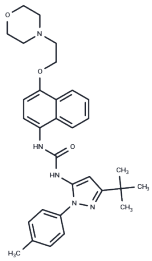
Data Sheet (Cat.No.T6277)

TargetMol

Doramapimod

Chemical Properties



CAS No. : 285983-48-4

Formula: C31H37N5O3

Molecular Weight: 527.66

Appearance: no data available

Storage: Powder: -20°C for 3 years | In solvent: -80°C for 1 year

Biological Description

Description	Doramapimod (BIRB 796) is a highly potent inhibitor of p38 MAPK (Kd: 0.1 nM), but weakly inhibits c-RAF, Fyn, Lck, ERK-1, SYK, IKK2, and ZAP-70.
Targets(IC50)	Raf,Autophagy,p38 MAPK
In vitro	Doramapimod (BIRB796) is a highly potent inhibitor of p38 MAPK (Kd: 0.1 nM) that blocks TNFα release in LPS-stimulated THP-1 cells (IC50: 18 nM) [1]. BIRB796 also inhibits the activity and the activation of SAPK3/p38gamma. BIRB796 blocks the stress-induced phosphorylation of the scaffold protein SAP97 [2]. BIRB 796 inhibited Hsp27 phosphorylation induced by 17-AAG plus bortezomib, thereby enhancing cytotoxicity. In bone marrow stromal cells (BMSC), BIRB 796 inhibited phosphorylation of p38 MAPK and secretion of IL-6 and vascular endothelial growth factor triggered by either tumour necrosis factor-alpha or tumour growth factor-beta1. BIRB 796 also inhibited IL-6 secretion induced in BMSCs by adherence to MM cells, thereby inhibiting tumour cell proliferation [3].
In vivo	Systolic blood pressure of untreated dTGRs was 204 mm Hg, but partially reduced after Doramapimod (30 mg/kg per day) treatment (166 mm Hg), whereas Sprague-Dawley rats were normotensive. The beta-myosin heavy chain expression of Doramapimod-treated hearts was significantly lower in Doramapimod compared with dTGRs. Doramapimod treatment significantly reduced cardiac fibrosis, connective tissue growth factor, tumor necrosis factor-alpha, interleukin-6, and macrophage infiltration [4].
Kinase Assay	Binding studies are conducted in a buffer containing 20 mM Bis-Tris Propane, pH 7.0, 2 mM EDTA, 0.01% (w/v) NaN3 and 0.15% (w/v) n-octylglucoside. Kinetic data for the association of SK&F 86002 to p38 MAP kinase is collected on a Kintech fluorescence detector system equipped with a stopped-flow controller. The data are fit simultaneously to an appropriate equation describing kinetic binding for a simple one-step binding mechanism. The data for the binding of the fluorescent analog of BIRB 796 is corrected for background fluorescence of unbound ligand. The exchange curve assays are run as two half-reactions using an SLM Aminco Bowman Series 2 Model SQ-340 fluorescence detector. In the first half-reaction, p38 MAP kinase and SK&F 86002 are preincubated for 3 min. In the second half-reaction, p38 MAP kinase is preincubated with Doramapimod for 60 min. A net dissociation of the fluoroprobe is observed for the first half-reaction, and a net association is observed for the second half-reaction. The raw data from both half reactions are fit simultaneously to an equation describing simple competitive inhibition. p38 is preactivated by treatment with constitutively active

	recombinant MKK6 (prepared by mutagenizing the two activation residues, Ser189 and Thr193, to Glu residues). Activated p38 is purified and used as a source of enzyme in a standard kinase activity assay monitoring the incorporation of radioactive phosphate into recombinant human MAPKAP k2. Cellular assays follow published procedures. Briefly, human THP.1 cells are stimulated with 1 μg/mL LPS, in the presence or absence of compound, followed by the determination of released TNF using a commercial ELISA kit [1].
Cell Research	Human embryonic kidney (HEK) 293 and HeLa cells were cultured in Dulbecco's modified Eagle's medium at 37 °C, supplemented with 10% fetal calf serum, 50 units of penicillin/ml, 50 μg/ml streptomycin, and 2 mM glutamine. Mouse embryonic fibroblasts were cultured as described previously, and C2C12 myoblasts were cultured. Cells were exposed to 0.5 M sorbitol for 30 min or 100 ng/ml EGF for 10 min and then lysed in buffer A (50 mM Tris-HCl, pH 7.5, 1 mM EGTA, 1 mM EDTA, 1 mM sodium orthovanadate, 10 mM sodium fluoride, 50 mM sodium β-glycerophosphate, 5 mM pyrophosphate, 0.27 M sucrose, 0.1 mM phenylmethylsulfonyl fluoride, 1% (v/v) Triton X-100) plus 0.1% (v/v) 2-mercaptoethanol and Complete proteinase inhibitor mixture from Roche Applied Science. Lysates were centrifuged at 18,000 × g for 5 min at 4 °C, and the supernatants were removed, quick-frozen in liquid nitrogen, and stored at -20 °C until use. When required, cells were preincubated for 1 h without or with 10 μM SB 203580 or 10 μM PD 184352 or with different concentrations of BIRB796 for the times indicated in the figures [2].
Animal Research	We studied male transgenic dTGRs and age-matched nontransgenic Sprague–Dawley (SD) rats (MDC). Local authorities approved the studies, and American Physiological Society guidelines for animal care were followed. We performed 2 different protocols. In protocol 2, untreated dTGR (n=15), dTGR+BIRB796 (30 mg/kg per day in the diet for 3 weeks; n=11), and SD (n=8 each group) rats were analyzed. Systolic blood pressure was measured weekly by tail cuff. Twenty-four- hour urine samples were collected in metabolic cages from weeks 5 to 7. Serum was collected at week 7. Serum creatinine and cystatin C were measured by clinical routine assays. Urinary rat albumin was determined by enzyme-linked immunosorbent assay. The aim of protocol 2 was to focus on electrophysiological alterations and mortality. Untreated dTGR (n=10), dTGR+BIRB796 (n=10), and SD (n=10) rats were studied up to week 8. Cardiac magnetic field mapping (CMFM) was performed at week 7 under isoflurane anesthesia. Echocardiography was performed as described earlier [4].

Solubility Information

Solubility	Ethanol: 26.4 mg/mL (50.03 mM),Sonication is recommended. DMSO: 20 mg/mL (37.9 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	1.8952 mL	9.4758 mL	18.9516 mL
5 mM	0.379 mL	1.8952 mL	3.7903 mL
10 mM	0.1895 mL	0.9476 mL	1.8952 mL
50 mM	0.0379 mL	0.1895 mL	0.379 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Reference

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Yuan F, Liu B, Xu Y, et al. TIPE3 is a regulator of cell apoptosis in glioblastoma. Cancer Letters. 2019, 446: 1-14
Zhao L, Wang Y, Xu Y, et al. BIRB796, an Inhibitor of p38 Mitogen-Activated Protein Kinase, Inhibits Proliferation and Invasion in Glioblastoma Cells. ACS Omega. 2021 Apr 22;6(17):11466-11473. doi: 10.1021/acsomega.1c00521.
Kuma Y, et al. BIRB796 inhibits all p38 MAPK isoforms in vitro and in vivo. J Biol Chem, 2005, 280(20), 19472-19479.
Yasui H, et al. BIRB 796 enhances cytotoxicity triggered by bortezomib, heat shock protein (Hsp) 90 inhibitor, and dexamethasone via inhibition of p38 mitogen-activated protein kinase/Hsp27 pathway in multiple myeloma cell lines and inhibits paracrine tumour growth. Br J Haematol. 2007 Feb;136(3):414-23.
Park JK, et al. p38 mitogen-activated protein kinase inhibition ameliorates angiotensin II-induced target organ damage. Hypertension. 2007 Mar;49(3):481-9.
Zhao L, Wang Y, Xu Y, et al. BIRB796, an Inhibitor of p38 Mitogen-Activated Protein Kinase, Inhibits Proliferation and Invasion in Glioblastoma Cells[J]. ACS Omega. 2021
Yuan F, Liu B, Xu Y, et al. TIPE3 is a regulator of cell apoptosis in glioblastoma[J]. Cancer letters. 2019 Apr 1;446:1-14.