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BIOLOGICAL EVALUATION OF A NOVEL POTENT ANGIOTENSIN II RECEPTOR 1 ANTAGONIST WITH ANTI-HYPERTENSION EFFECT

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The pharmacological activity of a novel angiotensin II type 1 receptor antagonist (**1a**) was evaluated. Radioligand binding assays *in vitro* suggested that **1a** display8ed nanomolar affinity for angiotensin II type 1 receptor with an IC_{50} value of 2.33 ± 4.89 nM.Antihypertensive experiments *in vivo* showed that compound **1a** showed an efficient and long-lasting effect in reducing blood pressure at 10mg/kg inspontaneously hypertensive rats. The pharmacokinetic experiments showed that **1a** was absorbed efficiently and metabolized smoothly in Wistar rats. These results demonstrated that **1a**was a potent AT_1 receptor antagonist which could be considered as a novel anti-hypertension drug candidate and deserved for further investigation.

Keywords: Hypertension, Anti-hypertension, AT_1 receptor antagonist, ARBs

1 INTRODUCTION

Hypertension, defined as raised blood pressure greater than orto 140 mmHg systolic or 90 mmHg diastolic,it is a serious health problem associated with an increased risk of death, stroke, andmetabolic syndromes including insulin resistance and lipid abnormalities (Bao et al., 2016).The renin-angiotensin-aldosterone system (RAAS) plays a pivotal role in blood pressure regulation and electrolyte homeostasis. Angiotensin II (Ang II), a vasoconstrictive peptide hormone, is the effector molecule of the renin-angiotensin system(Collins et*al*., 1994). Ang II receptor 1 (AT_1) antagonist is a novel class of antihypertension drug which is widely accepted for its little side effect and good therapeutic profiles (Bali et al., 2005). Losartan is the most widely used drug of this series, and numerous modifications to its chemical structure have generated a large number of Ang II antagonists including valsartan, irbesartan, telmisartan, candesartan, olmesartan and so on (Bakris et al., 2001).

In this study, the pharmacological profiles of a novel angiotensin II receptor 1 antagonist were investigated, including receptor binding studies *in vitro*, anti-hypertensive effect *in vivo*, and the pharmacokinetic characteristics in Wistar rats.

2 MATERIALS AND METHODS

Compound **1a** was designed and synthesized in our group. Vascular smooth muscle cells (VSMCs) were purchased from Abcore-inc, Co., Ltd, Shanghai. Losartan was obtained from Shanghai Zhong Kang Wei Ye Biological Technology Co., Ltd. DMSO was from Shanghai Ling Feng Chemical Reagent Co., Ltd. ^{125}I-Ang II was from Zhongshan Hospital, Shanghai. Spontaneously hypertensive rats (SHRs, 250 ± 20 g) were from Charles River Experimental Animal Technology Co. Ltd, Beijing. The non-linear regression program GraphPad Prism 5 software was obtained from Network of Science Software of China.

2.1 Binding affinities to Ang II (AT1) receptor *in vitro*

The affinity toward AT_1 receptor of **1a** was tested by its ability to displace [^{125}I]-Ang II from its specific binding sites in

vascular smooth muscle cells (VSMCs) line of rats. VSMCs of 3–6 generations were used for experiments. Losartan and **1a** were dissolved in DMSO and diluted to different concentrations (10^{-10} to 10^{-4} M) with PBS before experiments.^{125}I-Ang IIwas dissolved with PBS and diluted to 0.1 nM. VSMCs (10^6 cells/well, 500 μL) were seeded into 24-well plates and cultured in 37□, 5% CO_2. After the cells adhered to the wall, they were washed and incubated in PBS containing 0.1 nM ^{125}I-Ang II and **1a** atdifferent concentrations were then cultivated 4□ for 150 min. The final concentrations of the **1a** were 10^{-12} to 10^{-6} M. And then nonspecific binding represented 5–10% of total binding which was measured in presence of l μM Ang II. The resulting VSMCs were washed 3 times with PBS and digested for 10 min with0.1 M NaOH. These cells bound by ^{125}I-Ang II were counted by c-counter (SN-682, Ri Huan Company, Shanghai, China). IC_{50} value was estimated by the nonlinear portion of the competition curves.

2.2 Anti-hypertensive effect in spontaneously hypertensive rats

The anti-hypertensive effect of compound**1a**was investigated using spontaneously hypertensive rats (SHRs) (250 ± 20 g). The systolic blood pressure (SBP) and diastolic blood pressure (DBP) of SHRs were measured by noninvasive tail artery manometry under conscious state.18 male SHRs were divided into 3experimental groups randomly: negative control group, positive control group (losartan 10 mg/kg) and **1a** group(10 mg/kg). **1a**and losartan were suspended in DMSO and oleic acid(V_1 : V_2 = 1: 4).

Rats in positive control group and compound**1a** group were orally administered with Losartan(10 mg/kg) and **1a**(10 mg/kg) respectively. Rats in negative control group were administered with the same volume of the solvent. The blood pressure and heart rates were monitored at 0 h(before administration) and 1 -12h, 24 h after administration by a biological signal analysis system (MPA-2000, Alcott Biotech, Shanghai, China). Six determinations were made in every session of blood pressure measurements and the means of the six values were taken as the SBP level and DBP level, respectively. The mean blood pressure (MBP) was calculated by the formula: MBP =(SBP-DBP)/3 + DBP (Da et al., 2012) and results were expressed as mean ± SEM. A probability level of less than 0.05 was considered significant.

2.3.Pharmacokinetic assays-drug concentration in plasma

High performance liquid chromatography-mass Spectrometry (HPLC-MS/MS) method was used to analyze the drug concentrations in plasma.Agilent HPLC-MS electrospray ionization (ESI) single quadrupoles mass spectrometer using a C18 column 2.1 mm ×150 mm, 3 mm with following mobile phases: (A) H_2O 0.1% formic acid and (B) acetonitrile. The flow rate was 0.2 mL/min and the injection volume was 10 μL. The Gradient elution program of mobile phase A : B (v/v) were 55 : 45. HPLC-MS spectral data of each single sample were collected, Microsoft Excel program,Origin 8.0 and DAS 2.0 software were used to calculate thepharmacokinetic parameters.

6Wistar rats were administrated with compound **1a** at dose of 5 mg/kg. 0.5 mL venous blood was taken before administration (0 h) and 0.5, 1, 2, 4, 6, 8, 12, 24, 48 , 72 h after administration. Plasma was extracted by centrifuging at 4000g, 4 ℃for 10 min. Then 200 μL plasma samples for analysis were placed into a 1.5 mL polypropylene microfuge tube followed by 100μL of internal standard. Acetonitrile (200 μL) was added to precipitate proteins and mixed for 30 s by the tube vortex. Precipitated proteins were separated by centrifugation at 10000g for 5 min. The supernatant was filtered by needle filter of 0.45 μL and then 10 μL filtrationwas taken to analyze in HPLC-MS. Linearity for **1a** was tested by extracting plasma standards spiked at nominal concentrations of 1, 5, 10, 50, 100, 500 ng/mL (1, 5, 10, 50, 100, 500 ng/mL for **1a**). The calibration line was generated by least squares linear regression of the peak height ratio (PHR) of analyte/internal standard against nominal concentration with a weighting of concentration (Cao et al., 2015).

2.4. Statistics

Results were expressed as means ± standard error of the means. Data were analyzed by one-way analysis of variance.When overall statistical significance was achieved ($P < 0.05$), student's t-test was used to compare each of the doses to the vehicle control. Probability values less than 0.05 were considered to besignificant. Binding isotherms from competition studies were obtained using the non-linear regression program GraphPad Prism 5 software (Network of Science Software of China). The completed animal research here adhered to the Principles of Laboratory Animal Care and was approved by IACUC.

3. RESULTS

3.1. Binding affinities to Ang II (AT1) receptor *invitro*

Radioligand binding assay showed that compound**1a** had nanomolar affinity to angiotensin type 1 receptor (**Table 1**,**Fig. 1**). In competition experiments, **1a** and Losartan could compete dose-dependently with ^{125}I-Ang II. **1a** displayed the highest specific affinity to the AT_1 receptor with the IC_{50} value of 2.33±4.89nM and the Ki value of 1.69.±3.55nM.

Table 1. IC_{50} and Ki value of the tested compound 1a and Loartan

Compounds	IC_{50} ± SEM (nM)	Ki (nM)
1a	2.33 ± 4.89	1.69 ± 3.55
Losartan	12.19 ± 0.37	8.23 ± 0.27

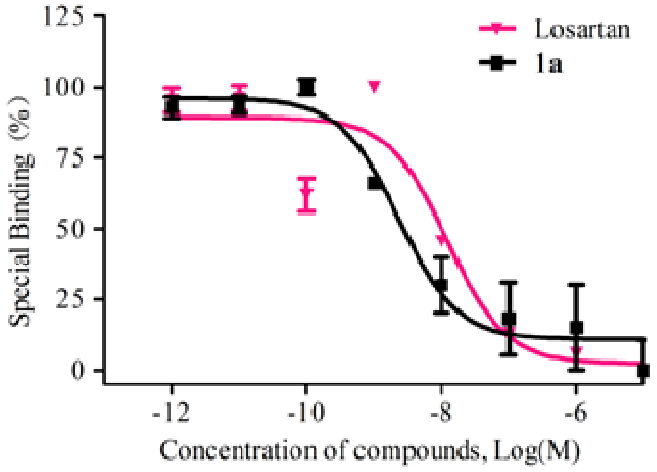

Fig. 1. Inhibitory effects of compounds 1a and losartan (10^{-5} – 10^{-12} M) onspecific binding of ^{125}I-Ang II to AT_1 receptors in VSMCs.

3. 2. Anti-hypertensive effects *invivo*

The effects of **1a** (10mg/kg), Losartan (10 mg/kg) on the mean blood pressure (MBP) *in vivo* after oral administration in SHRs were shown in **Fig. 2**. The results indicated that the **1a** could decrease the blood pressure significantly compared with the negative control group. The maximal response of compound **1a** (10 mg/kg) was observed at 2 h after dosing with reduction of 47 mmHg of MBP respectively which were superior than that of losartan at 10 mg/kg. The significant ($p < 0.05$) anti-hypertensive effect of compound **1a** lasted for at least 12 h and it did not influence heart rates of the rats.

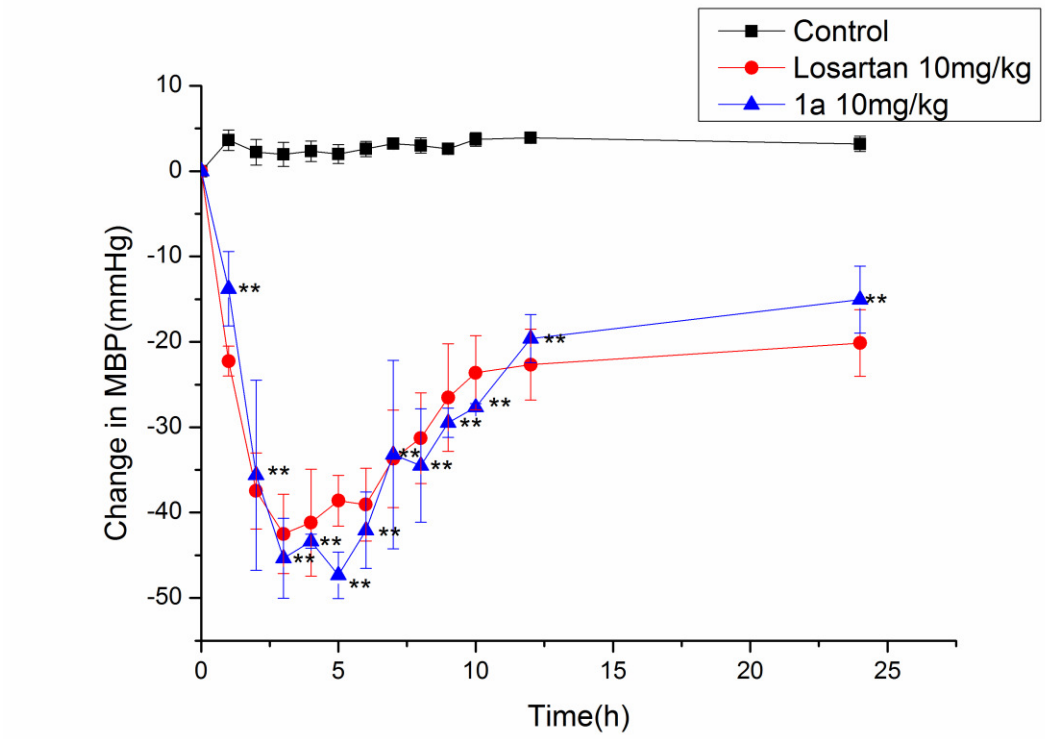

Fig 2. Effects of compound **1a** and Losartan on mean blood pressure (MBP) in spontaneously hypertensive rats. *and** Significantdifference from negative control, *$p < 0.05$ and **$p < 0.01$, respectively. (n=6)

The analytical procedures described were used to quantify compound in rat plasma samples obtained from 6 male Wistar rats which were orally administered with compound solution. Microsoft excel program, GraphPad Prism software and DAS 2.0 were used to calculate the pharmacokinetic parameters. The mean concentration–time curve of compound **1a** was shown in **Fig. 3**. The area under the concentration–time curve from 0 to 72 h (AUC_{0-72}) was estimated by linear trapezoidal rule. Maximum concentration (C_{max}) and time to reach C_{max} (T_{max}) were obtained directly from the observed concentration–time curve. Terminal half-life ($t_{1/2}$) was calculated as $t_{1/2}$ = 0.693/ke and ke was determined by linear regression of the logarithmical plasma concentration - time for the last four data points in the concentration–time curve(Yuan et al., 2013). The pharmacokinetic parameters of compound **1a** were shown in **Table 2**. The results showed that compound was absorbed quickly and metabolized slowly in animals.

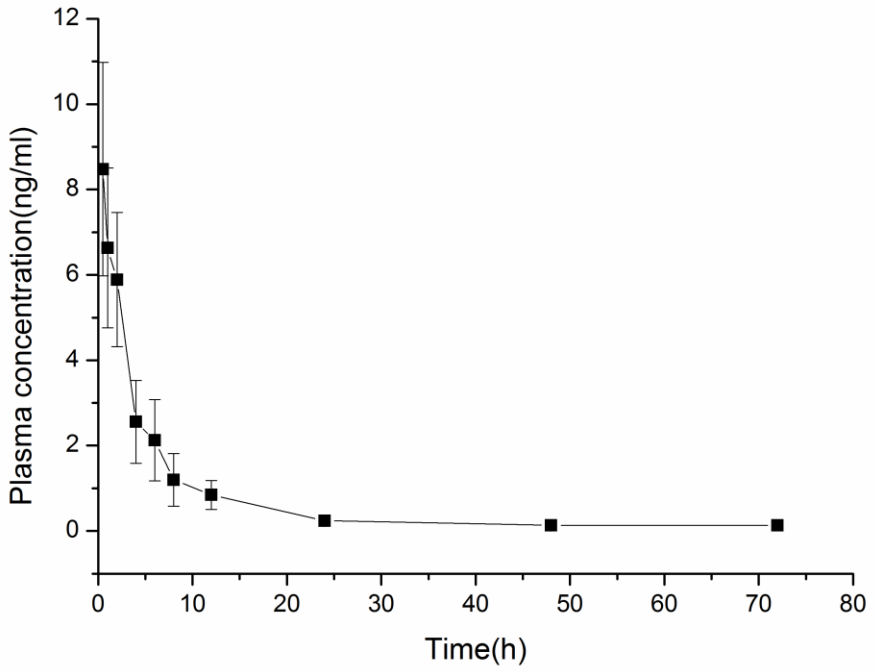

Fig 3. The plasma concentration-timecurve of **1a** in Wistar rats. (Data are average values of 6 experiments (Mean±SD).

Table 2 Pharmacokinetic parameters of compound **1a** in plasma of male Wistar rats after oral administration (5 mg/kg) (n=6).

Dose(mg/kg)	Pharmkinetic parameters
	AUC(0-72) (ng/mL h) AUC(0-∞) (ng/mL h) MRT(0-72) (h) MRT(0-∞) (h) T1/2 (h) Tmax(h) Cmax(ng/mL)
5	28.525±16.1128.755±16.5038.2±3.21810.233±5.452 6.54±5.5761±0.866 6.183±2.466

4.DISCUSSION AND CONCLUSION

In this study, a new AT_1 receptor antagonist **1a**was evaluated. The results showed**1a** had nanomolar affinity for the AT_1 receptor in radioligand binding assay *in vitro* and could cause significant decrease on MBP in a dose dependent manner in SHRs *in vivo*. **1a** showed highly competitive and specific affinity antagonist of AT_1 receptor, and also it caused significant and continuous reduction in blood pressure which could last more than 24 h in spontaneously hypertensive rats at 10 mg/kg. Pharmacokinetic experiments in Wistar rats showed that **1a**could be rapidly absorbed and gradually metabolized. From the present tests, **1a**could be considered as a novel candidate of anti-hypertensive drug with effective, long-lasting and worthy of further investigation.

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