Data Sheet (Cat.No.TQ0103)

INCB3344

Chemical Properties

CAS No.:	1262238-11-8
Formula:	C29H34F3N3O6
Molecular Weight:	577.24
Appearance:	N/A
Storage:	0-4℃ for short term (days to weeks), or -20℃ for long term (months).

Biological Description

Description	INCB3344 is an effective, specific and orally bioavailable CCR2 antagonist with IC50 values of 9.5 nM (mCCR2) and 5.1 nM (hCCR2) in binding antagonism and 7.8 nM (mCCR2) and in 3.8 nM (hCCR2) antagonism of chemotaxis activity.
In vitro	INCB3344 is also a potent antagonist towards rat and cynomolgus CCR2, 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 exhibit 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. The binding IC50 of INCB3344 in this assay is determined to be 10 nM, and inhibition of >90% binding is observed at a concentration of 90 nM [2].
In vivo	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 compared with sham animals; however, this level of CCR2 expression is significantly lower than that observed in the vehicle-treated group. Likewise, increased expression of its receptor-ligand CCL2 in DOCA/salt-treated mice is blunted in mice receiving INCB3344. By contrast, levels of CCL7, CCL8, and CCL12 are elevated to similar extents in DOCA/salt-treated mice receiving vehicle or INCB3344 [3]. 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 [1].
Kinase Assay	Cells (5×10^5) in RPMI 1640 (VWR), +0.1% BSA+20 mM HEPES (VWR), are added to various concentrations of INCB3344 in RPMI 1640 followed 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].

Cell Research	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% CO2. 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 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].
Animal Research	In a subset of experiments, DOCA/salt-treated mice are further randomly assigned to receive the CCR2 antagonist, INCB3344 (30 mg/kg per day) 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 consists of sham-treated mice that receive a vehicle from days 10 to 21 [3]. 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 is calculated for every time point [4].

Solubility Information

Solubility	DMSO: 236 mg/mL (408.59 mM) Water: Insoluble (< 1 mg/ml refers to the product slightly soluble or insoluble)

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	1.732 mL	8.662 mL	17.324 mL
5 mM	0.346 mL	1.732 mL	3.465 mL
10 mM	0.173 mL	0.866 mL	1.732 mL
50 mM	0.035 mL	0.173 mL	0.346 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. The storage conditions and period of the stock solution: - 80 ℃ for 6 months; - 20 ℃ for 1 month. Please use it as soon as possible.

Reference

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

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.

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