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Dinor- and 17-nor-pimaranes from *Icacina trichantha*

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Abstract

Six new pimarane derivatives, including two *dinor*-pimaranes (**1**, **2**), two *17-nor*-pimaranes (**3**, **4**), and two *17-nor*-(9 β -H)-pimaranes (**5**, **6**) were isolated from the tuber of *Icacina trichantha*. Their structures were elucidated based on spectroscopic and HRMS data. The absolute configurations of **1** and **5** were determined by single-crystal X-ray diffraction, and that of **2** was established by electronic circular dichroism data analysis. Compound **3** possesses a unique C-20 acetal moiety. It is the first report of the isolation of *dinor*-(9 β -H)-pimarane derivatives from *Icacina* plants. Compounds **5** and **6** showed moderate cytotoxicity against MDA-MB-435, MDA-MB-231, and OVCAR3 cell lines, with IC₅₀ values of 2.91–7.60 μ M and 1.48–3.23 μ M, respectively.

Graphical abstract

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ASSOCIATED CONTENT

Supporting Information

IR, UV, 1D and 2D NMR spectroscopic data for compounds **1**–**6**. ECD calculations for **1** and **2**. This material is available free of charge via Internet at <http://pubs.acs.org>.

The authors declare no competing financial interest.

Icacin trichantha Oliv. (Icacinaceae) is a medicinal plant in Nigeria and other regions of western Africa.^{1, 2} Its tuber is used to treat food poisoning, constipation, and malaria.¹ It was also found to show hypoglycemic, sedative, analgesic, and bacterial inhibitory effects.^{3–5} (9β-H)-Pimaranes, 17-*nor*-(9β-H)-pimaranes, and 17-*nor*-pimaranes have been reported previously from *I. trichantha*, *I. claessensis*, *I. mannii*, and *I. guesfeldtii*.^{6–11} In the course of continuing efforts to discover novel compounds from *I. trichantha*,^{6, 7, 10, 11} isolation of 16,17-*dinor*- and 17,19-*dinor*-pimaranes, 17-*nor*-pimaranes, and 17-*nor*-(9β-H)-pimaranes from the tuber of the plant is herein reported. This is the first report of the isolation of 16,17-*dinor*- and 17,19-*dinor*-pimaranes from *Icacin* species. The cytotoxic properties of the isolated compounds were evaluated using MDA-MB-435, MDA-MB-231, and OVCAR3 cell lines.

RESULTS AND DISCUSSION

The tuber of *I. trichantha* was extracted with 80% aqueous MeOH by percolation, followed by solvent partition into petroleum ether-soluble, EtOAc-soluble, and *n*-BuOH-soluble fractions, successively. Six new pimarane derivatives, icacinlactone K (**1**), icacintrichanone (**2**), icacinlactone L (**3**), 7α-hydroxyicacinlactone B (**4**), 7α-hydroxyicacenone (**5**), and 2β-hydroxyhumirianthenolide C (**6**), were purified from the EtOAc- and *n*-BuOH-soluble fractions.

Compound **1** was obtained as a needle-like crystal. A molecular formula of $C_{19}H_{20}O_7$ with 10 indices of hydrogen deficiency was suggested by HRESIMS (m/z 361.1292 $[M + H]^+$; calcd for $C_{19}H_{21}O_7^+$, 361.1282) with the aid of ^{13}C NMR spectroscopic data. IR absorptions at 1730 and 1630 cm^{-1} indicated the presence of γ -lactone and conjugated formyl moieties, respectively. In the 1H NMR spectrum, signals for formyl (δ_H 10.31, H-15), methyl (δ_H 1.45, CH_3 -18), methoxy (δ_H 3.95, 12- OCH_3), and an isolated aromatic proton (δ_H 6.54, H-11) were observed (Table 1). Nineteen carbons were displayed in the ^{13}C NMR spectrum (Table 2). A comparison of the ^{13}C NMR data of **1** with those of icacinlactone A⁷ revealed similarities except for those of the benzofuranyl moiety of the latter. Using 1H - 1H COSY, HSQC, and HMBC analyses (Figure 1), connectivities across the C-1-C-10 bicyclic ring, as well as the presence of 3,20-epoxy and 19,6-lactone moieties, became obvious. The observation of HMBC correlations from H-7 (δ_H 4.00, dd, $J = 9.7, 17.3\text{ Hz}$) to C-5 (δ_C 51.3), C-6 (δ_C 75.7), C-8 (δ_C 114.4), C-9 (δ_C 150.5), as well as an oxygenated tertiary carbon at δ_C 161.5, led to the assignment of the latter signal to C-14. The olefinic carbon at δ_C 99.3 was assignable to C-11 since its proton (δ_H 6.54, H-11) displayed HMBC correlations with both C-8 and C-10 (δ_C 37.7). Subsequently, the assignments of C-12 (δ_C 162.8), C-13 (δ_C 110.8), and C-15 (δ_C 195.4) were possible on the basis of the following HMBC correlations: H-11 with C-12 and C-13; H-15 (δ_H 10.31, s) with C-13 and C-14. The 2D structure of **1** was thus proposed as shown. The relative configuration of **1** was established on the basis of NOESY results (Figure 2). Finally the absolute configuration was determined as (3*S*, 4*R*, 5*R*, 6*R*, 10*R*) by single-crystal X-ray diffraction (Figure 3). Furthermore, calculations of the electronic circular dichroism (ECD) spectra of **1** and its enantiomer were performed using the time-dependent density functional theory (TDDFT) method. The calculated ECD spectrum for **1** matched its experimental data, showing similar Cotton effects (Figure 4). Collectively, these permitted definition of the structure of compound **1**, icacinlactone K, as (3*S*, 4*R*, 5*R*, 6*R*, 10*R*)-3 β ,20-epoxy-13-formyl-3 α ,14-dihydroxy-12-methoxy-16,17-*dinor*-pimar-8(9),11,13(14)-trien-19,6 β -olide. It is the first report of a 16,17-*dinor*-pimarane from *Icacina* plants.

Compound **2** was obtained as a colorless amorphous powder. HRESIMS suggested a molecular formula of $C_{19}H_{20}O_6$ with 10 indices of hydrogen deficiency (m/z 327.1246 $[M + H - H_2O]^+$; calcd for $C_{19}H_{19}O_5^+$, 327.1227). The presence of a dialkylketo group was indicated by the IR absorption at 1739 cm^{-1} . The 1H NMR spectrum displayed three olefinic protons at δ_H 7.67 (d, $J = 2.2\text{ Hz}$, H-16), 6.84 (d, $J = 2.2\text{ Hz}$, H-15), and 6.63 (H-11), and a methyl at δ_H 1.26 (d, $J = 6.3\text{ Hz}$, CH_3 -18) (Table 1). The ^{13}C NMR spectrum exhibited 19

carbon signals corresponding to a methyl, a methoxy, three methylenes, six methines, a dioxygenated secondary carbon, two oxygenated tertiary carbons, a carbonyl carbon, and four quaternary carbons (Table 2). Three coupled spin systems were revealed by the following ^1H - ^1H COSY correlations: H-1a (δ_{H} 2.72–2.79)/H-2b (δ_{H} 2.46), CH₃-18/H-4 (δ_{H} 2.79–2.85)/H-5 (δ_{H} 2.27), and H-15/H-16, allowing the establishment of fragments corresponding to C-1/C-2, C-18/C-4/C-5, and C-15/C-16 (Figure 1). In the HMBC spectrum, both H-1a and CH₃-18 correlated to the carbonyl carbon at δ_{C} 215.1, leading to the connectivity between C-2 and C-4 via C-3 (δ_{C} 215.1). A benzofuran moiety was proposed based on the HMBC correlations as shown in Figure 1. A methoxy group was attached to C-12 (δ_{C} 154.7). The proton at δ_{H} 4.85 showing HMBC correlations with C-5 (δ_{C} 54.0), C-9 (δ_{C} 143.9), and C-14 (δ_{C} 157.0) was assigned to H-7. On the other hand, observation of HMBC correlations from H-20b (δ_{H} 3.35) to C-5, C-6 (δ_{C} 109.9), C-9, and C-10 (δ_{C} 49.9) revealed the presence of 6,20-epoxy and C-6 hemiketal functional groups. The 2D structure of **2** was thus elucidated as shown. In the NOESY spectrum, H-5 correlated with both H-7 (δ_{H} 4.85) and CH₃-18, while H-4 correlated with H-20a (δ_{H} 4.66), indicating the α -orientations of H-5, H-7, and CH₃-18, as well as the β -orientations of H-4 and the 6,20-epoxy group (Figure 3). To determine the absolute configuration of **2**, the ECD spectra for (4*S*, 5*S*, 6*S*, 7*R*, 10*R*)-**2** and its enantiomer were calculated using the TDDFT method (Figure 4). The experimental ECD spectrum of **2** revealed the absolute configuration of **2** as (4*S*, 5*S*, 6*S*, 7*R*, 10*R*). Consequently, the structure of **2**, icacintrichanone, was defined as (4*S*, 5*S*, 6*S*, 7*R*, 10*R*)-6 β ,20:14,16-diepoxy-6 α ,7 β -dihydroxy-12-methoxy-3-oxo-17,19-dinor-pimar-8(9),11,13(14),15-tetraene. It is the first example of a 17,19-dinor-pimarane from *Icacina* plants. Another similar but simpler compound, 17,19-dinor-pimar-8(9),11,13(14)-triene, was detected in the sediments of a deltaic environment.¹²

Compound **3** was obtained as a colorless amorphous powder. Its HRESIMS showed a protonated molecular ion at m/z 371.1138 [$\text{M} + \text{H}$]⁺ (calcd for C₂₀H₁₉O₇⁺, 371.1125), suggesting a molecular formula of C₂₀H₁₈O₇ with 12 indices of hydrogen deficiency. The IR absorption at 1739 cm⁻¹ indicated the presence of a γ -lactone moiety. The ^1H NMR spectrum displayed resonances for three olefinic protons at δ_{H} 6.70 (s, H-11), 6.91 (d, J = 2.2 Hz, H-15), and 7.69 (d, J = 2.2 Hz, H-16), and a methyl at δ_{H} 1.42 (s, CH₃-18) (Table 1). The ^{13}C NMR spectrum exhibited 20 carbon signals corresponding to a methyl, a methoxy, two methylenes, seven methines, a dioxygenated secondary carbon, two oxygenated tertiary carbons, a carbonyl carbon, and five quaternary carbons (Table 2). With the aid of an HSQC experiment, all proton signals were assignable to their attached carbons (Table 1). The ^1H - ^1H COSY spectrum displayed three coupled spin systems, i.e. H-1 (δ_{H} 2.24–2.32, 1.92)/H-2 (δ_{H} 2.38), H-5 (δ_{H} 2.29)/H-6 (δ_{H} 4.67)/H-7 (δ_{H} 5.61), and H-15 (δ_{H} 6.91)/H-16 (δ_{H} 7.69), allowing the assignment of connectivity of these fragments (Figure 2). The presence of benzofuranyl and 6,19- γ -lactone moieties were proposed based on the interpretation of the HMBC data (Figure 2). The methoxy group was attached at C-12 (δ_{C} 155.7). The dioxygenated secondary carbon (δ_{C} 100.1) exhibited HMBC correlation to CH₃-18 (δ_{H} 1.42, s) and was, therefore, assignable to C-3. C-10 (δ_{C} 36.8) showed HMBC correlations with H-2, H-11, and H-6. It is noteworthy that the proton at δ_{H} 4.99 (H-20) correlated to C-3 (δ_{C} 100.1), C-5 (δ_{C} 45.0), C-7 (δ_{C} 68.1), and C-10 (δ_{C} 36.8) in the HMBC spectrum, indicating the presence of an acetal moiety at C-20 (δ_{C} 101.1). The 2D structure

of **3** was thus elucidated as shown. To determine the relative configuration, a NOESY experiment was performed. The observation of the following NOESY correlations indicated the α -orientations of H-5, H-6, H-7, and CH₃-18, and β -orientations of the 3,20- and 7,20-epoxy groups: CH₃-18 correlated to H-5 and H-1a; H-6 correlated with H-5 and H-7; and H-20 correlated with H-1b (Figure 2). Consequently, the structure of **3**, icacinlactone L, was defined as 3 β ,20:7 β ,20:14,16-triepoxy-3 α -hydroxy-12-methoxy-17-*nor*-pimar-8(9), 11,13(14),15-tetraen-19,6 β -olide. It is the first example of a 17-*nor*-pimarane bearing a unique C-20 acetal group from *Icacina* plants.

Compound **4** was obtained as a colorless amorphous powder. A molecular formula of C₂₀H₂₀O₇ with 11 indices of hydrogen deficiency was deduced from HRESIMS (m/z 355.1204 [M + H – H₂O]⁺; calcd for C₂₀H₁₉O₆⁺, 355.1176) and ¹³C NMR spectroscopic data. The IR absorption at 1729 cm^{–1} indicated the presence of a γ -lactone moiety. The ¹H NMR spectrum displayed resonances for three olefinic protons at δ_H 7.69 (d, J = 2.2 Hz, H-16), δ_H 6.85 (d, J = 2.2 Hz, H-15), 6.71 (s, H-11), a methyl group at δ_H 1.46 (s, CH₃-18), and a methoxy group at δ_H 3.94 (s) (Table 1). Twenty carbon signals were observed in the ¹³C NMR spectrum (Table 2). A comparison of the ¹³C NMR data of **4** with those of icacinlactone B⁷ and interpretation of the 2D NMR data indicated that **4** was the 7-hydroxy derivative of icacinlactone B. The observation of the following NOESY correlations (Figure 2) revealed the presence of 5 α -H, 6 α -H, and 4 α -CH₃, as well as 7 β -H and 3 β ,20-epoxy: H-5 (δ_H 2.54) correlated with H-1b (δ_H 1.94), H-6 (δ_H 4.93), and CH₃-18 (δ_H 1.46); H-7 (δ_H 5.33) correlated with H-20a (δ_H 3.84); and H-1a (δ_H 2.75) correlated with H-20b (δ_H 3.73). The structure of **4**, 7 α -hydroxyicacinlactone B, was defined as 3 β ,20:14,16-diepoxy-3 α ,7 α -dihydroxy-12-methoxy-17-*nor*-pimar-8(9),11,13(14),15-tetraen-19,6 β -olide.

Compound **5** was obtained as a needle-like crystal. A molecule formula of C₁₉H₂₀O₈ with 10 indices of hydrogen deficiency was deduced from HRESIMS (m/z 377.1232 [M + H]⁺; calcd for C₁₉H₂₁O₈⁺, 377.1231) and the ¹³C NMR data. The IR spectrum showed two strong absorptions at 1771 and 1660 cm^{–1}, corresponding to γ -lactone and conjugated carbonyl functionalities, respectively. The ¹H NMR spectrum displayed two olefinic protons at δ_H 7.54 (d, J = 2.0 Hz, H-16) and 6.67 (d, J = 2.0 Hz, H-15), and a methyl group at δ_H 1.37 (s, CH₃-18) (Table 1). The ¹³C NMR spectrum exhibited resonances for 19 carbons (Table 2). A comparison of the ¹³C NMR data of **5** with those of icacenone,⁶ as well as interpretation of the 2D NMR data indicated that **5** was the 7-hydroxy derivative of icacenone. In the NOESY spectrum, the following correlations were observed: between CH₃-18 and H-1a (δ_H 2.41), H-2 (δ_H 3.99), H-5 (δ_H 2.52), and H-6 (δ_H 4.64); between H-20b (δ_H 3.63) and H-1b (δ_H 1.46) and H-9 (δ_H 2.19); between H-8 (δ_H 3.29–3.33) and H-20a (δ_H 4.31); and between H-8 and H-9. The orientations of 2 α -H, 5 α -H, 6 α -H, 4 α -CH₃, as well as 8 β -H, 9 β -H, and 3 β ,20-epoxy were thus proposed (Figure 2). However, the orientation of H-7 could not be unambiguously determined from the NOESY data. Finally, single crystal X-ray diffraction was used to establish the absolute configuration as (2*S*, 3*R*, 4*R*, 5*R*, 6*S*, 7*S*, 8*S*, 9*R*), and 10*S* (Figure 3). The structure of **5**, 7 α -hydroxyicacenone, was thus defined as (2*S*, 3*R*, 4*R*, 5*R*, 6*S*, 7*S*, 8*S*, 9*R*, 10*S*)-3 β ,20:14,16-diepoxy-2 β ,3 α ,7 α -trihydroxy-12-oxo-17-*nor*-(9 β -H)-pimar-13(14),15-dien-19,6 β -olide.

Compound **6** was obtained as a colorless amorphous powder. The HRESIMS data suggested a molecular formula of $C_{19}H_{22}O_7$ with nine indices of hydrogen deficiency (m/z 363.1466 $[M + H]^+$; calcd for $C_{19}H_{23}O_7^+$, 363.1438). Two strong IR absorption bands at 1767 and 1691 cm^{-1} suggested the presence of γ -lactone and conjugated carbonyl functionalities, respectively. All 1H NMR signals were readily assignable to their attached carbons using HSQC data (Table 1). The ^{13}C NMR spectrum displayed 19 carbon resonances (Table 2). The 1H - 1H COSY spectrum revealed two coupled spin systems: H-1/H-2 and H-5/H-6/H-7/H-8/H-9/H-11/H-12 (Figure 1). On the basis of HMBC results (Figure 1), compound **6** was shown to be the 2-hydroxy derivative of humirianthenolide C.⁶ In the NOESY spectrum (Figure 2), H-2 (δ_H 3.96) correlated with CH_3 -18 (δ_H 1.33), suggesting the presence of a 2 β -OH group. The remaining relative configurations were confirmed by the observation of the following NOESY correlations: H-6 (δ_H 4.75) with H-5 (δ_H 2.27), CH_3 -18, and H-7b (δ_H 1.90); H-8 (δ_H 2.79) with H-7a (δ_H 2.51) and H-9 (δ_H 1.66); H-20b (δ_H 3.62) with H-1b (δ_H 1.41) and H-9; H-8 with H-20a (δ_H 4.31); and H-1a (δ_H 2.45) with H-2 (δ_H 3.96) (Figure 2). The structure of **6**, 2 β -hydroxyhumirianthenolide C, was thus defined as 3 β ,20:14,16-diepoxy-2 β ,3 α -dihydroxy-15-oxo-17-*nor*-(9 β -H)-pimar-13(14)-en-19,6 β -olide.

Compounds **1–6** were evaluated for cytotoxic activity against MDA-MB-435 (human melanoma cancer), MDA-MB-231 (human breast cancer), and OVCAR3 (human ovarian cancer) cell lines. Compound **6** was the most active, with IC_{50} values of 1.48–3.23 μM ; compound **5** showed IC_{50} values of 2.91–7.60 μM . Compounds **1–4** did not show significant cytotoxicity at concentrations up to 20 μM (Table 3). Collectively, the reported cytotoxicity data of (9 β -H)-pimarane and derivatives^{6, 7, 10} showed that compounds possessing a 9 β -hydrogen are cytotoxic while those with an aromatic C-ring are inactive. It is thus proposed that the presence of a 9 β -hydrogen or an alicyclic C-ring in these structures is essential for their cytotoxicity, though more detailed supportive evidence are still required.

Icacina plants are rich in (9 β -H)-pimarane, 17-*nor*-(9 β -H)-pimarane, and 17-*nor*-pimarane diterpenoids,^{6–9} and a (9 β -H)-pimarane pathway instead of pimarane pathway has been proposed for their biosynthesis.¹¹ This is the first report of the isolation of a 16,17-*dinor*-pimarane (**1**) and a 17,19-*dinor*-pimarane (**2**) from *Icacina* plants. Theoretically, compound **1** could have been derived from the loss of either C15/C-16 or C16/C-17. The latter case (i.e. 16,17-*dinor*-pimarane structure) is preferred because several other compounds obtained from the same plant belong to 17-*nor*-pimaranes.

In summary, six new *Icacina* diterpenoids were isolated from the tuber of *I. trichantha*. Among these, 7 α -hydroxyicacenone (**5**) and 2 β -hydroxyhumirianthenolide C (**6**) displayed cytotoxicity against MDA-MB-435, MDA-MB-231, and OVCAR3 cell lines.

EXPERIMENTAL SECTION

General Experimental Procedures

The melting points were measured on a Thomas-Hoover capillary melting point apparatus (Arthur H. Thomas Company, Philadelphia, PA., U.S.A.). Optical rotations at the sodium D line were measured with a Perkin-Elmer 241 digital polarimeter using quartz cell with a path

length of 100 mm at room temperature. Concentrations (*c*) are given in g/100 mL. IR spectra were measured on a Jasco Fourier Transform IR Spectrometer (FT-IR model 410) loaded with an OMNIC software. UV spectra were collected on a Shimadzu UFLC system with a PDA detector. NMR spectra were recorded on a Bruker AV-400 spectrometer. All chemical shifts were quoted on the δ scale in ppm using residual solvent as the internal standard (methanol-*d*₄: δ_{H} 3.30 for ¹H NMR, δ_{H} 49.90 for ¹³C NMR). Coupling constants (*J*) are reported in Hz. For HPLC purification, a C₁₈ semi-preparative HPLC column (Phenomenex C₁₈ column, 250 × 10 mm, 5 μ m) and a Shimadzu UFLC system were used. HRESIMS were measured on a Waters SYNAPT hybrid quadrupole/time of flight spectroscopy using positive electrospray ionization. Human melanoma cancer cells MDA-MB-435, human breast cancer cells MDA-MB-231, and human ovarian cancer cells OVCAR3 were purchased from the American Type Culture Collection (Manassas, VA). Molecular models in Figure 2 were generated by Chem3D Pro 14.0 using MM2 force field calculation.

Plant Material

Tubers of *Icacina trichantha* Oliv. were collected in June, 2011 from the Orba village in Nsukka of the Enugu State, Nigeria, and authenticated by Prof. B.O. Olorede of the Botany Department, University of Abuja, Nigeria, and Mr. A. Ozioko, botanist at the BDCP laboratories, Nsukka, Nigeria. A voucher specimen (UNN/FVM 456) was deposited in the pharmacology laboratory at the University of Nigeria, Nsukka, Nigeria.

Extraction and Isolation

The tubers of *I. trichantha* (1.5 kg) were milled and extracted with 80% aqueous MeOH by percolation to yield 166 g of crude extract. The crude extract was partitioned into petroleum ether-soluble (11 g), EtOAc-soluble (17 g), *n*-BuOH-soluble (15 g), and H₂O-soluble (128 g) fractions. The EtOAc fraction (17 g) was separated into 88 sub-fractions on a silica gel column (5 × 60 cm) eluted with gradient petroleum ether and EtOAc mixtures (from 100:0 to 0:100, v/v; 600 mL each). The combined sub-fractions 29–32 was chromatographed by semipreparative HPLC eluted with MeOH–H₂O (48:52 v/v; 3.5 mL/min) to afford icacinlactone L (**3**, 2.9 mg, *t_R* = 18.4 min, soluble in MeOH). 7 α -Hydroxyicacinlactone B (**4**, 1.8 mg, *t_R* = 7.6 min, soluble in MeOH) and icacinalactone K (**1**, 1.5 mg, *t_R* = 16.0 min, soluble in MeOH) were purified from subfractions 33–35 by semipreparative HPLC (MeOH–H₂O, 60:40 v/v; 3.5 mL/min). The combined sub-fractions 36–42 was further separated into five fractions using Sephadex LH-20 column chromatography (1 × 80 cm, eluted with MeOH). The 2nd and 3rd fractions were purified by HPLC (MeOH–H₂O, 55:45 v/v; 3.5 mL/min) to afford icacintrichanone (**2**, 2.1 mg, *t_R* = 10.7 min, soluble in MeOH). The *n*-BuOH-soluble fraction (19 g) was passed through a macroporous resin column (4 × 30 cm) eluted with aqueous MeOH (from 5 to 80%, 800 mL each). The fractions obtained from 20–30% aqueous MeOH were combined and further separated into 30 sub-fractions using MCI CHP20P resin column chromatography (2.5 × 20 cm; eluted by aqueous MeOH, from 10 to 50%). The 3rd and 4th fractions were applied to semipreparative HPLC (MeCN–H₂O, 8:92 v/v; 3.5 mL/min) to afford 7 α -hydroxyicacenone (**5**, 2.3 mg, *t_R* = 18.7 min, soluble in MeOH). In a similar manner, 2 β -hydroxyhumirianthenolide C (**6**, 0.8 mg, *t_R* = 16.0 min, soluble in MeOH) was purified from the 6th and 7th fractions by HPLC (MeCN–H₂O, 12:88 v/v; 3.5 mL/min).

Icacinlactone K (1): needle-like crystal; mp 188–189 °C; $[\alpha]_D^{20} = +39$ (*c* 0.1, MeOH); IR (film) $\nu_{\max}$ 3500, 2916, 2849, 1730, 1630, 1440, 1418, 1352, 1234, 1180, 1128, 1113, 1044, 960 cm^{-1} ; UV $_{\max}$: 211, 284 nm; ^1H NMR (methanol-*d*₄, 400 MHz), see Table 1; ^{13}C NMR (methanol-*d*₄, 100 MHz), see Table 2; (+)–HRESIMS m/z 361.1292 $[\text{M} + \text{H}]^+$; calcd for $\text{C}_{19}\text{H}_{21}\text{O}_7^+$, 361.1282.

Icacintrichanone (2): Colorless amorphous powder; $[\alpha]_D^{20} = -36$ (*c* 0.1, MeOH); IR (film) $\nu_{\max}$ 3394, 2916, 2849, 1739, 1708, 1497, 1463, 1371, 1304, 1239, 1206, 1165, 1118, 1032, 988 cm^{-1} ; UV $_{\max}$: 217, 250, 260 nm; ^1H NMR (methanol-*d*₄, 400 MHz), see Table 1; ^{13}C NMR (methanol-*d*₄, 100 MHz), see Table 2; (+)–HRESIMS m/z 327.1246 $[\text{M} + \text{H} - \text{H}_2\text{O}]^+$ (calcd for $\text{C}_{19}\text{H}_{19}\text{O}_5$, 327.1227).

Icacinlactone L (3): Colorless amorphous powder; $[\alpha]_D^{20} = -13$ (*c* 0.1, MeOH); IR (film) $\nu_{\max}$ 3382, 2910, 2848, 1739, 1602, 1355, 1238, 1179, 1117, 1029, 986 cm^{-1} ; UV $_{\max}$: 218, 252, 260 nm; ^1H NMR (methanol-*d*₄, 400 MHz), see Table 1; ^{13}C NMR (methanol-*d*₄, 100 MHz), see Table 2; (+)–HRESIMS m/z 371.1138 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{20}\text{H}_{19}\text{O}_7^+$, 371.1125).

7 α -Hydroxyicacinlactone B (4): Colorless amorphous powder; $[\alpha]_D^{20} = -2$ (*c* 0.1, MeOH); IR (film) $\nu_{\max}$ 3470, 2916, 2849, 1729, 1609, 1464, 1378, 1330, 1293, 1224, 1102, 1047 cm^{-1} ; UV $_{\max}$: 218, 252, 262 nm; ^1H NMR (methanol-*d*₄, 400 MHz), see Table 1; ^{13}C NMR (methanol-*d*₄, 100 MHz), see Table 2; (+)–HRESIMS m/z 355.1204 $[\text{M} + \text{H} - \text{H}_2\text{O}]^+$ (calcd for $\text{C}_{20}\text{H}_{19}\text{O}_6^+$, 355.1176).

7 α -Hydroxyicacenone (5): needle-like crystal; mp 244–245 °C; $[\alpha]_D^{20} = -3$ (*c* 0.2, MeOH); IR (film) $\nu_{\max}$ 3409, 2916, 1771, 1660, 1452, 1390, 1239, 1194, 1126, 1072, 1003 cm^{-1} ; UV $_{\max}$: 266 nm; ^1H NMR (methanol-*d*₄, 400 MHz), see Table 1; ^{13}C NMR (methanol-*d*₄, 100 MHz), see Table 2; (+)–HRESIMS m/z 377.1232 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{19}\text{H}_{21}\text{O}_8^+$, 377.1231).

2 β -Hydroxyhumirianthenolide C (6): Colorless amorphous powder; $[\alpha]_D^{20} = +8$ (*c* 0.1, MeOH); IR (film) $\nu_{\max}$ 3427, 2919, 1767, 1691, 1605, 1434, 1357, 1296, 1232, 1174, 1066, 1048, 998, 907 cm^{-1} ; UV $_{\max}$: 270 nm; ^1H NMR (methanol-*d*₄, 400 MHz), see Table 1; ^{13}C NMR (methanol-*d*₄, 100 MHz), see Table 2; (+)–HRESIMS m/z 363.1466 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{19}\text{H}_{23}\text{O}_7^+$, 363.1438).

Single Crystal X-Ray Structure Determination

X-Ray diffraction intensity data were collected at the Advanced Photon Source, Argonne National Laboratory, at LS-CAT (beamline 21-ID-D), with a 50 μm x-ray beam, at a distance of 100 mm and temperature of 100K, using a MAR CCD 300 mm area detector. For compound **1**, a needle-like crystal, roughly 10 $\times$ 10 $\times$ 50 μm , was used for data collection. The space group was determined to be $\text{P}2_12_12_1$ (No. 19) by identification of the systematic absences. The absolute configuration of the structure was inferred from an analysis of the Bijvoet differences, with a Flack parameter of 0.1(4) (1.1 for the inverted structure), Parsons

z parameter = 0.19(16), and a Bayesian Bijvoet probability of $P_2(\text{true}) = 0.999$. For compound **5**, a needle-like crystal, roughly $10 \times 10 \times 50 \mu\text{m}$, was used for data collection. The space group was determined to be $P2_1$ (No. 4) by the systematic absences. The absolute configuration of the structure was inferred from an analysis of Bijvoet differences, with the Flack parameter of 0.10(19) (1.1 for the inverted structure), and a probability of $P_2(\text{true}) = 1.000$. Both structures were solved by SHELXS, and refined with SHELX-2014. The data for X-ray diffraction analyses of compounds **1** and **5** have been deposited at the Cambridge Crystallographic Data Centre. CCDC 1424346 (for **1**) and 1424347 (for **5**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via <http://www.ccdc.cam.ac.uk/conts/retrieving.html> (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: +44 1223 336033; depositccdc.cam.ac.uk).

Computational Section for ECD Calculation

Conformational analysis was performed for the enantiomers of **1** and **2** by the Sybyl 8.1 program using Random Searching together with the MMFF94s molecular mechanics force field charged with MMFF94. Two and six conformers for **1** and **2** were obtained, respectively. Optimization followed by ECD calculation of the resulted conformers were performed over the Gaussian09 software at B3LYP/6-31+G(d,p)//B3LYP/6-31G(d) level in the gas phase.

Cytotoxicity Assays

The MDA-MB-435, MDA-MB-231, and OVCAR3 cell lines were propagated at 37°C in 5% CO₂ in RPMI 1640 medium, supplemented with fetal bovine serum (10%), penicillin (100 units/mL), and streptomycin (100 µg/mL). Cells in log phase growth were harvested by trypsinization followed by two washings to remove all traces of enzyme. A total of 5,000 cells were seeded per well of a 96-well clear, flat-bottom plate (Microtest 96®, Falcon) and incubated overnight (37°C in 5% CO₂). Samples dissolved in DMSO were then diluted and added to the appropriate wells (concentrations: 20, 4, 0.8, 0.16, and 0.032 µM; total volume: 100 µL; DMSO: 0.5%). The cells were incubated in the presence of test substance for 72 h at 37°C and evaluated for viability with a commercial absorbance assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega Corp, Madison, WI) that measured viable cells. IC₅₀ values are expressed in µM relative to the solvent (DMSO) control. Vinblastine was used for positive control.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
 ^1H - ^1H COSY and selective HMBC correlations for 1-6

Figure 2.
Key NOESY correlations for **1–6**

Figure 3.
ORTEP representations of **1** and **5**

Figure 4.

Experimental ECD spectra for **1** and **2**, and the calculated ECD spectra for **1** and **2**, and their enantiomers

Table 1

¹H (400 MHz) NMR Spectroscopic Data for Compounds 1–6 (δ in ppm)^a

position	1	2	3	4	5	6
	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
1	2.71, ddd (4.4, 12.6, 12.6)	2.72–2.79, m ^b	2.24–2.32, m ^d	2.75, ddd (4.6, 12.3, 12.3)	2.41, ddd (3.1, 10.2, 13.2)	2.45, dd (3.5, 13.2) ^f
	1.92, dddd/tt-like (3.7, 4.5, 11.9, 12.6)	2.33, ddd (4.9, 13.7, 13.7)	1.92, m	1.94, dddd (3.3, 3.7, 12.1, 12.5)	1.46, dd (4.8, 13.1)	1.41, dd (4.8, 13.2)
2	2.29, ddd (4.4, 11.9, 16.6)	2.80–2.90, m ^b	2.38, m	2.29, ddd (4.6, 12.0, 14.0)	3.99, dd (4.8, 9.9)	3.96, dd (4.8, 10.0)
3	2.07, ddd (4.5, 12.6, 16.6)	2.46, ddd (2.1, 4.9, 13.7)		2.05, ddd (4.2, 11.9, 13.8)		
4		2.79–2.85, m				
5	2.36, dd (2.0, 8.0)	2.27, d (10.9)		2.54, dd (2.0, 8.4)	2.52, brd (5.3)	2.27, br d (4.5)
6	5.10, ddd (5.8, 8.0, 9.7)		2.29, dd (2.3, 9.7) ^d	4.93, dd (4.1 8.4)	4.64, dd (2.1, 5.4)	4.75, ddd/br t-like (0.6, 5.3, 5.4)
7	4.00, dd (9.7, 17.3)	4.85	5.61, br d (2.2)	5.33, d (4.0)	4.38, dd (2.1, 3.6)	2.51, br dd (5.2, 15.8) ^f
	2.58, dd (5.8, 17.3)					1.90, ddd (5.2, 12.8, 16.0) ^g
8					3.29–3.33 ^e	2.79, m
9					2.19, ddd/dt-like (4.7, 4.7, 13.6)	1.66, m/br d-like
11	6.54, s	6.63, s	6.70, s	6.71, s	2.68, dd (13.6, 16.8)	1.84, br dd (5.0, 12.8) ^g
12					2.35, dd (4.7, 16.8)	1.49, ddd (5.8, 12.9, 12.9)
						2.34, ddd (1.1, 4.6, 16.1)
						2.07, ddd (6.1, 11.5, 16.1)
15	10.31, s	6.84, d (2.2)	6.91, d (2.2)	6.85, d (2.2)	6.67, d (2.0)	
16		7.67, d (2.2)	7.69, d (2.2)	7.69, d (2.2)	7.54, d (2.0)	4.57, d (4.7)
18	1.45, s	1.26, d (6.3)	1.42, s	1.46, s	1.37, s	1.33, s
20	4.12, dd (3.7, 9.5)	4.66, d (7.6)	4.99, d (2.2)	3.84, dd (3.7, 9.8)	4.31, dd (3.5, 9.7)	4.31, dd (3.5, 9.6)
	3.80, dd (2.0, 9.5)	3.35 ^c		3.73, dd (2.1, 9.7)	3.63, dd (1.4, 9.7)	3.62, dd (1.2, 9.6)
OCH ₃	3.95, s	3.93, s	3.98, s	3.94, s		

^aData were measured in methanol-*d*₄. For CH₂, the deshielded signal was assigned as H_a, and the shielded signal as H_b.

Signal was partially obscured.

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Table 2

¹³C (100 MHz) NMR Spectroscopic Data for Compounds 1–6 (δ in ppm)^a

	1	2	3	4	5	6
position	δ_C , type	δ_C , type	δ_C , type	δ_C , type	δ_C , type	δ_C , type
1	30.2, CH ₂	29.7, CH ₂	28.0, CH ₂	30.9, CH ₂	41.7, CH ₂	41.9, CH ₂
2	28.3, CH ₂	37.8, CH ₂	22.4, CH ₂	28.6, CH ₂	67.1, CH	67.2, CH
3	98.2, C	215.1, C	100.1, C	97.9, C	97.9, C	98.0, C
4	50.7, C	41.7, CH	49.0, C	50.9, C	51.6, C	52.0, C
5	51.3, CH	54.0, CH	45.0, CH	50.2, CH	43.5, CH	45.5, CH
6	75.7, CH	109.9, C	78.0, CH	85.7, CH	80.2, CH	75.4, CH
7	25.0, CH ₂	72.7, CH	68.1, CH	70.4, CH	66.3, CH	27.2, CH ₂
8	114.4, C	115.0, C	113.8, C	115.3, C	34.8, CH	30.6, CH
9	150.5, C	143.9, C	137.6, C	135.5, C	38.4, CH	38.4, CH
10	37.7, C	49.9, C	36.8, C	36.4, C	34.2, C	33.8, C
11	99.3, CH	99.2, CH	99.6, CH	101.3, CH	38.2, CH ₂	17.9, CH ₂
12	162.8, C	154.7, C	155.7, C	154.5, C	196.8, C	18.7, CH ₂
13	110.8, C	118.0, C	118.3, C	119.6, C	124.1, C	112.8, C
14	161.5, C	157.0, C	152.5, C	155.8, C	168.8, C	194.4, C
15	195.4, CH	104.8, CH	105.4, CH	104.4, CH	107.2, CH	204.1, C
16		145.3, CH	145.7, CH	145.4, CH	145.0, CH	76.6, CH ₂
18	19.9, CH ₃	14.6, CH ₃	21.5, CH ₃	19.8, CH ₃	17.1, CH ₃	17.2, CH ₃
19	181.4, C		181.9, C	181.2, C	179.5, C	180.2, C
20	71.5, CH ₂	74.2, CH ₂	101.1, CH	73.2, CH ₂	73.8, CH ₂	73.8, CH ₂
OCH ₃	56.5, CH ₃	56.1, CH ₃	56.4, CH ₃	56.1, CH ₃		

^aData were measured in methanol-*d*₄

Table 3

Cytotoxic Activity in Cancer Cell Lines

compound	IC ₅₀ (μM)		
	MDA-MB-435	MDA-MB-231	OVCAR3
1	> 20	> 20	> 20
2	> 20	> 20	> 20
3	> 20	> 20	> 20
4	> 20	> 20	> 20
5	2.91	7.60	7.53
6	1.48	2.85	3.23
Vinblastine	0.49 <i>nM</i>	8.78 <i>nM</i>	1.82 <i>nM</i>