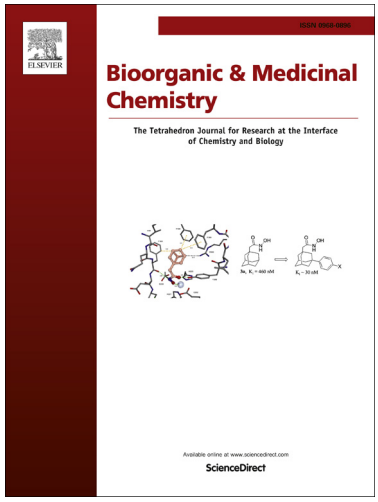

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Design, Synthesis and Biological Evaluation of New 5-Nitro Benzimidazole Derivatives as AT_1 Antagonists with Anti-Hypertension Activities

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ABSTRACT: The design, synthesis, *in vitro* and *in vivo* evaluation of 5-nitro benzimidazole with 1, 4-disubsituted or 1, 5-disubsituted indole derivatives as novel angiotensin II receptor antagonist is outlined. Radioligand binding assays showed that 2-(4-((2-butyl-5-nitro-1H-benzo[d]imidazol-1-yl)methyl)-1H-indol-1-yl)benzoic acid, compound **3**, displayed a high affinity for the angiotensin II type 1 receptor with IC_{50} value of 1.03 ± 0.26 nM. The biological evaluation on spontaneously hypertensive rats and renal hypertensive rats showed that **3** could cause significant decrease on MBP in a dose dependent manner, whose maximal response lowered 30 mmHg of MBP at 5 mg/kg and 41 mmHg of MBP at 10 mg/kg after oral administration, and the significant antihypertensive effect lasted beyond 24 h, which is better than Losartan. Taken together **3** could be considered as an effective and durable anti-hypertension drug candidate. These encouraging results are deserved of further investigation towards its use for therapeutic benefit.

Keywords: 5-Nitro benzimidazole derivatives; AT_1 antagonist; Synthesis; Anti-hypertension

1. Introduction

The pivotal role of the rennin-angiotensin system (RAS) in the regulation of blood pressure and fluid volume homeostasis has been well established. This system consists of a cascade of enzymatic reactions, leading to the production of angiotensin II (AII) [1] which is the final site of action in the RAS, elicits multiple biological effects such as increase in blood pressure, vascular contraction and modulation of central drinking behavior [2]. Angiotensin II has become the most common target in the treatment of cardiovascular disease, such as diabetic nephropathy, cardiac hypertrophy, arrhythmia and heart failure. Losartan is the first non-peptide orally active angiotensin II receptor antagonist [3]. Since then, a series of angiotensin II receptor blockers (ARBs) were used in clinics for the treatment of hypertension. These ARBs share a common mechanism of action, which block the effects of angiotensin II by selectively antagonizing the angiotensin II type 1 receptor [4]. Recent researches suggest that ARBs are also useful for treating congestive heart failure and reducing the cardiac and vascular remodeling associated with cardiovascular disease.

As the active AII receptor antagonists were widely developed, a lot of receptor binding models were proposed based on the structure-activity relationships. Alka Bali [5] proposed a drug–receptor interaction model that 5-substituted benzimidazole nucleus coupled through a methylene linker to pendent biphenyl system bearing a carboxyl group, and nitro group at 5-position, which had been found to be favorable for AII antagonism. Among this series compound **7** displayed higher activities than Losartan. Its binding profile proposed in literature (**Fig.1a**) depicted that n-butyl chain and biphenyl system respectively interacted with lipophilic pockets **L1** and **L2** through van der Waal interactions. *N*-3 of benzimidazole nucleus and terminal carboxyl group interacted with receptor through H-bonding interactions, while nitro group at 5-position interacted with pocket **L3** through van der Waal and/or H-bonding interactions.

We designed a series of compounds based on 5-nitrobenzimidazole as nucleus and N-phenylindole groups as rigid structure bearing a carboxyl group. 1, 4- and 1, 5- disubstituted

indoles were introduced to show which one could display a higher affinity. In addition, the appropriate alkyl groups were optimized through altering the length of alkyl chain. The binding profiles of **3** (**Fig.1b**) and **6** (**Fig.1c**) are similar to that of **7**. The dominant conformation of **7**, **3** and **6** were shown as **Fig.2** using Spartan 8. The energy-minimized stereo-conformation of **3**, **6** showed a high degree of fit with **7** and as such warranted biological evaluation.

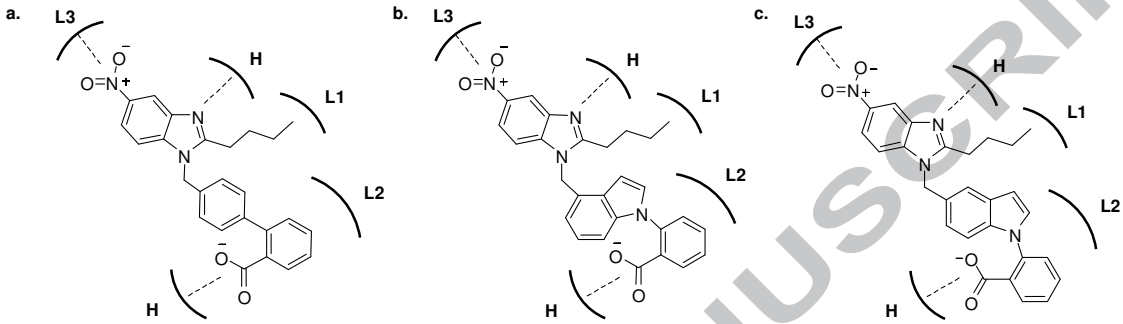


Fig.1 (a) Binding profile of **7** where **L1–L2** are lipophilic pockets, **L3** is a lipophilic or H-bond donor pocket, and **H** is H-bond donor site. (b) Binding profile of **3** where **L1–L2** are lipophilic pockets, **L3** is a lipophilic or H-bond donor pocket, and **H** is H-bond donor site. (c) Binding profile of **6** where **L1–L2** are lipophilic pockets, **L3** is a lipophilic or H-bond donor pocket, and **H** is H-bond donor site.

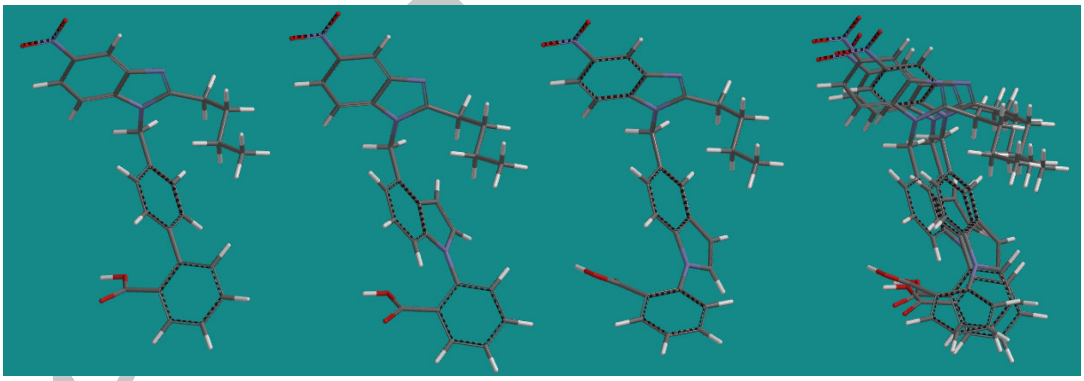


Fig.2 Energy-minimized conformation of **7**, **3**, **6** and their overlay conformation

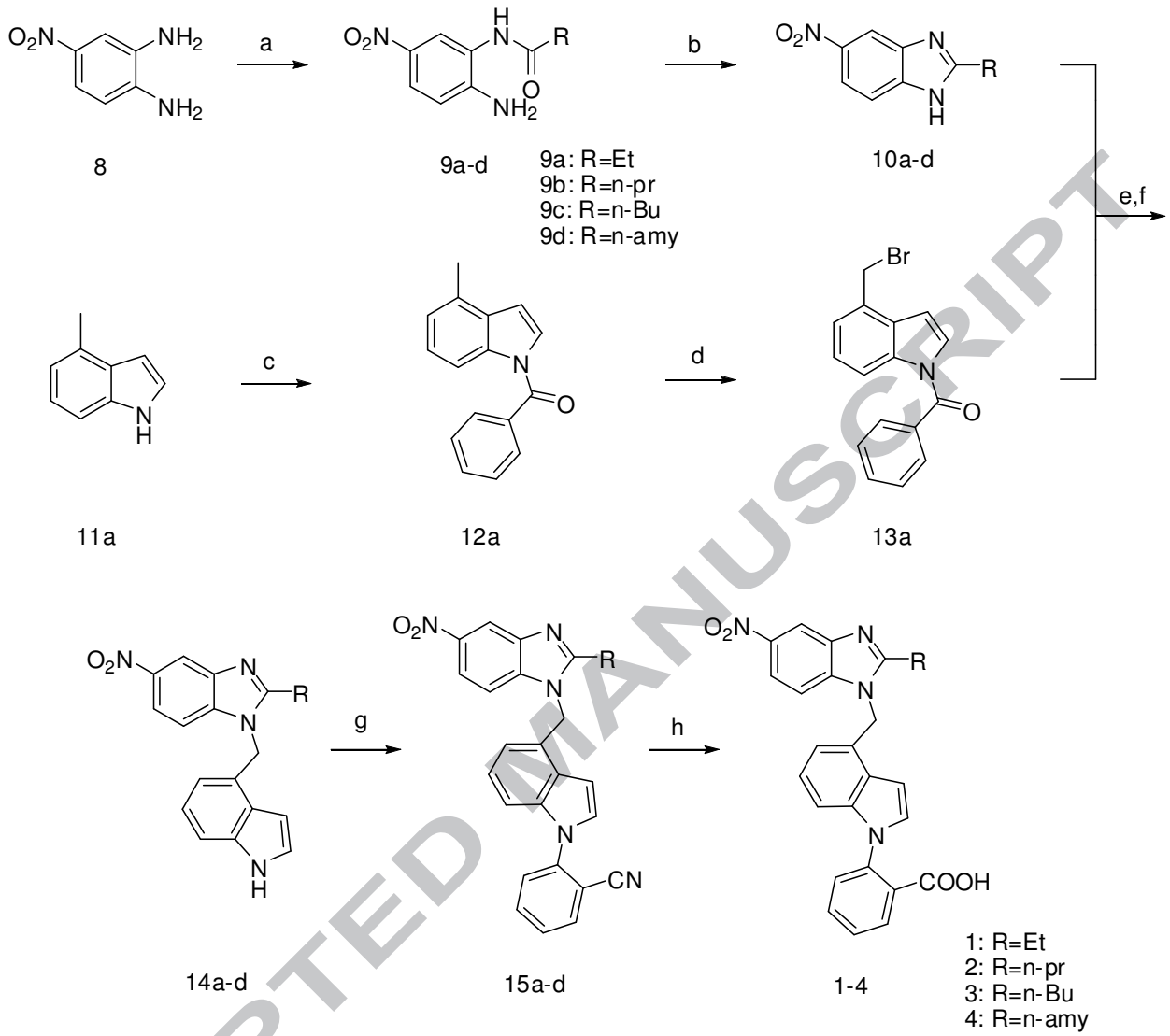
Six new designed compounds were synthesized, which were measured for their affinity to the AT_1 receptor to displace ^{125}I-AII from its specific binding sites in rat vascular smooth muscle cells (VSMC). The *in vivo* anti-hypertension properties were evaluated in spontaneously hypertensive rats and renal hypertensive rats.

2. Results and discussion

2.1 Chemistry

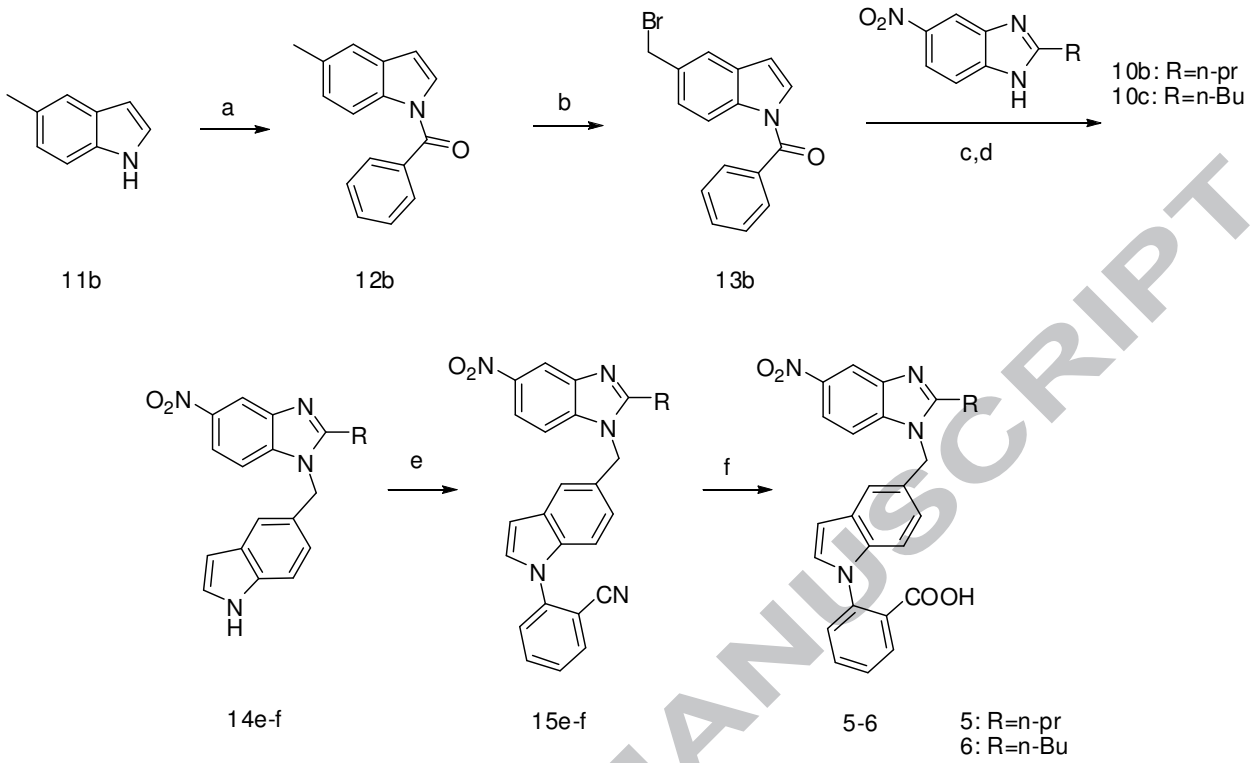
The preparation of indole derivatives **1-6** was performed by means of a multistep procedure described in **Scheme 1-2**.

The synthesis of **1-4** was accomplished starting from the suitable commercially available 4-Nitro-benzene-1, 2-diamine (**8**), which reacted with different acyl chlorides in THF using Et_3N as base to give amide **9a-d**. They were cyclized to produce benzoimidazoles **10a-d** with con. HCl in EtOH. 4-Methylindole **11a** was *N*-protected by acylation with benzoyl chloride in the presence of Et_3N and 4-(dimethylamino)pyridine (DMAP) to give (4-methyl-1H-indol-1-yl) (phenyl) methanone **12a**, which was brominated with NBS in the presence of AIBN to produce (4-bromomethyl-1H-indol-1-yl) (phenyl)methanone **13a**. **14a-d** was obtained after reaction of **13a** with **10a-d** in the presence of K_2CO_3, and then hydrolyzed with 2M aqueous NaOH. **14a-d** reacted with 2-fluoro-benzonitrile using K_2CO_3 as base in DMF yielded **15a-d**. The final products **1-4** were acquired after hydrolysis of **15a-d**. At each stage of the synthetic sequence the product was isolated, purified by column chromatography and characterized by NMR and mass spec techniques.



Scheme 1. Reagents and conditions: **(a)** RCOCl, Et_3N, THF; **(b)** HCl, C_2H_5OH, reflux; **(c)** benzoyl chloride, Et_3N, DMAP, CH_2Cl_2; **(d)** NBS, AIBN, CCl_4, reflux; **(e)** K_2CO_3, CH_3COCH_3, reflux; **(f)** NaOH, H_2O, CH_3OH, reflux; **(g)** 2-fluorobenzonitrile, K_2CO_3, DMF, reflux; **(h)** NaOH, H_2O, CH_3OH, reflux.

The preparation of **5** and **6** were similar to **1-4**.



Scheme 2. Reagents and conditions: **(a)** benzoyl chloride, Et_3N, DMAP, CH_2Cl_2; **(b)** NBS, AIBN, CCl_4, reflux; **(c)**K_2CO_3, CH_3COCH_3, reflux; **(d)** NaOH, H_2O, CH_3OH, reflux; **(e)** 2-fluorobenzonitrile, K_2CO_3, DMF, reflux; **(f)** NaOH, H_2O, CH_3OH, reflux.

One of the committed steps was the preparation of **14a-f**. In this step two compounds were obtained, namely, target compound and its regioisomer caused by the tautomeric forms of **10a-d** (**Fig.3**) under refluxing of acetone.

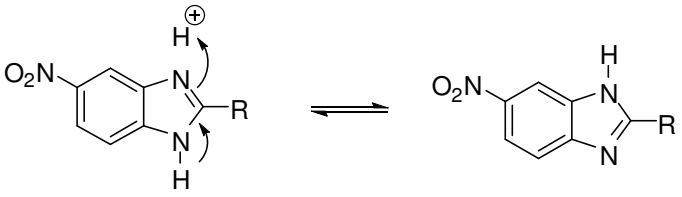


Fig.3 Tautomeric forms of **10a-d**

14b was selected as an example to identify the position of nitro group. **14b** and its regioisomer **16** were separated by silica gel column chromatography. Nuclear Overhauser Effect Spectroscopy (NOESY) was used to confirm the structure of **14b** and **16**. Irradiation of the benzylic CH_2 gave enhancement of the aromatic protons at position 7 of the benzimidazole

moiety (**Fig.4**). The difference between proton at position 7 in **14b** and **16** was that chemical shift of the former (at δ 7.26ppm, red circle in **Fig.5**) was lower than the latter (at δ 8.21ppm, red circle in **Fig.5**). This indicates that the nitro group is at position 5 of benzimidazole in **14b** while at position 6 in **16**. The structures of **14a, c-f** could also be confirmed as the identification of **14b**.

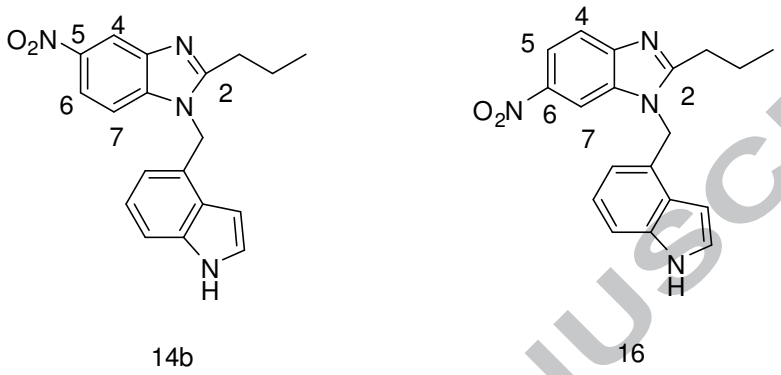


Fig.4 compounds **14b** and **16**

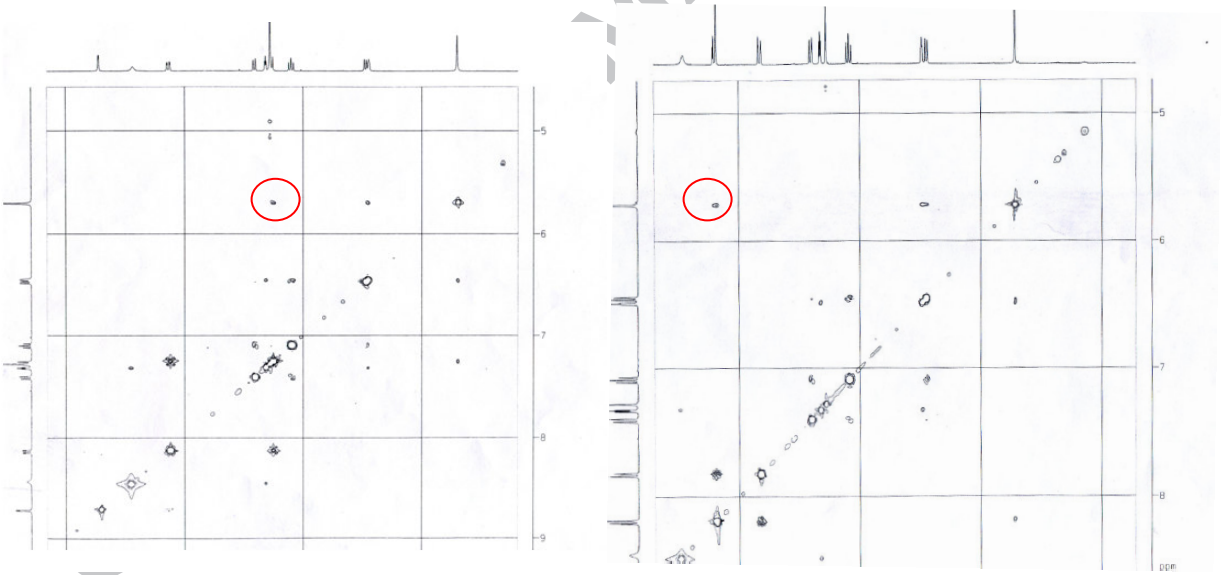


Fig.5 The NOESY of **14b** (left) and **16**(right).

2.2 Biological evaluation

2.2.1 Radioligand binding assays

The prepared compounds were evaluated for their activities to competitively inhibit ^{125}I-AII

binding to the AT_1 receptor by a conventional ligand-binding assay with VSMC. The specific binding of ^{125}I-AII was inhibited in a concentration-dependent manner by compounds **1**, **2**, **3**, **4**, **5**, **6** and Losartan, as exemplified by **3** and **4** in **Fig.6**. The IC_{50} and Ki values were shown in **Table 1**. Compound **3** and **5** exhibited more affinity to AT_1 receptors than other compounds. **4** didn't compete well in the receptor assay. The result indicated that **3** and **5** might have better *in vivo* antihypertensive activity.

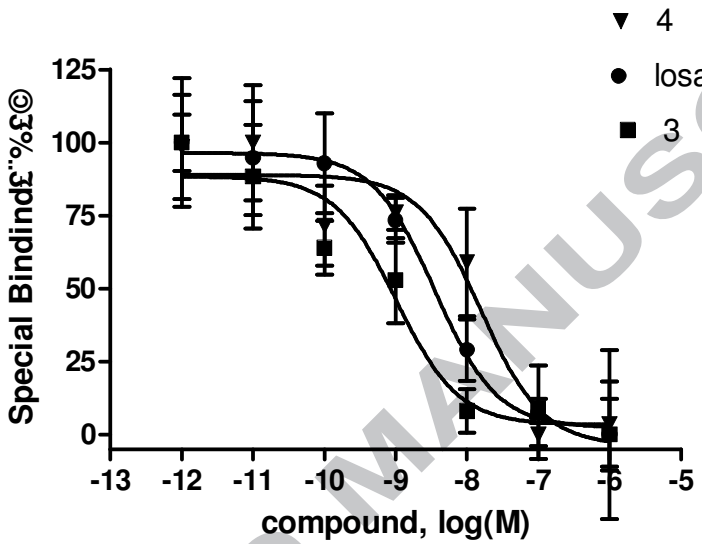
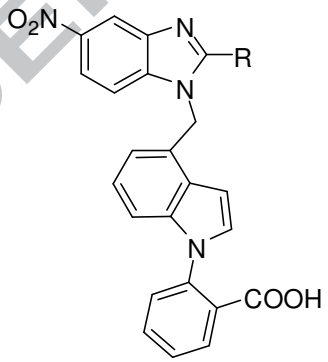
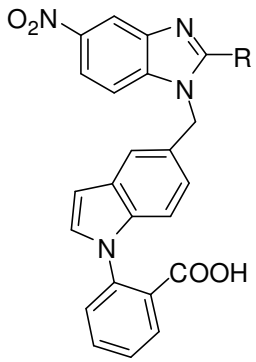


Fig.6 Inhibitory effects of **3**, **4** and Losartan (10^{-6}-10^{-12}M) on the specific binding of ^{125}I-AII to AT_1 receptors in VSMCs

Table 1. IC_{50} and Ki value of the tested compounds



1-4



5-6

Compound	R	Mp (°C)	Formula	IC_{50}±SEM (nM)	K_i (nM)
1	Et	191-194	$C_{25}H_{20}N_4O_4$	4.12±1.15	3.04±1.17
2	n-pr	196-199	$C_{26}H_{22}N_4O_4$	5.43±0.27	4.23±0.47
3	n-Bu	200-203	$C_{27}H_{24}N_4O_4$	1.03±0.26	0.97±0.43
4	n-amyl	205-208	$C_{28}H_{26}N_4O_4$	15.74±0.32	13.42±2.78
5	n-pr	197-200	$C_{26}H_{22}N_4O_4$	3.32±0.78	2.22±1.21
6	n-Bu	201-204	$C_{27}H_{24}N_4O_4$	5.11±0.89	4.89±0.34
Losartan				3.54±0.34	2.53±1.12

2.2.2 *In vivo* antihypertensive activities

In spontaneously hypertensive rats (SHR), the effects of compounds **1**, **2**, **3**, **4**, **5**, **6** (5, 10 mg/kg) and Losartan (10 mg/kg) on the mean blood pressure (MBP) after oral administration are shown in **Fig.7**. It displayed that under the dose of 5 mg/kg or 10 mg/kg, **4** could not decrease blood pressure significantly compared with the negative control group. **2** could decrease blood pressure but not obviously enough. **1**, **5**, **6** had similar anti-hypertensive effects with Losartan at the dose of 10 mg/kg. The maximal reduction of MBP of **1**, **5**, **6** and Losartan were 30 mmHg, 28 mmHg, 32 mmHg and 31 mmHg, respectively. **3** decreased the MBP with the maximal reduction reached 4 to 5 h following administration. A dose related response was observed for **3**(5, 10 mg/kg) with giving a MBP of 30 mmHg and 41 mmHg respectively. In addition, the duration of antihypertensive action of **3** was seen to last for at least 24 h. Compared with Losartan, **3** has a higher and longer antihypertensive effect at the same dose.

In renal hypertensive rats (RHR), the effects of compounds **1**, **2**, **3**, **4**, **5**, **6** (5, 10 mg/kg) and Losartan (10 mg/kg) on the mean blood pressure (MBP) after oral administration are shown in **Fig.8**. In RHR, the same effects were observed as in SHR. **2**, **4** had no significant antihypertensive effect. **1**, **5**, **6** at 10 mg/kg showed the similar effects to Losartan at the same dose. **3** at 5 mg/kg showed the equal effect to that of Losartan at 10 mg/kg. At 10 mg/kg, **3** had a more effective and durable antihypertensive effect.

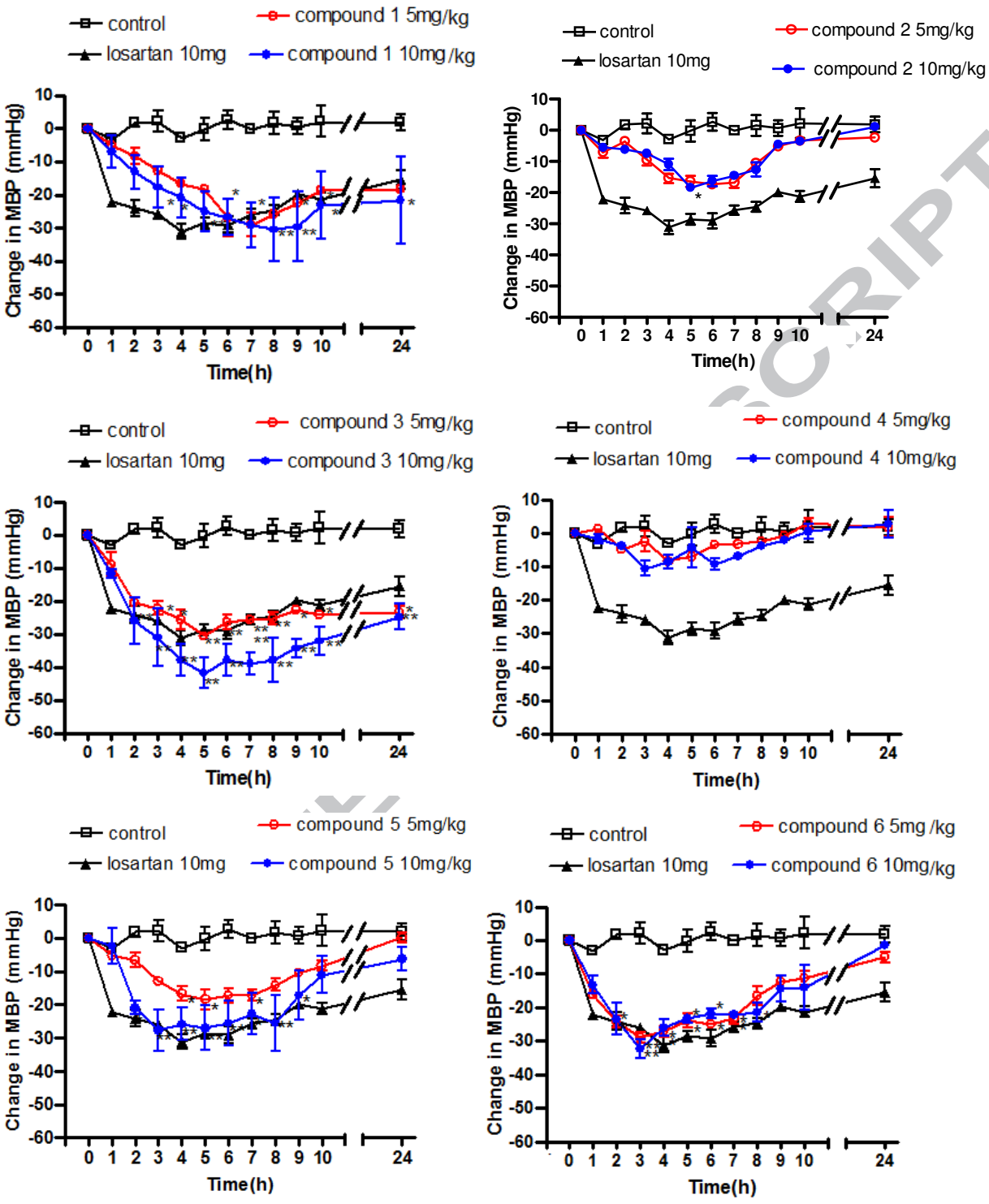


Fig.7 Effects of compounds 1, 2, 3, 4, 5, 6, and Losartan on mean blood pressure (MBP) in spontaneously hypertensive rats. *,** Significant difference from the control, $p<0.05$ and $p<0.01$, respectively.

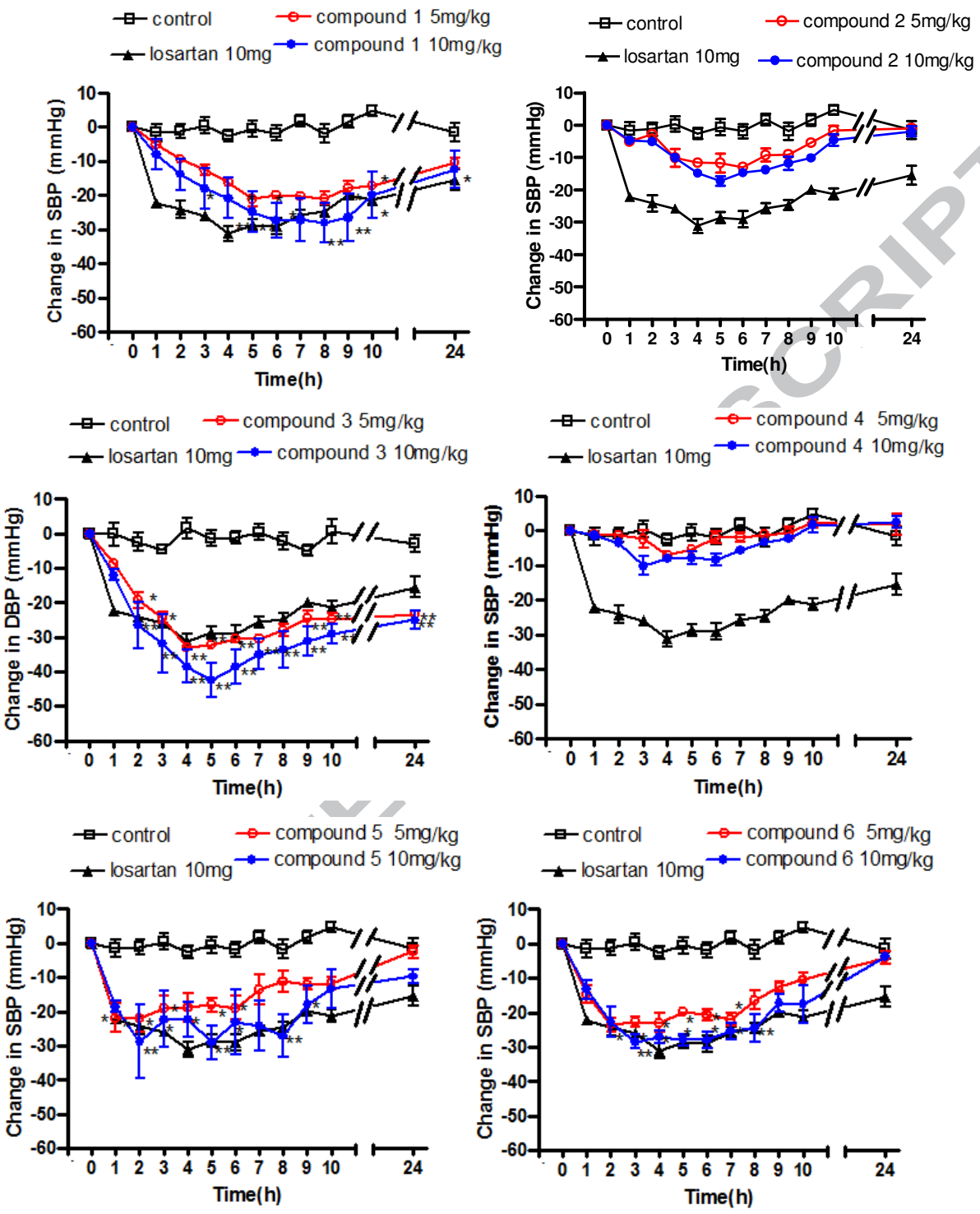


Fig.8 Effects of compounds 1, 2, 3, 4, 5, 6, and Losartan on mean blood pressure (MBP) in renal hypertensive rats. *, ** Significant difference from the control, $p<0.05$ and $p<0.01$, respectively.

3. Conclusions

A series of 5-nitro benzimidazole with 1, 4-disubsituted or 1, 5-disubsituted indole derivatives were designed and synthesized. Their *in vitro* AII antagonistic activities as well as *in vivo* antihypertensive activities were evaluated. Among them, **3** with n-butyl chain at 2-position of benzimidazole and 1, 4-disubstituted indole group was found to be more potent than Losartan. A binding profile for target compounds was proposed where 1, 4-disubstituted or 1, 5-disubstituted indole group could interact with **L2** lipophilic pocket, and 5-nitro benzimidazole could interact with the receptor through binding sites. The length of alkyl chain on 2-position of benzimidazole is very important, and the compound with butyl chain has the highest activity. The persuasive explanation is that alkyl chain with suitable length could interact more quickly and bind more tightly with **L1** lipophilic pocket. 1, 4-disubstituted and 1, 5-disubstituted indole group were introduced to form different spatial conformations, and the former showed better activity. In addition, a simple method for synthesis of 1, 4-disubstituted and 1, 5-disubstituted indole compounds had been developed. In summary, **3** could be considered as a novel effective anti-hypertension drug candidate with long-lasting blood pressure-lowering effect, and deserved for further investigation.

4. Experimental section

4.1 Chemistry

All chemical reagents were of highest commercially available quality and applied without further purification. Yields refer 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).

4.2 Preparations

4.2.1 *N*-(2-amino-5-nitrophenyl) butyramide(9b)

9b was prepared by the general procedure described by Katritzky [6].

4.2.2 *N*-(2-amino-5-nitrophenyl)propionamide(9a)

9a was prepared by the general procedure described by Katritzky [6].

4.2.3 *N*-(2-amino-5-nitrophenyl) valeramide(9c)

9c was prepared by the general procedure described by Katritzky [6].

4.2.4 *N*-(2-amino-5-nitrophenyl)hexanamide (9d)

9d was prepared by the general procedure described by Katritzky [6].

4.2.5 2-propyl-5-nitro-1H-benzo[d]imidazole (10b)

10b was prepared by the general procedure described by Olguin [7].

4.2.6 2-ethyl-5-nitro-1H-benzo[d]imidazole (10a)

10a was prepared by the general procedure described by Olguin [7].

4.2.7 2-butyl -5-nitro-1H-benzo[d]imidazole(10c)

10c was prepared by the general procedure described by Olguin [7].

4.2.8 2-pentyl-5-nitro-1H-benzo[d]imidazole（10d)

10d was prepared by the general procedure described by Olguin [7].

4.2.9 (4-methyl-1H-indol-1-yl) (phenyl) methanone (12a)

12a was prepared by the general procedure described by Dhanoa [8].

4.2.10 (5-methyl-1H-indol-1-yl) (phenyl) methanone (12b)

12b was prepared by the general procedure described by Dhanoa [8].

4.2.11 ((4-bromomethyl)-1H-indol-1-yl)(phenyl) methanone (13a)

13a was prepared by the general procedure described by Dhanoa [8].

4.2.12 ((5-bromomethyl)-1H-indol-1-yl)(phenyl) methanone (13b)

13b was prepared by the general procedure described by Dhanoa [8].

4.2.13 1-((1H-indol-4-yl)methyl)-2-propyl-5-nitro-1H-benzo[d]imidazole (14b)

A solution of **10b** (500 mg, 2.44 mmol) in acetone (50 ml) was treated with K_2CO_3(435 mg, 3.15 mmol) and **13a**（765 mg，2.44 mmol), and the mixture stirred at 60^{o}C for 6h. The resulting mixture was diluted with water and extracted with ethyl acetate (50 ml×3). The organic layer was washed with saturated salt water (75 ml) and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The residue was treated with the solution of 2M NaOH (12 ml) in 12ml MeOH, the mixture stirred and heated under reflux for 3h. After completed, the solvent was removed in vacuo. The resulting mixture was diluted with water and extracted with DCM (15 ml×4). The organic layer was washed with saturated salt water (20 ml) and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The residue was purified by CC to give **14b** (249 mg) as a yellow solid. Yield: 30.6%. m.p.: 172-174 ^{o}C. ^{1}H NMR (400MHz, $CDCl_3$) δ: 8.72 (1H, d, J=1.9Hz, Ph-H), 8.44 (1H, d, N-H), 8.13 (1H, dd, J_1=2.0Hz, J_2=8.8Hz, Ph-H), 7.41 (1H, d, J=8.1Hz, Ph-H), 7.32 (1H, t, J=2.8,Hz, Ph-H), 7.26 (1H, s, Ph-H), 7.10 (1H, t, J=7.0Hz, indo-H), 6.47 (1H, d, J=7.2Hz, Ph-H), 6.45 (1H, s, indo-H), 5.70 (2H, s,

Ph-C<u>H</u>$_2$), 2.93 (2H, t, J=7.5Hz, -C<u>H</u>$_2$CH$_2$CH$_3$), 1.92(2H, m, J=7.4Hz, -CH$_2$C<u>H</u>$_2$CH$_3$), 1.03 (3H, t, J=7.3Hz, -CH$_2$CH$_2$ C<u>H</u>$_3$). MS(ESI): $[M+H]^+$ calcd 335.1; found 335.0.

4.2.14 1-((1H-indol-4-yl)methyl)-2-ethyl-5-nitro-1H-benzo[d]imidazole(14a)

14a was prepared according to the procedure described for the preparation of **14b.** Yield: 37.0%. m.p.: 168-171 ^{o}C. ^{1}H NMR (400 MHz, CDCl$_3$) δ: 8.73 (d, 1H, J=1.81Hz, Ph-H), 8.47 (s, 1H, N-H), 8.14 (dd, 1H, J_1=1.74Hz, J_2=8.92Hz, Ph-H), 7.41 (d, 1H, J=8.22Hz, Ph-H), 7.32 (d,1H, J=2.78Hz, Ph-H), 7.27 (d, 1H, J=6.45Hz, indo-H), 7.10 (t,1H, J=7.86Hz, Ph-H), 6.49 (d, 1H, J=7.32, Ph-H), 6.44 (s,1H, indo-H), 5.32 (s, 2H, PhC<u>H</u>$_2$), 2.96 (q, 2H, J=7.48Hz, -C<u>H</u>$_2$CH$_3$), 1.47 (t, 3H, J=7.59Hz, -CH$_2$C<u>H</u>$_3$). MS(ESI): $[M+H]^+$ calcd 321.1; found 321.0.

4.2.15 1-((1H-indol-4-yl)methyl)-2-butyl-5-nitro-1H-benzo[d]imidazole (14c)

14c was prepared according to the procedure described for the preparation of **14b.** Yield: 32.7%. m.p.: 173-175 ^{o}C. ^{1}H NMR (400MHz, CDCl$_3$) δ: 8.67（d, 1H, J=2.10Hz, Ph-H）, 8.46 (s, 1H, N-H), 8.09 (dd, 1H, J_1=2.15Hz, J_2=8.90Hz, Ph-H), 7.38 (d, 1H, J=8.12Hz, Ph-H), 7.29 (t, 1H, J=2.96Hz, Ph-H), 7.22 (d, 1H, J=8.90Hz, indo-H), 7.07 (t, 1H, J=7.76Hz, Ph-H), 6.44 (m, 2H, Ph-H, indo-H), 5.67 (s, 2H, Ph-CH$_2$-), 2.89 (t, 2H, J=7.72Hz, -C<u>H</u>$_2$CH$_2$CH$_2$CH$_3$), 1.83 (quin, 2H, J=7.56Hz, -CH$_2$C<u>H</u>$_2$CH$_2$CH$_3$), 1.40 (sext, 2H, J=7.56Hz, -CH$_2$CH$_2$C<u>H</u>$_2$CH$_3$), 0.90 (t, 3H, J=7.32Hz, -CH$_2$CH$_2$CH$_2$C<u>H</u>$_3$). MS(ESI): $[M+H]^+$ calcd 349.2; found 349.3.

4.2.16 1-((1H-indol-4-yl)methyl)-2-pentyl-5-nitro-1H-benzo[d]imidazole (14d)

14d was prepared according to the procedure described for the preparation of **14b.** Yield: 18.6%. m.p.: 175-178 ^{o}C. ^{1}H NMR (400MHz,CDCl$_3$) δ: 8.68 (d, 1H, J=2.16Hz, Ph-H), 8.41 (s, 1H, N-H), 8.09 (dd, 1H, J_1=2.10Hz, J_2=8.84Hz, Ph-H), 7.38 (d, 1H, J=8.16Hz, Ph-H), 7.29 (t, 1H, J=2.64Hz, Ph-H), 7.23 (d, 1H, J=8.76Hz, indo-H), 7.08 (t, 1H, J=7.48Hz, Ph-H), 6.45 (m, 2H, Ph-H, indo-H), 5.67 (s, 2H, Ph-CH$_2$), 2.89 (t, 2H, J=7.72Hz, -C<u>H</u>$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 1.83 (sext, 2H, J=7.76Hz, -CH$_2$C<u>H</u>$_2$CH$_2$CH$_2$CH$_3$), 1.32 (m, 4H, -CH$_2$CH$_2$C<u>H</u>$_2$C<u>H</u>$_2$CH$_3$), 0.85 (t, 3H, J=7.12Hz, -CH$_2$CH$_2$CH$_2$CH$_2$C<u>H</u>$_3$). MS(ESI): $[M+H]^+$ calcd 363.2; found 363.4.

4.2.17 1-((1H-indol-5-yl)methyl)-2-propyl-5-nitro-1H-benzo[d]imidazole(14e)

14e was prepared according to the procedure described for the preparation of **14b.** Yield: 30.3%. m.p.: 171-173 ^{o}C. ^{1}H NMR (400MHz, CDCl$_3$) δ: 8.67 (d, 1H, J=2.10Hz, Ph-H), 8.40 (s, 1H, N-H), 8.13 (dd, 1H, J_1=2.15Hz, J_2=8.88Hz, Ph-H), 7.36 (d, 1H, J=8.40Hz, Ph-H), 7.30 (m, 2H, Ph-H, indo-H), 7.25 (d, 1H, J=2.87Hz, Ph-H), 6.90 (d, 1H, J=8.40Hz, Ph-H) , 6.49 (s, 1H, indo-H), 5.49 (s, 2H, Ph-CH$_2$), 2.92 (t, 2H, J=7.51Hz, -C<u>H</u>$_2$CH$_2$CH$_3$), 2.05-1.89(m, 2H, -CH$_2$C<u>H</u>$_2$CH$_3$), 1.03(t, 3H, -CH$_2$C<u>H</u>$_2$CH$_3$). MS(ESI): $[M+H]^+$ calcd 335.1; found 335.1.

4.2.18 1-((1H-indol-5-yl)methyl)-2-butyl-5-nitro-1H-benzo[d]imidazole (14f)

14f was prepared according to the procedure described for the preparation of **14b.** Yield: 26.4%. m.p.: 172-174 ^{o}C. ^{1}H NMR (400MHz, $CDCl_3$) δ: 8.66（d, 1H, J=1.92Hz, Ph-H）, 8.23 (s, 1H, N-H), 8.12 (dd, 1H, J_1=1.98Hz, J_2=8.96Hz, Ph-H), 7.35 (d, 1H, J=8.4Hz, Ph-H), 7.29 (m, 2H, Ph-H, indo-H), 7.24 (t, 1H, J=2.92Hz, Ph-H), 6.89 (d, 1H, J=8.36Hz, Ph-H), 6.48 (t, 1H, J=2.04Hz, indo-H), 5.48 (s, 2H, Ph-CH_2-), 2.92 (t, 2H, J=7.68Hz, -C$\underline{H}_2$$CH_2CH_2CH_3$), 1.85 (quin, 2H, J=8.16Hz, -CH_2C$\underline{H}_2$$CH_2CH_3$), 1.45-1.37 (m, 2H, -$CH_2CH_2C\underline{H}_2$$CH_3$), 0.91 (t, 3H, J=7.36Hz, -$CH_2CH_2CH_2$C$\underline{H}_3$). MS(ESI): $[M+H]^+$ calcd 349.2; found 349.3.

4.2.19 2-(4-((2-propyl-5-nitro-1H-benzo[d]imidazol-1-yl)methyl)-1H-indol-1-yl) benzonitrile (15b)

A solution of **14b (**158 mg, 0.47 mmol) and K_2CO_3(135 mg , 0.98 mmol) in DMF (10 ml) was treated with 2-fluorobenzonitrile (0.08 ml, 0.74 mmol), and the mixture stirred and heated under reflux for 5h under nitrogen. After completed, the resulting mixture was diluted with water and extracted with EA (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 CC to give **15b** (163 mg) as a yellow solid. Yield: 79.3%. m.p.: 183-186 ^{o}C. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 8.67（1H, d, J=2.0Hz, Ph-H）, 8.09（1H, dd, J_1= 2.1Hz, J_2= 6.7Hz, Ph-H）, 7.87 (1H, d, J=7.8Hz, Ph-H), 7.78 (1H, t, J=7.7Hz, Ph-H), 7.60 (1H, d, J=8.1Hz, Ph-H), 7.56 (1H, t, J=7.6Hz, Ph-H), 7.47 (1H, d, J=3.4Hz, Ph-H) , 7.26 (1H, d, J=2.1Hz, Ph-H), 7.24 (1H, s, Ph-H), 7.09 (1H, t, J=7.6Hz, indo-H), 6.98 (1H, d, J=2.1Hz, Ph-=3.4Hz, Ph-H), 6.46 (1H, d, J=7.3Hz, indo-H), 5.71 (2H, s, Ph-C$\underline{H}_2$), 2.90 (2H, t, J=7.4Hz, -C$\underline{H}_2$$CH_2CH_3$), 1.91 (2H, q, J=7.4Hz, -CH_2C$\underline{H}_2$$CH_3$), 1.03 (3H, t, J=7.3Hz, -CH_2CH_2C$\underline{H}_3$). MS(ESI): $[M+H]^+$ calcd 436.2; found 436.5.

4.2.20 2-(4-((2-ethyl-5-nitro-1H-benzo[d]imidazol-1-yl)methyl)-1H-indol-1-yl) benzonitrile (15a)

15a was prepared according to the procedure described for the preparation of **15b.**Yield: 74.9%. m.p.: 179-181 ^{o}C. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 8.71 (1H, s, Ph-H), 8.13 (1H, s, J=8.8Hz, Ph-H), 7.88 (1H, d,J=7.8Hz, Ph-H), 7.78 (1H, s, J=7.7Hz, Ph-H), 7.61 (1H, q, J=7.9Hz, Ph-H), 7.56 (1H, d,J=7.6Hz, Ph-H), 7.47 (1H, d, J=3.3Hz, Ph-H), 7.28 (2H, t, J=9.0Hz, Ph-H), 7.11 (1H, t, J=7.4Hz, indo-H), 6.68 (1H, d, J=3.3Hz, Ph-H), 6.47 (1H, d, J=7.2Hz, indo-H), 5.71 (2H, s, Ph-CH_2), 2.95 (2H, q, J=7.4Hz, -C$\underline{H}_2$$CH_3$), 1.47 (3H, t, J=7.4Hz, -CH_2C$\underline{H}_3$). MS(ESI): $[M-H]^-$ calcd 420.2; found 420.6.

4.2.21 2-(4-((2-butyl-5-nitro-1H-benzo[d]imidazol-1-yl)methyl)-1H-indol-1-yl) benzonitrile(15c)

15c was prepared according to the procedure described for the preparation of **15b.**Yield: 84.8%. m.p.: 184-187 °C. ^{1}H NMR (400MHz, $CDCl_3$) δ: 8.68 (1H, d, J=2.0Hz, Ph-H), 8.12 (1H, dd, J_1=2.0Hz, J_2=9.0Hz, Ph-H), 7.88 (1H, d, J=7.7Hz, Ph-H), 7.78 (1H, t, J=8.0Hz, Ph-H), 7.61 (1H, d, J=8.0Hz, Ph-H), 7.56 (1H, t, J=7.6Hz, Ph-H), 7.47 (1H, d, J=3.4Hz, Ph-H), 7.28 (1H, s, Ph-H), 7.25 (1H, s, indo-H), 7.10 (1H, t, J=7.4Hz, Ph-H), 6.69 (1H, q, J=3.3Hz, Ph-H), 6.47 (1H, q, J=7.2Hz, indo-H), 5.71 (2H, s, Ph-C$\underline{H}_2$), 2.92 (2H, t, J=7.6Hz, C$\underline{H}_2$$CH_2CH_2CH_3$), 1.90-1.83 (2H, m, J=7.4Hz, $CH_2C\underline{H}_2CH_2CH_3$), 1.43 (2H, s, J=7.5Hz, $CH_2CH_2C\underline{H}_2CH_3$), 0.92 (3H, t, J=7.3Hz, $CH_2CH_2CH_2C\underline{H}_3$). MS(ESI): $[M+H]^+$ calcd 450.2; found 450.5.

4.2.22 2-(4-((2-pentyl-5-nitro-1H-benzo[d]imidazol-1-yl)methyl)-1H-indol-1-yl) benzonitrile (15d)

15d was prepared according to the procedure described for the preparation of **15b.**Yield: 84.3%. m.p.: 186-188°C. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 8.67 (1H, d, J=2.04Hz, Ph-H), 8.13 (1H, dd, J_1=2.1Hz, J_2=8.7Hz, Ph-H), 7.88 (1H, d, J=7.7Hz, Ph-H), 7.78 (1H, t, J=8.0Hz, Ph-H), 7.60 (1H, m, Ph-H), 7.56 (1H, s, Ph-H), 7.46 (1H, d, J=3.4Hz, Ph-H), 7.25 (2H, m, Ph-H, indo-H), 7.10 (1H, s, Ph-H), 6.68 (1H, d, J=3.3Hz, Ph-H), 6.47 (1H, d, J=7.8Hz, indo-H), 5.70 (2H, s, Ph-CH_2), 2.91 (2H, t, J=7.5Hz, -C$\underline{H}_2CH_2CH_2CH_2CH_3$), 1.89-1.85 (2H, m,-$CH_2C\underline{H}_2CH_2CH_2CH_3$), 1.40-1.33 (4H, m,-$CH_2CH_2C\underline{H}_2C\underline{H}_2CH_3$), 0.86 (3H, t, J=7.0Hz, -$CH_2CH_2CH_2CH_2C\underline{H}_3$). MS(ESI): $[M+H]^+$ calcd 464.2; found 464.5.

4.2.23 2-(5-((2-propyl-5-nitro-1H-benzo[d]imidazol-1-yl)methyl)-1H-indol-1-yl) benzonitrile(15e)

15e was prepared according to the procedure described for the preparation of **15b.** Yield: 79.5%. m.p.: 182-184 °C. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 8.66 (1H, d, J=2.0Hz, Ph-H), 8.12 (1H, d, J_1=2.1Hz, J_2=8.9Hz, Ph-H),7.84 (1H, d, J=0.8Hz, Ph-H), 7.74 (1H, d, J=0.7Hz, Ph-H), 7.54 (2H, t, J=8.4Hz, Ph-H), 7.40 (1H, d, J=3.3Hz, Ph-H), 7.31 (2H, d, J=2.2Hz, indo-H, Ph-H), 7.26 (1H, m, Ph-H), 6.95 (1H, s, Ph-H), 6.68 (1H, d,J=3.3Hz, indo-H), 5.51 (2H, s, Ph-CH_2-), 2.91 (2H, q, J=8.6Hz, -C$\underline{H}_2CH_2CH_3$), 1.92 (2H, q, J=7.5Hz, -$CH_2C\underline{H}_2CH_3$), 1.06 (3H, t, J=7.3Hz, -$CH_2CH_2C\underline{H}_3$). MS(ESI): $[M+H]^+$ calcd 436.2; found 436.2.

4.2.24 2-(5-((2-butyl-5-nitro-1H-benzo[d]imidazol-1-yl)methyl)-1H-indol-1-yl) benzonitrile (15f)

15f was prepared according to the procedure described for the preparation of **15b.** Yield: 77.1%. m.p.: 183-185 °C. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 8.66 (1H, d, J=2.0Hz, Ph-H), 8.13 (1H, dd, J_1=2.1Hz， J_2=8.9Hz, Ph-H),8.02 (1H, d, J=7.7Hz,Ph-H), 7.85 (1H, t, J=7.6Hz, Ph-H), 7.54 (2H, m, Ph-H), 7.40 (1H, d, J=3.4Hz, Ph-H), 7.32 (1H, d, J=2.6Hz， Ph-H)， 7.30-7.25 (2H, m,

Ph-H, indo-H), 6.95 (1H, d, J=3.5Hz, Ph-H), 6.68 (1H, s, indo-H), 5.51 (2H, s, Ph-CH_2) , 2.95 (2H, t, J=7.6Hz, -C<u>H</u>$_2$$CH_2CH_2CH_3$), 1.92-1.84(2H, m, -$CH_2$C<u>H</u>$_2$$CH_2CH_3$), 1.51-1.44 (2H, m, -$CH_2CH_2$C<u>H</u>$_2$$CH_3$), 0.95 (3H, t, J=7.3Hz, -$CH_2CH_2CH_2$C<u>H</u>$_3$). MS(ESI): $[M+H]^+$ calcd 450.2; found 450.3.

4.2.25 2-(4-((2-propyl-5-nitro-1H-benzo[d]imidazol-1-yl)methyl)-1H-indol-1-yl) benzoic acid (2)

A solution of 5M NaOH(10 ml) in 10ml methanol was treated with **15b**（107 mg，0.25 mmol), and the mixture stirred and heated under reflux for 34 h. After completed, the solvent was removed in vacuo. The pH value of the mixture was adjusted to 5-6 by careful addition of 6M aqueous hydrochloride. The resulting mixture was extracted with DCM (20 ml×5). The organic layer was washed with saturated salt water (40 ml×1) and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The residue was purified by CC to give **2** as a yellow solid 57 mg. Yield: 51.2%. m.p.: 196-199 ^oC. ^{1}H-NMR (DMSO，400MHz,) δ: 12.19 (1H, s, -COOH), 8.52(1H, d, J=2.15Hz, Ph-H), 8.12-8.09 (1H, dd, J_1=2.2Hz, J_2=8.9Hz, Ph-H), 7.70-7.68 (1H, m，Ph-H) , 7.58(1H, s, Ph-H), 7.52(1H, m, indo-H), 7.37-7.35(2H, m, Ph-H), 7.28-7.27(1H, m, Ph-H), 7.17-7.15 (1H, m, Ph- H), 6.97-6.94 (1H, m, Ph-h), 6.54 (1H, d, J=3.03Hz, Ph-H), 6.30 (1H, d, J=4.3Hz, indo-H), 5.87 (2H, s，Ph-CH_2-), 2.88(2H, t, J=7.4Hz, -C<u>H</u>$_2$$CH_2CH_3$)，1.82(2H, m, -$CH_2$C<u>H</u>$_2$$CH_3$), 0.96 (3H, t, J=7.4Hz, -CH_2CH_2C<u>H</u>$_3$). ^{13}C-NMR (DMSO, 125 MHz) δ: 170.56, 160.42, 143.08, 143.08, 142.02, 140.68, 140.68, 139.31, 135.67, 131.13, 127.72, 127.48, 127.31, 126.49, 121.85, 118.07, 116.64, 115.08, 111.21, 110.76, 105.08, 99.80, 45.64, 29.22, 20.37, 14.13. HRMS (ESI): *m/z* calculated for $C_{26}H_{22}N_4O_4$ $[M+Na]^+$: 477.15387; found 477.15106. Anal. Calcd for $C_{26}H_{22}N_4O_4$: C, 68.71; H, 4.88; N, 12.33. Found: C, 68.83; H, 4.96; N, 12.29.

4.2.26 2-(4-((2-ethyl-5-nitro-1H-benzo[d]imidazol-1-yl)methyl)-1H-indol-1-yl) benzoic acid (1)

1 was prepared according to the procedure described for the preparation of **2.** Yield: 47.2%. m.p.: 191-194 ^oC. ^{1}H-NMR (400MHz, DMSO) δ: 12.9 (1H, s, -COOH), 8.54 (1H, d, J=2.15Hz, Ph-H), 8.13(1H, dd, J_1=2.2Hz, J_2=8.9Hz, Ph-H), 7.94 (1H, d, J=7.9Hz, Ph-H), 7.74 (2H, t, J=8.8Hz, Ph-H), 7.60 (1H, t, J=7.5Hz, Ph-H), 7.53 -7.51 (2H, m, Ph-H, indo-H), 7.02 (2H, d, J=8.5, Ph-H), 6.64 (1H, d, J=3.2Hz, Ph-H), 6.42 (1H, d, J=4.3Hz, indo-H), 5.91 (2H, s, Ph-CH_2-), 2.91 (2H, q, J_1=7.4Hz, J_2=14.8Hz, -C<u>H</u>$_2$$CH_3$), 1.30 (3H, t, J=7.4Hz, -CH_2C<u>H</u>$_3$). ^{13}C-NMR: 167.18, 161.04, 142.59, 141.49, 140.37, 137.18, 136.79,132.58, 130.69, 130.24, 128.46, 128.09, 127.72, 126.14, 122.08, 117.71, 116.76, 114.68, 110.66, 109.53, 100.46, 45.00,

40.12, 20.48, 11.04. HRMS (ESI): *m/z* calculated for $C_{25}H_{20}N_4O_4$ $[M+H]^+$: 441.15628; found 441.15573. Anal. Calcd for $C_{25}H_{20}N_4O_4$: C, 68.17; H, 4.58; N, 12.72. Found: C, 68.06; H, 4.43; N, 12.54.

4.2.27 2-(4-((2-butyl-5-nitro-1H-benzo[d]imidazol-1-yl)methyl)-1H-indol-1-yl) benzoic acid (3)

3 was prepared according to the procedure described for the preparation of **2**.Yield: 56.8%. m.p.: 200-203 °C. ^{1}H NMR (400MHz, DMSO) δ: 12.8 (1H, s,-COOH), 8.51 (1H, d, J=2.2Hz, Ph-H), 8.11 (1H, dd, J_1=2.2Hz, J_2=8.8Hz, Ph-H), 7.94 (1H, d, J=7.7Hz, Ph-H), 7.71-7.69 (2H, m, Ph-H), 7.60 (1H, t, J=7.6Hz, Ph-H), 7.51-7.48 (2H, m, Ph-H, indo-H), 7.03-7.00（2H, m, Ph-H）, 6.64 (1H, d, J=3.3Hz, Ph-H), 6.42 (1H, m, indo-H), 5.91 (2H, s, Ph-CH_2-), 2.88 (2H, t, J=7.4Hz, -C$\underline{H}_2$CH$_2$CH$_2$CH$_3$), 1.70 (2H, m, -CH$_2$C$\underline{H}_2$CH$_2$CH$_3$), 1.34 (2H, m, -CH$_2$CH$_2$C$\underline{H}_2$CH$_3$), 0.83 (3H, t, J=7.3Hz,-CH$_2$CH$_2$CH$_2$C$\underline{H}_3$).^{13}C-NMR: 169.81, 162.90, 145.40, 144.31, 143.02, 140.04, 139.58, 135.51, 133.52, 132.99, 132.69, 131.28, 130.90, 130.57, 128.96, 124.86, 120.44, 119.59, 117.41,113.49, 112.29, 103.29, 47.85, 31.76, 29.34, 24.50, 16.38. HRMS (ESI): *m/z* calculated for $C_{27}H_{24}N_4O_4$ $[M+H]^+$: 469.18758; found 469.18703. Anal. Calcd for $C_{27}H_{24}N_4O_4$: C, 69.22; H, 5.16; N, 11.96. Found: C, 69.16; H, 5.03; N, 11.89.

4.2.28 2-(4-((2-pentyl-5-nitro-1H-benzo[d]imidazol-1-yl)methyl)-1H-indol-1-yl) benzoic acid (4)

4 was prepared according to the procedure described for the preparation of **2.** Yield: 47.7%. m.p.: 205-208 °C. ^{1}H NMR (400MHz, DMSO) δ: 12.85 (1H, s, -COOH), 8.52 (1H, d，J=2.1Hz, Ph-H), 8.11 (1H, dd，J_1=2.1Hz, J_2=8.9Hz, Ph-H), 7.93 (1H, d, J=1.4Hz, Ph-H), 7.74-7.69 (2H, m，Ph-H), 7.60 (1H, m，Ph-H), 7.51-7.48 (2H, m，Ph-H, indo-H), 7.02-7.00 (2H, m，Ph-H), 6.63 (1H, d，J=3.2Hz, Ph-H), 6.31 (1H, m，indo-H), 5.90 (2H, s，Ph-CH_2-), 2.88 (2H, t，J=6.7Hz, C$\underline{H}_2$CH$_2$CH$_2$CH$_2$CH$_3$), 1.73 (2H, m，CH$_2$C$\underline{H}_2$CH$_2$CH$_2$CH$_3$), 1.26-1.24 (4H, m，CH$_2$CH$_2$C$\underline{H}_2$C$\underline{H}_2$CH$_3$), 0.81 (3H, t，J=7.0Hz, CH$_2$CH$_2$CH$_2$CH$_2$C$\underline{H}_3$).^{13}C-NMR: 162.92, 145.40, 144.30, 143.01, 139.52, 134.51, 133.21, 130.85, 128.92, 124.69, 120.43, 119.45, 117.41, 113.49, 112.48, 103.01, 47.87, 42.89, 42.69, 42.48, 42.06, 41.85, 33.54, 29.59, 28.92, 24.53, 16.54. HRMS (ESI): *m/z* calculated for $C_{28}H_{26}N_4O_4$ $[M+H]^+$: 483.20323; found 483.20268. Anal. Calcd for $C_{28}H_{26}N_4O_4$: C, 69.70; H, 5.43; N, 11.61. Found: C, 69.78; H, 5.51; N, 11.56.

4.2.29 2-(5-((2-propyl-5-nitro-1H-benzo[d]imidazol-1-yl)methyl)-1H-indol-1-yl) benzoic acid (5)

5 was prepared according to the procedure described for the preparation of **2.** Yield: 61.2%. m.p.: 197-200 °C. ^{1}HNMR (400MHz, DMSO) δ: 12.84(1H, s, -COOH), 8.49 (1H, d，J=2.1Hz,

Ph-H), 8.12 (1H, dd，J_1=2.1Hz, J_2=8.9Hz, Ph-H), 7.91 (1H, d，J=1.4Hz, Ph-H), 7.77-7.76 (2H, m，J=8.9Hz, Ph-H), 7.57 (1H, d, J=0.8Hz, Ph-H), 7.48 (1H, d，J=7.8Hz, indo-H), 7.44 (1H, d，J=3.2Hz, Ph-H), 7.35 (1H, s, Ph-H), 7.05 (1H, s，Ph-H), 6.98 (1H, d，J=2.8Hz, Ph-H), 6.58 (1H, d，indo-H), 5.66 (2H, s, Ph-CH_2), 2.94 (2H, t, J=7.4Hz, -$CH_2CH_2CH_3$), 1.85-1.79 (2H, m，-$CH_2CH_2CH_3$), 0.98 (3H, t，J=7.3Hz, -$CH_2CH_2CH_3$). ^{13}C-NMR: 167.72, 160.36, 143.21, 142.03, 140.44, 137.83, 136.72, 133.34, 131.28, 131.10, 130.31, 129.13, 128.64, 128.18, 121.45, 119.29, 118.28, 115.09, 111.47, 103.43, 47.52, 40.31, 39.90, 29.34, 20.61, 14.28. HRMS (ESI): *m/z* calculated for $C_{26}H_{22}N_4O_4$ $[M+H]^+$: 455.17193; found 455.17138. Anal. Calcd for $C_{26}H_{22}N_4O_4$: C, 68.71; H, 4.88; N, 12.33. Found: C, 68.80; H, 4.92; N, 12.30.

4.2.30 2-(5-((2-butyl-5-nitro-1H-benzo[d]imidazol-1-yl)methyl)-1H-indol-1-yl) benzoic acid (6)

6 was prepared according to the procedure described for the preparation of **2.** Yield: 52.5%. m.p.: 201-204 ^{o}C. ^{1}H NMR (400MHz, DMSO-d_6) δ: 12.80 (1H, s，-COOH), 8.48 (1H, d，J=1.9Hz, Ph-H), 8.12 (1H, dd，J_1=2.0 Hz, J_2=8.9Hz, Ph-H), 7.91 (1H, d，J=7.7 Hz, Ph-H), 7.77 -7.71 (2H, m, Ph-H), 7.57 (1H, t，J=7.5 Hz, Ph-H), 7.46 (1H, d，J=7.8 Hz, indo-H), 7.42(1H, d, J=3.1 Hz, Ph-H), 7.36 (1H, s, Ph-H), 7.05 (1H, d，J=8.4 Hz, Ph-H), 6.97 (1H, d，J=8.5 Hz, Ph-H), 6.57 (1H, d, J=3.1 Hz, indo-H), 5.66 (2H, s, -PhCH_2-), 2.96 (2H, t, J=7.6 Hz, C<u>H</u>$_2$$CH_2CH_2CH_3$), 1.78-1.74 (2H, m, CH_2C<u>H</u>$_2$$CH_2CH_3$), 1.40-1.37 (2H, m，$CH_2CH_2$C<u>H</u>$_2$$CH_3$), 0.88(3H, t, J=7.3 Hz, $CH_2CH_2CH_2$C<u>H</u>$_3$). ^{13}C-NMR: 159.81, 142.56, 141.57, 139.96, 137.09, 136.07, 130.53, 128.53, 128.21, 127.86, 127.60, 120.77, 118.62, 117.58, 114.54, 110.81, 110.35, 102.66, 46.91, 40.12, 39.91, 39.08, 38.87, 28.67, 26.58, 21.80, 13.68. HRMS (ESI): *m/z* calculated for $C_{27}H_{24}N_4O_4$ $[M+H]^+$: 469.18758; found 469.18703. Anal. Calcd for $C_{27}H_{24}N_4O_4$: C, 69.22; H, 5.16; N, 11.96. Found: C, 69.11; H, 5.22; N, 11.88.

4.3 Radioligand binding assay

The vascular smooth muscle cells (VSMCs) were gained from thoracic aorta of SD rats (Second Military Medical University, China) and cultured by the tissue explants methods [9]. 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 1h, at 37^{o}C. The homogenate was centrifuged at 10000×g for 5 mins. Then they were incubated with 1ml of DMEM supplemented with 15% fetal bovine serum (FBS) at 37^{o}C in 95% air 5% CO_2. Cells at

passage 3-7 were used for the experiments.

Each 250μL incubate contained the following: 0.1nM ^{125}I-Angiotensin II (Zhongshan Hospital, Shanghai, China) and concentration of test compounds. The final concentrations were 1×10^{-6}-1×10^{-12}M. They were incubated in 24-well plates with VSMCs for 60 min at 37°C. Nonspecific binding was measured in the presence of 1 μM AII and represented 5-10% of total binding. After the reaction, removed the liquid immediately, washed five times with PBS, and digested cells with 0.1M NaOH for 10 min. The radioactivity was counted with a γ-counter (Wallac 1470 Wizard, PerkinElmer, Finland). The IC_{50} value (concentration for 50% displacement of the specifically bound ^{125}I-Angiotensin II) was determined by regression analysis of displacement curves [10]. The inhibition constant (K_i value) was calculated from the formula $K_i = IC_{50}/ (1+ [L]/k_d)$ [11], where [L] was the concentration of radioligand present in tubes.

4.4 *In vivo* study of anti-hypertensive effect

For preparing renal hypertensive rats, the left renal arteries of Sprague-Dawley rats (250-350 g, Second Military Medical University, China) were completely ligated under sodium pentobarbital (40 mg/kg) anesthesia. Thereafter，the rats with SBP higher than 160 mmHg were selected and used as renal hypertensive rats. Spontaneous hypertensive rats (250-300 g) came from Second Military Medical University, China. Both spontaneous and renal hypertensive rats were randomly divided into different experimental groups of 10 animals (negative control group, positive control groups, 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 5 mg/kg and 10 mg/kg separately. Losartan (10 mg/kg, SanXin Zhujiang Chemical Engineering Company) was used as positive control group. The negative control group was administered the same volume of sodium carboxymethyl cellulose solution. To measure the blood pressure, the animals were anesthetized with sodium pentobarbital (40 mg/kg, i.p.). The right carotid artery and jugular vein were cannulated for arterial pressure measurement and drug administration, respectively. The arterial catheter was connected to a pressure transducer and displayed on a computer and analyzed with a biological signal analysis system (MPA-2000, Alcott Biotech,

China). The parameters were measured continuously for 10 h, and again at 24 h [12].

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Abbreviations used

RAS, rennin-angiotensin system; AII, angiotensin II; ARBs, angiotensin II receptor blockers; FDA, US Food and Drug Administration; DMAP, 4-(dimethylamino)pyridine; NMR, nuclear magnetic resonance; HRMS, high-resolution mass spectrometry; ESI, electrospray ionization; MS, mass spectrometry; DMSO, dimethylsulfoxide; DMF, N,N-dimethylformamide; THF, tetrahydrofuran; AIBN, azodiisobutyronitrile; NBS, bromosuccinimide; NOESY, nuclear overhauser effect spectroscopy; VSMC, vascular Smooth Muscle Cell; IC_{50}, half maximal inhibitory concentration; SHR, spontaneously hypertensive rats; MBP, mean blood pressure; RHR, renal hypertensive rats; DMEM, Dulbecco's Modified Eagle's Medium; FBS, fetal bovine serum.

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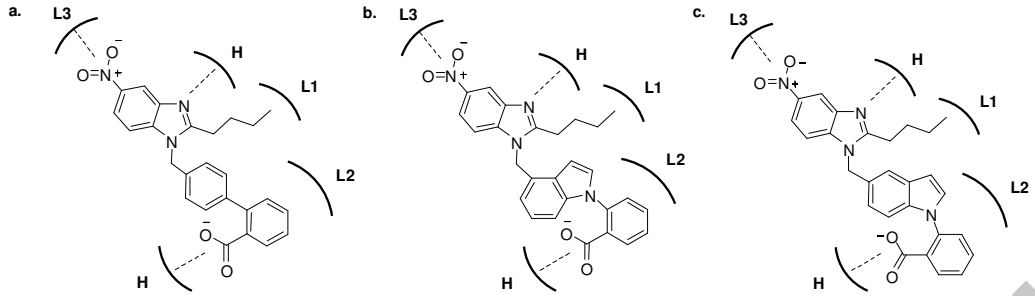


Fig.1 (a) Binding profile of **7** where **L1–L2** are lipophilic pockets, **L3** is a lipophilic or H-bond donor pocket, and **H** is H-bond donor site. (b) Binding profile of **3** where **L1–L2** are lipophilic pockets, **L3** is a lipophilic or H-bond donor pocket, and **H** is H-bond donor site. (c) Binding profile of **6** where **L1–L2** are lipophilic pockets, **L3** is a lipophilic or H-bond donor pocket, and **H** is H-bond donor site.

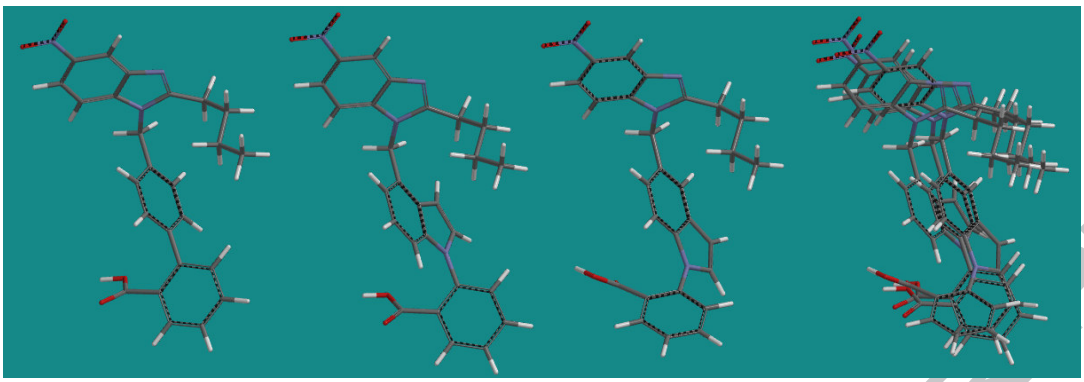


Fig.2 Energy-minimized conformation of **7**, **3**, **6** and their overlay conformation

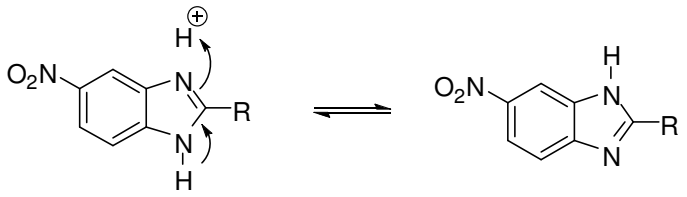
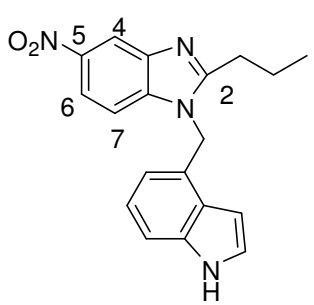
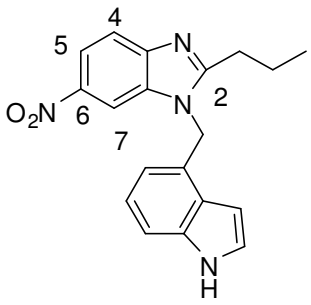


Fig.3 Tautomeric forms of **10a-d**



14b



16

Fig.4 compounds **14b** and **16**

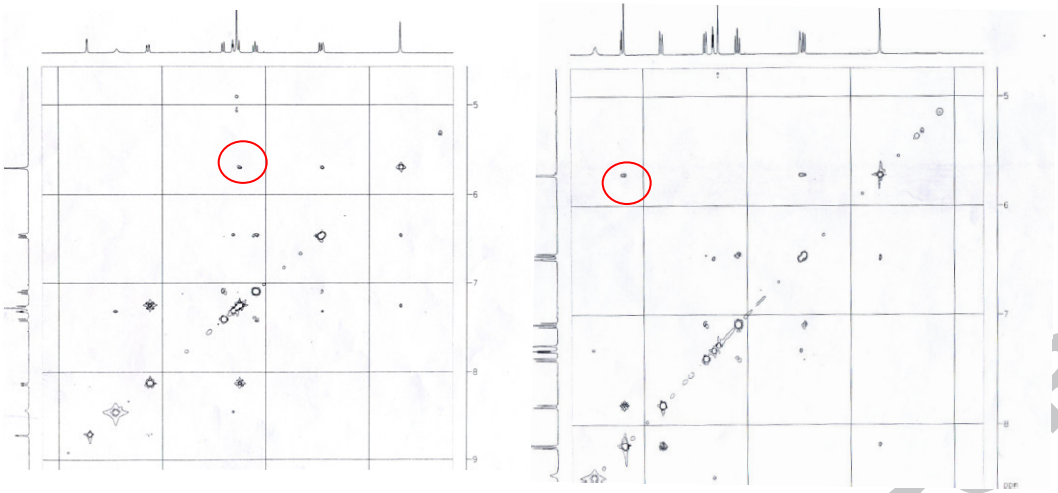


Fig.5 The NOESY of **14b** (left) and **16**(right).

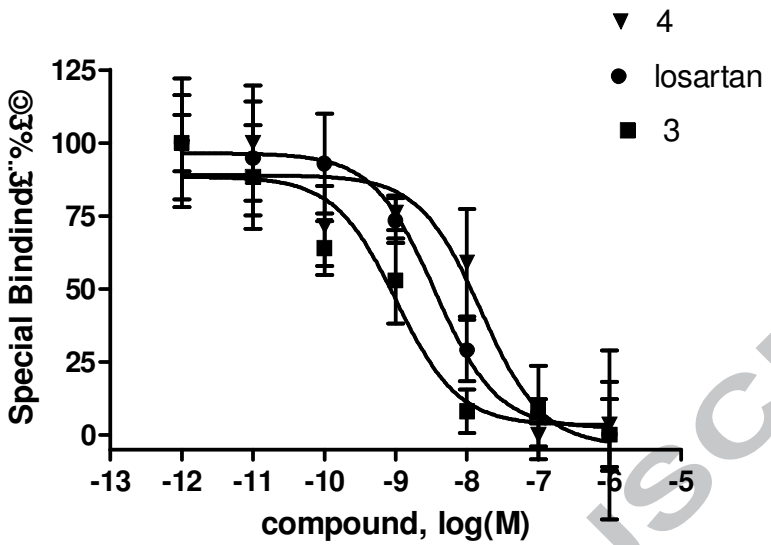


Fig.6 Inhibitory effects of **3**, **4** and Losartan (10^{-6}-10^{-12}M) on the specific binding of ^{125}I-AII to AT_1 receptors in VSMCs

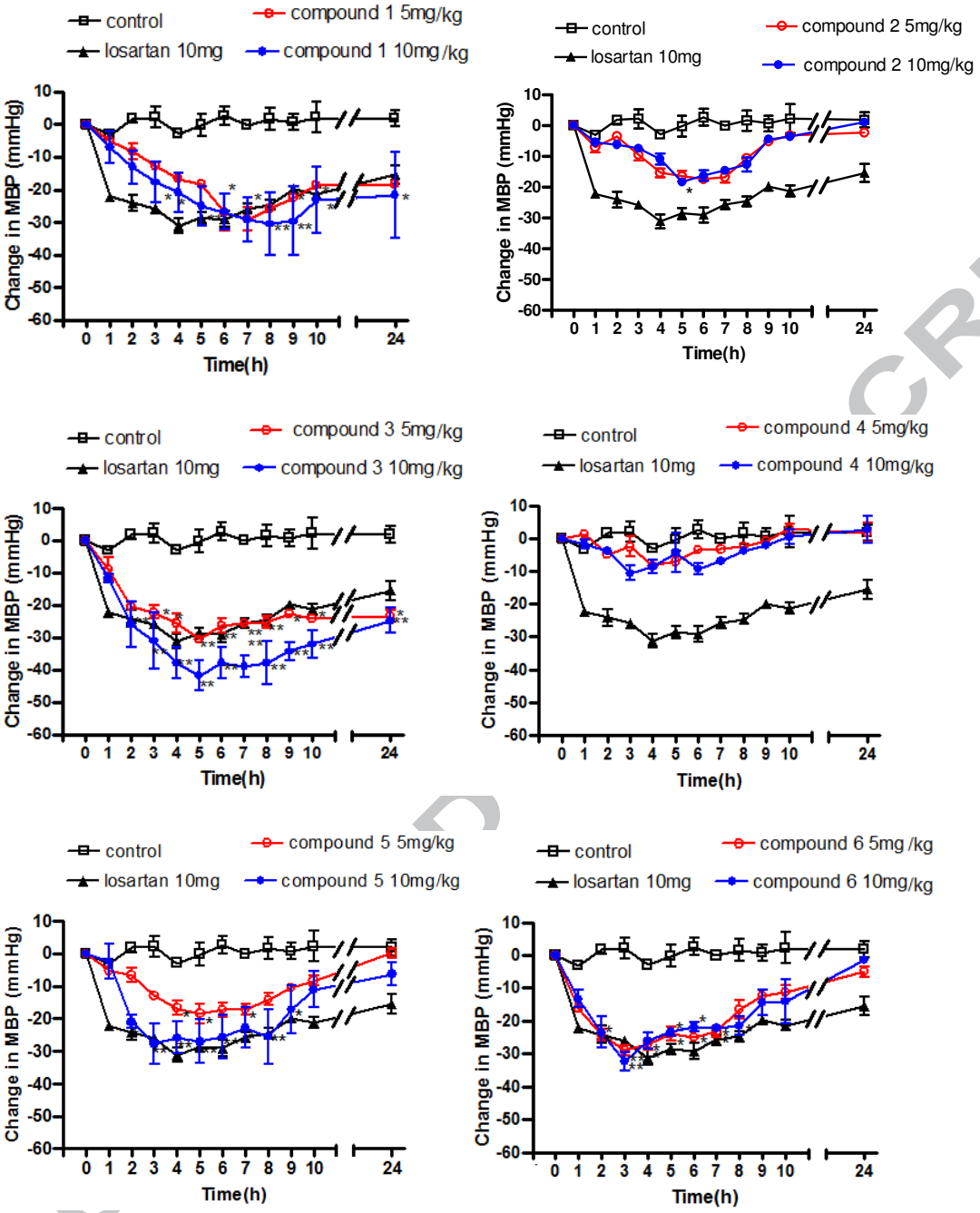


Fig.7 Effects of compounds 1, 2, 3, 4, 5, 6, and Losartan on mean blood pressure (MBP) in spontaneously hypertensive rats. *,** Significant difference from the control, $p<0.05$ and $p<0.01$, respectively.

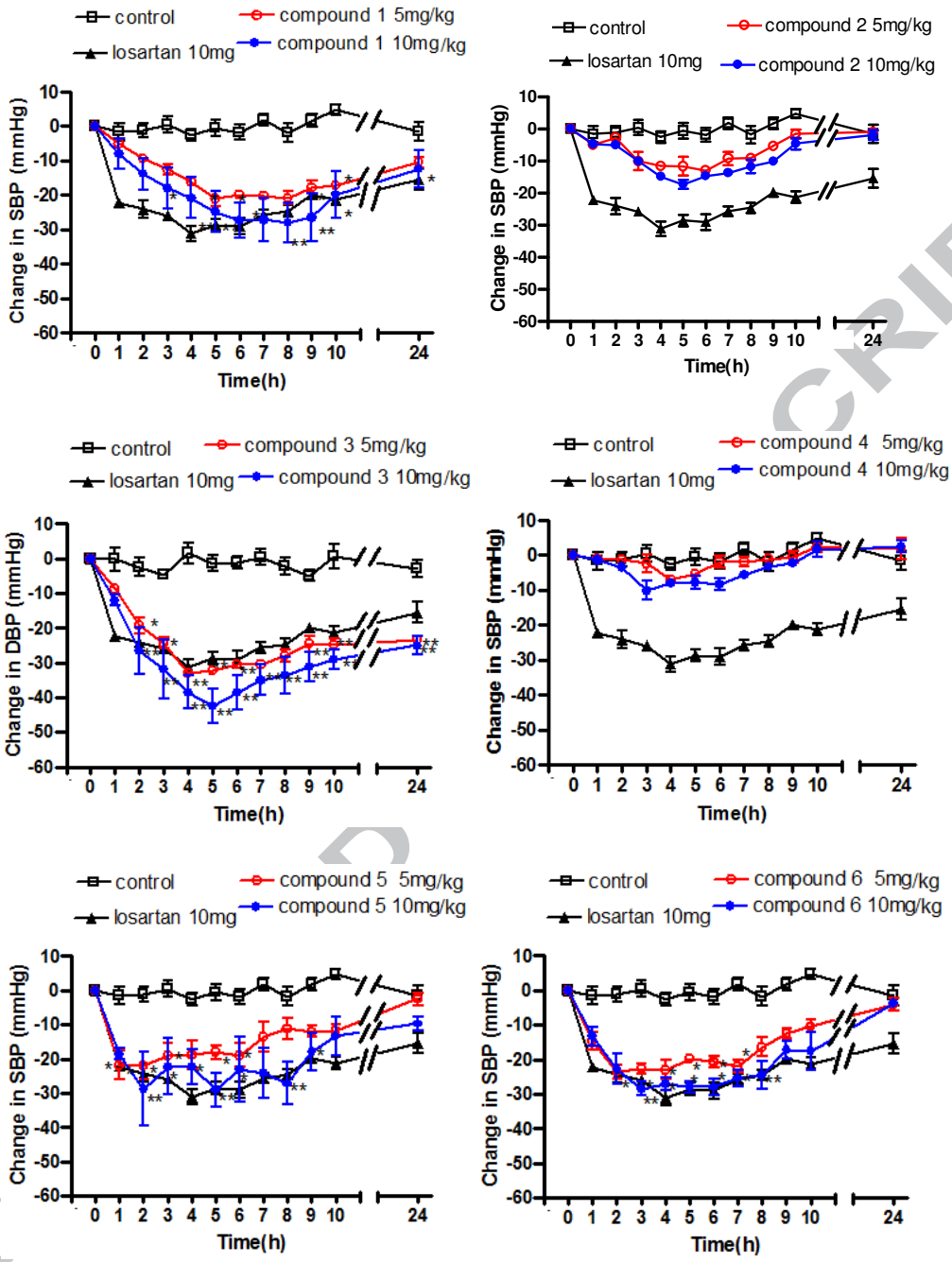
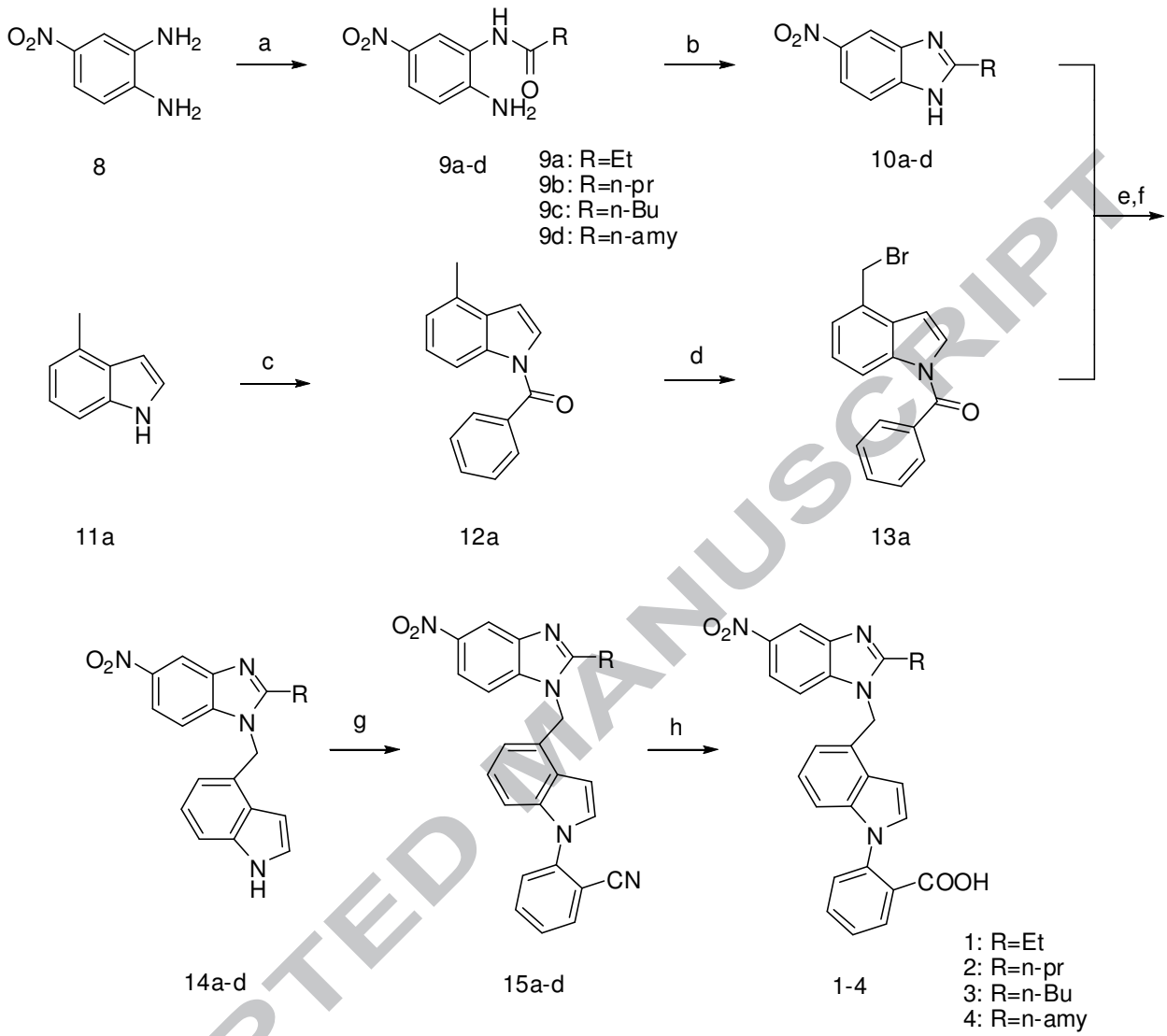
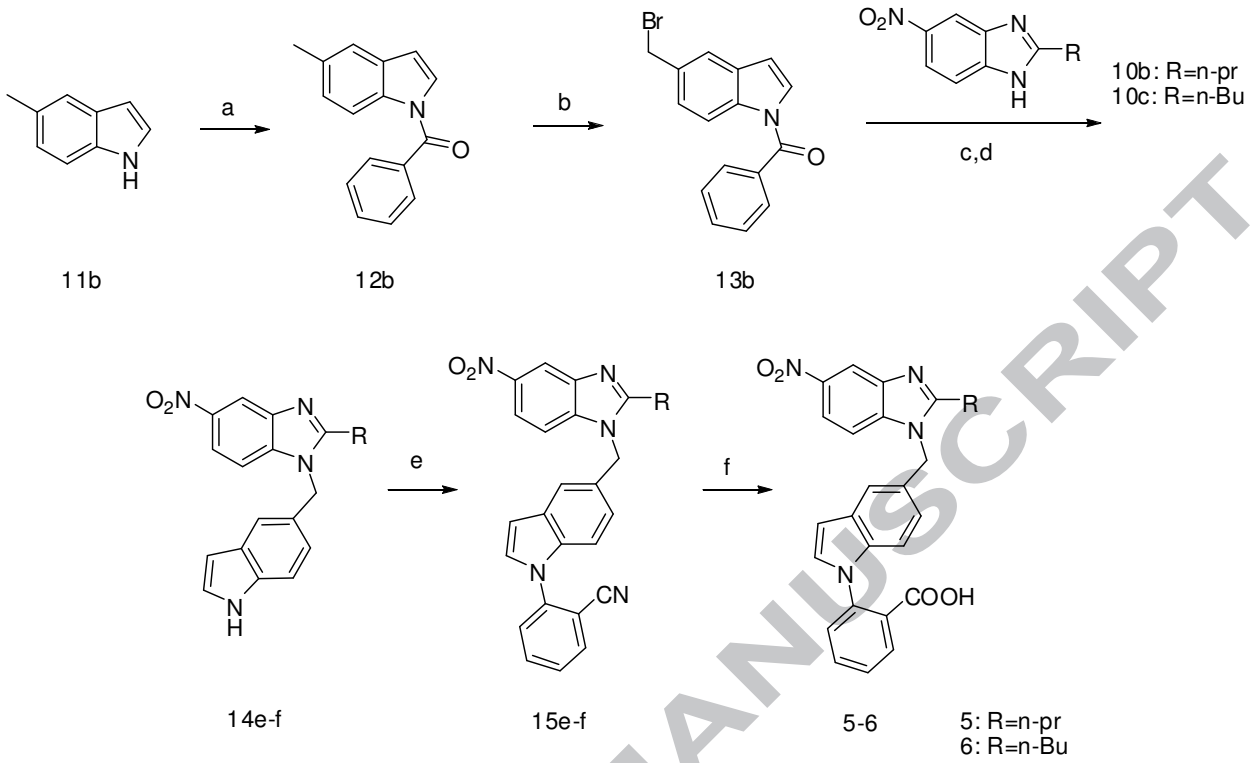


Fig.8 Effects of compounds 1, 2, 3, 4, 5, 6, and Losartan on mean blood pressure (MBP) in renal hypertensive rats. *,** Significant difference from the control, $p<0.05$ and $p<0.01$, respectively.

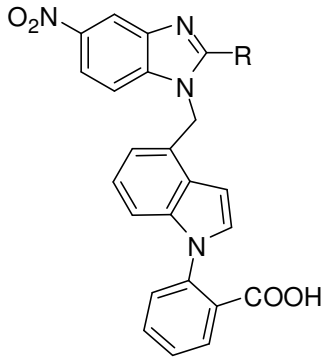


Scheme 1. Reagents and conditions: **(a)** RCOCl, Et_3N, THF; **(b)** HCl, C_2H_5OH, reflux; **(c)** benzoyl chloride, Et_3N, DMAP, CH_2Cl_2; **(d)** NBS, AIBN, CCl_4, reflux; **(e)** K_2CO_3, CH_3COCH_3, reflux; **(f)** NaOH, H_2O, CH_3OH, reflux; **(g)** 2-fluorobenzonitrile, K_2CO_3, DMF, reflux; **(h)** NaOH, H_2O, CH_3OH, reflux.

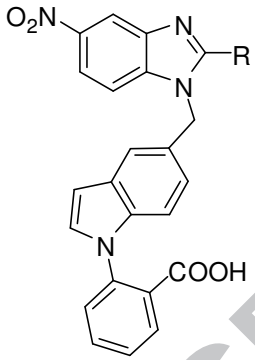


Scheme 2. Reagents and conditions: **(a)** benzoyl chloride, Et_3N, DMAP, CH_2Cl_2; **(b)** NBS, AIBN, CCl_4, reflux; **(c)**K_2CO_3, CH_3COCH_3, reflux; **(d)** NaOH, H_2O, CH_3OH, reflux; **(e)** 2-fluorobenzonitrile, K_2CO_3, DMF, reflux; **(f)** NaOH, H_2O, CH_3OH, reflux.

Table 1. IC_{50} and Ki value of the tested compounds



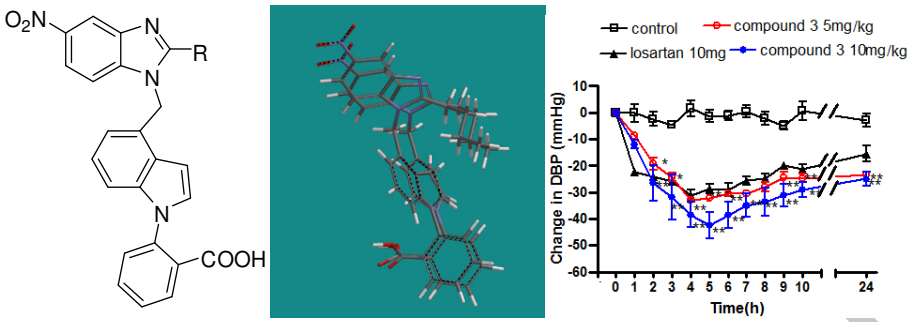
1-4



5-6

Compound	R	Mp (^oC)	Formula	IC_{50}±SEM (nM)	K_i (nM)
1	Et	191-194	$C_{25}H_{20}N_4O_4$	4.12±1.15	3.04±1.17
2	n-pr	196-199	$C_{26}H_{22}N_4O_4$	5.43±0.27	4.23±0.47
3	n-Bu	200-203	$C_{27}H_{24}N_4O_4$	1.03±0.26	0.97±0.43
4	n-amyl	205-208	$C_{28}H_{26}N_4O_4$	15.74±0.32	13.42±2.78
5	n-pr	197-200	$C_{26}H_{22}N_4O_4$	3.32±0.78	2.22±1.21
6	n-Bu	201-204	$C_{27}H_{24}N_4O_4$	5.11±0.89	4.89±0.34
Losartan				3.54±0.34	2.53±1.12

Graphical abstract



R=n-Bu