

产品名称：**SB225002**

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生物活性:				
Description	SB225002, a potent, selective and non-peptide CXCR2 antagonist, inhibits ¹²⁵ I-IL-8 binding to CXCR2 with an IC ₅₀ of 22 nM.			
IC ₅₀ & Target	¹²⁵ I-IL-8-CXCR2			
	22 nM (IC ₅₀ , in CHO cell membrane)			
In Vitro	SB225002 (SB 225002) is an antagonist of ¹²⁵ I-IL-8 binding to CXCR2 with an IC ₅₀ =22 nM. SB225002 shows >150-fold selectivity over CXCR1 and four other 7-TMRs tested. SB225002 is a potent antagonist of rabbit CXCR2, inhibiting rabbit PMN chemotaxis in response to optimal concentrations of human IL-8 or GRO α (IC ₅₀ values of 30 and 70 nM, respectively. In these cells (PMN, HL60, CXCR1-RBL-2H3), SB225002 produces a concentration-dependent inhibition of both IL-8- and GRO α -mediated calcium mobilization with IC ₅₀ values of 8 and 10 nM, respectively. In 3ASubE cells stably transfected with CXCR2, SB 225002 dose-dependently inhibits calcium mobilization induced by both GRO α and IL-8, with IC ₅₀ values of 20 and 40 nM, respectively[1]. WHCO1 cells treated with SB225002 exhibits a 40% reduction in cell proliferation. Blocking CXCR2 signaling in WHCO1 cells with 400 nM SB225002 (SB 225002) significantly decreases cell proliferation by ~40% to 50%[2].			
In Vivo	SB225002 (SB 225002) selectively blocks IL-8-induced neutrophil margination in rabbits[1]. CXCR2 is blocked using the selective antagonist SB225002 (2 mg/kg) or neutralizing CXCR2 antiserum. The CXCR2 antagonist SB225002 decreases neutrophil counts in ischemic hemispheres of ApoE ^{-/-} mice on Western diet and wildtype mice on normal diet[3]. SB225002 significantly attenuates microglial activation and BBB damage, increases myelination, and reduces astrogliosis in the white matter after LPS-sensitized HI[4].			
Solvent&Solubility	In Vitro: DMSO : \geq 100 mg/mL (283.98 mM) * "≥" means soluble, but saturation unknown.			
	Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg
		1 mM	2.8398 mL	14.1989 mL
		5 mM	0.5680 mL	2.8398 mL
		10 mM	0.2840 mL	1.4199 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℃，6 months；-20℃，1 month。-80℃ 储存时，请在 6 个月内使用，-20℃ 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: \geq 2.5 mg/mL (7.10 mM); Clear solution 此方案可获得 \geq 2.5 mg/mL (7.10 mM，饱和度未知) 的澄清溶液。			

	<p>以 1 mL 工作液为例，取 100 μL 25.0 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。</p> <p>2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline) Solubility: \geq 2.5 mg/mL (7.10 mM); Clear solution 此方案可获得 \geq 2.5 mg/mL (7.10 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀。</p> <p>3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: \geq 2.5 mg/mL (7.10 mM); Clear solution 此方案可获得 \geq 2.5 mg/mL (7.10 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 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。</p>
References	<p>[1]. White JR, et al. Identification of a potent, selective non-peptide CXCR2 antagonist that inhibits interleukin-8-induced neutrophil migration. <i>J Biol Chem</i>. 1998 Apr 24;273(17):10095-8.</p> <p>[2]. Wang B, et al. A growth-related oncogene/CXC chemokine receptor 2 autocrine loop contributes to cellular proliferation in esophageal cancer. <i>Cancer Res</i>. 2006 Mar 15;66(6):3071-7.</p> <p>[3]. Herz J, et al. Role of Neutrophils in Exacerbation of Brain Injury After Focal Cerebral Ischemia in Hyperlipidemic Mice. <i>Stroke</i>. 2015 Oct;46(10):2916-25.</p> <p>[4]. Wang LY, et al. CXCL5 signaling is a shared pathway of neuroinflammation and blood-brain barrier injury contributing to white matter injury in the immature brain. <i>J Neuroinflammation</i>. 2016 Jan 6;13:6.</p> <p>[5]. Shi ZR, et al. Decrease of galectin-3 in keratinocytes: A potential diagnostic marker and a critical contributor to the pathogenesis of psoriasis. <i>J Autoimmun</i>. 2018 May;89:30-40.</p>
实验参考：	
Cell Assay	<p>Three esophageal squamous cell carcinoma cell lines WHCO1, WHCO5, and WHCO6 originally established from surgical biopsies of primary esophageal squamous cell carcinomas are cultured in DMEM containing 10% FCS at 37°C in a humidified atmosphere of 5% CO₂. MTT assays are carried out using the Cell Proliferation kit. Briefly, 1.5×10^3 cells are plated in 96-well plates in a final volume of 180 μL DMEM per well. SB 225002 (400 nM) is added to cells and 0.001% DMSO (solvent) is added as a control. After the indicated incubation period, 18 μL of the MTT labeling reagent (final concentration 0.5 mg/mL) is added to each well and incubated for 4 hours in a humidified atmosphere. One hundred eighty microliters of the solubilization solution are added to each well and the plates are left overnight at 37°C. The spectrophotometric absorbance of samples is measured at 595 nm using a microtiter plate reader[2].</p>
	<p>Mice[3]</p> <p>Male 7-8 weeks old wildtype (C57BL/6J, Harlan) and ApoE^{-/-} mice, which are generated on the same C57BL/6 background, are either fed with a normal chow or a cholesterol rich chow for 6 weeks and submitted to 20 min of left-sided middle cerebral artery occlusion (MCAO) or sham surgery. Animals are randomly attributed to treatment paradigms, and experimenters are blinded at all stages of interventions and data analysis. The selective CXCR2 antagonist SB225002 (2 mg/kg) or vehicle (1% DMSO in PBS) is injected intraperitoneally (i.p.) at 0, 24 and 48 hours post-ischemia. In other</p>

<p>Animal Administration</p>	<p>experiments, CXCR2 is specifically blocked by i.p. injection of a neutralizing rabbit anti-CXCR2 serum (300 μL) at 0 hours, 24 hours and 48 hours post-ischemia. In the latter studies, normal rabbit serum (NRS) served as control. In some experiments, neutrophils are depleted by i.p. injection of 200 μg anti-mouse Ly6G 24 hours before and 24 hours after ischemia. In these experiments, 200 μg of an isotype control antibody is delivered as control.</p> <p>Rats[4]</p> <p>In this study, 10-12 Sprague-Dawley rat pups per dam are used. The pups receive intraperitoneal injections of SB225002 (1 or 3 mg/kg, diluted in NS containing 0.33 % Tween 80) or vehicle (NS solution containing 0.33 % Tween 80) 30 min before lipopolysaccharide (LPS) administration and immediately after hypoxic ischemia (HI). The pups are randomly assigned to four groups: control (pups unexposed to LPS or HI, N=14), vehicle (NS injections 30 min before LPS administration and immediately after HI, N=18), and SB-1 (1 mg/kg, N=14) and SB-3 (3 mg/kg, N=18) (SB225002 injections 30 min before LPS administration and immediately after HI).</p>
<p>Kinase Assay</p>	<p>CHO-CXCR1 and CHO-CXCR2 membranes are prepared. Assays are performed in 96-well microtiter plates where the reaction mixture contained 1.0 μg/mL membrane protein in 20 mM Bis-Tris-propane, pH 8.0, with 1.2 mM $MgSO_4$, 0.1 mM EDTA, 25 mM NaCl, and 0.03% CHAPS and SB 225002 (10 mM stock in Me_2SO) added at the indicated concentrations, the final Me_2SO concentration is <1% under standard binding conditions. Binding is initiated by addition of 0.25 nM ^{125}I-IL-8 (2,200 Ci/mmol). After 1-h incubation at room temperature the plate is harvested using a Tomtec 96-well harvester onto a glass fiber filtermat blocked with 1% polyethyleneimine, 0.5% BSA and washed three times with 25 mM NaCl, 10 mM Tris•HCl, 1 mM $MgSO_4$, 0.5 mM EDTA, 0.03% CHAPS, pH 7.4. The filter is dried, sealed in a sample bag containing 10 mL of Wallac 205 Betaplate liquid scintillation fluid, and counted with a Wallac 1205 Betaplate liquid scintillation counter[1].</p>
<p>References</p>	<p>[1]. White JR, et al. Identification of a potent, selective non-peptide CXCR2 antagonist that inhibits interleukin-8-induced neutrophil migration. <i>J Biol Chem.</i> 1998 Apr 24;273(17):10095-8.</p> <p>[2]. Wang B, et al. A growth-related oncogene/CXC chemokine receptor 2 autocrine loop contributes to cellular proliferation in esophageal cancer. <i>Cancer Res.</i> 2006 Mar 15;66(6):3071-7.</p> <p>[3]. Herz J, et al. Role of Neutrophils in Exacerbation of Brain Injury After Focal Cerebral Ischemia in Hyperlipidemic Mice. <i>Stroke.</i> 2015 Oct;46(10):2916-25.</p> <p>[4]. Wang LY, et al. CXCL5 signaling is a shared pathway of neuroinflammation and blood-brain barrier injury contributing to white matter injury in the immature brain. <i>J Neuroinflammation.</i> 2016 Jan 6;13:6.</p> <p>[5]. Shi ZR, et al. Decrease of galectin-3 in keratinocytes: A potential diagnostic marker and a critical contributor to the pathogenesis of psoriasis. <i>J Autoimmun.</i> 2018 May;89:30-40.</p>