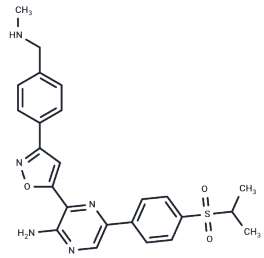
Data Sheet (Cat.No.T2669)

TargetMol

Berzosertib

Chemical Properties



CAS No. : 1232416-25-9

Formula: C24H25N5O3S

Molecular Weight: 463.55

Storage: Keep away from direct sunlight
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	Berzosertib (VE-822) is an ATR inhibitor (Ki<0.2 nM) that also inhibits ATM (Ki=34 nM). Berzosertib has antitumor activity and has been used in trials investigating the treatment of ovarian tumors, plasmacytoid tumors of the ovary, solid tumors in adults, advanced solid tumors, and advanced solid tumors.
Targets(IC50)	ATM/ATR
In vitro	**METHODS**: Human pancreatic cancer cells PSN-1 and MiaPaCa-2 were treated with Berzosertib (80 nmol/L) and gemcitabine (100 nM), XRT (6 Gy), and the expression levels of target proteins were detected by Western Blot. **RESULTS**: Berzosertib reduced phosphorylated Ser345-Chk1. Berzosertib did not inhibit ATM, Chk2 or DNA-PK phosphorylation in response to radiation, which further supports the selectivity of Berzosertib for ATR. [1] **METHODS**: Osteosarcoma cells MNNG/HOS and 143B were treated with Berzosertib (0-100 μM) for 48 h. Cell viability was measured by MTT assay. **RESULTS**: Berzosterib caused a dose-dependent decrease in MNNG/HOS and 143B cell viability. [2]
In vivo	**METHODS**: To assay antitumor activity in vivo, Nude mice bearing PSN-1 xenografts were treated with Berzosertib (60 mg/kg, once daily by gavage) and XRT (6 Gy, once) for six days. **RESULTS**: Berzosertib alone had no effect on tumor growth, but XRT plus Berzosertib administered for six or four days more than doubled the TV600 of XRT alone. [1]
Kinase Assay	A375 cells are pre-treated with 1 μM JNK-IN-8 for the indicated amounts of time. Remove the medium and wash 3 times with PBS. Resuspend the cell pellet with 1 mL Lysis Buffer (1% NP-40, 1% CHAPS, 25 mM Tris, 150 mM NaCl, Phosphatase Inhibitor Cocktail, and Protease Inhibitor Cocktail). Rotate end-to-end for 30 min at 4°C. Lysates are cleared by centrifugation at 14000 rpm for 15 min in the Eppendorf. The cleared lysates gel filtered into Kinase Buffer (0.1% NP-40, 20 mM HEPES, 150 mM NaCl, Phosphatase Inhibitor Cocktail, Protease Inhibitor Cocktail) using Bio-Rad 10DG colums. The total protein concentration of the gel-filtered lysate should be around 5-15 mg/mL. Cell lysate is labeled with the probe from ActivX at 5 μM for 1 hour. Samples are reduced with DTT, and cysteines are blocked with iodoacetamide and gel filtered to remove excess reagents and exchange the buffer. Add 1 volume of 2X Binding Buffer (2% Triton-100, 1% NP-40, 2 mM EDTA, 2X PBS) and 50 μL streptavidin bead slurry and rotate end-to-end

Kinase Assay	for 2 hours, centrifuge at 7000 rpm for 2 min. Wash 3 times with 1X Binding Buffer and 3 times with PBS. Add 30 μL 1X sample buffer to beads, heat samples at 95°C for 10 min. Run samples on an SDS-PAGE gel at 110V. After transferred, the membrane is immunoblotted with JNK antibody[1].
Cell Research	VE-822 is dissolved in DMSO and stored, and then diluted with appropriate media before use[1]. Gemcitabine (10 nM) is added 24 h pre-XRT and is replaced with fresh medium before addition of VE-822. PSN-1 cells are treated with VE-822 (80 nM) for 1 h before, through to 18 h after, XRT (6 Gy). Apoptosis is analyzed 48 h after XRT by flow cytometry using an Annexin V-FITC kit with PI[1].

Solubility Information

Solubility	DMSO: 28 mg/mL (60.4 mM),Sonication is recommended. Ethanol: < 1 mg/mL (insoluble or slightly soluble), (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 2 mg/mL (4.31 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	2.1573 mL	10.7863 mL	21.5726 mL
5 mM	0.4315 mL	2.1573 mL	4.3145 mL
10 mM	0.2157 mL	1.0786 mL	2.1573 mL
50 mM	0.0431 mL	0.2157 mL	0.4315 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

Fokas E, et al. Targeting ATR in vivo using the novel inhibitor VE-822 results in selective sensitization of pancreatic tumors to radiation. Cell Death Dis. 2012 Dec 6;3(12):e441.

Li X, et al. Inhibition of ATR-Chk1 signaling blocks DNA double-strand-break repair and induces cytoplasmic vacuolization in metastatic osteosarcoma. Ther Adv Med Oncol. 2020 Sep 14;12:1758835920956900.

Konstantinopoulos P A , Cheng S C , Hendrickson A E W , et al. Berzosertib plus gemcitabine versus gemcitabine alone in platinum-resistant high-grade serous ovarian cancer: a multicentre, open-label, randomised, phase 2 trial[J]. The Lancet Oncology, 2020, 21(7).