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产品名称：(2R,3R,4S)-4-(1,3-苯并二氧戊环-5-基)-1-[2-(二丁基氨基)-2-氧代乙基]-2-(4-甲氧基苯基)吡咯烷-3-羧酸盐酸盐

产品别名：盐酸阿曲生坦；Atrasentan hydrochloride；ABT-627 hydrochloride; (+)-A 127722 hydrochloride; A-147627 hydrochloride

生物活性：

Description	Atrasentan hydrochloride (ABT-627 hydrochloride) is an endothelin receptor antagonist with IC50 of 0.0551 nM for ET_A[1].
IC_{50} & Target	IC50: 0.055 nM (ET_A)
In Vitro	Atrasentan hydrochloride (ABT-627 hydrochloride) (0-50 μM) significantly inhibits LNCaP and C4-2b prostate cancer cell growth. ABT-627 in conbination with Taxotere elicits a significantly greater loss of viable prostate cancer cells relative to either agent alone and shows greater degree of down-regulation of the NF-κB DNA binding activity[2]. Atrasentan profoundly induces several CYPs and drug transporters (e.g. 12-fold induction of CYP3A4 at 50 μM). It is a moderate P-gp inhibitor (IC_{50} in P388/dx cells=15.1±1.6 μM) and a weak BCRP inhibitor (IC_{50} in MDCKII-BCRP cells=59.8±11 μM)[3].
In Vivo	Atrasentan hydrochloride (ABT-627 hydrochloride) (3 mg/kg, p.o.) inhibits the pressor response induced by big endothelin-1 (1 nmol/kg) in pithed rats[1]. Aatrasentan (ABT-627, 10 mg/kg, i.p.) as well as Taxotere alone inhibited the C4-2b tumor growth within the bone environment to some extent in the SCID-hu model[2].
Solvent&Solubility	***In Vitro:*** **DMSO :≥ 33.3 mg/mL (60.87 mM)** * "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	1.8279 mL	9.1394 mL	18.2789 mL
5 mM	0.3656 mL	1.8279 mL	3.6558 mL
10 mM	0.1828 mL	0.9139 mL	1.8279 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：0.5% CMC-Na/saline water

Solubility: 0.75 mg/mL (1.37 mM); Clear solution; Need ultrasonic and warming

[1]. Yuyama H, et al. Superiority of YM598 over atrasentan as a selective endothelin ETA receptor

References	antagonist. Eur J Pharmacol. 2004 Sep 13;498(1-3):171-7. [2]. Banerjee S, et al. In vitro and in vivo molecular evidence for better therapeutic efficacy of ABT-627 and taxotere combination in prostate cancer. Cancer Res. 2007 Apr 15;67(8):3818-26. [3]. Weiss J, et al. Interaction potential of the endothelin-A receptor antagonist atrasentan with drug transporters and drug-metabolising enzymes assessed in vitro. Cancer Chemother Pharmacol. 2011 Oct;68(4):1093-8.

实验参考：

Cell Assay	All three prostate cancer cell lines (LNCaP, C4-2b, and PC-3 cells) are seeded at a density of 3 × 10^3 cells per well in 96-well microtiter culture plates. After overnight incubation, the medium is removed and replaced with a fresh medium containing different concentrations of ABT-627 (0-50 μM) diluted from a 10-mM stock. After 72 h of incubation with drug, 20 μL of MTT solution (5 mg/mL in PBS) are added to each well and incubated further for 2 h. Upon termination, the supernatant is aspirated and the MTT formazan formed by metabolically viable cells is dissolved in isopropanol (100 μL). The plates are mixed for 30 min on a gyratory shaker, and the absorbance is measured at 595 nm on a plate reader.[2]
AnimalAdministration	YM598 (0.3, 1, and 3 mg/kg), atrasentan (0.3, 1, and 3 mg/kg), or 0.5% methyl cellulose as vehicle is orally administered to rats with a dosing cannula. Dosing volume of the test substances and vehicle is set at 5 mL/kg. Approximately 20 min after administration of compounds, the rats are anesthetized with sodium pentobarbital, and then pithed and ventilated 30 min after dosing. Approximately 1 h after oral administration of compounds, big endothelin-1 (1 nmol/kg) is intravenously administered, and blood pressure is measured. In these two experiments, the dose of test compound that cause 50% inhibition (ID_{50}) of the big endothelin-1-induced increase in diastolic blood pressure is determined by linear regression analysis.[1]
Kinase Assay	Cells are incubated and treated with Atrasentan. They are then washed twice with PBS and lysed in ice-cold lysis buffer [20 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 2.5 mM sodium PPi, 1 mM β-glycerophosphate, 1 mM sodium orthovanadate, 1 μg/mL leupeptin, and 1 mM PMSF]. The extracts are centrifuged to remove cellular debris, and the protein content of the supernatants is determined using the bicinchoninic acid (BCA) protein assay reagent. Proteins (150 μg) are incubated with gentle rocking at 4° C overnight with immobilized Akt antibody cross-linked to agarose hydrazide beads. After the Akt is selectively immunoprecipitated from the cell lysates, the immunoprecipitated products are washed twice with lysis buffer and twice with kinase assay buffer [25 mM Tris (pH 7.5), 10 mM MgCl2, 5 mM β-glycerol phosphate, 0.1 mM sodium orthovanadate, 2 mM DTT] and then resuspended in 40 μL of kinase assay buffer containing 200 μM ATP and 1 μg GSK-3α/β fusion protein. The kinase assay reaction is allowed to proceed at 30° C for 30 min and stopped by the addition of Lamelli SDS sample buffer. Reaction products are resolved by 10% SDS-PAGE, followed by Western blotting with antiphosphorylated GSK-3α/β antibody. For analysis of the total amount of Akt, 40 μg of protein from the lysate samples are resolved by 10% SDS-PAGE, followed by Western blotting with anti-Akt antibody.[2]
	[1]. Yuyama H, et al. Superiority of YM598 over atrasentan as a selective endothelin ETA receptor antagonist. Eur J Pharmacol. 2004 Sep 13;498(1-3):171-7.

References	[2]. Banerjee S, et al. In vitro and in vivo molecular evidence for better therapeutic efficacy of ABT-627 and taxotere combination in prostate cancer. Cancer Res. 2007 Apr 15;67(8):3818-26. [3]. Weiss J, et al. Interaction potential of the endothelin-A receptor antagonist atrasentan with drug transporters and drug-metabolising enzymes assessed in vitro. Cancer Chemother Pharmacol. 2011 Oct;68(4):1093-8.