Arch. Pharm. Res. (2015) 38:2066–2075
DOI 10.1007/s12272-015-0613-8

Archives of Pharmacal Research
www.springer.com/12272

RESEARCH ARTICLE

Isoprenylated flavonoids from the root bark of *Morus alba* and their hepatoprotective and neuroprotective activities

Jae-Woo Jung[1] · Won-Min Ko[2] · Ji-Hae Park[1] · Kyeong-Hwa Seo[1] · Eun-Ji Oh[1] · Dae-Young Lee[4] · Dong-Sung Lee[3] · Youn-Chul Kim[2] · Dong-Wook Lim[5] · Daeseok Han[5] · Nam-In Baek[1]

Received: 4 February 2015 / Accepted: 30 April 2015 / Published online: 16 May 2015

Abstract A new isoprenylated flavonoid, 2*S*-5,7,2′,4′-tetrahydroxy-3′,5′-di-(γ,γ-dimethylallyl)flavanone, sanggenol Q (**1**), along with seven known isoprenylated flavonoids, sanggenol A (**2**), sanggenol L (**3**), kuwanon T (**4**), cyclomorusin (**5**), sanggenon F (**6**), sanggenol O (**7**), and sanggenon N (**8**), three known Diels–Alder type adducts, sanggenon G (**9**), mulberrofuran G (**10**), and mulberrofuran C (**11**), and a known benzofuran, moracin E (**12**), were isolated from the root bark of *Morus alba* using silica gel, ODS, and Sephadex LH-20 column chromatography. Chemical structures were determined based on spectroscopic data analyses including NMR, MS, CD, and IR. For the first time, compounds **1** and **7** were isolated from the root bark of *M. alba*. All compounds were evaluated for hepatoprotective activity on *t*-BHP-induced oxidative stress in HepG2 cells and neuroprotective activity on glutamate-induced cell death in HT22 cells. Compounds **1**, **4**, **8**, **10**, and **11** showed protective effects on *t*-BHP-induced oxidative stress with EC_{50} values of 6.94 ± 0.38, 30.32 ± 6.82, 23.45 ± 4.72, 15.31 ± 2.21, and 0.41 ± 0.48 μM, respectively, and compounds **1**, **2**, **10**, **11**, and **12** showed protective effects on glutamate-induced cell death with EC_{50} values of 5.54 ± 0.86, 34.03 ± 7.71, 19.71 ± 0.71, 16.50 ± 7.82, and 1.02 ± 0.13 μM, respectively.

Keywords Hepatoprotective activity · HepG2 · HT22 · Isoprenylated flavonoid · *Morus alba* L. · Neuroprotective activity

Introduction

The mulberry tree (*Morus alba* L.) is plant native to Thailand and is widely distributed throughout Europe, America, Africa, and Asia (Park et al. 2011). Most parts of this plant have been widely used for medicinal purpose in south Asia and elsewhere. The root bark, named Sang-Bai-Pi, has been used for treating diabetes, relieving asthma, and protecting the liver (Ahn 2012). Phytochemical studies on Sang-Bai-Pi extracts resulted in the isolation of phenolic compounds including isoprenylated flavonoids (Jiang et al. 2003), Diels–Alder type adducts (Hano et al. 1988), triterpenoids (Jung et al. 2014), coumarins (Piao et al. 2009), benzofurans (Piao et al. 2009), and stilbenes (Piao et al. 2009), which were reported to show anti-oxidant (Zhang et al. 2012), tyrosinase inhibitory (Lee et al. 2004), anti-inflammatory (Yang et al. 2011), anti-hepatitis B virus (Geng et al. 2012), anti-cancer (Dat et al. 2010), and anti-microbial (Naik et al. 2015) activities. The EtOAc soluble

Electronic supplementary material The online version of this article (doi:10.1007/s12272-015-0613-8) contains supplementary material, which is available to authorized users.

✉ Nam-In Baek
nibaek@khu.ac.kr

1 Graduate School of Biotechnology and Department of Oriental Medicine Biotechnology, Kyung Hee University, Yongin 446-701, Republic of Korea

2 Institute of Pharmaceutical Research and Development, College of Pharmacy, Wonkwang University, Iksan 570-749, Republic of Korea

3 Inha Research Institute for Medical Sciences, Inha University School of Medicine, Incheon 400-712, Republic of Korea

4 Department of Herbal Crop Research, National Institute of Horticultural and Herbal Science, RDA, Eumseong 369-873, Republic of Korea

5 Division of Matabolism and Functionality Research, Korea Food Research Institute, Sungnam 463-746, Republic of Korea

fraction of *M. alba* root bark showed antidepressant effects in vivo (Lim et al. 2014). In this study, we isolated and identified pharmacologically-active compounds from the root bark of *M. alba* and evaluated them for hepatoprotective and neuroprotective effects.

A new isoprenylated flavonoid (**1**) and 11 known compounds (**2**–**12**) were isolated from the EtOAc fraction. Structural determination of the compounds was carried out on the basis of spectroscopic data analyses. The isolated compounds were evaluated for their protective effects on *t*-BHP-induced cytotoxicity in HepG2 cells and on glutamate-induced cell death in HT22 cells.

Materials and methods

General experimental procedures

The silica gel (SiO_2), octadecyl SiO_2 (ODS), and Sephadex LH- 20 resins used for column chromatography (c.c.) were Kiesel gel 60 (Merck, Darmstadt, Germany), Lichroprep RP-18 (Merck), and Sephadex^{TM} LH-20 (GE Healthcare Bio-Sciences, Piscataway, NJ, USA), respectively.

Thin layer chromatography (TLC) analysis was carried out using Kiesel gel 60 F_{254} (Merck) and RP-18 F_{254S} (Lichroprep RP-18, Merck) plates. The spots on TLC were detected using a Spectroline Model ENF-240 C/F UV lamp (Westbury, NY, USA) and a 10 % H_2SO_4 solution by spraying and heating. Optical rotation was measured on a JASCO P-1010 digital polarimeter (Tokyo, Japan). Infrared (IR) spectra were obtained using a Perkin-Elmer Spectrum model 599B (Waltham, MA, USA). The melting points were determined on a Fisher-John's apparatus (Miami, FL, USA). Fast atom bombardment mass spectrometry (FAB/MS) and electronic ionization mass spectrometry (EI/MS) spectra were obtained from a JEOL JMSAX-700 (Tokyo, Japan). ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Varian Inova AS 400 FT-NMR spectrometer (Palo Alto, CA, USA). Circular dichroism (CD) spectra were obtained with a Chirascan Plus Instrument (Applied Photophysics, Surrey, UK).

RPMI medium 1640, Dulbecco's modified Eagle's medium (DMEM), penicillin, fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Gibco-BRL (Grand Island, NY, USA). DMSO, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and L-glutamate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tissue culture and 96-well plates were purchased from Falcon (Grand Island, NY, USA). All other chemicals and reagents of analytical grade were obtained from Sigma-Aldrich, unless indicated otherwise.

Plant materials

The dried root bark of *M. alba* were supplied from the Korea Food Research Institute, Sungnam, Korea, in January 2012, and identified by Professor Dae-Keun Kim, College of Pharmacy, Woosuk University, Jeonju, Korea. A voucher specimen (KHU-NPCL-201204) is deposited at the Laboratory of Natural Products Chemistry, Kyung Hee University, Yongin, Korea.

Extraction and isolation of compounds from the root bark of *M. alba*

The dried and powdered root bark of *M. alba* were extracted with 80 % MeOH (68 L × 3) at room temperature for 24 h. The extracts were filtered through filter paper and concentrated in a rotary vacuum evaporator to yield of a residue (1.7 kg). The concentrated residue was suspended using 2 L water and successively extracted with ethyl acetate (EtOAc, 2 L × 2) and *normal*-butanol (*n*-BuOH, 1.8 L × 3). The organic and aqueous layers were concentrated to produce the residues of the EtOAc fraction (MRE, 580 g), the *n*-BuOH fraction (MRB, 114 g), and the H_2O fraction (MRW, 1006 g), respectively. The EtOAc fraction was fractionated by the SiO_2 c.c. (12.5 × 17 cm) eluting with *n*-hexane–EtOAc (4:1 → 2:1 → 1:1, 27 L of each) and $CHCl_3$-MeOH (10:1, 27 L) to yield 41 fractions (MRE-1 to MRE-41). Fraction MRE-6 [1.8 g, elution volume/total volume (V_e/V_t) 0.05–0.06] was subjected to the ODS c.c. (3 × 15 cm) and was eluted with MeOH-H_2O (6:1 → 8:1, 1 L of each), yielding 16 fractions (MRE-6-1 to MRE-6-16). Fraction MRE-6-6 (80 mg, V_e/V_t 0.13–0.17) was subjected to the SiO_2 c.c. (2.5 × 14 cm) and eluted with *n*-hexane–EtOAc (6:1, 0.8 L) to obtain five fractions (MRE-6-6-1 to MRE-6-6-5) along with a purified compound **7** [MRE-6-6-3, 25 mg, V_e/V_t 0.18–0.71, TLC (ODS) R_f 0.50, MeOH-H_2O = 20:1]. Fraction MRE-8 (3.0 g, V_e/V_t 0.07–0.08] was subjected to the ODS c.c. (6.5 × 12 cm) and was eluted with MeOH-H_2O (10:1, 1.8 L), yielding 12 fractions (MRE-8-1 to MRE-8-12). Fraction MRE-8-1 (160 mg, V_e/V_t 0.00–0.05) was subjected to the Sephadex LH-20 c.c. (1.5 × 60 cm) and was eluted with MeOH-H_2O (4:1, 0.25 L) to obtain six fractions (MRE-8-1-1 to MRE-8-1-6) along with a purified compound **1** [MRE-8-1-3, 12 mg, V_e/V_t 0.42–0.53, TLC (ODS) R_f 0.50, acetone-H_2O = 3:1]. Fraction MRE-8-2 (1.5 g, V_e/V_t 0.05–0.08) was subjected to the ODS c.c. (4.5 × 8 cm) and was eluted with acetone-H_2O (2:1, 1.2 L), yielding seven fractions (MRE-8-2-1 to MRE-8-2-7). Fraction MRE-8-2-2 (520 mg, V_e/V_t 0.19–0.26) was purified through the ODS c.c. (2.5 × 5.5 cm) eluting with MeOH-H_2O (7:2, 1 L), yielding eight fractions (MRE-8-2-2-1 to MRE-8-2-2-8) along with a purified compound **8**

[MRE-8-2-2-6, 220 mg, V_e/V_t 0.55–0.63, TLC (ODS) R_f 0.38, MeOH-H_2O = 8:1]. Fraction MRE-8-8 (500 mg, V_e/V_t 0.30–0.39) was subjected to the ODS c.c. (4.5 × 8 cm) and was eluted with acetone-H_2O (2:1, 1.2 L), yielding four fractions (MRE-8-8-1 to MRE-8-8-4) along with a purified compound **5** [MRE-8-8-1, 58 mg, V_e/V_t 0.00–0.40, TLC (ODS) R_f 0.51, acetone-H_2O = 6:1]. Fraction MRE-14 (8.0 g, V_e/V_t 0.12–0.21) was subjected to the ODS c.c. (5.5 × 10 cm) and was eluted with MeOH-H_2O (3:1, 13 L), yielding 18 fractions (MRE-14-1 to MRE-14-18). Fraction MRE-14-3 (424 mg, V_e/V_t 0.05–0.07) was purified using the ODS c.c. (4 × 7 cm) and was eluted with MeOH-H_2O (2:1, 1.6 L) to obtain nine fractions (MRE-14-3-1 to MRE-14-3-9) along with a purified compound **2** [MRE-14-3-7, 79 mg, V_e/V_t 0.58–0.82, TLC (ODS) R_f 0.64, MeOH-H_2O = 8:1]. Fraction MRE-14-3-1 (120 mg, V_e/V_t 0.00–0.23) was subjected to the Sephadex LH-20 c.c. (2 × 50 cm) and eluted with MeOH-H_2O (4:1, 1.4 L) to obtain six fractions (MRE-14-3-1-1 to MRE-14-3-1-6) along with a purified compound **6** [MRE-14-3-1-3, 46 mg, V_e/V_t 0.44-0.53, TLC (ODS) R_f 0.51, MeOH–H_2O = 7:1] and a purifited compound **12** [MRE-14-3-1-5, 23 mg, V_e/V_t 0.65–0.76, TLC (ODS) R_f 0.49, MeOH-H_2O = 7:1]. Fraction MRE-14-3-2 (78 mg, V_e/V_t 0.24–0.39) was purified through the Sephadex LH-20 c.c. (2 × 50 cm) eluting with MeOH–H_2O (4:1, 0.6 L) to obtain six fractions (MRE-14-3-2-1 to MRE-14-3-2-6) along with a purified compound **4** [MRE-14-3-2-3, 23 mg, V_e/V_t 0.34–0.46, TLC (ODS) R_f 0.53, MeOH–H_2O = 7:1]. Fraction MRE-17 (4.0 g, V_e/V_t 0.21–0.22) was subjected to the ODS c.c. (4 × 10 cm) and was eluted with MeOH-H_2O (3:1, 5.7 L), yielding 18 fractions (MRE-17-1 to MRE-17-18). Fraction MRE-17-12 (960 mg, V_e/V_t 0.30–0.54) was purified through the SiO_2 c.c. (5 × 14 cm) eluting with $CHCl_3$-MeOH (12:1, 4.8 L) yielding 13 fractions (MRE-17-12-1 to MRE-17-12-13) along with a purified compound **3** [MRE-17-12-6, 130 mg, V_e/V_t 0.52–0.63, TLC (ODS) R_f 0.45, MeOH–H_2O = 8:1]. Fraction MRE-36 (5.88 g, V_e/V_t 0.69–0.72) was subjected to the SiO_2 c.c. (5 × 14 cm) and eluted with $CHCl_3$–MeOH (12:1, 4.8 L) yielding 13 fractions (MRE-36-1 to MRE-36-13). Fraction MRE-36-2 (1.2 g, V_e/V_t 0.05–0.08) was subjected to the ODS c.c. (4.5 × 9 cm) and was eluted with MeOH-H_2O (3:2, 2.3 L) yielding 12 fractions (MRE-36-2-1 to MRE-36-2-12) along with a purified compound **10** [MRE-36-2-5, 450 mg, V_e/V_t 0.64–0.73, TLC (ODS) R_f 0.38, MeOH-H_2O = 3:1]. Fraction MRE-37 (1.5 g, V_e/V_t 0.80–0.86) was purified through the ODS c.c. (4 × 10 cm) eluting with MeOH-H_2O (2:1, 4.2 L) to obtain 23 fractions (MRE-37-1 to MRE-37-23) along with a purified compound **11** [MRE-37-10, 50 mg, V_e/V_t 0.14–0.15, TLC (ODS) R_f 0.58, MeOH–H_2O = 4:1] and a purified compound **9** [MRE-37-17, 350 mg, V_e/V_t 0.28–0.44, TLC (ODS) R_f 0.45, MeOH–H_2O = 4:1].

Sanggenol Q (1)

Yellow amorphous powder ($CHCl_3$); $[\alpha]_D^{20}$ + 0.3° (*c* 0.55, MeOH); m.p. 118–120 °C; negative high-resolution FAB-MS *m/z* 423.1818 $[M-H]^-$ (calcd 423.1808 for $C_{25}H_{27}O_6$); IR (CaF_2 plate, ν) 3371, 2924, 2853, 1652, 1613, 1572 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, δ_H) 6.86 (1H, s, H-6′), 5.96 (1H, br.s, H-8), 5.95 (1H, br.s, H-6), 5.56 (1H, dd, *J* = 13.2, 2.8 Hz, H-2), 5.25 (1H, t, *J* = 7.2 Hz, H-2″), 5.21 (1H, t, *J* = 7.2 Hz, H-2‴), 3.41 (2H, d, *J* = 7.2 Hz, H-1″), 3.26 (2H, d, *J* = 7.2 Hz, H-1‴), 3.17 (1H, dd, *J* = 17.2, 13.2 Hz, H-3a), 2.77 (1H, dd, *J* = 17.2, 2.8 Hz, H-3b), 1.79 (3H, s, H-4″), 1.75 (3H, s, H-4‴), 1.75 (3H, s, H-5‴), 1.73 (3H, s, H-5″); ^{13}C-NMR (100 MHz, $CDCl_3$, δ_C) 196.62 (C-4), 165.05 (C-7), 164.24 (C-5), 162.84 (C-8a), 153.76 (C-4′), 151.72 (C-2′), 135.54 (C-3″), 135.18 (C-3‴), 125.47 (C-6′), 121.85 (C-2‴), 121.38 (C-2″), 119.06 (C-5′), 116.01 (C-1′), 114.97 (C-3′), 103.01 (C-4a), 97.00 (C-6), 95.72 (C-8), 76.68 (C-2), 41.73 (C-3), 29.72 (C-1‴), 25.78 (C-5‴), 25.76 (C-4‴), 22.65 (C-1″), 17.87 (C-5″), 17.84 (C-4″).

Sanggenol A (2)

Yellow amorphous powder (CH_3OH); $[\alpha]_D^{22}$ + 11° (*c* 0.16, MeOH); m.p. 105–110 °C; EI/MS *m/z* 424 $[M]^+$; IR (CaF_2 plate, ν) 3381, 2918, 2873, 1668, 1608, 1568 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD, δ_H) 7.06 (1H, d, *J* = 8.4 Hz, H-6′), 6.40 (1H, d, *J* = 8.4 Hz, H-5′), 5.88 (1H, d, *J* = 2.4 Hz, H-8), 5.85 (1H, d, *J* = 2.4 Hz, H-6), 5.63 (1H, dd, *J* = 13.2, 2.8 Hz, H-2), 5.17 (1H, t, *J* = 6.8 Hz, H-2″), 5.03 (1H, t, *J* = 6.8 Hz, H-2‴), 3.34 (2H, d, *J* = 6.8 Hz, H-1″), 3.06 (1H, dd, *J* = 17.2, 13.2 Hz, H-3a), 2.68 (1H, dd, *J* = 17.2, 2.8 Hz, H-3b), 2.03 (2H, dt, *J* = 6.8, 7.2 Hz, H-1‴), 1.94 (2H, t, *J* = 7.2 Hz, H-5″), 1.75 (3H, s, H-4″), 1.58 (3H, s, H-5‴), 1.53 (3H, s, H-4‴); ^{13}C-NMR (100 MHz, CD_3OD, δ_C) 198.30 (C-4), 168.66 (C-5), 165.50 (C-7), 162.21 (C-8a), 157.49 (C-4′), 154.11 (C-2′), 136.03 (C-3″), 132.16 (C-3‴), 125.53 (C-6′), 125.40 (C-2‴), 124.00 (C-2″), 118.87 (C-1′), 117.46 (C-3′), 108.48 (C-5′), 103.27 (C-4a), 97.12 (C-6), 96.33 (C-8), 76.66 (C-2), 43.20 (C-3), 40.91 (C-5″), 27.67 (C-1‴), 25.86 (C-5‴), 23.24 (C-1″), 17.72 (C-4‴), 16.31 (C-4″).

Sanggenol L (3)

Yeollow amorphous powder (CH_3OH); $[\alpha]_D^{22}$ − 18° (*c* 0.1, MeOH); m.p. 168–172 °C; EI/MS *m/z* 422 $[M]^+$; IR (CaF_2 plate, ν) 3361, 2898, 2863, 1661, 1609, 1554 cm^{-1}; ^{1}H-NMR

(400 MHz, CD_3OD, δ_H) 7.19 (1H, d, $J = 8.8$ Hz, H-6′), 6.56 (1H, d, $J = 10.4$ Hz, H-1″), 6.33 (1H, dd, $J = 8.8$, 2.4 Hz, H-5′), 6.32 (1H, d, $J = 2.4$ Hz, H-3′), 5.86 (1H, s, H-6), 5.58 (1H, dd, $J = 13.2$, 2.8 Hz, H-2), 5.38 (1H, d, $J = 10.4$ Hz, H-2″), 4.99 (1H, m, H-2‴), 2.99 (1H, dd, $J = 17.2$, 13.2 Hz, H-3a), 2.68 (1H, dd, $J = 17.2$, 2.8 Hz, H-3b), 2.00 (2H, m, H-1‴), 1.63 (2H, overlapped, H-5″), 1.57 (3H, s, H-5‴), 1.49 (3H, s, H-4‴), 1.30 (3H, s, H-4″); ^{13}C-NMR (100 MHz, CD_3OD, δ_C) 198.57 (C-4), 164.45 (C-5), 163.38 (C-7), 159.40 (C-4′), 159.13 (C-8a), 156.55 (C-2′), 132.41 (C-3‴), 128.79 (C-6′), 125.89 (C-2″), 124.92 (C-2‴), 117.60 (C-1′), 116.61 (C-1″), 107.67 (C-5′), 103.50 (C-8), 103.40 (C-4a), 103.23 (C-3′), 96.75 (C-6), 81.70 (C-3″), 75.81 (C-2), 42.97 (C-3), 42.59 (C-5″), 27.53 (C-4″), 25.91 (C-5‴), 23.63 (C-1‴), 17.76 (C-4‴).

Kuwanon T (4)

Yellow amorphous powder (CH_3OH); m.p. 191–193 °C; EI/MS *m/z* 422 $[M]^+$; IR (CaF_2 plate, ν) 3394, 2932, 2892, 1658, 1609, 1588 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD, δ_H) 6.86 (1H, d, $J = 8.4$ Hz, H-6′), 6.41 (1H, d, $J = 8.4$ Hz, H-5′), 6.24 (1H, d, $J = 2.0$ Hz, H-8), 6.14 (1H, d, $J = 2.0$ Hz, H-6), 5.21 (1H, t, $J = 7.2$ Hz, H-2″), 5.06 (1H, t, $J = 7.2$ Hz, H-2‴), 3.35 (2H, d, $J = 7.2$ Hz, H-1″), 3.04 (2H, d, $J = 7.2$ Hz, H-1‴), 1.75 (3H, s, H-4″), 1.64 (3H, s, H-5″), 1.54 (3H, s, H-5‴), 1.31 (3H, s, H-4‴); ^{13}C-NMR (100 MHz, CD_3OD, δ_C) 183.97 (C-4), 165.87 (C-7), 163.80 (C-5), 163.41 (C-2), 160.16 (C-8a), 159.51 (C-4′), 154.96 (C-2′), 132.98 (C-3‴), 132.09 (C-3″), 129.06 (C-6′), 124.22 (C-2″), 122.82 (C-2‴), 122.28 (C-3), 117.95 (C-3′), 114.08 (C-1′), 108.35 (C-5′), 105.67 (C-4a), 99.75 (C-6), 94.85 (C-8), 26.20 (C-5″), 26.06 (C-5‴), 25.06 (C-1‴), 23.54 (C-1″), 18.20 (C-4″), 17.82 (C-4‴).

Cyclomorusin (5)

Yellow amorphous powder ($CHCl_3$); m.p. 256–258 °C; EI/MS *m/z* 422 $[M]^+$; IR (CaF_2 plate, ν) 3389, 2942, 2860, 1662, 1620, 1574 cm^{-1}; ^{1}H-NMR (400 MHz, acetone-d_6, δ_H) 7.74 (1H, d, $J = 8.8$ Hz, H-6′), 6.86 (1H, d, $J = 10.4$ Hz, H-1‴), 6.58 (1H, dd, $J = 8.8$, 2.4 Hz, H-5′), 6.37 (1H, d, $J = 2.4$ Hz H-3′), 6.17 (1H, d, $J = 9.2$ Hz, H-1″), 6.13 (1H, s, H-6), 5.76 (1H, d, $J = 10.4$ Hz, H-2‴), 5.43 (1H, d, $J = 9.2$ Hz, H-2″), 1.93 (3H, s, H-4″), 1.68 (3H, s, H-5″), 1.45 (3H, s, H-4‴), 1.45 (3H, s, H-5‴); ^{13}C-NMR (100 MHz, acetone-d_6, δ_C) 177.14 (C-4), 164.86 (C-4′), 163.66 (C-7), 158.53 (C-8a), 158.41 (C-2), 157.67 (C-2′), 155.42 (C-5), 138.23 (C-3″), 128.19 (C-2‴), 125.78 (C-6′), 121.02 (C-2″), 114.25 (C-1‴), 111.44 (C-5′), 110.35 (C-1′), 106.33 (C-3), 105.89 (C-3′), 103.74 (C-8), 101.31 (C-4a), 99.48 (C-6), 78.23 (C-3‴), 68.81 (C-1″), 27.79 (C-4‴), 27.79 (C-5‴), 18.33 (C-4″), 17.83 (C-4″).

Sanggenon F (6)

Yellow amorphous powder (CH_3OH); $[\alpha]_D^{20}$ − 24° (*c* 0.32, $CHCl_3$); m.p. 138–140 °C; EI/MS *m/z* 354 $[M]^+$; IR (CaF_2 plate, ν) 3397, 2924, 2886, 1656, 1612, 1566 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD, δ_H) 7.16 (1H, d, $J = 8.4$ Hz, H-6′), 6.65 (1H, d, $J = 10.4$ Hz, H-1″), 6.35 (1H, d, $J = 8.4$ Hz, H-5′), 5.89 (1H, d, $J = 2.4$ Hz, H-8), 5.86 (1H, d, $J = 2.4$ Hz, H-6), 5.63 (1H, dd, $J = 13.2$, 2.8 Hz, H-2), 5.62 (1H, d, $J = 10.4$ Hz, H-2″), 3.02 (1H, dd, $J = 17.2$, 13.2 Hz, H-3a), 2.68 (1H, dd, $J = 17.2$, 2.8 Hz, H-3b), 1.36 (3H, s, H-5″), 1.35 (3H, s, H-4″); ^{13}C-NMR (100 MHz, CD_3OD, δ_C) 198.24 (C-4), 168.42 (C-5), 165.55 (C-7), 165.22 (C-8a), 155.28 (C-4′), 151.18 (C-2′), 130.56 (C-2″), 127.94 (C-6′), 120.41 (C-1′), 117.85 (C-1″), 111.95 (C-3′), 109.88 (C-5′), 103.38 (C-4a), 97.16 (C-6), 96.32 (C-8), 76.71 (C-3″), 76.18 (C-2), 43.18 (C-3), 27.97 (C-4″), 27.92 (C-5″).

Sanggenol O (7)

White amorphous powder ($CHCl_3$); $[\alpha]_D^{20}$ − 40° (*c* 0.2, MeOH); m.p. 157–159 °C; EI/MS *m/z* 420 $[M]^+$; IR (CaF_2 plate, ν) 3371, 2922, 2874, 1648, 1567, 1436 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, δ_H) 6.97 (1H, s, H-6′), 6.63 (1H, d, $J = 10.4$ Hz, H-1″), 6.25 (1H, d, $J = 10.4$ Hz, H-1‴), 5.98 (2H, br.s, H-6,8), 5.63 (1H, dd, $J = 13.2$, 2.8 Hz, H-2), 5.56 (1H, d, $J = 10.4$ Hz, H-2″), 5.48 (1H, d, $J = 10.4$ Hz, H-2‴), 2.96 (1H, dd, $J = 17.2$, 13.2 Hz, H-3a), 2.77 (1H, dd, $J = 17.2$, 2.8 Hz, H-3b), 1.41, 1.41, 1.39, 1.37 (12H, s, H-4″, 5″, 4‴, 5‴); ^{13}C-NMR (100 MHz, $CDCl_3$, δ_C) 197.02 (C-4), 164.99 (C-8a), 164.26 (C-7), 163.85 (C-5), 150.12 (C-2′), 148.77 (C-4′), 129.18 (C-2″), 128.19 (C-2‴), 123.28 (C-6′), 122.01 (C-1‴), 117.89 (C-1′), 116.50 (C-1″), 114.54 (C-5′), 109.91 (C-3′), 103.09 (C-4a), 96.53 (C-6), 95.52 (C-8), 76.78 (C-3″), 76.68 (C-3‴), 73.98 (C-2), 42.38 (C-3), 27.92, 27.92, 27.79, 27.74 (C-4″, 5″, 4‴, 5‴).

Sanggenon N (8)

Yellow amorphous powder (CH_3OH); $[\alpha]_D^{20}$ − 4° (*c* 0.35, $CHCl_3$); m.p. 102–105 °C; EI/MS *m/z* 422 $[M]^+$; IR (CaF_2 plate, ν) 3369, 2932, 2869, 1689, 1622, 1543, 1285 cm^{-1}; ^{1}H-NMR (400 MHz, acetone-d_6, δ_H) 7.17 (1H, d, $J = 8.4$ Hz, H-6′), 6.75 (1H, d, $J = 10.4$ Hz, H-1″), 6.35 (1H, d, $J = 8.4$ Hz, H-5′), 5.89 (1H, s, H-6), 5.89 (1H, s, H-8), 5.68 (1H, dd, $J = 13.2$, 2.8 Hz, H-2), 5.62 (1H, d, $J = 10.4$ Hz, H-2″), 5.05 (1H, m, H-2‴), 3.13 (1H, dd, $J = 17.2$, 13.2 Hz, H-3a), 2.65 (1H, dd, $J = 17.2$, 2.8 Hz, H-3b), 1.97 (2H, m, H-5″), 1.62 (2H, m, H-1‴), 1.57 (3H, s, H-4‴), 1.49 (3H, s, H-5‴), 1.29 (3H, s, H-4″); ^{13}C-NMR (100 MHz, acetone-d_6, δ_C) 197.65 (C-4), 167.76 (C-7),

165.55 (C-5), 164.72 (C-8a), 155.43 (C-2′), 151.21 (C-4′), 132.20 (C-3‴), 129.52 (C-6′), 128.28 (C-2″), 125.20 (C-2‴), 119.35 (C-1′), 118.04 (C-1″), 111.31 (C-3′), 109.54 (C-5′), 103.34 (C-4a), 97.17 (C-6), 96.24 (C-8), 78.95 (C-3″), 76.08 (C-2), 42.60 (C-3), 41.90 (C-5″), 26.72 (C-4″), 26.01 (C-4‴), 23.56 (C-1‴), 17.88 (C-5‴).

Sanggenon G (9)

Brown amourphous powder (CH_3OH); $[\alpha]_D^{20}$ − 277° (*c* 0.09, MeOH); m.p. 188–190 °C; EI/MS *m/z* 694 $[M]^+$; IR (CaF_2 plate, ν) 3427, 2984, 2892, 1633, 1482 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD, δ_H) 7.59 (1H, d, *J* = 8.0 Hz, H-33), 7.15 (1H, d, *J* = 8.8 Hz, H-27), 6.82 (1H, d, *J* = 8.0 Hz, H-6′), 6.31 (1H, d, *J* = 2.4 Hz, H-30), 6.29 (1H, dd, *J* = 8.0, 2.4 Hz, H-32), 6.13 (1H, d, 2.4 Hz, H-24), 6.06 (1H, dd, *J* = 8.8, 2.4 Hz, H-26), 6.06 (1H, dd, *J* = 8.0, 2.0 Hz, H-5′), 5.96 (1H, d, *J* = 2.0 Hz, H-3′), 5.87 (1H, s, H-8), 5.45 (1H, m, H-2), 5.27 (1H, m, H-14), 5.18 (1H, m, H-10), 4.27 (2H, m, H-12), 2.98 (1H, m, H-18a), 2.96 (1H, m, H-3a), 2.60 (1H, m, H-18b), 2.57 (1H, m, H-3b), 2.15 (1H, m, H-19), 2.14 (1H, m, H-20), 2.14 (2H, m, H-13), 2.04 (1H, m, H-9), 1.68 (3H, s, H-16), 1.61 (3H, s, H-17); ^{13}C-NMR (100 MHz, CD_3OD, δ_C) 210.58 (C-21), 198.25 (C-4), 165.80 (C-7), 165.80 (C-5), 165.61 (C-23), 165.61 (C-25), 163.28 (C-8a), 159.58 (C-4′), 157.12 (C-29), 157.11 (C-31), 156.60 (C-2′), 136.84 (C-11), 134.15 (C-33), 131.90 (C-15), 128.80 (C-27), 128.80 (C-6′), 125.54 (C-14), 125.12 (C-10), 122.90 (C-28), 116.23 (C-22), 110.38 (C-1′), 108.16 (C-5′), 107.77 (C-6), 107.77 (C-32), 107.34 (C-26), 103.79 (C-24), 103.79 (C-4a), 103.40 C-30), 102.82 (C-3′), 96.12 (C-8), 76.01 (C-2), 47.40 (C-20), 43.14 (C-3), 38.50 (C-12), 38.10 (C-18), 38.10 (C-9), 36.77 (C-19), 27.37 (C-13), 25.95 (C-16), 17.83 (C-17).

Mulberrofuran G (10)

Brown amorphous powder (CH_3OH); $[\alpha]_D^{22}$ + 546° (*c* 0.033, MeOH); m.p. 182–183 °C; EI/MS *m/z* 564 $[M]^+$; IR (CaF_2 plate, ν) 3400, 1633, 1620, 1600 cm^{-1}; ^{1}H-NMR (400 MHz, acetone-d_6, δ_H) 7.38 (1H, d, *J* = 8.4 Hz, H-4), 7.24 (1H, d, *J* = 8.8 Hz, H-14″), 7.13 (1H, d, *J* = 8.4 Hz, H-20″), 7.02 (1H, s, H-3), 6.98 (1H, d, *J* = 1.6 Hz, H-6′), 6.96 (1H, d, *J* = 2.0 Hz, H-7), 6.93 (1H, d, *J* = 1.6 Hz, H-2′), 6.80 (1H, dd, *J* = 8.4, 2.0 Hz, H-5), 6.50 (1H, dd, *J* = 8.4, 2.4 Hz, H-19″), 6.44 (1H, d, *J* = 1.2 Hz, H-2″), 6.41 (1H, d, *J* = 2.4 Hz, H-17″), 6.37 (1H, d, *J* = 2.4 Hz, H-11″), 6.23 (1H, dd, *J* = 8.8, 2.4 Hz, H-13″), 3.48 (1H, overlapped, H-5″), 3.32 (1H, overlapped, H-3″), 2.98 (1H, ddd, *J* = 17.2, 10.8, 5.2 Hz, H-4″), 2.70 (1H, dd, *J* = 17.2, 5.2 Hz, H-6″a), 2.02 (1H, overlapped, H-6″b), 1.77 (3H, s, H-7″); ^{13}C-NMR (100 MHz, acetone-d_6, δ_C) 159.71 (C-10″), 157.74 (C-18″), 157.53 (C-12″), 157.32 (C-6), 156.53 (C-2), 156.48 (C-5′), 154.84 (C-3′), 154.33 (C-16″), 153.15 (C-7a), 133.57 (C-1″), 130.88 (C-1′), 130.18 (C-14″), 127.71 (C-20″), 122.67 (C-2″), 122.36 (C-3a), 121.81 (C-4), 117.38 (C-4′), 116.75 (C-15″), 113.26 (C-5), 113.08 (C-9″), 109.70 (C-19″), 107.03 (C-13″), 105.27 (C-6′), 104.92 (C-2′), 104.46 (C-17″), 103.75 (C-11″), 102.44 (C-8″), 102.10 (C-3), 98.21 (C-7), 37.03 (C-3″), 36.05 (C-6″), 34.96 (C-5″), 28.33 (C-4″), 23.70 (C-7″).

Mulberrofuran C (11)

Brown amorphous powder (CH_3OH);); $[\alpha]_D^{22}$ + 211° (*c* 0.11, MeOH); m.p. 192–194 °C; EI/MS *m/z* 580 $[M]^+$; IR (CaF_2 plate, ν) 3311, 1680,1601, 1584 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD, δ_H) 8.43 (1H, d, *J* = 8.8 Hz, H-14″), 7.27 (1H, d, *J* = 8.4 Hz, H-4), 6.91 (1H, d, *J* = 8.4 Hz, H-20″), 6.84 (1H, d, *J* = 2.0 Hz, H-7), 6.80 (1H, s, H-3), 6.68 (1H, dd, *J* = 8.4, 2.0 Hz, H-5), 6.69 (1H, s H-2′), 6.69 (1H, s H-6′), 6.34 (1H, d, *J* = 2.4 Hz, H-17″), 6.32 (1H, dd, *J* = 8.8, 2.4 Hz, H-13″), 6.22 (1H, dd, *J* = 8.4, 2.4 Hz, H-19″), 6.17 (1H, d, *J* = 2.4 Hz, H-11″), 5.71 (1H, br.s, H-5″), 4.55 (1H, dd, *J* = 5.2, 4.4 Hz, H-4″), 4.08 (1H, m, H-5″), 3.70 (1H, m, H-3″), 2.44 (1H, br.d, *J* = 18.4 Hz, H-6″a), 2.18 (1H, br.d, *J* = 18.4 Hz, H-6″b), 1.89 (3H, s, H-7″); ^{13}C-NMR (100 MHz, CD_3OD, δ_C) 209.95 (C-8″), 166.90 (C-10″), 166.50 (C-12″), 157.94 (C-18″), 157.94 (C-6), 157.72 (C-16″), 157.10 (C-7a), 156.99 (C-3′), 156.56 (C-2), 155.99 (C-5′), 135.39 (C-14″), 134.32 (C-1″), 131.36 (C-1′), 128.98 (C-20″), 124.77 (C-2″), 123.10 (C-3a), 122.69 (C-15″), 121.84 (C-4), 116.88 (C-4′), 113.96 (C-9″), 113.08 (C-5), 108.92 (C-19″), 107.23 (C-13″), 104.84 (C-2′), 104.84 (C-6′) 103.53 (C-11″), 103.45 (C-17″), 101.75 (C-3), 98.40 (C-7), 48.36 (C-4″), 36.80 (C-5″), 33.71 (C-3″), 33.01 (C-6″), 23.78 (C-7″).

Moracin E (12)

Brown amorphous powder (CH_3OH); EI/MS *m/z* 354 $[M]^+$; m.p. 184–185 °C; IR (CaF_2 plate, ν) 3398, 1598, 1574 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD, δ_H) 7.31 (1H, d, *J* = 8.8 Hz, H-4), 6.89 (1H, s, H-3), 6.87 (1H, d, *J* = 2.0 Hz, H-7), 6.78 (1H, d, *J* = 1.6 Hz, H-6′), 6.71 (1H, dd, *J* = 8.8, 2.0 Hz, H-5), 6.69 (1H, d, *J* = 1.6 Hz, H-4′), 6.63 (1H, d, *J* = 10.4 Hz, H-1″), 5.58 (1H, d, *J* = 10.4 Hz, H-2″), 1.39 (6H, s, H-4″, 5″); ^{13}C-NMR (100 MHz, CD_3OD, δ_C) 157.29 (C-6), 156.92 (C-7a), 155.86 (C-2), 155.46 (C-3′), 154.64 (C-5′), 132.48 (C-1′), 129.71 (C-2″), 123.07 (C-3a), 122.01 (C-4), 118.03 (C-1″), 113.30 (C-5), 110.93 (C-2′), 104.95 (C-4′), 104.54 (C-6′), 102.34 (C-7), 98.44 (C-3), 76.94 (C-3″), 28.03 (C-4″), 28.03 (C-5″).

Cell culture

HepG2 cells were obtained from the American Type Culture Collection (Manassas, VA, USA), and Mouse hippocampal HT22 cells were received from Dr. Inhee-Mook (Seoul National University). They were respectively maintained at 5×10^5 cells/mL in RPMI medium and HT22 cells were maintained at 5×10^5 cells/mL in DMEM medium. Each cell type was supplemented with penicillin G (100 units/mL), streptomycin (100 mg/mL), L-glutamine (5 mM), and 10 % heat-inactivated FBS followed by incubation under a humidified atmosphere containing 5 % CO_2 and 95 % air at 37 °C.

Hepatoprotective activity assay

HepG2 cells were pretreated with either compound (**1**–**12**) or positive control (curcumin, 20 μM) for 2 h. Subsequently, cells were exposed to *tert*-butylhydroperoxide (*t*-BHP, 50 μM) for 4 h. *t*-BHP-mediated toxicity was evaluated using the MTT assay (Mosmann 1983). The compounds were tested at various concentrations (5, 10, 20, 40, and 80 μM). The results express the half maximal effective concentration (EC_{50}) values as a percentage of viability versus control.

Cytoprotective activity assay

HT22 cells were pretreated for 2 h with trolox as a positive control (100 μM) or one of the compounds (**1**-**12**) at various concentrations (1, 5, 10, 20, 40, and 80 μM), and the cells were exposed to glutamate (5 mM) for 12 h followed by an assessment of cell viability. Results expressed are the EC_{50} values as a percentage of viability versus control.

Statistical analysis

All experiments were performed with triplicate samples and repeated at least three times. The data are presented as mean ± SD, and statistical comparisons between groups were performed using one-way analysis of variance (ANOVA) compared by Newman-Keuls post hoc test. Statistical analysis was performed with GraphPad Prism software version 3.03 (GraphPad Software, Inc., San Diego, CA, USA).

Results and discussion

The root bark of *M. alba* was extracted in aqueous methanol, and the obtained extracts were successively partitioned into EtOAc, *n*-BuOH, and H_2O. Repeated SiO_2, ODS, and Sephadex LH-20 column chromatography of the EtOAc fraction afforded one new flavonoid (**1**) and 11 known ones (**2**–**12**). The chemical structures of the known compounds were determined based on analysis of 1D-NMR (^1H ^{13}C, and DEPT) and 2D-NMR (HSQC, HMBC, and COSY) experiments and confirmed by comparison of spectroscopic data with those reported in the literature. The known compounds (**2**–**12**) were identified to be sanggenol A (**2**, Fukai et al. 1996), sanggenol L (**3**, Shi et al. 2001a), kuwanon T (**4**, Fukai et al. 1985b), cyclomorusin (**5**, Lin et al. 1996), sanggenon F (**6**, Nomura et al. 1983), sanggenol O (**7**, Shi et al. 2001b), sanggenon N (**8**, Hano et al. 1984), sanggenon G (**9**, Fukai et al. 1983), mulberrofuran G (**10**, Fukai et al. 1985a), mulberrofuran C (**11**, Fukai et al. 1985a), and moracin E (**12**, Takasugi et al. 1979) (Fig. 1).

Compound **1** was obtained as a yellow amorphous powder and showed yellow color on the TLC plate after spraying with 10 % sulfuric acid and heating. The molecular weight was determined to be 424 from the molecular ion peak *m/z* 423 $[M\text{-}H]^-$ in the negative FAB/MS spectrum with a molecular formula of $C_{25}H_{28}O_6$ according to the high-resolved molecular ion peak *m/z* 423.1818 $[M\text{-}H]^-$ (calculated for 423.1808, $C_{25}H_{27}O_6$) in the HR/FAB/MS. The IR absorbance bands of hydroxyl (3371 cm^{-1}), carbonyl (1652 cm^{-1}), and aromatic (1613, 1572 cm^{-1}) groups were detected. The ^1H-NMR spectrum demonstrated one aromatic signal at δ_H 6.86 (1H, s, H-6′) owing to a penta-substituted benzene ring B and two aromatic signals at δ_H 5.96 (1H, br.s, H-8) and 5.95 (1H, br.s, H-6) due to a 1,2,3,5-tetrasubstituted benzene ring A. In addition, the oxygenated methine signal at δ_H 5.56 (1H, dd, $J = 13.2$, 2.8 Hz, H-2) and the methylene signals at δ_H 3.17 (1H, dd, $J = 17.2$, 13.2 Hz, H-3a) and 2.77 (1H, dd, $J = 17.2$, 2.8 Hz, H-3b) indicated the AMX system as typical of flavanone ring C. The other proton signals of the two olefin methines at δ_H 5.25 (1H, t, $J = 7.2$, H-2″) and 5.21 (1H, t, $J = 7.2$, H-2‴), two allyl methylenes at δ_H 3.41 (2H, d, $J = 7.2$, H-1″) and 3.26 (2H, d, $J = 7.2$, H-1‴), and four methyls at δ_H 1.79 (3H, s, H-4″), 1.75 (6H, s, H-4‴, 5‴), and 1.73 (3H, s, H-5″) signals indicated the presence of two isoprenyl moieties. The above-mentioned evidence suggested that compound **1** was a flavanone compound with two isoprenyl groups. The ^{13}C NMR spectrum showed 25 carbon signals. The chemical shifts of the flavanone moieties including a conjugated ketone signal at δ_C 196.62 (C-4), five oxygenated olefin quaternary signals at δ_C 165.05 (C-7), 164.24 (C-5), 162.84 (C-8a), 153.76 (C-4′), and 151.72 (C-2′), four olefin quaternary signals at δ_C 119.06 (C-5′), 116.01 (C-1′), 114.97 (C-3′), and 103.01 (C-4a), three olefin methine signals at δ_C 125.47 (C-6′), 97.00 (C-6), and 95.72 (C-8), one oxygenated methine signal at δ_C 76.68 (C-2), and one methylene signal at δ_C 41.73 (C-3) were identified as the signals of a highly substituted flavanone moiety. Moreover,

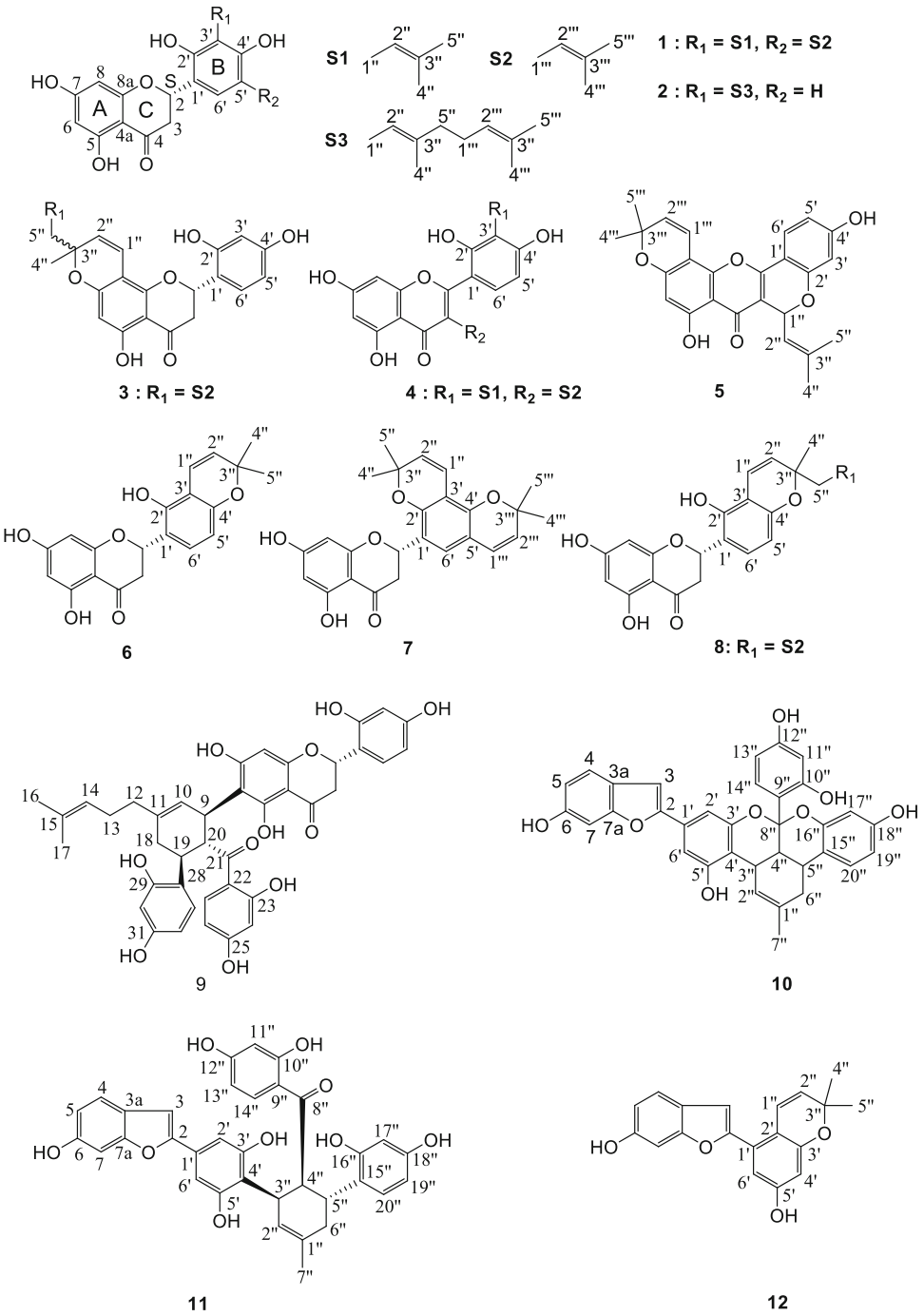

Fig. 1 Chemical structures of compounds **1–12** isolated from the root bark of *M. alba*

two olefin quaternary signals at δ_C 135.54 (C-3″) and 135.18 (C-3‴), two olefin methine signals at δ_C 121.85 (C-2‴) and 121.38 (C-2″), two methylene signals at δ_C 29.72 (C-1‴) and 22.65 (C-1″), four methyl signals at δ_C 25.78 (C-5‴), 25.76 (C-4‴), 17.87 (C-5″), and 17.84 (C-4″) were observed owing to two isoprenyl groups. The location of the two isoprenyl groups in compound **1** was determined by heteronuclear multiple bond connectivity (HMBC) experiments. Two methyl proton signals (δ_H, 1.79, H-4″; δ_H, 1.73, H-5″) were correlated with the olefin methine carbon signal (δ_C, 121.38, C-2″), and the olefin methine proton signal (δ_H, 5.25, H-2″) showed cross peaks with the allyl methylene carbon signal (δ_C, 22.65, C-1″) and the olefin quaternary carbon signal (δ_C, 114.97, C-3′). The allyl methylene proton signal (δ_H, 3.41, H-1″) was correlated with two oxygenated olefin quaternary carbon signals (δ_C, 153.76, C-4′; δ_C, 151.72, C-2′) and two olefin quaternary carbon signals (δ_C, 135.54, C-3″; δ_C, 114.97, C-3′). HMBC

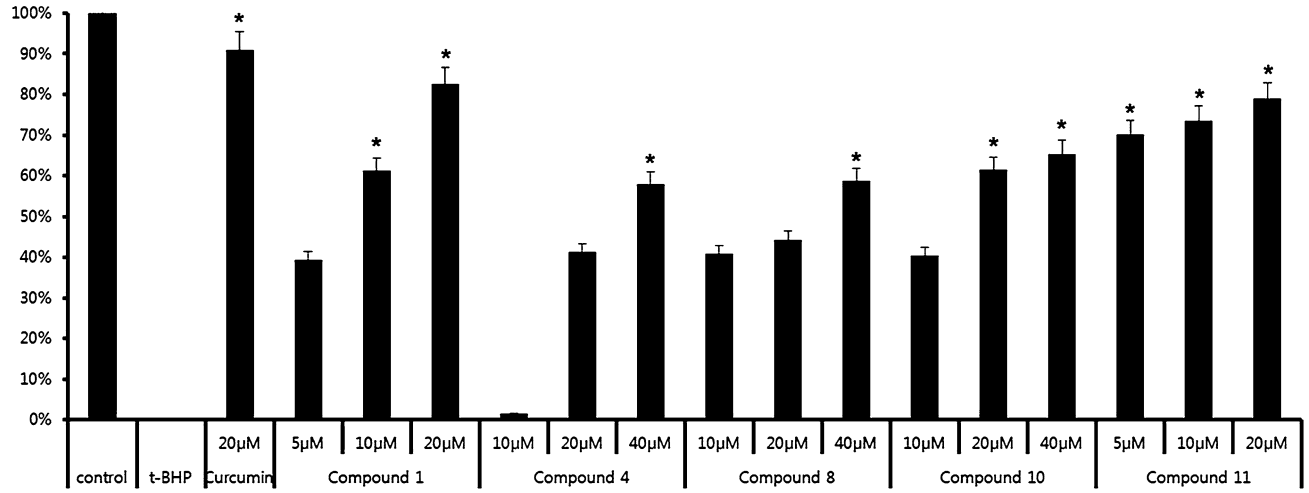

Fig. 2 Hepatoprotective effects of compounds **1**, **4**, **8**, **10**, and **11** on *t*-BHP-induced cytotoxicity in HepG2 cells. Each *bar* represents the mean ± SD of three independent experiments. $^{*}p < 0.05$, compared to the group treated with *t*-BHP. Curcumin (20 μM) was used as a positive control

correlations in the other isoprenyl group were observed and included H-4‴, 5‴ (δ_H 1.75)/C-2‴ (δ_C 121.85); H-2‴ (δ_H 5.21)/C-5′ (δ_C 119.06) and C-1‴ (δ_C 29.72); H-1‴ (δ_H 3.26)/C-4′ (δ_C 153.76), C-3‴ (δ_C 135.18), C-5′ (δ_C 116.06), and C-6′ (δ_C 125.47). From the HMBC correlations, two isoprenyl groups were determined to be located at C-5′ and C-3′ in the flavanone B ring. The absolute configuration of C-2 was suggested to be *S* from the large coupling constant ($J = 13.2$ Hz) between the axial protones of H-2 and H-3a and was confirmed according to the positive cotton effect at 330 nm ($\Delta\varepsilon + 1.72$) and the negative cotton effect at 288 nm ($\Delta\varepsilon - 0.74$) in the CD spectrum (Slade et al. 2005). From these data, we determined that compound **1** was 2*S*-5,7,2′,4′-tetrahydroxy-3′,5′-di-(γ,γ-dimethylallyl)flavanone, a new isoprenylated flavonoid, named sanggenol Q (Fig. 1).

Many researchers have reported that flavonoids and phenolic compounds have a wide range of biological activities, and many flavonoids have been isolated from plant materials. Mulberry root bark is a rich source of natural isoprenoid substituted phenolic compounds such as isoprenylated flavonoids, Diels–Alder type adducts, and benzofurans. This plant is attractive to biochemists because it has important materials of structural, biological, and pharmacological value (Kumar and Chauhan 2008). Accordingly, in this study the isolated compounds (**1**–**12**) from mulberry root bark were evaluated for their pharmacological activities.

t-BHP is a well-known toxic agent that can induce oxidative stress. Oxidative stress has been recognized to be a factor in several diseases including liver diseases (Hix et al. 2000; Lee et al. 2014). The search for active compounds with hepatoprotective effects on *t*-BHP-induced HepG2 was carried out. Compounds **1**, **4**, **8**, **10**, and **11** exhibited protective effects in a dose-dependent manner, and the EC_{50} values were determined to be 6.94 ± 0.38, 30.32 ± 6.82, 23.45 ± 4.72, 15.31 ± 2.21, and 0.41 ± 0.48 μM, respectively (Fig. 2). Curcumin, which is well known as hepatoprotective material, was used as a positive control; its EC_{50} value was 6.81 ± 0.76 μM (Song et al. 2001). Compound **11** showed a higher protective effect than curcumin, while compounds **1** and **10** showed similar activity as the positive control. Previously, An et al. (2006) reported isoprenylated flavonoids from the roots of *Cudrania tricuspidata* showing hepatoprotective effects on tacrine-induced HepG2 cells. These results suggest compounds **1**, **10,** and **11** can be used as hepatoprotective agents; further studies are required to verify the mechanism of the effect.

Glutamate is an endogenous excitatory neurotransmitter in the central nervous system. Glutamate-induced oxidative stress in neuronal cells contributes to epilepsy, ischemia, Alzheimer's, and Parkinson's diseases (Fukai et al. 2009). The isolated compounds from mulberry root bark were evaluated for protective effects against glutamate-induced oxidative stress in HT22 cells using a previously described method (Jeong et al. 2010). Compounds **1**, **2**, **10**, **11**, and **12** exhibited protective effects in a dose-dependent manner, and the EC_{50} values were determined to be 5.54 ± 0.86, 34.03 ± 7.71, 19.71 ± 0.71, 16.50 ± 7.82, and 1.02 ± 0.13 μM, respectively (Fig. 3, Table 1). Compounds **1**, **2**, **10**, **11**, and **12** showed higher protective effects than the positive control, Trolox (EC_{50}: 72.44 ± 3.62 μM). Previously, several studies reported that prenoid substituted flavonoids and benzofurans from the root bark of *M. alba* showed protective effects against nitric oxide-induced cell

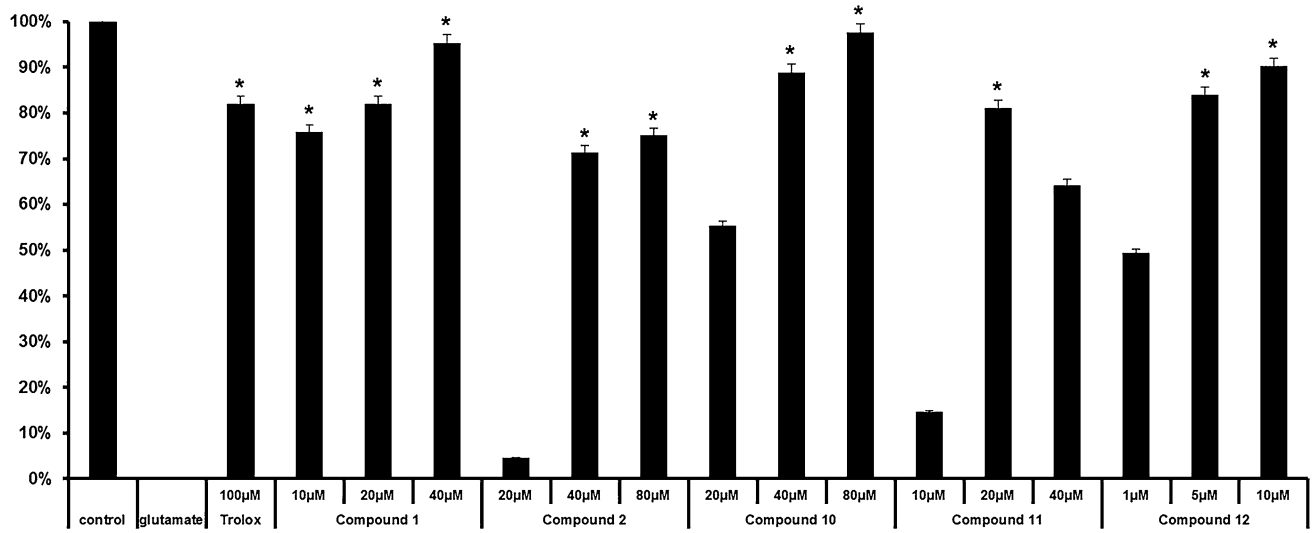

Fig. 3 Neuroprotective effects of compounds **1**, **2**, and **10–12** on glutamate-induced HT22 cells. Each *bar* represents the mean ± SD of three independent experiments. *$p < 0.05$, compared to the group treated with glutamate. Trolox (100 μM) was used as a positive control

Table 1 Hepatoprotective effects of compounds **1–12** from the root bark of *M. alba* on *t*-BHP-induced cytotoxicity in HepG2 cells and neuroprotective effects on cell death in glutamate-induced HT22 cells

Compound	HepG2 (EC_{50}) (μM)[a]	HT22 (EC_{50}) (μM)[b]
1	6.94 ± 0.38*	5.54 ± 0.86*
2	>80	34.03 ± 7.71*
3	>80	>80
4	30.32 ± 6.82	>80
5	>80	>80
6	>80	>80
7	>80	>80
8	23.45 ± 4.72	>80
9	>80	>80
10	15.31 ± 2.21*	19.71 ± 0.71*
11	0.41 ± 0.48*	16.50 ± 7.82
12	>80	1.02 ± 0.13*
Positive control	Curcumin, 6.81 ± 0.76	Trolox, 72.44 ± 3.62

[a] The EC_{50} value of each compound was defined as the concentration (μM) that produced a 50 % protective effect against cytotoxicity in *t*-BHP induced HepG2. * $p < 0.05$ compared to the group treated with *t*-BHP

[b] The EC_{50} value of each compound was defined as the concentration (μM) that produced a 50 % protective effect against cell death in glutamate-induced HT22 cells. * $p < 0.05$ compared to the group treated with glutamate

death in neuroblastoma SH-SY5Y cells (Lee et al. 2012). These results suggest that compounds **1**, **10**, **11**, and **12** may be useful as neuroprotective agents, but further intensive investigation is needed to determine how to best use these compounds in clinical practice.

Acknowledgments This study was financially supported from the Korea Food Research Institute (Grant Number 20140398U0054 101S00100) and the Kyung Hee University (S&P project: and sabbatical year project), Republic of Korea.

References

Ahn, D.K. 2012. *Sinnongbonchogyung, 525*. Seoul: Euiseungdang.

An, R.B., D.H. Sohn, and Y.C. Kim. 2006. Hepatoprotective compounds of the roots of *Cudrania tricuspidata* on tacrine-induced cytotoxicity in HepG2 cells. *Biological and Pharmaceutical Bulletin* 29: 838–840.

Dat, N.T., P.T.X. Binh, L.T.P. Quynh, C.V. Minh, H.T. Huong, and J.J. Lee. 2010. Cytotoxic prenylated flavonoids from *Morus alba*. *Fitoterapia* 81: 1224–1227.

Fukai, M., J.H. Song, J. Choi, H.J. Choi, and B.T. Zhu. 2009. Mechanism of glutamate-induced neurotoxicity in HT22 mouse hippocampal cells. *European Journal of Pharmacology* 617: 1–11.

Fukai, T., Y. Hano, K. Hirakura, T. Nomura, J. Uzawa, and K. Fukushima. 1985a. Structures of two natural hypotensive Diels-Alder type adducts, mulberrofurans F and G, from the cultivated mulberry tree (*Morus ihou* Koidz.). *Chemical & Pharmaceutical Bulletin* 33: 3195–3204.

Fukai, T., Y. Hano, K. Hirakura, T. Nomura, and J. Uzawa. 1985b. Structures of a novel 2-arylbenzofuran derivative and two flavone derivatives from the cultivated mulberry tree (*Morus ihou* Koidz.). *Chemical & Pharmaceutical Bulletin* 33: 4288–4295.

Fukai, T., Y.H. Pei, T. Nomura, C.Q. Xu, L.J. Wu, and Y.J. Chen. 1996. Components of the root bark of *Morus cathayana*. 1. Structures of five new isoprenylated flavonoids, sanggenols A-E and a diprenyl-2-arylbenzofuran, mulberrofuran V. *Heterocycles* 43: 425–436.

Fukai, T., Y. Hano, T. Fujimoto, and T. Nomura. 1983. Structure of sanggenon G, a new diels-alder adduct from the Chinese crude drug "Sang-Bai-Pi" (*Morus* root barks). *Heterocycles* 20: 611–615.

Geng, C.A., Y.B. Ma, X.M. Zhang, S.Y. Yao, D.Q. Xue, R.P. Zhang, and J.J. Chen. 2012. Mulberrofuran G and isomulberrofuran G from *Morus alba* L.: anti-hepatitis B virus activity and mass spectrometric fragmentation. *Journal of Agricultural and Food Chemistry* 60: 8197–8202.

Hano, Y., M. Itoh, N. Koyama, and T. Nomura. 1984. Constituents of the Chinese crude drug "Sang-Bai-Pi" (*Morus* root barks). V. Structures of three new flavanones, sanggenons L, M, and N. *Heterocycles* 22: 1791–1800.

Hano, Y., S. Suzuki, T. Nomura, and Y. Iitaka. 1988. Absolute configuration of natural Diels-Alder type adducts from the *Morus* root bark. *Heterocycles* 27: 2315–2325.

Hix, S., M.B. Kadiiska, R.P. Mason, and O. Augusto. 2000. In vivo metabolism of *tert*-butyl hydroperoxide to methyl radicals. EPR spin-trapping and DNA methylation studies. *Chemical Research in Toxicology* 13: 1056–1064.

Jeong, G.S., E. Byun, B. Li, D.S. Lee, R.B. An, and Y.C. Kim. 2010. Neuroprotective effects of constituents of the root bark of *Dictamnus dasycarpus* in mouse hippocampal cells. *Archives of Pharmacal Research* 33: 1269–1275.

Jiang, D., Z.D. He, R.W. Jiang, W.C. Ye, H.X. Xu, and P.P.H. But. 2003. Antiviral flavonoids from the root bark of *Morus alba* L. *Phytochemistry* 62: 1235–1238.

Jung, J.W., J.H. Park, Y.J. Jung, C.H. Lee, D.S. Han, and N.I. Baek. 2014. Isolation and identification of triterpenoids from the Mulberry (*Morus alba*) root bark. *Journal of Applied Biological Chemistry* 57: 295–299.

Kumar, R.V., and S. Chauhan. 2008. Mulberry: Life enhancer. *Journal of Medicinal Plants Research* 2: 271–278.

Lee, D.S., K.S. Kim, W. Ko, B. Li, G.S. Jeong, J.H. Jang, H. Oh, and Y.C. Kim. 2014. The cytoprotective effect of sulfuretin against *tert*-butyl hydroperoxide-induced hepatotoxicity through Nrf2/ARE and JNK/ERK MAPK-mediated heme oxygenase-1 expression. *International Journal of Molecular Sciences* 15: 8863–8877.

Lee, H.J., D.H. Lyu, U.K. Koo, K.W. Nam, S.S. Hong, K.O. Kim, K.H. Kim, D. Lee, and W. Mar. 2012. Protection of prenylated flavonoids from Mori cortex radicis (Moraceae) against nitric oxide-induced cell death in neuroblastoma SH-SY5Y cells. *Archives of Pharmacal Research* 35: 163–170.

Lee, N.K., K.H. Son, H.W. Chang, S.S. Kang, H. Park, M.Y. Heo, and H.P. Kim. 2004. Prenylated flavonoids as tyrosinase inhibitors. *Archives of Pharmacal Research* 27: 1132–1135.

Lim, D.W., Y.T. Kim, J.H. Park, N.I. Baek, and D. Han. 2014. Antidepressant-like effects of the ethyl acetate soluble fraction of the root bark of *Morus alba* on the immobility behavior of rats in the forced swim test. *Molecules* 19: 7981–7989.

Lin, C.N., P.H. Chiu, S.C. Fang, B.J. Shieh, and R.R. Wu. 1996. Revised structure of broussoflavonol G and 2D nmr spectra of some related prenyflavonods. *Phytochemistry* 41: 1215–1217.

Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* 65: 55–63.

Naik, R., D.S. Harmalkar, X. Xuezhen, K. Jang, and K. Lee. 2015. Bioactive benzofuran derivatives: moracins A-Z in medicinal chemistry. *European Journal of Medicinal Chemistry* 90: 379–393.

Nomura, T., T. Fukai, Y. Hano, and K. Tsukamoto. 1983. Constituent of the Chinese crude drug "Sang-Bai-Pi" (*Morus* root barks). III. Structure of a new flavanone derivative, sanggenon *F*. *Heterocycles* 20: 661–666.

Park, J.H., D.G. Lee, S.W. Yeon, H.S. Kwon, J.H. Ko, D.J. Shin, H.S. Park, Y.S. Kim, M.H. Bang, and N.I. Baek. 2011. Isolation of megastigmane sesquiterpenes from the silkworm (*Bombyx mori* L.) droppings and their promotion activity on HO-1 and SIRT1. *Archives of Pharmacal Research* 34: 533–542.

Piao, S.J., F. Qiu, L.X. Chen, Y. Pan, and D.Q. Dou. 2009. New stilbene, benzofuran, and coumarin glycosides from *Morus alba*. *Helvetica Chimica Acta* 92: 579–587.

Shi, Y.Q., T. Fukai, H. Sakagami, W.J. Chang, P.Q. Yang, F.P. Wang, and T. Nomura. 2001a. Cytotoxic flavonoids with isoprenoid groups from *Morus mongolica*. *Journal of Natural Product* 64: 181–188.

Shi, Y.Q., T. Fukai, W.J. Chang, P.Q. Yang, F.P. Wang, and T. Nomura. 2001b. Phenolic constituents of the root bark of Chinese *Morus australis*. *Natural Medicine* 55: 143–146.

Slade, D., D. Ferreira, and P.J. Marais. 2005. Circular dichroism, a powerful tool for the assessment of absolute configuration of flavonoids. *Phytochemistry* 66: 2177–2215.

Song, E.K., H. Cho, J.S. Kim, N.Y. Kim, N.H. An, J.A. Kim, S.H. Lee, and Y.C. Kim. 2001. Diarylheptanoids with free radical scavenging and hepatoprotective activity in vitro from *Curcuma longa*. *Planta Medica* 67: 876–877.

Takasugi, M., S. Nagao, T. Masamune, A. Shirata, and K. Takahashi. 1979. Structures of moracins E, F, G, and H, new phytoalexins from diseased mulberry. *Tetrahedron Letters* 48: 4675–4678.

Yang, Z.G., K. Matsuzaki, S. Takamatsu, and S. Kitanaka. 2011. Inhibitory effects of constituents from *Morus alba* var. *multicaulis* on differentiation of 3T3-L1 cells and nitric oxide production in RAW 264.7 cells. *Molecules* 16: 6010–6022.

Zhang, Z.P., H.Y. Tan, and M. Wang. 2012. Tyrosinase inhibition constituents from the roots of *Morus australis*. *Fitoterapia* 83: 1008–1013.