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Novel anti-tumour barringenol-like triterpenoids from the husks of *Xanthoceras sorbifolia* Bunge and their three dimensional quantitative structure activity relationships analysis

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ABSTRACT

The high edible oil content of *Xanthoceras sorbifolia* Bunge seeds contributes to its economic value. In this study, we analysed the barrigenol-like triterpenoids derived from *X. sorbifolia* husks. We also identified anti-tumour agents that could enhance the health benefits and medicinal value of *X. sorbifolia*. We isolated 10 barrigenol triterpenoids, including six new compounds (**1**–**6**) and four known compounds (**7**–**10**). New compounds **3** and **5** showed significant inhibitory activity against the proliferation of three human tumour cell lines, namely, HepG2, HCT-116 and U87-MG. We determined the relationship between the structures and inhibitory activity of 25 barrigenol triterpenoids and 15 penta-cyclic triterpenoids through analysis of three-dimensional quantitative structure activity relationships (3D-QSAR). The isolation of novel barrigenol derivatives with anti-tumour activity from *X. sorbifolia* implied that husks of this plant may be a good source of anti-tumour agents.

1. Introduction

Primary prevention of cancer in daily life has been extensively investigated. Cancer development may be prevented through suppression of tumour promotion by using functional constituents of plant resources. Barrigenol-like triterpene is an outstanding derivative of oleanane-type penta-cyclic triterpene and is considered a promising plant-derived anti-tumour agent [1]. The C-3, C-21 and C-28 positions of this triterpene are replaced with sugar chains, which usually contain one or more molecules of D-glucose, D-glucuronic acid, D-galactose, D-fucose, L-rhamnose and L-arabinose [2,3]. C-16, C-21, C-22, C-28 and the carbon of D-glucose may be linked by alkyl derivatives, such as acetoxy, angeloyl, tigloyl and 2-methylbutanoyl groups [3,4].

Xanthoceras sorbifolia Bunge belongs to the Sapindaceae family and is widely distributed throughout northern regions in China, particularly in the Inner-Mongolia autonomous region [2,5]. *X. sorbifolia* is an important woody oil crop with an oil content of >55%. *X. sorbifolia* seed oil has also been used in the food industry. The total fatty acids of *X. sorbifolia* kernel contains >90% unsaturated fatty acids as well as >40%, 30% and 10% 9,12-octadecadienoic acid, 9-octadecadienoic acid and 11-eicosenoic acid, respectively [6]. Oil production from *X. sorbifolia* generates a large amount of waste, including husks and seed meals. Extracts from *X. sorbifolia* husks exhibit anti-inflammatory, anti-HIV, anti-tumour and anti-Alzheimer activities. Chemical studies have revealed that barrigenol-type triterpenoids are the main active components responsible for these inhibitory activities [7,8].

As part of our on-going search for anti-tumour agents derived from secondary metabolites of medicinal and food plants [9–11], this study aims to chemically characterize barrigenol-type triterpenoids in *X. sorbifolia* Bunge husks and evaluate their anti-tumour activities. Our study also aims to establish scientific knowledge on this economically and pharmaceutically important nutritional crop. The 3D quantitative

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structural activity relationships (3D-QSAR) of 40 compounds were also analysed. The relationship of the structures of barrigenol derivatives and other penta-cyclic triterpenoids to their inhibitory activities were determined through comparative molecular field analysis (CoMFA) and comparative molecular similarity index analysis (CoMSIA).

2. Experimental details

2.1. Apparatus and instruments

Nuclear magnetic resonance (NMR) spectra were obtained with Bruker ARX-300, AV-400, or AV-600 spectrometers (Bruker, Billerica, MA, USA). Chemical shifts were expressed in δ (ppm) with tetramethylsilane as an internal standard. HR-ESI-MS spectra were recorded on a Bruker micro-TOF-Q mass spectrometer, whereas IR spectra were recorded on a Bruker IFS-55 Fourier transform infrared spectrometer (Bruker). Rotation spectra were obtained with a JASCO PU-4100 pump and JASCO ORD-4090 ORD spectrophotometric detector with an NH2P-50 4E column (20 × 250 mm). Specific rotations were obtained with an Anton-Paar MCP 200 polarimeter (Anton-Paar, AT). Silica gel (200-300 mesh, Qingdao Marine Chemical Co., Ltd.), macroporous adsorption resin D101 (Langfang Nanda Resin Co., Ltd.) and Sephadex LH-20 (Pharmacia Biotech, USA) were used for open-column chromatography. The compounds were purified on an Agela P1050 pump and Agela UV1000D UV detector (wavelength: 210 nm) by using a Mightysil RP-18 GP 250-20 column (5 μm, 20 × 250 mm). Flash chromatography was conducted on an Agela Cheetah Flash System with an ODS Flash Column (spec: C-18, 80 g, 120 g, 20–45 μm; Tianjin Agela Technologies Co., Ltd.). Cytotoxicity assay was performed with a microplate spectrophotometer (Gemini EM; Molecular Devices).

2.2. Plant material

The husks of *X. sorbifolia* were collected from the Chifeng City Inner-Mongolia autonomous region, China in 2011 and were identified by Associate Professor Jiu-Zhi Yuan of Shenyang Pharmaceutical University. A voucher specimen (ZB-11-XS001A) was deposited in the Department of Natural Products Chemistry, Shenyang Pharmaceutical University, Shenyang, China.

2.3. Extraction and isolation

Extraction was performed using methods reported in our previous articles [11]. The air-dried husks of *X. sorbifolia* (15.0 kg) were powdered and extracted three times with 70% (v/v) ethanol under reflux for 2 h each time. The solution was concentrated under vacuum to obtain the crude extract (1050 g). The product was dissolved and suspended in 15 L of water and successively extracted with ethyl acetate (EtOAc) and *n*-butyl alcohol (*n*-BuOH) three times to yield EtOAc soluble fraction (185 g), *n*-BuOH soluble fraction (390 g) and aqueous residues. The *n*-BuOH extract (350 g) was then subjected to silica gel column chromatography and eluted with $CH_2Cl_2/CH_3OH/H_2O$ (30:1:1–1:1:1) to obtain fractions 1–12 based on TLC analysis. Fraction 6 (8 g) was further subjected to silica gel column chromatography and eluted with CH_2Cl_2/CH_3OH (15:1–1:1) to obtain eight sub-fractions (sub-fraction 6.1–6.8). Compounds **1** (15 mg), **2** (19 mg), **3** (28 mg), **4** (15 mg), **5**(14 mg) and **6** (15 mg) were obtained from sub-fraction 6.5 (2.8 g) by RP-18 HPLC (CH_3OH-H_2O, 78:22). Fraction 11 (75.9 g) was isolated by ODS flash column chromatography system (CH_3OH-H_2O, 10:90–80:20) to yield nine sub-fractions (sub-fraction 11.1–11.9). Furthermore, compounds **7** (17 mg), **8** (116 mg), **9** (21 mg) and **10** (12 mg) were isolated from sub-fraction 11.7 (1500 mg) by preparative RP-18 HPLC (CH_3OH-H_2O, 68:32).

Compound **1**: 3-*O*-[α-L-arabinofuranosyl (1 → 3)]-[β-D-galactopyranosyl (1 → 2)]-β-D-(6-*O*-*n*-butyl)-glucuronopyranosyl-21-*O*-(3,4-*O*-diangeloyl)-β-D-fucopyranosyl-22-*O*-acetyl-barringtogenol C; white amorphous powder (CH_3OH), $[\alpha]_D^{20}$ + 42.3 (*c* 0.52, CH_3OH); IR (KBr) ν_{max} 1072, 1722, 2929, and 3425 cm^{-1}; ^1H NMR (C_5D_5N, 600 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz) were shown in Table 1; HR-ESI-MS (positive-ion model) *m/z* 1391.6949 $[M + Na]^+$ (calcd for $C_{69}H_{108}O_{27}Na$, 1391.6976).

Compound **2**: 3-*O*-[α-L-arabinofuranosyl (1 → 3)]-[β-D-galactopyranosyl(1 → 2)]-β-D-(6-*O*-*n*-butyl)-glucuronopyranosyl-21-*O*-(3,4-*O*-diangeloyl)-β-D-fucopyranosyl-28-*O*-acetyl- barringtogenol C; white amorphous powder (CH_3OH); $[\alpha]_D^{20}$ + 97.1 (*c* 0.53, CH_3OH); IR (KBr) ν_{max} 1040, 1642, 1725, 2924, and 3436 cm^{-1}; ^1H NMR (C_5D_5N, 300 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz) were shown in Table 1; HR-ESI-MS (positive-ion model) *m/z* 1391.6952 $[M + Na]^+$ (calcd for $C_{69}H_{108}O_{27}Na$, 1391.6976).

Compound **3**: 3-*O*-[α-L-arabinofuranosyl (1 → 3)]-[β-D-galactopyranosyl (1 → 2)]-β-D-(6-*O*-*n*-butyl)-glucuronopyranosyl-21, 22-*O*-diangeloyl-R_1-barrigenol C; white amorphous powder (CH_3OH); $[\alpha]_D^{20}$ + 27.1 (*c* 1.18, CH_3OH); IR (KBr) ν_{max} 1081, 1722, 2959, and 3432 cm^{-1}; ^1H NMR (C_5D_5N, 300 MHz) and ^{13}C NMR (C_5D_5N, 75 MHz) were shown in Table 2; HR-ESI-MS (positive-ion model) *m/z* 621.3087 1/2 $[M + 2Na]^+$ (calcd for $C_{61}H_{96}O_{23}NaNa$, 1242.6138).

Compound **4**: 3-*O*-[α-L-arabinofuranosyl (1 → 3)]-[β-D-galactopyranosyl (1 → 2)]-β-D-(6-*O*-*n*-butyl)-glucuronopyranosyl-21, 22-*O*-diangeloyl-barringtogenol C; white amorphous powder (CH_3OH); $[\alpha]_D^{20}$ + 34.7 (*c* 0.72, CH_3OH); IR (KBr) ν_{max} 1112, 1632, 2923, and 3427 cm^{-1}; ^1H NMR (C_5D_5N, 300 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz) were shown in Table 2; HR-ESI-MS (positive-ion model) *m/z* 1203.6226 $[M + Na]^+$ (calcd for $C_{61}H_{96}O_{22}Na$, 1203.6291).

Compound **5**: 3-*O*-[α-L-arabinofuranosyl (1 → 3)]-[β-D-galactopyranosyl (1 → 2)]-β-D-(6-*O*-*n*-butyl)-glucuronopyranosyl-21-*O*-angeloyl-22-*O*-(2-methyl) butyryl-R_1-barrigenol C; $[\alpha]_D^{20}$ + 48.0 (*c* 0.63, CH_3OH); IR (KBr) ν_{max} 1075, 1639, 1711, 1927, 1960, and 3423 cm^{-1}; ^1H NMR (C_5D_5N, 300 MHz) and ^{13}C NMR (C_5D_5N, 75 MHz) were shown in Table 2; HR-ESI-MS (positive-ion model) *m/z* 1221.6309 $[M + Na]^+$ (calcd for $C_{61}H_{98}O_{23}Na$, 1221.6397).

Compound **6**: 3-*O*-[α-L-arabinofuranosyl (1 → 3)]-[β-D-glucopyranosyl (1 → 2)]-β-D-(6-*O*-*n*-butyl)-glucuronopyranosyl-21, 22-*O*-diangeloyl-24-hydroxy-R_1-barrigenol; $[\alpha]_D^{20}$ + 18.7 (*c* 0.80, CH_3OH); IR (KBr) ν_{max} 1081, 1644, 1721, 2926, 3420 cm^{-1}; ^1H NMR (C_5D_5N, 300 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz) are shown in Table 2; HR-ESI-MS (positive-ion model) *m/z* 1235.6187 $[M + Na]^+$ (calcd for $C_{61}H_{96}O_{24}Na$, 1235.6189).

2.4. Acid hydrolysis

The sugars present in the compounds were identified by the method reported in our previous work [11]. Briefly, 4 mg each of compounds **1**–**6** were incubated in 5 mL of 2 M HCl and refluxed for 5 h at 90 °C. The mixture was extracted with 8 mL of chloroform three times. The upper aqueous layers were repeatedly evaporated to remove HCl. The residues were analysed by HPLC with an NH_2P-50 4E column (4.6 × 250 mm, Showa Denko K.K., Japan) and an optical rotation detector (JASCO ORD-4090, JASCO International Co, Ltd., Japan) under the following chromatographic conditions: mobile phase, acetonitrile-water (75:25, v/v); flow rate, 0.8 mL/min. Monosaccharides from the isolated compounds were identified using standard samples. L-Arabinose (Tokyo Chemical Industry Co. Ltd., Lot. LQAGA-A1), D-glucose (ChromaDex Inc., Lot. 00,007,265-1JA), D-galactose (Tokyo Chemical Industry Co. Ltd., Lot. ISDKF-DH), and D-fucose (Aladdin Co. Ltd., Lot. #D1503126) were detected at 11.4 min (positive), 15.3 min (positive), 15.6 min (positive), and 9.7 min (positive), respectively.

2.5. Cell culture

All cell lines were purchased from the Stem Cell Bank, Chinese Academy of Science, China. HCT-116 and HepG2 cells were cultured in DEME (Gibco, USA) supplemented with 10% FBS (Gibco) and 0.5% penicillin/