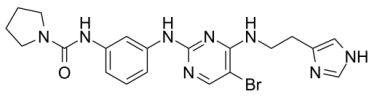
Data Sheet

Product Name:	BX-912
Cat. No.:	CS-0079
CAS No.:	702674-56-4
Molecular Formula:	$C_{20}H_{23}BrN_8O$
Molecular Weight:	471.35
Target:	Apoptosis; PDK-1
Pathway:	Apoptosis; PI3K/Akt/mTOR
Solubility:	DMSO : ≥ 100 mg/mL (212.16 mM)



BIOLOGICAL ACTIVITY:

BX-912 is a direct, selective, and ATP-competitive **PDK1** inhibitor (**IC_{50}**=26 nM). BX-912 blocks PDK1/Akt signaling in tumor cells and inhibits the anchorage-dependent growth of a variety of tumor cell lines in culture or induces **apoptosis**[1]. IC50 & Target: IC50: 26 nM (PDK1)[1] **In Vitro:** BX-912 promotes a block at the G2/M phase of the cell cycle in MDA-468 cells[1].

BX-912 binds to the ATP binding site of PDK1, and is 9-fold selective for PDK1 relative to PKA. BX-912 blocks PDK1 activity in PTEN-negative PC-3 cells. PTEN-negative PC-3 cells display constitutive activation of Akt which is reflected in high levels of the PDK1 product, phospho-Thr^{308}-Akt[1].

BX-912 is identified in a coupled assay measuring PDK1- and PtdIns-3,4-P_2-mediated Akt activation, which can detect inhibitors of PDK1, AKT2, or other steps critical for activation of AKT2[1].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay: [1]PDK1 is assayed in a direct kinase assay and a coupled assay format measuring PDK1 and PtdIns-3,4-P_2 mediated activation of AKT2. For the coupled assay, the final assay mixture (60 μL) contains: 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-P_2-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 can sensitively detect inhibitors of AKT2 activation as well as direct inhibitors of PDK1 or AKT2[1].

Cell Assay: BX-912 is dissolved in DMSO and then diluted with growth medium (final concentration of DMSO, 0.1%)[1].[1]**The cell lines MDA-468, MDA-453, HCT-116, U87-MG, U2OS, PC-3, B16F10, and MiaPaCa; LOX amelanotic human melanoma cells; and HeLa 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 **BX-912 (1, 10, 100 and 1000 nM)** in 1% DMSO and growth medium (final concentration of DMSO, 0.1%), followed by brief shaking. Treated cells are incubated for 72 h, 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[1].

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.

CAIndexNames:

1-Pyrrolidinecarboxamide, N-[3-[[5-bromo-4-[[2-(1H-imidazol-5-yl)ethyl]amino]-2-pyrimidinyl]amino]phenyl]-

SMILES:

BrC1=C(NCCC2=CNC=N2)N=C(NC3=CC(NC(N4CCCC4)=O)=CC=C3)N=C1

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