产品名称：4-Piperidinecarboxamide, 4-amino-N-[(1S)-1-(4-chlorophenyl)-3-hydroxypropyl]-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-

产品别名：Capivasertib ；AZD5363

生物活性：

Description	Capivasertib (AZD5363) is a potent pan-AKT kinase inhibitor with IC50 of 3, 7 and 7 nM for Akt1,Akt2 and Akt3, respectively.

IC_{50} & Target

Akt1	Akt2	Akt3	P70S6K	PKA	ROCK2
3 nM (IC_{50})	7 nM (IC_{50})	7 nM (IC_{50})	6 nM (IC_{50})	7 nM (IC_{50})	60 nM (IC_{50})
ROCK1	Autophagy				
470 nM (IC_{50})					

In Vitro

Capivasertib, a novel pyrrolopyrimidine-derived compound, inhibits all AKT isoforms with a potency of 10 nM or less and inhibits phosphorylation of AKT substrates in cells with a potency of approximately 0.3 to 0.8 μM. Capivasertib inhibits phosphorylation of these substrates with an IC_{50} value of 0.06 to 0.76 μM in the 3 cell lines. Capivasertib effectively inhibits phosphorylation of S6 and 4E-BP1 in these cell lines, whereas it increases phosphorylation of AKT at both ser^{473} and thr^{308}. In BT474c cells, Capivasertib induces FOXO3a nuclear translocation with EC_{50}value of 0.69 μM; a concentration of 3 μM is sufficient to almost completely localize FOXO3a to the nucleus. AZD5363Capivasertibhibitor MK-2206 is much less active (IC_{50}>30 μM) [1].

In Vivo

Oral dosing of Capivasertib (AZD5363) to nude mice causes dose- and time-dependent reduction of PRAS40, GSK3β, and S6 phosphorylation in BT474c xenografts (PRAS40 phosphorylation EC_{50} ~0.1 μM total plasma exposure), 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 Capivasertib caused dose-dependent growth inhibition of xenografts derived from various tumor types, including $HER2^+$ breast cancer models. Capivasertib also significantly enhances the antitumor activity of RP-56976 and GW572016 in breast cancer xenografts[1].

Solvent&Solubility

In Vitro:

DMSO : ≥ 21.5 mg/mL (50.13 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	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

*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: ≥ 2.08 mg/mL (4.85 mM); Clear solution 此方案可获得 ≥ 2.08 mg/mL (4.85 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 20.8 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: ≥ 2.08 mg/mL (4.85 mM); Clear solution 此方案可获得 ≥ 2.08 mg/mL (4.85 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 20.8 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: ≥ 2.08 mg/mL (4.85 mM); Clear solution 此方案可获得 ≥ 2.08 mg/mL (4.85 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 20.8 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Davies BR, et al. Preclinical pharmacology of AZD5363, an inhibitor of AKT: pharmacodynamics, antitumor activity, and correlation of monotherapy activity with genetic background. Mol Cancer Ther. 2012 Apr;11(4):873-87.

实验参考：

Cell Assay	Cell proliferation assay is determined by 2 methods, MTS and Sytox Green. Briefly, cells are seeded in 96-well plates (at a density to allow for logarithmic growth during the 72-hour assay) and incubated overnight at 37°C, 5% CO_2. Cells are then exposed to concentrations of Capivasertib ranging from 30 to 0.003 μM for 72 hours. For the MTS endpoint, cell proliferation is measured by the CellTiter AQueous Non-Radioactive Cell Proliferation Assay reagent. Absorbance is measured with a Tecan Ultra instrument. 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 (GI_{50}) values are determined using absorbance readings (MTS) or live cell counts[1]
Animal Administration	Mice[1] Specific, pathogen-free, female nude mice (nu/nu: Alpk) and male SCID mice (SCID/CB17; 786-0 xenograft studies) are used. When mean tumor sizes reach approximately 0.2 cm3, the mice are randomized into control and treatment groups. The treatment groups received varying dose schedules of Capivasertib (AZD5363) solubilized in a 10% DMSO 25% w/v Kleptose HPB (Roquette) buffer by oral gavage, RP-56976 solubilized in 2.6% ethanol in injectable water by intravenous injection once on day 1 at 15 or 5 mg/kg once weekly. When administered in combination, RP-56976 is administered 1 hour before the oral dose of Capivasertib (AZD5363). The

	control group received the DMSO/Kleptose buffer alone, twice daily by oral gavage. Tumor volumes (measured by caliper), animal body weight, and tumor condition are recorded twice weekly for the duration of the study. Mice are sacrificed by CO2 euthanasia. The tumor volume is calculated (taking length to be the longest diameter across the tumor and width to be the corresponding perpendicular diameter) using the formula: (length×width)×√(length×width)×(π/6). Growth inhibition from the start of treatment is assessed by comparison of the differences in tumor volume between control and treated groups.
References	[1]. Davies BR, et al. Preclinical pharmacology of AZD5363, an inhibitor of AKT: pharmacodynamics, antitumor activity, and correlation of monotherapy activity with genetic background. Mol Cancer Ther. 2012 Apr;11(4):873-87.