Product Data Sheet

Product Name: BX471 hydrochloride
Cat. No.: GC35570

Chemical Properties

Cas. No.	288262-96-4		
SMILES	O=C(N)NC1=CC(Cl)=CC=C1OCC(N2[C@H](C)CN(CC3=CC=C(F)C=C3)CC2)=O.[H]Cl		
Formula	$C_{21}H_{25}Cl_2FN_4O_3$	M.Wt	471.35
Solubility	DMSO: 150 mg/mL (318.23 mM)	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 ℃ and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20℃ for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		
Structure	BX471 hydrochloride		

Protocol

Caution: Product has not been fully validated for medical applications. For research use only.
Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com
Address: 10292 Central Ave. #205, Montclair, CA, USA

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Cell experiment:

Briefly, dermal microvascular endothelial cells grown to confluence in Petri dishes are stimulated with IL-1β (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 ×20 and ×40 phase-contrast objectives. Isolated human blood monocytes are isolated and resuspended at 5×105?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 Mg2+?and 1 mM?Ca2+?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/cm2?for 5 min. For inhibition experiments, monocytes are preincubated with BX471 at different concentrations (0.1-10 μM) or a Me2SO 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/mm2. The type of adhesion analyzed is restricted to primary,?i.e.?direct interactions of monocytes with endothelium.

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Animal experiment:

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× 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× 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 FISONS, 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 (4.6×250 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 µL/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.

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[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.

Product Data Sheet

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.

Background

The CC chemokine receptor-1 (CCR1), whose ligands include macrophage inflammatory protein-1α (MIP-1α), RANTES, and monocyte chemotactic protein-3 (MCP-3), has a central role in leukocyte trafficking and is highly expressed in certain autoimmune diseases. BX 471 is a nonpeptide CCR1 antagonist that has been shown to displace MIP-1α, RANTES, and MCP-3 with K_i values of 1, 2.8, and 5.5 nM, respectively.[1] It demonstrates >10,000-fold selectivity for CCR1 over 28 additional G protein-coupled receptors, including related chemokine receptors.[1] In a rat experimental allergic encephalomyelitis model of multiple sclerosis, BX 471 at 50 mg/kg was shown to significantly reduce the severity of the disease.[1] BX 471 also decreases the inflammatory response during sepsis, blocks migration of monocytes isolated from rheumatoid arthritis patients, and prevents macrophage and T-cell recruitment in a mouse model of lupus nephritis.[2,3,4]

1.Liang, M., Mallari, C., Rosser, M., et al.Identification and characterization of a potent, selective, and orally active antagonist of the CC chemokine receptor-1J. Biol. Chem.275(25)19000-19008(2000) 2.He, M., Horuk, R., Moochhala, S., et al.Treatment with BX471, a CC chemokine receptor 1 antagonist, attenuates systemic inflammatory response during sepsisAm. J. Physiol. Gastrointest. Liver Physiol.292(4)G1173-G1180(2007) 3.Lebre, M.C., Vergunst, C.E., Choi, I.Y.K., et al.Why CCR2 and CCR5 blockade failed and why CCR1 blockade might still be effective in the treatment of

rheumatoid arthritisPLoS One6(7)(2011) 4.Anders, H.J., Belemezova, E., Eis, V., et al.Late onset of treatment with a chemokine receptor CCR1 antagonist prevents progression of lupus nephritis in MRL-Fas(lpr) miceJ. Am. Soc. Nephrol.15(6)1504-1513(2004)