Bioorganic & Medicinal Chemistry 20 (2012) 2747–2761

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and biological evaluation of novel potent angiotensin II receptor antagonists with anti-hypertension effect

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ARTICLE INFO

Article history:
Received 21 October 2011
Revised 31 January 2012
Accepted 1 February 2012
Available online 11 February 2012

Keywords:
Angiotensin II receptor antagonists
Hypertension
Synthesis
Acute toxicity

ABSTRACT

A series of novel angiotensin II type 1 receptor antagonists were prepared. Radioligand binding assay suggested that compounds **1b** and **1c** could be recognized by the AT_1 receptor with an IC_{50} value of 1.6 ± 0.09 nM and 2.64 ± 0.7 nM, respectively. In vivo anti-hypertension experiments showed that compounds (**1a, 1b, 1c, 1e**) elicited a significant decrease in SBP and DBP of spontaneous hypertensive rats (SHRs). The antihypertensive effects maintained for 10 h, which indicated that these compounds had a favorable blood pressure-lowering effect. Acute toxicity testing suggested that the LD_{50} value of compound **1b** was 2316.8 mg/kg which was lower than valsartan (LD_{50} = 307.50 mg/kg) but higher than losartan (LD_{50} = 2248 mg/kg). So they could be considered as novel anti-hypertension candidates and deserved for further investigation.

1. Introduction

The rennin angiotensin system (RAS) plays a central role in the regulation of blood pressure and electrolyte/fluid homeostasis. It consists of a cascade of enzymatic reactions producing angiotensin II (Ang II), which is an octapeptide that presents a wide range of physiological actions on cardiovascular, renal, endocrine and central nervous systems.[1,2]

Ang II is produced in vivo from angiotensin I by the angiotensin converting enzyme (ACE). ACE inhibitors, such as Enalapril and Captopril, are widely used in clinic to control hypertension. But ACE has its limitation in clinical use for some of the major side effects, such as dry cough, angioedema and rashes, resulted from lack of specificity to ACE for Ang I.[3] However, angiotensin II type 1 (AT_1) receptor antagonists, namely the angiotensin II type 1 receptor blockers (ARBs), are selective for AT_1 receptors, and act independently on the Ang II synthetic pathway.[4] AT_1 receptor antagonists are the most specific means presently available to block the renin-angiotensin enzymatic cascade. In addition, studies in clinic and experiments have demonstrated the protective effect of AT_1 blockers on cardiovascular morbidity and mortality.[5] The discovery of potent and orally active nonpeptide Ang II antagonists with few side effects such as valsartan, and losartan has encouraged the design of a large number of similar compounds. Among them, candesartan, irbesartan, telmisartan, and olmesartan are also being used in the clinics and lots of other congeners are being developed.

Most of ARBs have acidic group tetrazole ring, such as valsartan, losartan, candesartan, ibersartan. The tetrazole group had some disadvantages in chemical synthesis and biometabolism. For example, the synthesis of tetrazole derivatives could be dangerous due to the use of toxic and explosive azide compounds such as sodium azide or trialkytin azide. So it is important to find an acidic group in place of tetrazole. 5-Oxo-1,2,4-oxadiazol was found to be a suitable substitute because it is easy to be prepared with high yield, and friendly to environment. So another new ARB azilsartan medoxomil (Edarbi-Takeda) was developed and approved in clinics by FDA in 2011 for oral treatment of hypertension, either alone or combined with other drugs. azilsartan medoxomil at its maximal dose had superior efficacy to both valsartan and olmesartan at their maximal approved doses without increasing adverse events.[6] Bakris also reported that azilsartan medoxomil was well tolerated and more efficacious at its maximal dose than the highest dose of olmesartan.[7]

In the present work 5-oxo-1,2,4-oxadiazol was used to displace tetrazole group of valsartan which is appeared in some ARBs in clinics.[8] So a series of new compounds **1a–g** with N-acylated aminoacid moiety and biphenyl moiety were synthesized (Table 1). Fluorine atoms was also introduced in *para*-position of 5-oxo-1,2,4-oxadiazol to raise the acidity of the molecular **2**. In addition, the preliminary pharmacological characteristics including the effect on hypertension, and acute toxicity of compound **1b** was also presented to the development of novel anti-hypertension.

The dominant conformations of compound **1a** and valsartan are shown as Figure 1 using software Spartan 8. We found that the

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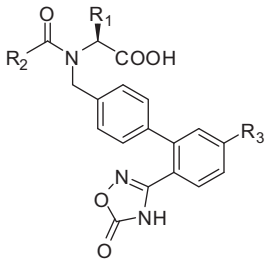
doi:10.1016/j.bmc.2012.02.003

Table 1
Oxadiazole compounds **1a–f**



Compound	R_1	R_2	R_3	Mp (°C)	$[\alpha]_D^{24}$ (c3, EtOH)	Formula
1a	^{i}Pr	^{n}Bu	H	112–113	−55	$C_{25}H_{29}N_3O_5$
1b	^{i}Pr	^{n}Pr	H	86–88	−48	$C_{24}H_{27}N_3O_5$
1c	^{i}Pr	Et	H	79–81	−45	$C_{23}H_{25}N_3O_5$
1d	^{i}Bu	^{n}Bu	H	123–124	−67	$C_{26}H_{31}N_3O_5$
1e	^{i}Bu	^{n}Pr	H	115–117	−86	$C_{25}H_{29}N_3O_5$
1f	$PhCH_2$	^{n}Bu	H	166–167	−101	$C_{29}H_{29}N_3O_5$
1g	$PhCH_2$	^{n}Pr	H	153–154	−92	$C_{28}H_{27}N_3O_5$
2	^{i}Pr	^{n}pr	F	157–159	−47	$C_{24}H_{26}FN_3O_5$

minimal energy of **1a** is lower than that of varsartan. The energy-minimized stereo-conformation of **1a** fits perfectly with valsartan.

2. Result and discussion

2.1. Chemistry

Compounds with biphenylyloxadiazol group (**1a–g**) were prepared according to the route described in Scheme 1. Natural amino acid, Val, Leu and Phe, were reacted with methanol and acetyl chloride to give aminoacid esters (**4a–c**). Alkylation of **4a–c** in DMF with 4′-(bromomethy)-biphenyl-2-carbonitrile using triethylamine as base gave the secondary amines **5a–c**.[9] Subsequently compounds **6a–g** were obtained by the acylation of **5a–c** with different acyl chlorides in the presence of 4-*N*,*N*-dimethylamino pyridin and triethylamine.

In order to obtain the oxadiazole compounds, the cyano compounds **6a–g** were firstly converted into the amidoximes **7a–g** in DMSO by reacting with hydroxylamine hydrochloride in presence of triethylamine.[10] Then the amidoximes **7a–g** were reacted with isobutyl chloroformate using pyridine as a base in DMF to give the *o*-acyl amidoxime compounds **8a–g**. The cyclized oxadiazole compounds **9a–g** were easily obtained from **8a to 8g** in xylene under refluxing. These ester derivatives **9a–g** were hydrolyzed with aqueous LiOH to provide the final biphenylyloxadiazol compounds **1a–g**.[11]

For the 5′-fluoro-biphenylyloxadiazol compound **2**, we chose a different synthetic route because the key intermediate 4′-(bromomethyl)-5-fluoro-biphenyl-2-carbonitrile **12** was commercially unavailable. As outlined in Scheme 2, compound **11** was prepared from 2-bromo-4-fluorobenzonitrile which was subjected to palladium coupling with *p*-toylboronic acid in the presence of a base under the conditions described by Suzuki in 81.1% yield. After radical bromination of **11** with NBS in chloroform 4′-(bromomethyl)-5-fluoro-biphenyl-2-carbonitrile **12** was obtained in 78% yield after recrystallization.[12] The following synthetic steps was similar to that of **1a–g**.

2.2. Biological evaluation

2.2.1. Effects of the new compounds on specific Ang II binding to AT_1 receptors in VSMCs

In vascular smooth muscle cell (VSMC) cultures, the specific binding of ^{125}I-Ang II was inhibited in a concentration-dependent manner by compounds **1a**, **1b**, **1c**, **1d**, **1e**, **1f**, **1g**, compounds **2** and valsartan, irbesartan (Fig. 2). The IC_{50} values of compounds **1b**, 1c and valsartan were 0.49 ± 0.1 nM, 1.6 ± 0.09 nM and 2.64 ± 0.7 nM, respectively (Table. 2). It showed that compounds **1b**, **1c** exhibited more affinity to AT_1 receptors than valsartan because their IC_{50} values were significantly lower compared with valsartan's (P <0.05). So compounds **1b** and **1c** showed much stronger inhibitory ability than compounds**1a, 1d, 1e, 1f, 1g** and **2** in receptor binding assay which indicated that compounds **1b** and **1c** might have better antihypertensive activity in vivo antihypertensive experiments.

2.2.2. Effects of the compounds on blood pressure of SHR

Compounds **1a**, **1b**, **1c**, **1e** and **2** caused significant reduction of SBP (Fig. 3A) and DBP (Fig. 3B) in spontaneously hypertensive rats, but compounds **1d**, **1f** and **1g** did not decreased blood pressure significantly, there were no differences between the experimental groups and the negative control group (Fig. 3C). Compounds **1a**, **1b**, **1c** and **1e** at the dose of 15 mg/kg had the antihypertensive activities equal to valsartan and better than irbesartan. But compound **2** at 15 mg/kg had little effect on SBP and DBP. Biological study of compounds **1a**, **1b**, **1c**, **1e** and **2** at 30 mg/kg on hypertensive rats also revealed the higher antihypertensive activity of these compounds than irbesartan. The maximal effects of the compounds on the reduction of SBP and DBP were presented in 3–4 h after administration. The antihypertensive effects maintained for 10 h, which indicated that the compounds had a favorable blood

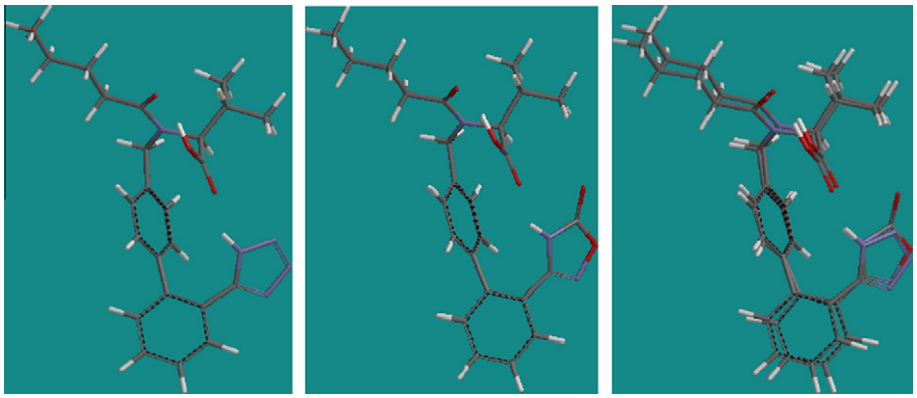
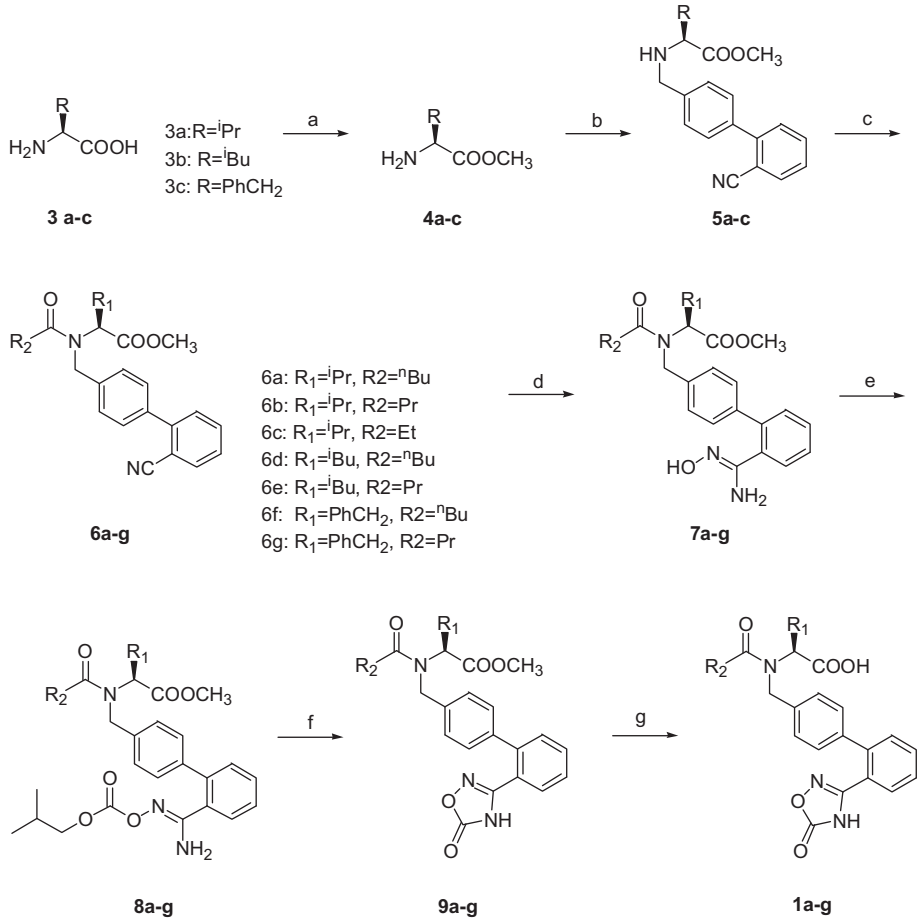


Figure 1. Energy-minimized conformation of valsartan (135.0750 kJ/mol), compound **1a** (112.3563 kJ/mol) and their overlay conformation.



Scheme 1. Reagents and conditions: (a) CH_3OH, $SOCl_2$, 0 °C; (b) 2′-cyano-4-bromomethylbiphenyl, DMF, Et_3N, reflux; (c) R_2COCl (R_2 = ^{n}Bu, Pr, Et), DMAP, Et_3N, CH_2Cl_2, reflux; (d) $NH_2OH{\cdot}HCl$, DMSO, Et_3N, reflux; (e) isobutyl chloroformate, pyridine, DMF; (f) xylene, reflux; (g) LiOH, CH_3OH, H_2O.

pressure-lowering effect. All the compounds did not influence heart rate of the rats.

2.2.3. Toxicity of compound 1b via intragastric administration

Acute toxicity testing suggested that the LD_{50} value of compound **1b** was 2316.8 mg/kg and the 95% confidence interval was 1878.8–2856.8 mg/kg (Table 3). It was well known that the LD_{50} of losartan was 2248 mg/kg and the LD_{50} of valsartan was 307.50 mg/kg. At all dose levels except for 1000.0 mg/kg, rats were observed to be hypokinetic beginning at approximately 5 h after intragastric administration, and some of them died. Rats treated at all doses survived through the two weeks observation period could maintain weight without obvious untoward effects. The results showed that compound **1c** was a potent AT_1-receptor antagonist with low acute toxicity.

3. Conclusions

In this work a series of new compounds with 5-oxo-1,2,4-oxadiazole group were designed and synthesized avoiding the use of hazardous azide and tin compounds existing in the preparing of losartan, valsartan and some other ARBs.

The binding assay in the receptor affinity presented that compounds **1b**, **1c** exhibited more affinity to AT_1 receptors than valsartan, which suggested that compounds **1b** and **1c** could specifically and competitively antagonize Ang II to AT_1 receptors. Its tight receptor binding ability might be expected to produce potent and long-lasting antihypertensive effects in preclinical and clinical settings. The anti-hypertension test showed that compounds **1a**, **1b**, **1c**, **1e** had obvious reduction in the blood pressure in spontaneously hypertensive rats. Acute toxicity test revealed that the highly active compound **1b** showed higher LD_{50} values compared to losartan, which suggested that the new compound was safer. So the new compounds could be considered as candidate with high performance and fewer adverse effects for novel anti-hypertension drugs.

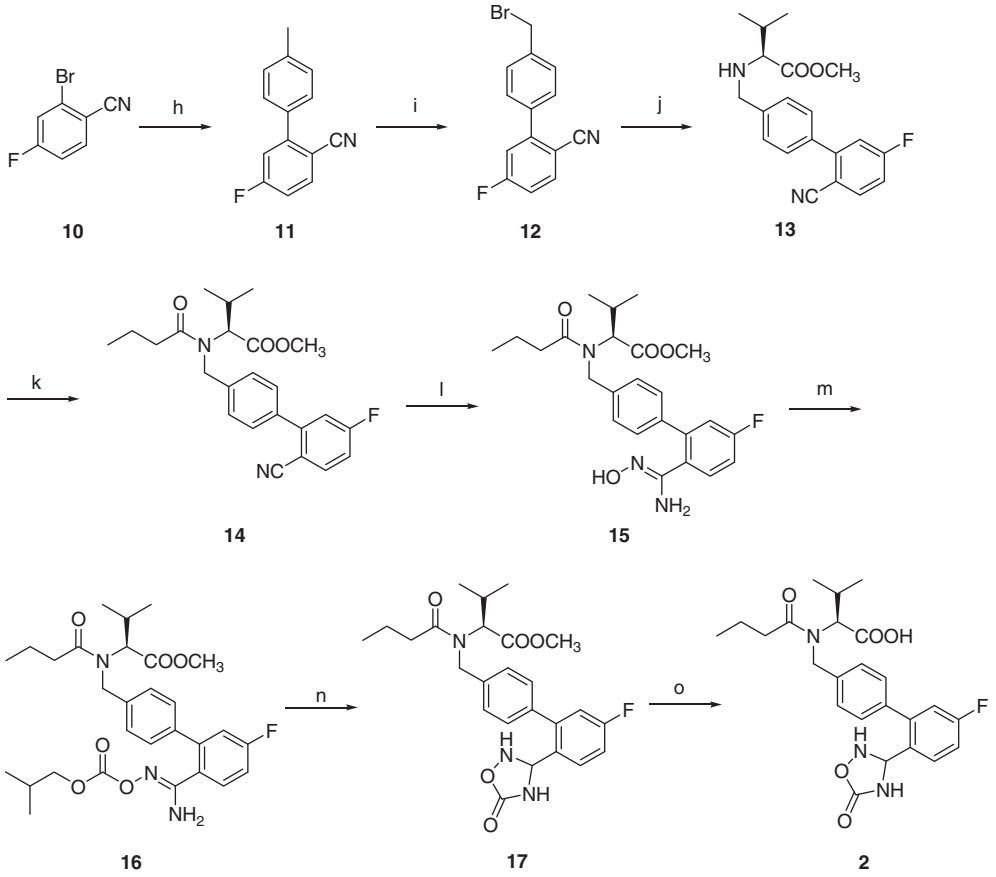
From both the in vitro and in vivo data it can be seen that the nature of the amino acid side chain is crucial for activity. The compounds with Val as side chain was better than the compounds with Leu and Phe. These results were in agreement with the molecular modeling studies.

4. Experimental section

All chemicals used were of reagent grade. Yields refer to purified products and are not optimized. All melting points were measured using an Electrothermal 9200 apparatus and are uncorrected. ^1H NMR spectra were measured on a Bruker 400 MHz spectrometer using Me_4Si as internal standard. ESI-MS spectra were recorded on a Micromass triple quadrupole mass spectrometer. Optical rotations were measured at 25 °C on a Jasco-P2000 polarimeter. Column chromatography was performed using silica gel H (300–400).

4.1. L-Valine methyl ester hydrochloride (4a)

To 5 mL of ice-bath cooled methanol was added dropwise 1 mL of $SOCl_2$, the resulting mixture was stirred for 0.5 h and 1.01 g



Scheme 2. Reagents and conditions: (h) *p*-tolylboronic acid, $Pd(OAc)_2$, K_2CO_3, PPh_3, 1,4-dioxane, H_2O, reflux; (i) NBS, AIBN, $CHCl_3$, reflux; (j) DIPEA, CH_2Cl_2, reflux; (k) butyryl chloride, DIPEA, CH_2Cl_2, Et_3N; (l) $NH_2OH{\cdot}HCl$, DIPEA, CH_3CH_2OH, reflux; (m) isobutyl chloroformate, DIPEA, CH_2Cl_2; (n) xylene, reflux; (o) LiOH, H_2O, CH_3OH, reflux.

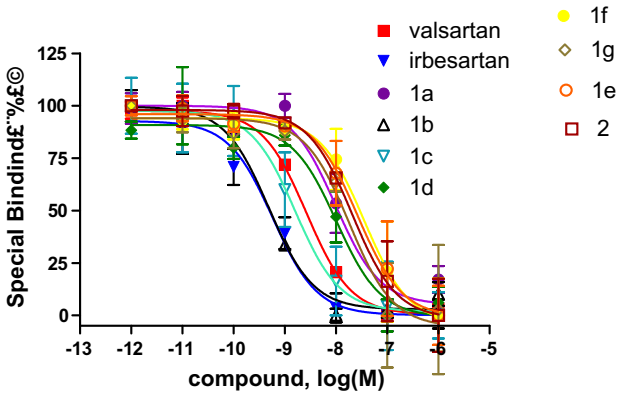


Figure 2. Inhibitory effects of compounds**1a**, **1b**, **1c**, **1d**, **1e**, **1f**, **1g**, **2**, valsartan and irbesartan (10^{-6} to 10^{-12} M) on the specific binding of ^{125}I-Ang II to AT_1 receptors in VSMCs.

(8.55 mmol) of L-Valine was added. The stirring was continued overnight, and the solvent was removed under reduced pressure. The residue was purified by flash column chromatograph (eluent: methanol/ether = 1/5, v/v) to afford a colorless pinch-like crystal. Mp: 128–132 °C. Yield 98.8%. The spectral data were consistent with that reported in the literature.[8]

4.2. L-Isoleucine methyl ester hydrochloride (4b)

Compound 4b was prepared according to the procedure for the preparation of **4a**. Yield 97.5%. Mp: 158–160 °C. The spectral data were consistent with that reported in the literature.[8]

Table 2
Radioligand binding assay (binding IC_{50}) of compounds **1a–f** and **2**.

	Binding IC_{50}	
Compounds	IC_{50} ± SEM (nM)	K_i (nM)
1a	9.82 ± 4.20	6.65 ± 2.50
1b	0.49 ± 0.10	0.33 ± 0.12
1c	1.60 ± 0.09	1.03 ± 0.59
1d	10.18 ± 0.02	6.88 ± 0.11
1e	35.97 ± 0.21	24.30 ± 0.25
1f	17.99 ± 0.13	12.16 ± 0.47
1g	26.52 ± 0.09	17.92 ± 0.17
2	20.81 ± 0.12	14.06 ± 0.33
Valsartan	2.64 ± 0.70	2.22 ± 0.50
Irbesartan	0.58 ± 0.22	0.41 ± 0.12

4.3. L-Phenylalanine methyl ester hydrochloride (4c)

Compound 4c was prepared according to the procedure described for the preparation of **4a**. Yield 96.4%. Mp: 173.2–175.3 °C. The spectral data were consistent with that reported in the literature.[8]

4.4. *N*-((2′-Cyanobiphenyl-4-yl) methyl)-L-Valine methyl ester (5a)

4.6 mL Triethylamine (36.00 mmol) was added dropwise to an ice-bath cooled solution of **4a** (2.01 g, 12.0 mmol) in 15 mL of DMF under nitrogen followed by 2′-cyano-4-bromomethylbiphenyl (2.99 g, 11.00 mmol). The mixture was heated to 70 °C and was monitored by TLC. After completion of the reaction, the

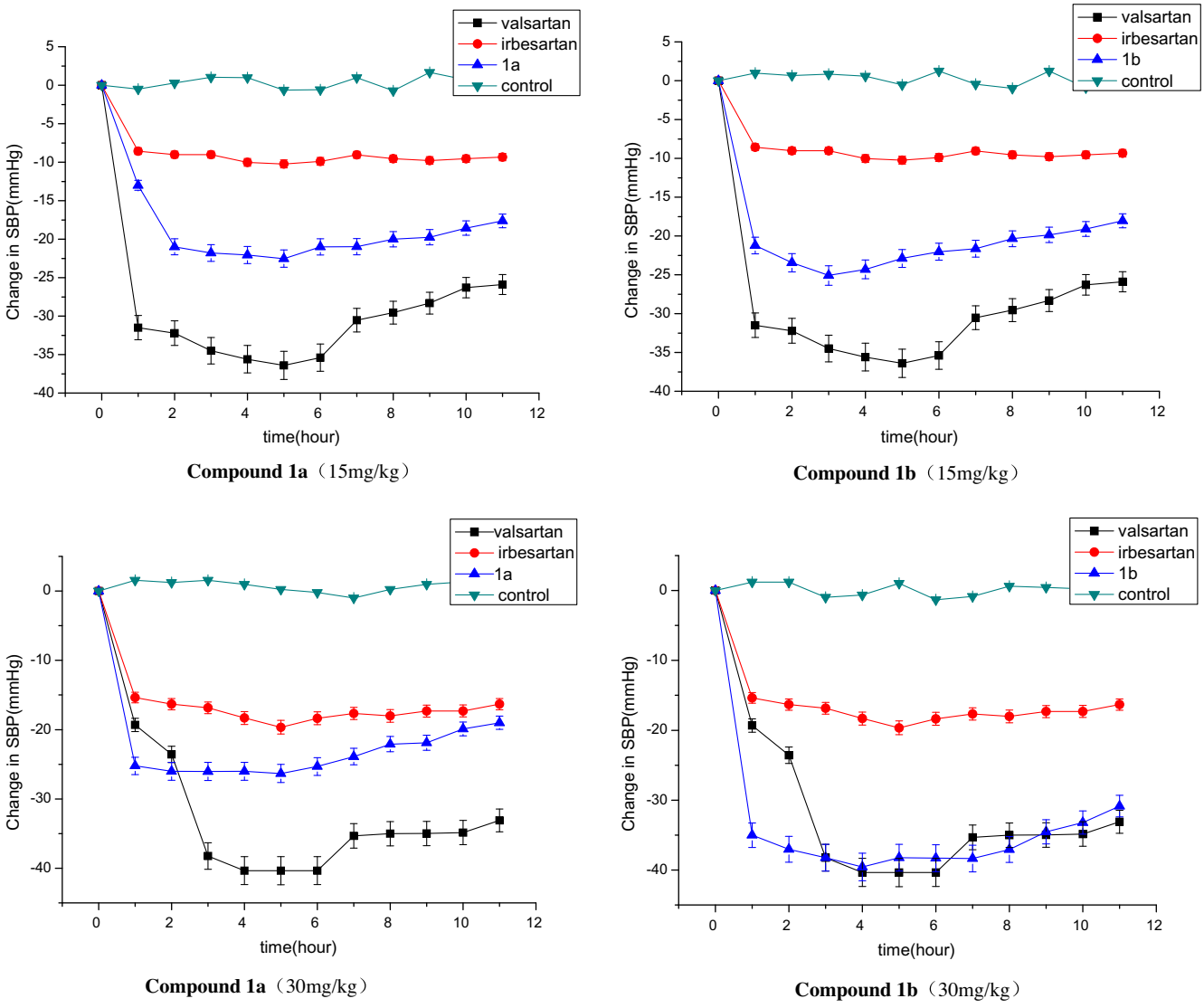


Figure 3A. Systolic Blood Pressure (SPB) and diastolic blood pressure (DBP) development during the experiment. Compounds **1a**, **1b**, **1c**, **1e** (15 mg/kg and 30 mg/kg) and **2** (30 mg/kg) presented favorable antihypertensive activities in SPB of spontaneous hypertensive rats (SHRs). The equal doses of valsartan and irbesartan were taken as positive controls.

solution was washed with water and the organic layer was evaporated. The residue was purified by column chromatograph to afford a colorless oil. Yield 81.8%.^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.78–7.25 (8H, m, Ph), 3.90, 3.65 (2H, two d, J = 13.5 Hz, $PhCH_2$), 3.74 (s, OCH_3), 3.05 (1H, d, J = 6.3 Hz, NCH), 1.94 (1H, m, Me_2CH), 0.96, 0.97 (3H, two d, 6H, J = 6.6 Hz, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 175.5, 145.2, 140.7, 136.7, 133.6, 132.6, 129.9, 128.5, 128.3, 127.3, 118.6, 111.0, 66.6, 52.0, 51.3, 31.6, 19.2, 18.5; ESI-MS (m/z): 323.2[M+1]$^+$, 345.2[M+Na]$^+$.

4.5. *N*-((2′-Cyanobiphenyl-4-yl)methyl)-L-Isoleucine methyl ester (5b)

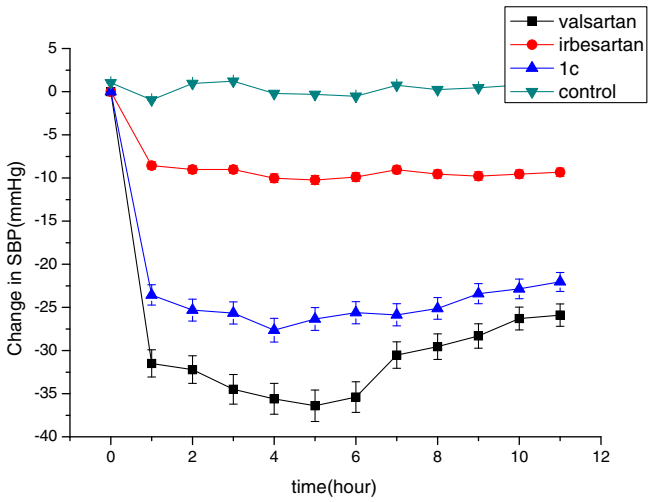
Compound 5b was prepared according to the procedure described for the preparation of **5a**. Yield 62.1%, ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.78–7.23 (8H, m, Ph-H), 3.89, 3.62 (2H, two d, J = 13.5 Hz, J = 13.7 Hz, –NH–CH_2–), 3.72 (3H, s, –OCH_3), 3.03 (1H, d, J = 6.3 Hz, –CO–CH–N–), 1.95 (1H, m, –CH–CH_3), 1.26 (2H, m, –CH–C$H_2$$CH_3$), 0.96, 0.97 (6H, two d, J = 6.6 Hz, –CHCH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 177.0, 145.1, 141.1, 139.9, 138.3, 137.5, 133.6, 133.6, 133.1, 133.1, 132.2, 132.2, 121.5, 116.4, 70.3, 57.5, 55.4, 38.6, 29.6, 19.4, 16.2 ESI-MS (m/z): 337.2[M+1]$^+$, 359.2[M+Na]$^+$.

4.6. *N*-((2′-Cyanobiphenyl-4-yl) methyl)-L-Phenylalanine methyl ester (5c)

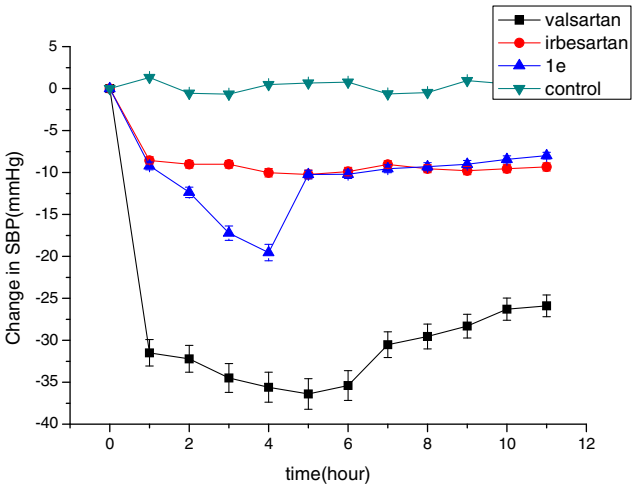
Compound 5c was prepared according to the procedure described for the preparation of **5a**. Yield 67.3%, ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.67 (1H, d, J = 8.56 Hz, Ph), 7.54 (1H, t, J = 7.68 Hz, Ph), 7.45–7.41 (3H, m, Ph), 7.35–7.31 (3H, m, Ph), 7.28–7.25 (2H, m, Ph), 7.21–7.16 (3H, m, Ph), 3.85 (1H, d, J = 13.6 Hz, NCH_2), 3.67 (d, J = 13.6 Hz, NCH_2), 3.63 (3H, s, $COOCH_3$), 3.56 (2H, t, J = 6.8 Hz, NCH), 2.97 (2H, m, $PhCH_2$), 1.96 (1H, s, NH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 174.5, 144.8, 140.1, 137.0, 136.5, 133.4, 132.5, 129.6, ESI-MS (m/z): 371.2[M+1]$^+$, 393.2[M+Na]+.

4.7. *N*-Butyryl-*N*-((2′-cyanobiphenyl-4-yl)methyl)-L-Valine methyl ester (6b)

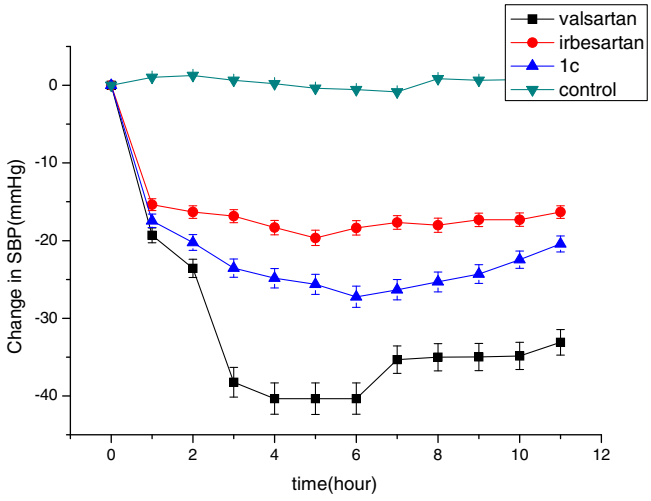
To 31 mL of dichloromethane was added methyl ester **5b** (3.97 g, 11.8 mmol), TEA (5 mL, 39.10 mmol) and DMAP (0.09 g, 0.7 mmol). To the mixture was added dropwise carbonyl chloride



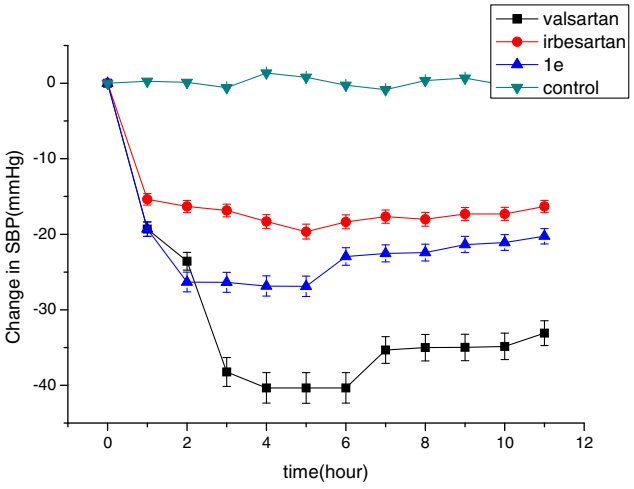
Compound 1c（15mg/kg）



Compound 1e（15mg/kg）



Compound 1c（30mg/kg）



Compound 1e（30mg/kg）

Fig. 3A (*continued*)

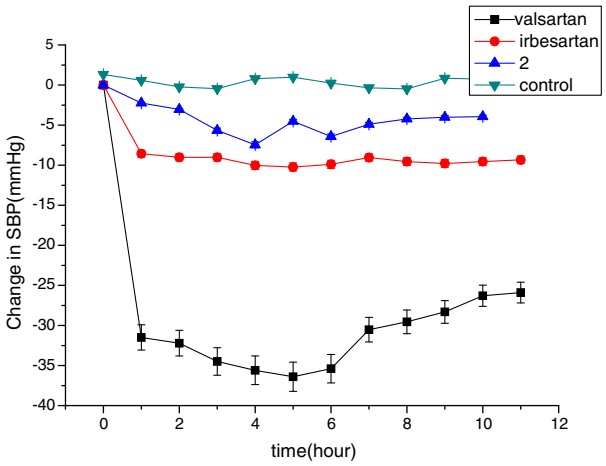
(2.1 mL, 35.60 mmol) under nitrogen. After stirring for 1 h, the temperature was raised to 45 °C, and the mixture was monitored by TLC till completion. To the mixture was added 10 mL of 1 M dilute aqueous hydrochloride. The organic phase was separated, washed with saturated $NaHCO_3$ solution, and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure, and the residue was purified by flash column chromatograph to afford a colorless oil. Yield 74.9%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.72–7.26 (8H, m, Ph-H), 5.08 (d, J = 15.6 Hz), 5.00 (1H, d, J = 10.5 Hz, –CO–CH–N–), 4.68 (d, J = 17.9 Hz), 4.26 (d, J = 15.6 Hz), 4.04 (2H, d, J = 10.8 Hz, –N–CH_2–), 3.43 (s), 3.35 (3H, s, –OCH_3), 2.43–2.30 (3H, m, –CO–CH_2–, –$CHCH_3$), 1.28–0.84 (9H, m,); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 178.2, 175.0, 145.1, 141.1, 139.9, 138.3, 137.5, 130.6, 130.6, 130.1, 130.1, 129.2, 129.2, 121.5, 116.4, 66.8, 55.8, 55.4, 39.3, 30.2, 21.1, 20.9, 20.9, 18.6. ESI-MS (m/z): 379.2[M+1]$^+$, 401.2[M+Na]$^+$; HR-MS: 378.1944, $C_{23}H_{26}N_2O_3$.

4.8. *N*-Valeryl-*N*-((2′-cyanobiphenyl-4-yl)methyl)-L-Valine methyl ester (6a)

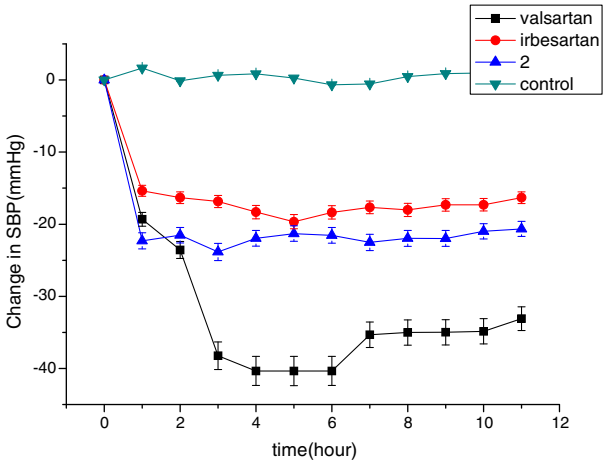
Compound 6a was prepared according to the procedure described for the preparation of **6b**. Yield 75.5%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.75–7.67 (1H, m, Ph-H), 7.63–7.58 (1H, m, Ph-H), 7.54–7.37 (4H, m, Ph-H), 7.34–7.28 (2H, m, Ph-H), 5.08 (d, J = 15.4 Hz), 4.97 (1H, d, J = 10.3 Hz, –CO–CH–N–), 4.72 (d, J = 17.9 Hz), 4.26 (d, J = 15.4 Hz), 4.11 (2H, d, J = 11.1 Hz, –N–CH_2–), 3.40 (s), 3.35 (3H, s, –OCH_3), 2.58–2.18 (3H, m, –CO–CH_2–, –$CHCH_3$), 1.85–1.13 (4H, m, –$CH_2CH_2CH_3$), 0.83–0.99 (9H, m,); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 180.2, 177.0, 145.1, 141.1, 139.9, 138.3, 137.5, 133.6, 133.6, 136.1, 136.1, 135.2, 135.2, 121.5, 116.4, 68.8, 55.8, 55.4, 35.8, 33.5, 30.2, 27.1, 21.9, 21.9, 18.3. ESI-MS (m/z): 407.3[M+1]$^+$, 429.3[M+Na]$^+$; HR-MS: 406.2261, $C_{25}H_{30}N_2O_3$.

4.9. *N*-Propionyl-*N*-((2′-cyanobiphenyl-4-yl)methyl)-L-Valine methyl ester (6c)

Compound 6c was prepared according to the procedure described for the preparation of **6b**. Yield 74.9%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.72–7.26 (8H, m, Ph-H), 5.08 (d, J = 15.6 Hz), 5.00 (d, 1H, J = 10.5 Hz, –CO–CH–N–), 4.68 (d, J = 17.9 Hz), 4.26 (d, J = 15.6 Hz), 4.04 (2H, d, J = 10.8 Hz, –N–CH_2–), 3.43 (s), 3.35 (3H, s, –OCH_3), 2.43–2.30 (3H, m, –CO–CH_2–, –$CHCH_3$), 1.28–0.84 (9H, m,); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 178.9, 177.0, 145.1, 141.4, 139.9, 138.3, 137.5, 133.6, 133.6, 133.1, 133.1, 132.2, 132.2, 121.5, 116.4, 68.8, 55.8, 55.4, 30.2 29.9, 21.9, 21.9, 15.0. ESI-MS (m/z): 379.2[M+1]$^+$, 401.2[M+Na]$^+$; HRMS: 378.1944, $C_{23}H_{26}N_2O_3$



Compound 2（15mg/kg）



Compound 2（30mg/kg）

Fig. 3A (*continued*)

4.10. *N*-Valeryl-*N*-((2′-cyanobiphenyl-4-yl)methyl)-L-Isoleucine methyl ester (6d)

Compound 6d was prepared according to the procedure described for the preparation of **6b**. Yield 74.4%. The spectral data were consistent with that reported in the literature.[8]

4.11. *N*-Butyryl-*N*-((2′-cyanobiphenyl-4-yl)methyl)-L-Isoleucine methyl ester (6e)

Compound 6e was prepared according to the procedure described for the preparation of **6b**. Yield 75.2%. The spectral data were consistent with that reported in the literature.[8]

4.12. *N*-Valeryl-*N*-((2′-cyanobiphenyl-4-yl)methyl)-L-Phenylal anine methyl ester (6f)

Compound **6f** was prepared according to the procedure described for the preparation of **6b**. Yield 75.5%, ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.77 (1H, m, Ph-H), 7.50–7.43 (4H, m, Ph-H), 7.31–7.08 (8H, m, Ph-H) 4.50 (1H, d, J = 17.1 Hz, –CO–CH–N–), 4.37 (m), 3.90 (2H, d, J = 17.2 Hz, –N–CH_2–), 3.67 (3H, s, –OCH_3), 3.40–3.36 (m), 3.29–3.24 (2H, m, –N–CHCH_2), 2.35–2.25 (2H, m, –CO–CH_2–), 1.63 (2H, m, –$CH_2CH_2CH_2$–), 1.35–1.24 (m, 2H, –$CH_2CH_2CH_3$), 0.85 (3H, t, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 178.2, 177.0, 145.2, 145.1, 141.1, 139.9, 138.3, 137.5, 133.6, 133.6, 133.4, 133.4, 133.1, 133.1, 132.9, 132.9, 132.2, 132.2, 130.7, 121.5, 116.4, 64.1, 55.5, 55.4, 39.8, 36.8, 33.5, 27.1, 18.3. ESI-MS (m/z): 455.3[M+1]$^+$, 477.2[M+Na]$^+$; HR-MS: 454.2256, $C_{29}H_{30}N_2O_3$.

4.13. *N*-Butyryl-*N*-((2′-cyanobiphenyl-4-yl)methyl)-L-Phenylal anine methyl ester (6g)

Compound **6g** was prepared according to the procedure described for the preparation of **6b**, Yield 75.5%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.77 (1H, d, Ph-H), 7.50–7.43 (4H, m, Ph-H), 7.31–7.08 (8H, m, Ph-H), 4.49 (d, 1H, J = 17.7 Hz, –CO–CH–N–), 4.37 (m), 3.87 (2H, d, J = 16.5 Hz, –N–CH_2–), 3.67 (3H, s, –OCH_3), 3.40–3.36 (m), 3.29–3.24 (2H, m, –N–$CHCH_2$), 2.35–2.25 (2H, m, –CO–CH_2–), 1.69 (2H, m, –$CH_2CH_2CH_3$), 0.89 (3H, t, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 173.7, 170.8, 144.8, 138.0, 137.4, 137.1, 133.7, 132.8, 130.0, 129.9, 129.2, 129.1, 128.9, 128.7, 128.5, 128.3, 127.6, 127.4, 127.3, 126.6, 118.5, 111.2, 77.3, 77.0, 76.7, 61.1, 52.0, 51.8, 35.4, 35.2, 18.5, 13.8; ESI-MS (m/z): 441.3[M+1]$^+$, 463.3[M+Na]$^+$.

4.14. *N*-Propionyl-*N*-[(2′-(hydroxycarbamimidoyl)biphenyl-4-yl) methyl]-L-Valine methyl ester (7c)

To a solution of nitrile **6c** (3.75 g, 9.90 mmol) in 60 mL of DMSO was added hydroxylamine hydrochloride (2.76 g, 39.70 mmol) and TEA (10.1 mL, 61.20 mmol). The mixture was stirred for 16 h at 90 °C. After cooling to rt, the mixture was diluted with water, extracted with ethyl acetate, dried, the solvent was removed under reduced pressure. The residue was purified by flash column chromatograph to afford a white solid. Yield 34.4%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.71–7.15 (8H, m, Ph-H), 4.99 (d, J = 15.3 Hz), 4.92 (1H, d, J = 10.3 Hz, –CO–CH–N–), 4.76 (m), 4.26 (d, J = 15.3 Hz), 4.04 (2H, d, J = 10.8 Hz –N–CH_2–), 4.43 (2H, s, –NH_2), 3.39 (s), 3.36 (3H, s, –OCH_3), 2.62–2.25 (3H, m, –CO–CH_2–, –$CHCH_3$), 1.27–0.86 (9H m,); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 178.9, 175.0, 169, 141.1, 139.9, 139.0, 136.8, 135.4, 133.6, 133.6, 132.5, 132.5, 132.2, 132.2, 131.4, 68.8, 55.8, 55.4, 30.2, 29.9, 21.9, 21.9, 15.0. LRMS (m/z): 410[M−1]$^-$; ESI-MS: 411.2152, $C_{23}H_{29}N_3O_4$.

4.15. *N*-Valeryl-*N*-[(2′-(hydroxycarbamimidoyl)biphenyl-4-yl) methyl]-L-Valine methyl ester (7a)

Compound 7a was prepared according to the procedure described for the preparation of **7c**. Yield 75.5%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.58 (m, 1H, Ph-H), 7.57–7.37 (5H, m, Ph-H), 7.21 (2H, m, Ph-H), 5.02 (d, J = 15.3 Hz), 4.92 (d, 1H, J = 10.3 Hz, –CO–CH–N–), 4.67 (m), 4.30 (d, J = 15.3 Hz), 4.07 (2H, d, J = 10.8 Hz –N–CH_2–), 4.43 (2H, s, –NH_2), 3.46 (s), 3.40 (3H, s, –OCH_3), 2.55–2.24 (3H, m, –CO–CH_2–, –$CHCH_3$), 1.82–1.58 (2H, m, –$CH_2CH_2CH_3$), 1.48–1.25 (2H, m, –$CH_2CH_2CH_3$), 1.03–0.82 (9H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 178.2, 177.0, 169, 141.1, 139.9, 139, 136.8, 135.4, 133.6, 133.6, 132.5, 132.5, 132.2, 132.2, 131.4, 68.8, 55.8, 55.4, 36.8, 31.5, 30.2, 27.1, 21.1, 21.9, 21.9, 18.3. LRMS (m/z): 440.3[M+1]$^+$; HR-MS: 439.2446, $C_{25}H_{33}N_3O_4$.

4.16. *N*-Butyryl-*N*-[(2′-(hydroxycarbamimidoyl)biphenyl-4-yl) methyl]-L-Valine methyl ester (7b)

Compound **7b** was prepared according to the procedure described for the preparation of **7c**. Yield 75.5%. NMR spectra are complicated due to amide rotomers. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.68–7.15 (8H, m, Ph-H), 5.03 (d, J = 15.3 Hz), 4.97 (1H, d, J = 10.8 Hz, –N–CH–), 4.45 (2H, s, –NH_2), 4.75–4.57 (m), 4.28 (d, J = 15.3 Hz), 4.06 (d, J = 10.8 Hz, –CH_2–N–), 3.45 (s), 3.39 (3H, s, –OCH_3), 2.65–2.21 (3H, m, –CO–CH_2–, –CH–CH_3), 1.70 (2H, m), 1.07–0.91 (9H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 173.6, 170.8, 153.0, 139.8, 139.4, 137.9, 135.4, 131.4, 130.1, 129.9, 129.6, 129.1,

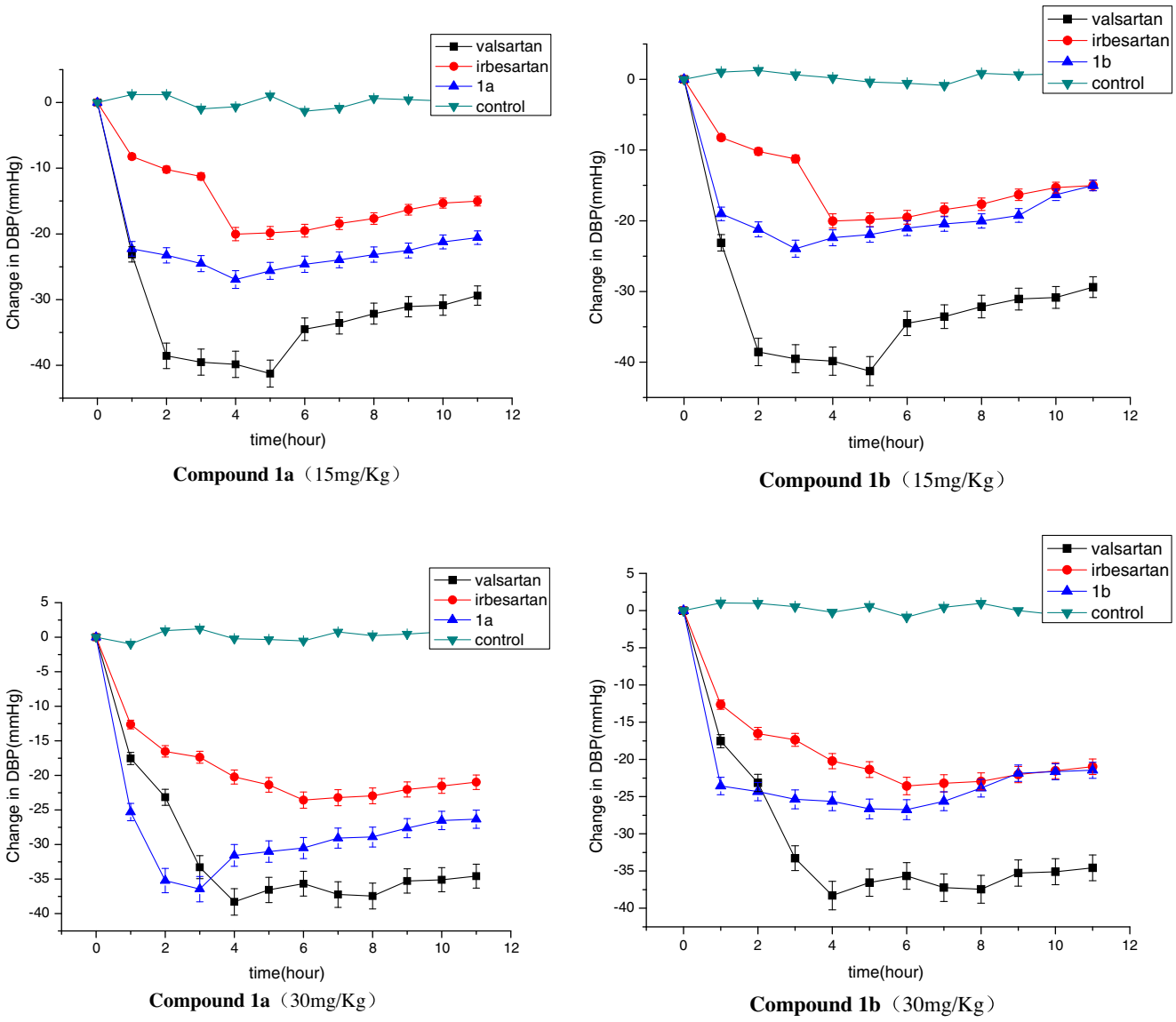


Figure 3B. Systolic Blood Pressure (SPB) and diastolic blood pressure (DBP) development during the experiment. Compounds **1a**, **1b**, **1c**, **1e** (15 mg/kg and 30 mg/kg) and **2** (30 mg/kg) also caused obvious reduction in DBP of SHRs.

128.9, 128.6, 128.3, 127.4, 127.1, 126.5, 60.7, 51.9, 51.8, 35.3, 35.1, 29.5, 18.4, 14.0, 13.7; ESI-MS (*m*/*z*): 426.2[M+1]$^+$, 448.3[M+Na]$^+$.

4.17. *N*-Valeryl-*N*-[(2′-(hydroxycarbamimidoyl)biphenyl-4-yl) methyl]-L-Isoleucine methyl ester (7d)

Compound 7d was prepared according to the procedure described for the preparation of **7c**. Yield 46.5%. Mp 98.2–100.6 °C. NMR spectra are complicated due to amide rotomers. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 11.08 (1H, s), 7.29–7.89 (8H, m), 6.90 (2H, s), 4.46 (2H, s), 4.41 (1H, d), 3.68 (3H, s), 2.53 (1H, m), 2.05 (2H, t), 1.31–1.55 (6H, m), 1.11 (3H, d), 0.90 (6H, t). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 178.2, 177.0, 169, 141.1, 139.9, 139.0, 136.8, 135.4, 133.6, 133.6, 132.5, 132.5, 132.2, 132.2, 131.4, 66.3, 55.8, 55.4, 36.8, 35.9, 33.5, 29.5, 27.1, 19.4, 18.3, 16.2. ESI-MS (*m*/*z*): 454.26[M+1]$^+$.

4.18. *N*-Butyryl-*N*-[(2′-(hydroxycarbamimidoyl)biphenyl-4-yl) methyl]-L-Isoleucine methyl ester (7e)

Compound 7e was prepared according to the procedure described for the preparation of **7c**. Yield 43.2%. Mp 97.5–99.7 °C. NMR spectra are complicated due to amide rotomers. ^{1}HNMR ($CDCl_3$, 500 MHz) δ: 11.08 (1H, s), 7.29–7.89 (8H, m), 6.90 (2H, s), 4.46 (2H, s), 4.41 (1H, d), 3.68 (3H, s), 2.53 (1H, m), 2.05 (2H, t), 1.55–1.68 (4H, m), 1.11 (3H, d), 0.90 (6H, t). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 178.2, 177.0, 169, 131.1, 139.9, 139.0, 136.8, 135.4, 133.6, 133.6, 132.5, 132.5, 132.2, 132.2, 131.2, 66.4, 55.8, 55.4, 37.3, 35.9, 29.6, 24.1, 19.4, 18.0, 16.2. ESI-MS (*m*/*z*): 440.25[M+1]$^+$, $C_{25}H_{34}N_3H_4$.

4.19. *N*-Valeryl-*N*-[(2′-(hydroxycarbamimidoyl)biphenyl-4-yl) methyl]-L-Phenylalanine methyl ester (7f)

Compound 7f was prepared according to the procedure described for the preparation of **7c**. Yield 46.5%. NMR spectra are complicated due to amide rotomers.^{1}H NMR ($CDCl_3$, 300.75 MHz) δ: 7.57–7.13 (13H, m, Ph-H), 4.47–4.40 (4H, m, –CO–CH–N–, $-NH_2$, $-N-CH_2-$), 3.90 (1H, d, J = 16.9 Hz, $-N-CH_2-$), 3.65 (3H, s, $-OCH_3$), 3.42–3.24 (2H, m, $-N-CHCH_2$), 2.31–2.28 (2H, m, $-CO-CH_2-$), 1.64–1.60 (2H, m, $-CH_2CH_2CH_3$), 1.34–1.30 (2H, m, $-CH_2CH_2CH_3$), 1.05–0.87 (3H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 178.2, 177.0, 169, 145.2, 141.1,

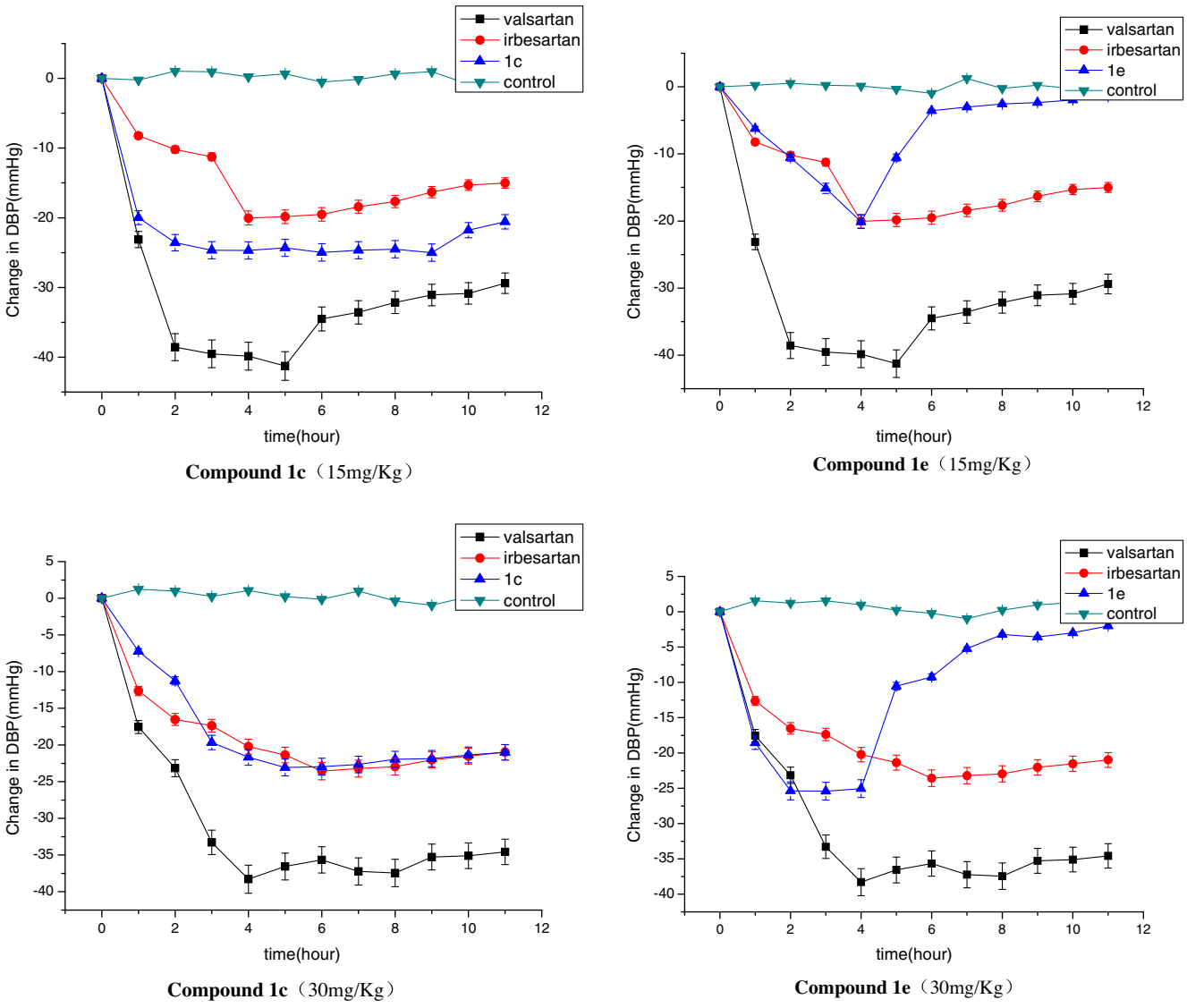


Fig. 3B (*continued*)

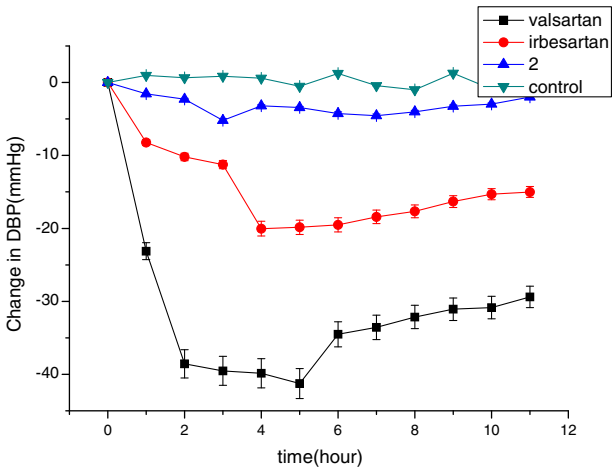
139.9, 139.0, 136.8, 136.0, 135.4, 133.6, 133.6, 133.4, 133.4, 132.9, 132.9, 132.5, 132.5, 132.2, 132.2, 131.2, 131.4, 64.1, 55.5, 55.4, 36.8, 33.5, 27.1, 18.3. ESI-MS (*m*/*z*): 488.3[M+1]$^+$, 510.3[M+Na]$^+$; HR-MS: 487.2468, $C_{29}H_{33}N_3O_4$.

4.20. *N*-Butyryl-*N*-[(2′-(hydroxycarbamimidoyl)biphenyl-4-yl)methyl]-L-Phenylalanine methyl ester (7g)

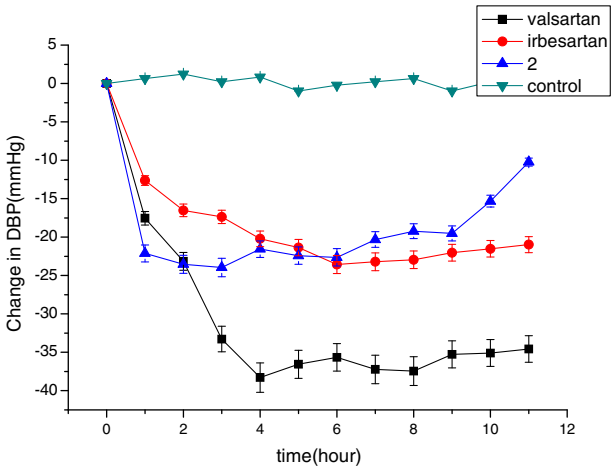
Compound 7g was prepared according to the procedure described for the preparation of **7c**. Yield 46.5%. NMR spectra are complicated due to amide rotomers.^{1}H NMR ($CDCl_3$, 300.75 MHz) δ: 7.58–7.19 (13H, m, Ph-H), 4.98 (d, J = 16.0 Hz), 4.97 (1H, d, J = 10.7 Hz, –CO–CH–N–), 4.66 (m), 4.31 (d), 4.05 (2H, d, J = 17.9 Hz, –N–CH_2–), 3.63 (3H, s, –OCH_3), 3.40–3.20 (2H, m, –N–CHCH_2), 2.61–2.18 (m, 2H, –CO–CH_2–), 1.69–1.61 (2H, m, –$CH_2CH_2CH_3$), 1.04–0.86 (3H m,); ^{13}C–NMR ($CDCl_3$, 125 MHz) δ: 171.2, 153.4, 140.0, 139.4, 138.7, 136.6, 131.6, 130.3, 129.9, 129.8, 128.8, 128.4, 127.6, 127.4, 126.1, 77.3, 77.0, 76.7, 65.8, 61.6, 51.8, 51.6, 48.1, 46.2, 45.4, 35.7, 35.5, 30.9, 27.7, 27.5, 19.9, 18.8, 18.7, 13.9, 11.5; ESI-MS (*m*/*z*): 474.2[M+1]$^+$, 496.3[M+Na]$^+$.

4.21. *N*-propionyl-*N*-[(2′-(*N*′-(isobutoxycarbonyloxy)carbamimidoyl)biphenyl-4-yl)methyl]-L-Valine methyl ester (8c)

To an ice-bath cooled solution of hydroxide **7c** (1.81 g, 4.4 mmol) and pyridine (0.43 mL, 5.30 mmol) dissolved in 20 mL of DMF was added dropwise isobutyl carbonochloridate (0.53 mL, 4.40 mmol). After stirring for 2 h, the mixture was diluted with water and extracted with ethyl acetate. The organic phase was dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The residue was purified by flash column chromatography to afford a white solid. Yield 87.4%. NMR spectra are complicated due to amide rotomers. ^{1}H NMR ($CDCl_3$, 300.75 MHz) δ: 7.64–7.15 (8H, m, Ph-H), 5.01–4.96 (1H, m, –CO–CH–N–), 4.69–4.64 (m), 4.28 (2H, d, J = 15.6 Hz, –N–CH_2–), 4.11–4.00 (m, 2H, –OCH_2–), 3.44 (s), 3.37 (3H, s, –OCH_3), 2.38–2.25 (2H, m, –$CHCH_3$ × 2), 2.03–1.99 (2H, m, –CO–CH_2–), 1.27–0.83 (m, 15H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 178.9, 177.0, 169, 156.5, 141.1, 139.9, 139.0, 136.8, 135.4, 133.6, 133.6, 132.5, 132.5, 132.2, 132.2, 131.4, 84.6, 68.8, 55.8, 55.4, 32.8, 30.2, 29.9, 23.5, 23.5, 21.9, 21.9, 15.0. ESI-MS (*m*/*z*): 512[M+1]$^+$.

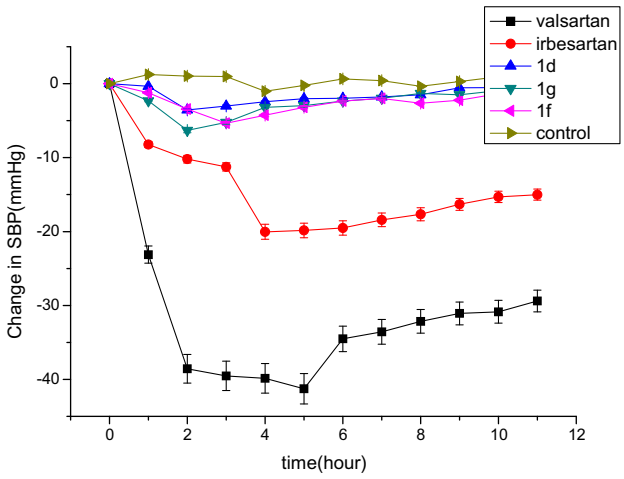


Compound 2（15mg/Kg）

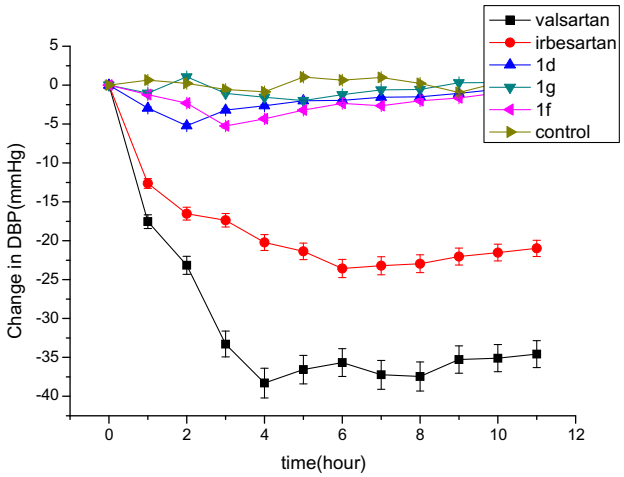


Compound 2（30mg/Kg）

Fig. 3B (*continued*)



Compound 1d、1f、1g(15mg/Kg)



Compound 1d、1f、1g(15mg/Kg)

Figure 3C. Systolic Blood Pressure (SPB) and diastolic blood pressure (DBP) development during the experiment. No significant alteration between the negative control group and the groups treated with 15 mg/kg (ig) of compounds **1d**, **1f**, **1g** ($^{*}P$ <0.05; ^{+}P <0.01; versus control; &: P <0.05 vs irbesartan).

4.22. *N*-Valeryl-*N*-[(2′-(*N*′-(isobutoxycarbonyloxy)carbamimidoyl)biphenyl-4-yl)methyl]-L-Valine methyl ester (8a)

Compound 8a was prepared according to the procedure described for the preparation of **8c**. Yield 87.6%. NMR spectra are complicated due to amide rotomers. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.64–7.20 (8H, m, Ph-H), 4.97–4.94 (1H, m, –CO–CH–N–), 4.65–4.61 (m), 4.07–4.04 (2H, m, –N–CH_2–), 4.02 (2H, d, J = 6.8 Hz, –OCH_2–), 3.45 (s), 3.39 (3H, s, –OCH_3), 2.48–2.02 (4H, m, –CO–CH_2–, –$CHCH_3$ × 2), 1.78–1.60 (2H, m, –$CH_2CH_2CH_3$), 1.32 (2H, m, –$CH_2CH_2CH_3$), 1.03–0.84 (15H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 178.2, 177.0, 169, 156.5, 141.1, 139.9, 139.0, 136.8, 135.4, 133.6, 133.6, 132.5, 132.5, 132.2, 132.2, 131.4, 84.6, 68.8, 55.8, 55.4, 36.8, 33.5, 32.8, 30.2, 27.1, 23.5, 23.5, 21.9, 21.9, 18.3. ESI-MS (m/z): 540.4[M+1]$^{+}$, 562.4[M+Na]$^{+}$.

4.23. *N*-Butyryl-*N*-[(2′-(*N*′-(isobutoxycarbonyloxy)carbamimidoyl)biphenyl-4-yl)methyl]-L-Valine methyl ester (8b)

Compound 8b was prepared according to the procedure described for the preparation of **8c**. Yield 87.6%. NMR spectra are complicated due to amide rotomers. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.65–7.19 (8H, m, Ph-H), 4.98–4.96 (1H, m, –CO–CH–N–), 4.66–4.62 (m), 4.31 (d, J = 15.4 Hz, –N–CH_2–), 4.07–4.03 (2H, m, –OCH_2), 3.46 (s), 3.40 (3H, s, –OCH_3), 2.63–2.03 (4H, m, –CO–CH_2–, –$CHCH_3$ × 2),

Table 3
Acute toxicity test of compound **1c** (ig) determined by median lethal dose (LD_{50})

Compound	Dose (mg/kg)	Mortality (%)	LD_{50} (mg/kg)	95% confidence interval (mg/kg)
1b	1000.0	0	2573.2	2167.1–3061.5
	1710.0	30		
	2236.1	50		
	2924.0	60		
	3823.6	80		
	5000.0	100		

1.84–1.65 (2H, m, –CH_2CH_3), 1.05–0.85 (15H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 174.4, 171.2, 157.2, 140.0, 138.8, 136.9, 130.6, 130.3, 129.9, 128.8, 128.4, 127.6, 127.4, 126.2, 77.3, 77.0, 76.7, 74.4, 61.7, 51.8, 51.5, 48.2, 35.7, 35.5, 27.8, 27.7, 27.5, 19.9, 18.9, 18.7, 13.9, 13.8; LRMS (m/z): 526.5[M+1]$^+$, 548.4[M+Na]$^+$.

4.24. *N*-Valeryl-*N*-[(2′-(*N*′-(isobutoxycarbonyloxy)carbamimidoyl) biphenyl-4-yl)methyl]-L-Isoleucine methyl ester (8d)

Compound 8d was prepared according to the procedure described for the preparation of **8c**. Yield 85.6%. NMR spectra are complicated due to amide rotomers. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.29–7.89 (8H, m), 6.90 (2H, s), 4.46 (2H, s), 4.41 (1H, d), 3.94 (2H, d), 3.68 (3H, d), 2.53 (1H, m), 1.97 (1H, m), 1.31–1.55 (6H, m), 1.11 (3H, d), 0.91 (6H, d), 0.90 (6H, t). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 178.2, 177.0, 169, 156.5, 141.1, 139.0, 136.8, 135.4, 133.6, 133.6, 132.5, 132.5, 132.2, 131.4, 84.6, 66.9, 55.8, 55.4, 36.8, 35.9, 32.8, 29.6, 27.1, 23.5, 23.5, 19.4, 18.3, 16.2. ESI-MS (m/z): 554.32[M+1]$^+$.

4.25. *N*-Butyryl-*N*-[(2′-(*N*′-(isobutoxycarbonyloxy)carbamimidoyl) biphenyl-4-yl)methyl]-L-Isoleucine methyl ester (8e)

Compound 8e was prepared according to the procedure described for the preparation of **6c**. Yield 84.9%. NMR spectra are complicated due to amide rotomers. ^{1}H NMR ($CDCl_3$, 500.12 MHz) δ: 7.29–7.89 (8H, m), 6.90 (2H, s), 4.46 (2H, s), 4.41 (1H, d), 3.94 (2H, d), 3.68 (3H, d), 2.53 (1H, m), 2.05 (2H, t), 1.97 (1H, m), 1.55–1.68 (4H, m), 1.11 (3H, d), 0.91 (6H, d), 0.90 (6H, t). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 178.2, 177.0, 169, 156.5, 141.1, 139.9, 136.8, 135.4, 133.6, 133.6, 132.5, 132.5, 132.2, 132.2, 131.4, 84.6, 66.3, 55.8, 55.4, 39.3, 35.9, 29.6, 32.8, 29.6, 24.1, 23.5, 23.5, 19.4, 18.0, 16.2. ESI-MS (m/z): 540.30[M+1]$^+$

4.26. *N*-Valeryl-*N*-[(2′-(*N*′-(isobutoxycarbonyloxy)carbamimidoyl) biphenyl-4-yl)methyl]-L-Phenylalanine methyl ester (8f)

Compound 8f was prepared according to the procedure described for the preparation of **8c**. Yield 85.6%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.76–7.14 (13H, m, Ph-H), 4.49 (1H, d, J = 17.1 Hz, –CO–CH–N–), 4.39 (m), 3.88 (2H, d, J = 17.0 Hz, –N–CH_2–), 4.02 (d, 2H, J = 6.8 Hz, –O–CH_2–), 3.65 (3H, s, –OCH_3), 3.41–3.33 (m), 3.26–3.19 (2H, m, –N–CH–CH_2), 2.33–2.27 (2H, m, –CO–CH_2–), 2.02 (1H, m, –$CH$$CH_3$), 1.62 (2H, m, –$CH_2CH_2CH_3$), 1.36–1.24 (2H, m, –$CH_2CH_2CH_3$), 0.95–0.87 (9H, m); ESI-MS (m/z): 588.4[M+1]$^+$, 610.4[M+Na]$^+$.

4.27. *N*-Butyryl-*N*-[(2′-(*N*′-(isobutoxycarbonyloxy)carbamimidoyl) biphenyl-4-yl)methyl]-L-Phenylalanine methyl ester (8g)

Compound 8g was prepared according to the procedure described for the preparation of **8c**. Yield 85.6%. NMR spectra are complicated due to amide rotomers. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.76–7.14 (13H, m, Ph-H), 4.50 (d, J = 17.0 Hz, –CO–CH–N–), 4.40–4.37 (m), 3.90 (2H, d, J = 16.9 Hz, –N–CH_2–), 4.02 (2H, d, J = 6.7 Hz, –OCH_2–), 3.66 (3H, s, –OCH_3), 3.42–3.35 (m), 3.26–3.21 (2H, m, –N–CH–CH_2), 2.30–2.26 (2H, m, –CO–CH_2–), 2.04–2.00 (1H, m, –$CH$$CH_3$), 1.70–1.64 (m, 2H, –$CH_2CH_2CH_3$), 0.98–0.86 (9H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 173.7, 170.9, 157.2, 153.9, 145.8, 140.0, 139.0, 138.0, 136.0, 130.8, 130.6, 130.3, 129.8, 129.1, 128.8, 128.5, 128.0, 127.6, 127.3, 119.8, 65.2, 61.0, 52.0, 51.9, 42.0, 35.5, 35.3, 31.9, 29.7, 27.8, 23.8, 22.7, 18.9, 18.5, 15.4, 13.8, 10.9; ESI-MS (m/z): 574.5[M+1]$^+$, 596.5[M+Na]$^+$.

4.28. *N*-Propionyl-*N*-[(2′-(2,5-dihydro-5-oxo-1,2,4-oxadiazol-3-yl)biphenyl-4-yl)methyl]-L-Valine methyl ester (9c)

To 10 mL of xylene dried over anhydrous calcium dichloride was added **8c** (1.46 g, 2.85 mmol). The solution was stirred over reflux for 5 h. The solvent was removed under reduced pressure to afford a yellow oil. The residue was recrystallized to afford the product as a white crystal. Yield 78.7%. ^{1}H NMR ($CDCl_3$, 300.75 MHz, doubling due to amide rotamers) δ: 9.26 (1H, s, –NH–), 7.76–7.18 (8H, m, Ph-H), 4.88–4.83 (1H, m, –CO–CH–N–), 4.63 (s), 4.27 (d, J = 15.6 Hz), 4.02 (2H, d, J = 10.5 Hz, –N–CH_2–), 3.43 (s), 3.41 (3H, s, –OCH_3), 2.57–2.03 (3H, m, –CO–CH_2–, –$CH$$CH_3$), 1.15–0.82 (9H, m);ESI-MS (m/z): 438[M+1]$^+$, 460[M+Na]$^+$.

4.29. *N*-Valeryl-*N*-[(2′-(2,5-dihydro-5-oxo-1,2,4-oxadiazol-3-yl)biphenyl-4-yl)methyl]-L-Valine methyl ester (9a)

Compound 9a was prepared according to the procedure described for the preparation of **9c**. Yield 78.3%. NMR spectra are complicated due to amide rotomers. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 9.60 (s, 1H, –NH–), 7.70–7.16 (m, 8H, Ph-H), 4.85–4.80 (m, 1H, –CO–CH–N–), 4.63–4.62 (m), 4.27 (d, J = 15.4 Hz), 4.12 (d, J = 10.1 Hz, –N–CH_2–), 3.49 (s), 3.43 (3H, s, –OCH_3), 2.40–2.34 (3H, m, –CO–CH_2–, –$CH$$CH_3$), 1.67–1.58 (2H, m, –$CH_2CH_2CH_3$), 1.34–1.25 (2H, m, –$CH_2CH_2CH_3$), 1.03–0.82 (9H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 174.8, 174.6, 170.9, 170.1, 159.6, 159.5, 157.9, 157.7, 141.2, 141.0, 137.9, 137.8, 137.1, 131.8, 130.9, 129.7, 129.0, 128.6, 127.8, 127.6, 127.5, 126.1, 121.9, 65.9, 61.8, 51.9, 51.6, 48.1, 45.4, 33.2, 27.6, 27.5, 27.4, 27.1, 22.3, 22.2, 19.7, 18.5, 13.7; ESI-MS (m/z): 466.5[M+1]$^+$, 488.4[M+Na]$^+$.

4.30. *N*-Butyryl-*N*-[(2′-(2,5-dihydro-5-oxo-1,2,4-oxadiazol-3-yl)biphenyl-4-yl)methyl]-L-Valine methyl ester (9b)

Compound 9b was prepared according to the procedure described for the preparation of **9c**. Yield 78.3%. NMR spectra are complicated due to amide rotomers. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 9.43 (1H, s, –NH–), 7.76–7.19 (8H, m, Ph-H), 4.87–4.86 (1H, m, –CO–CH–N–), 4.72–4.05 (2H, m, –NCH_2), 3.48–3.42 (3H, m, –OCH_3), 2.58–2.17 (3H, m, –CO–CH_2–, –$CH$$CH_3$), 1.84–1.65 (2H, m, –$CH_2CH_3$), 1.04–0.92 (m, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 174.5, 174.4, 171.1, 170.3, 159.4, 159.3, 157.7, 157.6, 141.1, 140.9, 138.5, 137.7, 137.6, 137.0, 132.0, 131.9, 130.9, 129.8, 129.1, 128.7, 128.1, 127.9, 127.7, 126.4, 121.9, 66.0, 61.8, 52.0, 51.7, 48.1, 45.6, 35.6, 35.4, 27.7, 19.8, 19.7, 18.8, 18.7, 18.6, 13.9, 13.8; ESI-MS (m/z): 452.2[M+1]$^+$, 474.2[M+Na]$^+$.

4.31. *N*-Valeryl-*N*-[(2′-(2,5-dihydro-5-oxo-1,2,4-oxadiazol-3-yl)biphenyl-4-yl)methyl]-L-Isoleucine methyl ester (9d)

Compound 9d was prepared according to the procedure described for the preparation of **9c**. Yield 71.8%. ^{1}H NMR ($CDCl_3$, 500 MHz) 7.29–7.89 (8H, m), 4.46 (2H, s), 4.41 (1H, d), 3.68 (3H, s), 2.53 (1H, m), 2.05 (2H, t), 2.0 (1H, s), 1.31–1.55 (6H, m), 1.11 (3H, d), 0.90 (6H, t). ESI-MS (m/z): 479. 6[M+1]$^+$.

4.32. *N*-Butyryl-*N*-[(2′-(2,5-dihydro-5-oxo-1,2,4-oxadiazol-3-yl)biphenyl-4-yl)methyl]-L-Isoleucine methyl ester (9e)

Compound 9e was prepared according to the procedure described for the preparation of **9c**. Yield71.8%. ^{1}H NMR ($CDCl_3$, 500 MHz)7.29–7.89 (8H, m), 4.46 (2H, s), 4.41 (1H, d), 3.68 (3H, s), 2.53 (1H, m), 2.05 (2H, t), 2.0 (1H, s), 1.55–1.68 (6H, m), 1.11 (3H, d), 0.90 (6H, t). ESI-MS (m/z): 465.54[M+1]$^+$.

4.33. *N*-Valeryl-*N*-[(2′-(2,5-dihydro-5-oxo-1,2,4-oxadiazol-3-yl)biphenyl-4-yl)methyl]-L-Phenylalanine methyl ester (9f)

Compound 9f was prepared according to the procedure described for the preparation of **9c**. Yield 78.3%. NMR spectra are complicated due to amide rotomers. ^{1}H NMR ($CDCl_3$, 500.12 MHz) δ: 7.78–7.15 (m, 13H, Ph-H), 4.64–4.44 (m, 1H, –CO–CH–N–), 4.27 (m), 3.75 (2H, d, J = 17.1 Hz, –N–CH_2–), 3.64 (3H, s, –OCH_3), 3.35–3.24 (2H, m, –N–CH–CH_2), 2.31 –2.21 (2H, m, –CO–CH_2–), 1.65–1.59 (2H, m, –$CH_2CH_2CH_3$), 1.35–1.30 (2H, m, –$CH_2CH_2CH_3$), 0.92–0.85 (3H m,); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 175.9, 174.9, 172.5, 171.8, 160.7, 157.9, 157.7, 140.9, 140.7, 138.5, 137.9, 136.5, 136.3, 132.1, 131.9, 130.7, 130.5, 129.7, 129.4, 129.2, 128.6, 128.3, 127.9, 127.8, 127.0, 121.9, 121.4, 68.0, 65.2, 60.3, 38.5, 33.6, 33.2, 29.5, 28.7, 27.2, 26.8, 22.8, 27.2, 27.1, 26.8, 22.8, 22.3, 19.6, 19.5, 18.7, 18.4, 14.0, 13.7, 13.6; ESI-MS (m/z): 514.3[M+1]$^+$, 536.3 [M+Na]$^+$

4.34. *N*-Butyryl-*N*-[(2′-(2,5-dihydro-5-oxo-1,2,4-oxadiazol-3-yl)biphenyl-4-yl)methyl]-L-Phenylalanine methyl ester (9g)

Compound 9g was prepared according to the procedure described for the preparation of **9c**. Yield 78.7%. ^{1}H NMR ($CDCl_3$, 500.12 MHz) δ: 9.26 (1H, s, –NH–), 7.76–7.18 (8H, m, Ph-H), 4.88–4.83 (1H, m, –CO–CH–N–), 4.63 (s), 4.27 (d, J = 15.6 Hz), 4.02 (2H, d, J = 10.5 Hz, –N–CH_2–), 3.43 (s), 3.41 (s, 3H, –OCH_3), 2.57–2.03 (m, 3H, –CO–CH_2–, –C$H$$CH_3$), 1.15–0.82 (m, 9H); ESI-MS (m/z): 438[M+1]$^+$, 460[M+Na]$^+$

4.35. *N*-Propionyl-*N*-[(2′-(2,5-dihydro-5-oxo-1,2,4-oxadiazol-3-yl)biphenyl-4-yl)methyl]-L-Valine (1c)

To a solution of **9c** (0.96 g, 2.20 mmol) dissolved in DMF was added 10 mL of 2 M aqueous sodium hydroxide and slight amount of methanol. The mixture was stirred for 3 h at 70 °C. The pH value of the mixture was adjusted to 3 by careful addition of 2 M aqueous hydrochloride. The mixture was extracted with ethyl acetate, and the organic phase was dried over $MgSO_4$. After filtration, the residue was purified by flash column chromatograph to afford a white solid. Yield 16.3%. Mp: 79.1–82.4 °C. ^{1}H NMR ($CDCl_3$, 300.75 MHz, doubling due to amide rotamers) (CM: major rotamer; Cm: minor rotamer) δ: 7.98–7.20 (m, 8H, CM, Cm), 4.70–4.58 (d, J = 15.0 Hz, 1H, CM, Cm), 4.22–4.10 (m), 3.98–3.71 (2H, m, CM, Cm), 2.81–2.42 (m, 3H, CM, Cm), 1.23–0.96 (m, 9H, CM, Cm) ESI-MS (m/z): 424[M+1]$^+$, 446[M+Na]$^+$.

4.36. *N*-Valeryl-*N*-[(2′-(2,5-dihydro-5-oxo-1,2,4-oxadiazol-3-yl)biphenyl-4-yl)methyl]-L-Valine (1a)

Compound 1a was prepared according to the procedure described for the preparation of **1c**. Yield 78.7%. NMR spectra are complicated due to amide rotomers. ^{1}H NMR ($CDCl_3$, 400 MHz) (CM: major rotamer; Cm: minor rotamer) δ: 8.14 (br s, 1H), 7.56–7.52 (m, 2H, CM, Cm), 7.41–7.39 (m, 1H, CM, Cm), 7.35–7.33 (m, 1H, CM, Cm), 7.25–7.23 (m, 1H, CM, Cm), 4.76–4.60 (d, J = 17.2 Hz, 1H, CM, Cm), 4.02–3.73 (m, 2H, CM, Cm), 2.75–2.55 (m, 2H, CM, Cm) 1.77 (m, 2H, CM, Cm), 1.50–1.43 (M, 2H, CM, Cm), 1.27 (M, 1H, CM, Cm), 1.01–0.96 (9H, m, CM, Cm); IR (KBr, cm^{-1}): 3432.21, 2962.79, 2873.10, 1775.81, 1620.17, 1466.11, 1207.30, 945.89, 762.93, 660.77, 577.82. ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 175.9, 174.9, 172.5, 171.8, 160.7, 160.1, 157.9, 141.0, 138.6, 137.9, 136.5, 132.1, 131.9, 130.8, 130.6, 129.8, 129.4, 129.2, 128.6, 128.3, 127.9, 127.8, 126.9, 121.9, 121.4, 68.0, 65.2, 60.3, 38.6, 33.6, 33.2, 29.5, 28.8, 27.2, 27.1, 26.8, 22.8, 22.3, 22.2, 19.6, 19.5, 18.8, 18.4, 14.0, 13.7, 13.6 ESI-MS (m/z): 452.3[M+1]$^+$, 474.3[M+Na]$^+$; HR-MS: 474.2007.

4.37. *N*-Butyryl-*N*-[(2′-(2,5-dihydro-5-oxo-1,2,4-oxadiazol-3-yl)biphenyl-4-yl)methyl]-L-Valine (1b)

Compound 1b was prepared according to the procedure described for the preparation of **1c**. Yield 85.6%. NMR spectra are complicated due to amide rotomers. ^{1}H NMR ($CDCl_3$, 400 MHz) (CM: major rotamer; Cm: minor rotamer) δ: 9.96 (d, J = 7.8 Hz), 9.17 (d, 1H, J = 7.6 Hz), 7.66–7.56 (m, 2H, CM, Cm, 7.46–7.41 (m, 2H, CM, Cm), 7.25–7.23 (m, 1H, CM, Cm), 7.21–7.18 (m, 2H, CM, Cm), 7.14–7.12 (m, 1H, CM, Cm), 5.24–5.20 (d, J = 15.2HZ, 1H, CM, Cm), 4.70–3.96 (2H, m, CM, Cm), 2.61–2.30 (m, 3H, CM, Cm), 1.67–1.52 (m, 2H), 1.52–0.0.81 (m, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 176.0, 175.2, 172.2, 160.9, 160.3, 158.2, 141.0, 138.2, 136.8, 136.4, 132.4, 132.2, 130.8, 129.5, 129.0, 128.2, 127.3, 122.1, 121.6, 66.5, 65.5, 65.4, 51.6, 45.2, 35.9, 35.7, 27.4, 20.0, 18.8, 18.7, 14.0; ESI-MS (m/z): 438.2[M+1]$^+$, 460.2[M+Na]$^+$. HR-MS: 460.1835.

4.38. *N*-Valeryl-*N*-[(2′-(2,5-dihydro-5-oxo-1,2,4-oxadiazol-3-yl)biphenyl-4-yl)methyl]-L-Isoleucine (1d)

Compound 1d was prepared according to the procedure described for the preparation of **1c**. Yield 87.2%. NMR spectra are complicated due to amide rotomers. ^{1}H NMR ($CDCl_3$, 400 MHz) (CM: major rotamer; Cm: minor rotamer) δ: 10.08–9.44 (m, 2H,), 7.64–7.55 (m, 2H, CM, Cm), 7.44–7.39 (m, 2H, CM, Cm), 7.24–7.21 (m, 4H, CM, Cm), 5.25–4.68 (d, J = 15.4 Hz, 1H, –CM, Cm, 4.53–4.03 (m, 2H, CM, Cm), 2.58–2.33 (m, 2H, CM, Cm), 2.10–1.93 (m, 1H, CM, Cm), 1.57–1.45 (m, 2H, CM, Cm), 1.36–1.27 (m, 2H, CM, Cm) 1.25–1.20 (m, 2H, CM, Cm) 0.89–0.78 (m, 9H, (m, 2H CM, Cm); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 176.5, 175.5, 173.3, 172.2, 160.7, 160.2, 158.1, 158.0, 141.1, 140.9, 138.5, 138.1, 136.8, 136.4, 129.9, 128.8, 126.9, 121.9, 121.5, 64.6, 51.0, 33.8, 33.4, 33.3, 27.3, 25.1, 24.8, 22.4, 22.3, 15.9, 15.7, 14.9, 13.9, 13.8, 11.2, 11.0, 10.9; ESI-MS (m/z): 466.4[M+1]$^+$, 488.4[M+Na]$^+$. HR-MS: 488.2155.

4.39. *N*-Butyryl-*N*-[(2′-(2,5-dihydro-5-oxo-1,2,4-oxadiazol-3-yl)biphenyl-4-yl)methyl]-L-Isoleucine (1e)

Compound 1e was prepared according to the procedure described for the preparation of **1c**. Yield 83.6%. NMR spectra are complicated due to amide rotomers. ^{1}H NMR ($CDCl_3$, 400 MHz) (CM: major rotamer; Cm: minor rotamer) δ: 10.15–9.44 (br s, 2H), 7.67–7.54 (m, 2H, CM, Cm), 7.38–7.37 (m, 2H, CM, Cm), 7.20–7.08 (m, 4H, CM, Cm), 4.94–4.91 (d, J = 16.2 Hz, 1H, CM, Cm), 4.50–4.01 (m, 2H, CM, Cm) 2.26 (m, 2H, CM, Cm), 2.08 (m, 2H, CM, Cm) 1.58–1.48 (m, 2H, CM, Cm), 1.33 (m, 2H, CM, Cm), 0.87–0.77 (m, 9H, CM, Cm); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 176.23, 176.0, 175.4, 173.4, 173.2, 160.7, 160.2, 158.1, 158.0, 141.2, 141.0, 138.4, 138.0, 136.8, 136.4, 136.3, 132.2, 132.1, 130.8, 129.9, 129.3, 128.8, 128.3, 127.9, 127.0, 126.9, 122.0, 121.6, 64.1, 53.6, 51.0, 45.5, 35.8, 35.7, 35.6, 33.6, 33.3, 31.0, 26.5, 25.1, 24.8, 18.7, 15.9, 15.8, 14.9, 14.6, 13.9, 13.7, 11.2, 11.0; ESI-MS (m/z): 452.3[M+1]$^+$, 474.3[M+Na]$^+$. HR-MS: 474.2009.

4.40. *N*-Valeryl-*N*-[(2′-(2,5-dihydro-5-oxo-1,2,4-oxadiazol-3-yl)biphenyl-4-yl)methyl]-L-Phenylalanine (1f)

Compound 1f was prepared according to the procedure described for the preparation of **1c**. Yield 89.5%. NMR spectra are complicated due to amide rotomers. ^{1}H NMR ($CDCl_3$, 400 MHz) (CM: major rotamer; Cm: minor rotamer) δ: 7.74–7.72 (m, 1H, CM, Cm), 7.64–7.60 (m, 1H, CM, Cm), 7.51–7.43 (m, 2H, CM, Cm), 7.32–7.25 (m, 5H, CM, Cm), 7.18–7.13 (m, 4H, CM, Cm), 4.44–4.40 (d, J = 16.8HZ, 1H, CM, Cm), 4.17 (m, 1H, CM, Cm), 3.57–3.52

(m, 1H, CM, Cm), 3.27–3.26 (m, 2H, CM, Cm), 2.33–2.28 (m, 2H, CM, Cm), 1.54–1.50 (m, 2H, CM, Cm)1.31–1.25 (m, 2H, CM, Cm), 0.89–0.85 (m, 3H, CM, Cm); ^{13}C NMR ($CDCl_3$, 125 MHz,) δ: 175.8, 174.9, 174.8, 174.7, 173.5, 160.1, 157.9, 157.8, 140.9, 137.9, 137.5, 135.6, 131.8, 130.6, 129.7, 129.0, 128.9, 128.5, 128.4, 127.7, 127.6, 126.6, 121.7, 61.3, 52.6, 34.5, 33.1, 31.7, 31.2, 29.9, 29.4, 29.2, 29.1, 28.7, 27.0, 26.9, 22.4, 22.1, 20.5, 13.9, 13.5; IR (KBr, cm^{-1}): 3431.01, 3029.60, 2957.90, 2925.82, 2858.36, 1772.16, 1620.14, 1492.83, 1464.04, 1215.21, 947.01, 759.88, 703.20. ESI-MS (m/z): 500.3[M+1]$^+$, 522.4[M+Na]$^+$; HR-MS: 522.2011.

4.41. *N*-Butyryl-*N*-[(2′-(2,5-dihydro-5-oxo-1,2,4-oxadiazol-3-yl)biphenyl-4-yl)methyl]-L-Phenylalanine (1g)

Compound 1g was prepared according to the procedure described for the preparation of **1c**. Yield 91.2%. NMR spectra are complicated due to amide rotomers. ^{1}H NMR ($CDCl_3$, 400 MHz) (CM: major rotamer; Cm: minor rotamer) δ: 7.93–7.77 (m, 1H, CM, Cm), 7.67–7.60 (m, 1H, CM, Cm), 7.51–7.49 (m, 1H, CM, Cm), 7.44–7.40 (m, 1H, CM, Cm), 7.32–7.30 (m, 2H, CM, Cm), 7.27–7.24 (M, 3H, CM, Cm), 7.20–7.16 (m, 4H, CM, Cm), 4.44–4.40 (d, J = 16.8HZ, 1H, CM, Cm), 4.26–4.11 (m, 1H, 4.44–4.40 (d, J = 16.8HZ, 1H, CM, Cm)), 3.58–3.54 (d, 1H, 4.44–4.40 (d, J = 16.8HZ, 1H, CM, Cm), 3.66–3.54 (m, 1H, CM, Cm), 3.26 (m, 2H, CM, Cm), 2.43–2.05 (m, 2H, CM, Cm), 1.59–1.26 (m, 2H, CM, Cm), 0.92–0.86 (m, 3H, CM, Cm); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 174.6, 172.2, 161.5, 157.2, 140.8, 139.6, 136.6, 136.4, 132.5, 132.3, 130.6, 130.5, 129.8, 129.6, 129.5, 128.9, 128.5, 128.3, 128.2, 88.3, 36.3, 35.6, 29.7, 26.6, 26.2, 19.8, 19.4, 18.8, 18.7, 18.4, 14.0, 13.9, 36.3, 35.6, 29.7, 26.6, 26.2, 19.8, 19.5, 18.8, 18.7, 18.4, 14.0, 13.9; ESI-MS (m/z): 486.1[M+1]$^+$, 508.2[M+Na]$^+$. HR-MS: 508.1849.

4.42. 5-Fluoro-4′-methylbiphenyl-2-carbonitrile (11)

To a solution of 2-bromo-4-fluorobenzonitrile (6.82 g, 34.10 mmol), *p*-tolylboronic acid (5.75 g, 42.29 mmol), PPh_3 (1.76 g, 6.33 mmol) and $Pd(OAc)_2$ (28 mg, 0.13 mmol) in 1,4-dioxane (96 mL) was added K_2CO_3 (11.67 g, 84.60 mmol) in 24 mL H_2O. The mixture was refluxed for 4 h under nitrogen. Then the mixture was diluted with EtOAc, washed with 5% NaOH and brine, dried with $MgSO_4$, and concentrated in vacuo. Chromatographied with petroleum ether/ethyl acetate (150:1) to yield the desired product as white solid (5.84 g, 81.1%). ^{1}HNMR (400 MHz, $CDCl_3$) δ: 7.79 (1H, dd, J_1 = 8.4 Hz, J_2 = 5.2 Hz, Ph′-H), 7.40 (2H, d, J = 8.4 Hz, Ph-H), 7.29 (2H, d, J = 8.4 Hz, Ph-H), 7.22 (1H, dd, J_1 = 9.6 Hz, J_2 = 2.8 Hz, Ph′-H), 7.14–7.10 (1H, m, Ph′-H), 2.44 (3H, s, Ph-CH_3). ESI-MS (m/z): 211.23[M+1]$^+$.

4.43. 4′-(Bromomethyl)-5-fluorobiphenyl-2-carbonitrile (12)

A solution of **11** (5.83 g, 27.60 mmol), NBS (5.40 g, 30.33 mmol), AIBN (0.68 g, 4.14 mmol) in CCl_4 (100 mL) was refluxed for 3 h, then the mixture was diluted with CH_2Cl_2, washed with brine, dried with $MgSO_4$, and concentrated in vacuo. The product was purified by chromatography with petroleum ether/ethyl acetate (150:1) as eluant. A yellow viscous oil 6.25 g was obtained. Yield: 78%. ^{1}HNMR (400 MHz, $CDCl_3$) δ: 7.79 (1H, dd, J_1 = 8.4 Hz, J_2 = 5.6 Hz, Ph′-H), 7.55 (4H, m, Ph-H), 7.28–7.15 (1H, m, Ph′-H), 4.56 (2H, s, Ph-CH_2-Br). ESI-MS (m/z): 290.13[M+1]$^+$.

4.44. (*S*)-Methyl 2-((2′-cyano-5′-fluorobiphenyl-4-yl) methylamino)-3-methylbutanoate (13)

A solution of **12** (6.14 g, 21.16 mmol), Valine methyl ester (7.80 g, 46.52 mmol), DIPEA (10.48 mL, 63.44 mmol) in CH_2Cl_2 (100 mL) was stirred at 50 °C for 11 h under nitrogen. The mixture was washed with water for 5 times (50 mL × 5) and brine for 1 time (50 mL), then organic layer dried with $MgSO_4$, and concentrated in vacuo. The product was purified by chromatography with petroleum ether/ethyl acetate (40:1) as eluant. A yellow solid 3.65 g was obtained. Yield: 50.7%. ^{1}HNMR (400 MHz, $CDCl_3$) δ: 7.77 (1H, dd, J_1 = 8.8 Hz, J_2 = 5.6 Hz, Ph′-H), 7.53–7.48 (4H, m, Ph-H), 7.22 (1H, dd, J_1 = 9.2 Hz, J_2 = 2.4 Hz, Ph′-H), 7.14 (1H, dt, J_1 = 2.6 Hz, J_2 = 8.0 Hz, Ph′-H), 3.92 and 3.66 (2H, two d, J = 13.6 Hz, –NH–CH_2–), 3.75 (3H, s, –OCH_3), 3.06 (1H, d, J = 6.0 Hz, –CO–CH–N–), 2.00–1.89 (2H, m, –$CH(CH_3)_2$, –NH–), 0.99–0.96 (6H, m, $CH(CH_3)_2$); ESI-MS (m/z): 341.1[M+H]$^+$.

4.45. (*S*)-Methyl-2-(*N*-((2′-cyano-5′-fluorobiphenyl-4-yl) methyl)butyramido)-3-methylbutanoate (14)

To a solution of **12** (3.65 g, 10.72 mmol), DIPEA (1.95 mL, 11.76 mmol) in CH_2Cl_2 (60 mL) was added *n*-butyryl chloride (1.66 mL, 16.04 mmol) at 0 °C, and the mixture was stirred for 6 h at room temperature. Then the reaction solution was poured into the ice water, the organic layer was washed with diluted hydrochloric acid, $KHCO_3$ aqueous and brine, dried by $MgSO_4$, and concentrated in vacuo. The product was purified by chromatography with petroleum ether/ethyl acetate (15:1) as eluant. A yellow oil 2.80 g (yield 63.6%) was obtained. ^{1}HNMR (400 MHz, $CDCl_3$) δ: 7.78–7.16 (m, 7H, Ph-H), 5.08 and 4.70 (1H, two d, J_1 = 18.4 Hz, J_2 = 16.0 Hz, –NCH_2–), 4.98 and 4.07 (1H, two d, J = 10.8 Hz, –N–CH–), 4.65 and 4.28 (1H, two d, J_1 = 18.4 Hz, J_2 = 15.6 Hz, –NCH_2–), 3.46 and 3.38 (3H, two s, –OCH_3), 2.34–2.25 (3H, m, –$CH_2CH_2CH_3$ and –$CH(CH_3)_2$), 1.71–1.68 (2H, m, –$CH_2CH_2CH_3$), 1.05–0.87 (9H, m); ESI-MS (m/z): 433.2[M+H]$^+$.

4.46. (*S*)-Methyl 2-(*N*-((5′-fluoro-2′-(*N*′-hydroxycarbamimidoyl) biphenyl-4-yl)methyl) butyramido)-3-methylbutanoate (15)

A solution of **13** (2.80 g, 6.82 mmol), hydroxylamine hydrochloride (1.42 g, 20.46 mmol) and DIPEA (3.57 mL, 20.46 mmol) in EtOH (50 mL) was refluxed for 23 h under nitrogen. After removal of solvent in *vacuo*, the residue was dissolved in EtOAc, then washed with water and brine, dried by $MgSO_4$, and concentrated in vacuo. The product was purified by chromatography with petroleum ether/ethyl acetate (1:1) as eluant. A yellow oil 1.02 g was obtained. Yield 33.7%. ^{1}HNMR (400 MHz, $CDCl_3$) δ: 7.54–7.01 (7H, m, Ph-H), 5.01 and 4.65 (1H, two d, J_1 = 18.4 Hz, J_2 = 15.6 Hz, –NCH_2–), 4.95 and 4.07 (1H, two d, J = 10.4 Hz, –N–CH–), 4.63 and 4.28 (1H, two d, J_1 = 18.4 Hz, J_2 = 15.6 Hz, –NCH_2–), 4.46 (2H, d, J = 8.4 Hz, –NH_2–), 3.42 and 3.37 (3H, two s, –OCH_3), 2.34–2.25 (3H, m, –$CH_2CH_2CH_3$ and –$CH(CH_3)_2$), 1.71–1.68 (m, 2H, –$CH_2CH_2CH_3$), 1.05–0.87 (m, 9H); ESI-MS (m/z): 444.2[M+H]$^+$.

4.47. (*S*)-Methyl-2-(*N*-((5′-fluoro-2′-(*N*′-(isobutoxycarbonyloxy) carbamimidoyl) biphenyl-4-yl) methyl)butyramido)-3-methyl butanoate (16)

To a solution of **15** (1.02 g, 2.30 mmol), DIPEA (0.45 mL, 2.53 mmol) in 50 mL CH_2Cl_2 was added isobutyl carbonochloridate at 0 °C, and the mixture was stirred for 4 h at room temperature. The reaction was washed with water and brine, dried by $MgSO_4$, and concentrated in vacuo. The product was purified by chromatography with petroleum ether/ethyl acetate (2.5:1) as eluant. A yellow viscous oil 1.02 g was obtained. Yield 81.6%. ^{1}HNMR (400 MHz, $CDCl_3$) δ: 7.58–7.00 (m, 7H, Ph-H), 4.96 and 4.63 (1H, two d, J_1 = 18.4 Hz, J_2 = 15.6 Hz, –NCH_2–), 4.89 and 4.06 (1H, two d, J = 10.4 Hz, –N–CH–), 4.62 and 4.25 (1H, two d, J_1 = 18.4 Hz, J_2 = 15.6 Hz, –NCH_2–), 3.98 (2H, d, J = 6.8 Hz, –NH_2–), 3.41 and 3.36 (two s, 3H, –OCH_3), 3.82 (d, 2H, J = 4.0 Hz, –OCH_2–CH–), 2.27–1.98 (4H, m, –$CH_2CH_2CH_3$ and 2 × –$CH(CH_3)_2$), 1.77–1.62

(2H, m, $-CH_2CH_2CH_3$), 1.00–0.82 (15H, m); ESI-MS (m/z): 544.3$[M+H]^+$.

4.48. (*S*)-Methyl 2-(*N*-((5′-fluoro-2′-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)biphenyl-4-yl)methyl) butyramido)-3-methyl butanoate (17)

The solution of **16** (1.02 g, 1.88 mmol) in 50 mL xylene was refluxed for 5 h under nitrogen. After the reaction was completed, the solvent was removed in *vacuo*, the residue was dissolved in EtOAc, then washed with water and brine, dried by $MgSO_4$, and concentrated in vacuo. The product was purified by chromatography with petroleum ether/ethyl acetate (1:1) as eluant. A yellow oil748 mg was obtained. Yield 88.9%. ESI-MS m/z: 468.2$[M-H]^-$. 8.0 (1H, s), 7.20–7.87 (8H, m), 4.46 (2H, s), 4.41 (1H, d), 3.06 (1H, m), 2.05 (2H, t), 1.68 (2H, m), 0.91 (6H, d), 0.90 (3H, t) ESI-MS (m/z): 469.51$[M+H]^+$.

4.49. (*S*)-2-(*N*-((5′-Fluoro-2′-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)biphenyl-4-yl)methyl)butyramido)-3-methylbutanoic acid (2)

A solution of **17** (748 mg, 1.59 mmol) in methanol (21 mL) was added LiOH (234 mg) in 7 mL H_2O. The mixture was refluxed for 4.5 h under nitrogen. After the reaction was completed, the solvent was removed in vacuo, the residue was dissolved in 15 mL H_2O, then washed with ether. The water layer was added dropwise 1 mol/L hydrochloric acid until the white solid was precipitated. The precipitate was filtered and was purified by chromatography with petroleum CH_2Cl_2/CH_3OH (15:1) as eluant. A white solid 230 mg was obtained. Yield: 31.7 %. NMR spectra are complicated due to amide rotomers. ^{1}HNMR (400 MHz, $CDCl_3$) (CM: major rotamer; Cm: minor rotamer) δ: 8.31 (brs, 1H), 7.85–8.00 (m, 1H, CM, Cm), 7.20–7.37 (m, 6H, CM, Cm), 3.75–3.79 and 4.56–4.71 and 5.86–5.91 (m, 2H, CM, Cm), 4.16 (brs, 1H), 3.39 and 3.99 (d, 1H, J = 11.2 Hz, CM, Cm), 2.69 (m, 1H, CM, Cm), 2.48–2.54 (m, 2H, CM, Cm), 1.77 (m, 2H, CM, Cm), 0.89–1.05 (m, 9H, CM, Cm). ESI-MS (m/z): 478.2$[M+Na]^+$. ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 174.7, 172.4, 163.5, 161.4, 156.7, 140.0, 137.1, 135.2, 132.3, 131.8, 129.4, 128.8, 128.0, 117.8, 117.5, 117.4, 115.9, 115.6, 64.6, 36.1, 35.6, 26.8, 26.3, 19.8, 18.8, 18.7, 18.5, 14.0, 13.9,

4.50. Cell lines and cell culture

The vascular smooth muscle cells (VSMCs) were obtained from thoracic aorta of SD rats and cultured by the tissue explants methods. One section of aorta was removed and placed in Dulbecco's Modified Eagle's Medium (DMEM). Adherent fat and connective tissue were gently removed with fine sterile forceps. The aorta was minced into small cube-shaped specimens and digested with collagenase for 1 h at 37 °C. The homogenate was centrifuged at 10000 g for 5 min and then incubated with 1 ml of DMEM supplemented with 15% fetal bovine serum (FBS) at 37 °C in 95% air 5% CO_2. Cells were characterized morphologically as smooth muscle by phase contrast microscopy and by immunostaining with α-actin.[13]

4.51. Radioligand binding assay

The new compounds, valsartan and irbesartan were dissolved in DMSO and diluted to different concentration with PBS before the experiment. The final concentrations were 1×10^{-10} M to 1×10^{-4} M.[14] ^{125}I-Ang II (Northern Biotechnology Company) was dissolved in phosphate buffered saline (PBS) and diluted to 0.1 nM. Quiescent and confluent cells were cultured in 24-well plates. After the cells adhibited to the walls, they were washed and incubated for 1 h in PBS containing 0.1 nM ^{125}I-Ang II and new compounds of different concentrations. The final concentrations were 1×10^{-6} to 1×10^{-12} M. Binding of ^{125}I-Ang II to VSMCs was performed at 4 °C for 2.5 h.[15] Total reaction volume was 500μL consisting of VSMCs, ^{125}I-Ang II and various concentrations of compounds. Nonspecific binding was measured in the presence of 1×10^{-6} M and represented 5–10% of total binding.[16] The reaction was terminated by removing the PBS and washing the cells thrice. The attached cells were dissolved in 0.5 ml of 0.1 M NaOH. The cells bound by Ang II were quantified by radioactivity counting in a gamma spectrometer (SN-682, Ri Huan Company, Shanghai). The IC_{50} value (concentration for 50% displacement of the specifically bound ^{125}I-Ang II) was estimated by the linear portion of the competition curves.

4.52. In vivo study of antihypertensive activity

The new compounds were subjected to biological evaluation for their effects on systolic blood pressure (SBP) and diastolic blood pressure (DBP) of spontaneous hypertensive rats (SHRs) (6 weeks old, purchased from Second military medical university, China). Male SHRs were randomly divided into different experimental groups of ten animals (negative control group, positive control groups, compounds 1–8 low-dose groups and high-dose groups). Each compound was suspended in a 0.5% solution of sodium carboxymethyl cellulose and administered orally at the dose of 15 mg/kg and 30 mg/kg separately. Valsartan and irbesartan (15 mg/kg and 30 mg/kg) were taken as positive controls. The negative control group was administered the same volume of sodium carboxymethyl cellulose solution. Blood pressure and heart rate were monitored at 0–10 h after the administration by a biological signal analysis system (MPA-2000, Alcott Biotech, China). Ten determinations were made in every session of blood pressure measurements and the means of ten values were taken as the systolic blood pressure level and diastolic blood pressure level, respectively. Results of the study were expressed as mean ± SD. A probability level of less than 0.05 was considered significant. The completed animal research here adhered to the 'Principles of Laboratory Animal Care' and was approved by IACUC.

4.53. Toxicity studies

The toxicity of compound **1b** was determined in normal ICR rats (6–8 weeks, Academia Sinica, China), which were weighed individually. LD_{50} was delivered via intragastric administration (ig) at doses of 5000.0, 3823.6, 2924.0, 2236.1, 1710.0 and 1000.0 mg/kg. Survival was assessed daily for two weeks. The lethal dose (LD_{50}) 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 and respiratory movements. Finally, the survivals were dissected and examined pathological changes of organs.

Acknowledgments

This work was supported by Grant of Chinese Government (30976611, 81102407, 81101298), Shanghai Government (10DZ0502300, 10ZZ45), and Donghua University (11D10501, 11D10518, 105-10-0044030).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2012.02.003.

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