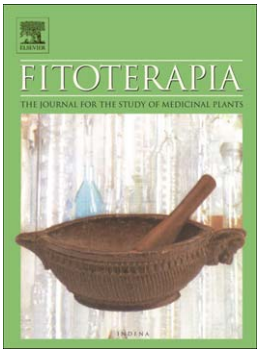

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Isolation, modification, and aldose reductase inhibitory activity of rosmarinic acid derivatives from the roots of *Salvia grandifolia*

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Abbreviated running title: **Aldose reductase inhibitors from *S. grandifolia***

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ABSTRACT

To find aldose reductase inhibitors, two previously unreported compounds, grandifolias H and I, and five known compounds, including rosmarinic acid and rosmarinic acid derivatives, were isolated from the roots of *Salvia grandifolia.* A series of rosmarinic acid derivatives was obtained from rosmarinic acid using simple synthetic methods. The aldose reductase inhibitory activity of the isolated and synthesized compounds was assessed. Seven of the tested compounds showed moderate aldose reductase inhibition (IC_{50} = 0.06–0.30 μM). The structure-activity relationship of aldose reductase inhibitory activity of rosmarinic acid derivatives was discussed for the first time. This study provided useful information that will facilitate the development of aldose reductase inhibitors.

Keywords

Salvia grandifolia; isolation; modification; grandifolias H and I; rosmarinic acid derivative; aldose reductase inhibition

1. Introduction

Diabetes is a chronic disease characterized by an impaired response to insulin and/or progressively reduced function of pancreatic β-cells [1]. All forms of diabetes are characterized by chronic hyperglycemia and the development of diabetes-specific microvascular pathology in the retina, renal glomerulus, and peripheral nerves, in addition to other tissues. Diabetes is also associated with accelerated atherosclerotic macrovascular disease, which affects arteries that supply the heart, brain, and lower extremities [2,3]. Diabetes and its complications are a major cause of morbidity and mortality worldwide and currently affect almost 400 million people. The number of patients afflicted with type 2 diabetes is increasing rapidly in developed and developing countries [1,4].

Aldose reductase is a central enzyme in the polyol pathway that belongs to the aldo-keto reductase superfamily and is implicated in aberrant glucose metabolism and diabetic complications [4-6]. Several studies have demonstrated the important role of aldose reductase in accelerating atherosclerosis and vascular injury associated with diabetes and aging. In hyperglycaemic conditions, aldose reductase catalyses NADPH-dependent reduction of glucose to sorbitol, which in turn is oxidized to fructose by an NAD^+-dependent sorbitol dehydrogenase. Once sorbitol is accumulated inside the cells, it can not diffuse easily across the cell membrane as a result osmotic pressure increases causing cellular damage. Aldose reductase inhibitors have been shown to reduce tissue sorbitol accumulation in diabetic animals and there are evidences that blockage of aldose reductase can have beneficial effect in diabetic

complications [7-8]. Up to now, various small molecule aldose reductase inhibitors have been evaluated in preclinical and clinical trials [9-12]. However, epalrestat is the only commercially available aldose reductase inhibitor in Japan in 2015 [13]. Hence, there is an urgent need for new and effective aldose reductase inhibitors.

Salvia, the largest genus in the family Lamiaceae, comprises over 900 species that are distributed globally [14-16]. The genus *Salvia* has been assessed in many studies because it is a rich source of polyphenol compounds, of which more than 160 have been isolated from plants in the genus, some of which are unique to *Salvia* [15]. The majority of polyphenolics isolated from *Salvia* probably originate from condensation events on caffeic acid, their common building block [17,18]. The roots of *Salvia grandifolia*, a plant endemic to China, have been used to treat cardiovascular diseases in Sichuan and Yunnan Provinces of China for centuries.

Rosmarinic acid is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid that is found in a variety of plants, but it is especially abundant in the subfamily Nepetoideae of the Lamiaceae [19]. Rosmarinic acid has been identified in more than 77 species of Lamiaceae at a concentration ranging from 0.01 to 9.30 mg rosmarinic acid per gram of plant material [20,21]. Rosmarinic acid has several biological activities with potential therapeutic applications, including antiviral, antibacterial, anti-inflammatory, antioxidant, and aldose reductase inhibitory effects [22-24].

To our knowledge, there have been no reports on water soluble polyphenol compounds in *S. grandifolia*. We found that the EtOAc extract of *S. grandifolia* was rich in rosmarinic acid and showed good aldose reductase inhibition (65% at 10

μg/mL), which inspired us to further study hydrosoluble polyphenols in the extract, especially rosmarinic acid derivatives. According to the functional groups of rosmarinic acid, semi-synthetic compounds were prepared. All of the rosmarinic acid derivatives both from nature and synthesis were investigated the relationship between the structures and aldose reductase inhibitory effects.

2. Experimental part

2.1. General

Optical rotation was measured with a Jasco P-2000 polarimeter (Tokyo, Japan). UV spectra were collected in MeOH on a Jasco V-650 spectrophotometer (Tokyo, Japan). IR spectra were recorded on a Nicolet 5700 spectrometer (Madison, WI, USA) by the FT-IR transmission electron microscopy method. ^{1}H and ^{13}C NMR spectra were acquired using a Varian MP-400 spectrometer (Palo Alto, CA, USA) or an INOVA (500 or 600 M) spectrometer (Varian, Palo Alto, CA, USA). HRESIMS were recorded on an Agilent 1200 SL series LC/6520 QTOF spectrometer (Boeblingen, Germany). Column chromatography (CC) purification was performed using silica gel (160–200 mesh, Qingdao Marine Chemical Factory, Qingdao, China), Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden), and C-18 (50 μm, YMC, Kyoto, Japan). CC fractions were analyzed by TLC (silica gel GF254, Qingdao Marine Chemical Factory, Qingdao, China). A μQuant MQX200 microplate

reader (BIO-TEK, USA) was used to assay aldose reductase inhibitory activity. NADPH-Na_4, D,L-glyceraldehyde, and epalrestat were obtained from Sigma-Aldrich (Shanghai) Trading Co., Ltd. (Shanghai, China). All analytical grade solvents used for isolation and separation were obtained from Beijing Chemical Factory (Beijing, China).

2.2. Plant materials

Roots of *Salvia grandifolia* W. W. Smith (Liabiatae) were collected from Yunnan Province, China, in October 2007, and identified by Professor Lin Ma of the Institute of Materia Medica, Chinese Academy of Medical Science and Peking Union Medical College, China. A voucher specimen (ID-21587) was deposited in the Institute of Materia Medica, Chinese Academy of Medical Science and Peking Union Medical College, China.

2.3. Extraction and isolation

Air dried, powdered roots of *S. grandifolia* (13.3 kg) were extracted with EtOH-H_2O (95:5, v/v, ×3, 150 L each time) under reflux for 2 h. Evaporation of the solvent under reduced pressure gave a residue (1.05 kg), which was suspended in H_2O and successively partitioned with $CHCl_3$, EtOAc, and *n*-BuOH, respectively. The EtOAc extract (290 g) was applied to a silica gel column (160–200 mesh, 2.0 kg) and subjected to gradient elution (petroleum ether : acetone = 9:1–1:1, v/v) to yield nine

fractions (A–I). Fraction D (25.4 g) was subjected to C-18 CC (50 μm, 800 g) and eluted in a gradient eluent (MeOH:H_2O = 3:7–9:1, v/v) to give **1** (10 mg), **4** (3.1 g), and **5** (2.9 g). Fraction F (24.3 g) was applied to a C-18 column (50 μm, 800 g) and eluted with an isocratic eluent (MeOH:H_2O = 2:8, v/v) to give **3** (4.2 g) and **6** (11 mg). Fraction H (19.0 g) was applied to a C-18 column (50 μm, 500 g) and eluted with an isocratic eluent (MeOH:H_2O = 6:4, v/v) to give seven subfractions, H1–H7. Subfraction H4 was applied to a Sephadex LH-20 column and eluted with MeOH:H_2O (6:4, v/v) to yield **2** (11 mg) and **7** (12 mg).

2.4. Absolute sugar configuration

The absolute configuration of glucose was determined according to a reported procedure [25]. Compound **2** (2.2 mg) was hydrolyzed with 1 M HCl (1 mL) at 100 °C for 2 h, diluted with H_2O, and extracted with EtOAc (3 × 5 mL). After the H_2O layer was dried under vacuum, the residue was dissolved in pyridine (0.5 mL) containing L-cysteine methyl ester hydrochloride (2 mg) and heated at 60 °C for 2 h. *o*-Tolylisothiocyanate (2 μL) was added and the mixture was heated at 60 °C for 2 h. The reaction mixture was directly analyzed by an Agilent 1260 HPLC with a DAD detector at 254 nm. A Cosmosil 5C18-AR-II HPLC column (5 μm, 150 mm × 4.6 mm, Nacalai Tesque Inc., Nakagyo-ku, Japan) was used at 35 °C. Each sample was eluted in an isocratic step of CH_3CN-H_2O (25:75) containing 50 mM H_3PO_4 with a flow rate of 0.8 mL/min. The HPLC column was washed with MeOH after each injection. The

reaction conditions described above were also used for the analysis of D- and L-glucose. The retention times of D-glucose (12.65 min) and L-glucose (11.62 min) were compared with those of the reaction mixtures. The sugar derivatives from **2** showed a peak at 12.56 min, which coincided with derivatives of D-glucose.

*2.5. HRESIMS, ^{1}H and ^{13}C NMR spectral data of compounds **1**–**2***

*Grandifolia H (**1**)*

Red amorphous powder; $[\alpha]^{25}_{D}$ -119 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 205 (2.83) nm, 219 (2.73) nm, 251 (2.54) nm, 274 (2.60) nm, 339 (2.32) nm; ECD (*c*, 1×10^{-3} M, MeOH) λ_{max} ($\Delta\ \varepsilon$) 330 (-0.02), 367 (+0.03), 435 (+0.02), 501 (-0.04); IR ν_{max} 3432, 2939, 1740, 1670, 1602, 1284; for ^{1}H NMR (MeOD, 500 MHz) and ^{13}C NMR (MeOD, 125 MHz) spectroscopic data, see Tables 1 and 2; HRESIMS (negative ion) *m/z* 653.1659 $[M - H]^-$ (calcd. for $C_{36}H_{29}O_{12}$, 653.1664).

*Grandifolia I (**2**)*

White amorphous powder; $[\alpha]^{25}_{D}$ -30 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 206 (2.34) nm, 228 (2.41) nm, 268 (2.19) nm, 291 (2.36) nm, 318 (2.28) nm; IR ν_{max} 3357, 2974, 1694, 1601, 1516, 1285; for ^{1}H NMR (MeOD, 600 MHz) and ^{13}C NMR (MeOD, 150 MHz) spectroscopic data, see Tables 1 and 2; HRESIMS (negative ion) *m/z* 671.1621 $[M - H]^-$ (calcd. for $C_{32}H_{31}O_{16}$, 671.1618).

2.6. Synthesis

*2.6.1. Synthesis of compounds **8**-**17***

*2.6.1.1. Preparation of compound **8***

A stirred solution of **3** (180 mg, 0.5 mmol) in DMF (5 mL) was added to anhydrous K_2CO_3 (520 mg, 3.75 mmol) followed by methyl iodide (0.24 mL, 3.75 mmol). The reaction mixture was heated to 60 ºC for 2 d, after which methyl iodide (0.24 mL, 3.75 mmol) was added and heating was continued for a further 2 d. The mixture was cooled to room temperature and quenched by the addition of H_2O (30 mL), after which the resulting mixture was extracted with Et_2O (3 × 40 mL) and the combined organic phases were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc:petroleum ether = 2:5) to afford **8** as a colorless oil (160 mg, 74% yield).

*2.6.1.2. Preparation of compounds **12** and **13***

Rosmarinic acid (**3**) (300 mg) was resolved in acetone (5 mL), after which 5% palladium on activated carbon (40 mg) was added to the solution. The reaction mixture was stirred under a hydrogen atmosphere at room temperature for 3 h and filtered through celite, affording **12** as colorless oil (296 mg, 98% yield).

Using compound **8** as a starting material, compound **13** was obtained as a colorless oil (95% yield) by the same procedure.

*2.6.1.3. Preparation of compounds **9–11** and **14–17***

A mixture of rosmarinic acid (**3**) or 7,8-saturated rosmarinic acid (**12**) (1 equiv.) was stirred overnight at room temperature with different amine substituents (1.2 equiv.), N,N-Diisopropylethylamine (DIEA, 2.5 equiv.), and 2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU, 1.2 equiv.) in CH_2Cl_2 and concentrated *in vacuo*. The residue was resolved in EtOAc and washed with H_2O. The organic phase was separated and concentrated *in vacuo* to give a residue, which was purified by silica gel column chromatography (CH_2Cl_2:CH_3OH:CH_3COOH = 45:1:0.02) to afford target compounds (**9–11** and **14–17**, the yields of all amino compounds about 85%-95%).

*2.6.2. HRESIMS, ^1H, and ^{13}C NMR spectral data of compounds **8**–**17***

Compound **8**, HRESIMS (positive ion) *m/z* 453.1525 $[M+Na]^+$ (calcd. for $C_{23}H_{26}O_8Na$, 453.1520). ^1H NMR (400 MHz, DMSO-d_6) δ: 7.62 (1H, d, *J* = 16.0 Hz), 7.36 (1H, d, *J* = 2.0 Hz), 7.25 (1H, dd, *J* =8.0, 2.0 Hz), 6.99 (1H, d, *J* = 8.0 Hz), 6.91 (1H, d, *J* = 2.0 Hz), 6.87 (1H, d, *J* = 8.0 Hz), 6.80 (1H, dd, *J* = 8.0, 2.0 Hz), 6.57 (1H, d, *J* = 16.0 Hz), 5.25 (1H, m), 3.81 (3H, s), 3.80 (3H, s), 3.73 (3H, s), 3.71 (3H, s),

3.67 (3H, s), 3.10 (2H, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 169.9, 165.8, 151.2, 149.0, 148.4, 147.7, 145.9, 128.3, 126.6, 123.3, 121.3, 114.4, 113.0, 111.6, 111.5, 110.4, 72.7, 55.6, 55.5, 55.4, 55.3, 52.0, 36.3.

Compound **9**, HRESIMS (positive ion) *m/z* 450.1543 $[M+H]^+$ (calcd. for $C_{25}H_{24}NO_7$, 450.1547). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.62 (1H, s, OH), 9.15 (1H, s, OH), 8.75 (1H, s, OH), 8.71 (1H, s, OH), 8.57 (1H, br s, NH), 7.46 (1H, d, J = 15.6 Hz), 7.21 (4H, m), 7.00 (3H, m), 6.76 (1H, m), 6.63 (2H, m), 6.51 (1H, m), 6.25 (1H, d, J = 15.6 Hz), 5.15 (1H, br s), 4.29 (2H, br s), 2.90 (2H, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 169.0, 165.8, 148.5, 145.6 (2), 144.9, 143.9, 139.1, 128.2 (2), 127.4, 126.9 (2), 126.6, 125.4, 121.5, 120.1, 116.8, 115.7, 115.3, 114.8, 113.6, 74.3, 41.8, 36.9.

Compound **10**, HRESIMS (positive ion) *m/z* 480.1649 $[M+H]^+$ (calcd. for $C_{26}H_{26}NO_8$, 480.1653). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.63 (1H, s, OH), 9.17 (1H, s, OH), 8.77 (1H, s, OH), 8.72 (1H, s, OH), 8.51 (1H, br s, NH), 7.47 (1H, d, J = 16.0 Hz), 7.08 (4H, m), 6.86 (3H, m), 6.65 (2H, m), 6.50 (1H, d, J = 7.2 Hz), 6.25 (1H, d, J = 16.0 Hz), 5.15 (1H, br s), 4.29 (2H, br s), 3.74 (3H, s), 2.90 (2H, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.9, 165.8, 158.1, 148.5, 145.6 (2), 144.9, 143.9, 131.0, 128.3 (2), 127.4, 125.4, 121.5 (2), 120.0, 116.7, 115.7, 115.3, 114.8, 113.6 (2), 74.2, 55.0, 41.2, 36.9.

Compound **11**, HRESIMS (positive ion) *m/z* 464.1704 $[M+H]^+$ (calcd. for $C_{26}H_{26}NO_7$, 464.1715). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.62 (1H, s, OH), 9.16 (1H, s, OH), 8.75 (1H, s, OH), 8.71 (1H, s, OH), 8.49 (1H, d, *J* = 4.4 Hz, NH), 7.46 (1H, d, *J* = 16.0 Hz), 7.28 (2H, m), 7.22 (3H, m), 7.05 (1H, s), 7.00 (1H, d, *J* = 7.6 Hz), 6.77 (1H, d, *J* = 8.0 Hz), 6.69 (1H, s), 6.62 (1H, d, *J* = 8.0 Hz), 6.49 (1H, d, *J* = 7.6 Hz), 6.25 (1H, d, *J* = 16.0 Hz), 4.94 (1H, m), 4.55 (1H, m), 2.84 (2H, m), 1.36 (3H, d, *J* = 6.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.1, 165.9 148.5, 145.6, 145.5, 144.9, 144.2, 143.9, 128.2 (2), 127.4, 126.5, 125.8 (2), 125.4, 121.5, 120.0, 116.7, 115.7, 115.3, 114.8, 113.6, 74.2, 47.6, 36.9, 22.2.

Compound **12**, HRESIMS (positive ion) *m/z* 385.0893 $[M+Na]^+$ (calcd. for $C_{18}H_{18}O_8Na$, 385.0894). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.88 (1H, br s, COOH), 8.79 (1H, s, OH), 8.70 (2H, s, OH), 8.63 (1H, s, OH), 6.62 (2H, m), 6.56 (2H, m), 6.46 (1H, dd, *J* = 8.0, 1.6 Hz), 6.36 (1H, dd, *J* = 8.0, 1.6 Hz), 4.93 (1H, m), 2.83 (2H, m), 2.62 (2H, m), 2.51 (2H, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.7, 170.6, 145.0, 144.9, 144.0, 143.5, 131.1, 127.0, 120.0, 118.7, 116.6, 115.6, 115.4, 115.3, 72.8, 35.9, 35.4, 29.5.

Compound **13**, HRESIMS (positive ion) *m/z* 455.1681 $[M+Na]^+$ (calcd. for $C_{23}H_{28}O_8Na$, 455.1676). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.86 (2H, m), 6.80 (2H, m), 6.72 (1H, dd, *J* = 8.0, 1.6 Hz), 6.62 (1H, dd, *J* = 8.0, 1.6 Hz), 5.13 (1H, m), 3.73 (3H, s), 3.72 (3H, s), 3.71 (6H, s), 3.64 (3H, s), 3.01 (2H, m), 2.76 (2H, m), 2.63 (2H,

m). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.7, 169.6, 148.6, 148.4, 147.6, 147.2, 132.6,128.1, 121.3, 119.8, 113.0, 112.1, 111.8, 111.6, 72.7, 55.5, 55.4, 55.3 (2), 52.0, 36.1, 35.0, 29.6.

Compound **14**, HRESIMS (positive ion) *m/z* 452.1714 $[M+H]^+$ (calcd. for $C_{25}H_{26}NO_7$, 452.1704). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.78 (1H, s, OH), 8.70 (2H, s, OH), 8.65 (1H, s, OH), 8.46 (1H, m, NH), 7.31 (2H, m), 7.22 (1H, m), 7.11 (2H, d, *J* = 7.2 Hz), 6.60 (4H, m), 6.45 (1H, dd, *J* = 8.0, 2.0 Hz), 6.36 (1H, dd, *J* = 8.0, 2.0 Hz), 5.06 (1H, m), 4.27 (2H, m), 2.85 (2H, m), 2.58 (4H, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.7, 168.8, 145.0, 144.9, 143.9, 143.4, 139.0, 131.1, 128.2 (2), 127.2, 126.9 (2), 126.6, 120.1, 118.7, 116.8, 115.6, 115.4, 115.3, 74.2, 41.7, 36.8, 35.4, 29.5.

Compound **15**, HRESIMS (positive ion) *m/z* 482.1807 $[M+H]^+$ (calcd. for $C_{26}H_{28}NO_8$, 482.1809). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.70 (4H, br s, OH), 8.35 (1H, br s, NH), 7.00 (2H, d, *J* = 8.0 Hz), 6.82 (2H, d, *J* = 8.0 Hz), 6.58 (4H, m), 6.41 (1H, d, *J* = 8.0 Hz), 6.33 (1H, d, *J* = 8.0 Hz), 5.03 (1H, m), 4.16 (2H, m), 3.70 (3H, s), 2.80 (2H, m), 2.54 (4H, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.6, 168.6, 158.1, 145.0, 144.9, 143.9, 143.4, 131.1, 131.0 128.2 (2), 127.2, 120.1, 118.7, 116.8, 115.6, 115.4, 115.3, 113.6 (2), 74.2, 55.0, 41.2, 36.8, 35.5, 29.5.

Compound **16**, HRESIMS (positive ion) *m/z* 466.1864 $[M+H]^+$ (calcd. for $C_{26}H_{28}NO_7$, 466.1860). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.76 (1H, s, OH), 8.71 (1H,

s, OH), 8.70 (1H, s, OH), 8.64 (1H, s, OH), 8.38 (1H, d, *J* = 7.6 Hz, NH), 7.29 (2H, m), 7.20 (3H, m), 6.61 (4H, m), 6.44 (1H, dd, *J* = 8.0, 2.0 Hz), 6.36 (1H, dd, *J* = 8.0, 2.0 Hz), 5.04 (1H, m), 4.91 (1H, m), 2.85 (1H, m), 2.83 (1H, m), 2.60 (2H, m), 2.51 (2H, overlapped), 1.34 (3H, d, *J* = 6.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.6, 167.9, 145.0, 144.9, 144.2, 143.9, 143.4, 131.1, 128.1 (2), 127.2, 126.5, 125.8 (2), 120.0, 118.7, 116.7, 115.6, 115.4, 115.3, 74.3, 47.6, 36.8, 35.5, 29.6, 22.2.

Compound **17**, HRESIMS (positive ion) *m/z* 524.1923 $[M+H]^+$ (calcd. for $C_{28}H_{30}NO_9$, 524.1915). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.73 (1H, s, OH), 8.69 (1H, s, OH), 8.65 (1H, s, OH), 8.61 (1H, s, OH), 8.47 (1H, d, *J* = 8.0 Hz, NH), 7.20 (4H, m), 6.56 (4H, m), 6.33 (3H, m), 4.92 (1H, m), 4.46 (1H, m), 3.62 (3H, s), 3.03 (1H, m), 2.89 (1H, m), 2.65 (6H, m, overlapped). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.6, 171.3, 168.9, 145.0, 144.9, 143.9, 143.4, 137.1, 131.1, 129.1 (2), 128.1 (2), 127.4, 126.5, 119.9, 118.6, 116.6, 115.5, 115.4, 115.3, 73.8, 55.8, 53.3, 36.8, 36.5, 35.4, 29.6.

2.7. Aldose reductase inhibitory assay

Measurements were based on a previously reported method [26]. Crude aldose reductase was prepared from rat lens. Two lenses were homogenized in 1 mL of 100 mM sodium phosphate buffer at pH 6.2. The homogenate was centrifuged at 15000 × *g* for 30 min at 4 °C and the resulting supernatant was used as the source of aldose reductase. The assay mixture contained 10 mM D,L-glyceraldehyde and 0.16 mM NADPH-Na_4 in 1 mL of 50 mM sodium phosphate buffer (pH 6.2), with or without

the test compound and enzyme preparation (rat lens enzyme). Appropriate blanks that contains sodium phosphate buffer and enzyme preparation were employed for corrections. The assay mixture was incubated at 25 °C. After 10 min of incubation, the plate was immediately cooled at -20 °C for 5 min to stop the reaction. The change in the absorbance at 340 nm due to NADPH oxidation was measured in a plate reader. All data are given as the mean ± standard deviations.

3. Results and discussion

3.1. Structural analysis of compounds

The EtOAc fraction of the 95% EtOH extract of the roots of *S. grandifolia* was subjected to column chromatography over silica gel, C-18, and Sephadex LH-20, respectively, with purification using preparative HPLC to yield two new and five known rosmarinic acid derivatives (including rosmarinic acid) (Fig. 1). The known compounds were identified through a comparison of their NMR spectroscopic data with those reported in the literature, and they were totally matched: rosmarinic acid (**3**) [27], methyl rosmarinate (**4**) [28], ethyl rosmarinate (**5**) [28], 3′-*O*-methyl-rosmarinic acid (**6**) [28], salviaflaside (**7**) [27].

Compound **1**, had the molecular formula $C_{36}H_{30}O_{12}$, as established by HRESIMS $[M - H]^-$ *m*/*z* 653.1659, which indicated 22 degrees of unsaturation. The IR spectrum

of **1** indicated the presence of hydroxyl (3432 cm^{-1}), carbonyl (1740, 1670 cm^{-1}), and phenyl ring (1602 cm^{-1}) functionalities. The ^{1}H NMR spectrum showed resonances (Table 1) for two methyl (δ_H 2.19, H_3-17″; 1.43, H_3-18″), three methylene (δ_H 3.13 (2H, t), H-7′; 2.95 (m), 2.60 (m), H-1″; 1.91 (m), 1.84 (m), H-2″), two oxymethine (δ_H 5.23 (t), H-3″; 5.17 (t), H-8′), and 11 methine (δ_H 7.90 (d), H-6″; 7.48 (d), H-7″; 7.26 (d), H-15″; 6.95 (d), H-7; 6.82 (d), H-5′; 6.74 (d), H-2′; 6.65 (dd), H-6′; 6.52 (d), H-2; 6.50 (dd), H-6; 6.48 (d), H-5; 5.61 (d), H-8) groups. The ^{13}C NMR spectrum displayed a total of 36 carbon resonances (Table 2), assignable to four ketocarbonyl (δ_C 184.8, C-11″; 176.7, C-12″; 171.3, C-9; 170.5, C-9′), two methyl (δ_C 30.2, C-18″; 9.7, C-17″), three methylene (δ_C 38.0, C-7′; 25.7, C-2″; 25.5, C-1″), two oxymethine (δ_C 76.9, C-3″; 75.9, C-8′), 11 methine (δ_C 148.3, C-7; 143.9, C-15″; 135.3, C-6″; 124.2, C-6; 123.0, C-6′; 122.5, C-7″; 118.9, C-2′; 117.1, C-5, 5′; 115.3, C-2; 113.4, C-8), and 14 quaternary carbon (δ_C 163.0, C-14″; 150.7, C-4; 147.4, C-3; 147.1, C-5″; 146.9, C-3′; 146.5, C-4′; 144.6, C-10″; 131.1, C-8″; 129.0, C-1′; 127.5, C-1; 126.9, C-9″; 122.5, C-13″; 121.5, C-16″; 73.2, C-4″) groups (Table 2). The ^{1}H and ^{13}C NMR spectra of **1** showed rosmarinic acid and tanshinone parts in its structure. The HMBC correlation of H-3″/C-9′ (Fig. 2) suggested an ester group formed by the hydroxyl group of tanshinone and the carboxyl group of rosmarinic acid. Thus, the planar structure of **1** was established as shown in Fig. 1. NOEs were observed between H-3″(5.23, t, J = 2.0 Hz) and CH_3-18″ (1.43, 3H, s) suggested those protons on the same side of the molecule (Fig. 2), which determined the structure of tanshinone part is the same as that of tanshindiol C [29]. However, NOEs of H-8□ and H-3″ were not

shown. Because the absolute configuration of natural rosmarinic acid is *R* (C-8□), it was concluded that the absolute configuration of C-8□ is *R*. Since the absolute configuration of C-8□ was determined, the structure of compound **1** was symplified to **1-A** and **1-B** (Fig. 3a) to make the calculation of the other chiral carbons easier. Conformational analysis of **1-A** (Fig. 3a) showed four lowest energy conformers whose relative energy within 4 kcal/mol. The conformers were further optimized at the B3LYP/6-31G(d) level. Rotational strengths of the 100 lowest electronic transitions were calculated. ECD spectra of different conformers were simulated with a half-bandwidth of 0.2 eV. The overall theoretical ECD spectra were then obtained according to the Boltzmann weighting of each conformers. The ECD spectrum of **1** was recorded and showed positive (367, 435 nm) and negative (330, 501 nm) Cotton effects, which was similar as the calculated ECD curve of **1-A** (3″*S*, 4″*R*), but was opposite to that of **1-B** (3″*R*, 4″*S*) (Fig. 3a and b). Therefore the absolute configurations of C-8□, 3″ and 4″ were determined to be *R*, *S* and *R*. The structure of **1** (grandifolia H) was established as shown (Fig. 1). This is the first report of an ester compound formed by tanshinone and rosmarinic acid.

Compound **2**, obtained as a white amorphous powder, had the molecular formula $C_{32}H_{32}O_{16}$, as determined by HRESIMS ($[M - H]^-$ *m*/*z* 671.1621), which indicated 17 degrees of unsaturation. The 1D (Tables 1 and 2) and 2D NMR spectroscopic data of **2** were similar to that of known compound **7** [27], except for an extra 3-methoxy-4-hydroxy-benzoyl group connecting the hydroxyl group at C-6″ in **2**, which was further confirmed by the HMBC correlations of H-6″, 2‴, and 6‴/C-7‴, as

well as NOE difference experiments (Fig. 2). Irradiation of the methoxy group at δ 3.76 enhanced hydrogen signal H-2‴ at δ 7.44 (Fig. 2), which was indicative of the methoxy group at C-3‴, but not C-4‴. The coupling constant (J = 6.5 Hz) of anomeric hydrogen (δ 4.95) suggested a β-glucopyranosyl moiety. The identity of D-glucose was confirmed by a reported procedure [25]. Because the absolute configuration of natural rosmarinic acid is *R* (C-8□), and the optical rotation value of **2** is negative, which is similar as that of hedyotoside E [30], so the absolute configuration of C-8□ was determined to be *R*. The structure of **2** (grandifolia I) was established as shown (Fig. 1).

*3.2. Synthesis of compounds **8**–**17***

To determine the aldose reductase inhibitory activity of different substituents of rosmarinic acid derivatives, ten rosmarinic acid derivatives (**8**–**17**) (Fig.1) were synthesized according to the method described in Fig. 4. Among the synthesized rosmarinic acid derivatives, compounds **9**, **10**, and **14**–**17** have not been reported previously. The absolute configurations of C-8□ of rosmarinic acid derivatives (**8**–**17**) are *R*, due to no configurations changed during the synthetic process.

*3.3. Aldose reductase inhibitory activities of compounds **1**–**17***

Aldose reductase is the first enzyme of the polyol pathway, which converts

glucose into sorbitol and is therefore considered to be responsible for diabetic complications [31]. Rosmarinic acid (**3**) inhibits aldose reductase at relatively low concentration [32-34]. Herein, the structure-activity relationship of rosmarinic acid derivatives with regard to aldose reductase inhibition was discussed for the first time.

The aldose reductase inhibitory activities of seven natural (**1**–**7**) and ten modified (**8**–**17**) rosmarinic acid derivatives were evaluated by comparing their effects with that of positive control epalrestat. Seven compounds (**1**, **3**, **4**, **5**, **9**, **10**, and **11**) had IC_{50} values within the range of 0.06–0.30 μM (Fig. 5, Table 3), indicating greater potency than that of natural aldose reductase inhibitor quercetin (IC_{50} = 5.20 μM) [32]. The other ten tested compounds showed very weak aldose reductase inhibitory activities (inhibition rates at a concentration of 10 μM were 5.7–24.2%). Compounds **2** and **7** are rosmarinic acid derivative glycosides with aldose reductase inhibitory rates of 28.6% and 26.9% (10 μM), respectively, suggesting that glycosylation attenuated aldose reductase inhibition. 7,8-Saturated rosmarinic acid derivatives **12**–**17** also showed weak aldose reductase inhibitory rates (9.3%–30.6%) at a concentration of 10 μM, indicating that the double bond of the α,β-unsaturated ketone is the key moiety for aldose reductase inhibition. The aldose reductase inhibitory rates of compounds **6** and **8**, containing phenolic hydroxyl groups with partial (**6**) or total (**8**) methylation, were 46.3% and 18.9%, respectively, at a concentration of 10 μM. These results showed that the degree of methylation of the phenolic hydroxyl groups of rosmarinic acid derivatives is inversely related to aldose reductase inhibitory activity.

The results described above suggest that the presence of a double bond in the

α,β-unsaturated ketone unit of rosmarinic acid derivatives is crucial for aldose reductase inhibition. Glycosylation and methylation of phenolic hydroxyl groups of rosmarinic acid derivatives were inactive.

4. Conclusion

Two new rosmarinic acid derivatives grandifolias H and I (**1**-**2**) were isolated from the roots of *S. grandifolia*, with a series of rosmarinic acid derivatives (**8**-**17**) obtained from rosmarinic acid (**3**). To the best of our knowledge, this is the first report of an ester compound (**1**) formed by tanshinone and rosmarinic acid. Bioactivity experiments showed that seven rosmarinic acid derivatives (**1**, **3**, **4**, **5**, **9**, **10**, and **11**) had moderate concentration-dependent aldose reductase inhibitory effects. The structure-activity relationship of rosmarinic acid derivatives with regard to aldose reductase inhibition was discussed for the first time. This study provided useful information that will facilitate the development of aldose reductase inhibitors. Further studies are warranted to reveal the mechanisms underlying the aldose reductase inhibitory effects of rosmarinic acid derivatives.

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There are no conflicts of interest to declare.

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Figure Legends

Figure 1. The structures of seven compounds (**1**–**7**) isolated from *S. grandifolia* and ten derivatives (**8**–**17**) of rosmarinic acid (**3**).

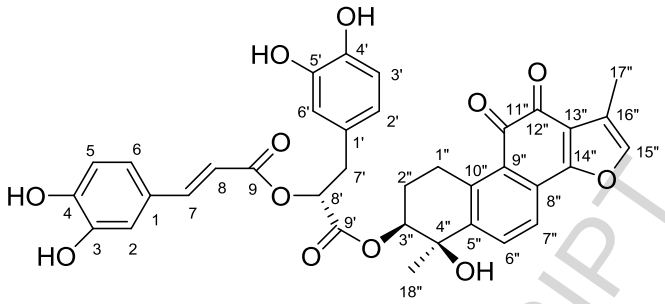
Figure 2. Key HMBC correlations ( ) and NOEs ( ) of compounds **1** and **2**.

Figure 3. Structures of **1-A** and **1-B** (a), experimental ECD spectra of **1** in MeOH and calculated ECD spectra of **1-A** and **1-B** (b).

Figure 4. Synthetic routes of compounds **8**–**17**.

Figure 5. The AR inhibitory values of seven test compounds and positive control epalrestat (A: **1**, **3**, **4**, **5**; B: **9**, **10**, **11**).

Fig. 1



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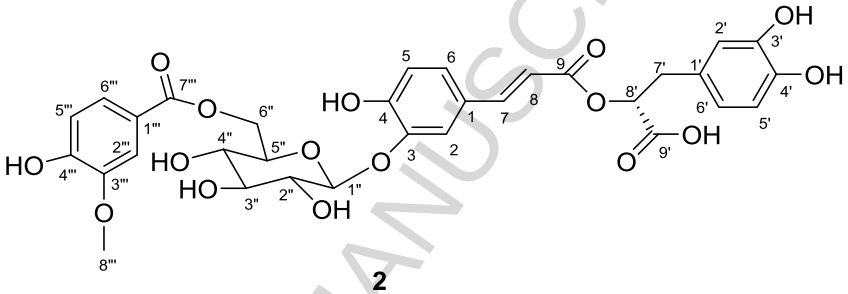

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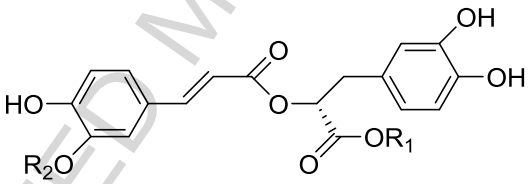
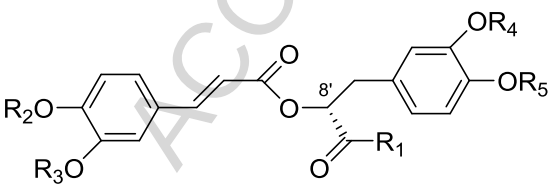
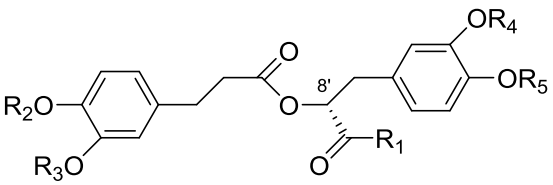


3 R_1=H, R_2=H
4 R_1=CH_3, R_2=H
5 R_1=C_2H_5, R_2=H
6 R_1=H, R_2=CH_3
7 R_1=H, R_2= -glc





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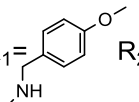
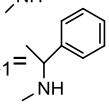
9 R_1=  R_2=R_3=R_4=R_5=H

10 R_1=  R_2=R_3=R_4=R_5=H

11 R_1=  R_2=R_3=R_4=R_5=H

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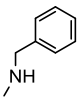
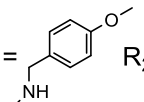
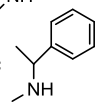
14 R_1=  R_2=R_3=R_4=R_5=H

15 R_1=  R_2=R_3=R_4=R_5=H

16 R_1=  R_2=R_3=R_4=R_5=H

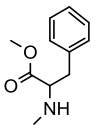
17 R_1=  R_2=R_3=R_4=R_5=H

Fig.2.

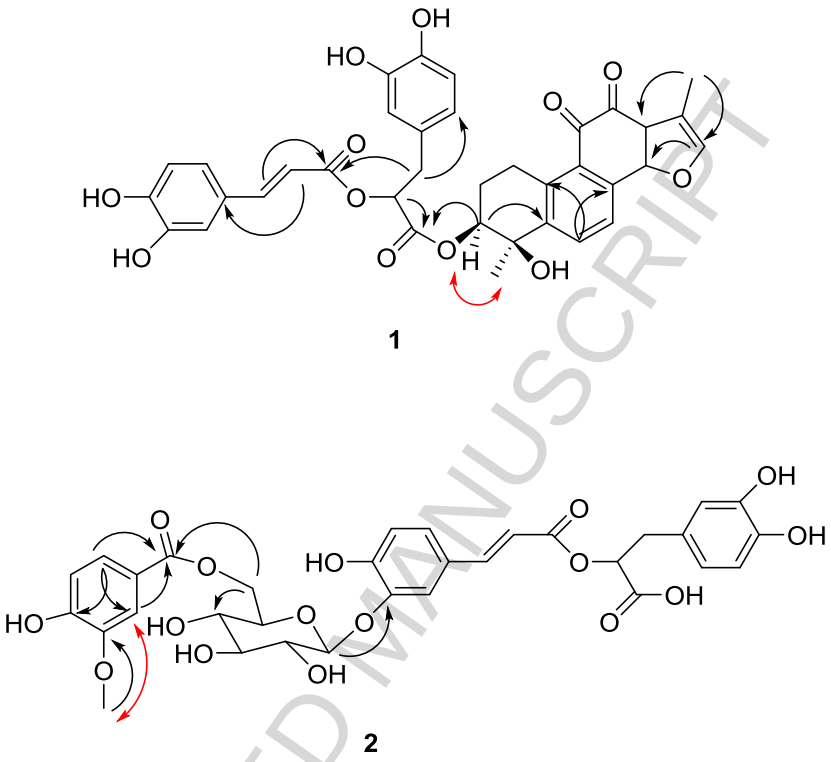
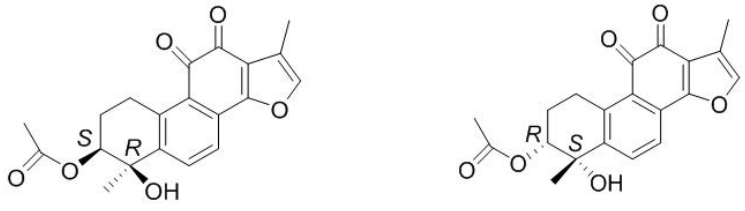
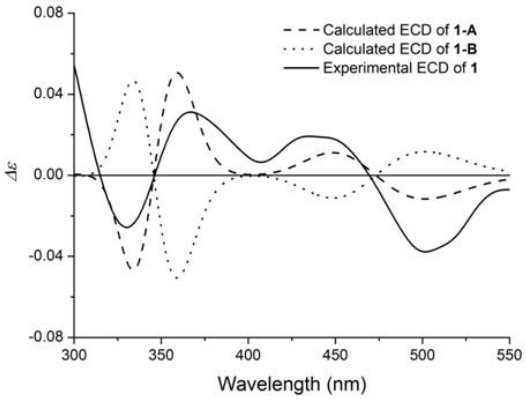


Fig. 3



1-A **1-B**

a



b

Fig.4.

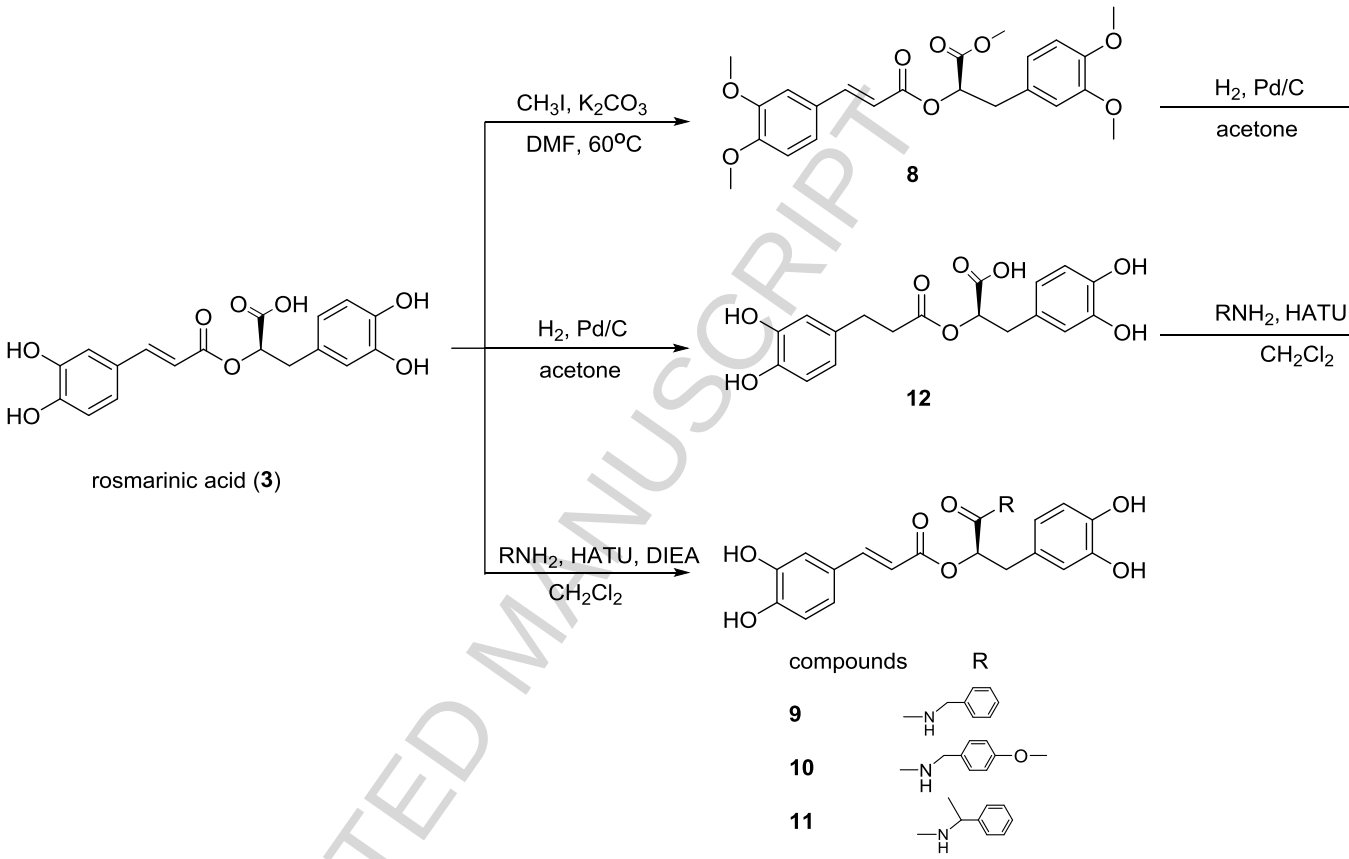
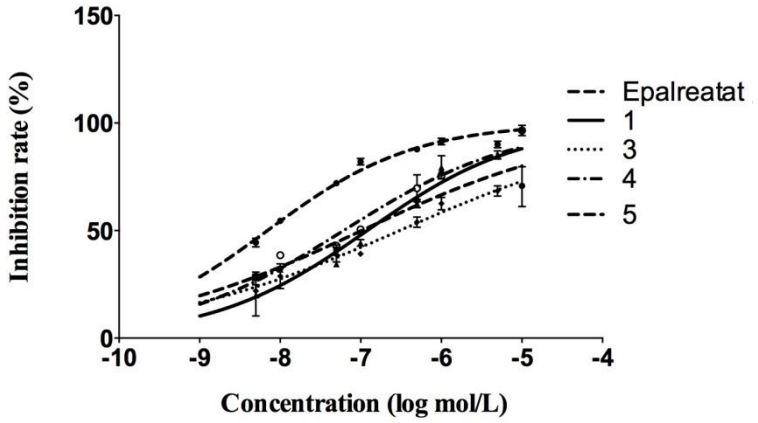
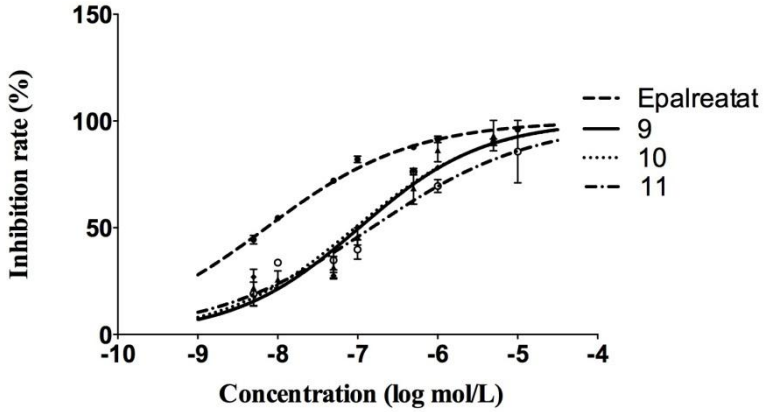


Fig.5.



A



B

Table 1

^{1}H-NMR spectroscopic data of compounds **1–2** (in MeOD)

Position	1	2
2	6.52(d, J = 2.0Hz)	7.27(d, J=1.8Hz)
5	6.48(d, J = 8.0Hz)	6.83(d, J=8.4Hz)
6	6.50(dd, J = 8.0,2.0Hz)	7.16(dd, J = 8.4,1.8Hz)
7	6.95(d, J = 16.0Hz)	7.46(d, J = 15.6Hz)
8	5.61(d, J = 16.0Hz)	6.27(d, J = 15.6Hz)
2′	6.74(d,J = 2.0H)	6.75(d, J = 1.8Hz)
5′	6.82(d,J = 8.0Hz)	6.66(d, J = 7.8Hz)
6′	6.65(dd,J = 8.0,2.0Hz)	6.59(dd, J = 7.8,1.8Hz)
7′	3.13 (2H,t,J = 5.0Hz)	2.94(m),3.05(m)
8′	5.17(t,J = 5.0Hz)	5.1(m)
1″	2.95(m),2.60(m)	4.95(d,J = 6.5Hz)
2″	1.91(m),1.84(m)	3.44(m)
3″	5.23(t,J = 2.0 Hz)	3.54(m)
4″		3.85(m)
5″		3.54(m)
6″	7.90(d,J = 8.0Hz)	4.39(m),4.73(m)
7″	7.48(d,J = 8.0Hz)	
15″	7.26(d,J = 2.0Hz)	
17″	2.19(3H,s)	
18″	1.43(3H,s)	
2‴		7.44(d, J = 1.8Hz)
5‴		6.75(d, J = 8.4Hz)
6‴		7.47(dd,J = 8.4,1.8Hz)
8‴		3.76(3H,s)

Table 2

^{13}C-NMR spectroscopic data of compounds **1–2** (in MeOD)

Position	1	2	Position	1	2
1	127.5	128.0	5″	147.1	74.8
2	115.3	118.0	6″	135.3	65.1
3	147.4	146.4	7″	122.5	
4	150.7	151.1	8″	131.1	
5	117.1	117.7	9″	126.9	
6	124.2	125.5	10″	144.6	
7	148.3	146.6	11″	184.8	
8	113.4	116.0	12″	176.7	
9	171.3	168.6	13″	122.5	
1′	129.0	130.2	14″	163.0	
2′	118.9	117.7	15″	143.9	
3′	146.9	146.1	16″	121.5	
4′	146.5	145.1	17″	9.7	
5′	117.1	116.2	18″	30.2	
6′	123.0	121.9	1‴		122.3
7′	38.0	38.3	2‴		125.2
8′	75.9	76.1	3‴		148.8
9′	170.5	176.2	4‴		153.0
1″	25.5	103.2	5‴		116.3
2″	25.7	72.1	6‴		113.8
3″	76.9	77.5	7‴		168.1
4″	73.2	75.8	8‴		56.4

Table 3

Aldose reductase inhibitory activities (IC_{50}, μM)

Sample number	IC_{50}
1	0.12 ± 0.007
3	0.30 ± 0.022
4	0.06 ± 0.002
5	0.10 ± 0.002
9	0.11 ± 0.003
10	0.10 ± 0.001
11	0.12 ± 0.011
positive control (epalrestat)	0.007 ± 0.0001

Data are means (n = 6) ± standard deviations

Graphical abstract

Isolation, modification, and aldose reductase inhibitory activity of rosmarinic acid derivatives from the roots of *Salvia grandifolia*

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