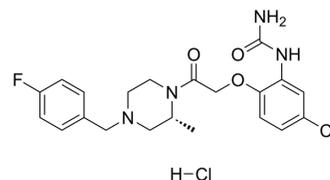


BX471 hydrochloride

Cat. No.:	HY-12080A
CAS No.:	288262-96-4
Molecular Formula:	C ₂₁ H ₂₅ Cl ₂ FN ₄ O ₃
Molecular Weight:	471.35
Target:	CCR
Pathway:	GPCR/G Protein; Immunology/Inflammation
Storage:	-20°C, sealed storage, away from moisture * In solvent : -80°C, 6 months; -20°C, 1 month (sealed storage, away from moisture)



SOLVENT & SOLUBILITY

In Vitro	DMSO : 120 mg/mL (254.59 mM; Need ultrasonic)						
	Preparing Stock Solutions	Solvent Concentration	Mass	1 mg	5 mg	10 mg	
				1 mM	2.1216 mL	10.6078 mL	21.2157 mL
				5 mM	0.4243 mL	2.1216 mL	4.2431 mL
				10 mM	0.2122 mL	1.0608 mL	2.1216 mL
Please refer to the solubility information to select the appropriate solvent.							
In Vivo	1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline Solubility: ≥ 3 mg/mL (6.36 mM); Clear solution						
	2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: ≥ 3 mg/mL (6.36 mM); Clear solution						
	3. Add each solvent one by one: 10% DMSO >> 90% corn oil Solubility: ≥ 3 mg/mL (6.36 mM); Clear solution						

BIOLOGICAL ACTIVITY

Description	BX471 hydrochloride (ZK-811752 hydrochloride) is a potent, selective non-peptide CCR1 antagonist with K _i of 1 nM for human CCR1, and exhibits 250-fold selectivity for CCR1 over CCR2, CCR5 and CXCR4.		
IC ₅₀ & Target	MIP-1α-CCR1 1 nM (K _i)	RANTES-CCR1 2.8 nM (K _i)	MCP-3-CCR1 5.5 nM (K _i)
In Vitro	BX471 is a potent functional antagonist based on its ability to inhibit a number of CCR1-mediated effects including Ca ²⁺ mobilization, increase in extracellular acidification rate, CD11b expression, and leukocyte migration. BX471 demonstrates a greater than 10,000-fold selectivity for CCR1 compared with 28 G-protein-coupled receptors ^[1] . BX471 is also able to displace		

^{125}I -MIP-1 α /CCL3 binding to mouse CCR1 in a concentration-dependent manner with a K_i of 215 ± 46 nM. Increasing concentrations of BX471 inhibits the Ca^{2+} transients induced by MIP-1 α /CCL3 in both human and mouse CCR1 with IC_{50} of 5.8 ± 1 nM and 198 ± 7 nM, respectively^[2]. BX471 (0.1-10 μM) shows a dose-dependent inhibition of RANTES-mediated and shear-resistant adhesion on IL-1 β -activated microvascular endothelium in shear flow in isolated blood monocytes. BX471 also inhibits the RANTES-mediated adhesion of T lymphocytes to activated endothelium^[4].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

In Vivo

BX471 (4 mg/kg, p.o. or i.v.) is orally active with a bioavailability of 60% in dogs. Furthermore, BX471 effectively reduces disease in a rat experimental allergic encephalomyelitis model of multiple sclerosis^[1]. BX471 (20 mg/kg, s.c.) reaches peak plasma levels of 9 μM by around 30 minutes, and this rapidly declines to approximately 0.4 μM after 2 hours. From 4 to 8 hours the drug plasma levels drops to 0.1 μM or lower. Mice treated with 20 mg/kg of BX471 for 10 days shows a reduction of interstitial CD45 positive leukocytes of approximately 55%. BX471 has a borderline significant effect on the number of CCR5-positive CD8 cells in the peripheral blood. BX471 reduces the amount of FSP1-positive cells by 65% in UUO kidneys as compared with vehicle control^[2]. Pretreatment with BX471 reduces macrophage and neutrophil accumulation in kidney after ischemia-reperfusion injury^[3].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Cell Assay^[4]

Briefly, dermal microvascular endothelial cells grown to confluence in Petri dishes are stimulated with IL-1 β (10 ng/mL) for 12 h followed by pre-incubation with RANTES (10 nM) for 30 min at 37°C just prior to assay. The plates are assembled as the lower wall in a parallel wall flow chamber and mounted on the stage of an Olympus IMT-2 inverted microscope with $\times 20$ and $\times 40$ phase-contrast objectives. Isolated human blood monocytes are isolated and resuspended at 5×10^5 cells/mL in assay buffer (HBSS) containing 10 mM HEPES, pH 7.4 and 0.5% human serum albumin. Shortly before the assay, 1 mM Mg^{2+} and 1 mM Ca^{2+} are added. The cell suspensions are kept in a heating block at 37°C during the assay and perfused into the flow chamber at a rate of 1.5 dyn/cm^2 for 5 min. For inhibition experiments, monocytes are preincubated with BX471 at different concentrations (0.1-10 μM) or a Me_2SO control for 10 min at 37°C. The number of firmly adherent cells after 5 min is quantitated in multiple fields (at least five per experiment) by analysis of images recorded with a long integration JVC 3CCD video camera and a JVC SR L 900 E video recorder and are expressed as cells/ mm^2 . The type of adhesion analyzed is restricted to primary, i.e. direct interactions of monocytes with endothelium.

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Animal Administration^[1]

Fasted male beagle dogs (n=3 per treatment group) are given BX471 either by oral gavage or by intravenous injection via the cephalic vein at a dose of 4 mg/kg. The compound is dissolved in a vehicle of 40% aqueous cyclodextrin. Serial blood samples are collected utilizing an in-dwelling catheter in the jugular vein at the indicated time points up to 6 h post-dosing. EDTA is used as an anticoagulant. The samples are centrifuged (1000 \times g for 10 min at 4°C), and plasma is stored frozen until analyzed for drug levels by HPLC-MS (electrospray mode operated under a positive ion mode). Plasma samples are thawed and denatured by the addition of four parts of ice-cold methanol containing a fixed amount of an internal standard to one part of plasma. The resulting protein precipitate is removed by centrifugation at 5000 \times g, and the supernatants are analyzed directly. Concurrently plasma calibration standards of BX471 are prepared over the range of quantification, processed, and analyzed under identical conditions. A FISIONS, VG Platform single quadrupole instrument is used in these analyses with an electrospray inlet operated at 3.57 kV. Chromatographic separation is accomplished using a YMC AQ octadecyl silane reversed phase column (4.6 \times 250 mm) following a short isocratic elution method (35% methanol, 65% water containing 0.1% trifluoroacetic acid). The total column flow (1 mL/min) is split post-column to infuse 50 $\mu\text{L}/\text{min}$ into the mass spectrometer. The chromatograms are collected over a total run time of 7.5 min/sample following a 50- μL injection on the column. The ions are collected in a single ion positive ionization mode. A calibration curve for quantification is generated by plotting ion current ratios between the internal standard peak and the analyte in the plasma standards over the quantification range. Calculations of percent oral availability is deduced from the area under curve measurements. Pharmacokinetic parameters are calculated using WinNonLin version 3.0.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Cancer Cell. 2021 Mar 8;39(3):423-437.e7.
- Cell Res. 2018 Mar;28(3):323-335.
- Signal Transduct Target Ther. 2021 Feb 28;6(1):91.
- Sci Adv. 2021 May 26;7(22):eabb5943.
- J Exp Clin Cancer Res. 2022 Mar 3;41(1):81.

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REFERENCES

- [1]. Liang M, et al. Identification and characterization of a potent, selective, and orally active antagonist of the CC chemokine receptor-1. J Biol Chem. 2000 Jun 23;275(25):19000-8.
 - [2]. Anders HJ, et al. A chemokine receptor CCR-1 antagonist reduces renal fibrosis after unilateral ureter ligation. J Clin Invest. 2002 Jan;109(2):251-9.
 - [3]. Furuichi K, et al. Chemokine receptor CCR1 regulates inflammatory cell infiltration after renal ischemia-reperfusion injury. J Immunol. 2008 Dec 15;181(12):8670-6.
 - [4]. Horuk R, et al. A non-peptide functional antagonist of the CCR1 chemokine receptor is effective in rat heart transplant rejection. J Biol Chem. 2001 Feb 9;276(6):4199-204.
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Caution: Product has not been fully validated for medical applications. For research use only.

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