Accepted Manuscript

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PII: S0968-0896(16)30177-8
DOI: http://dx.doi.org/10.1016/j.bmc.2016.03.028
Reference: BMC 12877

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 17 February 2016
Revised Date: 16 March 2016
Accepted Date: 16 March 2016

Please cite this article as: Bao, X., Zhu, W., Zhang, R., Wen, C., Wang, L., Yan, Y., Tang, H., Chen, Z., Synthesis and Evaluation of Novel Angiotensin II Receptor 1 Antagonists as Anti-hypertension Drugs, *Bioorganic & Medicinal Chemistry* (2016), doi: http://dx.doi.org/10.1016/j.bmc.2016.03.028

Synthesis and Evaluation of Novel Angiotensin II Receptor 1 Antagonists as Anti-hypertension Drugs

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Abstract:

Three new angiotensin II receptor 1 antagonists, **1**, **2** and **3** were designed, synthesized and evaluated. The AT_1 receptor-binding assays *in vitro* showed that all the synthesized compounds had nanomolar affinity for the AT_1 receptor. From which compound **3** was found to be the most potent ligands with an IC_{50} value of 2.67 ± 0.23 nM. Biological evaluation *in vivo* revealed that all the compounds could cause significant decrease on MBP in a dose dependent manner in spontaneously hypertensive rats, and compound **3** especially showed an efficient and long-lasting effect in reducing blood pressure, whose maximal response lowered 41 mmHg of MBP at 10 mg/kg and 62 mmHg at 15 mg/kg after oral administration, the significant anti-hypertensive effect lasted beyond 12 h, which is better than the reference compound losartan. The pharmacokinetic experiments showed that compound **3** could be absorbed efficiently and metabolized smoothly both in blood and in tissues in Wistar rats. The acute toxicity assay suggested that it has low toxicity with the LD_{50} value of 2974.35 mg/kg. These results demonstrate that compound **3** is a potent angiotensin AT_1 receptor antagonist which could be considered as a novel anti-hypertension candidate and deserved for further investigation.

Key words

Hypertension; Anti-hypertension; Angiotensin II; AT_1 receptor antagonist

1. Introduction

Hypertension is a complex multifactorial, polygenic disease that worsens with age, and leads to target-organ complications which span a spectrum comprising cardiac hypertrophy, heart failure, exacerbation of coronary heart disease, stroke and hypertensive chronic kidney disease[1]. Although, there are a number of pharmaceuticals such as diuretics, beta blockers, angiotensin converting enzyme inhibitors (ACE inhibitors), calcium channel antagonists, angiotensin receptor blockers (ARBs) have been invented for treating this disease, approximately one-third of the hypertensive population is still not adequately treated, and it remains one of the largest unmet medical needs in the 21st century[2, 3].

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. Classically, Ang II mediates its action via two major receptors, namely type 1 (AT_1) and type 2 (AT_2)[4, 5]. The angiotensin AT_1 receptor is closely associated with the regulation of blood pressure and electrolyte balance. The search for Angiotensin II receptor 1 antagonists as potential antihypertensive agents started 20 years ago[6], nowadays, several Ang II receptor 1 (AT_1) antagonists have been discovered and widely accepted as novel antihypertensive drug clinically because of its less side effects and good therapeutic profiles than ACE inhibitors[7].

Losartan (Figure 1) is the first Ang II receptor 1 antagonist launched which is widely used in reducing blood pressure. Based on the structure-activity relationship of losartan, we designed and synthesized 3 new compounds **1**, **2**, **3** (Figure 1) in which 5-oxo-1, 2, 4-oxadiazole in the structure formed the electron transport system and C- terminal carboxylic acid which is the charge area, n-butyl is the drain water area, diphenyl methyl is the intermediate connecting part, the fifth position of imidazole ring is alcohol aldehyde, acid, respectively. And then, the pharmacological profile of these compounds was investigated by receptor binding studies, antihypertension effect in spontaneously hypertensive rats (SHRs) *in vivo*. What's more, the pharmacokinetic characteristics and acute toxicity of selected compound were further investigated.

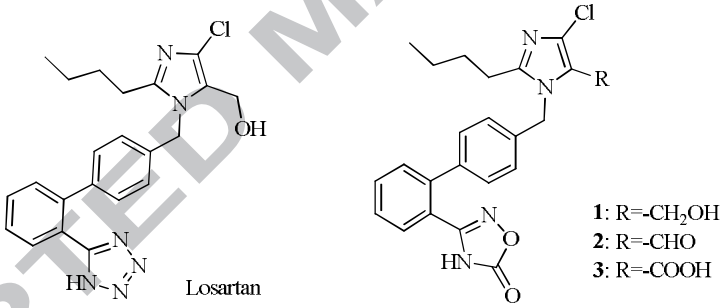


Figure 1. The chemical structure of losartan, compound **1**, **2** and **3**

2. Materials and methods

2.1 Chemistry

All chemical reagents were of highest commercially available quality and applied without further purification. Yields referring to purified products were not optimized. Melting points (m.p.) were measured on an electro thermal melting point apparatus and were uncorrected. ^{1}H-NMR spectra were measured on a Bruker 400 MHz Spectrometer using TMS (Me_4Si) as internal standard. ESI-MS spectra were recorded on a Micromass Triple Quadrupole Mass Spectrometer. Column chromatography was performed using silica gel H (300-400 meshes).

4'-((2-butyl-4-chloro-5-formyl-1H-imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-carbonitrile (6)

A solution of compound **4** (87.5 mg, 0.47 mmol) and K_2CO_3 (135 mg, 0.98 mmol) in DMF (10 mL) was treated with compound **5** (200.5 mg, 0.74 mmol), and the mixture was stirred at -10 ^{o}C under nitrogen for 12 h. The resulting mixture was diluted with water and extracted with ethyl acetate (30 mL × 4). The organic layer was washed with saturated salt water (40

mL × 4) and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The resulting residue was purified by column chromatography to give **6** (163 mg) as a yellow solid. Yield: 73.2%. ^{1}H NMR (400MHz, $CDCl_3$, ppm) δ: 9.78 (s, 1H), 7.76-7.43 (m, 6H), 7.18 (d, 2H), 5.62 (s, 2H), 2.68 (t, 2H), 1.71 (m, 2H), 1.36 (m, 2H), 0.89 (t, 3H). MS (ESI): $[M + H]^+$ calcd 378.1; found 378.2.

4'-((2-butyl-4-chloro-5-(hydroxymethyl)-1H-imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-carbonitrile (**7**)

A solution of compound **6** (500 mg, 1.33 mmol) in MeOH was treated with $NaBH_4$ (13 mg, 0.34 mmol) at 0 °C and stirred for 0.5 h. After the reaction was completed, the resulting mixture was diluted with water and extracted with ethyl acetate (30 mL × 4). The organic layer was washed with saturated salt water (40 mL × 4) and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The residue was purified by column chromatography to give 422 mg compound **7** as a yellow solid. Yield: 83.7%. ^{1}H NMR (400MHz, $CDCl_3$) δ: 7.77-7.44 (m, 6H), 7.12 (d, 2H), 5.29 (s, 2H), 4.52 (s, 2H), 2.59 (t, 2H), 2.03 (s, 1H), 1.68 (m, 2H), 1.33 (m, 2H), 0.88 (t, 3H). ^{13}C NMR (100MHz, $CDCl_3$) δ: 148.6, 144.6, 137.8, 136.8, 133.8, 132.9, 129.9, 129.4, 127.8, 127.3, 126.3, 124.9, 118.5, 111.2, 77.3, 77.1, 76.7, 53.0, 47.2, 29.7, 26.7, 22.4, 13.7. MS (ESI): $[M + H]^+$ calcd 380.2; found 380.3.

4'-((2-butyl-4-chloro-5-((methoxymethoxy)methyl)-1H-imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-carbonitrile (**8**)

A solution of compound **7** (500 mg, 1.32 mmol) in DCM was treated with chloromethyl methyl ether (126 mg, 1.58 mmol) and DIPEA (340 mg, 2.64 mmol) at r.t. and stirred for 3.5 h. The resulting mixture was diluted with water and extracted with DCM (30 mL × 4). The organic layer was washed with saturated salt water (40 mL × 4) and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The residue was purified by column chromatography to give 403 mg compound **8** as a yellow solid. Yield: 72.2%. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 7.72-7.42(m, 6H), 7.09(d, 2H), 5.22(s, 2H), 4.57(s, 2H), 4.45(s, 2H), 3.33(s, 3H), 2.57(t, 2H), 1.65(m, 2H), 1.33(m, 2H); 0.85(t, 3H). MS (ESI): $[M + H]^+$ calcd 424.2; found 424.2 [M + 1].

4'-((2-butyl-4-chloro-5-((methoxymethoxy)methyl)-1H-imidazol-1-yl)methyl)-N'-hydroxy-[1,1'-biphenyl]-2-carboximidamide (**9**)

A solution of compound **8** (500 mg, 1.18 mmol) in EtOH (50 mL) was treated with hydroxylamine hydrochloride (244 mg, 3.54 mmol) and K_2CO_3 (651 mg, 4.72 mmol) at 90 °C and stirred for 24 h. The resulting mixture was diluted with water (100 mL) and extracted with ethyl acetate (30 mL × 4). The organic layer was washed with saturated salt water (40 mL × 4) and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The resulting residue was purified by column chromatography to give 400 mg compound **9** as a yellow solid. Yield: 74.4%. MS (ESI): $[M + H]^+$ calcd 457.2; found 457.2 [M + 1].

4'-((2-butyl-4-chloro-5-((methoxymethoxy)methyl)-1H-imidazol-1-yl)methyl)-N'-((isobutoxycarbonyl)oxy)-[1,1'-biphenyl]-2-carboximidamide(**10**)

A solution of compound **9** (500 mg, 1.10 mmol) in DMF was treated with isobutyl chloroformate (180 mg, 1.32 mmol) and pyridine (174 mg, 2.20 mol) at 0 °C and stirred for 6 h under nitrogen. After the reaction was completed, the resulting mixture was diluted with water and extracted with ethyl acetate (30 mL × 4). The organic layer was washed with

saturated salt water (40 mL × 4) and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The residue was purified by column chromatography to give 481 mg compound **10** as a yellow solid. Yield: 78.7%. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 7.63-7.30 (m, 6H), 7.01 (d, 2H), 5.18 (s, 2H), 4.68 (s, 2H), 4.57 (s, 2H), 4.42 (s, 2H), 4.00 (d, 2H), 3.34 (s, 3H), 2.55 (t, 2H), 2.00 (m, 1H), 1.64 (m, 2H), 1.36-1.27 (m, 2H), 0.95 (d, 6H), 0.84 (t, 3H). MS (ESI): $[M + H]^+$ calcd 557.2; found 557.4 $[M + 1]^+$.

3-(4'-((2-butyl-4-chloro-5-((methoxymethoxy)methyl)-1H-imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-yl)-1,2,4-oxadiazol-5(4H)-one (**11**)

A solution of compound **10** (500 mg, 0.90 mmol) in 50 mL xylene was refluxed for 6 h under nitrogen. The solvent was removed under reduced pressure and the residue was purified by column chromatography to give 420 mg compound **11** as a yellow solid. Yield: 96.8%. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 7.72-7.25 (m, 6H), 6.88 (d, 2H), 5.18 (s, 2H), 4.48 (s, 2H), 4.27 (s, 2H), 3.26 (s, 3H), 2.33 (t, 2H), 1.52 (m, 2H), 1.28 (m, 2H); 0.84 (t, 3H). MS (ESI): $[M + H]^+$ calcd 483.2; found 483.3.

2-butyl-4-chloro-1-((2'-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-[1,1'-biphenyl]-4-yl)methyl)- 5-hydroxymethyl-1H-imidazole(**1**)

A solution of compound **11** (500 mg, 1.04 mmol) in 50 mL DCM was treated with TFA (10 mL) and stirred at r.t. for 28 h. The resulting mixture was diluted with water and extracted with ethyl acetate (30 mL × 4). The organic layer was washed with saturated salt water (40 mL × 4) and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The residue was purified by column chromatography to give 228 mg compound **1** as a yellow solid. Yield: 50.1%. ^{1}H NMR (400MHz, dimethyl sulfoxide(DMSO), ppm) δ: 7.73-7.38 (m, 6H), 7.16 (d, 2H), 5.88 (s, 2H, -NCH_2-), 2.67 (t, 2H), 2.61 (s, 2H), 1.62 (m, 2H), 1.36 (m, 2H), 0.92 (t, 3H). MS (ESI): $[M + H]^+$ calcd 439.2; found 439.3. HRMS (ESI): m/z calculated for $C_{23}H_{24}ClN_4O_3$ $[M + H]^+$: 439.1537; Found: 438.1459. IR (KBr, cm^{-1}): 3462.37, 2959.32, 2870.49, 1758.72, 1464.03, 1258.02, 1016.75, 756.46.

4'-((2-butyl-4-chloro-5-(1,3-dioxolan-2-yl)-1H-imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-carbonitrile(**12**)

A solution of compound **6** (500 mg, 1.33 mmol) in toluene was treated with ethylene glycol (247 mg, 3.99 mmol) and PPTS (33 mg, 0.13 mmol), the solution was refluxed for 7 h. The resulting mixture was diluted with water and extracted with ethyl acetate (30 mL × 4). The organic layer was washed with saturated salt water (40 mL × 4) and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The residue was purified by column chromatography to give 542 mg compound **12** as a yellow solid. Yield: 96.8%. ^{1}H NMR (400MHz, $CDCl_3$, ppm) δ: 7.75-7.42 (m, 6H), 7.20 (d, 2H), 5.92 (s, 1H), 5.26 (s, 2H), 3.98-3.93 (m, 4H), 2.47 (t, 2H), 1.59 (m, 2H), 1.28 (m, 2H), 0.82 (t, 3H). MS (ESI): $[M + H]^+$ calcd 422.2; found 422.2.

4'-((2-butyl-4-chloro-5-(1,3-dioxolan-2-yl)-1H-imidazol-1-yl)methyl)-N'-hydroxy-[1,1'-biphenyl]-2-carboximidamide(**13**)

A solution of compound **12** (500 mg, 1.19 mmol) in ethanol (50 mL) was treated with hydroxylamine hydrochloride (246 mg, 3.57 mmol) and K_2CO_3 (657 mg, 4.76 mmol) at 90 ^{o}C and stirred for 24 h. The resulting mixture was diluted with water (100 mL) and extracted with ethyl acetate (30 mL × 4). The organic layer was washed with saturated salt water (40 mL × 4) and dried over $MgSO_4$. After filtration, the solvent was removed under reduced

pressure. The resulting residue was purified by column chromatography to give 390 mg compound **13** as a yellow solid. Yield: 72.3%. ^{1}H NMR (400MHz, $CDCl_3$, ppm) δ: 7.65-7.33 (m, 6H), 7.13 (d, 2H), 5.92 (s, 1H), 5.22 (s, 2H), 4.41 (s, 2H), 3.98-3.92 (m, 4H), 2.47 (t, 2H), 1.57 (m, 2H), 1.26 (m, 2H), 0.87 (t, 3H). MS (ESI): $[M + H]^+$ calcd 455.2; found 455.3.

4'-((2-butyl-4-chloro-5-(1,3-dioxolan-2-yl)-1H-imidazol-1-yl)methyl)-N'-((isobutoxycarbonyl)oxy)-[1,1'-biphenyl]-2-carboximidamide(14)

A solution of compound **13** (500 mg, 1.10 mmol) in DMF (50 mL) was treated with isobutyl chloroformate (180 mg, 1.32 mmol) and pyridine (174 mg, 2.20 mol) at 0 ^{o}C and stirred for 6 h under nitrogen. After the reaction was completed, the resulting mixture was diluted with water and extracted with ethyl acetate (30 mL × 4). The organic layer was washed with saturated salt water (40 mL × 4) and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The residue was purified by column chromatography to give 252 mg compound **14** as a yellow solid. Yield: 41.3%. ^{1}H NMR (400MHz, $CDCl_3$, ppm) δ: 7.67-7.34 (m, 6H), 7.14 (d, 2H), 5.92 (s, 1H), 5.24 (s, 2H), 4.03 (d, 2H), 4.04-3.95 (m, 4H), 2.54 (t, 2H), 2.04-2.00 (m, 1H), 1.59 (m, 2H), 1.28 (m, 2H), 0.97 (d, 6H), 0.83 (t, 3H). MS (ESI): $[M + H]^+$ calcd 455.2; found 555.3.

3-(4'-((2-butyl-4-chloro-5-(1,3-dioxolan-2-yl)-1H-imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-yl)-1,2,4-oxadiazol-5(4H)-one(15)

A solution of compound **14** (500 mg, 0.90 mmol) in xylene (50 mL) was refluxed for 6 h under nitrogen. After the reaction was completed, the solvent was removed under reduced pressure. The residue was purified by column chromatography to give 400 mg compound **15** as a yellow solid. Yield: 92.8%. ^{1}H NMR (400 MHz, $CDCl_3$,) δ: 7.82-7.00 (m, 8H), 5.79 (s, 1H), 5.22 (s, 2H), 3.89 (s, 4H), 2.38 (t, 2H), 1.52 (m, 2H), 1.25 (m, 2H), 0.82 (t, 3H). MS (ESI): $[M + H]^+$ calcd 481.2; found 481.3.

2-butyl-4-chloro-1-((2'-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-[1,1'-biphenyl]-4-yl)methyl)-1H-imidazole-5-carbaldehyde(2)

A solution of compound **15** (500 mg, 1.04 mmol) in DCM (50 mL) was treated with PPTS (261 mg, 1.04 mmol) and stirred at r.t. for 3 h. After the reaction was completed, the resulting mixture was diluted with water and extracted with ethyl acetate (30 mL × 4). The organic layer was washed with saturated salt water (40 mL × 4) and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The residue was purified by column chromatography to give 303 mg **2** as a yellow solid. Yield: 66.8%. ^{1}H NMR (400 MHz, DMSO, ppm) δ: 9.65 (s, 1H), 8.95 (s, H), 7.78-7.08 (m, 8H), 5.54 (s, 2H), 2.66 (t, 2H), 1.69 (m, 2H), 1.36 (m, 2H), 0.90 (t, 3H). MS(ESI): $[M + H]^+$ calcd 437.2; found 437.3. IR (KBr, cm^{-1}): 3431.17, 2959.71, 2860.32, 2745.12, 2667.68, 1773.81, 1670.76, 1598.95, 1522.89, 1492.38, 1462.05, 1378.28, 1278.41, 1221.56, 941.26, 868.10, 763.50, 723.22.

2-butyl-4-chloro-1-((2'-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-[1,1'-biphenyl]-4-yl)methyl)-1H-imidazole-5-carboxylic acid(3)

A solution of compound **2** (500 mg, 1.14 mmol) in butanol (50 mL) was treated with $NaClO_2$ (1.03 g, 11.4 mmol) and NaH_2PO_4 (1.37 g, 11.4 mmol) in 20 mL water, and the solution was stirred at r.t. for 5 h. After the reaction was completed, the resulting mixture was diluted with water and extracted with ethyl acetate (30 mL × 4). The organic layer was washed with saturated salt water (40 mL × 4) and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The residue was purified by column chromatography to give

435 mg **3** as a yellow solid. Yield: 84.6%. ^{1}H NMR (400 MHz, DMSO, ppm) δ: 12.42 (s, 1H); 7.72-7.14 (m, 8H), 5.62 (s, 2H), 2.58 (t, 2H), 1.46 (m, 2H), 1.24 (m, 2H), 0.80 (t, 3H). MS (ESI): $[M + H]^+$ calcd 453.1; found 453.3. HRMS (ESI): m/z calculated for $C_{23}H_{22}N_4O_4Cl$ $[M + H]^+$: 453.1330; found 452.1244. IR (KBr, cm^{-1}): 3461.39, 2958.23, 2933.47, 2872.61, 1756.6, 1499.70, 1464.10, 1259.31, 1020.62, 1007.46, 757.19.

2.2 Biological evaluation

2.2.1 Binding affinities to Ang II (AT_1) receptor *in vitro*

The affinity toward AT_1 receptor of the new compounds were tested by their ability to displace [^{125}I]-Ang II from its specific binding sites in vascular smooth muscle cells(VSMCs, Abcore-inc, co., Ltd, Shanghai, China) line of rats. 3 - 6 generations of VSMCs were used for experiments. Losartan (Shanghai Zhongkang Weiye Biological Technology Co., Ltd) and new compounds **1**, **2**, **3** were dissolved in DMSO and diluted to different concentrations (10^{-10} - 10^{-4} M) with PBS before experiments. ^{125}I-Ang II (Zhongshan Hospital, Fudan University, Shanghai, China) was 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 ^{o}C, 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 compounds of diffident concentrations and then cultivated 4 □ for 150 min. The final concentrations of the compounds were 10^{-12} - 10^{-6} M. And then nonspecific binding represented 5 - 10% of total binding which was measured in presence of 1 μM Ang II. The resulting VSMCs were washed 3 times with PBS and digested for 10 min with 0.1 M NaOH[8, 9]. These cells bound by ^{125}I-Ang II were counted by γ - counter (SN - 682, Ri Huan Company, Shanghai, China). IC_{50} value and the half inhibition constant of the combination of compounds **1**, **2**, **3** with AT_1 receptor were estimated by the nonlinear portion of the competition curves.

2.2.2 Anti - hypertension effect in spontaneously hypertensive rats

Compounds **1**, **2**, **3** were subjected to biological evaluation for their effects on blood pressure of spontaneous hypertensive rats (SHRs) (250 ± 20g, Charles River Experimental Animal Technology Co. Ltd., Beijing, China). The systolic blood pressure (SBP) and diastolic blood pressure (DBP) of SHRs were measured by noninvasive tail artery manometry under conscious state. 48 male SHRs were divided into eight experimental groups randomly: negative control group, positive control group (losartan 10 mg/kg), compound **1** low-dose and high-dose groups, compound **2** low-dose and high-dose groups, compound **3** low-dose and high-dose groups. Compounds **1**, **2**, **3** and losartan were suspended in DMSO and oleic acid (V_1 : V_2 = 1 : 4).

Rats in compound **1**, **2**, **3** groups were administered orally with compound at the dose of 10 mg/kg and 15 mg/kg respectively. Rats in positive control group were administrated with losartan (10 mg/kg). And rats in negative control group were administered with the same volume of the solvent. The blood pressure and heart rates were monitored before and after the 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

[10], and results were expressed as mean ± SEM. A probability level of less than 0.05 was considered significant.

2.2.3 Pharmacokinetic assays

High performance liquid chromatography (HPLC) method was used to analyze the drug concentrations in plasma. The method was developed, validated and operates on two separate but similar Waters-brand systems. The primary system comprised an Alliance 2489 separations module, equipped with quaternary pump, on-line degasser and auto sampler, and a 600 photodiode array ultraviolet detector. Instrument control, data acquisition and data processing were achieved with Waters Empower software. The flow rate was 0.8 mL/min and the injection volume was 20 μL. Separations were performed at ambient temperature on a reverse phase WondasiliconC18-WR column (150 mm × 4.6 mm, 5 μm). Mobile phases A was 0.1% formic acid contained 2 mM ammonium acetate (20%) and Mobile phases B was methanol (80%).

2.2.3.1 Drug concentration in plasma

8 wistar rats were administrated with compound **3** at dose of 10 mg/kg. 0.5 mL venous blood was taken before administration and 0.5, 1, 2, 4, 6, 8, 12, 24, 48 and 72h after administration. Plasma was extracted by centrifuging at 4000 × g, 4□ for 10 minutes. Then 200 μL plasma samples for analysis were placed into a 1.8 mL polypropylene microfuge tube followed by 100 μL of internal standard. Acetonitrile (200 μL) was added to precipitate proteins and the tube vortex mixed for 30s. Precipitated proteins were separated by centrifugation for 5 min at 8,000 × g. The supernatant was filted by needle filter of 0.45 μL and then 20 μL filtrate was taken to analyze in HPLC. Linearity for compound **3** was tested by extracting plasma standards spiked at nominal concentrations of 1, 5, 10, 50, 100, 500 μg/mL (1, 5, 10, 50, 100, 500 μg/mL for compound **3**). 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^{-2}[11].

2.2.3.2 Drug distribution in tissues

66 Wistar rats were administrated with compound *c* at the dose of 10 mg/kg. Rats were killed by cutting the abdominal aorta, and the heart, spleen, kidney, brain and stomach were quickly excised at each designated time point (0.5, 1, 2, 4, 6, 8, 12, 24, 48 and 72h after administration). The tissues were cut into small pieces and then grinding at 4°C with normal saline using a Polytron homogeniser (PRO, USA) to prepare a 25% (w/v) homogenate dilution. Compound **3** and its metabolites in solution (final concentration of 0.01, 0.05, 0.1, 0.5, 1 and 5 μg/mL) were added to the tissue homogenate and vortexed at 4 °C for 5 min. Then the tissue homogenate dilution were extracted by centrifuging at 3000 × g, 4 □ for 10 min, supernatant fluid was collected and analyzed by HPLC[12, 13].

2.2.4 Acute toxicity experiments

The toxicity of compound **3** was determined in sixty normal ICR mice with half males and half females (20 ± 2 g, Academia Sinica, Shanghai, China). The lethal dose (LD_{50}) was delivered via intragastric administration at doses of 1200, 1600, 2133.3, 2844.40, 5056.79,

6742.38 mg/kg respectively. Survival rate was assessed daily for two weeks. The LD_{50} value and 95% confidence limits were determined from logistic regression analysis (GLM) curve fitting of the 14 days mortality data. They were observed continuously and recorded systematically for the physical signs of toxicity including skin changes, mobility, aggressiveness, respiratory movements and so on. The survivals were dissected and their organs and tissues were examined for pathological changes on the fifteenth day.

2.5 Statistics

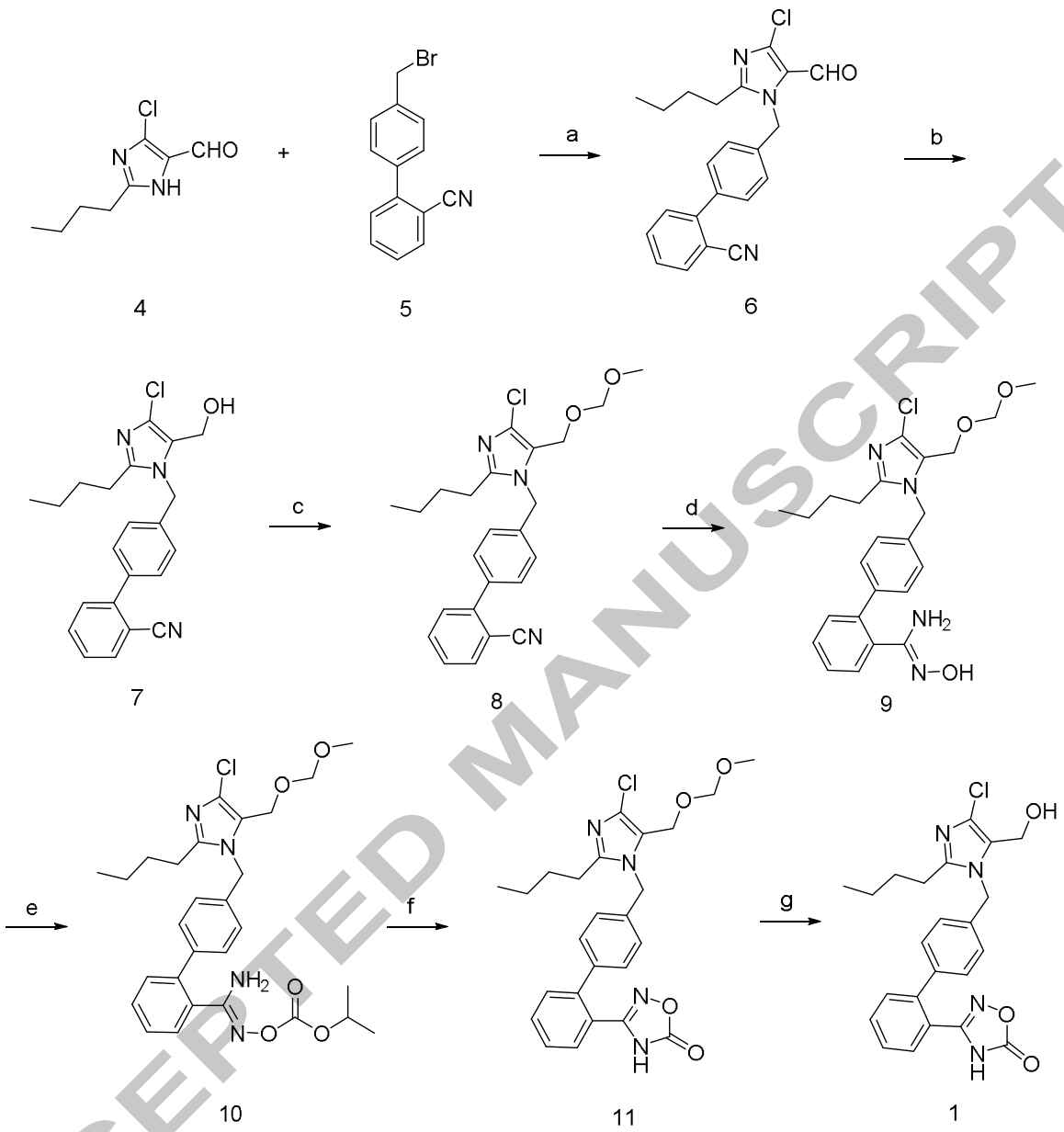
Results were expressed as means ± standard error of the means. Data were analyzed by one-way analysis of variance. When overall statistical significance was achieved ($P < 0.05$), student's t-test was used to compare each of the doses to the vehicle control. Probability values less than 0.05 were considered to be significant. Binding isotherms from competition studies were obtained using the nonlinear regression program GraphPad Prism 5 software (Network of Science Software of China) and LD_{50} were obtained using SPSS Statistics 20 (International Business Machines Corporation, New York, USA). The completed animal research here adhered to the "Principles of Laboratory Animal Care" and was approved by IACUC.

3 Results

3.1 Chemistry

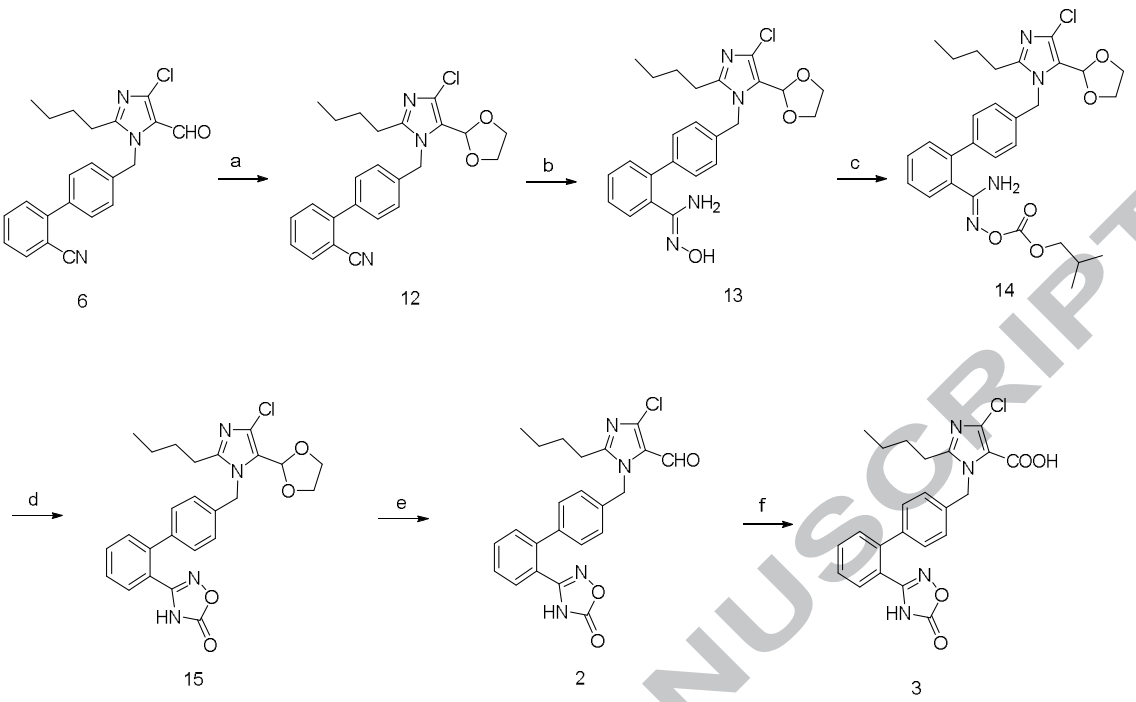
The preparation of imidazole derivatives **1**-**3** were performed by means of a multistep procedure described in **Scheme 1**-**2**.

The synthesis of compound **1** was accomplished starting from the suitable commercially available 2-butyl-4-chlorine-5-formyl imidazole (**4**), which reacted with 2- 4'- bromomethyl phenyl benzonitrile (**5**) in DMF using K_2CO_3 as base to give imidazole **6**. Compound **6** was hydrogenated and the resulting hydroxy group was protected by dimethoxymethane to give compound **8**. After reaction with hydroxylamine and then isobutyl chloroformate, the cyano group of **8** was transferred into 5-oxo-1, 2, 4-oxadiazol group of **11**. The protecting group methoxymethoxy of **11** was removed with TFA to generate target compound **1**.



Scheme 1. Reagents and reaction conditions: (a) K_2CO_3, DMF, -10 ^oC, 12h, 73.2%; (b) $NaBH_4$, CH_3OH, 0 ^oC, 0.5h, 83.7%; (c) $ClCH_2OCH_3$, $((CH_3)_2CH)_2NCH_2CH_3$, CH_2Cl_2, R.T., 3.5h, 72.2%; (d) $NH_2OH{\cdot}HCl$, K_2CO_3, EtOH, 90^oC, 24h, 74.4%; (e) $ClCOOCH_2CH(CH_3)_2$, C_6H_5N, DMF, 0 ^oC, 6h, 78.7% ; (f) xylene, reflux, 6h, 96.8%; (g)CF_3COOH, CH_2Cl_2, R.T., 28h, 50.1%.

The synthesis of compounds **2** and **3** was started from the protection reaction of aldehyde group of compound **6** with ethylene glycol. The resulting compound **12** was reacted with hydroxylamine and then isobutyl chloroformate to give 5-oxo-1,2,4-oxadiazol **15**. Compound **15** was deprotected by PPTS in acetone to generate **2**. Compound **2** was then oxidized to give the target compound **3**.



6 12 13 14 15 2 3

Scheme 2. Reagents and reaction conditions: (a) $HOCH_2CH_2OH$, PPTS, toluene, reflux, 7 h, 96.8%; (b) $NH_2OH \cdot HCl$, K_2CO_3, EtOH, 90 ^oC, 24 h, 72.3%; (c) $ClCOOCH_2CH(CH_3)_2$, pydridine, DMF, 0 ^oC, 6 h, 41.3%; (d) xylene, reflux, 6 h, 92.8%; (e) PPTS, CH_2Cl_2, reflux, 3 h, 66.8%; (f) $NaClO_2$, NaH_2PO_4, n-BuOH, H_2O, 84.6%

3.2 Biological evaluation

3.2.1 Binding affinities to Ang II (AT_1) receptor *in vitro*

Radioligand binding assay showed that the compounds **1**, **2**, **3** had nanomolar affinity to angiotensin type 1 receptor (Table 1, Figure 2). In competition experiments, compounds **1**, **2**, **3** and losartan could compete dose **-** dependently with ^{125}I **-** Ang II. Compound **3** displayed the highest specific affinity to the AT_1 receptor with the IC_{50} value of 2.67 ± 0.23 nM and the Ki value of 2.0 ± 0.17 nM which was more affinity than losartan (IC_{50} = 19.15 ± 0.15 nM, Ki = 14.36 ± 0.11 nM).

Table 1. Radioligand binding assay (binding IC_{50}) of new compounds **1**, **2**, **3** and losartan.

Compound	IC_{50} ± SEM (nM)	Ki (nM)
1	5.94 ± 0.22	4.30 ± 0.16
2	4.32 ± 0.15	3.13 ± 0.11
3	3.16 ± 0.19	2.29 ± 0.01
Losartan	19.15 ± 0.21	13.86 ± 0.15

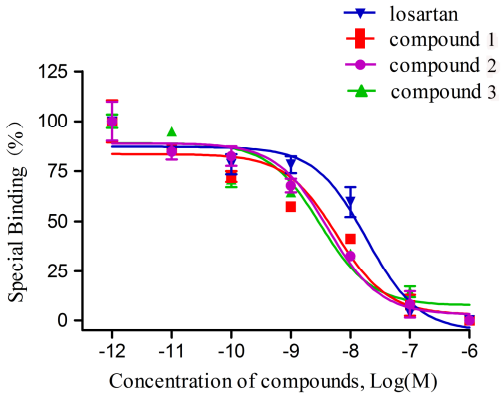
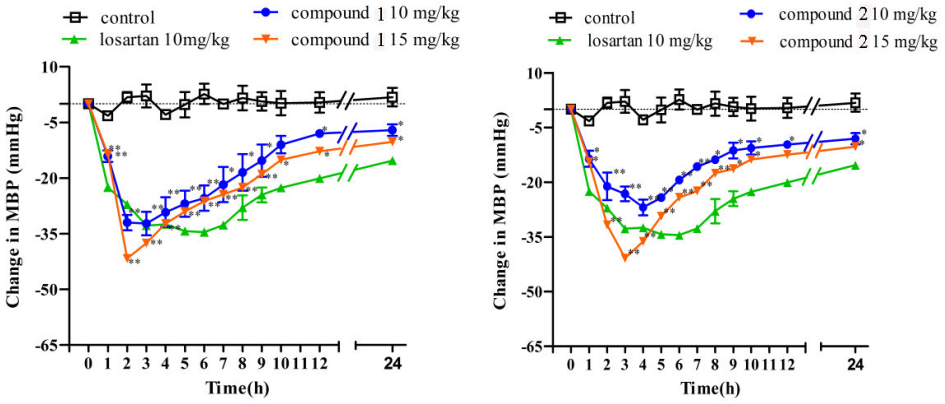


Figure 2. Inhibitory effects of new compounds **1**, **2**, **3** and losartan (10^{-6} - 10^{-12} M) on the specific binding of ^{125}I-Ang II to AT_1 receptors in VSMCs.

3.2.2 Antihypertensive effects *in vivo*

The effects of compound **1**, **2**, **3** (10, 15 mg/kg), losartan(10 mg/kg) on the mean blood pressure (MBP) *in vivo* after oral administration in spontaneously hypertensive rats (SHRs) were shown in Figure 3. The results indicated that all the compounds could decrease the blood pressure significantly compared with the negative control group, among which compound **3** played more effective than compound **1** and **2**. The maximal response of compound **3** (10, 15 mg/kg) was observed at 2h after dosing with reduction of 41 mmHg and 62 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 **3** lasted for at least 12h. Besides, compound **3** (15 mg/kg) was more effective than losartan (10 mg/kg), and it did not influence heart rates of the rats. Thus, compound **3** may have the potent to be a novel anti-hypertension candidate and deserved for further investigation.



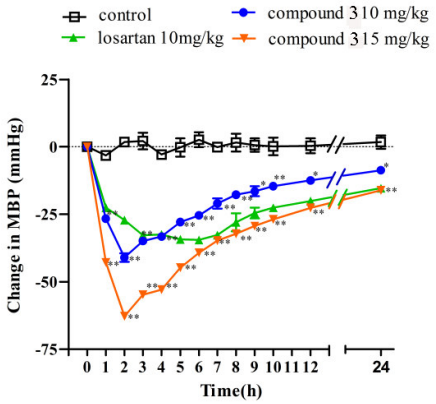


Figure 3. Effects of compound **1**, **2**, **3** (10 mg/kg, 15 mg/kg) and losartan (10 mg/kg) on mean blood pressure (MBP) in spontaneously hypertensive rats (n = 6). *, **Significant difference from the control, $p < 0.05$ and $p < 0.01$, respectively.

3.2.3 Pharmacokinetic study

3.2.3.1 Drug concentration in plasma

The analytical procedures described were used to quantify compound **3** in rat plasma samples obtained from 6 male Wistar rats which were orally administered with compound **3** 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 **3** was shown in Figure 4. The area under the concentration - time curve from 0 **-** 72 h ($AUC_{0\sim72}$) 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 curves. Terminal half **-** life ($t_{1/2}$) was calculated as $t_{1/2} = 0.693/k_e$ and k_e was determined by linear regression of the logarithmical plasma concentration vs time for the last four data points in the concentration-time curve[14]. The pharmacokinetic parameters of compound **3** were shown in table 2. The results showed that compound **3** absorbed quickly and metabolized slowly in animals and worthy of further researching.

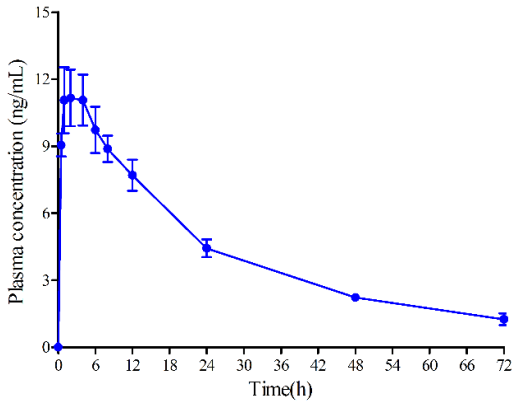


Figure 4. The mean concentration **-** time curve in rat plasma after oral administration of compound **3**.

Table 2. Pharmacokinetic parameters of compound **3** on plasma after oral administration.

Parameter	Unit	Compound **3**
T_{max}	h	2.00 ± 0.20
C_{max}	ng/mL	10.30 ± 0.05
k_e	h^{-1}	0.04 ± 0.005
$t_{1/2}$	h	18.17 ± 0.36
$AUC_{0\sim72}$	ng/mL·h	295.80 ± 2.35

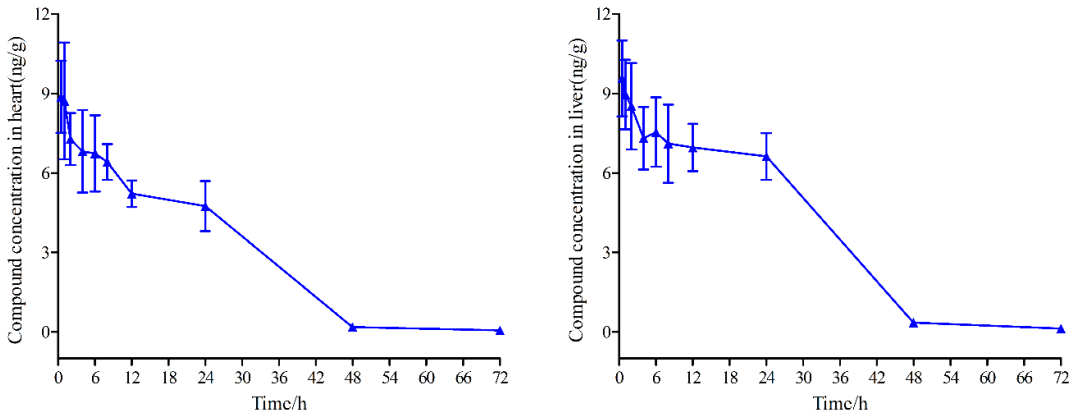
3.2.3.2 Drug distribution in tissues

The time courses of the concentration of compound **3** and its metabolites in the heart, liver, spleen, lung, kidney, brain and stomach following oral administration to rats were shown in Figure 5. The main parameters including AUC values in each tissue were shown in Table 3. The AUC value of compound **3** in the liver was larger than those in other tissues, and the AUC values in brain were the minimum (table 3). The distribution of compound **3** to the heart, liver, kidney and stomach tissues was rapid, and these concentrations peaked within 0.5 h (Figure 5). From the results shown in table 4, the compound **3** was highly distributed in spleen and liver.

Table 3. Pharmacokinetic parameters of compound **3** on tissue after oral administration to rats

Parameter	Unit	Heart	Liver	Spleen	Lung	Kidney	Brain	Stomach
T_{max}	h	0.5±0.32	0.5±0.21	4±0.36	1±0.29	0.5±0.15	2±0.59	0.5±0.16
C_{max}	ng/g	8.87±0.53	9.56±0.84	8.84±0.12	9.16±0.59	10.46±0.49	5.80±0.54	8.91±0.21
ke	h^{-1}	0.076±0.01	0.07±0.02	0.079±0.01	0.059±0.03	0.04±0.005	0.041±0.01	0.05±0.004
$t_{1/2}$	h	9.13±0.89	10.69±0.03	8.74±0.03	12.36±2.09	15.61±0.05	16.9±0.01	15.06±0.005
$AUC_{0\sim72}$	ng/g·h	200.41±0.69	260.05±1.99	275.21±0.64	213.96±7.67	202.97±7.34	123.07±2.61	221.03±107.6

(A) (B)



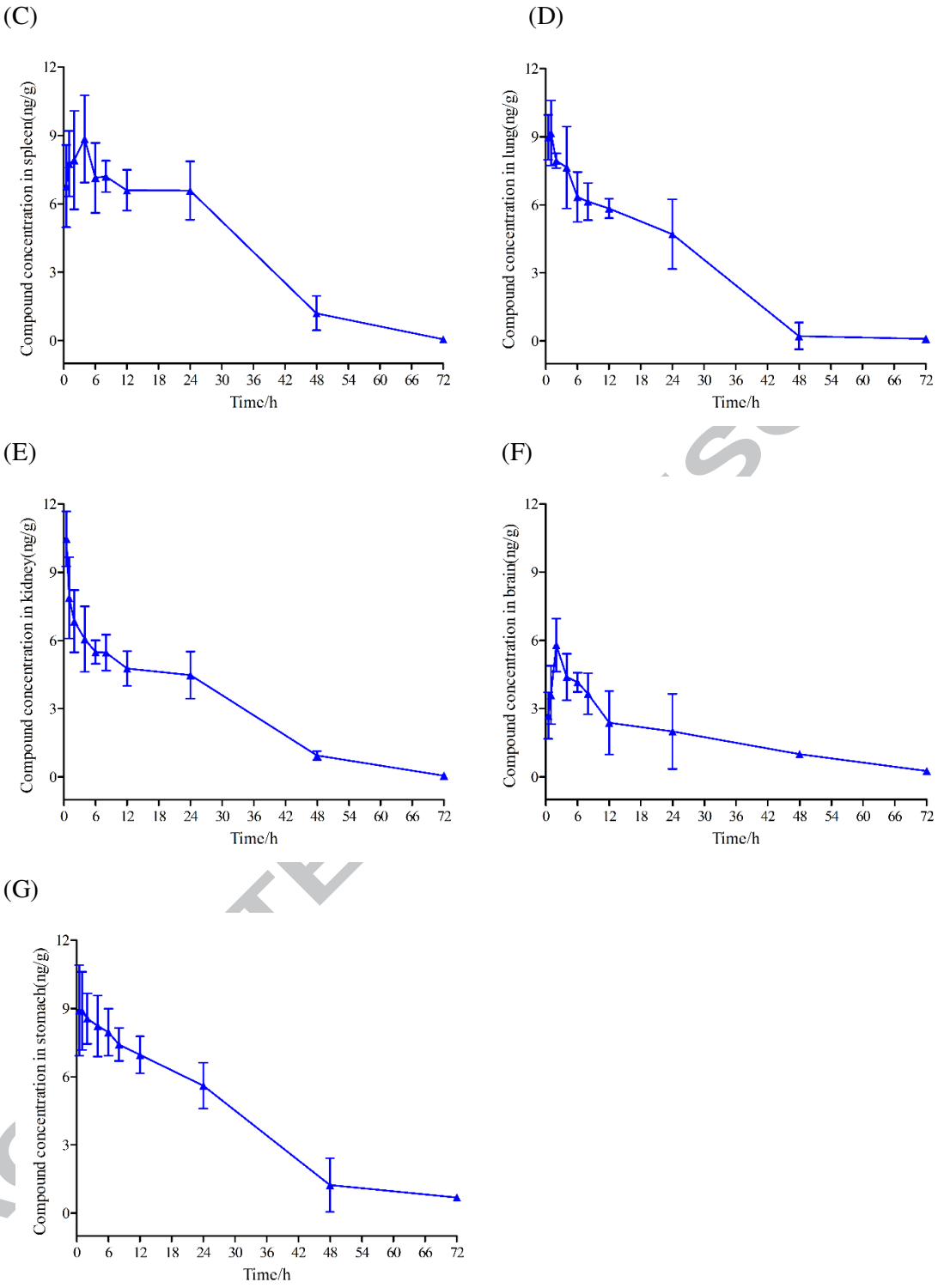


Figure 5. The mean concentration-time curves in the heart tissue (A), liver tissue (B), spleen tissue (C), lung tissue (D), kidney tissue (E), brain tissue (F), stomach tissue (G), of rats after administration. Each point represents the mean ± SEM (n = 5).

3.2.4 Acute toxicity experiments

The acute toxicity assay of compound **3** proved its low acute toxicity. The LD_{50} value of compound **3** was 2974.35 mg/kg and the 95% confidence interval was 2479.79 **-** 3694.45 mg/kg (Table 4). There was no physiological abnormalities appeared in mice administered at dose of 1200 mg/kg. However, hyperthyroidism at 5 h after administration appeared in others groups, and some mice died. There was no significant change in weight of survival mice after two weeks observation and no obvious untoward reaction. Besides, no abnormality was observed with the naked eye of the survival mice after dissection.

Table 4. The lethal dose (LD_{50}) of compound **3** determined by acute toxicity test

Dose(mg/kg)	Log(dose)	Mortality (%)	LD_{50} and 95% confidence interval (mg/kg)
1200.00	3.08	0	2974.35 (2479.79 - 3694.45)
1600.00	3.20	10	
2133.30	3.33	20	
2844.40	3.45	40	
5056.79	3.70	90	
6742.38	3.83	100	

4. Discussion and Conclusion

In this study, three new AT_1 receptor antagonists（**1**, **2**, **3**）were designed, synthesized and evaluated. All the synthesized compounds showed nanomolar affinity for the AT_1 receptor in radioligand binding assay *in vitro* and could cause decrease on MBP in a dose dependent manner in spontaneously hypertensive rats *in vivo*. Among them compound **3** 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 12 h in spontaneously hypertensive rats at 10 mg/kg. Pharmacokinetic experiments in Wistar rats showed that compound **3** could be absorbed rapidly and metabolized gradually. Besides, compound **3** was safe with an LD_{50} value of 2974.35 mg/kg in acute toxicity assay. From the present tests, compound 3 could be considered as a candidate of antihypertensive drug with effective, long-lasting and low toxic characteristics, and worthy of further investigation.

5. Acknowledgement

This work was supported by funds of Science and Technology Commission of Shanghai Municipality (Grants No. 13431900700, 14431906200), found of Science and Technology Bureau of Ningbo (Grant No. 2015A610274), and fundamental research fund of the Central Universities (Grant No. 16D310611).

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Tables

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Table 1

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Losartan	19.15 ± 0.21	13.86 ± 0.15

Table 2

Parameter	Unit	Compound **3**
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k_e	h^{-1}	0.04 ± 0.005
$t_{1/2}$	h	18.17 ± 0.36
$AUC_{0\sim72}$	ng/mL·h	295.80 ± 2.35

Table 3

Parameter	Unit	Heart	Liver	Spleen	Lung	Kidney	Brain	Stomach
T_{max}	h	0.5±0.32	0.5±0.21	4±0.36	1±0.29	0.5±0.15	2±0.59	0.5±0.16
C_{max}	ng/g	8.87±0.53	9.56±0.84	8.84±0.12	9.16±0.59	10.46±0.49	5.80±0.54	8.91±0.21
ke	h^{-1}	0.076±0.01	0.07±0.02	0.079±0.01	0.059±0.03	0.04±0.005	0.041±0.01	0.05±0.004
$t_{1/2}$	h	9.13±0.89	10.69±0.03	8.74±0.03	12.36±2.09	15.61±0.05	16.9±0.01	15.06±0.005
$AUC_{0\sim72}$	ng/g·h	200.41±0.69	260.05±1.99	275.21±0.64	213.96±7.67	202.97±7.34	123.07±2.61	221.03±107.

Table 4.

Dose(mg/kg)	Log(dose)	Mortality (%)	LD_{50} and 95% confidence interval

			(mg/kg)
1200.00	3.08	0	
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2844.40	3.45	40	(2479.79 - 3694.45)
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Graphical abstract