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产品名称：BML-277
产品别名：Chk2 Inhibitor II

生物活性：

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].

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

*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。

储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

In Vivo:

请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 In Vitro 方式配制澄清的储备液，再依次添加助溶剂：

——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶

1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline

Solubility: ≥ 1.67 mg/mL (4.59 mM); Clear solution

此方案可获得 ≥ 1.67 mg/mL (4.59 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 16.699999 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀；向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。

2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline)

Solubility: ≥ 1.67 mg/mL (4.59 mM); Clear solution

此方案可获得 ≥ 1.67 mg/mL (4.59 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 16.699999 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的

	SBE-β-CD 生理盐水水溶液中，混合均匀。 3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 1.67 mg/mL (4.59 mM); Clear solution 此方案可获得 ≥ 1.67 mg/mL (4.59 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的实验。 以 1 mL 工作液为例，取 100 μL 16.699999 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
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.

实验参考：

Cell Assay	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].
Kinase Assay	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].
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.