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产品名称：
(S)-N-[[3-Amino-1-(5-ethyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-yl]methyl]-2,4-difluorobenza
产品别名：PF-AKT400

生物活性：

Description	PF-AKT400 is a broadly selective, potent, ATP-competitive Akt inhibitor, displays 900-fold greater selectivity for PKBα (IC50=0.5 nM) than PKA (IC50=450 nM).	
IC_{50} & Target	PKBα	PKA
	0.5 nM (IC_{50})	450 nM (IC_{50})
In Vitro	PF-AKT400 (Compound 42) provides significantly enhanced selectivity for Akt relative to earlier leads such as spiroindoline 2. Free IC50 and EC50 values are estimated for phospho-S6 reduction (110 nM) and Akt hyperphosphorylation (216 nM), respectively. These values corresponded well to the cellular IC50 for PF-AKT400 in U87 cells measuring p-GSK-3α (310 nM)[2].	
In Vivo	PF-AKT400 is subsequently evaluated for modulation of Akt in tumors and in multiple in vivo mouse models of antitumor efficacy. It is active in a PC3 prostate carcinoma xenograft experiment, with 75% TGI observed at 100 mg/kg b.i.d. dosing for 10 days. In a colorectal carcinoma (Colo205) xenograft study, PF-AKT400 produces 60% TGI at 150 mg/kg b.i.d. after 10 days. Most intriguingly, in combination with Rapamycin (10 mg/kg, ip), 75 mg/kg b.i.d. (10 days) of PF-AKT400 results in 98% TGI in an additional PC3 prostate carcinoma xenograft study compared to 56% TGI and 66% TGI with PF-AKT400 and Rapamycin as single agents. To define the in vivo potency of PF-AKT400 (Compound 42) in the PC3 xenograft model, oral administration of 25, 75, and 100 mg/kg PF-AKT400 is performed with blood and tumor sampling over time. Immunoblot analysis of detergent-soluble extracts derived from PC3 tumors shows a significant reduction of S6 phosphorylation, and hyperphosphorylation of Akt upon treatment at doses that produced significant tumor growth inhibition. Plasma drug concentrations peak rapidly after oral administration of doses between 25-100 mg/kg (T_{max}=0.5 h). Peak PD responses of phospho-S6 and phospho-Akt are observed at approximately 2-4h and 1h post-administration of PF-AKT400, respectively. The time-course of PD marker response is well described by a PK/PD model at doses that ranged from no efficacy (25 mg/kg) to maximal efficacy (100 mg/kg)[2].	

In Vitro:

DMSO : ≥ 100 mg/mL (249.73 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	**1 mg**	**5 mg**	**10 mg**
	1 mM	2.4973 mL	12.4866 mL	24.9732 mL
	5 mM	0.4995 mL	2.4973 mL	4.9946 mL
	10 mM	0.2497 mL	1.2487 mL	2.4973 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。

Solvent&Solubility	***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: ≥ 2.5 mg/mL (6.24 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.24 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 25.0 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: ≥ 2.5 mg/mL (6.24 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.24 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 25.0 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: ≥ 2.5 mg/mL (6.24 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.24 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 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Chen SF, et al. Binding selectivity studies of PKBα using molecular dynamics simulation and free energy calculations. J Mol Model. 2013 Nov;19(11):5097-5112. [2]. Freeman-Cook KD, et al. Design of selective, ATP-competitive inhibitors of Akt. J Med Chem. 2010 Jun 24;53(12):4615-4622.

实验参考：

Animal Administration	Mice[2] Studies to describe the PK/PD relationship for PF-AKT400 are performed in male SCID/Beige mice bearing subcutaneous PC3 prostate carcinoma xenografts. Once tumors reach about ~300mm^3 in size, PF-AKT400 is formulated in 0.5% methylcellulose vehicle and administered orally to 3 mice per dose group. Plasma and tumors are harvested over time, tumor lysates prepared, and the levels of phospho S6 reduction and phospho Akt induction are evaluated by immunoblot.
Kinase Assay	A fluorescence polarization IMAP type assay is used. An amount of 15 μL of diluted PF-AKT400 (Compound 42) in DMSO is mixed with 60 μL of reaction buffer (10 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 0.1 mM EGTA, 0.01% Triton-X100, 1 mM DTT). Then 5 μL of the compound/buffer mixture, 10 μL of a solution containing 4 μM ATP and 40 nM fluorescent-labeled Crosstide (Tamara-labeled GRPRTSSFAEG peptide), and 5 μL of Akt1 protein (lacking the pleckstrin homology (PH) domain, containing an Asp at position 473, and prephosphorylated at Thr 308) in reaction buffer are

	combined. After a 90 min incubation, IMAP beads are added and plates are read (lamp filter, 544 nm; emission filter, 615 nm). The same procedure can be applied to full length Akt1 to provide similar results. All IC_{50} values are the geometric mean of at least n=2 determinations[2].
References	[1]. Chen SF, et al. Binding selectivity studies of PKBα using molecular dynamics simulation and free energy calculations. J Mol Model. 2013 Nov;19(11):5097-5112. [2]. Freeman-Cook KD, et al. Design of selective, ATP-competitive inhibitors of Akt. J Med Chem. 2010 Jun 24;53(12):4615-4622.