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产品名称：[4'-[3-甲基-4-[[[((R)-1-苯基乙基)氧基]羰基]氨基]异恶唑-5-基]联苯-4-基]乙酸
产品别名：AM095 free acid

生物活性：

Description	AM095 (free acid) is a potent LPA1 receptor antagonist with IC50 values of 0.98 and 0.73 μM for recombinant human or mouse LPA1 respectively.
IC_{50} & Target	IC50: 0.98 μM (human LPA1), 0.73 μM (mouse LPA1)
In Vitro	AM095 inhibits the LPA-induced calcium flux of CHO cells stably transfected with human or mouse LPA1. The IC50 for AM095 antagonism of LPA-induced calcium flux of human or mouse LPA1-transfected CHO cells is 0.025 and 0.023 μM, respectively[1]. AM095 reduces LPA-induced vasorelaxation by appr 90% at 10 μM as compared to vehicle control[2]. AM095 inhibits LPA-driven chemotaxis of CHO cells overexpressing mouse LPA1 (IC50=778 nM) and human A2058 melanoma cells (IC50=233 nM)[3].
In Vivo	Pharmacological antagonism of LPA1 with AM095 significantly attenuates bleomycin-induced dermal fibrosis[1]. AM095 has high oral bioavailability and a moderate half-life and is well tolerated at the doses tested in rats and dogs after oral and intravenous dosing. AM095 dose-dependently reduces LPA-stimulated histamine release. AM095 attenuates bleomycin-induced increases in collagen, protein, and inflammatory cell infiltration in bronchalveolar lavage fluid. AM095 decreases kidney fibrosis in a mouse unilateral ureteral obstruction model[3].

Solvent&Solubility

In Vitro:

DMSO： 67.3 mg/mL (147.43 mM; Need ultrasonic and warming)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.1906 mL	10.9531 mL	21.9063 mL
	5 mM	0.4381 mL	2.1906 mL	4.3813 mL
	10 mM	0.2191 mL	1.0953 mL	2.1906 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 →90% corn oil

Solubility: ≥ 2.25 mg/mL (4.93 mM); Clear solution

此方案可获得 ≥ 2.25 mg/mL (4.93 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 22.5 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Castelino FV, et al. Amelioration of dermal fibrosis by genetic deletion or pharmacologic antagonism of lysophosphatidic acid receptor 1 in a mouse model of scleroderma. Arthritis Rheum. 2011 May;63(5):1405-15. [2]. Ruisanchez E, et al. Lysophosphatidic acid induces vasodilation mediated by LPA1 receptors, phospholipase C, and endothelial nitric oxide synthase. FASEB J. 2014 Feb;28(2):880-90. [3]. Swaney, J. S., et al. Pharmacokinetic and pharmacodynamic characterization of an oral lysophosphatidic acid type 1 receptor-selective antagonist. Journal of Pharmacology and Experimental Therapeutics (2011), 336(3), 693-700.

实验参考：

Animal Administration	Mice underwent UUO or sham surgery to the left kidney. In brief, a longitudinal, upper left incision is performed to expose the left kidney. The renal artery is located and a 6/0 silk thread is passed between the artery and the ureter. The thread is looped around the ureter and knotted three times insuring full ligation of ureter. The kidney is returned to abdomen, the abdominal muscle is sutured, and the skin is closed with staples. The contralateral (right) kidney served as an uninjured control. AM095 (30 mg/kg) or vehicle (water) is given 1 to 4 h before UUO and then b.i.d. thereafter by oral gavage. After 8 days, mice are euthanized using inhaled CO_2, and the kidneys are harvested and cut in half for histopathological and biochemical analysis of fibrosis. To assess fibrosis, half of the kidney is fixed in 10% neutral buffered formalin and stained using Masson's trichrome. The other half of the kidney is frozen at −80°C for subsequent biochemical analysis of collagen content. [3]
Kinase Assay	Assays are conducted using both hLPA1/CHO and mLPA1/CHO cells. A cell pellet of hLPA1/CHO or mLPA1/CHO cells is resuspended in appr 20 mL of ice-cold membrane buffer containing 10 mM HEPES, pH 7.4, 1 mM dithiothreitol, and protease inhibitors. Cells are sonicated, and the cell lysate is centrifuged at 2000 rpm for 10 min at 4°C. The supernatant is further centrifuged at 25,000 rpm for 70 min at 4°C. The membrane pellet is resuspended in 5 mL of ice-cold membrane buffer and homogenized using a Potter-Elvehjem tissue grinder. Final protein concentration is determined using the Bradford Protein Assay Kit. Known amounts of AM095 (diluted in dimethyl sulfoxide) or vehicle (dimethyl sulfoxide) are added to 25 to 40 μg of hLPA1/CHO or mLPA1/CHO membranes and 0.1 nM [^{35}S]-GTPγS in buffer (50 mM HEPES, 0.1 mM NaCl, 10 mM $MgCl_2$, 50 μg/mL saponin, pH 7.5) containing 0.2% fatty acid-free human serum albumin and 5 μM GDP. To test for LPA1 antagonist activity, the ability of AM095 to inhibit GTPγS binding stimulated by 900 nM LPA (18:1) is measured. Alternatively, to test for agonist effects, the ability of AM095 to stimulate GTPγS binding in the absence of LPA is measured. Reactions are incubated for 30 min at 30°C, before harvesting membranes onto glass filter binding plates and washing three times with cold buffer containing 50 mM HEPES, pH 7.4, 100 mM NaCl, 10 mM $MgCl_2$ using a Brandel 96-tip cell harvester. Plates are dried and then cpm are evaluated by using a Packard TopCount NXT microplate scintillation counter. [3]
References	[1]. Castelino FV, et al. Amelioration of dermal fibrosis by genetic deletion or pharmacologic antagonism of lysophosphatidic acid receptor 1 in a mouse model of scleroderma. Arthritis Rheum. 2011 May;63(5):1405-15.

	[2]. Ruisanchez E, et al. Lysophosphatidic acid induces vasodilation mediated by LPA1 receptors, phospholipase C, and endothelial nitric oxide synthase. FASEB J. 2014 Feb;28(2):880-90. [3]. Swaney, J. S., et al. Pharmacokinetic and pharmacodynamic characterization of an oral lysophosphatidic acid type 1 receptor-selective antagonist. Journal of Pharmacology and Experimental Therapeutics (2011), 336(3), 693-700.