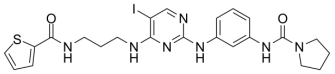
BX795

Cat. No.:	HY-10514
CAS No.:	702675-74-9
Molecular Formula:	$C_{23}H_{26}IN_7O_2S$
Molecular Weight:	591.47
Target:	PDK-1; IKK; Autophagy
Pathway:	PI3K/Akt/mTOR; NF-κB; Autophagy
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 6 months -20°C 1 month



SOLVENT & SOLUBILITY

In Vitro

DMSO : 33.33 mg/mL (56.35 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.6907 mL	8.4535 mL	16.9070 mL
	5 mM	0.3381 mL	1.6907 mL	3.3814 mL
	10 mM	0.1691 mL	0.8454 mL	1.6907 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: ≥ 2.5 mg/mL (4.23 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (4.23 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

BX795 is a potent and selective inhibitor of PDK1, with an IC_{50} of 6 nM. BX795 is also a potent and relatively specific inhibitor of TBK1 and IKKε, with an IC_{50} of 6 and 41 nM, respectively. BX795 blocks phosphorylation of S6K1, Akt, PKCδ, and GSK3β, and has lower selectivity over PKA, PKC, c-Kit, GSK3β etc. BX795 modulates autophagy[1][2][3][4].

IC_{50} & Target

PDK1	TBK1	IKKε
6 nM (IC_{50})	6 nM (IC_{50})	41 nM (IC_{50})

In Vitro

BX795 effectively blocks PDK1 activity in PC-3 cells, as shown by their ability to block phosphorylation of S6K1, Akt, PKCδ, and GSK3β. BX795 potently inhibits tumor cell growth on plastic with IC_{50} of 1.6, 1.4, and 1.9 μM for MDA-468, HCT-116, and MiaPaca cells, respectively.

In soft agar, BX795 displays higher growth inhibition with IC_{50} of 0.72, and 0.25 μM for MDA-468, and PC-3 cells, respectively [1].

In addition, BX795, as an inhibitor of the TBK1/IKKε, blocks TBK1- and IKKε-mediated activation of IRF3 and production of IFN-β[2]. In platelet physiological responses, BX795 produces inhibitory effect on 2-MeSADP-induced or collagen-induced aggregation, ATP secretion, and thromboxane generation[3].

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

PROTOCOL

Kinase Assay [1]

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-^{33}P]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 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-^{33}P]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.

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Cell Assay [1]

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.

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

CUSTOMER VALIDATION

- Nature. 2022 Oct;610(7933):761-767.
- Autophagy. 2017 Jan 2;13(1):133-148.
- Pharmacol Res. 2022 Nov 23;106577.
- Cell Rep. 2022 Sep 13;40(11):111342.
- Cell Chem Biol. 2022 Jun 9;S2451-9456(22)00201-X.

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REFERENCES

[1]. Feldman RI, et al. Novel small molecule inhibitors of 3-phosphoinositide-dependent kinase-1. J Biol Chem. 2005 May 20;280(20):19867-74.

[2]. Clark K, et al. Use of the pharmacological inhibitor BX795 to study the regulation and physiological roles of TBK1 and IkappaB kinase epsilon: a distinct upstream kinase mediates Ser-172 phosphorylation and activation. J Biol Chem. 2009 May 22;284(21):141

[3]. Dangelmaier C, et al. PDK1 selectively phosphorylates Thr(308) on Akt and contributes to human platelet functional responses. Thromb Haemost. 2014 Mar 3;111(3):508-17.

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