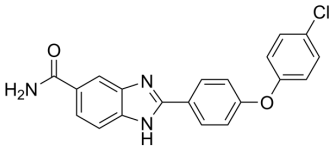
BML-277

Cat. No.:	HY-13946
CAS No.:	516480-79-8
Molecular Formula:	$C_{20}H_{14}ClN_3O_2$
Molecular Weight:	363.8
Target:	Checkpoint Kinase (Chk); Apoptosis
Pathway:	Cell Cycle/DNA Damage; Apoptosis
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 2 years -20°C 1 year



SOLVENT & SOLUBILITY

In Vitro

DMSO : 22.22 mg/mL (61.08 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.7488 mL	13.7438 mL	27.4876 mL
	5 mM	0.5498 mL	2.7488 mL	5.4975 mL
	10 mM	0.2749 mL	1.3744 mL	2.7488 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: ≥ 1.67 mg/mL (4.59 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 1.67 mg/mL (4.59 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 1.67 mg/mL (4.59 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

BML-277 is a selective checkpoint kinase 2 (Chk2) inhibitor with an IC_{50} of 15 nM.

IC_{50} & Target

Chk2
15 nM (IC_{50})

In Vitro

BML-277 is an ATP-competitive inhibitor of Chk2 that dose dependently protects human $CD4^+$ and $CD8^+$ T-cells from apoptosis due to ionizing radiation. BML-277 efficiently rescues both T-cell populations from radiation-induced apoptosis in

a dose-dependent manner with an observed EC_{50} of 3−7.6 μM. The concentration of BML-277 required for radioprotection is consistent with the biochemical measurement of chk2 inhibition. Providing theK_m of ATP for Chk2 is determined to be 99 μM and the K_i for BML-277 is 37 nM, and assuming that the intracellular ATP concentration is 10 mM, a 5 μM concentration of BML-277 would be expected to produce 42% inhibition of intracellular chk2[1].

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

PROTOCOL

Kinase Assay [1]

Activity of inhibitors of chk2 is determined by incubating inhibitory compounds with recombinant full-length chk2: 5 nM recombinant human Chk2, 50 mM HEPES (pH 7.4), 100 mM NaCl, 10 mM $MgCl_2$, 25 μM synthetic peptide substrate (biotin-SGLYRSPSMPENLNRPR, 1 μM ATP, 50 μCi/mL [γ-^{33}P] ATP, and a protease inhibitor mixture. The reaction mixtures are incubated at 37°C for 3 h, and the peptide substrate is captured on streptavidin conjugated to agarose beads. The agarose beads are washed repeatedly with a 0.1% solution of Tween-20 in phosphate-buffered saline, pH 7.4. Enzyme activity at different BML-277 concentrations (6.25, 12.5, 25, 50, 100, and 200 nM) is determined by measuring the amount of radioactive phosphate bound to the substrate peptide by scintillation counting. In kinetic experiments ATP concentration is varied while the ratio between unlabeled and [γ-^{33}P] labeled ATP is kept constant. Reactions are stopped at different time points by addition of 50 mM cold ATP and samples are kept on ice during further processing[1].

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Cell Assay [1]

To determine the radioprotective effect of Chk2 inhibitors, purified T-cells are incubated at 100 000 cells per well in BML-277 ($10^{2.5}$ nM, 1 μM, $10^{0.5}$ μM, 10 μM, and $10^{1.5}$ μM) or vehicle (DMSO) at varying concentrations in 96-well stripwells for 1 h. Cells are then exposed to a dose of 0 or 10 Gy gamma irradiation from a ^{137}Cs source at a dose rate of 3.65 Gy/min and then returned to the incubator for a further 24 h. Cells are stained with Annexin V-FITC and propidium iodide, according to the manufacturers protocol. Apoptotic and surviving cells are quantitated with a FACSCalibur FACS machine. Data are reported as percent recovery-or the number of survivors from treatment groups minus the number of cells surviving in the irradiated control group divided by the number of surviving cells in the untreated control groups[1].

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CUSTOMER VALIDATION

- Adv Mater. 2023 Mar;35(11):e2210017.
- Sci Adv. 2022 Jan 21;8(3):eabj8357.
- Cell Death Dis. 2020 Jun 15;11(6):464.
- Hum Reprod. 2023 Jul 14;dead145.
- Research Square Preprint. 2023 Jun 9.

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REFERENCES

[1]. Arienti KL, et al. Checkpoint kinase inhibitors: SAR and radioprotective properties of a series of 2-arylbenzimidazoles. J Med Chem. 2005 Mar 24;48(6):1873-85.

Caution: Product has not been fully validated for medical applications. For research use only.

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