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产品名称：吡菲他林-α
产品别名：Pifithrin-α hydrobromide

生物活性：

Description	Pifithrin-α hydrobromide is a p53 inhibitor which blocks its transcriptional activity and prevents cells from apoptosis. Pifithrin-α hydrobromide is also an aryl hydrocarbon receptor (AhR) agonist.
IC_{50} & Target	p53[1] AhR[2]
In Vitro	Pifithrin-α (PFT-α) hydrobromideis a water-soluble compound that could suppress p53 protein transcription. Pifithrin-α can suppress glucose oxidase (GOX)-induced p53 protein increase in whole cell lysates, but cyclosporine A (CsA) fails to show such an inhibition effect. Notably, Pifithrin-α is able to block the GOX-induced Bcl-2 protein reduction. Similarly, it is Pifithrin-α rather than CsA that able to prevent the Bax increasing in whole cell lysates[1]. Pifithrin-α inhibits p53-dependent apoptosis through an undetermined mechanism. Pifithrin-α also acts as an aryl hydrocarbon receptor (AhR) agonist and. Pifithrin-α is a potent AhR agonist as determined by its ability to bind the AhR, induce formation of its DNA binding complex, activate reporter activity, and up-regulate the classic AhR target gene CYP1A1[2].
In Vivo	When the experiment is performed with Pifithirin-α (PFT-α) hydrobromide, a pharmacological p53 inhibitor, the percentage of annexin V-positive *Foxe3*$^{-/-}$ SMCs decreases to WT levels. Pifithrin-α (2.2 mg/kg, i.p.) significantly reduces the incidence of aortic rupture and intramural hematomas in *Foxe3*$^{-/-}$ mice that underwent transverse aortic constriction (TAC) (50% to 17%, P<0.05). After Pifthirin-α treatment, the mean diameter of the ascending aorta and the percentage of TUNEL-positive cells in the aortic media are also normalized to WT levels in surviving *Foxe3*$^{-/-}$animals (P<0.05)[3].
Solvent&Solubility	***In Vitro:*** **DMSO : ≥ 50 mg/mL (136.13 mM)** * "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.7226 mL	13.6129 mL	27.2257 mL
5 mM	0.5445 mL	2.7226 mL	5.4451 mL
10 mM	0.2723 mL	1.3613 mL	2.7226 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 (6.81 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.81 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 (6.81 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.81 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 (6.81 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.81 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]. Yu W, et al. Cyclosporine A Suppressed Glucose Oxidase Induced P53 Mitochondrial Translocation and Hepatic Cell Apoptosis through Blocking Mitochondrial Permeability Transition. Int J Biol Sci. 2016 Jan 1;12(2):198-209. [2]. Hoagland MS, et al. The p53 Inhibitor Pifithrin-α Is a Potent Agonist of the Aryl Hydrocarbon Receptor. J Pharmacol Exp Ther. 2005 Aug;314(2):603-10. [3]. Kuang SQ, et al. FOXE3 mutations predispose to thoracic aortic aneurysms and dissections. J Clin Invest. 2016 Mar 1;126(3):948-61.

实验参考：

Cell Assay	The human hepatoma cell lines HepG2 (p53++) are cultured in RMPI 1640 medium with 10% fetal bovine serum (FBS), and 1% penicillin/streptomycin at 37°C in an atmosphere containing 5% CO_2. Cells are exposed to GOX (0-5 0U) for 0-8 hours with or without Pifithrin-α (20 μM/L), Pifithrin-μ (5 μM/L), CsA (10 μM/L), Sanglifehrin A (20 μM/L) and NAC (5 mM/L) for 1 hour, respectively. After treatment, cells are collected and processed for further experiments[1].
Animal Administration	Mice[3] The *Foxe3*-null (*Foxe3*$^{-/-}$) mice are used. To investigate the role of p53 in *Foxe3*-related apoptosis, Pifithrin-α is administered by i.p. injection at a dosage of 2.2 mg/kg, then dissolved in PBS 1 hour before TAC and then every 48 hours. Animals are euthanized 2 weeks after the surgery, and the ascending aortic tissues are harvested for either RNA, total protein, histomorphometric analysis, or TUNEL assay.
	The ligand binding competition assays are performed. Cytosolic cell extracts from Hepa-1 cells are generated by the resuspension of the cell pellets in HEDG buffer [25 mM Hepes, 1 mM EDTA, 1 mM dithiothreitol, and 10% (v/v) glycerol, pH 7.5] containing 0.4 mM leupeptin, 4 mg/mL aprotinin, and 0.3 mM phenylmethylsulfonyl fluoride, homogenization, and centrifugation at 100,000 g for 45 min. Aliquots of the supernatant (120 μg) are incubated at room temperature for 2 h with the indicated

Kinase Assay	concentrations of Pifithrin-α in the presence of 3 nM [^{3}H]TCDD in HEDG buffer. After incubation on ice with hydroxyapatite for 30 min, HEDG buffer with 0.5% Tween 80 is added. The samples are centrifuged, washed twice, resuspended in 0.2 mL of scintillation fluid, and subjected to scintillation counting. Nonspecific binding is determined using a 150-fold molar excess of TCDF and subtracted from the total binding to obtain the specific binding. The specific binding is reported relative to [^{3}H]TCDD alone[2].
References	[1]. Yu W, et al. Cyclosporine A Suppressed Glucose Oxidase Induced P53 Mitochondrial Translocation and Hepatic Cell Apoptosis through Blocking Mitochondrial Permeability Transition. Int J Biol Sci. 2016 Jan 1;12(2):198-209. [2]. Hoagland MS, et al. The p53 Inhibitor Pifithrin-α Is a Potent Agonist of the Aryl Hydrocarbon Receptor. J Pharmacol Exp Ther. 2005 Aug;314(2):603-10. [3]. Kuang SQ, et al. FOXE3 mutations predispose to thoracic aortic aneurysms and dissections. J Clin Invest. 2016 Mar 1;126(3):948-61.