Megastigmane glycosides from leaves of *Eucommia ulmoides* Oliver with ACE

inhibitory activity

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ABSTRACT

Four new megastigmane glycosides, **eucomegastigsides A-D (2, 3, 5 and 7)**, together with three known megastigmane glycosides, **(6*R*, 7*E*, 9*R*)-9-hydroxy-4, 7-megastigmadien-3-one-9-*O*-[α -L-arabinopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside (1)**, foliasalacioside **B₁ (4)** and eleganoside **A (6)**, were isolated from the leaves of *Eucommia ulmoides* Oliver. Their anti-hypertensive effect was investigated *in vitro* based on the inhibition of Angiotensin Converting Enzyme (ACE) using HPLC. The results showed that the isolates **(2, 3, 4, 5, 7)** had moderate inhibitory effects on ACE *in vitro* compared with captopril.

Key words:

Leaves of *Eucommia ulmoides* Oliver, Megastigmane glycosides, Eucomegastigsides, Angiotensin Converting Enzyme (ACE)

1. Introduction

Eucommia ulmoides Oliver is one of the oldest known and used tonic herbs in Asia. The medicinal parts of *E. ulmoides* are the bark and leaf as mentioned in the Pharmacopoeia of the People's Republic of China (2015) [1]. Leaves of *E. ulmoides* have been reported to possess anti-hypertensive [2-5] property *in vitro* and *in vivo*. In particular, the glycosidic ingredients play an important role in the anti-hypertensive effect. Previous studies have found many bioactive components in the leaves of *E. ulmoides*, including iridoids [6], phenylpropanoids [7] and flavonoids [8]. However, only few megastigmanes have been obtained from the leaves of *E. ulmoides* [7, 9].

Angiotensin Converting Enzyme (ACE) is the key enzyme that regulates the renin-angiotensin system (RAAS) which is a hormone system that is involved in the regulation of plasma sodium concentration and arterial blood pressure [10]. Inhibitors of ACE are the first line of therapy for hypertension. However, although all these drugs produce side effects such as digestive tract symptoms, dry cough or allergic phenomena, no relevant adverse effects were observed in the experimental group who took the extract of the leaves of *E. ulmoides* [5].

In this study, we explored the isolation and structure elucidation of four new megastigmane glycosides **2**, **3**, **5** and **7**, together with three known megastigmane glycosides, (6*R*, 7*E*, 9*R*)-9-hydroxy-4, 7-megastigmadien-3-one-9-*O*-[α -L-arabinopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside (**1**) [11], foliasalacioside B₁ (**5**) [12] and eleganoside A (**6**) [13] (Fig. 1), from the leaves of *E. ulmoides*. In addition, the *in vitro* ACE inhibitory activities of these compounds were measured by HPLC assay.

2. Experimental

2.1. General

Optical rotations were recorded on a Rudolph (Hackettstown, NJ) Autopol V automatic polarimeter, and CD spectra were measured on a JASCO J-815 spectrophotometer using MeOH as solvent. UV spectra were recorded on a UNICO UV-2102PCS spectrophotometer. IR spectra were obtained on a Perkin Elmer (Boston, MA) Spectrum 65 FT-IR Spectrometer. NMR spectra were carried out on a Bruker (Billerica, MA) AM-400/600 spectrometer at 25 °C. High-Resolution ESIMS (HRESIMS) was performed on a Waters (Milford, MA) Xevo G2-S UPLC-Q/TOF. A Waters e2695 equipped with a 2998 PDA detector and RP C₁₈ reversed-phase silica gel (4.6×250 mm, ODS-3 5 μ m, Japan) was used for High Performance Liquid Chromatography (HPLC). A Waters 2535 preparative High Performance Liquid Chromatography (pHPLC) equipped with RP C₁₈ reversed-phase silica gel (20×250 mm, ODS-3 5 μ m, Japan) and semi-preparative

High Performance Liquid Chromatography (semi-pHPLC) equipped with A chiral column (Daicel Chiralpak IE, 4.6×250 mm, Japan) were used for separation. Jasco LC Net II/ADC with a Jasco OR-4090 detector and Shodex Asahipak NH₂P-50 4E column was used for the analysis of the sugars. Silica gel (200-400 mesh, Qingdao Haiyang Chemical, China), C₁₈ reversed-phase silica gel (12 nm, S-50 μm, YMC, Wilmington, NC), Sephadex LH-20 gel (GE Healthcare (USA) Co. Ltd), were used for column chromatography.

ACE from rabbit lung, hippuryl-histidyl-leucine (HHL), hippuric acid (HA), captopril, orthoboric acid (H₃BO₃), were purchased from Sigma-Aldrich (Bornem, Belgium; St. Louis, MO). NaOH, NaCl, trifluoroacetic acid (FA) and other solvents used were of analytical grade (Concord Technology (Tianjin) Co. Ltd).

2.2. Plant materials

Leaves of *Eucommia ulmoides* Oliver were purchased from Anguo in Hebei Province, P.R. China in August 2014, and were authenticated by Prof. Lijuan Zhang of Tianjin University of Traditional Chinese Medicine. A voucher sample was deposited at the School of Chinese Materia Medica, Tianjin University of Traditional Chinese Medicine.

2.3. Extraction and isolation

The air-dried leaves of *Eucommia ulmoides* Oliver (9.5 kg) were extracted with 80% EtOH/H₂O (3×100 L) after 2-hour reflux to obtain 700 g of concentrated extract. The extract was suspended in water (5 L) and partitioned successively with CHCl₃ (4 × 1 L), EtOAc (4 × 1 L), and *n*-BuOH (4 × 1 L) to give the corresponding extracts.

The *n*-BuOH extract (180 g) was subjected to silica gel column (CH₂Cl₂-MeOH, 10:0 → 0:10) to yield eight fractions, B1-B8. Fraction B8 (24.0 g) was chromatographed over reversed-phase C18 (RP-C₁₈) silica gel CC eluted with MeOH-H₂O (0:10 → 10:0) to afford nineteen subfractions (B811-B815, B821-B825, B831-B835, B841-B844). B832 and B833 were separated by Sephadex LH-20 (MeOH) to give seven fractions (B8321-B8327) and nine fractions (B8331-B8339) respectively. B8321 was separated by preparative HPLC equipped with a semi-preparative column (MeOH-H₂O, 38:62, 6 mL/min) to obtain three fractions (B83211-B83213), with B83211 and B83213 as compounds **1** (360.0 mg) and **2** (120.0 mg), respectively. B83212 was further subjected to HPLC (MeCN-H₂O, 18:82, 6 mL/min) to give compound **3** (5.4 mg). B8331 was separated by preparative HPLC (MeOH-H₂O, 38:62, 6 mL/min) to give five fractions (B83311-B83315). B83315 was further separated by preparative HPLC (MeCN-H₂O, 18:82, 6 mL/min) to obtain compounds **4** (120.0 mg) and **5** (41.0 mg). B834 was separated by Sephadex LH-20 (MeOH) to give

six fractions (B8341-B8346). B8341 was separated by preparative HPLC (MeOH-H₂O, 35:65, 6 mL/min) to give eight fractions (B83411-B83418). B83415 was compound **6** (20.0 mg). B83416 (23.0 mg) was further separated by preparative HPLC (MeCN-H₂O, 18:82, 6 mL/min) to obtain compound **7** (7.1 mg).

Compounds **2**, **3**, **5** and **7** were identified as new compounds. The known compounds, by comparing with the published literature, were identified as (6*R*, 7*E*, 9*R*)-9-hydroxy-4, 7-megastigmadien-3-one 9-*O*-[α -L-arabinopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside (**1**), foliasalacioside B₁ (**4**) and eleganoside A (**6**). The structures of compounds **1–7** were shown in Fig. 1, and the ¹H NMR and ¹³C NMR data of **2**, **3**, **5** and **7** were listed in Table 1.

2.3.1. *Eucomegastigsides A (2)*

Pale yellow gum; [α]_D²⁵ + 37 (*c* 0.19, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 271 (0.122) nm, 236 (0.463) nm; ECD (*c* 3.18 $\times$ 10⁻³M, MeOH) $\lambda_{\max}$ ($\Delta\epsilon$) 261 (+74.52), 317 (-12.08); IR (KBr) $\nu_{\max}$ 2938, 1647, 1384, 1259 and 1053 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; positive HRESIMS *m/z* 503.2486 [M + H]⁺ (calcd. for C₂₄H₃₈O₁₁, 503.2492).

2.3.2. *Eucomegastigsides B (3)*

Pale yellow gum; [α]_D²⁵ - 90 (*c* 0.04, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 267 (0.116) nm, 234 (0.163) nm; ECD (*c* 1.47 $\times$ 10⁻²M, MeOH) $\lambda_{\max}$ ($\Delta\epsilon$) 251 (-42.40), 311 (+3.56); IR (KBr) $\nu_{\max}$ 2981, 2966, 1655, 1384, 1052 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; positive HRESIMS *m/z* 503.2492 [M + H]⁺ (calcd. for C₂₄H₃₈O₁₁, 503.2492).

2.3.3. *Eucomegastigsides C (5)*

Pale yellow gum; [α]_D²⁵ + 6 (*c* 0.10, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 272 (0.100) nm, 238 (0.626) nm; ECD (*c* 8.09 $\times$ 10⁻³M, MeOH) $\lambda_{\max}$ ($\Delta\epsilon$) 213 (+5.85), 233 (-10.10), 331 (+4.88); IR (KBr) $\nu_{\max}$ 2931, 1651, 1415, 1384, 1258 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; positive HRESIMS *m/z* 527.2463 [M + Na]⁺ (calcd. for C₂₄H₄₀O₁₁Na, 527.2468).

2.3.4. *Eucomegastigsides D (7)*

Pale yellow gum; [α]_D²⁵ - 9 (*c* 0.05, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 273 (0.078) nm, 239 (0.226) nm; IR (KBr) $\nu_{\max}$ 2966, 1641, 1384, 1033 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; positive HRESIMS *m/z* 527.2466 [M + Na]⁺ (calcd. for C₂₄H₄₀O₁₁Na, 527.2468).

2.4. Acid hydrolysis of compound **2,3, 5**, and **7**

A solution of **2**, **3**, **5**, **7** (1.0 mg, respectively) in 2 M HCl (5.0 mL) was heated for 5 h. The reaction mixture was neutralized with Amberlite IR-120, then the mixture was extracted with CH₂Cl₂. The aqueous layer was subjected to HPLC analysis with OR detector using the following conditions: Mobile phase: MeCN-H₂O (75 : 25), flow rate: 1.0 mL•min⁻¹, *t_R* = 6.43 min. (L-arabinose, $[\alpha]^{20}_D +29$), *t_R* = 8.61 min (D-glucose, $[\alpha]^{20}_D +27$). The sugars showed the retention times to be identical with those of authentic L-arabinose and D-glucose.

2.5. ACE Inhibitory Activity Assay

ACE activity was determined by HPLC assay according to the method described [14, 15]. In general, H₃BO₃ (6.18 g) and NaCl (17.53 g) were dissolved in Ultra Pure Water (which was adjusted by NaOH to pH 8.3) to 1000 mL (SBB). HHL (21.5 mg), HA (17.9 mg), captopril (50 mg) and ACE (1U) were dissolved in a 10 mL volumetric flask of the above-mentioned borate solution, respectively. All compounds were prepared by dissolving each of them in 2 mg/mL SBB, respectively. The experimental procedure is described in Table 2. Captopril was used as a positive control. Triplicate determinations were carried out for each compound. Samples (20 µL) were analyzed on a Waters e2695 equipped with a 2998 PDA detector and RP C₁₈ reversed-phase silica gel (4.6 × 250 mm, ODS-3 5 µm, Japan), and HA was detected on 227 nm. Acetonitrile and water (containing 0.01% TFA) (25:75, 1 mL/min) were used as the mobile phases A and B, respectively. The inhibitory activities of compounds were evaluated by determining ACE inhibition ratios. ACE inhibition ratio was obtained using the equation:

$$IR = (S1 - S2) / (S1 - S) \times 100\%$$

Where S1 is the mean peak area of HA in control group, S is the mean peak area of HA in blank group, and S2 is the mean peak area of HA in the sample

3. Results and discussion

3.1. Chemistry

Compound **2** was obtained as a pale yellow gum. The molecular formula C₂₄H₃₈O₁₁ was determined by HRESIMS with the [M + H]⁺ peak at *m/z* 503.2486 (calcd. for C₂₄H₃₉O₁₁, 503.2492). Its IR absorption bands at 2938, 1647, 1647 and 1260 cm⁻¹ indicated the presence of α, β-unsaturated carbonyl and olefin groups. The ¹H-NMR and ¹³C-NMR data of **2** (Table 1) indicated a glycosylated megastigmane skeleton. The ¹H-NMR spectrum displayed two singlet methyl proton signals at δ_H 1.03 (3H, s) and 0.99 (3H, s), a doublet methyl proton signal at δ_H 1.29 (3H, d, *J* = 6.4 Hz), a vinyl methyl proton signal at δ_H 1.98 (3H, d, *J* = 1.1

Hz), and three olefinic proton signals at δ_{H} 5.74 (1H, s), 5.77 (1H, dd, $J = 15.3, 9.4$ Hz), 5.60 (1H, dd, $J = 15.3, 7.3$ Hz) and two doublet signals at δ_{H} 4.29 (1H, d, $J = 7.8$ Hz) and 4.32 (1H, d, $J = 6.7$ Hz) which were assigned as two anomeric protons of sugar moieties. Comparing the ^{13}C -NMR data of **2** with the known compound **1** showed that they are similar to each other. The main differences were that the ^{13}C -NMR signals at δ_{C} 138.2 (C-8), 77.1 (C-9), 21.2 (C-10) and 102.6 (C-1') in **1** shifted to 137.1 (C-8), 74.9 (C-9), 22.4 (C-10) and 101.5 (C-1') in **2**, suggesting that the absolute configuration at C-9 in **2** was different from that in **1**. The chemical shift value of C-9 was important for deducing the configuration of C-9 in $\Delta^{7,8}$ -type of 9-hydroxymegastigmane 9-*O*- β -D-glucopyranoside in Methanol- d_4 . For the 9*S*, the chemical shift appears at around δ_{C} (74 ~ 76), and in the 9*R*, the carbon resonates near δ_{C} (77 ~ 79) [16]. The absolute configuration at C-9 in **2** was confirmed to be (*S*) based on chemical shift at C-9 δ_{C} (74.9). The absolute configuration at C-6 in **2** was further confirmed as (*R*) by CD spectrum, which showed positive Cotton effects at 261 nm ($\Delta\epsilon$ +74.52) and negative Cotton effects at 317 nm ($\Delta\epsilon$ -12.08) [12]. As shown in (Fig. 2), in the HMBC spectrum, some key long-range correlations were observed between 0.99 (11-CH₃) and C-2, C-3, C-6 and C-12, as well as 4.29 (1'-CH) and C-9, and between 4.32 (1''-CH) and C-6'. The positions of the glycoside linkages in **2** were determined unambiguously by HMBC experiment. The acid hydrolysis of **2** liberated D-glucose and L-arabinose, which were identified by HPLC analysis using an optical rotation detector by comparison of the retention times with the authentic samples [12]. The glucose and the arabinose moieties were deduced to be β and α configuration respectively by comparing coupling constants and chemical shift of signals of sugar moieties with those reported [11-13]. On the basis of the above analysis, compound **2** was elucidated as (6*R*, 7*E*, 9*S*)-9-hydroxy-4,7-megastigmadien-3-one-9-*O*-[α -L-arabinopyranosyl-(1 $\rightarrow$ 6)]- β -D-glucopyranoside. It was a new compound and named as **eucomegastigside A**.

Compound **3** was obtained as a pale yellow gum. Its molecular formula C₂₄H₃₈O₁₁ was determined by HRESIMS with the $[\text{M} + \text{H}]^+$ peak at m/z 503.24892 (calcd. for C₂₄H₃₉O₁₁, 503.2492). Comparing the proton and carbon signals of **3** with compound **1** showed that they are almost the same with little differences. The CD spectrum suggested that the absolute configuration at C-6 in **3** was different from that in **1**. The absolute configuration at C-6 in **3** was further confirmed as (*S*) by CD spectrum, which showed negative Cotton effects at 251 nm ($\Delta\epsilon$ -42.40) and positive Cotton effects at 311 nm ($\Delta\epsilon$ +3.56) [12]. Compound **3** was elucidated as (6*S*, 7*E*, 9*R*)-9-hydroxy-4,7-megastigmadien-3-one-9-*O*-[α -L-arabinopyranosyl-(1 $\rightarrow$ 6)]- β -D-glucopyranoside. It was a new compound and named as **eucomegastigside B**.

Compound **5** was obtained as a pale yellow gum. The molecular formula $C_{24}H_{40}O_{11}$ was determined by HRESIMS with the $[M + Na]^+$ peak at m/z 527.2463 (calcd. for $C_{24}H_{40}O_{11}Na$, 527.2468). Its IR spectrum showed absorption bands at 2930, 1651, 1415 and 1259 cm^{-1} assignable to α , β -unsaturated carbonyl and olefin groups. The 1H and ^{13}C -NMR data of **5** (Table 1) indicated a glycosylated megastigmane skeleton. The 1H -NMR spectrum displayed two singlet methyl proton signals at δ_H 1.03 (3H, s) and 0.95 (3H, s), a doublet methyl proton signal at δ_H 1.18 (3H, d, $J = 6.2\text{ Hz}$), a vinyl methyl proton signal at δ_H 1.99 (3H, s), and an olefinic proton signal at δ_H 5.74 (1H, s). The 1H and ^{13}C NMR data of **5** were very similar to those of **4**, with the differences being raised from the chiral centers C-9, indicating the absolute configuration at C-9 in **5** different from that in **4**. Based on the empirical rule and CD spectrum of **5**, the absolute configurations of C-6 and C-9 were confirmed as 6*R*,9*S*. On the basis of 2D-NMR spectra and above-mentioned evidence, the structure of **5** was characterized as shown (Fig. 1) and named **eucomegastigside C**.

Compound **7** was obtained as a pale yellow gum. The molecular formula $C_{24}H_{40}O_{11}$ was determined by HRESIMS with the $[M + Na]^+$ peak at m/z 527.2466 (calcd. for $C_{24}H_{40}O_{11}Na$, 527.2468). Its IR absorption bands at 2966, 1641 and 1384 cm^{-1} indicated the presence of α , β -unsaturated carbonyl. The 1H and ^{13}C -NMR data of **7** (Table 1) indicated a glycosylated megastigmane skeleton. The 1H -NMR spectrum displayed two singlet methyl proton signals at δ_H 1.13 (6H, s), a doublet methyl proton signal at δ_H 1.22 (3H, d, $J = 6.1\text{ Hz}$), a vinyl methyl proton signal at δ_H 1.70 (3H, s), and two doublet signals at δ_H 4.30 (1H, d, $J = 7.8\text{ Hz}$) and 4.26 (1H, d, $J = 6.6\text{ Hz}$) which were assigned as two anomeric protons of sugar moieties. Comparing the ^{13}C -NMR data of **7** with **5** showed that **7** was consistent with **5** in the saccharide part. The ^{13}C -NMR data of the aglycone part of **7** were similar with those of Foliasalaciosides **D** [12]. The main differences were that the carbon signals at δ_C 37.3 (C-8), 76.0 (C-9) and 20.0 (C-10) in Foliasalaciosides **D** shifted to δ_C 36.6 (C-8), 77.9 (C-9) and 22.1 (C-10) in **7**, suggesting that the absolute configuration at C-9 in **7** was different from that in Foliasalaciosides **D**. It is reported that the chemical shift at C-9 is indicative for 9*S* δ_C (77.7 ~ 78.1) and 9*R* δ_C (75.7 ~ 76.8) configuration for 9-hydroxymegastigmane 9-*O*- β -D-glucopyranoside in Methanol- d_4 [16]. The absolute configuration at C-9 in **7** was also confirmed to be (*S*) based on chemical shift at C-9 δ_C (77.9). The connection of glucosyl to C-9 and arabinosyl to glucosyl were determined unambiguously from key long-range correlations in HMBC experiment in (Fig. 2). Finally, the structure of **7** was characterized as shown (Fig. 1) and named **eucomegastigside D**.

3.2. ACE Inhibitory Activity

All the compounds (**1-7**) and control drug coptopril were evaluated for their ACE inhibitory activities using HPLC method. The inhibitory effects of **1-7** were computed using percentage inhibition ratio (%). All

isolates showed either moderate or weak inhibition ratios. Compounds **2**, **3**, **4**, **5**, **7** with inhibition ratios of $24.6 \pm 0.5 \%$, $29.1 \pm 0.6 \%$, $31.6 \pm 0.6 \%$, $31.2 \pm 0.2 \%$ and $29.7 \pm 0.4 \%$ respectively at the concentration of $240 \mu\text{g/mL}$ showed moderate activities compared with captopril ($98.0 \pm 0.1 \%$ at $240 \mu\text{g/mL}$).

4. Conclusion

Four new megastigmane glycosides, eucomegastigside A-D (**2**, **3**, **5** and **7**), together with three known megastigmane glycosides, (6*R*, 7*E*, 9*R*)-9-hydroxy-4,7-megastigmadien-3-one-9-*O*-[α -L-arabinopyranosyl-(1 $\rightarrow$ 6)]- β -D-glucopyranoside (**1**), foliasalacioside B₁ (**4**) and eleganoside A (**6**), were isolated from the leaves of *Eucommia ulmoides* Oliver. Their structures were elucidated by extensive spectroscopic examinations. All the isolates were evaluated for their ACE inhibitory activities using HPLC assays. Some of the isolates (**2**, **3**, **4**, **5**, **7**) showed moderate activities on ACE *in vitro*.

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Appendix A. Supplementary Data

The NMR, HRESIMS and ECD data of compounds (**1-7**) are available in the Supplementary data. Supplementary data associated with this article can be found in the online version.

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Table 1. ¹H-NMR and ¹³C-NMR data for compounds **2** (MeOH-*d*₄, 600/150 MHz) and **3**, **5** and **7** (MeOH-*d*₄, 400/100 MHz)

Position	2		3		5		7	
	δ_C	δ_H (mult, <i>J</i> in Hz)	δ_C	δ_H (mult, <i>J</i> in Hz)	δ_C	δ_H (mult, <i>J</i> in Hz)	δ_C	δ_H (mult, <i>J</i> in Hz)
1	37.2		37.3		37.5		37.8	
2	48.5	2.50 (1H, d, 16.8); 2.06 (1H, d, 16.7)	48.6	2.41 (1H, d, 16.8); 2.05 (1H, d, 16.6)	48.2	2.42 (1H, d, 17.4); 1.92 (1H, d, 17.4)	38.5	1.74 (2H, dd, 11.8, 5.1)
3	202.1		202.2		202.4		35.2	2.37 (2H, t, 6.8)
4	126.3	5.90 (1H, s)	126.2	5.87 (1H, s)	125.5	5.74 (1H, s)	201.6	
5	165.7		166.2		170.0		131.8	
6	57.0	2.69 (1H, d, 9.4)	56.9	2.66 (1H, d, 9.2)	52.6	1.91 (1H, m)	168.6	
7	131.1	5.77 (1H, dd, 15.3, 9.4)	129.1	5.73 (1H, dd, 15.3, 6.4)	26.8	1.66-1.54 (1H, m); 1.75-1.71 (1H, m)	27.7	2.29-2.17 (1H, m); 2.54-2.43 (1H, m)
8	137.1	5.60 (1H, dd, 15.3, 7.3)	138.3	5.62 (1H, dd, 15.3, 9.3)	37.6	1.66-1.54 (2H, m)	36.6	1.64-1.55 (2H, m)
9	74.9	4.45 (1H, m)	77.1	4.43-4.36 (1H, m)	77.9	3.81-3.74 (1H, m)	77.9	3.75-3.71 (1H, m)
10	22.4	1.29 (3H, d, 6.4)	21.2	1.28 (3H, d, 6.3)	22.2	1.18 (3H, d, 6.2)	22.1	1.22 (3H, d, 6.1)
11	27.6	0.99 (3H, s)	27.6	0.96 (3H, s)	27.6	1.03 (3H, s)	27.3	1.13 (3H, s)
12	28.1	1.03 (3H, s)	28.1	1.01 (3H, s)	29.1	0.95 (3H, s)	27.3	1.13 (3H, s)
13	24.0	1.98 (3H, d, 1.1)	24.1	1.95 (3H, s)	25.1	1.99 (3H, s)	11.9	1.70 (3H, s)
1'	101.5	4.29 (1H, d, 7.8)	102.8	4.33 (1H, d, 7.9)	104.2	4.26 (1H, d, 7.3)	104.4	4.30 (1H, d, 7.8)
2'	75.1	3.23-3.18 (1H, m)	75.3	3.16 (1H, t, 8.0)	75.3	3.11-3.07 (1H, m)	75.4	3.15-3.09 (1H, m)
3'	78.2	3.28 (1H, t, 8.9)	78.0	3.37-3.32 (1H, m)	78.1	3.28 (1H, m)	78.3	3.29-3.26 (1H, m)
4'	71.7	3.33 (1H, t, 3.4)	71.6	3.37-3.32 (1H, m)	72.4	3.55-3.51 (1H, m)	71.7	3.29-3.26 (1H, m)
5'	77.0	3.33 (1H, t, 3.4)	76.9	3.37-3.32 (1H, m)	76.8	3.36 (1H, br s)	76.9	3.36 (1H, d, 5.8)
6'	69.6	4.07 (1H, dd, 11.4, 1.6); 3.72 (1H, dd, 11.4, 5.3)	69.6	4.02 (1H, d, 10.8); 3.68 (1H, d, 11.3)	69.6	4.01 (1H, d, 11.0), 3.66 (1H, dd, 11.0, 5.3)	69.6	4.01 (1H, d, 9.6); 3.66 (1H, dd, 11.4, 5.5)
1''	105.3	4.32 (1H, d, 6.7)	105.3	4.25 (1H, d, 6.8)	105.2	4.26 (1H, d, 7.3)	105.2	4.26 (1H, d, 6.6)
2''	74.3	3.52-3.51 (1H, m)	74.3	3.53-3.47 (1H, m)	71.7	3.28 (1H, m)	72.4	3.52 (1H, t, 7.6)
3''	72.4	3.58 (1H, dd, 8.7, 6.8)	72.5	3.59-3.54 (1H, m)	74.2	3.48-3.45 (1H, m)	74.3	3.46 (1H, m)
4''	69.5	3.81 (1H, dd, 4.9, 3.2)	69.5	3.78 (1H, m)	69.5	3.76 (1H, s)	69.5	3.75-3.71 (1H, m)

5"	66.8	3.85 (1H, dd, 12.4, 3.3); 3.54 (1H, m)	66.8	3.84 (1H, dd, 12.3, 3.1); 3.53-3.47 (1H, m)	66.7	3.81-3.74 (1H, m); 3.48-3.45 (1H, m)	66.7	3.46 (1H, m); 3.87-3.75 (1H, m)
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Table 2. The experimental procedure of ACE inhibitory activity

	Sample (μL)	Control (μL)	Blank (μL)
SBB	350-(X+10)	340	350
ACE	10	10	0
ACEI	X (37 °C water-bath for 10 min)	0	0
HHL	50 (37 °C water-bath for 40 min)	50	50
1M HCl	600 (10000 g/min centrifugation for 10 min)	600	600

X=30, 60, 120 μL

fig 1

2

3

5

7

fig 2

Graphical Abstract