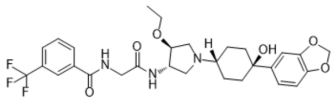
INCB3344

Cat. No.:	HY-50674
CAS No.:	1262238-11-8
Molecular Formula:	$C_{29}H_{34}F_3N_3O_6$
Molecular Weight:	577.59
Target:	CCR
Pathway:	GPCR/G Protein; Immunology/Inflammation
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 6 months -20°C 1 month



SOLVENT & SOLUBILITY

In Vitro

DMSO : 220 mg/mL (380.89 mM; Need ultrasonic)
Ethanol : 100 mg/mL (173.13 mM; Need ultrasonic)

Preparing Stock Solutions

Solvent / Mass / Concentration	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

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 5% DMSO >> 95% (20% SBE-β-CD in saline)
   Solubility: 6 mg/mL (10.39 mM); Clear solution; Need ultrasonic
2. Add each solvent one by one: 5% DMSO >> 95% corn oil
   Solubility: 6 mg/mL (10.39 mM); Clear solution; Need ultrasonic
3. Add each solvent one by one: 5% DMSO >> 40% PEG300 >> 5% Tween-80 >> 50% saline
   Solubility: ≥ 2.75 mg/mL (4.76 mM); Clear solution

BIOLOGICAL ACTIVITY

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 5.1 nM (IC_{50})	mCCR2 9.5 nM (IC_{50})

In Vitro	INCB3344 is a potent antagonist towards rat and cynomolgus CCR2 as well, displaying IC_{50} 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 IC_{50} 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 IC_{50} 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 ^{125}I-labeled mCCL2 as a tracer. The binding IC_{50} 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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
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/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 Deoxycorticosterone acetate/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 Deoxycorticosterone acetate/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 Deoxycorticosterone acetate/salt-treated mice receiving vehicle or INCB3344[3]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Cell Assay [2]	WEHI-274.1 cells (5×10^5) 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% CO_2. Filters are washed, stained with Wright-Giemsa, and the number of cells that migrate toward mCCL2 in the bottom chamber counted by microscopy. The ability of INCB3344 to antagonize CCR2-mediated chemotaxis is reported as the inhibitor concentration required for IC_{50} 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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [3][4]	Mice[3] In a subset of experiments, Deoxycorticosterone acetate/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. Rats[4] Adult male Sprague-Dawley rats (200-250 g) are used. 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. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- J Am Soc Nephrol. 2018 Oct;29(10):2471-2481.
- EMBO Mol Med. 2015 Mar 14;7(5):547-61.
- J Neuroinflammation. 2021 Sep 12;18(1):196.
- Hypertension. 2012 Nov;60(5):1207-12.
- Diabetes. 2019 Nov;68(11):2063-2073.

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[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.

[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.

[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.

[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.

[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.

[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.

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