



上海源叶生物科技有限公司
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产品名称: **INCB3344**
产品别名: **INCB3344**

生物活性:

Description	INCB3344 is a potent, selective and orally bioavailable CCR2 antagonist with IC ₅₀ values of 5.1 nM (hCCR2) and 9.5 nM (mCCR2) in binding antagonism and 3.8 nM (hCCR2) and 7.8 nM (mCCR2) in antagonism of chemotaxis activity.			
IC ₅₀ & Target	hCCR2	mCCR2		
	5.1 nM (IC ₅₀)	9.5 nM (IC ₅₀)		
In Vitro	INCB3344 is a potent antagonist towards rat and cynomolgus CCR2 as well, displaying IC50 values of 7.3 and 16 nM in binding antagonism and 2.7 and 6.2 nM in antagonism of chemotaxis activity, respectively. INCB3344 is a selective hCCR2 antagonist, exhibiting IC50 values of more than 1 μM against a panel of >50 ion channels, transporters, chemokine receptors and other selected GPCRs. It is also a selective mCCR2 antagonist, showing IC50 values of >1 μM and >3 μM against murine CCR1 and murine CCR5, respectively, the two most homologous chemokine receptors to mCCR2[1]. Characterization of the pharmacological activity of INCB3344 is first evaluated by testing its ability to inhibit CCL2 binding to CCR2 in a whole cell binding assay using a murine monocyte cell line, WEHI-274.1 and 125I-labeled mCCL2 as a tracer. The binding IC50 of INCB3344 in this assay is determined to be 10±5 nM, and inhibition of >90% binding is observed at a concentration of 90 nM[2].			
In Vivo	When administered intravenously to CD-1 mice, INCB3344 exhibits a high clearance and a moderate volume of distribution, resulting in a short half life of 1 h. Despite its high clearance, however, good oral exposure is achieved, with an AUC at 2664 nM h at a dose of 10 mg/kg. The oral bioavailability is 47%. By comparison, slightly better oral exposure (AUC=3888 nM h) is obtained when administered orally to Balb/c mice at the same dose. This PK property, couple with its potent anti-mCCR2 activity and good selectivity, makes this compound suitable for model studies in rodents[1]. INCB3344 prevents deoxycorticosterone acetate (DOCA)/salt-induced changes in vascular expression of CCR2. In a separate series of experiments, CCR2 expression is elevated (≈1.5-fold higher) in aortas from mice that receive INCB3344 from days 7 to 21 of the DOCA/salt treatment period compare with sham animals; however, this level of CCR2 expression is significantly lower than that observed in the vehicle-treated group (P<0.05, n=6). Likewise, increased expression of its receptor ligand CCL2 in DOCA/salt-treated mice is blunted in mice receiving INCB3344 (P<0.05, n=6). By contrast, levels of CCL7, CCL8, and CCL12 are elevated to similar extents in DOCA/salt-treated mice receiving vehicle or INCB3344[3].			
In Vitro: DMSO : 240 mg/mL (415.52 mM; Need ultrasonic) H₂O : < 0.1 mg/mL (insoluble)				
Preparing Stock Solutions	<div>Solvent / Mass / Concentration</div>	1 mg	5 mg	10 mg
	1 mM	1.7313 mL	8.6567 mL	17.3133 mL
	5 mM	0.3463 mL	1.7313 mL	3.4627 mL
	10 mM	0.1731 mL	0.8657 mL	1.7313 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				



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Solvent&Solubility	<p>复冻融造成的产品失效。</p> <p>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。</p> <p>In Vivo:</p> <p>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:</p> <p>——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</p> <p>1.请依序添加每种溶剂: 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline Solubility: 6 mg/mL (10.39 mM); Clear solution; Need ultrasonic</p> <p>2.请依序添加每种溶剂: 10% DMSO→ 90% (20% SBE-β-CD in saline) Solubility: 6 mg/mL (10.39 mM); Clear solution; Need ultrasonic</p> <p>3.请依序添加每种溶剂: 10% DMSO →90% corn oil Solubility: 6 mg/mL (10.39 mM); Clear solution; Need ultrasonic</p>
References	<p>[1]. Xue CB, et al. Discovery of INCB3344, a potent, selective and orally bioavailable antagonist of human and murine CCR2. Bioorg Med Chem Lett. 2010 Dec 15;20(24):7473-8</p> <p>[2]. Brodmerkel CM, et al. Discovery and pharmacological characterization of a novel rodent-active CCR2 antagonist, INCB3344. J Immunol. 2005 Oct 15;175(8):5370-8.</p> <p>[3]. Chan CT, et al. Reversal of vascular macrophage accumulation and hypertension by a CCR2 antagonist in deoxycorticosterone/salt-treated mice. Hypertension. 2012 Nov;60(5):1207-12.</p> <p>[4]. Dansereau MA, et al. Spinal CCL2 pronociceptive action is no longer effective in CCR2 receptor antagonist-treated rats. J Neurochem. 2008 Jul;106(2):757-69.</p> <p>[5]. Zhao W, et al. Enrichment of Ly6Chi monocytes by multiple GM-CSF injections with HBV vaccine contributes to viral clearance in a HBV mouse model. Hum Vaccin Immunother. 2017 Dec 2;13(12):2872-2882.</p> <p>[6]. Aye-Mon A, et al. CCR2 upregulation in DRG neurons plays a crucial role in gastric hyperalgesia associated with diabetic gastropathy. Mol Pain. 2018 Jan-Dec;14:1744806917751322.</p> <p>[7]. Cassini MF, et al. Mcp1 Promotes Macrophage-Dependent Cyst Expansion in Autosomal Dominant Polycystic Kidney Disease. J Am Soc Nephrol. 2018 Oct;29(10):2471-2481.</p>
实验参考:	
	<p>WEHI-274.1 cells (5×10⁵) in RPMI 1640 (VWR) with or without various concentrations of INCB3344 in RPMI 1640 are loaded in the wells on top of an 8-μm polycarbonate filter in a 96-well-modified Boyden chamber. Beneath the filter, 30 nM mCCL2 with or without INCB3344 or media is placed in a corresponding 96-well plate. The sealed chambers are incubated for 45 min at 37°C, 5% CO₂. Filters are washed, stained with Wright-Giemsa, and the number of cells that migrate toward mCCL2</p>



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Cell Assay	<p>in the bottom chamber counted by microscopy. The ability of INCB3344 to antagonize CCR2-mediated chemotaxis is reported as the inhibitor concentration required for IC₅₀ values of specific migration to mCCL2. Specific migration is defined as the total migration minus the background migration. A similar assay is used to determine the impact of INCB3344 on CCR1-mediated chemotaxis of WEHI-274.1 cells, by using mouse MIP-1α as a ligand. In addition C5a, FMLP and RANTES are similarly tested in the presence of INCB3344 for migration of WEHI-274.1 cells. For the studies on the impact of INCB3344 on CCR5-mediated chemotaxis, murine T cells are used as the cell system with mouse MIP-1β as the ligand^[2].</p>
Animal Administration	<p>Mice^[3]</p> <p>In a subset of experiments, DOCA/salt-treated mice are further randomly assigned to receive the CCR2 antagonist, INCB3344 (30 mg/kg per day; Haoyuan Chemexpress Co Ltd) or vehicle (10% DMSO/0.9% carboxymethylcellulose) via daily intraperitoneal injections commencing 10 days after induction of hypertension and continuing until the end of the 21-day treatment period. The normotensive control group for these experiments consist of sham-treated mice that receive vehicle from days 10 to 21.</p> <p>Rats^[4]</p> <p>Adult male Sprague-Dawley rats (200-250 g) are used. After t=0 baseline measurement, rats are lightly anesthetized under an isoflurane/oxygen (5%; 2 L/min) flow and 25 μL of either saline (vehicle), 1 μg of CCL2 and/or 1 mM of INCB3344 is administered intrathecally between L5 and L6 vertebrae. Animals are tested once at 30, 60, 90, 120, and 240 min following drug administration. The percentage of maximal potential effect (MPE) is calculated for every time point.</p>
Kinase Assay	<p>WEHI 274.1 (murine monocytic cell line) cells are used in a whole cell binding assay. Cells (5\times10⁵) in RPMI 1640 (VWR), +0.1% BSA+20 mM HEPES (VWR), are added to various concentrations of INCB3344 in RPMI 1640 follow immediately by the addition of 150 pM ¹²⁵I-labeled mCCL2 (mouse CCL2(JE)) and incubated for 30 min at room temperature (RT). For the nonspecific control, 0.3 μM mCCL2 is added in place of INCB3344. Cells are then harvested through 1.2-μm polyvinylidene difluoride filters, the filters are air-dried, and binding is determined by counting in a gamma counter. Antagonist activity is reported as the inhibitor concentration required for IC₅₀ of specific binding. Specific binding is defined as the total binding minus the nonspecific binding and typically represents 97% of the total binding^[2].</p>
References	<p>[1]. Xue CB, et al. Discovery of INCB3344, a potent, selective and orally bioavailable antagonist of human and murine CCR2. Bioorg Med Chem Lett. 2010 Dec 15;20(24):7473-8</p> <p>[2]. Brodmerkel CM, et al. Discovery and pharmacological characterization of a novel rodent-active CCR2 antagonist, INCB3344. J Immunol. 2005 Oct 15;175(8):5370-8.</p> <p>[3]. Chan CT, et al. Reversal of vascular macrophage accumulation and hypertension by a CCR2 antagonist in deoxycorticosterone/salt-treated mice. Hypertension. 2012 Nov;60(5):1207-12.</p> <p>[4]. Dansereau MA, et al. Spinal CCL2 pronociceptive action is no longer effective in CCR2 receptor antagonist-treated rats. J Neurochem. 2008 Jul;106(2):757-69.</p> <p>[5]. Zhao W, et al. Enrichment of Ly6Chi monocytes by multiple GM-CSF injections with HBV vaccine contributes to viral clearance in a HBV mouse model. Hum Vaccin Immunother. 2017 Dec 2;13(12):2872-2882.</p>



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