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Inhibitory effects of benzyl benzoate and its derivatives on angiotensin II-induced hypertension

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Abstract—Hypertension is a lifestyle-related disease which often leads to serious conditions such as heart disease and cerebral hemorrhage. Angiotensin II (Ang II) plays an important role in regulating cardiovascular homeostasis. Consequently, antagonists that block the interaction of Ang II with its receptors are thought to be effective in the suppression of hypertension. In this study, we searched for plant compounds that had antagonist-like activity toward Ang II receptors. From among 435 plant samples, we found that EtOH extract from the resin of sweet gum *Liquidambar styraciflua* strongly inhibited Ang II signaling. We isolated benzyl benzoate and benzyl cinnamate from this extract and found that those compounds inhibited the function of Ang II in a dose-dependent manner without cytotoxicity. An in vivo study showed that benzyl benzoate significantly suppressed Ang II-induced hypertension in mice. In addition, we synthesized more than 40 derivatives of benzyl benzoate and found that the *meta*-methyl and 3-methylbenzyl 2′-nitrobenzoate derivatives showed about 10-fold higher activity than benzyl benzoate itself. Thus, benzyl benzoate, its derivatives, and benzyl cinnamate may be useful for reducing hypertension.

1. Introduction

Hypertension is a lifestyle-related disease that is strongly influenced by excessive intake of salt, smoking, alcohol consumption, obesity, and stress. If the symptoms of hypertension continue long term, serious conditions such as heart disease and cerebral hemorrhage can occur. Thus, compounds that can suppress hypertension are important for treating hypertension itself and for preventing even more serious conditions. The renin–angiotensin system (RAS) plays a determinant role in the regulation of cardiovascular homeostasis. The octapeptide hormone angiotensin II (Ang II) produced by RAS is a potent vasoconstrictor, and thus plays an integral role in the pathophysiology of hypertension.[1–5] Ang II causes vasoconstriction when it binds to its receptors. Therefore, research efforts to control hypertension have recently focused on competition for Ang II binding to its receptors.

There are two types of Ang II receptors: Ang II type 1 (AT1) receptor and Ang II type 2 (AT2) receptor. AT1 receptor is closely associated with the regulation of blood pressure, fluid, and electrolyte balance in healthy adults, whereas AT2 receptor is expressed in fetal tissues, including brain, and plays a significant role in fetal development.[6,7] When Ang II binds to AT1 receptors that appear on the surface of smooth muscle cells in blood vessels, the vessel walls constrict and hypertensive effects occur.[8] Thus, antagonists that can selectively

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block the interaction between Ang II and AT1 receptors are thought to be effective for treating hypertension.

AT1 receptor antagonists have been shown to lower blood pressure effectively, and they are better-tolerated than other classes of drugs.[9,10] The search for such compounds led to the discovery of the first potent and orally active non-peptide Ang II antagonist: losartan.[11–15] Currently, several kinds of AT1 receptor antagonists, such as candesartan, eprosartan, and irbesartan, are used in the treatment of hypertension.[16–19] The antihypertensive efficacy of AT1 receptor antagonists has been demonstrated in mild to moderate hypertension as well as in severe essential hypertension and isolated systolic hypertension.

Considering that hypertension is a lifestyle-related disease, it is important to find ways to prevent hypertension by improving lifestyle factors, particularly diet. Therefore, we focused on plant components, since many of them can be used in foods. In this study, we constructed a new screening system and searched for plant compounds that show antagonistic activity toward Ang II receptors.

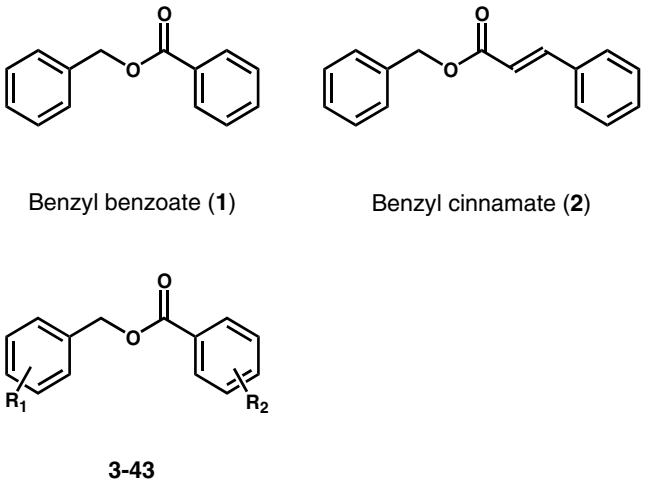

Figure 1. Structures of benzyl benzoate, benzyl cinnamate, and the derivatives of benzyl benzoate. Substituents of **3**–**43** are listed in Table 1.

2. Results

2.1. Inhibition of the function of Ang II by benzyl benzoate in Ang II receptor-overexpressing cells

To examine antagonistic activity toward Ang II receptor, we used human embryo kidney epithelial 293T cells (mAT1a(HA)/293T cells) that stably express AT1 receptors. By interacting with its receptors, Ang II induces the cellular uptake of calcium ion (Ca^{2+}), which acts as a second messenger. mAT1a(HA)/293T cells were pretreated with fura 2-AM, which is a specific fluorescent indicator of Ca^{2+}. The Ang II-induced increase in the Ca^{2+} concentration in cells was evaluated in the presence or absence of plant samples by spectrometric analysis.

We screened 435 plant extracts for compounds which have antagonistic activity toward Ang II receptors. We only considered samples that were not cytotoxic. In the course of our screening, we found that the EtOH extract of the resin of sweet gum *Liquidambar styraciflua* showed marked inhibitory activity toward the function of Ang II. From this resin extract, we isolated two active principles through several chromatographic separations. Spectroscopic analysis of the purified compounds revealed that they were identical to known aromatic compounds, benzyl benzoate (**1**) and benzyl cinnamate (**2**) (Fig. 1). In the present study, we used commercially available benzyl benzoate (Nacalai Tesque, Inc.) and benzyl cinnamate (Tokyo Chemical Industry Co.).

Treatment of mAT1a(HA)/293T cells with 1 μM Ang II increased the intracellular Ca^{2+} concentration. The addition of benzyl benzoate or benzyl cinnamate prior to Ang II markedly decreased the intracellular Ca^{2+} concentration in mAT1a(HA)/293T cells in a dose-dependent manner (Figs. 2B and C and 3B and C). The IC_{50} values were determined to be 107.2 μg/ml for benzyl benzoate and 47.2 μg/ml for benzyl cinnamate. As a positive control, losartan was also evaluated with this assay system. Losartan significantly suppressed Ang II-induced Ca^{2+} uptake in mAT1a(HA)/293T cells (IC_{50} value: 2.4 μg/ml) (Figs. 2A and 3A).

2.2. Benzyl benzoate is not cytotoxic toward mAT1a (HA)/293T cells

To exclude the possibility that the isolated compounds inhibited the function of Ang II by cytotoxicity toward mAT1a(HA)/293T cells, we examined cytotoxicity by the trypan blue dye exclusion assay. As shown in Figures 3B and C, benzyl benzoate and benzyl cinnamate did not show any cytotoxicity toward mAT1a(HA)/293T cells at up to 100 μg/ml after 24 h. Thus, they inhibited the function of Ang II without showing cytotoxicity.

2.3. Biological evaluation of derivatives of benzyl benzoate

To improve the activity of benzyl benzoate, we synthesized derivatives of benzyl benzoate that had various substituents on the benzyl alcohol moiety. The newly synthesized analogs **3**–**43** were assayed for inhibitory activity toward the function of Ang II in mAT1a(HA)/293T cells and for cytotoxicity. As shown in Table 1, many of the synthesized compounds inhibited the Ang II-induced uptake of Ca^{2+} in mAT1a(HA)/293T cells. In particular, derivatives with a methyl group at the 2-, 3-, or 4-position of the benzyl alcohol moiety (**18**, **19**, and **20**) showed about 5–10 times stronger activity than benzyl benzoate (for **19**, Figs. 2D and 3D). The 3-methylbenzyl 2′-nitrobenzoate derivative (**37**) also showed high activity (Figs. 2E and 3E). All of the synthesized compounds were confirmed not to show any cytotoxicity toward mAT1a(HA)/293T cells at up to 100 μg/ml after 24 h (data not shown).

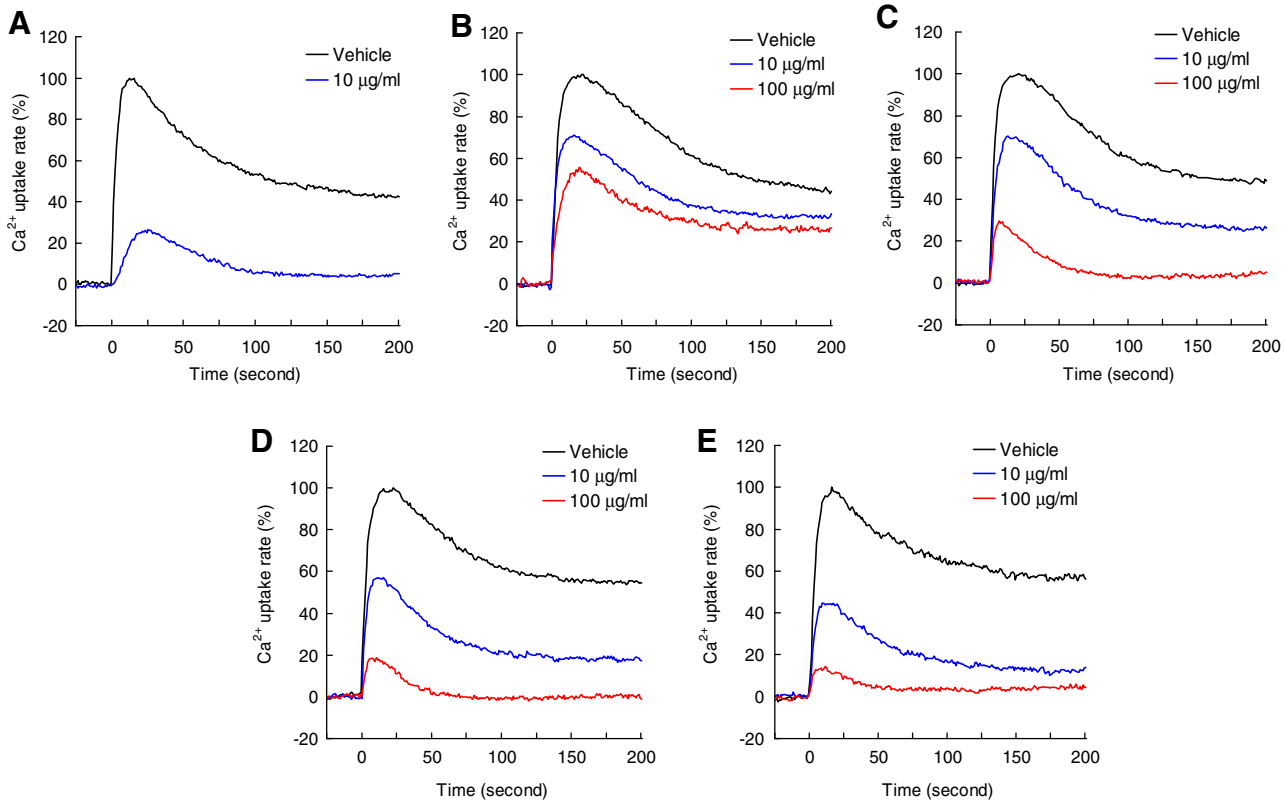

Figure 2. Inhibition of Ang II-stimulated Ca^{2+} uptake in Ang II receptor-overexpressing cells by benzyl benzoate, benzyl cinnamate, and the derivatives of benzyl benzoate. Fura 2-AM-loaded mAT1a(HA)/293T cells were treated with 1 μM Ang II for 200 s in the absence or presence of the indicated concentrations of losartan (A), benzyl benzoate (B), benzyl cinnamate (C), derivative **19** (D), or derivative **37** (E). Ang II was added at time 0, and the increase in the F340/F380 ratio (% of control) was monitored as the intracellular Ca^{2+} uptake rate. Results show a representative tracing of three separate experiments.

2.4. Selective inhibition of radio-labeled Ang II binding to AT1 receptors by benzyl benzoate

Next, we examined the effect of benzyl benzoate on the binding of [^{125}I] (Sar^1, Ile^8) Ang II to cells that overexpressed AT1 receptors. We used CHO cells that stably expressed AT1 receptors. A γ-ray counter analysis revealed the binding of [^{125}I] Ang II to the CHO transformants, and the addition of benzyl benzoate markedly decreased the binding of [^{125}I] Ang II at 100 μM and 300 μM (Table 2). One of the derivatives of benzyl benzoate (**9**) also inhibited this binding. On the other hand, benzyl benzoate did not inhibit the binding of [^{125}I] CGP-42112A, a specific radioligand of AT2 receptors, to HeLa cells that stably expressed AT2 receptors. Derivative **9** also did not inhibit this binding. An excess amount of non-labeled Ang II (cold Ang II) decreased fluorescence in both of the experiments (data not shown). Thus, benzyl benzoate was shown to block the binding of Ang II to AT1 receptors selectively.

2.5. Anti-hypertensive effect of benzyl benzoate in Ang II-induced hypertensive mice

Since benzyl benzoate was expected to suppress Ang II-induced hypertension, we subjected benzyl benzoate to an in vivo evaluation to determine its effect on Ang II-induced hypertension in mice. The systolic blood pressure was recorded before and 4 min after treatment with Ang II. As shown in Figure 4, oral administration of benzyl benzoate (2 and 10 mg/kg) significantly inhibited the elevation of systolic arterial pressure induced by Ang II treatment. This result suggested that benzyl benzoate may have a suppressive effect on Ang II-associated hypertension by blocking AT1 receptors in vivo. Benzyl cinnamate was also evaluated for its in vivo anti-hypertensive effect and it showed inhibitory activity (supplementary data).

3. Discussion

We constructed a screening system and searched for plant compounds that inhibit the function of Ang II. In the screening of 435 plant samples, we isolated benzyl benzoate and benzyl cinnamate (Fig. 1) from the resin of sweet gum *L. styraciflua* as antagonist-like compounds of Ang II receptors. Benzyl benzoate and benzyl cinnamate were shown to inhibit the function of Ang II in a dose-dependent manner without showing cytotoxicity (Figs. 2B and C and 3B and C).

For the analysis of structure–activity relationships and to improve upon the activity of benzyl benzoate, we synthesized more than 40 derivatives of the mother molecule. Synthesized analogs with various substituents in the benzyl alcohol moiety were tested to determine their biological activities. Among them, the *meta*-methyl derivative

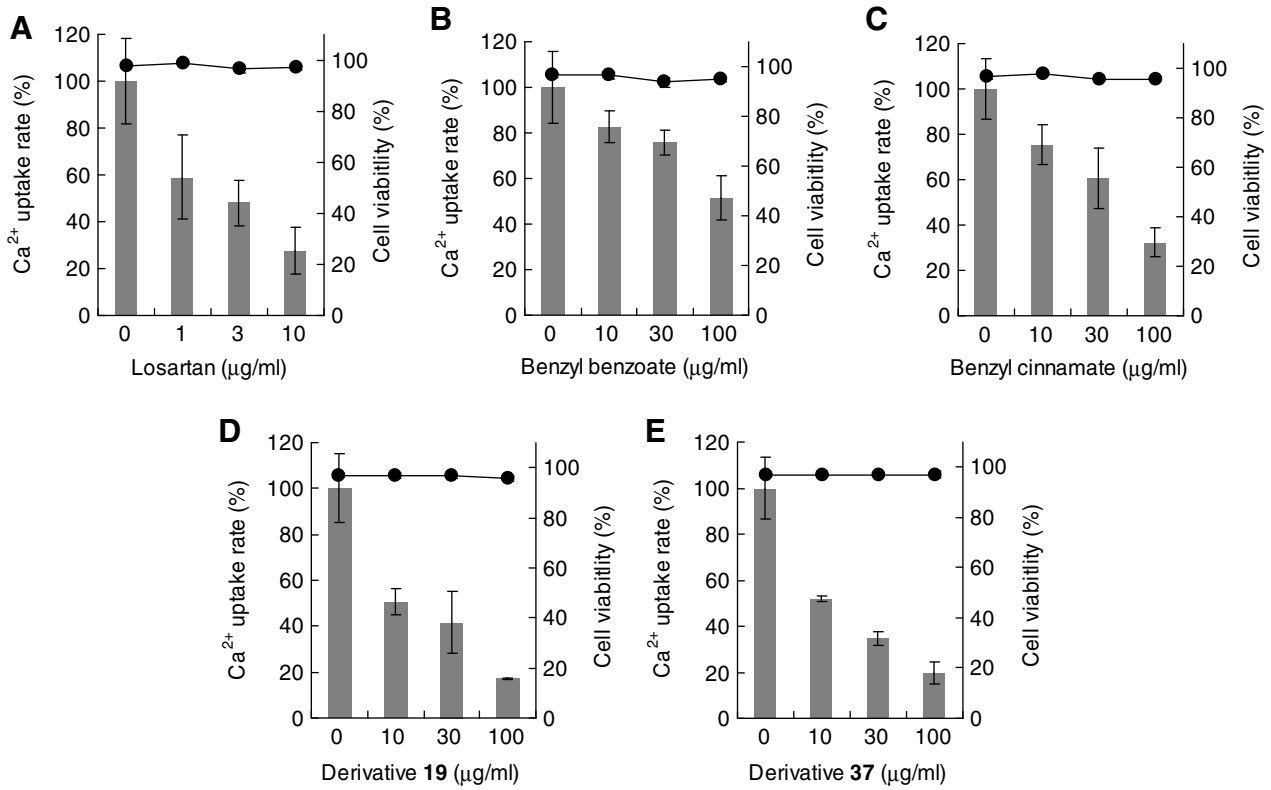

Figure 3. Effects of benzyl benzoate, benzyl cinnamate, and the derivatives of benzyl benzoate on Ang II-stimulated Ca^{2+} uptake and viability of mAT1a(HA)/293T cells. Columns: Fura 2-AM-loaded mAT1a(HA)/293T cells were pretreated with the indicated concentrations of losartan (A), benzyl benzoate (B), benzyl cinnamate (C), derivative **19** (D), or derivative **37** (E) for more than 50 s, and then treated with 1 μM Ang II for 200 s. The uptake rate of intracellular Ca^{2+} was then determined as described in Section 5. Values are means ± SD of triplicate determinations. Circles; mAT1a(HA)/293T cells were treated with the indicated concentrations of the above-mentioned compounds for 24 h, and cell viability was determined by trypan blue dye exclusion. Values are means ± SD of quadruplicate determinations.

(**19**) showed about 10-fold higher activity than benzyl benzoate itself (Figs. 2D and 3D). According to this observation, various derivatives at the benzoate moiety, including 3-methyl benzyl ester, were synthesized for biological trials. 3-Methylbenzyl 2′-nitrobenzoate (**37**) showed the best activity among them (Figs. 2E and 3E), but the results were not satisfactory compared to the positive control, losartan (Figs. 2A and 3A). The results are summarized in Table 1. From those results, it was estimated that substituents with a certain size, such as methyl and chloro groups, on benzyl alcohol moiety improved the potential of benzyl benzoate. In addition, electronegative substituents on *ortho*–portion of benzoate moiety were not thought to disturb the biological activity of **19**. Then, we tried to analyze the biological activities in terms of various parameters (electronegativity of the substituents, hydrophobicity and bulkiness) by numerical methods, but did not find any significant relationships. Meanwhile, we also synthesized β-phenylpropiophenone, which possesses alkyl-ketone connectivity between two aromatic rings, as a derivative of benzyl benzoate. It also inhibited Ang II-induced Ca^{2+} uptake in mAT1a(HA)/293T cells (IC_{50} value: 38.0 μg/ml, supplementary data). Thus, two aromatic rings in a molecule may be essential for its biological activity.

As mentioned above, many kinds of Ang II antagonists have been developed and are used clinically. Some of them show adequate potential, but improved compounds are sought to obtain a better understanding of the binding between Ang II and its receptors and to achieve better pharmacological profiles and lower cost. Recently, attempts have been made to design, synthesize, and evaluate several kinds of optimized compounds using computer modeling software. Among them, (5*S*)-1-benzylo-5-(1*H* imidazol-lylo-methylo-)-2-pyrrolidinone (MM1) and 2-metylsulfanyl-3-[2′-(*2H*-tetrazol-5-yl)biphenyl-4-ylmethyl]-*3H*-quinazolin-4-one were reported to show good activities similar to losartan.[20–22] Compared to these synthesized compounds, benzyl benzoate and its derivatives have much simpler structures. They also do not have as potent a biological activity as losartan. However, the modification of substituents in benzyl benzoate can enhance the activity to at least 10-fold stronger than that of the mother molecule. This result suggests that it may be possible to synthesize much stronger derivatives from benzyl benzoate. In addition, benzyl benzoate is a natural product. Thus, plant extracts which contain benzyl benzoate may themselves have an anti-hypertensive effect.

The inhibitory effects observed with regard to Ang II-induced intracellular Ca^{2+} uptake do not necessarily mean antagonistic activity toward Ang II receptors. Thus, a receptor binding assay was carried out by competitive displacement of the binding of specific ligands of AT1 or AT2 receptor to their receptors. This experiment was performed for benzyl benzoate. The results con-

Table 1. Synthetic derivatives of benzyl benzoate and their IC_{50} values against Ang II-stimulated Ca^{2+} uptake in mAT1a(HA)/293T cells

Compound	R_1	R_2	μg/ml
1	H	H	107.2
2	—	—	47.2
3	2-F	H	70.8
4	3-F	H	98.6
5	4-F	H	85.3
6	2-Cl	H	40.7
7	3-Cl	H	53.7
8	4-Cl	H	89.1
9	3-Br	H	97.1
10	4-Br	H	87.1
11	3-I	H	52.5
12	2-NO_2	H	186.2
13	3-NO_2	H	135.8
14	4-NO_2	H	280.4
15	2-NHAc	H	229.1
16	3-NHAc	H	120.2
17	4-NHAc	H	70.8
18	2-Me	H	20.4
19	3-Me	H	11.2
20	4-Me	H	20.9
21	2-OMe	H	117.4
22	3-OMe	H	39.2
23	4-OMe	H	42.3
24	2-Et	H	56.2
25	4-Et	H	81.3
26	4-*i*-Pr	H	81.3
27	3,4-$(OMe)_2$	H	50.1
28	3-Me	2-Me	24.0
29	3-Me	3-Me	32.4
30	3-Me	4-Me	26.3
31	3-Me	2-OMe	24.5
32	3-Me	3-OMe	44.7
33	3-Me	4-OMe	34.7
34	3-Me	2-Cl	56.2
35	3-Me	3-Cl	56.2
36	3-Me	4-C1	>100
37	3-Me	2-NO_2	11.2
38	3-Me	3-NO_2	91.2
39	3-Me	4-NO_2	93.3
40	3-Me	4-SO_2NH_2	42.7
41	3-Me	3-OH	24.5
42	3-Me	4-OH	20.9
43	3-Me	3,4-$(OH)_2$	26.3
Losartan	—	—	2.4

Table 2. Antagonistic activity of benzyl benzoate and its derivative on Ang II receptors

	Benzyl benzoate		Derivative **9**	
	100 μM	300 μM	100 μM	300 μM
AT1 receptor (%)	21	62	50	72
AT2 receptor (%)	0	9	10	23

firmed that benzyl benzoate blocked the binding of Ang II to AT1 receptors selectively (Table 2). One of the derivatives of benzyl benzoate (**9**) also inhibited the binding of Ang II to AT1 receptors. It was determined that a substituent on *meta*-portion of benzyl alcohol in benzyl benzoate did not diminish the inhibitory activity of benzyl benzoate against Ang II-AT1 receptors binding. Based on these results, benzyl benzoate and its derivatives were expected to have an anti-hypertensive effect in an animal model. Consistent with this expectation, benzyl benzoate reduced high blood pressure in Ang II-induced hypertensive mice (Fig. 4). In conclusion, benzyl benzoate, its derivatives, and benzyl cinnamate may suppress hypertension, and therefore they and plant components containing benzyl benzoate or benzyl cinnamate may have therapeutic potential for the treatment of hypertension.

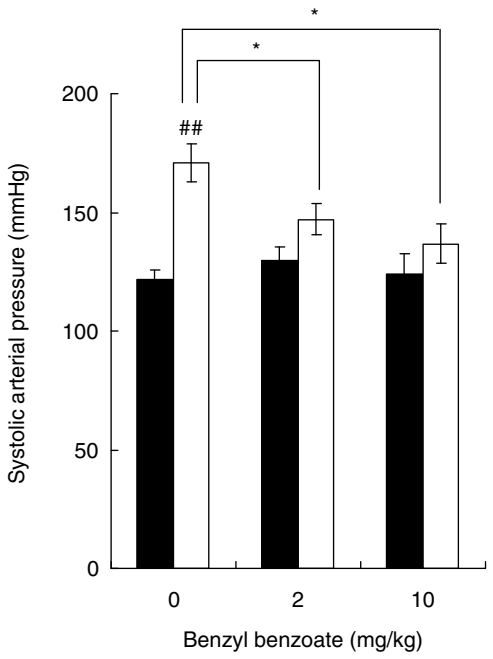

Figure 4. Anti-hypertensive effect of benzyl benzoate in Ang II-induced hypertensive mice. Male Std:ddY mice (6 weeks old) were injected with the indicated concentrations of benzyl benzoate by oral gavage, and then, 30 min later, received an intraperitoneal injection of 100 μg/kg Ang II. Systolic arterial pressure was then measured before (solid column) and after (open column) Ang II treatment as described in Section 5. Values are means ± SD ($n = 5$ per group). $^{*}p < 0.05$ versus vehicle group without benzyl benzoate. $^{\#\#}p < 0.005$ versus before Ang II treatment.

4. Conclusion

In the present study we constructed a new screening system for compounds that inhibit the function of Ang II, and isolated benzyl benzoate and benzyl cinnamate from the resin of sweet gum *L. styraciflua*, as antagonist-like compounds of Ang II receptors. Those compounds were shown to inhibit the function of Ang II in a dose-dependent manner without showing cytotoxicity. Next, we synthesized derivatives of benzyl benzoate, and tested their biological activities. Among them, the *meta*-methyl (**19**) and 3-methylbenzyl 2′-nitrobenzoate derivatives (**37**) showed about 10-fold higher activity than benzyl benzoate itself. We confirmed that benzyl benzoate and one of its derivatives block the binding of Ang II to AT1 receptors selectively. Benzyl benzoate showed an anti-hypertensive effect in Ang II-induced hypertensive mice. Thus, benzyl benzoate, its derivatives, and benzyl cinnamate were shown to suppress the function of Ang II, and therefore they and plant components containing benzyl benzoate or benzyl cinnamate may have therapeutic potential for the treatment of hypertension.

5. Experimental

5.1. Materials

Ang II and benzyl benzoate were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Fura 2-AM was procured from Wako (Tokyo, Japan). Losartan was kindly provided by Dr. T. Fukami, Banyu Pharmaceutical Co., Ltd. Benzyl cinnamate was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). All other substrates (substituted benzyl alcohols and benzoic acids) used to synthesize derivatives of benzyl benzoate were obtained from Sigma–Aldrich Co. (St. Louis, MO).

5.2. Cell culture

mAT1a(HA)/293T cells were prepared as described previously.[23] mAT1a(HA)/293T cells were cultured at 37 °C in a humidified atmosphere of CO_2–95% air in DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS; JRH Biosciences, Lenexa, KS), 2.25 mg/ml $NaHCO_3$, and 0.3 μg/ml hygromycin (Sigma–Aldrich Co.).

5.3. Measurement of intracellular Ca^{2+} concentration

mAT1a(HA)/293T cells were harvested and washed twice with solution A [9.8 g/L Hanks' solution (Nissui, Tokyo, Japan) supplemented with 5 mM HEPES (pH 7.4)]. The cells were then incubated with solution A containing 4 μg/ml fura 2-AM and 0.2% bovine serum albumin (BSA) for 1 h at 37 °C. After being washed twice with solution A, fura 2-AM-loaded cells were prepared at a concentration of 5×10^6 cells/ml and each group was used for fluorometry. Fluorescence was measured with a CAF-110 spectrofluorometer (Japan Spectroscopic Co., Tokyo, Japan) that detected 500 nm fluorescence emitted by excitation at 340 nm (F340) and 380 nm (F380) alternately at a frequency of 128 Hz, and the F340/F380 ratio from successive excitation periods was calculated. The increase in the F340/F380 ratio (% of control) was treated as the intracellular Ca^{2+} uptake rate. This was also calculated and transformed to the corresponding levels of Ca^{2+} concentration as described by Grynkiewicz and coworkers.[24] The control level in Figure 2A was calculated to be 306.8 ± 53.6 nM.

5.4. Trypan blue dye exclusion

mAT1a(HA)/293 T cells were seeded at 1×10^5 cells/well in 24-well plates (Iwaki, Tokyo, Japan) and cultured overnight. The cells were treated with various concentrations of benzyl benzoate and its derivatives for 24 h. They were then stained with trypan blue, and the number of stained cells was counted.

5.5. Binding assay

Ang II receptor binding assays were performed by MDS Pharma Services-Taiwan Ltd (Taipei, Taiwan) according to the method reported previously.[25,26] [^{125}I] (Sar^1, Ile^8) Ang II, which had a specific radioactivity of 2000 Ci/mmol, was purchased from Amersham Pharmacia Biotech (Uppsala, Sweden). The cells in 24-well plates were washed with an incubation buffer (50 mM Tris–HCl, pH 7.4, 5 mM $MgCl_2$ 0.1%BSA, 1 mM EDTA). The cells were incubated in incubation buffer with various concentrations of radio-labeled Ang II at 20 °C for 45 min. After the cells were washed twice with the incubation buffer, the cells were lysed in 1 N NaOH and cell-bound radioactivity was counted with a γ-ray counter (Aloka). Non-specific binding was determined by the addition of 100-fold unlabeled Ang II to the reactions. Specific binding was calculated as total binding minus non-specific binding. The same experiments were performed using [^{125}I] CGP-42112A, a specific radioligand of AT2 receptors, and HeLa cells that stably expressed AT2 receptors.

5.6. In vivo experiments

Male Std:ddY mice (6 weeks old, SLC, Tokyo, Japan) were used to examine in vivo anti-Ang II effects. They were housed in standard cages (21.5 × 32 × 14 cm, five mice/cage) under controlled conditions of temperature (24 ± 1 °C), humidity (50 ± 2%), and lighting (lights on from 08:00 to 20:00). Animal studies were performed in accordance with the 1980 guidelines, Notification No. 6 of the Prime Minister's Office of Japan.

After mice received a standard laboratory diet (Oriental Yeast, Tokyo, Japan) and water ad libitum for 1 week, they were divided into three groups. Mice were injected with several concentrations of benzyl benzoate by oral gavage, and then, 30 min later, received intraperitoneal injection of 100 μg/kg Ang II in 1% aqueous ethanol. After 4 min, systolic arterial pressure in trained conscious mice was recorded using an automated tail-cuff device (Model MK-2000, Muromachi Kikai Co., Ltd, Tokyo, Japan). The values from five consecutive readings were averaged and compared with ordinary blood pressure values obtained from each mouse before Ang II treatment.

5.7. Synthesis of derivatives of benzyl benzoate

5.7.1. Synthesis of 2-chlorobenzyl benzoate (6) (typical procedure with benzyl alcohol and benzoyl chloride; method A). To a solution of 2-chlorobenzyl alcohol (143 mg, 1.0 mmol) in pyridine (4.0 ml) was added benzoyl chloride (127 μl, 1.1 mmol) at 0 °C under a nitrogen atmosphere. The reaction mixture was kept at the same temperature for 1.5 h, and the excess reagent was destroyed by adding water (10 ml). The reaction mixture was extracted with ethyl acetate, and the organic layer was washed with saturated aq ammonium chloride followed by brine, and then evaporated. Purification of the residue by column chromatography (silicagel 60) with hexane/EtOAc (39:1) as an eluent yielded the benzyl benzoate derivative **6** (155 mg) as a colorless oil.

Yield 73%; R_f 0.83 (hexane/EtOAc = 5:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, J = 7.4 Hz, 2H), 7.56 (t, J = 7.4 Hz, 1H), 7.51–7.39 (m, 4H), 7.29–7.25 (m, 2H),

5.46 (s, 2H); HR-ESIMS *m/z* 269.0328 $[M+Na]^+$, calcd for $C_{14}H_{11}ClO_2Na$, 269.0345.

5.7.2. 2-Fluorobenzyl benzoate (3) [method A]. ^1H NMR (600 MHz, $CDCl_3$) δ 8.06 (d, $J = 7.5$ Hz, 2H), 7.54 (t, $J = 7.7$ Hz, 1H), 7.47 (t, $J = 7.5$ Hz, 1H), 7.42 (t, $J = 7.5$ Hz, 2H), 7.34–7.31 (m, 1H), 7.15 (dd, $J = \sim 6$, ~ 7 Hz, 1H), 7.09 (dd, $J = \sim 7$, ~ 9 Hz, 1H), 5.43 (s, 2H); HR-ESIMS *m/z* 253.0651 $[M+Na]^+$, calcd for $C_{14}H_{11}FO_2Na$, 253.0641.

5.7.3. 3-Fluorobenzyl benzoate (4) [method A]. ^1H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, $J = 7.4$ Hz, 2H), 7.58 (t, $J = 7.4$ Hz, 1H), 7.46 (t, $J = 7.4$ Hz, 2H), 7.38–7.33 (m, 1H), 7.22 (d, $J = 7.6$ Hz, 1H), 7.14 (d, $J = 9.6$ Hz, 1H), 7.03 (dd, $J = \sim 7$, ~ 9 Hz, 1H), 5.36 (s, 2H); HR-ESIMS *m/z* 253.0644 $[M+Na]^+$, calcd for $C_{14}H_{11}FO_2Na$, 253.0641.

5.7.4. 4-Fluorobenzyl benzoate (5) [method A]. ^1H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, $J = 7.4$ Hz, 2H), 7.57 (t, $J = 7.4$ Hz, 1H), 7.46–7.42 (m, 4H), 7.07 (dd, $J = \sim 7$, ~ 9 Hz, 2H), 5.33 (s, 2H); HR-ESIMS *m/z* 253.0645 $[M+Na]^+$, calcd for $C_{14}H_{11}FO_2Na$, 253.0641.

5.7.5. 3-Chlorobenzyl benzoate (7) [method A]. Yield 78%; R_f 0.73 (hexane/EtOAc = 1:1); ^1H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, $J = 8.0$ Hz, 2H), 7.58 (t, $J = 8.0$ Hz, 1H), 7.47–7.43 (m, 3H), 7.32 (m, 3H), 5.34 (s, 2H); HR-ESIMS *m/z* 269.0336 $[M+Na]^+$, calcd for $C_{14}H_{11}ClO_2Na$, 269.0345.

5.7.6. 4-Chlorobenzyl benzoate (8) [method A]. Yield 87%; R_f 0.75 (hexane/EtOAc = 1:1); ^1H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, $J = 7.3$ Hz, 2H), 7.57 (t, $J = 7.3$ Hz, 1H), 7.46–7.34 (m, 6H), 5.33 (s, 2H); HR-ESIMS *m/z* 269.0330 $[M+Na]^+$, calcd for $C_{14}H_{11}ClO_2Na$, 269.0345.

5.7.7. 3-Bromobenzyl benzoate (9) [method A]. Yield 94%; R_f 0.67 (hexane/EtOAc = 5:1); ^1H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, $J = 7.1$ Hz, 2H), 7.60–7.56 (m, 2H), 7.49–7.44 (m, 3H), 7.29 (d, $J = 7.8$ Hz, 1H), 7.26 (t, $J = 7.8$ Hz, 1H), 5.33 (s, 2H); IR ($CDCl_3$) 1718, 1572, 1271 cm^{-1}; HR-ESIMS *m/z* 312.9826 $[M+Na]^+$, calcd for $C_{14}H_{11}BrO_2Na$, 312.9840.

5.7.8. 4-Bromobenzyl benzoate (10) [method A]. Yield 93%; R_f 0.66 (hexane/EtOAc = 5:1); ^1H NMR (600 MHz, $CDCl_3$) δ 7.92 (d, $J = 7.5$ Hz, 2H), 7.56 (d, $J = 8.1$ Hz, 2H), 7.43 (d, $J = 8.1$ Hz, 2H), 7.38 (t, $J = 7.5$ Hz, 2H), 7.35–7.33 (m, 1H), 5.34 (s, 2H); IR ($CDCl_3$) 1718, 1590, 1269 cm^{-1}; HR-ESIMS *m/z* 312.9852 $[M+Na]^+$, calcd for $C_{14}H_{11}BrO_2Na$, 312.9840.

5.7.9. 3-Iodobenzyl benzoate (11) [method A]. Yield 100%; ^1H NMR (600 MHz, $CDCl_3$) δ 8.07 (d, $J = 7.3$ Hz, 2H), 7.79 (s, 1H), 7.66 (d, $J = 7.7$ Hz, 1H), 7.55 (t, $J = 7.3$ Hz, 1H), 7.44–7.39 (m, 3H), 7.10 (t, $J = 7.7$ Hz, 1H), 5.28 (s, 2H); HR-ESIMS *m/z* 360.9705 $[M+Na]^+$, calcd for $C_{14}H_{11}IO_2Na$, 360.9701.

5.7.10. 2-Nitrobenzyl benzoate (12) [method A]. Yield 75%; R_f 0.46 (hexane/EtOAc = 5:1); ^1H NMR (600 MHz, $CDCl_3$) δ 8.12 (d, $J = 8.0$ Hz, 1H), 8.08 (d, $J = 7.5$ Hz, 2H), 7.67–7.63 (m, 2H), 7.58 (t, $J = 7.5$ Hz 1H), 7.50–7.45 (m, 3H), 5.77 (s, 2H); IR ($CDCl_3$) 1724, 1529 cm^{-1}; HR-ESIMS *m/z* 280.0576 $[M+Na]^+$, calcd for $C_{14}H_{11}NO_4Na$, 280.0586.

5.7.11. 3-Nitrobenzyl benzoate (13) [method A]. Yield 100%; R_f 0.45 (hexane/EtOAc = 5:1); ^1H NMR (600 MHz, $CDCl_3$) δ 8.31 (s, 1H), 8.19 (d, $J = 7.7$ Hz, 1H), 8.07 (d, $J = 7.9$ Hz, 1H), 7.77 (d, $J = 7.7$ Hz, 1H), 7.59–7.54 (m, 2H), 7.45 (t, $J = 7.9$ Hz, 2H), 5.44 (s, 2H); IR ($CDCl_3$) 1719, 1534 cm^{-1}; HR-ESIMS *m/z* 280.0576 $[M+Na]^+$, calcd for $C_{14}H_{11}NO_4Na$, 280.0586.

5.7.12. 4-Nitrobenzyl benzoate (14) [method A]. Yield 94%; R_f 0.40 (hexane/EtOAc = 5:1); ^1H NMR (600 MHz, $CDCl_3$) δ 8.24 (d, $J = 8.3$ Hz, 2H), 8.07 (d, $J = 8.2$ Hz, 2H), 7.57–7.60 (m, 3H), 7.46 (t, $J = 8.2$ Hz, 2H), 5.45 (s, 2H); IR ($CDCl_3$) 1721, 1605, 1525 cm^{-1}; HR-ESIMS *m/z* 280.0575 $[M+Na]^+$, calcd for $C_{14}H_{11}NO_4Na$, 280.0586.

5.7.13. 2-Methylbenzyl benzoate (18) [method A]. Yield 85%; R_f 0.55 (hexane/EtOAc = 5:1); ^1H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, $J = 7.8$ Hz, 2H), 7.56 (t, $J = 7.8$ Hz, 1H), 7.46–7.42 (m, 3H), 7.27–7.23 (m, 3H), 5.38 (s, 2H), 2.42 (s, 3H); HR-ESIMS *m/z* 249.0899 $[M+Na]^+$, calcd for $C_{15}H_{14}O_2Na$, 249.0891.

5.7.14. 3-Methylbenzyl benzoate (19) [method A]. Yield 98%; R_f 0.55 (hexane/EtOAc = 5:1); ^1H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, $J = 7.8$ Hz, 2H), 7.56 (t, $J = 7.8$ Hz, 1H), 7.44 (t, $J = 7.8$ Hz, 2H), 7.27–7.23 (m, 3H), 7.14 (d, $J = 6.8$ Hz, 1H), 5.38 (s, 2H), 2.42 (s, 3H); HR-ESIMS *m/z* 249.0896 $[M+Na]^+$, calcd for $C_{15}H_{14}O_2Na$, 249.0891.

5.7.15. 4-Methylbenzyl benzoate (20) [method A]. Yield 97%; R_f 0.85 (hexane/EtOAc = 5:1); ^1H NMR (400 MHz, $CDCl_3$) δ 7.56 (d, $J = 7.3$ Hz, 2H), 7.54 (t, $J = 7.3$ Hz, 1H), 7.42 (t, $J = 7.3$ Hz, 2H), 7.34 (d, $J = 8.0$ Hz, 2H), 7.19 (d, $J = 8.0$ Hz, 2H), 5.32 (s, 2H), 2.36 (s, 3H); HR-ESIMS *m/z* 249.0903 $[M+Na]^+$, calcd for $C_{15}H_{14}O_2Na$, 249.0891.

5.7.16. 2-Methoxybenzyl benzoate (21) [method A]. Yield 92%; R_f 0.59 (hexane/EtOAc = 5:1); ^1H NMR (600 MHz, $CDCl_3$) δ 8.08 (d, $J = 7.3$ Hz, 2H), 7.54 (t, $J = 7.3$ Hz, 1H), 7.43–7.41 (m, 3H), 7.31 (t, $J = 8.0$ Hz, 1H), 6.97 (t, $J = 8.0$ Hz, 1H), 6.91 (d, $J = 8.0$ Hz, 1H), 5.42 (s, 2H), 3.85 (s, 3H); IR ($CDCl_3$) 1714, 1613, 1515 cm^{-1}; HR-ESIMS *m/z* 265.0835 $[M+Na]^+$, calcd for $C_{15}H_{14}O_3Na$, 265.0841.

5.7.17. 3-Methoxybenzyl benzoate (22) [method A]. Yield 100%; R_f 0.73 (hexane/EtOAc = 5:1); ^1H NMR (600 MHz, $CDCl_3$) δ 8.09 (d, $J = 7.3$ Hz, 2H), 7.55 (t, $J = 7.3$ Hz, 1H), 7.43 (t, $J = 7.3$ Hz, 2H), 7.30 (t, $J = 8.0$ Hz, 1H), 7.04 (d, $J = 8.0$ Hz, 1H), 7.00 (s, 1H), 6.89 (d, $J = 8.0$ Hz, 1H), 5.35 (s, 2H), 3.81 (s, 3H); IR ($CDCl_3$) 1715, 1602, 1490 cm^{-1}; HR-

ESIMS *m/z* 265.0840 $[M+Na]^+$, calcd for $C_{15}H_{14}O_3Na$, 265.0841.

5.7.18. 4-Methoxybenzyl benzoate (23) [method A]. Yield 84%; ^1H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, J = 7.3 Hz, 2H), 7.55 (t, J = 7.3 Hz, 1H), 7.45–7.40 (m, 4H), 6.94 (t, J = 8.5 Hz, 2H), 5.33 (s, 2H), 3.81 (s, 3H); IR ($CDCl_3$) 1714, 1603, 1495 cm^{-1}; HR-ESIMS *m/z* 265.0834 $[M+Na]^+$, calcd for $C_{15}H_{14}O_3Na$, 265.0841.

5.7.19. 2-Ethylbenzyl benzoate (24) [method A]. Yield 89%; R_f 0.75 (hexane/EtOAc = 5:1); ^1H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, J = 7.5 Hz, 2H), 7.54 (t, J = 7.5 Hz, 1H), 7.44–7.40 (m, 3H), 7.33–7.20 (m, 3H), 5.40 (s, 2H), 2.67 (q, J = 7.6 Hz, 2H), 1.26 (t, J = 7.6 Hz, 3H); HR-ESIMS *m/z* 263.1074 $[M+Na]^+$, calcd for $C_{16}H_{16}O_2Na$, 263.1048.

5.7.20. 4-Ethylbenzyl benzoate (25) [method A]. Yield 100%; R_f 0.73 (hexane/EtOAc = 5:1); ^1H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, J = 8.4 Hz, 2H), 7.55 (t, J = 7.4 Hz, 1H), 7.45–7.37 (m, 4H), 7.23 (d, J = 8.0 Hz, 2H), 5.34 (s, 2H), 2.67 (q, J = 7.6 Hz, 2H), 1.25 (t, J = 7.6 Hz, 3H); HR-ESIMS *m/z* 263.1068 $[M+Na]^+$, calcd for $C_{16}H_{16}O_2Na$, 263.1048.

5.7.21. 4-Isopropylbenzyl benzoate (26) [method A]. Yield 79%; R_f 0.70 (hexane/EtOAc = 5:1); ^1H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, J = 8.1 Hz, 2H), 7.54 (t, J = 8.1 Hz, 1H), 7.44–7.37 (m, 4H), 7.24 (d, J = 8.3 Hz, 2H), 5.34 (s, 2H), 2.92 (sept, J = 6.9 Hz, 1H), 1.26 (d, J = 6.9 Hz, 6H); HR-ESIMS *m/z* 277.1204 $[M+Na]^+$, calcd for $C_{17}H_{18}O_2Na$, 277.1204.

5.7.22. 3,4-Dimethoxybenzyl benzoate (27) [method A]. Yield 81%; R_f 0.58 (hexane/EtOAc = 2:1); ^1H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, J = 7.1 Hz, 2H), 7.57–7.53 (m, 1H), 7.43 (t, J = 7.1 Hz, 2H), 7.04–6.98 (m, 2H), 6.88 (d, J = 8.1 Hz, 1H), 5.30 (s, 2H), 3.90 (s, 3H), 3.89 (s, 3H); HR-ESIMS *m/z* 295.0946 $[M+Na]^+$, calcd for $C_{16}H_{16}O_4Na$, 295.0946.

5.7.23. 3-Methylbenzyl 2′-methylbenzoate (28) [method A]. Yield 100%; R_f 0.78 (hexane/EtOAc = 5:1); ^1H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, J = 7.3 Hz, 1H), 7.30 (t, J = 7.3 Hz, 1H), 7.21–7.12 (m, 5H), 7.06 (d, J = 6.6 Hz, 1H), 5.22 (s, 2H), 2.52 (s, 3H), 2.28 (s, 3H); HR-ESIMS *m/z* 263.1029 $[M+Na]^+$, calcd for $C_{16}H_{16}O_2Na$, 263.1048.

5.7.24. 3-Methylbenzyl 3′-methylbenzoate (29) [method A]. Yield 100%; R_f 0.75 (hexane/EtOAc = 5:1); ^1H NMR (400 MHz, $CDCl_3$) δ 7.81 (m, 2H), 7.29–7.16 (m, 5H), 7.07 (d, J = 6.8 Hz, 1H), 5.24 (s, 2H), 2.31 (s, 3H), 2.29 (s, 3H); HR-ESIMS *m/z* 263.1036 $[M+Na]^+$, calcd for $C_{16}H_{16}O_2Na$, 263.1048.

5.7.25. 3-Methylbenzyl 4′-methylbenzoate (30) [method A]. Yield 83%; R_f 0.70 (hexane/EtOAc = 5:1); ^1H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, J = 8.3 Hz, 2H), 7.22–7.14 (m, 5H), 7.07 (d, J = 6.8 Hz, 1H), 5.24 (s, 2H), 2.32 (s, 3H), 2.29 (s,3H); HR-ESIMS *m/z* 263.1065 $[M+Na]^+$, calcd for $C_{16}H_{16}O_2Na$, 263.1048.

5.7.26. 3-Methylbenzyl 2′-methoxybenzoate (31) [method A]. Yield 82%; R_f 0.40 (hexane/EtOAc = 5:1); ^1H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 7.6 Hz, 1H), 7.38 (t, J = 7.6 Hz, 1H), 7.19–7.17 (m, 3H), 7.05 (br s, 1H), 6.88 (t, J = 7.8 Hz, 2H), 5.23 (s, 2H), 3.83 (s, 3H), 2.29 (s, 3H); HR-ESIMS *m/z* 279.0992 $[M+Na]^+$, calcd for $C_{16}H_{16}O_3Na$, 279.0997.

5.7.27. 3-Methylbenzyl 3′-methoxybenzoate (32) [method A]. Yield 74%; R_f 0.53 (hexane/EtOAc = 5:1); ^1H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, J = 7.6 Hz, 1H), 7.61 (s, 1H), 7.35 (t, J = 7.9 Hz, 1H), 7.31–7.26 (m, 3H), 7.16 (d, J = 6.8 Hz, 1H), 7.11 (dd, J = 8.0, 2.7 Hz, 1H), 5.34 (s, 2H), 3.85 (s, 3H), 2.38 (s, 3H); HR-ESIMS *m/z* 279.0998 $[M+Na]^+$, calcd for $C_{16}H_{16}O_3Na$, 279.0997.

5.7.28. 3-Methylbenzyl 4′-methoxybenzoate (33) [method A]. Yield 86%; R_f 0.55 (hexane/EtOAc = 5:1); ^1H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 8.8 Hz, 2H), 7.19–7.15 (m, 3H), 7.06 (d, J = 6.8 Hz, 1H), 6.83 (d, J = 8.8 Hz, 2H), 5.22 (s, 2H), 3.77 (s, 3H), 2.93 (s, 3H); HR-ESIMS *m/z* 279.1012 $[M+Na]^+$, calcd for $C_{16}H_{16}O_3Na$, 279.0997.

5.7.29. 3-Methylbenzyl 2′-chlorobenzoate (34) [method A]. Yield 100%; R_f 0.53 (hexane/EtOAc = 5:1); ^1H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 7.8 Hz, 1H), 7.38–7.30 (m, 2H), 7.24–7.17 (m, 4H), 7.07 (d, J = 6.4 Hz, 1H), 5.26 (s, 2H), 2.29 (s, 3H); HR-ESIMS *m/z* 283.0494 $[M+Na]^+$, calcd for $C_{15}H_{13}ClO_2Na$, 283.0502.

5.7.30. 3-Methylbenzyl 3′-chlorobenzoate (35) [method A]. Yield 88%; R_f 0.78 (hexane/EtOAc = 5:1); ^1H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.97 (d, J = 7.7 Hz, 1H), 7.53 (d, J = 7.7 Hz, 1H), 7.38 (t, J = 7.7 Hz, 1H), 7.32–7.24 (m, 3H), 7.19 (d, J = 7.1 Hz, 1H), 5.34 (s, 2H), 2.39 (s, 3H); HR-ESIMS *m/z* 283.0497 $[M+Na]^+$, calcd for $C_{15}H_{13}ClO_2Na$, 283.0502.

5.7.31. 3-Methylbenzyl 4′-chlorobenzoate (36) [method A]. Yield 96%; R_f 0.65 (hexane/EtOAc = 5:1); ^1H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J = 8.5 Hz, 2H), δ 7.41 (d, J = 8.5 Hz, 2H), 7.31–7.28 (m, 3H), δ 7.17 (d, J = 7.1 Hz, 1H), 5.33 (s, 2H), 2.39 (s, 3H); HR-ESIMS *m/z* 283.0521 $[M+Na]^+$, calcd for $C_{15}H_{13}ClO_2Na$, 283.0502.

5.7.32. 3-Methylbenzyl 2′-nitrobenzoate (37) [method A]. Yield 92%; R_f 0.60 (hexane/EtOAc = 2:1); ^1H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, J = 8.0 Hz, 1H), 7.67 (d, J = 7.3 Hz, 1H), 7.60–7.51 (m, 2H), 7.21–7.07 (m, 4H), 5.24 (s, 2H), 2.29 (s, 3H); HR-ESIMS *m/z* 294.0743 $[M+Na]^+$, calcd for $C_{15}H_{13}NO_4Na$, 294.0742.

5.7.33. 3-Methylbenzyl 3′-nitrobenzoate (38) [method A]. Yield 93%; R_f 0.58 (hexane/EtOAc = 5:1); ^1H NMR (400 MHz, $CDCl_3$) δ 8.81 (s, 1H), 8.35–8.30 (m, 2H), 7.57 (t, J = 8.1 Hz, 1H), 7.24–7.17 (m, 3H), 7.11 (d, J = 7.1 Hz, 1H), 5.30 (s, 2H), 2.31 (s, 3H); HR-ESIMS *m/z* 294.0767 $[M+Na]^+$, calcd for $C_{15}H_{13}NO_4Na$, 294.0742.

5.7.34. 3-Methylbenzyl 4′-nitrobenzoate (39) [method A]. Yield 87%; R_f 0.75 (hexane/EtOAc = 5:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22–8.15 (m, 4H), 7.24–7.17 (m, 3H), 7.11 (d, J = 7.1 Hz, 1H), 5.29 (s, 2H), 2.31 (s, 3H); HR-ESIMS m/z 294.0746 $[M+Na]^+$, calcd for $C_{15}H_{13}NO_4Na$, 294.0742.

5.7.35. Synthesis of 2-acetamidobenzyl benzoate (15) (typical procedure by reductive acetylation of the corresponding nitro derivative; method B). To a solution of 2-nitrobenzyl benzoate (**12**, 47.5 mg, 0.2 mmol) in ethyl acetate (4.0 ml) was added 10% palladium on carbon (10 mg). The reaction mixture was stirred under a hydrogen atmosphere for 3 h after the addition of acetic anhydride 0.038 ml (0.4 mmol). The reaction mixture was filtered through a Celite (hi-flo super cel) pad, and concentrated. Purification of the residue by column chromatography (silicagel 60) with hexane/EtOAc (1:1) as an eluent yielded the benzyl benzoate derivative **15** (10.4 mg) as a crystalline solid.

Yield 20%; R_f 0.43 (hexane/EtOAc = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.96 (br s, 1H), 8.11–7.98 (m, 3H), 7.58 (t, J = 7.6 Hz, 1H), 7.49–7.43 (m, 3H), 7.38 (t, J = 7.3 Hz, 1H), 7.16 (t, J = 7.3 Hz, 1H), 5.37 (s, 2H), 2.27 (s, 3H); HR-ESIMS m/z 292.0967 $[M+Na]^+$, calcd for $C_{16}H_{15}NO_3Na$, 292.0950.

5.7.36. 3-Acetamidobenzyl benzoate (16) [method B]. Yield 50%; R_f 0.33 (hexane/EtOAc = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 7.3 Hz, 2H), 7.58–7.41 (m, 6H), 7.32 (t, J = 7.8 Hz, 1H), 7.18 (d, J = 7.3 Hz, 1H), 5.33 (s, 2H), 2.16 (s, 3H); HR-ESIMS m/z 292.0953 $[M+Na]^+$, calcd for $C_{16}H_{15}NO_3Na$, 292.0950.

5.7.37. 4-Acetamidobenzyl benzoate (17) [method B]. Yield 74%; R_f 0.50 (hexane/EtOAc = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 7.3 Hz, 2H), 7.77 (br s, 1H), 7.51 (d, J = 8.5 Hz, 2H), 7.42–7.35 (m, 5H), 5.28 (s, 2H), 2.14 (s, 3H); HR-ESIMS m/z 292.0961 $[M+Na]^+$, calcd for $C_{16}H_{15}NO_3Na$, 292.0950.

5.7.38. Synthesis of 3-methylbenzyl 4′-sulfonamoylbenzoate (40). To a solution of 4-carboxybenzene sulfonamide (20 mg, 0.1 mmol) in DMF (0.25 ml) were added, sequentially, 3-methylbenzyl alcohol (28 mg, 0.23 mmol), 1-hydroxybenzotriazole (16.8 mg, 0.11 mmol), and N,N'-diisopropylcarbodiimide (16.6 μl, 0.11 mmol) at room temperature under a nitrogen atmosphere, and the mixture was continuously stirred for 12 h. The reaction mixture was diluted with saturated aq NH_4Cl and extracted with ethyl acetate, and the organic layer was washed with brine and evaporated. Purification of the residue by column chromatography (silicagel 60) with toluene/acetone (4:1) as an eluent yielded the benzyl benzoate derivative **40** (15.7 mg) as a colorless oil.

Yield 51%; R_f 0.45 (toluene/acetone = 5:1); ^{1}H NMR (400 MHz, CD_3OD) δ 8.08 (d, J = 10.0 Hz, 2H), 7.90 (d, J = 10.0 Hz, 2H), 7.18–7.17 (m, 3H), 7.07 (d, J = 4.0 Hz, 1H), 5.38 (s, 2H), 5.25 (s, 2H), 2.26 (s, 3H); HR-ESIMS m/z 328.0640 $[M+Na]^+$, calcd for $C_{15}H_{15}NO_4SNa$, 328.0619.

5.7.39. Synthesis of 3-methylbenzyl 3′-hydroxybenzoate (41) (typical procedure with benzyl chloride and hydroxybenzoic acid; method C). To a solution of 3-hydroxybenzoic acid (27.6 mg, 0.2 mmol) in DMF (0.5 ml) were added 3-methylbenzyl chloride (15.8 μl, 0.24 mmol) and potassium fluoride 11.2 mg (0.2 mmol) under a nitrogen atmosphere. The reaction mixture was refluxed for 4 h. The reaction mixture was filtered through a cotton pad after being diluted with ethyl acetate, and washed with saturated aq ammonium chloride followed by brine, and then evaporated. Purification of the residue by column chromatography (silicagel 60) with hexane/EtOAc (39:1) as an eluent yielded the benzyl benzoate derivative **41** (155 mg) as a colorless oil.

Yield 73%; R_f 0.48 (hexane/EtOAc = 3:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65–7.62 (m, 2H), 7.32–7.06 (m, 6H), 6.26 (br s, 1H), 5.32 (s, 2H), 2.37 (s, 3H); HR-ESIMS m/z 265.0864 $[M+Na]^+$, calcd for $C_{15}H_{14}O_3Na$, 265.0841.

5.7.40. 3-Methylbenzyl 4′-hydroxybenzoate (42) [method C]. Yield 58%; R_f 0.45 (hexane/EtOAc = 3:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, J = 8.8 Hz, 2H), 7.21–7.14 (m, 3H), 7.07 (d, J = 7.1 Hz, 1H), 6.78 (d, J = 8.8 Hz, 2H), 6.22 (br s, 1H), 5.23 (s, 2H), 2.28 (s, 3H); HR-ESIMS m/z 265.0841 $[M+Na]^+$, calcd for $C_{15}H_{14}O_3Na$, 265.0841.

5.7.41. 3-Methylbenzyl 3′,4′-dihydroxybenzoate (43) [method C]. Yield 53%; R_f 0.25 (hexane/EtOAc = 3:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59–7.57 (m, 2H), 7.28–7.15 (m, 6H), 6.88 (d, J = 8.5 Hz, 1H), 5.29 (s, 2H), 2.38 (s, 3H); HR-ESIMS m/z 281.0818 $[M+Na]^+$, calcd for $C_{15}H_{14}O_4Na$, 281.0790.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.03.056.

References and notes

1. Burnier, M.; Brunner, H. R. *J. Am. Soc. Nephrol.* **1999**, *10*, S278.

2. Gavras, H.; Brunner, H. R.; Turini, G. A.; Kershaw, G. R.; Tifft, C. P.; Cuttelod, S.; Gavras, I.; Vukovich, R. A.; McKinstry, D. N. *N. Engl. J. Med.* **1978**, *298*, 991.
3. Corvol, P. *Clin. Exp. Hypertens. A* **1989**, *11*, 463.
4. Brunner, H. R.; Nussberger, J.; Waeber, B. *Adv. Nephrol. Necker. Hosp.* **1993**, *22*, 305.
5. Goodfriend, T. L.; Elliott, M. E.; Catt, K. J. *N. Engl. J. Med.* **1996**, *334*, 1649.
6. Whitebread, S.; Mele, M.; Kamber, B.; de Gasparo, M. *Biochem. Biophys. Res. Commun.* **1989**, *163*, 284.
7. Wu, X.; Wan, Y.; Mahalingam, A. K.; Murugaiah, A. M.; Plouffe, B.; Botros, M.; Karlén, A.; Hallberg, M.; Gallo-Payet, N.; Alterman, M. *J. Med. Chem.* **2006**, *49*, 7160.
8. Basso, N.; Terragno, N. A. *Hypertension* **2001**, *38*, 1246.
9. Singh, R. K.; Barker, S. *Curr. Opin. Invest. Drugs* **2005**, *6*, 269.
10. Mancia, G.; Seravalle, G.; Grassi, G. *Am. J. Hypertens.* **2003**, *16*, 1066.
11. Adang, A.; Hermkens, P.; Linders, J.; Ottenheijm, H.; Staveren, C. *J. R. Neth. Chem. Soc.* **1994**, *113*, 63.
12. Carini, D. J.; Duncia, J. V.; Johnson, A. L.; Chiu, A. T.; Price, W. A.; Wong, P. C.; Timmermans, P. B. *J. Med. Chem.* **1990**, *33*, 1330.
13. Duncia, J. V.; Carini, D. J.; Chiu, A. T.; Johnson, A. L.; Price, W. A.; Wong, P. C.; Wexler, R. R.; Timmermans, P. B. *Med. Res. Rev.* **1992**, *12*, 149.
14. Matsoukas, J. M.; Agelis, G.; Wahhab, A.; Hondrelis, J.; Panagiotopoulos, D.; Yamdagni, R.; Wu, Q.; Mavromoustakos, T.; Maia, H. L.; Ganter, R.; Moore, G. J. *J. Med. Chem.* **1995**, *38*, 4660.
15. Matsoukas, J. M.; Polevaya, L.; Ancans, J.; Mavromoustakos, T.; Kolocouris, A.; Roumelioti, P.; Vlahakos, D. V.; Yamdagni, R.; Wu, Q.; Moore, G. J. *Bioorg. Med. Chem.* **2000**, *8*, 1.
16. McClellan, K. J.; Goa, K. L. *Drugs* **1998**, *56*, 847.
17. McClellan, K. J.; Balfour, J. A. *Drugs* **1998**, *55*, 713.
18. Markham, A.; Spencer, C. M.; Jarvis, B. *Drugs* **2000**, *59*, 1187.
19. Goa, K. L.; Wagstaff, A. J. *Drugs* **1996**, *51*, 820.
20. Moutevelis-Minakakis, P.; Gianni, M.; Stougiannou, H.; Zoumpoulakis, P.; Zoga, A.; Vlahakos, A. D.; Iliodromitis, E.; Mavromoustakos, T. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1737.
21. Mavromoustakos, T.; Moutevelis-Minakakis, P.; Kokotos, C. G.; Kontogianni, P.; Politi, A.; Zoumpoulakis, P.; Findlay, J.; Cox, A.; Balmforth, A.; Zoga, A.; Iliodromitis, E. *Bioorg. Med. Chem.* **2006**, *14*, 4353.
22. Ismail, M. A.; Barker, S.; Abou el-Ella, D. A.; Abouzid, K. A.; Toubar, R. A.; Todd, M. H. *J. Med. Chem.* **2006**, *49*, 1526.
23. Ishida, J.; Asada, S.; Daitoku, H.; Fujiwara, K.; Kon, Y.; Sugaya, T.; Murakami, K.; Nakajima, T.; Kasuya, Y.; Fukamizu, A. *Int. J. Mol. Med.* **1999**, *3*, 263.
24. Grynkiewicz, G.; Poenie, M.; Tsien, R. Y. *J. Biol. Chem.* **1985**, *260*, 3440.
25. Webb, M. L.; Liu, E. C.; Monshizadegan, H.; Hedberg, A.; Misra, R. N.; Goldenberg, H.; Harris, D. N. *J. Pharmacol. Exp. Ther.* **1993**, *264*, 1387.
26. Whitebread, S. E.; Taylor, V.; Bottari, S. P.; Kamber, B.; de Gasparo, M. *Biochem. Biophys. Res. Commun.* **1991**, *181*, 1365.