receptor is determined to be 0.4 nM in multiple experiments.
IC_{50} & Target	Human CXCR3
	0.4 nM (Ki)
In Vitro	The affinity of SCH 546738 binding to human CXCR3 receptor is determined by competition binding analysis using ^{35}S radiolabeled SCH 535390 (a sulfonamide analog of the CXCR3 compound series with a K_d of 0.6 nM) as a competitive tracer. In addition, SCH 546738 displaces radiolabeled CXCL10 and CXCL11 from human CXCR3 with IC_{50} ranging from 0.8 to 2.2 nM in a non-competitive manner. SCH 546738 potently and specifically inhibits CXCR3-mediated chemotaxis in human activated T cells with IC_{90} about 10 nM. Competition of human CXCL10 and CXCL11 binding to human CXCR3 by SCH 546738 is determined at various concentrations of [^{125}I]hCXCL10 and [^{125}I]hCXCL11 around the K_d (50-100 pM) for the receptor. The IC_{50} of SCH 546738 is constant (~1 or 2 nM) and independent of the input concentrations of either [^{125}I]hCXCL10 (25-500 pM) or [^{125}I]hCXCL11 (12.5-250 pM), respectively[1].
In Vivo	SCH 546738 has strong cross-species activities with IC_{50} of 1.3 nM, 6.4 nM, 5.9 nM and 4.2 nM in inhibiting the binding of [^{125}I]hCXCL10 to CXCR3 of monkey, dog, mouse and rat origin, respectively. SCH 546738 is a selective and potent CXCR3 antagonist with a good PK for in vivo studies. In addition, SCH 546738 has a favourable pharmacokinetic profile in rodents, the plasma concentrations of SCH 546738 in Lewis rat and C57BL/6 mouse over 24 hr post-dose. The AUC (0-24 hr) is 7.7 μM.hr in Lewis rat 10 mg/kg (mpk) and is 12.6 μM.hr in C57BL/6 mouse 30 mpk[1].
Solvent&Solubility	***In Vitro:***

DMSO : 4.5 mg/mL (9.14 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.0307 mL	10.1535 mL	20.3070 mL
	5 mM	0.4061 mL	2.0307 mL	4.0614 mL
	10 mM	---	---	---

*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： corn oil Solubility: 1 mg/mL (2.03 mM); Suspended solution; Need ultrasonic and warming
References	[1]. Jenh CH, et al. A selective and potent CXCR3 antagonist SCH 546738 attenuates the development of autoimmune diseases and delays graft rejection. BMC Immunol. 2012 Jan 10;13:2. [2]. Zhang X, et al. CXC chemokine receptor 3 promotes steatohepatitis in mice through mediating inflammatory cytokines, macrophages and autophagy. J Hepatol. 2016 Jan;64(1):160-70. [3]. Yue C, et al. STAT3 in $CD8^+$ T Cells Inhibits Their Tumor Accumulation by Downregulating CXCR3/CXCL10 Axis. Cancer Immunol Res. 2015 Aug;3(8):864-870.

实验参考：

Cell Assay	The preparation of human activated T cells is performed. Human peripheral blood lymphocytes are prepared by Ficoll-Hypaque centrifugation, depleted of monocytes, and stimulated for 2 days with 1 μg/mL PHA and 100 U/mL IL-2 in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 2 mM L-Glutamine, 100 μg/mL Streptomycin, 100 U/mL Penecillin, 1% non-essential amino acids and 2 mM HEPES. Following stimulation, peripheral blood lymphocytes are cultured in above media containing 5% conditioned media for up to 15 days. uman activated T cell chemotaxis assays ae performed using 96-well ChemoTx microplates with a 3 μm filter. Activated T cells are washed with RPMI medium twice, and then resuspended in the medium containing 20% FBS. 1.25×10^5cells/reaction are mixed with indicated concentrations of SCH 546738 (1, 10 or 100 nM) and placed on the filter. SCH 546738 and chemokines are mixed and placed in the bottom well of the ChemoTx system. After 2.5 hours incubation at 37°C/5% CO_2, the cells are scraped off and the plate system is centrifuged for 5 minutes at 1000 RPM. The filter screen is then removed and the ChemoTx plate is inverted into a 96 well plate with a funnel plate. The plate system is centrifuged for 5 minutes at 1000 RPM. The volume in the wells is brought to 100 μL with assay buffer and the plates are rested for approximately 15 minutes at room temperature. The number of migrated cells is measured using the Cell Titer Glo Luminescent Assay. Chemotaxis is expressed as a chemotactic index, which is a ratio versus the one without chemokines[1]
Animal Administration	Mice[1] Female C57BL/6 mice are used. For immunization, 150 μg MOG35-55 peptide prepared by Princeton Biomolecules and 300 μg killed Mycobacterium tuberculosis are mixed in CFA and injected s.c. in two 50-μL injections over the flanks on day 1. Also, 200 ng of pertussis toxin is injected i.v. on days 0 and 2. SCH 546738 is administered orally 30 mpk in C57BL/6 mice twice daily. Dosing with SCH 546738 started at day 0, 24 h prior to MOG35-55 immunization (day 1). Mice are monitored daily and assessed for clinical signs of disease in a blinded fashion according to the following criteria: 0, no signs of disease; 1, tail paralysis; 2, limp tail and hind limb weakness; 3, hind limb paralysis; 4, hind limb plus forelimb paralysis; and 5, moribund or dead. Cumulative clinical scores are calculated by adding daily scores from the day of immunization until the end of the experiment. Mean clinical scores at separate days and mean maximal scores are calculated by adding the scores of individual mice and dividing with the number of mice in each group, including mice not developing signs of EAE. Rats[1]

	Male Lewis rats challenged by injection of 50 μL (30 mg) of a guinea pig spinal cord homogenate in complete Freund's adjuvant (CFA) into one footpad. SCH 546738 or 0.4% methylcellulose (vehicle) is orally administered at the indicated dose (0.2 mL) twice a day, starting on the day before transplantation until the day of graft rejection. SCH 546738 is administered orally at 10 mg/kg (mpk) in Lewis rats. To test whether SCH 546738 enhances the effect of conventional immunosuppressive reagent, the recipients are received treatment with subtherapeutic dose of CsA for one week combined with treatment with SCH 546738. Graft survival is analyzed using the log-rank test. The parametric data are analyzed by Student t test (2-tailed) using GraphPad InStat version.
Kinase Assay	A scintillation proximity assay is used for radioligand competition binding assays with some modifications. For each assay point, 1 μg of membrane is preincubated for 1 hr with 300 μg wheat germ agglutinin (WGA) coated SPA beads in the binding buffer (50 mM HEPES, 1 mM $CaCl_2$, 5 mM $MgCl_2$, 125 mM NaCl, 0.002% NaN_3, 1.0% BSA) at room temperature. The beads are spun down, resuspended in the binding buffer and transferred to a 96-well Isoplate. The indicated concentrations of ^{125}I-hCXCL10, ^{125}I-hCXCL11 or ^{35}S-SCH 535390 with a series of titrations of SCH 546738 (1-10 μM) are added to start the reaction. After indicated reaction times at room temperature, the amount of radioactivity bound to the SPA beads is determined with a Wallac 1450 Microbeta counter[1].
References	[1]. Jenh CH, et al. A selective and potent CXCR3 antagonist SCH 546738 attenuates the development of autoimmune diseases and delays graft rejection. BMC Immunol. 2012 Jan 10;13:2. [2]. Zhang X, et al. CXC chemokine receptor 3 promotes steatohepatitis in mice through mediating inflammatory cytokines, macrophages and autophagy. J Hepatol. 2016 Jan;64(1):160-70. [3]. Yue C, et al. STAT3 in $CD8^+$ T Cells Inhibits Their Tumor Accumulation by Downregulating CXCR3/CXCL10 Axis. Cancer Immunol Res. 2015 Aug;3(8):864-870.