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产品名称：AM966
产品别名：AM966

生物活性：

Description	AM966 is a high affinity, selective, oral LPA1-antagonist, inhibits LPA-stimulated intracellular calcium release (IC50=17 nM).
IC_{50} & Target	LPA1[1]
In Vitro	AM966 is a potent, selective, orally bioavailable LPA_1 receptor antagonist. AM966 inhibits LPA_1-mediated chemotaxis of human A2058 melanoma cells (IC_{50}=138±43 nM), IMR-90 human lung fibroblasts (IC_{50}=182±86 nM) and CHO mLPA$_1$ cells (IC_{50}=469±54 nM)[1]. LPA-induced ERK1/2 activation is completely blocked by AM966 (100 nM), which selectively antagonizes LPA_1 over LPA_{2-5}, with an IC_{50} value of 3.8±0.4 nM. Pre-treatment with AM966 (100 nM) completely blocks ERK1/2 phosphorylation induced by either amitriptyline or mianserin[2].
In Vivo	AM966 (30 mg/kg, BID) reduces vascular leakage, inflammation and lung injury and inflammation in a 3 day bleomycin model. AM966 inhibits lung fibrosis, maintains mouse body weight and decreases lung inflammation 14 days after bleomycin lung injury. AM966 reduces vascular leakage, tissue injury and pro-fibrotic cytokine production in the 14 day bleomycin study. AM966 demonstrates greater efficacy compared to pirfenidone in the 14 day bleomycin model. AM966 decreases mortality and fibrosis at late time points after bleomycin injury[1].
Solvent&Solubility	***In Vitro:*** **DMSO : 100 mg/mL** (**203.70 mM;** Need ultrasonic)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.0370 mL	10.1848 mL	20.3695 mL
5 mM	0.4074 mL	2.0370 mL	4.0739 mL
10 mM	0.2037 mL	1.0185 mL	2.0370 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 (5.09 mM); Suspended solution; Need ultrasonic

此方案可获得 2.5 mg/mL (5.09 mM)的均匀悬浊液，悬浊液可用于口服和腹腔注射。

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% corn oil Solubility: ≥ 2.5 mg/mL (5.09 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (5.09 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]. Swaney, JS, et al. A novel, orally active LPA1 receptor antagonist inhibits lung fibrosis in the mouse bleomycin model. Br J Pharmacol. 2010 Aug;160(7):1699-713. [2]. Olianas MC, et al. Antidepressants activate the lysophosphatidic acid receptor LPA(1) to induce insulin-like growth factor-I receptor transactivation, stimulation of ERK1/2 signaling and cell proliferation in CHO-K1 fibroblasts. Biochem Pharmacol. 2015 Jun 15;95(4):311-23.

实验参考：

Cell Assay	CHO-K1 cells are grown to 80% confluency in 12-well plates, serum-starved for 24 h and incubated in serum-free medium with AM966. After 21 h, [^{3}H]thymidine (0.5 μCi/well) is added and the incubation is continued for 3 h. The medium is then removed, and the cells are placed on ice and washed twice with 1 mL of ice-cold PBS containing 5% trichloroacetic acid. Cells are solubilized and [^{3}H]thymidine incorporation is determined by liquid scintillation counting. Assays are performed in triplicate[2].
Animal Administration	Mice[1] The oral exposure of AM966 is determined in fasted mice. Animals received AM966 (10 mg/kg) in vehicle (water) by oral gavage and are then killed by CO_2 inhalation at 1, 2, 4, 8 and 24 h post dose (n=2 animals per time point for each test compound). Blood (approximately 300 μL) is collected via cardiac puncture into EDTA-containing tubes and centrifuged at 1450×g for 10 min. The plasma is removed and analysed for AM966 content by liquid chromatography-mass spectrometry (LCMS). Briefly, known amounts of AM966 are added to thawed mouse plasma to yield a concentration range from 0.8 to 4000 ng/mL. Mouse plasma samples are precipitated using acetonitrile (1:4, v:v) containing the internal standard buspirone. A 10 μL aliquot of the analyte mixture is injected using a Leap PAL autosampler. Analyses are performed using an Agilent Zorbax SB-C8 column (2.1×50 mm; 5 μm) linked to a Shimadzu LC-10AD VP with SCL-10A VP system controller. Tandem mass spectrometric detection is carried out on a PE Sciex API3200 in the positive ion mode (ESI) by multiple reaction monitoring. The calibration curves are constructed by plotting the peak-area ratio of analysed peaks against known concentrations. The lower limit of quantitation is 0.8 ng/mL. The data are subjected to linear regression analysis with $1/x^2$weighting.
References	[1]. Swaney, JS, et al. A novel, orally active LPA1 receptor antagonist inhibits lung fibrosis in the mouse bleomycin model. Br J Pharmacol. 2010 Aug;160(7):1699-713. [2]. Olianas MC, et al. Antidepressants activate the lysophosphatidic acid receptor LPA(1) to induce insulin-like growth factor-I receptor transactivation, stimulation of ERK1/2 signaling and cell proliferation in CHO-K1 fibroblasts. Biochem Pharmacol. 2015 Jun 15;95(4):311-23.