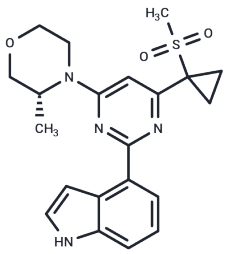
Data Sheet (Cat.No.T1958)

TargetMol

AZ20

Chemical Properties



CAS No. : 1233339-22-4

Formula: C21H24N4O3S

Molecular Weight: 412.51

Storage: 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	AZ20 is an effective and specific inhibitor of ATR kinase (IC50: 5 nM, in a cell-free assay), 8-fold selectivity over mTOR.
Targets(IC50)	ATM/ATR,mTOR
In vitro	AZ20 (10 μM) inhibits 50% of the metabolism of midazolam mediated by CYP3A4. In female nude mice with LoVo tumors, treatment with AZ20 (25 mg/kg, twice daily, orally; or 50 mg/kg/day, orally) for 13 days significantly suppresses tumor growth. This effect is associated with a sustained and extensive increase in γH2AX nuclear staining in xenografted tissues.
In vivo	In vitro, the concentration of AZ20 correspondingly decreases the levels of pChk1 Ser345/317/296. Extending the treatment duration with AZ20 intensifies γH2AX nuclear staining due to replication stress, associated with S phase arrest and an increase in histone H3 phosphorylation levels. The ability of AZ20 to inhibit cellular growth and induce cell death is entirely distinct from other cytotoxic drugs. The cytotoxic effects are enhanced when AZ20 is used in combination with the ATM inhibitor KU-60019.
Kinase Assay	ATR for use in the in vitro enzyme assay is obtained from HeLa nuclear extract by immunoprecipitation with rabbit polyclonal antiserum raised to amino acids 400-480 of ATR contained in the following buffer: 25 mM HEPES (pH 7.4), 2 mM MgCl2, 250 mM NaCl, 0.5 mM EDTA, 0.1 mM Na3VO4, 10% v/v glycerol, and 0.01% v/v Tween 20. ATR-antibody complexes are isolated from nuclear extract by incubating with protein A-Sepharose beads for 1 h and then through centrifugation to recover the beads. In the well of a 96-well plate, 10 μL ATR-containing Sepharose beads are incubated with 1 μg of substrate glutathione?S-transferase-p53N66 (NH2-terminal 66 amino acids of p53 fused to glutathione?S-transferase are expressed in?E. coli) in ATR assay buffer (50 mM HEPES (pH 7.4), 150 mM NaCl, 6 mM MgCl2, 4 mM MnCl2, 0.1 mM Na3VO4, 0.1 mM DTT, and 10% (v/v) glycerol) at 37°C in the presence or absence of inhibitor. After 10 min with gentle shaking, ATP is added to a final concentration of 3 μM and the reaction continued at 37°C for an additional 1 h. The reaction is stopped by addition of 100 μL of PBS, and the reaction is transferred to a white opaque glutathione coated 96-well plate and incubated overnight at 4°C. This plate is then washed with PBS/0.05% (v/v) Tween 20, blotted dry, and analyzed by a standard ELISA technique with a phosphoserine 15 p53 antibody. The detection of phosphorylated glutathione?S-transferase-p53N66 substrate is performed in combination with a goat anti-mouse horseradish peroxidase-

Kinase Assay	conjugated secondary antibody. Enhanced chemiluminescence solution is used to produce a signal, and chemiluminescent detection is carried out via a TopCount plate reader. The resulting calculated % enzyme activity is then used to determine the IC50? values for the compounds (IC50?taken as the concentration at which 50% of the enzyme activity is inhibited).
Cell Research	AZ20 is dissolved in 100% DMSO. Compound dose ranges are created by diluting in 100% DMSO and then further into assay medium (EMEM, 10% FCS, 1% glutamine) using a Labcyte Echo acoustic dispensing instrument. Cells are plated in 384-well Costar plates at 9×104?cells per mL in 40 µL of EMEM, 10% FCS, 1% glutamine and grown for 24 h. Following addition of compound the cells are incubated for 60 min. A final concentration of 3 µM?4NQO?(prepared in 100% DMSO) is then added using the Labcyte Echo, and the cells are incubated for a further 60 min. The cells are fixed by adding 40 µL of 3.7% v/v formaldehyde solution for 20 min. After removal of fix, cells are washed with PBS and permeabilized in 40 µL of PBS containing 0.1% Triton X-100. The cells are then washed, and 15 µL primary antibody solution (pChk1 Ser345) is added. The plates are incubated at 4°C overnight. The primary antibody is then washed off, and 20 µL of secondary antibody solution and 1 µM Hoechst 33258 added for 90 min at room temperature. The plates are washed and left in 40 µL of PBS. Plates are then read on an ArrayScan VTI instrument to determine staining intensities, and dose responses are obtained and used to determine the IC50?values for the compounds.

Solubility Information

Solubility	Ethanol: 3 mg/mL (7.27 mM),Sonication is recommended. H2O: < 1 mg/mL (insoluble or slightly soluble), DMSO: 50 mg/mL (121.21 mM),Sonication is recommended. (< 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 (4.85 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	2.4242 mL	12.1209 mL	24.2418 mL
5 mM	0.4848 mL	2.4242 mL	4.8484 mL
10 mM	0.2424 mL	1.2121 mL	2.4242 mL
50 mM	0.0485 mL	0.2424 mL	0.4848 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

Xavier Jacq, et al. Cancer Res 2012;72(8 Suppl):Abstract nr 1823.
Foote KM, et al. J Med Chem, 2013, 56(5), 2125-2138.

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