上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话：021-61312973　　传真：021-55068248
网址：www.shyuanye.com
邮箱：shyysw@sina.com

产品名称：AVE 0991
产品别名：AVE 0991

生物活性：

Description	AVE 0991 is a nonpeptide and orally active angiotensin-(1-7) receptor agonist with an IC50 of 21 nM.
IC_{50} & Target	IC50: 21±35 nM (Ang-(1-7) receptor)[1]
In Vitro	AVE 0991 is a nonpeptide compound that evokes effects similar to Ang-(1-7) on the endothelium. AVE 0991 and unlabeled Ang-(1-7) compete for high-affinity binding of [^{125}I]-Ang-(1-7) to bovine aortic endothelial cell membranes with **IC_{50}**s of 21±35 and 220±280 nM, respectively. Peak concentrations of NO and O_2^- release by AVE 0991 sodium salt and Ang-(1-7) (both 10 μM) are not significantly different (NO: 295±20 and 270±25 nM; O_2^-: 18±2 and 20±4 nM). However, the released amount of bioactive NO is ≈5 times higher for AVE 0991 in comparison to Ang-(1-7)[1].
In Vivo	AVE 0991 (0.58 nmol/g) produces a significant decrease of water diuresis in WT mice compared with vehicle-treated animals (0.06±0.03 mL versus 0.27±0.05; n=9 for each group; P<0.01). The antidiuretic effect of AVE 0991 (AVE) is associated with an increase in urine osmolality (1669±231.0 mOsm/KgH_2O versus 681.1±165.8 mOsm/KgH_2O in vehicle-treated mice; P<0.01). The genetic deletion of *Mas* abolishes the antidiuretic effect of AVE 0991 during water loading (0.37±0.10 mL [n=9] versus 0.27±0.03 mL [n=11] in AVE 0991-treated mice). As observed with C57BL/6 mice, administration of AVE 0991 (0.58 nmol/g) in water-loaded Swiss mice also produces a significant decrease of the urinary volume compared with vehicle-treated animals (0.13±0.05 mL [n=16] versus 0.51±0.04 mL [n=40]; P<0.01)[2]. One week of treatment with AVE-0991 produces a significant decrease in perfusion pressure (56.55±0.86 vs. 68.73±0.69 mmHg in vehicle-treated rats) and an increase in systolic tension (11.40±0.05 vs. 9.84±0.15 g in vehicle-treated rats), rate of tension rise (+dT/dt; 184.30±0.50 vs. 155.20±1.97 g/s in vehicle-treated rats), rate of tension fall (−dT/dt; 179.60±1.39 vs. 150.80±2.42 g/s in vehicle-treated rats). A slight increase in heart rate (HR) is also observed (220.40±0.71 vs. 214.20±0.74 beats/min in vehicle-treated rats[3].
Solvent&Solubility	***In Vitro:*** **DMSO : 41.67 mg/mL** (**71.76 mM;** Need ultrasonic) **H_2O : < 0.1 mg/mL** (insoluble)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.7220 mL	8.6100 mL	17.2200 mL
	5 mM	0.3444 mL	1.7220 mL	3.4440 mL
	10 mM	0.1722 mL	0.8610 mL	1.7220 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 (4.31 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.31 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 (4.31 mM); Suspended solution; Need ultrasonic 此方案可获得 2.5 mg/mL (4.31 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 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 (4.31 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.31 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]. Wiemer G, et al. AVE 0991, a nonpeptide mimic of the effects of angiotensin-(1-7) on the endothelium. Hypertension. 2002 Dec;40(6):847-52. [2]. Pinheiro SV, et al. Nonpeptide AVE 0991 is an angiotensin-(1-7) receptor Mas agonist in the mouse kidney. Hypertension. 2004 Oct;44(4):490-6. [3]. Ferreira AJ, et al. The nonpeptide angiotensin-(1-7) receptor Mas agonist AVE-0991 attenuates heart failure induced by myocardial infarction. Am J Physiol Heart Circ Physiol. 2007 Feb;292(2):H1113-9. [4]. Mo J, et al. AVE 0991 attenuates oxidative stress and neuronal apoptosis via Mas/PKA/CREB/UCP-2 pathway after subarachnoid hemorrhage in rats. Redox Biol. 2018 Sep 28;20:75-86.

实验参考：

Cell Assay	Monkey kidney (COS) cells and Chinese hamster ovary (CHO) cells are stably transfected with rat Mas cDNA driven by a cytomegalovirus promoter and selected by neomycin. ^{125}I-Ang-(1-7) (0.5×10^{-9} mol/L) is incubated in 24-well plates for 60 minutes at 4°C in 0.3 mL of serum-free medium (DMEM) supplemented with 0.2% BSA, 0.005% bacitracin, 0.1 mol/L PMSF, and 0.5 mol/L orthophenanthroline with Mas-transfected COS cells in the presence or absence of AVE 0991 (AVE, 10^{-10} to $^{-5}$ mol/L). After 2 ishes with ice-cold serum-free DMEM, cells are disrupted with 0.1% Triton X-100. Bound radioactivity is measured in a gamma counter. Binding of rhodamine-Ang-(1-7) in Mas-transfected CHO cells is performed under similar conditions using 2×10^{-9} mol/L rhodamine-labeled-Ang-(1-7) in the presence or absence of AVE (10^{-6} mol/L), CV11974 (10^{-6} mol/L), or PD123319 (10^{-6} mol/L). NSB is determined in the presence of 10^{-6} mol/L Ang-(1-7)[1].
	Mice[2] Swiss male mice, *Mas*-KO (*Mas*$^{-/-}$) male mice on the pure genetic background C57BL/6, and WT C57BL/6 control mice (*Mas*$^{+/+}$) are used. Water diuresis is induced by intraperitoneal water injection

Animal Administration	(0.05 mL/g of body weight [BW]) in conscious mice. Drugs are administered in the same injection with water load at prefixed volumes (0.01 mL/g BW). In the first set of experiments, WT mice (C57BL/6, control group) or *Mas*-KO mice are treated with: (1) 0.58 nmol/g AVE 0991 (n=9, control; n=11, *Mas*-KO mice); or (2) vehicle for AVE 0991 (10 μM KOH, 0.01 mL/g; n=9, control; n=9, *Mas*-KO). In the second set, Swiss mice are treated with: (1) vehicle (n=36); (2) 0.58 nmol/g AVE 0991 (n=16); (3) 46 pmol/g Ang-(1-7) antagonist A-779 (n=4); (4) 2 nmol/g losartan or valsartan (n=5); (5) 2 nmol/g AT_2 receptor antagonists PD123319 or PD123177 (n=9); (6) AVE 0991 combined with A-779; (7) AVE 0991 combined with losartan or valsartan (n=4 for each); (8) or AVE 0991combined with PD123319 (n=5) or PD123177 (n=4). The urinary volume is measured for 60 minutes after water loading, and urine samples are obtained to determine the osmolality. The dose of AVE 0991is based in preliminary experiments performed in Swiss mice. Rats[3] Male Wistar rats weighting 250-300 g are used. Rats are treated either with AVE-0991 (1 mg/kg, n=9) or vehicle (0.9% NaCl, n=11) administered orally by gavage. At the end of the 7 day period of AVE-0991 treatment, the animals are decapitated 10-15 min after intraperitoneal injection of 400 IU of heparin. After the thorax is opened, the heart is carefully dissected, removed from the thoracic cavity, and placed in a plate containing ice-cold Krebs-Ringer solution (KRS) to attenuate any potential cardiac damage during dissection of aorta artery.
Kinase Assay	Binding of [^{125}I]-Ang-(1-7) is performed. Briefly, 100 μg of membranes from primary cultured bovine aortic endothelial cells (BAECs, passage 1) are incubated in a total volume of 200 μL for 45 minutes at 25°C in HEPES-buffered saline (10 mM HEPES, 0.1 M NaCl, 5 mM $MgCl_2$) containing 0.2% BSA and protease inhibitor cocktail Complete (Boehringer Mannheim). Saturable binding of [^{125}I]-Ang-(1-7) is calculated by subtracting nonspecific binding (40% to 50%), determined in the presence of 10 μM unlabeled Ang-(1-7) from total binding. Competition experiments with increasing concentrations of AVE 0991 and unlabeled Ang-(1-7) are performed in the presence of 10 nM [^{125}I]-Ang-(1-7). Assays are terminated by vacuum filtration (≤15 mm Hg) over Durapore filters (0.65 μm, Opak 96-well plates) presoaked with 1% BSA. The filters are washed 3 times with each 100 μL of PBS (50 mM, $NaHPO_4$ and 0.15 M NaCl, pH 7.2). Radioactivity on dried filters is quantified with a gamma counter[1].
References	[1]. Wiemer G, et al. AVE 0991, a nonpeptide mimic of the effects of angiotensin-(1-7) on the endothelium. Hypertension. 2002 Dec;40(6):847-52. [2]. Pinheiro SV, et al. Nonpeptide AVE 0991 is an angiotensin-(1-7) receptor Mas agonist in the mouse kidney. Hypertension. 2004 Oct;44(4):490-6. [3]. Ferreira AJ, et al. The nonpeptide angiotensin-(1-7) receptor Mas agonist AVE-0991 attenuates heart failure induced by myocardial infarction. Am J Physiol Heart Circ Physiol. 2007 Feb;292(2):H1113-9. [4]. Mo J, et al. AVE 0991 attenuates oxidative stress and neuronal apoptosis via Mas/PKA/CREB/UCP-2 pathway after subarachnoid hemorrhage in rats. Redox Biol. 2018 Sep 28;20:75-86.