Synthesis, Anti-hypertensive Effect of a Novel Angiotensin II AT_1 Receptor Antagonist and its Anti-tumor Activity in Prostate Cancer

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Key words
- angiotensin II
- AT_1 receptor
- synthesis
- hypertension
- prostate
- cancer

Abstract

Since the first non-peptide Ang II receptor antagonist was originally reported, it has become the most common target in the development of new treatments for hypertension. In recent years, all components of the classical RAS have been reported in the prostate, these results suggest the possibility that ARB is a novel therapeutic class of agents for prostate cancer. In this study, a new compound 2-(4-((2-propyl-5-nitro-1H-benzo[d] imidazol-1-yl) methyl)-1H-indol-1-yl) benzoic acid was synthesized and evaluated as a novel angiotensin II AT_1 receptor antagonist by radioligand binding assays, anti-hypertensive assays in vivo and oral acute toxicity test. MTT assays and tests in nude mice were used to demonstrate its anti-tumor activity. This new compound showed high affinity to AT_1 receptor and anti-hypertensive activity in spontaneously hypertensive rats and renal hypertensive rats. Moreover, in human prostate cancer cells and in athymic nude mice bearing human prostate cancer cells, we observed this new compound had an efficient antiproliferative activity in vitro and anti-tumor activity in vivo. The preliminary pharmacological characteristics with oral acute toxicity test suggested that this new compound can be considered as a candidate for both anti-hypertensive and anti-tumor drug.

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Introduction

The renin-angiotensin system (RAS) is known to play a pivotal role in our body balance. In this system, angiotensin II (Ang II) receptor is the final site of action. It plays an important pharmacological part in the treatment of cardiovascular disorders [1]. Such as hypertension, cardiac hypertrophy, diabetic nephropathy, arrhythmia and heart failure. 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 [2]. This provides a more specific attempt to inhibit the activity of the RAS and has become the main pharmacological approach [3].

Since a low prevalence of cancer in hypertensive patients receiving angiotensin converting enzyme inhibitors was reported, a biological effect of Ang II on the development or progression of cancer has been drawn attention [4]. There is emerging evidence that Ang II is involved in the development of various cancers. For instance, AT_1 receptor antagonist suppress the growth of human pancreatic cancer cells [5] and, like ACE inhibitors, inhibit tumor angiogenesis growth and lung metastasis resulting from injection of lung cancer cell lines [6]. Rivera et al. also revealed that AT_1 receptor antagonist reduced tumor volume, vascular density, mitotic index and cell proliferation by injection of glicoma cells [7]. It is also reported that the AT_1 receptor antagonist had the potential to inhibit the growth of prostate cancer cells and tumors through the AT_1 receptor [8, 9].

Most of ARBs came from the transformation of Losartan's structure. In the structure of Losartan, the acidic group tetrazole have some disadvantages in chemical synthesis. For example, it could be dangerous due to the use of toxic such as sodium azide. So we tried to find an acidic group in place of tetrazole. Carboxyl group was then found to be used as a suitable substitute. Because carboxyl group is friendly to environment, moreover synthesis of carboxyl group has a higher yield. Poss developed a series of N-phenyl groups

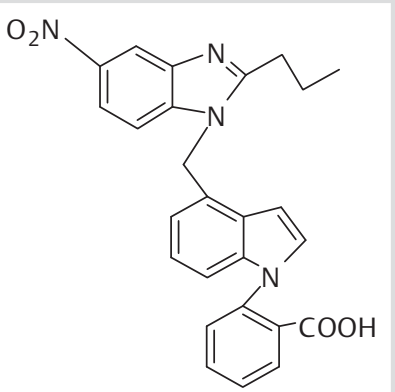

Fig. 1 The chemical structure of compound 1.

instead of the structure indole groups. And indole groups can extend the duration of the drug in human body. We can understand them through the patents EP0488532, US5208235, US5212177 and US5374615 [10–13]. Through benzimidazole structure in place of imidazole structure of losartan, candesartan and pomisartan [14,15] were developed. At last we used 5-nitro-1H-benzo[d]imidazole to replace the imidazole. Based on the above design solutions, a novel angiotensin II AT_1 receptor antagonist compound **1** (**Fig. 1**), **2**-(**4**-((**2**- propyl -5-nitro-1H-benzo[d]imidazol-1-yl) methyl)-1H-indol-1-yl) benzoic acid was designed and synthesized.

In radioligand binding assays, this compound showed high affinity to AT_1 receptor. Then its antihypertensive activity was investigated in spontaneously hypertensive rats and renal hypertensive rats. In these assays, compound **1** showed efficient, stable and durable antihypertensive effects. In addition, antiproliferative activity in prostate cancer cells and anti-tumor activity in nude mice were observed then. All these preliminary pharmacological characteristics with oral acute toxicity test suggested that compound **1** can be considered as a candidate for anti-hypertensive and anti-tumor drug.

Materials and Methods

Chemical

All chemicals used were of reagent grade. Yields refer to purified products are not optimized. ^{1}H 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. Column chromatography was performed using silic gel H (300–400).

(4-methyl-1H-indol-1-yl) (phenyl)methanone (3) **3** was prepared according to the procedure described by Daljit [16].

(4-bromomethyl)-1H-indol-1-yl)(phenyl)methanone (4) **4** was prepared according to the procedure described by Daljit [16].

N-(2-amino-5-nitrophenyl) butyramide (6) **6** was prepared by the general procedure described by Baker [17].

2-propyl-5-nitro-1H-benzo[d]imidazole (7) **7** was prepared by the general procedure described by Baker [17].

(4-((2-propyl-5-nitro-1H-benzo[d]imidazol-1-yl)methyl)-1H-indol-1-yl)(phenyl)methanone (8) To a solution of **7** (100 mg, 0.487 mmol) in acetone (10 mL) was added K_2CO_3 (87 mg, 0.630 mmol), and stirred for 30 min at 50 °C, then **4** (153 mg, 0.487 mmol) was added. The resulting mixture was refluxed for 6 h. The mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with water, dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The residue was purified by flash column chromatography with petroleum ether/ethyl acetate (7: 2) as eluant. A yellow solid 76 mg was obtained. Yield: 35.7%. mp: 97.5–99.7 °C.

1-((1H-indol-4-yl)methyl)-2-propyl-5-nitro-1H-benzo[d]imidazole (9) To a solution of **8** (318 mg, 0.73 mmol) in methanol (12 mL) was added 12 mL of 2M sodium hydroxide, and refluxed for 3 h. The solvent was removed in vacuo. The mixture was diluted with water and extracted with dichloromethane. The organic layer was washed with brine, dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The residue was purified by flash column chromatography with dichloromethane/methanol (250: 1) as eluant. A yellow solid 207 mg was obtained. Yield: 85.7%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.72 (1H, d, J = 1.9 Hz, Ph-H), 8.44 (1H, d, N-H), 8.13 (1H, dd, J_1 = 2.0 Hz, J_2 = 8.8 Hz, Ph-H), 7.41 (1H,d, J = 8.1 Hz, Ph-H), 7.32 (1H, t, J = 2.8 Hz, Ph-H), 7.26 (1H, s, Ph-H), 7.10 (1H, t, J = 7.0 Hz, indo-H), 6.47 (1H, d, J = 7.2 Hz, Ph-H), 6.45 (1H, s, indo-H), 5.70 (2H, s, Ph-CH_2), 2.93 (2H, t, J = 7.5 Hz, -$CH_2CH_2CH_3$), 1.92 (2H, m, J = 7.4 Hz, -$CH_2CH_2CH_3$), 1.03 (3H, t, J = 7.3 Hz, -$CH_2CH_2CH_3$). MS (ESI) m/z: 335.6[M + H]$^+$.

2-(4-((2-propyl-5-nitro-1H-benzo[d]imidazol-1-yl) methyl)-1H-indol-1-yl) benzonitrile (10) To a solution of **9** (158 mg, 0.47 mmol) K_2CO_3 (135 mg, 0.98 mmol) in DMF (10 mL) was added 2-Fluorobenzonitrile (0.08 mL, 0.74 mmol) under nitrogen, and the mixture was refluxed for 5 h. The resulting mixture was washed with water and brine, and then the organic layer was dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The residue was purified by flash column chromatograpy with petroleum ether/ethyl acetate (2: 1) as eluant. A yellow solid 163 mg was obtained. Yield: 79.3%. ^{1}H -NMR (400 MHz, $CDCl_3$) δ: 8.67 (1H, d), 8.09 (1H, dd, J_1 = 2.1 Hz, J_2 = 6.7 Hz, Ph-H), 7.87 (1H, d, J = 7.8 Hz, Ph-H), 7.78 (1H, t, J = 7.7 Hz, Ph-H), 7.60 (1H, d, J = 8.1 Hz, Ph-H), 7.56 (1H, t, J = 7.6 Hz, Ph-H), 7.47 (1H, d, J = 3.4 Hz, Ph-H), 7.26 (1H, d, J = 2.1 Hz, Ph-H), 7.24 (1H, s, Ph-H), 7.09 (1H, t, J = 7.6 Hz, indo-H), 6.98 (1H, d, J_1 = 2.1 Hz, J_2 = 3.4 Hz, Ph-H), 6.46 (1H, d, J = 7.3 Hz, indo-H), 5.71 (2H, s, Ph-CH_2), 2.90 (2H, t, J = 7.4 Hz, -$CH_2CH_2CH_3$), 1.91 (2H, q, J = 7.4 Hz, -$CH_2CH_2CH_3$), 1.03 (3H, t, J = 7.3 Hz, -$CH_2CH_2CH_3$). MS(ESI)m/z: 436.5[M + H]$^+$.

2-(4-((2-propyl-5-nitro-1H-benzo[d]imidazol-1-yl) methyl)-1H-indol-1-yl) benzoic acid (1) A solution of 10 (107 mg, 0.25 mmol) and 10 mL of 5M NaOH in methanol (10 mL) were refluxed for 34 h. The solvent was removed in vacuo. The pH value of the mixture was adjusted to 6 by careful addition of 6M aqueous hydrochloride. The mixture was extracted with dichloromethane, washed with water. 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 with dichloromethane/methanol (40: 1) as eluant. A yellow solid 57 mg was obtained. Yield: 51.2%. ^{1}H-NMR(DMSO, 400 MHz)δ: 12.9(1H, s, -COOH), 8.52(1H, d, J = 2.15 Hz, Ph-H), 8.13(1H, dd, J_1 = 2.2 Hz, J_2 = 8.9 Hz, Ph-H),

8.09(1H, d, Ph-H), 7.70–7.51 (4H, t, Ph-H),7.36(1H, indo-H), 7.28–7.14(3H, t, Ph-H), 6.30(1H, J=4.3 Hz, indo-H), 5.87(2H, s, Ph-CH_2-), 2.88(2H, t, J=7.4 Hz, -$CH_2CH_2CH_3$), 1.82(2H, m, -$CH_2CH_2CH_3$), 0.96 (3H,t, J=7.4 Hz, -$CH_2CH_2CH_3$). ^{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. ESI-MS (m/z): 455.2$[M+H]^+$, 477.1[M+Na]+; HR-MS: 477.15106.

Radioligand binding assays in VSMC

Cell lines and cell culture

Primary vascular smooth muscle cells (VSMCs) were obtained from thoracic aorta of SD rats and cultured by the tissue explants methods [18]. 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. They were then incubated with 1 ml of DMEM supplemented with 15% fetal bovine serum (FBS) at 37 °C in 95% air 5% CO_2. Cells at passage 3–7 were used for the experiments.

Saturation study

50 µL test compounds (1nM) (Department of Medicinal Chemistry, DongHua University) and 50 µL $^{[125]}$ Ang II (0.03–10.00 nM) (Northern Biotechnology Company) were incubated in 24-well plates with VSMCs for 60 min at 37 °C. Nonspecific binding was defined as $^{[125]}$ Ang II bounding in the presence of unlabeled 10 µM $^{[Sar1, Ile8]}$ Ang II (GL Biochem, Shanghai). After the reaction, removed the liquid immediately, washed 5 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 dissociation constant (K_d value) and the receptor density (Bmax value) were estimated by Scatchard analysis [19].

Competition study

The inhibitory efficacy of the antagonists was determined under the same conditions as in the saturation study. The VSMCs containing 0.1 nM $^{[125]}$ Ang II were incubated with various concentrations of competitors for 60 min at 37 °C. The final concentrations were 1×10^{-6}~1×10^{-12}M. The inhibitory concentration of test compound that caused 50% inhibition if the specific binding of Ang II (IC_{50}) was determined by regression analysis of displacement curves. The inhibition constant (K_i value) was calculated from the formula $K_i = IC_{50}/(1+[L]/k_d)$ [20], where [L] was the concentration of radioligand present in tubes.

Antihypertensive effects in rats

The compound **1** were subjected to biological evaluation for their effects on systolic blood pressure (SBP) and diastolic blood pressure (DBP) in spontaneous hypertensive rats (SHRs) (250–300 g, Second military medical university, China) and renal hypertensive rats. For preparing renal hypertensive rats, the left renal arteries of Sprague-Dawley rats (300–350 g) 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. Than both spontaneous and renal hypertensive rats were randomly divided into different experimental groups of 10 animals (negative control group, positive control groups, compound 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 taken as positive control. 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, and China). Blood pressure and heart rate were monitored at 0–10 h and 24 h after the administration in spontaneous hypertensive rats and renal hypertensive rats. MAP was calculated using the formula: MAP=DBP+(SBP−DBP)/3. 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.

Antiproliferative activity in prostate cancer cells

The MTT assays were performed as previously described [21]. Briefly, LNCap cells (Institute of Biochemistry and Cell Biology, China) were seeded in 24-well culture plates at a density of 10^4 to 10^5 cells/well. Cells were cultured in phenol red-free RPMI+0.1% BSA for 18–24 h at 37 °C, 5% CO_2 atmosphere before experiments and then treated with Ang II (0.1 µM), Ang II (1 µM), losartan (10 µM), compound **1** (10 µM), AngII (1 µM)+losartan (10 µM), AngII (1 µM)+ compound **1** (10 µM), respectively. The control group received drug-free medium with 0.05% v/v DMSO. Subsequently, MTT (5 mg/mL) was added to each well on day 2. After incubation at 37 °C for 4 h, the medium was discarded and 150 µL DMSO was added into each well. The optical density was measured by a micro-plate reader (Bio-Rad, California, USA) at a test wavelength of 570 nm.

Antitumor activity in nude mice

The antitumor activity of compound **1** was determined in athymic nude mice bearing LNCap tumors. LNCap cells (10^7) were injected into the flank region of athymic nude mice (4–6 weeks old), and treatment was started on day 8 when the tumor measured 5 mm in diameter [22]. Each mouse received one of 2 different doses of compound **1** (5.0 or 10.0 mg/kg/day) and losartan (5.0 or 10.0 mg/kg/day) was taken as positive group. The control group received water containing sodium hypochlorite (10 ppm). Each group consisted of 10 animals. Tumors were measured with a caliper every 7 days. The volume of the tumor was calculated using the formula: tumor volume (mm^3)=length×$(width)^2\times0.5$.

Acute toxicity studies

The toxicity of compound **1** 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, 3823.6.0, 2924.0, 2236.1, 1710.0, 1000.0 mg/kg. Survival was assessed daily for 2 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,

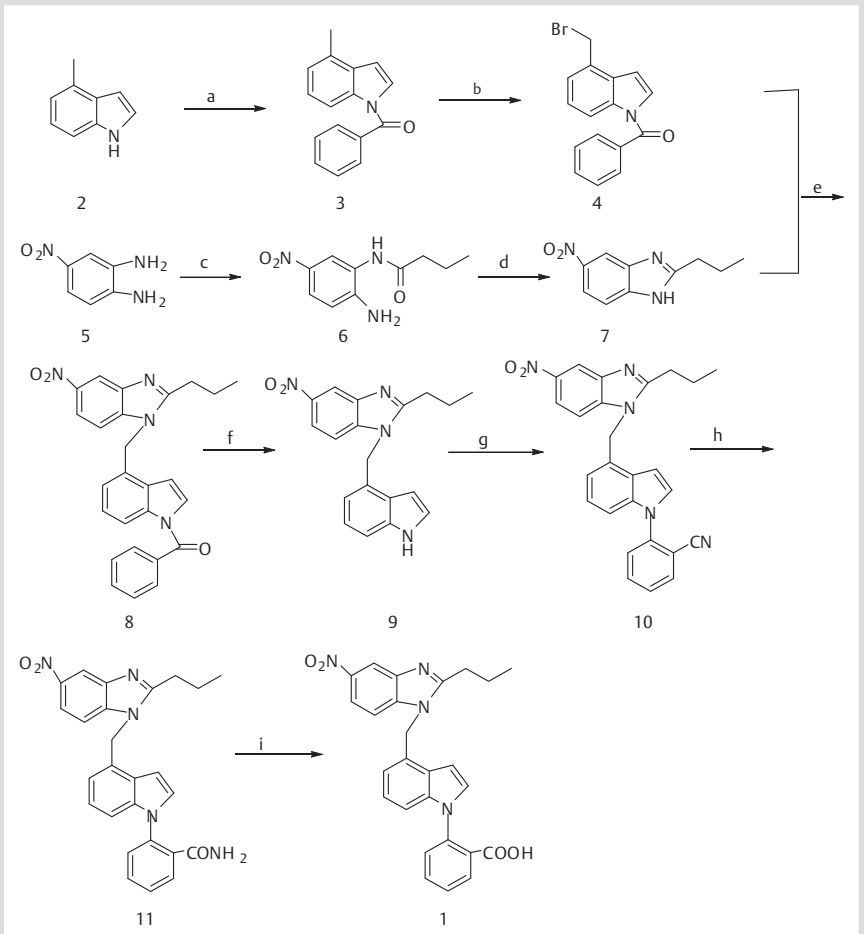

Fig. 2 Reagent and conditions: **a** benzoyl chloride, Et_3N, DMAP, CH_2Cl_2; **b** NBS, AIBN, CCl_4, reflux; **c** PrCOCl,Et_3N, THF; **d** HCl, C_2H_5OH, reflux; **e** K_2CO_3, CH_3COCH_3, reflux; **f** NaOH, H_2O, CH_3OH, reflux; **g** 2-fluorobenzonitrile, K_2CO_3, DMF, reflux; **h** NaOH, H_2O, CH_3OH, reflux; **i** NaOH, H_2O, CH_3OH, reflux.

mobility, aggressiveness and respiratory movements. Finally, the survivals were dissected and examined pathological changes of organs.

Statistics

Results are expressed as means ± standard error of the means. Data were analyzed by one-way analysis of variance. When overall statistical significance was achieved ($P < 0.05$). Student's t-test was used to compare each of the doses to the vehicle control. Probability values less than 0.05 were considered to be significant. Binding isotherms from competition studies were obtained using the nonlinear regression program Graphpad Prism 4 software.

Results

Chemistry

The target compound was prepared according to the route described in **Fig. 2**.

Treatment of **4-methyl-1H-indole 2** with benzoyl chloride in methylene chloride in the presence of triethylamine and 4-(dimethylamino) pyridine (DMAP) gave **(4-methyl-1H-indol-1-yl) (phenyl)methanone 3**, which was brominated to produce **(4-bromomethyl)-1H-indol-1-yl)(phenyl)methanone 4**. **4-nitrobenzene-1,2-diamine 5** in THF with butyryl chloride using triethylamine as base gave the secondary amines **6,** which was cyclized to produced benzoimidazoles **7.** Subsequently compound **8** was obtained using**4** and **7** in the presence of K_2CO_3. Then **8** was hydrolyzed with aqueous NaOH to provide compound **9**. Compound **9** with 2-fluorobenzonitrile using K_2CO_3 in DMF yielded the compound **10**, which was amidated to give the compound **11**. The final compound was obtained through the hydrolysis of compound **11**.

Biological evaluation

Radioligand binding assays in VSMC

Compound **1** displaced the specific binding of [^{125}I] [Sar^1, Ile^8] angiotensin II to a single population of binding site (angiotensin AT_1 receptor) in rat vascular smooth muscle cell (VSMC). The k_d and B_{max} values were 0.34 ± 0.023 nM and 5.242 ± 0.75 fmol/mg protein. In competition study, compound **1** and Losartan competed dose-dependently with ^{125}I-Ang II (**Fig. 3**). Compound **1** and Losartan displayed high specific affinity for the AT_1 receptor (K_i = 0.473 ± 0.28 nM, 1.71 ± 0.12 nM, IC_{50} = 0.277 ± 0.136 nM, 2.36 ± 0.091 nM, respectively). It showed that compound **1** exhibited more affinity to AT_1 receptors than Losartan because its K_i and IC_{50} values were significantly much lower compared with Losartan's.

Antihypertensive effects in rats

The effects of compound **1** (5, 10 mg/kg) and Losartan (10 mg/kg) on the reduction of mean arterial pressure (MAP) in spontaneously hypertensive rats (SHR) and renal hypertensive rats (RHR) are shown in **Fig. 4**, **5** respectively (n = 10). The results suggested that compounds **1** caused significant, durable and stable reduction in blood pressure in vivo.

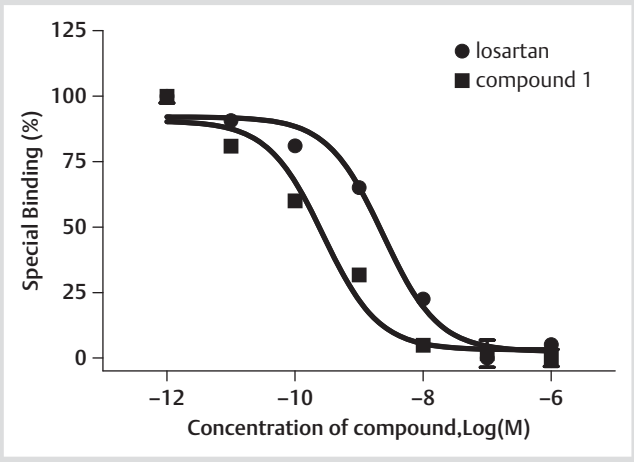

Fig. 3 Inhibitory effects of compound **1** and losartan (10^{-6}~10^{-12} M) on the specific binding of ^{125}I-Ang II to AT_1 receptors in VSMCs.

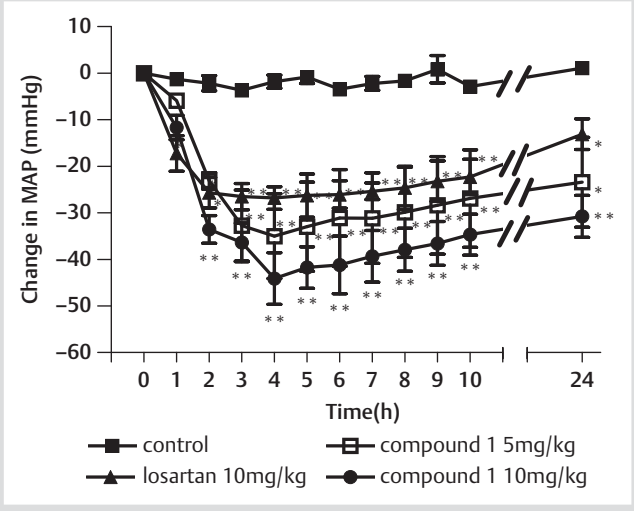

Fig. 4 Reduction of mean arterial pressure in spontaneously hypertensive rats (n = 10). Values are means ± standard error of means for experiments. *, ** Significant difference from the control, $p < 0.05$ and $p < 0.01$, respectively.

In SHR, compared to the control group, compound **1** (5 mg/kg, 10 mg/kg) decreased the MAP in a dose-dependent manner (▶ **Fig. 4**). The maximal reduction was observed at 4 h after intragastric administration, namely, 34 mmHg for 5 mg/kg and 51 mmHg for 10 mg/kg, respectively. This reduction can remain for more than 10 h. Then at 24 h compound **1** still had significant difference from the control. The reduction of compound **1** at 24 h was 23 mmHg for 5 mg/kg and 30 mmHg for 10 mg/kg, respectively. Losartan (10 mg/kg) decreased MAP at 4 h after taken to reach the maximum (26 mmHg) and than blood pressure returned slowly, at 24 h it only decreased MAP 13 mmHg. Compared to Losartan, compound **1** has both stronger and longer anti-hypertensive effect, and this effect maintained for more than 24 h. The results indicated that compound **1** had a more favorable, stable and durable blood pressure-lowering effect than Losartan. All the compounds did not influence heart rate of the rats (data not shown).

In RHR, compound **1** (5 mg/kg, 10 mg/kg) also decreased the MAP in a dose-dependent manner (▶ **Fig. 5**). The maximal reduction was observed at 4 h after administration, namely,

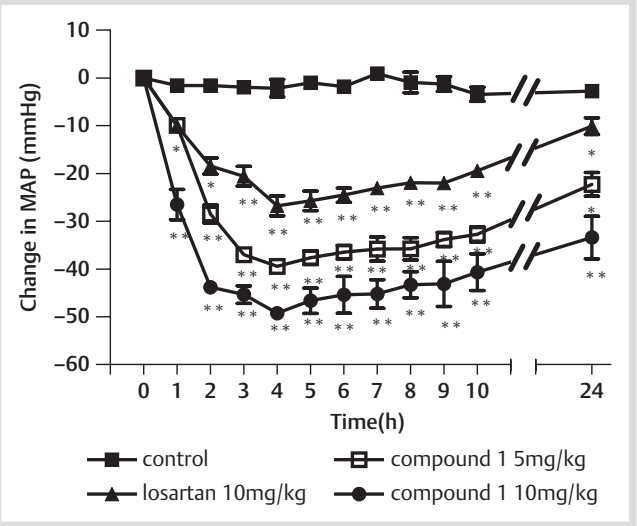

Fig. 5 Reduction of mean arterial pressure in renal hypertensive rats (n = 10). Values are means ± standard error of means for experiments. *, ** Significant difference from the control, $p < 0.05$ and $p < 0.01$, respectively.

39 mmHg for 5 mg/kg and 49 mmHg for 10 mg/kg, respectively. At 24 h, the reduction of compound **1** is 22 mmHg for 5 mg/kg and 33 mmHg for 10 mg/kg, respectively. It is a significant difference from the control. Losartan (10 mg/kg) decreased MAP at 4 h after taken to reach the maximum (26 mmHg). At 24 h, the reduction of Losartan returned to 10 mmHg. The result indicated that in RHR, compound **1** also had a better effect than Losartan. All the compounds did not influence heart rate of the rats (data not shown).

Antiproliferative activity in prostate cancer cells

The effect of the compound **1** on cell viability was assessed by MTT assay. Treatment of human prostate cancer cell line LNCaP cells with Ang II at the concentrations of 0.1 μM and 1 μM resulted in an increase in the viability of cells. The proliferative effect of Ang II is dose-manner. A significant reduction in the viability as compared to DMSO-treated controls was observed after 24 h of treatment with Losartan and compound **1** at the concentration of 10 μM treat with Ang II (1 μM). However, Losartan and compound **1** could not influence the growth of LNCaP cells without treated with Ang II (▶ **Fig. 6**). The result suggested that compound **1** has antiproliferative activity in prostate cancer cells.

Antitumor activity in nude mice

To determine whether the in vitro antiproliferative activity of compound **1** could be translated to antitumor activity in vivo, LNCap cells were established as xenografts in nude mice (n = 10). When the tumors reached about 5 mm in diameter, the animals were given compound **1** at 5.0 or 10 mg/kg/day. Losartan at 5.0 or 10 mg/kg/day were used as positive control group. The negative control group received water containing sodium hypochlorite (10 ppm). As shown in ▶ **Fig. 7**, at 4 weeks, control group had developed large tumor of 29.899 ± 2.011 relative volume compared with those at 0 week. Mice treated with compound **1** at 5.0 or 10 mg/kg/day show more inhibition of tumor relative volume at 4 weeks by 14.712 ± 2.129, 9.449 ± 3.871, respectively compared with tumor relative volume at 4 weeks by

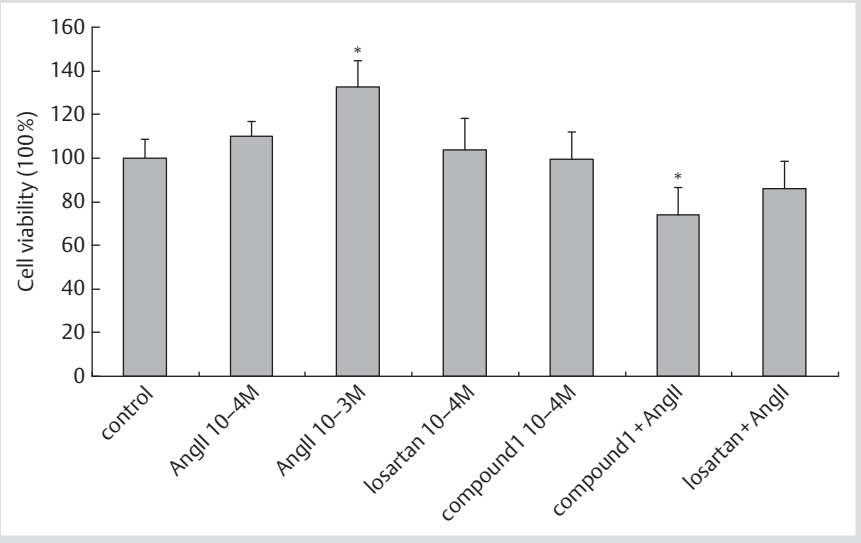

Fig. 6 Cell viability effect of AngII, losartan and compound 1 in prostate carcinoma cell lines (LNCaP) measured by MTT. Values are means ± standard error of means for experiments. * $P<0.05$, compared with control.

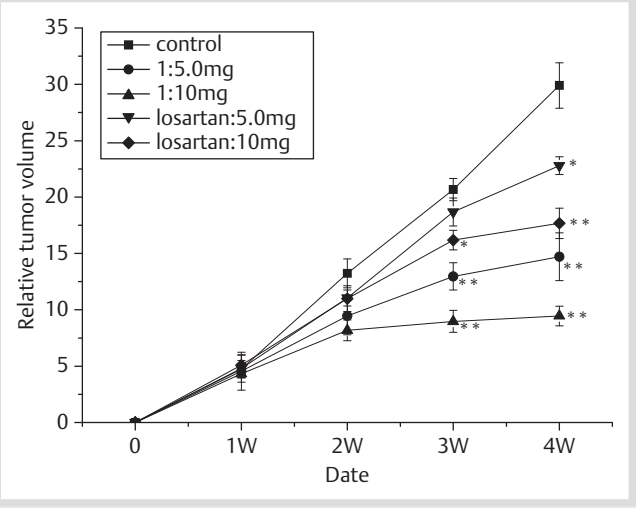

Fig. 7 Anti-tumor activity of compound 1 and losartan (5 mg/kg/day, 10 mg/kg/day) in nude mice, tumor growth of LNCap xenografts was measured at indicated times. Values are means ± standard error of means for experiments. *, ** Significant difference from the control, $p<0.05$ and $p<0.01$, respectively.

22.789 ± 5.782, 17.674 ± 1.344, resectively treated with losartan at 5.0 or 10 mg/kg/day.

Acute toxicity via intragastric administration

Acute toxicity testing suggested that the LD_{50} value of compound **1** was 2573.2 mg/kg and the 95% confidence interval was 2167.1~3061.5 (**Table 1**). The LD_{50} of losartan was 2248 mg/kg. At all dose levels except for 1000.0 mg/kg, rats were observed to be hypokinetic beginning at approximately 8 h after intragastric administration, and some of them died. Rats treated at all doses survived through the 2 weeks observation period could maintain weight without obvious untoward effects. The results showed that compound **1** was a potent AT_1-receptor antagonist with low acute toxicity.

Discussion

In this study, a new angiotensin II AT_1 receptor antagonist, compound **1**, 2-(4-((2-propyl-5-nitro-1H-benzo[d]imidazol-1-yl) methyl)-1H-indol-1-yl) benzoic acid was designed and synthesized.

The binding assay in the receptor affinity presented that compound **1** exhibited more affinity to AT_1 receptors than losartan, which suggested that compound **1** 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 tests in spontaneously and renal hypertensive rats showed that compound **1** had an obvious and continuous reduction in blood pressure. This reduction can last more than 24 h. And this anti-hypertension effect is dose-dependent. Acute toxicity test revealed that the highly active compound **1** showed higher LD_{50} values compared to losartan, which suggested that this new compound was safer.

Prostate cancer is highly prevalent in society, and its early stages can be treated by androgen ablation therapy, but there is no effective treatment for the cancer eventually regresses. Therefore, clinical development of agents that are relatively safe but can delay onset and progression of human prostate cancer is highly desirable. There is an increasing number of evidence that Ang II may have an important role in both normal prostatic growth and the development of a range of prostatic diseases in addition to its well known actions. In our study, the MTT assay in prostate cancer cells (LNCap) showed that compound **1** possessed the significant effects against carcinoma of prostate in vitrio. In vivo assay further displayed that compound **1** has anti-tumor effect in nude mice with LNCaP cells. The results of the present work further demonstrated that drugs target AT_1 receptor might be effective in the treatment of carcinoma of prostate in clinic.

Some evidence suggests that AT_1 receptor blockade can reduce tumor volume, vascular density, mitotic index and cell proliferation through the suppression of the mitogen-activated protein kinase (MAPK) or the Janus tyrosine kinase-signal transducer and activator of transcription (STAT) phosphorylation. Samir et al. considered Ang II might reduce the aberrant methylation and accordingly reduced the risk of cancer development [23], the

Table 1 Acute Toxicity Test of Compound 1 (ig) Determined by lethal dose (LD_{50}).

Compound	Dose (mg/kg)	Mortality (%)	LD50 (mg/kg)	95% confidence interval (mg/kg)
1	1000.0	0	2573.2	2167.1~3061.5
	1710.0	30		
	2236.1	50		
	2924.0	60		
	3823.6	80		
	5000.0	100		

mechanisms of the compound against prostate cancer need further investigation.

Briefly, this new compound 2-(4-((2-propyl-5-nitro-1H-benzo[d]imidazol-1-yl) methyl)-1H-indol-1-yl) benzoic acid could be considered as a candidate with high performance and fewer adverse effects for a novel anti-hypertension and anti-tumor drug.

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Conflict of Interest Statement

We declare that we have no conflict of interest.

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