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Isoprenylated flavonoids from *Morus nigra* and their PPAR γ agonistic activities

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ABSTRACT

A novel dihydroflavonol unprecedentedly with a prenyl group at C-2, nigragenon A (**1**), four new sanggenon-type flavonones, nigragenons B–E (**2–5**), along with six known isoprenylated flavonoids (**6–11**) were isolated from the twigs of *Morus nigra*. Their structures were elucidated through extensive analysis of spectroscopic data. Interestingly, compound **1** was the first reported biogenetic precursor of sanggenon-type flavanones and the biogenetic pathway from **1** to sanggenol F was proposed. The PPAR γ agonistic activity was investigated in HEK293 cells using dual luciferase reporter assay. Compounds **2**, **4**, **7**, and **9** showed obvious agonistic activities on PPAR γ, and compound **2** was a potential PPAR γ partial agonist. Moreover, the preliminary structure-activity relationships for the tested compounds were discussed.

1. Introduction

The genus *Morus* (Moraceae), containing around 16 species, has both economic and medicinal value [1,2]. The plants of this genus are rich in isoprenylated phenolic compounds and alkaloids, which exhibited important biological activities including hypoglycemic, hypolipidemic, hypotensive, anti-inflammatory, and -antitumor effects [3].

Morus nigra Linn., the only 22-ploid species in genus *Morus*, is mainly planted in the southeastern region of Xinjiang province in China, and recognized in folk as "Yao-Sang" [4], which has been widely used as antihypertensive, anti-diabetic, and anti-inflammatory agents in the Uygur medicine [5]. Some flavonoids, coumarins, stilbenes, and 2-arylbenzofurans have been reported from the plant previously [1–3]. In our program of seeking natural anti-diabetic constituents, phytochemical study on *M. nigra* was conducted and the activities of PPAR γ activation of isolates were investigated.

Peroxisome proliferators-activated receptor γ (PPAR γ), a ligand-activated nuclear receptor.

transcription factor, acts as the major regulator of adipocyte gene expression and insulin cell signaling [6,7]. PPAR γ is an important antidiabetic target, and its agonists, such as Thiazolidinediones (TZDs) drugs, are widely used as insulin sensitizers to treat type 2 diabetes medllitus (T2DM) [8].

In our study, five new compounds, nigragenons A–E (**1–5**), as well as six known compounds (**6–11**), were isolated from the 90% ethanol extract of the twigs of *M. nigra* (Fig. 1). All of these compounds except **3** were tested for their agonistic activities on PPAR γ. The results indicated that compounds **2**, **4**, **7**, and **9** had PPAR γ agonistic effects, and compound **2** was a potential PPAR γ partial agonist. We report herein the isolation, structure elucidation, and PPAR γ agonistic activities of these compounds.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured using a Rudolph AUTOPOL VI polarimeter, UV spectra in methanol were recorded on a UV-2500PC instrument, and IR spectra were obtained on a Thermo Scientific Nicolet iS5 FT-IR spectrometer using KBr pellets. NMR spectra were recorded on a Bruker AV III 400 MHz NMR spectrometer with TMS as internal standard and acetone-d_6 (δ_H 2.05, δ_C 29.8 ppm) as solvent. HRESIMS was performed on a Waters Xevo G2-XS Q Tof mass spectrometer. ECD spectra were determined on a JASCO-810 spectropolarimeter. Column chromatography (CC) was performed on silica gel (200–300 mesh), Sephadex LH-20 (50–80 μm), and RP-C_{18} (40–80 μm), respectively. HPLC was conducted on a Waters 2695 connected to a Waters 2998 PDA detector at 254 and 210 nm equipped with a C_{18} column (250 × 4.6 mm, 5 μm). Semi-preparative HPLC was carried out on an Agilent 1100 equipped with a C_{18} column (250 × 10 mm, 5 μm)

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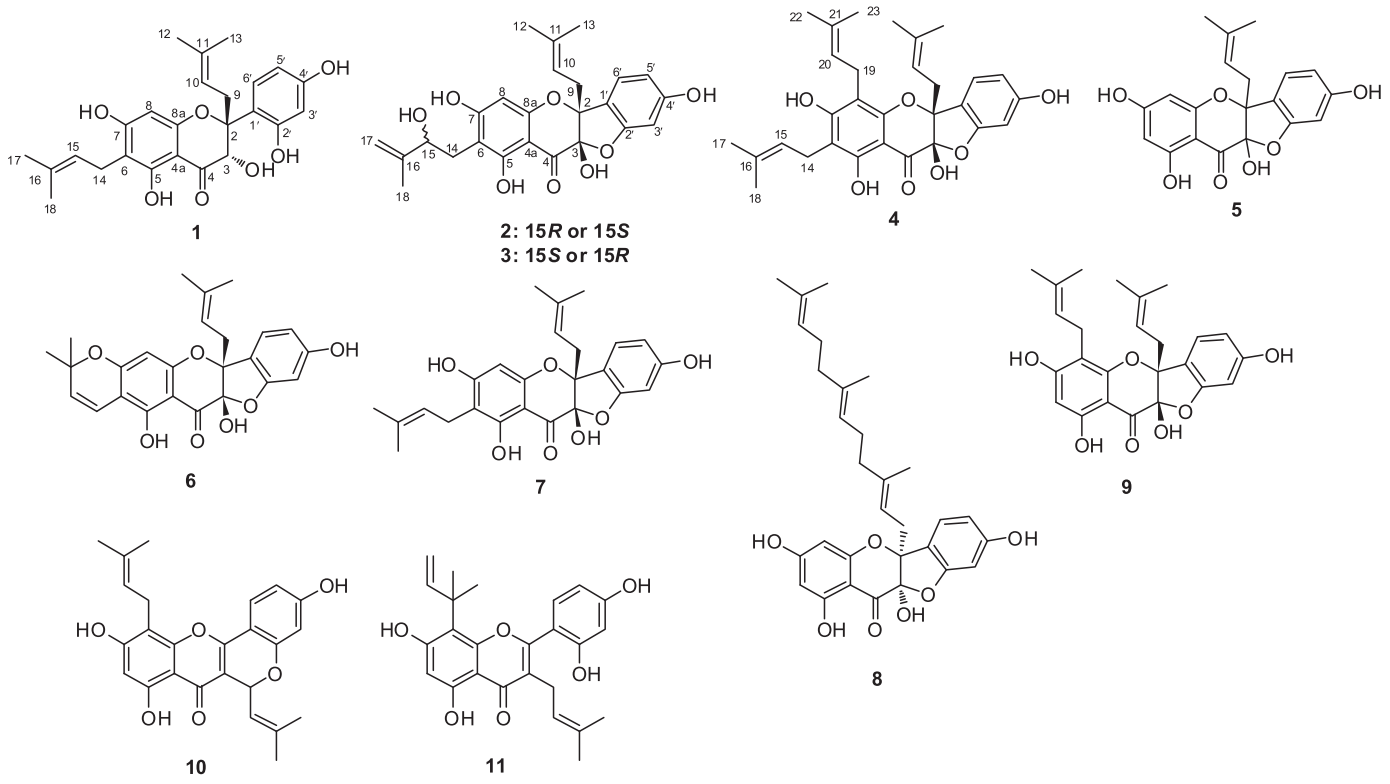

Fig. 1. The structures of compounds **1–11**.

using a UV detector at 254 and 210 nm. The chiral separation was performed on a Waters ACQUITY UPC^2 equipped with a Chiralcel OD-3 column (150 × 4.6 mm, 3 μm) using a PDA detector at 210 nm. Fractions were monitored by TLC, and spots were visualized by UV light (254 and 365 nm) and spraying with 10% H_2SO_4 in ethanol (v/v) followed by heating.

2.2. Plant material

The twigs of *M. nigra* were collected from Hetian town, in the Xinjiang province of China in Sep. 2016, and identified by Prof. Tong Wu, China State Institute of Pharmaceutical Industry, China. A voucher specimen (No. 201609001) has been deposited in our department.

2.3. Extraction and isolation

The dried and powdered twigs (15.0 kg) were extracted twice with 90% ethanol under hot reflux (1.5 h each time). After evaporation under vacuum, the extract was suspended in H_2O, and then partitioned with petroleum ether (PE), dichloromethane (DCM), ethyl acetate (EtOAc), and n-butanol (NBA), successively. The EtOAc portion (80.0 g) and DCM portion (120.0 g) were mingled and subjected to CC on silica gel with a gradient of CH_2Cl_2-MeOH (80:1, 50:1, 30:1, 20:1, 15:1, 10:1, 5:1, 1:1) to obtain six fractions (Fr.A–Fr.F). Fr.A (42.5 g) was fractionated on silica gel CC (PE-DMK, 15:1, 10:1, 7:1, 4:1, 3:1, 2:1, 1:1) to yield 18 sub-fractions (Fr.A1 − Fr.A18). Fr.A9 (5.8 g) was chromatographed on Sephadex LH-20 (CH_2Cl_2-MeOH, 1:1), followed by RP-C_{18} (MeOH-H_2O, 60:40 → 90:10) to obtain fractions (Fr.A9-1–Fr.A9-4). Fr.A9-4 (1.8 g) was purified by semi-preparative HPLC (CH_3OH-H_2O, 75:25) to afford compound **6** (84.9 mg). Fr.A10 (1.6 g) was treated with similar methods, firstly CC over Sephadex LH-20 (CH_2Cl_2-MeOH, 1:1), then on RP-C_{18} silica gel (CH_3OH-H_2O, 50:50 → 90:10), and finally semi-preparative HPLC eluted with CH_3CN-H_2O (60:40) to provide compounds **4** (9.0 mg) and **10** (2.0 mg). Fr.A12 (3.2 g) was purified by CC on Sephadex LH-20 (CH_2Cl_2-MeOH, 1:1), followed by ODS eluted with a gradient of CH_3OH-H_2O (60:40 → 90:10) to yield Fr.A12-1–Fr.A12-4. Fr.A12-1 (1.4 g) was subjected to semi-preparative HPLC (CH_3CN-H_2O, 58:42) to afford compound **7** (23.0 mg), and Fr.A12-2 (0.8 g) was purified by CC on Sephadex LH-20 (CH_2Cl_2-MeOH, 1:1) to provide compound **8** (70.0 mg). Fr.A15 (1.0 g) was subjected to CC over Sephadex LH-20 (CH_2Cl_2-MeOH, 1:1), followed by semi-preparative HPLC eluted with CH_3CN-H_2O (45:55) to give compounds **2** (22.0 mg) and **3** (17.2 mg). Fr·B (6.6 g) was separated by Sephadex LH-20 eluted with CH_2Cl_2-MeOH (1:1) to yield two sub-fractions (Fr·B1 and Fr·B2). Fr·B1 (4.5 g) was further purified by CC on RP-C_{18} (CH_3OH-H_2O, 20:80 → 70:30) to produce six fractions (Fr.B1-1–Fr.B1-6). Fr.B1-2 (0.9 g) gave compound **5** (4.6 mg) after being subjected to CC on silica gel (a gradient of CH_2Cl_2-MeOH, 70:1, 50:1, 30:1, 15:1, 2:1) and semi-preparative HPLC (CH_3CN-H_2O 40:60). Fr·B1-3 (1.2 g) was separated by CC on silica gel (a gradient of CH_2Cl_2-MeOH, 70:1, 50:1, 30:1, 15:1, 2:1) to provide Fr.B1-3-1–Fr.B1-3-4. Fr.B13-2 (87.2 mg) was further purified by semi-preparative HPLC (CH_3CN-H_2O, 48:52) to afford compounds **1** (3.9 mg) and **11** (6.0 mg). Compound **9** (8.0 mg) was obtained through Fr·B1-6 (0.8 g) being purified by CC on MCI (CH_3OH-H_2O, 30:70 → 100:0) and semi-preparative HPLC (CH_3CN-H_2O, 58:42).

2.4. Spectroscopic data

Nigragenon A (**1**): brownish-yellow powder; α_D^{25} −67.2 (*c* 0.25, MeOH); UV λ_{max} (MeOH) (log ε) 206 (2.87), 234 (sh) (1.24), 268 (1.35), 298 (2.35), 340 (sh) (1.20) nm; ECD (MeOH, nm) λ_{max} (Δε) 241 (−3.90), 263 (+0.16), 276 (+2.40), 301 (−2.74), 343 (+0.53); IR (KBr) ν_{max} 3398, 2955, 2924, 1632, 1454, 1121 cm^{-1}; ^{1}H and ^{13}C NMR data see Tables 1 and 2; negative ion HRESIMS *m/z*: 439.1754 [M–H]$^-$ (calcd for $C_{25}H_{27}O_7$, 439.1757).

Nigragenon B (**2**): yellow oil; α_D^{25} +103.9 (*c* 1.01, MeOH); UV λ_{max} (MeOH) (log ε) 206 (4.53), 233 (sh) (4.33), 287 (sh) (3.63), 307 (3.96) nm; ECD (MeOH, nm) λ_{max} (Δε) 216 (+3.60), 238 (−0.82), 252 (+0.85), 275 (−0.74), 291 (+1.68), 301 (+1.21); IR (KBr) ν_{max} 3384, 2955, 2924, 2853, 1635, 1458, 1095, 960, 756 cm^{-1}; ^{1}H and ^{13}C NMR