产品名称：PP242
产品别名：Torkinib

生物活性：

Description	Torkinib (PP 242) is a selective and ATP-competitive mTOR inhibitor with an IC_{50} of 8 nM. PP242 inhibits both mTORC1 and mTORC2 with IC_{50}s of 30 nM and 58 nM, respectively.

IC_{50} & Target

mTORC1	mTORC2	mTOR	p110δ	DNA-PK	PDGFR
30 nM (IC_{50})	58 nM (IC_{50})	8 nM (IC_{50})	100 nM (IC_{50})	410 nM (IC_{50})	410 nM (IC_{50})
p110α	p110β	p110γ	Abl	Hck	Scr
2 μM (IC_{50})	2.2 μM (IC_{50})	1.3 μM (IC_{50})	3.6 μM (IC_{50})	1.2 μM (IC_{50})	1.4 μM (IC_{50})
Scr(T338I)	VEGFR2	EGFR	EphB4	Autophagy	Mitophagy
5.1 μM (IC_{50})	1.5 μM (IC_{50})	4.4 μM (IC_{50})	3.4 μM (IC_{50})		

In Vitro

Torkinib (PP 242) potently inhibits mTOR (IC50=8 nM) but is much less active against other PI3K family members. Testing of Torkinib (PP 242) against 219 protein kinases reveals remarkable selectivity relative to the protein kinome: at a concentration 100-fold above its IC50 for mTOR, Torkinib (PP 242) inhibits only one kinase by more than 90% (Ret) and only three by more than 75% (PKCα, PKCβII and JAK2V617F)[1]. Torkinib (PP 242) has a dose-dependent effect on proliferation and at higher doses is much more effective than Rapamycin at blocking cell proliferation. The ability of Torkinib (PP 242) to block cell proliferation more efficiently than Rapamycin could be a result of its ability to inhibit mTORC1 and mTORC2, because Rapamycin can only inhibit mTORC1. In SIN1-/- mouse embryonic fibroblasts (MEFs), Rapamycin is also less effective at blocking cell proliferation than Torkinib. That Torkinib (PP 242) and Rapamycin exhibit very different anti-proliferative effects in SIN1-/- MEFs suggests that the two compounds differentially affect mTORC1[2].

In Vivo

In fat and liver, Torkinib (PP 242) is able to completely inhibit the phosphorylation of Akt at S473 and T308, consistent with its effect on these phosphorylation sites observed in cell culture. Surprisingly, Torkinib (PP 242) is only partially able to inhibit the phosphorylation of Akt in skeletal muscle and is more effective at inhibiting the phosphorylation of T308 than S473, despite it's ability to fully inhibit the phosphorylation of 4EBP1 and S6. These results will be confirmed by in vivo dose-response experiments, but, consistent with the partial effect of Torkinib (PP 242) on pAkt in skeletal muscle, a muscle-specific knockout of the integral mTORC2 component rictor resulted in only a partial loss of Akt phosphorylation at S473. These results suggest that a kinase other than mTOR, such as DNA-PK, may contribute to phosphorylation of Akt in muscle[2].

In Vitro:

DMSO : 50 mg/mL (162.16 mM; Need ultrasonic)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	3.2432 mL	16.2159 mL	32.4317 mL
5 mM	0.6486 mL	3.2432 mL	6.4863 mL
10 mM	0.3243 mL	1.6216 mL	3.2432 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

Solvent&Solubility	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 (8.11 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (8.11 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 (8.11 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (8.11 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 (8.11 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (8.11 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]. Apsel B, et al. Targeted polypharmacology: discovery of dual inhibitors of tyrosine and phosphoinositide kinases. Nat Chem Biol. 2008 Nov;4(11):691-9. [2]. Feldman ME, et al. Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. PLoS Biol. 2009 Feb 10;7(2):e38.

实验参考：

Cell Assay	Wild-type and SIN1-/- MEFs are plated in 96-well plates at approximately 30% confluence and left overnight to adhere. The following day cells are treated with Torkinib (PP 242) (1 nM, 10 nM, 100 nM, 1 μM, and 10 μM), Rapamycin, or vehicle (0.1% DMSO). After 72 h of treatment, 10 μL of 440 μM resazurin sodium salt is added to each well, and after 18 h, the florescence intensity in each well is measured using a top-reading florescent plate reader with excitation at 530 nm and emission at 590 nm[2].
Animal Administration	Mice[2] Six-wk-old male C57BL/6 mice are fasted overnight prior to drug treatment. Torkinib (PP 242) (0.4 mg), Rapamycin (0.1 mg), or vehicle alone is injected IP. After 30 min for the Rapamycin-treated mouse or 10 min for the Torkinib (PP 242) and vehicle-treated mice, 250 mU of insulin in 100 μL of saline is injected IP. 15 min after the insulin injection, the mice are killed by CO2 asphyxiation followed by cervical dislocation. Tissues are harvested and frozen on liquid nitrogen in 200 μL of cap lysis buffer. The frozen tissue is thawed on ice, manually disrupted with a mortar and pestle, and then further processed with a micro tissue-homogenizer. Protein concentration of the cleared lysate is

	measured by Bradford assay and 5-10 μg of protein is analyzed by Western blot[2].
References	[1]. Apsel B, et al. Targeted polypharmacology: discovery of dual inhibitors of tyrosine and phosphoinositide kinases. Nat Chem Biol. 2008 Nov;4(11):691-9. [2]. Feldman ME, et al. Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. PLoS Biol. 2009 Feb 10;7(2):e38.