产品名称：**PF-04691502**
产品别名：**PF-04691502**

生物活性：

Description	PF-04691502 is a potent and selective inhibitor of PI3K and mTOR. PF-04691502 binds to human PI3Kα, β, δ, γ and mTOR with K_is of 1.8, 2.1, 1.6, 1.9 and 16 nM, respectively.

IC_{50} & Target	PI3Kδ	PI3Kα	PI3Kγ	PI3Kβ	mTOR
	1.6 nM (Ki)	1.8 nM (Ki)	1.9 nM (Ki)	2.1 nM (Ki)	16 nM (Ki)

In Vitro	PF-04691502 inhibits recombinant mouse PI3Kα in an ATP-competitive inhibitor. PF-04691502 potently inhibits AKT phosphorylation on S473 and T308 in all the 3 cancer cell lines with IC50 values of 3.8 to 20 nM and 7.5 to 47 nM, respectively. Using a 96-well plate-based P-S6RP(S235/236) ELISA assay, PF-04691502 potently inhibits mTORC1 activity with an IC50 of 32 nM. PF-04691502 inhibits cell proliferation of BT20, SKOV3, and U87MG with IC50 values of 313, 188, and 179 nM, respectively. In PIK3CA-mutant and PTEN-deleted cancer cell lines, PF-04691502 reduces phosphorylation of AKT T308 and AKT S473 (IC50 of 7.5-47 nM and 3.8-20 nM, respectively) and inhibits cell proliferation (IC50 of 179-313 nM). PF-04691502 inhibits mTORC1 activity in cells as measured by PI3K-independent nutrient stimulated assay, with an IC50 of 32 nM and inhibits the activation of PI3K and mTOR downstream effectors including AKT, FKHRL1, PRAS40, p70S6K, 4EBP1, and S6RP[1].
In Vivo	Nude mice bearing U87MG tumors are administered orally once a day with PF-04691502 at 0.5, 1, 5, and 10 mg/kg (maximum tolerated dose, MTD). Treatment with 10 mg/kg results in a significant reduction of P-AKT(S473) levels at 1 hour postdosing, and persistent inhibition is observed for 8 hours. P-AKT(S473) recovers to above baseline 24 hours after 10 mg/kg treatment. For P-S6RP(S235/236), a similar inhibition time course is observed, but after 24 hours of treatment, P-S6RP levels remain lower than vehicle tumors. Modulation of the AKT downstream effector, P-PRAS40(T246), and mTOR downstream effector, P-4EBP1(T37/46), is observed. The PF-04691502-treated tumors are also evaluated by immunohistochemistry for levels of P-AKT(S473), total AKT, P-S6RP, and total S6RP. Phosphorylation of AKT and S6RP are significantly reduced at 4 hours after a single dose of PF-04691502 at 10 mg/kg. Dose-dependent tumor growth inhibition (TGI) is obtained in the U87MG xenograft model and approximately 73% TGI is observed at the MTD dose of 10 mg/kg[1].

Solvent&Solubility

In Vitro:

DMSO : 50 mg/mL (117.51 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	**1 mg**	**5 mg**	**10 mg**
	1 mM	2.3503 mL	11.7514 mL	23.5029 mL
	5 mM	0.4701 mL	2.3503 mL	4.7006 mL
	10 mM	0.2350 mL	1.1751 mL	2.3503 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: ≥ 2.5 mg/mL (5.88 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (5.88 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 (5.88 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (5.88 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 (5.88 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (5.88 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]. Yuan J, et al. PF-04691502, a potent and selective oral inhibitor of PI3K and mTOR kinases with antitumor activity. Mol Cancer Ther. 2011 Nov;10(11):2189-99.

实验参考：

Cell Assay	BT20, U87MG, and SKOV3 cells are plated at 3,000 cell/well in 96-well culture plates in growth medium with 10% FBS. Cells are incubated overnight and treated with DMSO (0.1% final) or serial diluted compound for 3 days. Resazurin is added to 0.1 mg/mL. Plates are incubated at 37°C in 5% CO2 for 3 hours. Fluorescence signals are read as emission at 590 nm after excitation at 530 nm. IC50 values are calculated by plotting fluorescence intensity to drug concentration in nonlinear curves. U87MG and SKOV3 cells are plated in 96-well plates overnight and caspase-3/caspase-7 activity is assessed with the Caspase-Glo 3/7 Assay Kit[1].
Animal Administration	Mice [1] Female nu/nu mice (6-8 weeks old) are used. Tumor cells for implantation are harvested and resuspended in serum-free medium mixed with matrigel (1:1). SKOV3, U87MG, or NSCLC cells ($2.5\text{-}4\times10^6$) are implanted subcutaneously into the hind flank region. Treatment started when average tumor size is 100 to 200 mm^3. PF-04691502 is formulated in 0.5% methylcellulose in water suspension and given orally once a day. Animal body weights and tumor volumes are measured every 2 to 3 days. Tumor volume is determined with Vernier calipers and calculated. Percentage of tumor growth inhibition (TGI) is calculated. Data are presented as mean±SE. Comparisons between treatment groups and vehicle group are done using 1-way ANOVA by Dunnett's tests. Student's t test is used to determine the P value for the comparison of 2 groups.
	The biochemical protein kinase assays for class I PI3K and mTOR are assessed. The fluorescence polarization assay for ATP competitive inhibition is done as follows: mPI3Kα dilution solution (90 nM) is prepared in fresh assay buffer (50 mM Hepes pH 7.4, 150 mM NaCl, 5 mM DTT, 0.05%

Kinase Assay	CHAPS) and kept on ice. The enzyme reaction contained 0.5 nM mouse PI3Kα (p110α/p85α complex purified from insect cells), 30 μM PIP2, PF-04691502 (0, 1, 4, and 8 nM), 5 mM MgCl2, and 2-fold serial dilutions of ATP (0-800 μM). Final DMSO is 2.5%. The reaction is initiated by the addition of ATP and terminated after 30 minutes with 10 mM EDTA. In a detection plate, 15 uL of detector/probe mixture containing 480 nM GST-Grp1PH domain and 12 nM TAMRA tagged fluorescent PIP3 in assay buffer is mixed with 15 uL of kinase reaction mixture. The plate is shaken for 3 minutes, and incubated for 35 to 40 minutes before reading on an LJL Analyst HT[1].
References	[1]. Yuan J, et al. PF-04691502, a potent and selective oral inhibitor of PI3K and mTOR kinases with antitumor activity. Mol Cancer Ther. 2011 Nov;10(11):2189-99.