AZD5363 (Capivasertib)

Catalog No.C13128 Batch:C1312803

Product Datasheet

Technical Data

Formula	$C_{21}H_{25}ClN_6O_2$					
Molecular Weight	428.92	**CAS No.**	1143532-39-1			
Solubility (25°C)*	In vitro	DMSO	86 mg/mL (200.5 mM)			
		Ethanol	22 mg/mL (51.29 mM)			
		Water	Insoluble			
	In vivo (Add solvents to the product individually and in order)	Oral Administration	Homogeneous suspension	CMC-NA	≥5mg/ml	Taking the 1 mL working solution as an example, add 5 mg of this product to 1 ml of CMC-Na solution, mix evenly to obtain a homogeneous suspension with a final concentration of 5 mg/ml.
		Injection	Clear solution	5%DMSO $\xrightarrow{1}$ 40%PEG300 $\xrightarrow{2}$ 5%Tween80 $\xrightarrow{3}$ 50%ddH2O Validated by Capsid Bio labs. Should you need adjustments to this formulation, contact our sales team for custom testing.	4.300mg/ml (10.03mM)	Taking the 1 mL working solution as an example, add 50 μL 86 mg/ml clarified DMSO stock solution to 400 μL PEG300, mix evenly to clarify it; add 50 μL Tween80 to the above system, mix evenly to clarify it; then continue to add 500 μL ddH2O to adjust the volume to 1 mL. The mixed solution should be used immediately for optimal results.

* <1 mg/ml means slightly soluble or insoluble.
* Please note that Capsid Bio tests the solubility of all compounds in-house, and the actual solubility may differ slightly from published values. This is normal and is due to slight batch-to-batch variations.
* Room temperature shipping (Stability testing shows this product can be shipped at 2-8°C for storage purposes.)

Preparing Stock Solutions

Concentration \ Volume / Mass	1 mg	5 mg	10 mg
1 mM	2.3314 mL	11.6572 mL	23.3144 mL
5 mM	0.4663 mL	2.3314 mL	4.6629 mL
10 mM	0.2331 mL	1.1657 mL	2.3314 mL
50 mM	0.0466 mL	0.2331 mL	0.4663 mL

Biological Activity

Description	Capivasertib (AZD5363) potently inhibits all isoforms of **Akt**(Akt1/Akt2/Akt3) with **IC50** of 3 nM/8 nM/8 nM in cell-free assays, similar to P70S6K/PKA and lower activity towards ROCK1/2. Phase 2.
Targets	Akt1 [1] (Cell-free assay): 3 nM Akt2 [1] (Cell-free assay): 8 nM Akt3 [1] (Cell-free assay): 8 nM ROCK2 [1] (Cell-free assay): 56 nM
In vitro	Capivasertib (AZD5363) is a potent Akt inhibitor with IC50 of 3 nM, 8 nM and 8 nM for Akt1, Akt2 and Akt3, respectively. [1] It inhibits phosphorylation of AKT substrates in cells with a potency of approximately 0.3 to 0.8 μM. This compound inhibits the proliferation of 41 of 182 solid and hematologic tumor cell lines with a potency of < 3 μM. [2] Activating mutations in PIK3CA, loss or inactivation of tumor suppressor PTEN, or HER2 amplification all are significantly predictive of responsiveness to AZD5363. Additionally, correlation is also seen between the RAS mutation status of cell lines and resistance to it. [1]
In vivo	Oral dosing of Capivasertib (AZD5363) (100, 300 mg/kg) to nude mice causes dose- and time-dependent reduction of PRAS40, GSK3β, and S6 phosphorylation in BT474c xenografts, reversible increases in blood glucose concentrations, and dose-dependent decreases in 2[^{18}F]fluoro-2-deoxy-d-glucose (^{18}F-FDG) uptake in U87-MG xenografts. Chronic oral dosing of this compound (130, 200, and 300 mg/kg) causes dose-dependent growth inhibition of xenografts derived from various tumor types, including HER2+ breast cancer models.
Features	Moderate preclinical tolerability, and PD characteristics of an AKT inhibitor. Distinct profile from other AKT inhibitors in clinical development.

Protocol (from reference)

Kinase Assay:[1]

- **Caliper Off-Chip Incubation Mobility Shift assay**

  The ability of Capivasertib (AZD5363) and other compounds to inhibit the activity of AKT1, AKT2, and AKT3 is evaluated by the Caliper Off-Chip Incubation Mobility Shift assay. Active recombinant AKT1, AKT2, or AKT3 are incubated with a 5-FAM-labeled custom-synthesized peptide substrate together with increasing concentrations of this compound. Final reactions contained 1 to 3 nM AKT1, AKT2, or AKT3 enzymes; 1.5 mM peptide substrate; ATP at K_m for each AKT isoform; 10 mM $MgCl_2$, 4 mM DTT, 100 mM HEPES, and 0.015% Brij-35. The reactions are incubated at room temperature for 1 hour and stopped by the addition of buffer containing 100 mM HEPES, 0.015% Brij-35 solution, 0.1% coating reagent, 40 mM EDTA, and 5% DMSO. Plates are then analyzed using a Caliper LC3000, allowing for separation of peptide substrate and phosphorylated product by electrophoresis with subsequent detection and quantification of laser induced fluorescence.

Cell Assay:[2]

- **Cell lines**

  182 solid and hematologic tumor cell lines

- **Concentrations**

  ~30 μM

- **Incubation Time**
  72 hours
- **Method**
  Cell proliferation assay is determined by 2 methods, MTS and Sytox Green, using Capivasertib (AZD5363). Briefly, cells are seeded in 96-well plates and incubated overnight at 37 ℃, 5% CO_2. Cells are then exposed to concentrations of this compound ranging from 30 to 0.003μM for 72 hours. For the MTS endpoint, cell proliferation is measured by the CellTiter AQueous Non-Radioactive Cell Proliferation Assay reagent in accordance with the manufacturer's protocol. For the Sytox Green endpoint, Sytox Green nucleic acid dye diluted in TBS-EDTA buffer is added to cells (final concentration of 0.13 μM) and the number of dead cells detected using an Acumen Explorer. Cells are then permeabilized by the addition of saponin (0.03% final concentration, diluted in TBS-EDTA buffer), incubated overnight and a total cell count measured. Predose measurements are made for both MTS and Sytox Green endpoints, and concentration needed to reduce the growth of treated cells to half that of untreated cells values are determined using absorbance readings (MTS) or live cell counts.

Animal Study:[2]

- **Animal Models**
  Female nude mice and male SCID mice with BT474c, U87MG, KPL-4, HCC-1187 xenografts.
- **Dosages**
  130 mg/Kg - 300 mg/Kg
- **Administration**
  p.o.

References

http://www.ncbi.nlm.nih.gov/pubmed/?term=23394218 http://www.ncbi.nlm.nih.gov/pubmed/?term=22294718

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