Cytotoxic cycloartane triterpenes from the roots of *Cimicifuga heracleifolia*

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**A B S T R A C T**

Phytochemical investigation on the roots of *Cimicifuga heracleifolia* afforded nine new 9,19-cycloartane triterpenes (1–9), along with seven known constituents (10–16). The new structures were elucidated by spectroscopic and chemical methods. Biological evaluation of the compounds against human HL-60, SMMC-7721, A549, MCF-7, and SW-480 cell lines indicated that eight cimigenol-type compounds (1–3, 5, 10–12, 14) showed different levels of cytotoxic activities, with IC_{50} values ranging from 0.83 to 23.94 μM. In addition, their structural and bioactive features enriched the structure–activity relationships we proposed before.

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1. Introduction

*Cimicifuga* (now *Actaea* in Europe and USA), belonging to the family Ranunculaceae, consists of 28 species and has a long history to be used as medicinal herb worldwide. Previous chemical and biological studies on *Cimicifuga* species led to isolation of various bioactive constituents, such as chromones, cinnamic acid derivatives, and 9,19-cycloartenol triterpenes. In China, the roots of *C. foetida*, *C. dahurica*, and *C. heracleifolia* are important elements of Traditional Chinese Medicine (TCM), namely ‘shengma’ and have been officially listed in the Chinese Pharmacopoeia as cooling and detoxification agents.

In the theory of TCM, tumor is defined as a kind of toxin, so it is of interest to study antitumor activities of plants used as detoxification agents. Intriguingly, we chose *C. foetida* as an object to investigate potential antitumor constituents and reported a number of cytotoxic 9,19-cycloartenol triterpenes both from the roots and aerial parts of this medicinal plant. Moreover, the preliminary structure–activity relationships (SAR) of the compounds with a cimigenol-skeleton were proposed based on the analyses of related bioassay results.

Although *C. heracleifolia* is a famous TCM, few works of its chemical constituents and bioactivities have been reported. Inspired by what our group achieved studies in *C. foetida*, using the theory of tumor in TCM as a guiding principle, we further undertook phytochemical and pharmacological investigations on the roots of *C. heracleifolia*. Nine new 9,19-cycloartenol triterpenes (1–9), together with seven known compounds, 7,8-didehydro-25-O-acetyl-17-dehydrocimigenol-3-O-α-L-arabinopyranoside (10), 25-anhydrocimigenol-3-O-β-D-xylopyranoside (11), 7,8-didehydro-25-anhydrocimigenol-3-O-β-D-xylopyranoside (12), 24-epi-7,8-didehydrocimigenol (13), acerol (14), 23-epi-26-deoxy-7,8-didehydroacteinol (15), and acerolin (16), were isolated and characterized (Scheme 1). The isolated compounds were tested for their cytotoxicities against human HL-60, SMMC-7721, A549, MCF-7, and SW480 cell lines, using the MTT method. Described herein are the isolation, structure elucidation, and biological activities of these compounds.

2. Results and discussion

Compound 1 was obtained as white powder and gave a molecular formula of C_{31}H_{50}O_{7}, as determined by HRTOF-ESIMS ([M+Cl]– m/z 569.3236, calcld 569.3245), requiring 7 carbons at 1741 cm\(^{-1}\), and carbon groups at 1741 cm\(^{-1}\), respectively. The ^1H NMR spectrum (Table 1) showed the presence of the characteristic cyclopropene methylene signals at δ_H 0.59 and 0.83 (1H each, d, J = 4.0 Hz), a sec-methyl signal at δ_H 0.85 (d, J = 6.5 Hz), and six tert-methyl groups at δ_H 1.16, 1.26, 1.37 (×2), 1.46, and 1.48, respectively. Above evidence, together with the diagnostic signals of two oxygen-bearing methine carbons at δ_C 90.7 (C-24), and 72.3 (C-23), and an oxygen-bearing quaternary carbon at δ_C 112.5 (C-16), suggesting 1 is a cimigenol-type triterpene. Apart from a carbonyl group, the remaining degrees of unsaturation in 1 is one less than those of cimigenol (17). Based on the evidence, we may deduce that one of the rings was opened in 1. Comparison of the NMR data of 1 with those of 17 suggested the two compounds are structurally similar, except for...
the major differences at ring A. In the $^{13}$C spectrum of 1, a hydroxymethine signal due to C-3 at $\delta_{C}$ 78.0 (d) was absent, showing instead an ester carbonyl group ($\delta_{C}$ 175.3). Besides, the signal due to C-4 exhibited a downfield shift from $\delta_{C}$ 41.1 to 75.6. The changes of these chemical shifts may be due to the oxidative cleavage of ring A through formation of a lactone between C-3 and C-4, and followed by hydrolysis and methyl esterification at C-3. The HMBC correlations of methoxy signal at $\delta_{H}$ 3.58 with the ester carbonyl group at $\delta_{C}$ 175.3, and CH$_3$-29 and CH$_3$-30 signals at $\delta_{H}$ 1.37 (2H) with the hydroxy bearing quaternary carbon at $\delta_{C}$ 75.6 further confirmed the deduction (Fig. 1). Significant ROESY correlations of H-5 with CH$_3$-28 suggested an $\alpha$-orientation of the proton. In addition, the associations of H-15 with H-8 and Me-18, indicated an $\alpha$-orientation of the hydroxy group at C-3. The configurations of H-15 and H-14 were assigned as R and S, respectively, by comparison of the coupling constants of H15-H16 and H14-H15 of 1 with those of known compounds. Therefore, the structure of 1 was determined as methyl 3,4-seco-4-hydroxy-3-cimigenolate.

Compounds 2 and 3 were isolated as white powder. The spectroscopic features of compounds 2 and 3 resembled each other. The HRTOF-ESIMS of both compounds exhibited a pseudo-molecular ion at m/z 703.40 [M–H]$^-$ (2, m/z 703.4042; 3, m/z 703.4066) for the same molecular formula of C$_{39}$H$_{60}$O$_{11}$ (calcld 703.4057 for C$_{39}$H$_{59}$O$_{11}$). The $^1$H NMR spectrum (Table 1) of 2 indicated presence of the characteristic cyclopropane methylene signals at $\delta_{H}$ 0.20 and 0.46 (each 1H, d, $J=3.5$ Hz), seven methyl groups at $\delta_{H}$ 0.97–1.48, two acetoxymethyl groups at $\delta_{H}$ 1.78 and 2.11, and an anemic proton at $\delta_{H}$ 4.75, suggesting 2 is a 9,19-cycloartane triterpene monoglycoside with two acetoxy groups. A correlation was observed between the anomeric proton at $\delta_{H}$ 4.75 (H-1$^0$, 1H, d, $J=7.5$ Hz) and the methine signal at $\delta_{C}$ 88.9 (C-3) in the HMBC spectrum, suggesting that a sugar moiety was attached at the C-3. In addition, H-4$^0$ signal of 2 was a broad singlet, the coupling constants of H1$^0$-H2$^0$ and H2$^0$-H3$^0$ were both 7.5 Hz. These evidence together with the correlations between H-1$^0$ and H-3, H-1$^0$ and H-3$^0$, and H-1$^0$ and H-4$^0$ in the ROESY spectrum indicated that the protons at C-1$^0$, C-2$^0$, C-3$^0$, and C-4$^0$ are in the $\alpha$-, $\beta$-, and $\beta$-positions, respectively, as found in $\alpha$-L-arabinopyranose. The sugar obtained after acid hydrolysis was confirmed as $\alpha$-L-arabinopyranose by comparing its TLC and specific rotation with the standard. The NMR spectroscopic data (Tables 1 and 2) of 2 and 3 were consistent with those of the known compounds. Therefore, the structure of 2 was determined as methyl 2-O-acetyl-$\alpha$-L-arabinopyranosyl.
and 3) of 2 were similar to those of cimigenol-3-O-[2'-O-acetyl]-α-L-arabinopyranoside (18), except for the sugar moiety. The molecular weight of 2 is 42 Da more than those of 18, which may ascribe to an acetyl group. In the 1H NMR spectrum of 2, the signal due to H-40 exhibited a downfield shift from δH 4.26 (m) to 5.54 (br s). In the 13C and DEPT spectra, the signal due to C-40 exhibited a downfield shift from δC 69.8 to 72.4, whereas the signal due to C-30 and C-50 showed an upfield shift from δC 72.5 to 70.3, and δC 67.3 to 64.5, respectively.

Based on above evidence, we deduced that another O-acetyl group is attached at C-40 of the arabinopyranose unit of 2, which was further confirmed by the presence of the HMBC correlation.
between the H-4' signal at δH 5.54 and the O-acetyl group at δC 170.7. Thus, the structure of 2 was assigned as cimigenol-3-O-[2',4'-diacetyl]-α-L-arabinopyranosyl. In the same way, two acetoy groups were determined to be at C-3' and C-4' of the α-L-arabinopyranosyl unit for 3, which was further confirmed by the presence of the HMBC correlations between the H-3' and H-4' signal at δH 5.54, 5.66 and the carbonyl group signal at δC 170.6 and 170.5, respectively. Therefore, 3 was identified as cimigenol-3-O-[3',4'-diacetyl]-α-L-arabinopyranosyl.

Compound 4 was isolated as white powder. The 13C NMR and HRTOF-ESIMS established the molecular formula of 4 as C73H58O10. The NMR spectroscopic data of 4 (Tables 1 and 3) are similar to those of 1-5, with the major differences at sugar unit. A significant downfield signal was observed at δH 5.52 (dd, J=2.5, 8.5 Hz) in the 1H NMR spectrum. In addition, the proton signals were correlated with the methine signal at δH 4.66 (H-2'), which, in turn, exhibited a correlation with an anumeric proton at δH 4.92 in the 1H-1H COSY spectrum. Based on above evidence, we deduced an acetoxy group attached at C-3'. This result was confirmed by the presence of the HMBC correlation between the H-3' signal at δH 5.52 and the formyl group signal at δC 170.9. The sugar was identified as α-L-arabinopyranosyl by the same way as that of 4. Therefore, 4 was elucidated as 25-anhydrocimigenol-3-O-[3'-O-acetyl]-α-L-arabinopyranosyl.

Compound 5 was isolated as white powder. In the high-resolution negative TOF-ESIMS, it showed a pseudo-molecular ion at m/z 485.3257 [M+Na]−, corresponding to the molecular formula of C30H48O6. Its IR spectrum showed hydroxy, carbonyl, and double bond absorptions at 3423, 1725, and 1635 cm−1, respectively. The 13C NMR data of 5 were similar to those of 25-anhydrocimigenol-3-O-[3'-O-acetyl]-α-L-arabinopyranosyl.

Compound 6 was isolated as white powder. The high-resolution negative TOF-ESIMS showed a pseudo-molecular ion at m/z 485.3257 [M+Na]−, corresponding to the molecular formula of C30H48O6. Its IR spectrum showed hydroxy, carbonyl, and double bond absorptions at 3423, 1725, and 1635 cm−1, respectively. The 13C NMR data of 6 was similar to those of 25-anhydrocimigenol-3-O-[3'-O-acetyl]-α-L-arabinopyranosyl. In the ROESY spectrum, CH3-28 showed a correlation with the formyl group at δC 215.0. Comparison of the 13C NMR data of 6 with those of 24-epi-cimigenol (20)10,16 suggested the two compounds are structurally similar and the differences may be deduced by a methine at δC 78.1 (C-3) was transformed to a carbonyl carbon at δC 215.0. This assignment was supported by the HMBC correlation between H-2/C-3, and CH3-29, CH3-30/C-3, as well as the upfield shift of C-2 about 7.6 ppm in the 13C spectrum. The configurations of C-23-C-24 are proposed as R and R, respectively, by comparing the coupling constants of H-23 (9.6 Hz) and H-24 (4.0 Hz) of 6 with those of known 9,19-cyclolanostane triterpenes.10,15 Ultimately, 6 was elucidated as 24-epi-cimigenol-3-one.

Compound 7 was isolated as white powder. The positive HRTOF-ESIMS showed a quasi-molecular ion at m/z 495.3206 [M+Na]+, corresponding to the molecular formula of C30H48O6. Its IR spectrum showed hydroxy, carbonyl, and double bond absorptions at 3423, 1725, and 1635 cm−1, respectively. The 13C NMR data of 7 were similar to the aglycone resonances of 15,16-seco-14-carboxyl-16-oxo-hydroshengmanol-3-O-β-D-xylanopyranoside (21)20 except for the presences of two olefinic carbons at δC 122.4 and 140.5 and a significant downfield carbonyl signal at δC 204.0. The correlations between H-6 at δH 1.97, 1.57 and olefinic proton at δH 5.58 in 1H COSY spectrum indicated the double bond at δC 7-8 and C-28 in HSQC spectrum, the proton at δH 9.85 (s) correlated with the downfield carbonyl signal, showing there is a formyl group in 7. In addition, the downfield shift of the carbonyl group by 21.8 ppm, as well as 18 Da of molecular weight less than those of aglycone part of 21 further confirmed above deduction. The correlations of CH3-28 at δH 1.62 with the carbonyl signal at δC 204.4, and the proton of the formyl group at δH 9.85 with C-14 at δC 59.5 in the HMBC spectrum revealed the formyl group was attached at C-14. In the ROESY spectrum, CH3-28 showed a correlation with H-5, while H-17 showed a correlation with Me-21, suggesting both CH3-28 and H-17 in an α-orientation. In addition, the ROESY correlation between H-23 and H-20 (β-orientation) indicated the configuration of C-23 as R. The configuration of C-24 is proposed as S by comparison of the coupling constants of H-24 (4.0 Hz) of 7 with the known compounds.20 Accordingly, compound 7 was characterized as 15,16-seco-7,8-didehydro-14-formyl-16-oxo-hydroshengmanol.

Compound 8 was isolated as white powder. The negative HRTOF-ESIMS showed a pseudo-molecular ion at m/z 505.3083 [M+Cl]−, leading to the molecular formula C38H46O4, which is 14 Da less than...
those of cimicidanol (22),21 All the NMR data of 8 resembled to those of 22 and suggested the configuration at C-24 was the same as that of 22, which was assigned as R. Moreover, for 8, a methine at δC 63.0 (C-11) was transformed to a methylene at δC 115.6 (C-7, d) and 147.1 (C-8, s), were reduced to a methylene at δC 26.6 (t) and a methine at δC 47.7 (d). The correlations of H-12 (δH 1.20 and 1.72, 1H each) with C-11 (δC 26.7) and H-6 (δH 0.72 and 1.60, 1H each) with C-7 (δC 26.6) and C-8 (δC 47.7) in HMBC spectrum and H-7/H-8 and H-11/H-12 correlations in 1H–1H COSY spectrum confirmed above conclusion. Thus, compound 8 was characterized as 7,8-dihydro-11-dehydrocimicidanol.

Compound 9 was isolated as white powder. In the high-resolution positive TOF-ESIMS, it showed a quasi-molecular ion at m/z 685.3930 [M+Na]+, for a molecular formula of C37H39OS10O. The 1H and 13C spectrum (Tables 2 and 3) exhibited signals very similar to those of shengmanol-3-O-α-L-arabinopyranoside (23)22 except for the sugar moiety. Using the same way as that of 2, an O-acetyl group was determined to be at C-2 of the sugar unit, which was further confirmed by the presence of the HMBC correlation between H-2′ (δH 5.84) and the carbonyl carbon at δC 170.3. The sugar was identified as α-L-arabinopyranoside after acid hydrolysis. Moreover, the ROESY correlation between H-23 and H-20 (β-orientation) assigned the configuration of C-23 as R. The configuration of C-24 of 9 was proposed as S by the same way as that of compound 1.10,22

Therefore, compound 9 was elucidated as shengmanol-3-O-[2′-O-acetyl]-α-L-arabinopyranoside.

All isolated compounds were evaluated against human HL-60, SMMC-7721, A549, MCF-7, and SW480 cell lines for their cytotoxicities. As summarized in Table 4, nine compounds (1–3, 5, 7, 10–12, 14) showed different levels of activities. Among the active triterpenes, compound 1 (did not have enough weight to test last two cell lines) showed as strong activity as positive control cisplatin against HL-60 cells with IC50 values of 0.83 μM, and more potent activities against SMMC-7721 and A549 cell lines, having IC50 values of 2.59 and 1.41 μM. While, compounds 5, 10, and 12 exhibited stronger cytotoxicities than cisplatin against all testing cell lines except HL-60, with IC50 values ranging from 5.02 to 11.40 μM. In addition, Compounds 2, 3, 7, 11, and 14 exhibited broad spectrum and weak activities with IC50 values around 15–25 μM. Above results further supported the theory of tumor in TCM as a guiding principle to explore potential antitumor agents from herb medicines and the roots of C. heracleifolia is a promising resource for anti-cancer agents.

In present study, another seven active glycosides (2, 3, 5, 10–12, 14) with a cimigenol skeleton were discovered. Either hydrophobic groups like acetoxy at the sugar unit or hydroxy group at C-25 besides last two compounds exhibited broad spectrum activities against SMMC-7721, A549, and SW480 cell lines, having IC50 values of 2.59 and 1.41 μM. In present study, another seven active glycosides (2, 3, 5, 10–12, 14) with a cimigenol skeleton were discovered. The ROESY correlation between H-23 and H-20 (β-orientation) assigned the configuration of C-23 as R. The configuration of C-24 of 9 was proposed as S by the same way as that of compound 1.10,22

Therefore, compound 9 was elucidated as shengmanol-3-O-[2′-O-acetyl]-α-L-arabinopyranoside.
potent activities than glycoside in present study. The main structural features of 1 being that C-3 and C-4 were cleaved and a methyl ester group was formed at C-3 through a flexible carbon chain (C-1 and C-2). The carbonyl group at C-3 showed different dimensional character as that of none ring opened active aglycon, actrin-3-one, which also contained a carbonyl group at C-3. Based on above analyses, we may suggest that the dimensional position of the carbonyl group at C-3 is critical to the activities of cimigenol-type triterpenes and afford a new perspective for chemical modification.

3. Experimental section

3.1. General experimental procedures

Optical rotations were measured in MeOH with a Horiba SEAP-300 polarimeter. 1H and 13C NMR spectrum were recorded in pyridine-d5 on Bruker DRX-500 and Avance III-600 MHz spectrometer (Bruker, Zürich, Switzerland). Unless otherwise specified, chemical shifts (δ) are expressed in ppm with respect to the solvent signals, ESIMS and HORTOF-ESIMS data were obtained using a VG Autospec-3000 spectrometer. Infrared spectra were recorded on a Shimadzu IR-450 instrument with KBr pellets. Thin-layer chromatography was performed on precoated TLC plates (200–250 µm thickness, Silica gel 60 F254, Qingdao Marine Chemical, Inc.) and spots were visualized by heating after spraying with 10% aq H2SO4 soln. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a VMAX-Pack Pro C18 RS 10 mm×250 mm column. Silica gel (200–300 mesh, Qingdao Marine Chemical, Inc.), Lichroprep RP-18 (40–63 µm, Merck), and Sephadex LH-20 (20–150 µm, Pharmacia) were used for column chromatography (cc).

3.2. Plant material

The roots of C. heraclefolia Kom. (1.0 kg) were collected from Qingyuan County, Liaoning Province, China, in September 2006 and identified by Prof. Zongyu Wang, Kunming Institute of Botany, Chinese Academy of Science. A voucher specimen (KUN No. 200609004) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, PR China.

3.3. Extraction and isolation

The dried and milled roots of C. heraclefolia (1.0 kg) were extracted with MeOH (3×3 L×24 h) at room temperature to give a residue (116 g) after evaporating in vacuum at 50 °C. The extract was subjected to silica gel cc (2 kg, 10×150 cm) and eluted with CHCl3/MeOH [100:0 (2 L), 50:1 (4 L), 20:1 (5 L), 10:1 (4 L), 0:100 (3 L)] to afford fractions A (24.5 g), B (11.1 g), C (12.8 g), D (17.1 g), and E (20.8 g). Fraction B (11.1 g) was divided into five sub-fractions (B-1 to B-5) after performing RP-18 cc (180 g, 5×25 cm), eluting with MeOH/H2O (gradient from 60:40 to 100:0, 10 L). Fraction B-3 (1.3 g) was subjected to repeated silica gel cc (40 g, 4×40 cm), eluted with CHCl3/Me2CO (gradient from 20:1 to 10:1, 4 L) and then semipreparative HPLC (eluted with CH3CN/H2O, gradient from 60:40 to 85:15) to yield 6 (4.0 mg), 13 (4.0 mg), 14 (5.1 mg). Compounds 8 (0.5 mg), 15 (2.3 mg), and 16 (1.5 mg) were purified from fraction 2.4 (1.3 g) by conducting silica gel cc (30 g, 3.5×40 cm), eluted with CHCl3/Me2CO (20:1, 3 L), followed by semipreparative HPLC (eluted with CH3CN/H2O, gradient from 65:35 to 85:15). Fraction B-5 (1.1 g) was applied to a silica gel (30 g) column (3×40 cm), eluted with CHCl3/Me2CO (20:1, 3 L), then purified on semipreparative HPLC (eluted with CH3CN/H2O, gradient from 65:35 to 90:10) to afford 1 (0.7 mg), and 7 (1.5 mg). Fraction C (12.8 g) was fractionated into three sub-fractions (C-1 to C-3) by performing RP-18 cc (180 g, 5×25 cm), eluting with MeOH/H2O (gradient from 50:40 to 90:10, 12 L). Fraction C-1 (2.8 g) was subjected to silica gel cc (50 g, 4×40 cm) eluted with CHCl3/Me2CO (gradient from 10:1 to 5:1, 4 L), then semipreparative HPLC (eluted with CH3CN/H2O, gradient from 60:40 to 75:25) to obtain 10 (3.3 mg), 11 (3.7 mg), and 12 (4.2 mg). Fraction C-2 (3.4 g) was chromatographed on a silica gel cc (50 g, 4×40 cm), eluting with CHCl3/Me2CO (10:1, 8 L), then repeated chromatography over semipreparative HPLC (eluted with CH3CN/H2O, gradient from 60:40 to 80:20) to yield 2 (6.2 mg), 3 (6.0 mg), 4 (5.0 mg), 5 (4.8 mg), and 9 (5.2 mg).

3.3.1. Methyl 3,4-seco-4-hydroxy-3-cimigenol (1). White powder; [α]D20 +20.00 (c 0.05, MeOH); IR (KBr) νmax 3430, 2965, 2828, 1714, 1456, 1383, 1027, 987 cm−1; 1H and 13C NMR data, see Tables 1 and 2; negative ESIMS m/z 569 [M+Cl]−; HORTOF-ESIMS m/z 569.3236 [M+Cl]− (calcd for C36H59O11, 569.3245).

3.3.2. Cimigenol-3-O-[2′,4′-diacetyl]-α-L-arabinopyranoside (2). White powder; [α]D20 +86.58 (c 0.17, MeOH); IR (KBr) νmax 3435, 2943, 2871, 1738, 1457, 1381, 1033, 987 cm−1; 1H and 13C NMR data, see Tables 1 and 2; negative ESIMS m/z 703 [M−H]−; HORTOF-ESIMS m/z 703.4042 [M−H]− (calcd for C43H66O19Cl, 703.4057).

3.3.3. Cimigenol-3-O-[3′,4′-diacetyl]-α-L-arabinopyranoside (3). White powder; [α]D20 +91.11 (c 0.18, MeOH); IR (KBr) νmax 3473, 2962, 2871, 1740, 1380, 1053, 987 cm−1; 1H and 13C NMR data, see Tables 1 and 2; negative ESIMS m/z 703 [M−H]−; HORTOF-ESIMS m/z 703.4066 [M−H]− (calcd for C43H66O19Na, 703.4057).

3.3.4. Cimigenol-3-O-[4′-O-acetyl]-α-L-arabinopyranoside (4). White powder; [α]D20 +26.52 (c 0.09, MeOH); IR (KBr) νmax 3449, 2932, 2867, 1735, 1459, 1382, 1030, 982 cm−1; 1H and 13C NMR data, see Tables 1 and 2; positive ESIMS m/z 685 [M+Na]+; HORTOF-ESIMS m/z 685.3929 [M+Na]+ (calcd for C42H65O18Na, 685.3927).

3.3.5. 25-Anhydrocimigenol-3-O-[3′-O-acetyl]-α-L-arabinopyranoside (5). White powder; [α]D20 +20.73 (c 0.18, MeOH); IR (KBr) νmax 3472, 2954, 2872, 1739, 1648, 1579, 1019, 901 cm−1; 1H and 13C NMR data, see Tables 1 and 2; negative ESIMS m/z 679 [M−Cl]−; HORTOF-ESIMS m/z 679.3613 [M−Cl]− (calcd for C42H64O18Cl, 679.3612).

3.3.6. 24-epi-Cimigenol-3-one (6). White powder; [α]D20 −2.11 (c 0.06, MeOH); IR (KBr) νmax 3430, 2962, 2868, 1715, 1452, 1383, 1027, 977 cm−1; 1H and 13C NMR data, see Tables 1 and 2; negative ESIMS
3.3.5. Cytotoxicity bioassay

implanted with 10% fetal bovine serum (Hyclone, USA), in 5% CO2. Cancer MCF-7, and colon cancer SW480, were used in the cytotoxicity assay. Hepatocellular carcinoma SMMC-7721, lung cancer A-549, breast cancer MDA-MB-231, and allowed to adhere for 12 h before addition of test compounds, a cell growth curve was graphed. IC50 values were calculated by Reed and Muench’s method.30

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2012.05.083. These data include MOL file and InChIKey of the most important compound described in this article.

References and notes