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产品名称：
6-Methyl-1'-(2-(5-methyl-2-phenyloxazol-4-yl)ethyl)spiro-[benzo[d][1,3]oxazine-4,4'-piperidin]-2(1H)-one
产品别名：RS 504393

生物活性：

Description	RS 504393 is a selective CCR2 chemokine receptor antagonist (IC_{50} values are 89 nM and > 100 μM for inhibition of human recombinant CCR2 and CCR1 receptors respectively).			
IC_{50} & Target	CCR2	Human α_{1a} receptor	Human α_{1d} receptor	5HT-1a receptor
	89 nM (IC_{50})	72 nM (IC_{50})	460 nM (IC_{50})	1070 nM (IC_{50})
In Vitro	RS 504393 inhibits the MCP-1-induced chemotaxis with an IC50 of 330 nM. RS 504393 treatment suppresses allergen induced β-hexosaminidase release significantly. Without allergen priming, MCP-1 induces mast cell degranulation, which is completely suppressed by RS 504393[4].			
In Vivo	RS504393 (0.3-3 μg) with CCL2 progressively blocks thermal hyperalgesia dose-dependently in mice[1]. RS 504393 (5 mg/kg, i.v.) supresses the elevated numbers of leukocytes and increased total protein content in BALF induced by The LPS. RS504393 significantly down regulates the LPS-induced elevation of IL-1β, PAI-1 mRNA and protein expressions. RS504393 significantly suppresses induced lung edema, protein-rich fluid, polymorphonuclear accumulation and bronchial wall thickening induced by LPS[2]. RS-504393 significantly reduces renal pathology, especially the extensive interstitial fibrosis mediated by decrease in type I collagen synthesis in a UUO model[3].			

Solvent&Solubility

In Vitro:

DMSO : 12.5 mg/mL (29.94 mM; Need ultrasonic)

H_2O : < 0.1 mg/mL (insoluble)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.3952 mL	11.9760 mL	23.9521 mL
	5 mM	0.4790 mL	2.3952 mL	4.7904 mL
	10 mM	0.2395 mL	1.1976 mL	2.3952 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→40% PEG300 →5% Tween-80 → 45% saline

Solubility: ≥ 1.25 mg/mL (2.99 mM); Clear solution

此方案可获得 ≥ 1.25 mg/mL (2.99 mM，饱和度未知) 的澄清溶液。

	以 1 mL 工作液为例，取 100 μL 12.5 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。 2 请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 1.25 mg/mL (2.99 mM); Clear solution 此方案可获得 ≥ 1.25 mg/mL (2.99 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 12.5 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。 3.请依序添加每种溶剂： 50% DMSO →15% EtOH → 35% PEG300 Solubility: 31.25 mg/mL (74.85 mM); Suspended solution; Need ultrasonic
References	[1]. Baamonde, Ana., et al. Involvement of glutamate NMDA and AMPA receptors, glial cells and IL-1β in the spinal hyperalgesia evoked by the chemokine CCL2 in mice. Neuroscience Letters (2011), 502(3), 178-181. [2]. Yang, Dong., et al. Roles of CC chemokine receptors (CCRs) on lipopolysaccharide-induced acute lung injury. Respiratory Physiology & Neurobiology (2010), 170(3), 253-259. [3]. Kitagawa, Kiyoki., et al. Blockade of CCR2 ameliorates progressive fibrosis in kidney. American Journal of Pathology (2004), 165(1), 237-246. [4]. Tominaga T, et al. Blocking mast cell-mediated type I hypersensitivity in experimental allergic conjunctivitis by monocyte chemoattractant protein-1/CCR2. Invest Ophthalmol Vis Sci. 2009 Nov;50(11):5181-8. [5]. Mirzadegan T, et al. Identification of the binding site for a novel class of CCR2b chemokine receptor antagonists: binding to a common chemokine receptor motif within the helical bundle. J Biol Chem. 2000 Aug 18;275(33):25562-71.

实验参考：

Animal Administration	Male C57BL/6J mice (n=30) and male homozygote CCR1, CCR2 and CCR3 knockout mice (n=12, in each phenotype), 6-8 weeks old and weighing 20±2 g. Intranasal administration of PBS or LPS (5 mg/kg) is performed in a volume of 1 mL/kg body weight. C57BL/6J mice are treated with vehicle or RS504393 (5 mg/kg) intraperitoneally 30 min before LPS challenge. Four hours after LPS challenge, mice are terminated by an intraperitoneal injection of an overdose of pentobarbitone. The mice are kept on a 12-h light/dark cycle with access to mice chow and water ad libitum. [2]
Kinase Assay	Isolated mast cells are sensitized by incubation with anti-DNP IgE in RPMI1640 containing 10 ng/mL of murine recombinant IL-3, 10 ng/mL of recombinant SCF, and 5% murine serum. The cells are then washed with HBSS containing 10 ng/mL of murine recombinant IL-3, 10 ng/mL of recombinant SCF, 0.04% BSA, and 10 mM HEPES. Resuspended cells at a concentration of 2 to 8×10^4 cells/100 μL are transferred into triplicate wells of a 96 well U-bottom plate and allowed to equilibrate at 37°C for 10 minutes before the addition of DNP-albumin or compound 48/80. After 45 minutes, the plate is centrifuged at 290 g for 5 minutes at 4°C. The β-hexosaminidase activity of the culture supernatant is determined using a Published protocol. Fifty-μL aliquots of the supernatant are placed in wells of another 96-well plate together with 100 μL of 2.5 mM p-nitrophenyl-N-acetyl β-d glucosaminide solubilized in 0.04mol/Lcitrate buffer adjusted to pH 4.5 with disodium phosphate. After incubation at

	37°C for 90 minutes, the reactions are terminated by addition of 50 µL of 0.4mol/Lglycin adjusted to pH 10.7 with sodium hydroxide. The colored product is measured at 405 nm with a reference filter of 570 nm. The relative release of β-hexosaminidase is defined as the activity in the supernatant of the tested cells divided by the activity in the positive control cell supernatant, multiplied by 100. Compound 48/80 stimulus is used for assay control. [4]
References	[1]. Baamonde, Ana., et al. Involvement of glutamate NMDA and AMPA receptors, glial cells and IL-1β in the spinal hyperalgesia evoked by the chemokine CCL2 in mice. Neuroscience Letters (2011), 502(3), 178-181. [2]. Yang, Dong., et al. Roles of CC chemokine receptors (CCRs) on lipopolysaccharide-induced acute lung injury. Respiratory Physiology & Neurobiology (2010), 170(3), 253-259. [3]. Kitagawa, Kiyoki., et al. Blockade of CCR2 ameliorates progressive fibrosis in kidney. American Journal of Pathology (2004), 165(1), 237-246. [4]. Tominaga T, et al. Blocking mast cell-mediated type I hypersensitivity in experimental allergic conjunctivitis by monocyte chemoattractant protein-1/CCR2. Invest Ophthalmol Vis Sci. 2009 Nov;50(11):5181-8. [5]. Mirzadegan T, et al. Identification of the binding site for a novel class of CCR2b chemokine receptor antagonists: binding to a common chemokine receptor motif within the helical bundle. J Biol Chem. 2000 Aug 18;275(33):25562-71.