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Seven new sesquineolignans isolated from the seeds of hawthorn and their neuroprotective activities

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ABSTRACT

Seven new sesquineolignans (**1–7**) were isolated from the 70% ethanolic extract of the hawthorn seeds. Their structures were established by comprehensive spectroscopic analyses including 1D, 2D NMR, CD and HRESIMS data. The neuroprotective activity of the isolated sesquilignans towards H_2O_2-induced damage in human neuroblastoma SH-SY5Y cells was investigated. All of these sesquineolignans exhibited significant neuroprotective activity towards damaged SH-SY5Y cells, compared with the positive control (Trolox). Among them, **6** displayed the most potent neuroprotective ability with the survival rate of 90.74% at the concentration of 50 μM. Moreover, Hoechst 33258 staining and Annexin V/PI analysis proved that **6** could protect damaged SH-SY5Y cells through inhibiting cellular apoptosis.

1. Introduction

Oxidative stress (OS) has long been implicated in the pathogenesis of various neurological disorders, including dysautonomia, Parkinson's disease, Alzheimer's disease and stroke [1,2]. In the central and peripheral nervous systems, several antioxidant ingredients such as glutathione peroxidase, superoxide dismutase and carotenoid can eliminate reactive oxygen species (ROS) and protect against oxidative stress [3,4]. With the increased oxidative damage and the weakened activities of antioxidant defense system in the brain of neurodegenerative diseases, pharmacological approach to attenuate oxidative stress may be one of the promising therapeutic strategies. Natural products are rich in diverse bioactive with suited health benefits, and some naturally occurring polyphenols, such as flavonoids and lignans, have been proved to possess promising neuroprotective effects on oxidative stress [5–7].

Crataegus pinnatifida, also called hawthorn, taxonomically belonging to the family of Rosaceae, is widely distributed in the Northern Hemisphere mostly in China, Europe and North America [8]. The pharmaceutical research revealed that hawthorn possessed quenching free radicals, inhibiting the oxidation of the low density lipoprotein, antityrosinase, anti-inflammatory effects [9]. Up to now, over 150 constituents have been identified in hawthorn [8]. Among them, the polyphenol compounds were the most key bioactive constituents. Our group is interested in the isolation and identification of the antioxidant constituents in the seeds of hawthorn. Previous investigations led to the isolation of a series of lignans, and some of them were proved to possess promising antioxidant activities [10,11].

In the search for antioxidant agents from the seeds of hawthorn, seven new sesquineolignans (**1–7**) with characteristic aryl glycerol and benzodihydrofuran moiety simultaneously (Fig. 1) were isolated. Their structures were elucidated by a combination of the systematic NMR and circular dichroism (CD) data analyses. The antioxidant activities of lignans from the seeds of hawthorn were proved in previous study. However, there are few reports addressing the neuromodulatory potential in terms of their underlying mechanisms. Therefore, the present study aimed to evaluate the neuroprotective potential of sesquineolignans from the seeds of hawthorn against H_2O_2-induced neurotoxicity in SH-SY5Y cells.

2. Materials and methods

2.1. General experimental procedures

The UV spectra were measured on a Shimadzu UV-1700 spectrometer (Shimadzu, Japan). The FT-IR spectra were provided on a Bruker IFS-55 spectrometer in KBr pellets (Bruker Co., Karlsruhe, Germany). Optical rotations were recorded by using a JASCO DIP-370 digital

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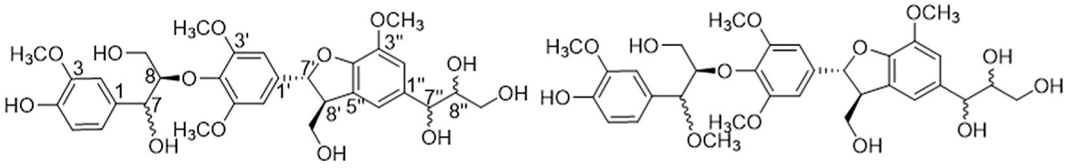

1: 7*R*; 7",8"-*threo*
2: 7*S*; 7",8"-*threo*
3: 7*R*; 7",8"-*erythro*

4: 7*S*; 7",8"-*threo*
5: 7*R*; 7",8"-*threo*
6: 7*R*; 7",8"-*erythro*
7: 7*S*; 7",8"-*erythro*

Fig. 1. Structures of compounds **1–7**.

polarimeter. The CD spectra were obtained using MOS 450 detector from BioLogic. The ^{1}H NMR, ^{13}C NMR, HMBC, HSQC spectra were obtained on Bruker ARX-300 and Bruker AV-600 (Bruker Corporation, Bremen, Germany) spectrometers with TMS as an internal standard. And the chemical shifts were recorded as δ values. HRESIMS experiments were performed on an Agilent G6520 Q-TOF spectrometer (Agilent Technologies Inc., Santa Clara, USA). Macroporous adsorption resin D101 was provided by Cangzhou Bon Adsorber Technology Co., Ltd. The chromatographic silica gel (200–300 meshes) was purchased from Qingdao Marine Chemical Factory. ODS (50 μm) was produced by YMC Co., Ltd. Semipreparative RP-HPLC isolation was conducted in an Agilent 1100 instrument using YMC 5 μm C_{18} column (250 mm × 10 mm). Peak detection was made using a refractive index detector (RID). MTT, trolox were purchased from Sigma-Aldrich (St. Louis, MO). The OD values were detected on a Varioskan Flash Multimode Reader (Thermo scientific). All solvents for extraction and chromatography were commercially purchased and routinely distilled prior to use. Dulbecco's modified Eagle's medium (DMEM) was provided from Beyotime Institute of Biotechnology (Haimen, China). Fetal bovine serum (FBS) was purchased from Gibco Company (Grand Island, N.Y., USA).

2.2. Plant material

The dried seeds of hawthorn (30 kg) were collected from Shijiazhuang, Hebei province, PR China, in June 2011, and identified by Professor Jin-Cai Lu (Department of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, PR China). A voucher specimen (No. 20110701) has been deposited in the Herbarium of Shenyang Pharmaceutical University, Liaoning, PR China.

2.3. Extraction and isolation

The air-dried seeds of hawthorn (30 kg) obtained were ground into pieces and extracted with 70% ethanol for 3 × 30 L × 4 h. The solvents were evaporated under reduced pressure using a rotary evaporator. Then, the extracts (1500 g) was suspended in H_2O (20 L) and partitioned successively with ethyl acetate, *n*-BuOH. The *n*-BuOH extract (1000 g) was suspended in H_2O (5 L) and then subjected to D101 macroporous resin column using an increasing gradient of EtOH-H_2O (from 40:60 to 90:10) as eluents. The 40% EtOH fraction was chromatographed over the silica gel column (200–300 meshes), which was eluted with CH_2Cl_2:MeOH (20:1, 10:1, 8:1, 6:1, 5:1, 3:1, 1:1, 0:1) to yield fraction 1 to fraction 7. Fraction 4 was further purified by ODS column eluted with H_2O, which was enriched with methanol (from 10:90 to 40:60) to obtain fraction 4.1–4.5. Fraction 4.3 was subjected to silica column with the mobile phase CH_2Cl_2: methanol (from 10:1 to 2:1) to yield fraction 4.3.1–4.3.9. Compounds **1** (11 mg, t_R = 29.4 min), **4** (26 mg, t_R = 25.3 min) were separated from fraction 4.3.4 by semipreparative HPLC eluted with CH_3CN-H_2O (30:70). Compounds **6** (9 mg, t_R = 31.1 min), **7** (20 mg, t_R = 35.9 min) were isolated from fraction 4.3.3 eluted with CH_3CN-H_2O (28:72). Fraction 4.3.7 was subjected to semipreparative HPLC eluted with CH_3CN-H_2O (33:67) to afford compounds **2** (41 mg, t_R = 23.2 min), **3** (13 mg, t_R = 26.3 min), **5** (8 mg, t_R = 30.9 min). The purity of compounds **1–7** was > 95% on the basis of the analytical results of TLC and NMR.

2.4. Physical and spectroscopic data of isolated compounds from the seeds of hawthorn

Compound **1**: Light yellow oil (MeOH); $[\alpha]_{20}^{D}$ − 45.21 (c 0.073, MeOH); UV (MeOH) λ_{max} (logε): 215 nm (2.26), 280 nm (0.45); IR (KBr) ν_{max} 3409, 1594, 1501, 833 cm^{-1}; CD (c = 0.6 mg/mL, MeOH) 233 nm (− 8.12), 283 nm (− 2.23); The ^{1}H (300 MHz, CD_3OD) and ^{13}C NMR data (150 MHz, CD_3OD), see Tables 1 and 2; HRESIMS (*m/z*): 641.2202 [M + Na]$^{+}$ (calcd for $C_{31}H_{38}O_{13}Na$, 641.2205).

Compound **2**: Light yellow oil (MeOH); $[\alpha]_{20}^{D}$ − 23.87 (c 0.243, MeOH); UV (MeOH) λ_{max} (logε): 213 nm (1.99), 280 nm (0.32); IR (KBr) ν_{max} 3385, 1602, 1512, 831 cm^{-1}; CD (c = 0.2 mg/mL, MeOH) 231 nm (− 1.61), 280 nm (− 0.66); The ^{1}H (300 MHz, CD_3OD) and ^{13}C NMR data (150 MHz, CD_3OD), see Tables 1 and 2; HRESIMS (*m/z*): 641.2205 [M + Na]$^{+}$ (calcd for $C_{31}H_{38}O_{13}Na$, 641.2205).

Compound **3**: Light yellow oil (MeOH); $[\alpha]_{20}^{D}$ − 30.34 (c 0.234, MeOH); UV (MeOH) λ_{max} (logε): 213 nm (2.54), 280 nm (0.71); IR (KBr) ν_{max} 3386, 1603, 1513, 832 cm^{-1}; CD (c = 0.4 mg/mL, MeOH) 238 nm (− 12.60), 281 nm (− 8.72); The ^{1}H (300 MHz, CD_3OD) and ^{13}C NMR data (150 MHz, CD_3OD), see Tables 1 and 2; HRESIMS (*m/z*): 641.2184 [M + Na]$^{+}$ (calcd for $C_{31}H_{38}O_{13}Na$, 641.2205).

Compound **4**: Light yellow oil (MeOH); $[\alpha]_{20}^{D}$ − 21.68 (c 0.226, MeOH); UV (MeOH) λ_{max} (logε): 215 nm (2.58), 280 nm (0.69); IR (KBr) ν_{max} 3397, 1605, 1512, 832 cm^{-1}; CD (c = 0.2 mg/mL, MeOH) 232 nm (− 8.06), 276 nm (− 0.59); The ^{1}H (300 MHz, CD_3OD) and ^{13}C NMR data (150 MHz, CD_3OD), see Tables 1 and 2; ^{1}H NMR in $CDCl_3$ (400 MHz), δ 6.82–6.85 (5H, m, H-2, 5,6,2″,6″), 6.56 (2H, s, H-2′,6′), 4.58 (1H, d, *J* = 5.5 Hz, H-7), 4.07 (1H, m, H-8), 5.44 (1H, d, *J* = 7.2 Hz, H-7′), 3.50 (1H, m, H-8′), 4.59 (1H, d, *J* = 5.2 Hz, H-7″), 3.60 (1H, m, H-8″), 3.48 (3H, s, 3-OCH_3), 3.30 (3H, s, 7-OCH_3), 3.85 (6H, s, 3′,5′-OCH_3), 3.71 (3H, s, 3″-OCH_3). HRESIMS (*m/z*): 655.2347 [M + Na]$^{+}$ (calcd for $C_{32}H_{40}O_{13}Na$, 655.2361).

Compound **5**: Light yellow oil (MeOH); $[\alpha]_{20}^{D}$ − 55.48 (c 0.146, MeOH); UV (MeOH) λ_{max} (logε): 220 nm (2.91), 280 nm (0.84); IR (KBr) ν_{max} 3395, 1605, 1512, 832 cm^{-1}; CD (c = 0.2 mg/mL, MeOH) 229 nm (− 1.92), 275 nm (− 0.30); The ^{1}H (300 MHz, CD_3OD) and ^{13}C NMR data (150 MHz, CD_3OD), see Tables 1 and 2; ^{1}H NMR in $CDCl_3$ (400 MHz), δ_H 6.88–6.94 (5H, m, H-2,5,6,2″,6″), 6.66 (2H, s, H-2′,6′), 4.64 (1H, d, *J* = 8.7 Hz, H-7), 4.30 (1H, m, H-8), 5.60 (1H, d, *J* = 6.0 Hz, H-7′), 3.68 (1H, m, H-8′), 4.69 (1H, d, *J* = 6.8 Hz, H-7″), 3.79 (1H, m, H-8″), 3.90 (3H, s, 3-OCH_3), 3.27 (3H, s, 7-OCH_3), 3.86 (6H, s, 3′,5′-OCH_3), 3.92 (3H, s, 3″-OCH_3). HRESIMS (*m/z*): 655.2343 [M + Na]$^{+}$ (calcd for $C_{32}H_{40}O_{13}Na$, 655.2361).

Compound **6**: Light yellow oil (MeOH); $[\alpha]_{20}^{D}$ − 63.71 (c 0.124, MeOH); UV (MeOH) λ_{max} (logε): 215 nm (2.51), 280 nm (0.46); IR (KBr) ν_{max} 3386, 1603, 1512, 820 cm^{-1}; CD (c = 0.2 mg/mL, MeOH) 230 nm (− 5.89), 277 nm (− 1.32). The ^{1}H (300 MHz, CD_3OD) and ^{13}C NMR data (150 MHz, CD_3OD), see Tables 1 and 2; ^{1}H NMR in $CDCl_3$ (400 MHz), δ_H 6.90–6.93 (5H, m, H-2,5,6,2″,6″), 6.66 (2H, s, H-2′,6′),