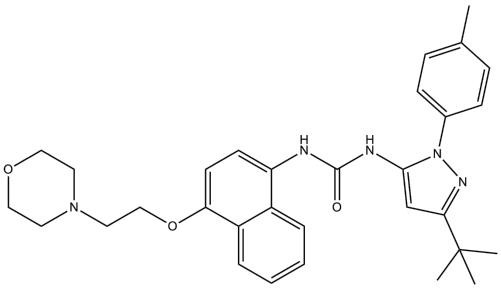
Product Data Sheet

Product Name: BIRB 796 (Doramapimod)
Cat. No.: GC11497

Chemical Properties

Cas. No.	285983-48-4		
Chemical Name	1-[5-tert-butyl-2-(4-methylphenyl)pyrazol-3-yl]-3-[4-(2-morpholin-4-ylethoxy)naphthalen-1-yl]urea		
SMILES	CC1=CC=C(C=C1)N2C(=CC(=N2)C(C)(C)C)NC(=O)NC3=CC=C(C4=CC=CC=C43)OCCN5CCOCC5		
Formula	$C_{31}H_{37}N_5O_3$	M.Wt	527.66
Solubility	≥ 26.4mg/mL in DMSO, ≥ 11.24 mg/mL in EtOH with ultrasonic	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20°C 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



Protocol

Cell experiment [1]:	
Cell lines	Human polymorphonuclear leukocytes (PMNs), MH-S cells (mouse alveolar macrophage cell line), MLE-12, MLE-15 cells (mouse lung epithelial cell line)

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Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com
Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

Preparation Method	Cells were seeded and treated with 10μM BIRB 796 or vehicle (0.01% DMSO) in the presence of 100ng/mL LPS (*E. coli* O111:B4) for PMNs and MH-S/MLE-15 cells, or 10μg/mL *Staphylococcus aureus* LTA for MLE-12 cells; cells were lysed at 0-240min for p38 MAPK phosphorylation Western blot, or supernatants harvested at 4 and 7h for cytokine ELISA (TNFα, KC).
Reaction Conditions	10μM; 0-240min (phosphorylation), 4-7h (cytokine release)
Applications	BIRB 796 reduced LPS-induced p38 MAPK phosphorylation in PMNs, MH-S, MLE-12 and MLE-15 cells. BIRB 796 reduced TNFα release from MH-S cells at 4 and 7h of LPS stimulation. BIRB 796 reduced KC release from MLE-12 cells under LTA stimulation. BIRB 796 showed a trend toward reduced KC release from MLE-15 cells under LPS stimulation.
Animal experiment [2]:	
Animal models	Female Balb/c mice (6-8 week old)
Preparation Method	Mice were sensitized on day 0 by a subcutaneous injection of 100μg KLH emulsified in IFA and CFA (1:1:1). On day 5, mice were challenged with an intradermal injection of 10μg KLH into the left ear. BIRB 796 in 0.4% methylcellulose was administered by intraperitoneal injection twice daily starting 24h prior to challenge. Ear thickness was measured using dial gauge calipers at various time points post-challenge. Ears were harvested at 24h post-challenge for molecular and cellular analyses, including kinase activation measurement, cytokine ELISA, chemokine mRNA/protein quantification, and flow cytometry for leukocyte infiltration.
Dosage form	50mg/kg; i.p.; twice daily from 24h before challenge to 24-48h post-challenge

Product Data Sheet

Applications	BIRB 796 reduced ear swelling. BIRB 796 inhibited p38 MAPK phosphorylation but not JNK or ERK phosphorylation in ear tissue. BIRB 796 decreased IL-1β and IL-10 protein levels in ear lysates. BIRB 796 reduced TNFα, IL-1β and IL-10 production in ear whole organ cultures. BIRB 796 decreased CXCL1, CXCL2, CCL2, CCL3 and CCL4 mRNA and protein levels in ear tissue. BIRB 796 reduced total $CD45^+$ leukocyte infiltration, with neutrophil infiltration decreased by approximately 60% and macrophage and $CD4^+$ T cell infiltration modestly reduced.

References:[1] Engstrom L, Pinzon-Ortiz MC, Li Y, et al. Characterization of a murine keyhole limpet hemocyanin (KLH)-delayed-type hypersensitivity (DTH) model: Role for p38 kinase. Int Immunopharmacol. 2009 Oct;9(11):1218-27.[2] Wei X, Zhang J, Niu J,et al. Evaluation of arecoline hydrobromide toxicity after a 14-day repeated oral administration in Wistar rats. PLoS One. 2015 Apr 16;10(4):e0120165.

Background

EC50: 18 nM for TNF-α in THP-1 cells

The signal transduction pathway leading to the production of TNF-α from stimulated inflammatory cells, while not fully understood, has been shown to be partially regulated by p38 mitogen activated protein (MAP) kinase. The p38 MAP kinase plays a crucial role in regulating the production of proinflammatory cytokines. Blocking this kinase may offer an effective therapy for the treatment of many inflammatory diseases. BIRB 796 is a highly potent inhibitor of p38 MAPK.

In vitro: BIRB 796 is a picomolar inhibitor of human p38 MAP kinase with a 12,000-fold increase in binding affinity. Moreover, BIRB 796 behavors as one of the most potent and slowest dissociating human p38 MAP kinase inhibitors now known [1].

In vivo: In a LPS-stimulated TNF-α synthesis mouse model, a 65% inhibition of TNF-α synthesis was observed when BIRB 796 was dosed orally at 10 mg/kg. In a model of established collagen-induced arthritis using B10.RIII mice, BIRB 796 showed a 63% inhibition of arthritis severity when dosed orally at 30 mg/kg qd [2].

Clinical trial: No clinical efficacy (Crohn's Disease Endoscopic Index of Severity) was seen for BIRB

Product Data Sheet

796 in comparison with placebo. A significant and dose-dependent decrease of C-reactive protein level was seen transiently after BIRB 796 after 1 week with a return to baseline level over time [3].

References:

[1] Pargellis C, Tong L, Churchill L, Cirillo PF, Gilmore T, Graham AG, Grob PM, Hickey ER, Moss N, Pav S, Regan J. Inhibition of p38 MAP kinase by utilizing a novel allosteric binding site. Nat Struct Biol. 2002 Apr;9(4):268-72.
[2] Regan J, Breitfelder S, Cirillo P, Gilmore T, Graham AG, Hickey E, Klaus B, Madwed J, Moriak M, Moss N, Pargellis C, Pav S, Proto A, Swinamer A, Tong L, Torcellini C. Pyrazole urea-based inhibitors of p38 MAP kinase: from lead compound to clinical candidate. J Med Chem. 2002 Jul 4;45(14):2994-3008.
[3] Schreiber S, Feagan B, D'Haens G, Colombel JF, Geboes K, Yurcov M, Isakov V, Golovenko O, Bernstein CN, Ludwig D, Winter T, Meier U, Yong C, Steffgen J; BIRB 796 Study Group. Oral p38 mitogen-activated protein kinase inhibition with BIRB 796 for active Crohn's disease: a randomized, double-blind, placebo-controlled trial. Clin Gastroenterol Hepatol. 2006 Mar;4(3):325-34.

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