Data Sheet (Cat.No.T1830)

TargetMol

BX795

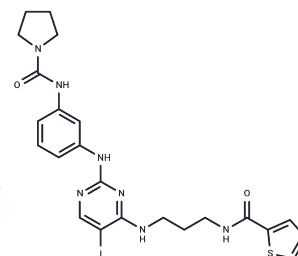
Chemical Properties

CAS No. : 702675-74-9

Formula: C23H26IN7O2S

Molecular Weight: 591.47

Storage: Powder: -20°C for 3 years | In solvent: -80°C for 1 year
Actual storage temperature shall be subject to the COA.



Biological Description

Description	BX795 is an effective and selective PDK1 inhibitor (IC50: 6 nM), and its selectivity is 140- and 1600-fold for PDK1 over PKA and PKC in cell-free assays, respectively. Meanwhile, the selectivity for PDK1 is 100-fold than GSK3β.
Targets(IC50)	CDK,PDK,Autophagy,Chk,c-Kit,IκB/IKK
In vivo	BX795 (1 μM) does not inhibit the following tyrosine protein kinases: hepatocyte receptor A2 and B3, Bruton's tyrosine kinase, Syk, and FGFR1. However, its inhibitory effect on the endothelial growth factor receptor is lower than on TBK1. BX795's ability to inhibit the phosphorylation of IRF3 at Ser396 (catalyzed by TBK1) decreases with increasing ATP concentration, indicating its role as an ATP-competitive inhibitor. Additionally, BX795 inhibits NUAK1, MARK1/2/4, VEGFR, and MLK1/2/3 with IC50s of 5, 55, 53, 19, 157, 50, 46, and 42 nM respectively. It also suppresses IRF3-dependent gene transcription and interferon β secretion in macrophages. BX795 blocks IRF3 activation regulated by IKKε and TBK1, reducing IFN-β production and does not activate p38α MAPK and JNK1/2 following TBK1/IKKε inhibition. It impedes IRF3 accumulation in the nucleus post-poly (I:C) treatment and does not affect LPS-induced phosphorylation at Thr229 on p70 ribosomal S6 kinase 1—a target of PDK1—nor does it affect NFκB-dependent gene transcription activation (stimulated by IKKα/β complex, LPS, poly (I:C), IL-1α, or TNFα). BX795 is effective in MEFs stimulated by IL-1α or TNFα, blocking the phosphorylation of p38α MAPK and JNK1/2.
Kinase Assay	Kinase assays: PDK1 is assayed in a direct kinase assay and a coupled assay format measuring PDK1- and PtdIns-3,4-P2-mediated activation of AKT2. For the coupled assay, the final assay mixture (60 μL) contained: 15 mM MOPS, pH 7.2, 1 mg/mL bovine serum albumin, 18 mM β-glycerol phosphate, 0.7 mM dithiothreitol, 3 mM EGTA, 10 mM MgOAc, 7.5 μM ATP, 0.2 μCi of [γ-33P]ATP, 7.5 μM biotinylated peptide substrate (biotin-ARRRDGGGAQPFRPRAATF), 0.5 μL of PtdIns-3,4-P2-containing phospholipid vesicles, 60 pg of purified recombinant human PDK1, and 172 ng of purified recombinant human AKT2. After incubation for 2 h at room temperature, the biotin-labeled peptide is captured from 10 μl of the assay mixture on streptavidin-coated SPA beads, and product formation is measured by scintillation proximity in a Wallac MicroBeta counter. The product formed is proportional to the time of incubation and to the amount of PDK1 and inactive AKT2 added. PDK1 is added at suboptimal levels so that the assay could sensitively detect inhibitors of AKT2 activation as well as direct inhibitors of PDK1 or AKT2. To measure PDK1 activity directly, the final assay mixture (60 μL) contained 50 mM

Kinase Assay	Tris-HCl, pH 7.5, 0.1 mM EGTA, 0.1 mM EDTA, 0.1% β-mercaptoethanol, 1 mg/mL bovine serum albumin, 10 mM MgOAc, 10 μM ATP, 0.2 μCi of [γ-33P]ATP, 7.5 μM substrate peptide (H2N-ARRRGVTTKTFCGT), and 60 ng of purified recombinant human PDK1. After 4 h at room temperature, we add 25 mM EDTA and spotted a portion of the reaction mixture on Whatman P81 phosphocellulose paper. The filter paper is washed three times with 0.75% phosphoric acid and once with acetone. After drying, the filter-bound labeled peptide is quantified using a Fuji phosphorimager.
Cell Research	Cells seeded at a low density (1,500-3,000 cells/well, 0.1 mL/well, 96-well plates) are incubated overnight. Compound treatments are made by adding 10 μL/well of the compound in 1% dimethyl sulfoxide and growth medium (final concentration of dimethyl sulfoxide, 0.1%), followed by brief shaking. Treated cells are incubated for 72 hours, and viability is measured by the addition of 10 μL of the metabolic dye WST-1. The WST-1 signal is read in a plate reader at 450 nm, and a no cell, or zero time cell, background is subtracted to calculate the net signal. Results are reported as the average ± S.E. of two or more replicates.(Only for Reference)

Solubility Information

Solubility	DMSO: 250 mg/mL (422.68 mM),Sonication is recommended. Ethanol: 59.2 mg/mL (100.09 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 5 mg/mL (8.45 mM),Sonication is recommended. *Please add the solvents sequentially, clarifying the solution as much as possible before adding the next one. Dissolve by heating and/or sonication if necessary. Working solution is recommended to be prepared and used immediately. The formulation provided above is for reference purposes only. In vivo formulations may vary and should be modified based on specific experimental conditions.*

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	1.6907 mL	8.4535 mL	16.907 mL
5 mM	0.3381 mL	1.6907 mL	3.3814 mL
10 mM	0.1691 mL	0.8454 mL	1.6907 mL
50 mM	0.0338 mL	0.1691 mL	0.3381 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.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

Reference

Feldman RI, et al. J Biol Chem. 2005, 280(20), 19867-119874.
Clark K, et al. J Biol Chem. 2009, 284(21), 14136-14146.
Dangelmaier C, et al. Thromb Haemost. 2013,111(4).

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