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17-Norpimaranes and (9*β*H)-17-Norpimaranes from the Tuber of *Icacina trichantha*

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Abstract

Seven new 17-norpimarane and (9*β*H)-17-norpimarane diterpenoids, icacinlactones A-G (**1**-**7**), were isolated from the tuber of *Icacina trichantha*. The structures were elucidated by spectroscopic and HRMS techniques, and the absolute configuration of **2** was determined by means of X-ray crystallographic analysis. Compounds **1**-**7**, as well as four known related structures, 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. Several of these natural products displayed significant cytotoxic activity, with humirianthenolide C being most active.

Icacina trichantha Oliv. (Icacinaceae) is a traditional herbal medicine used in Nigeria and other regions of western Africa.[1] The tuber of this plant is often prescribed by herbalists for the treatment of food poisoning, constipation, and malaria;[1] and the macerated tuber in alcohol is a common household first-aid medicine for emergency treatment of food poisoning.[2] Recent studies on *I. trichantha* have revealed its antihyperglycemic, anticonvulsion, sedative, analgesic, and antimicrobial properties.[3-5] In a previous study on this plant, five cytotoxic diterpenoids (17-hydroxyicacinol, icacinol, humirianthol, humirianthenolide C, and icacenone), belonging to the small subclasses of (9*β*H)-pimarane and (9*β*H)-17-norpimarane, were isolated.[6] Further investigation now led to the purification

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The authors declare no competing financial interest.

Supporting Information

IR, 1D and 2D NMR spectra data for compounds **1**-**7**. This material is available free of charge via Internet at http://pubs.acs.org.

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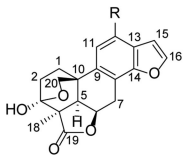
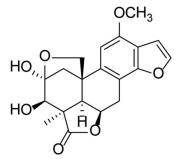
of seven new 17-norpimaranes and (9*β*H)-17-norpimaranes. In the present report, the isolation, structural elucidation, and cytotoxic properties of these diterpenoids are described.

RESULTS AND DISCUSSION

The 80% aqueous MeOH extract of the tubers of *I. trichantha* was successively partitioned into petroleum ether, EtOAc, and *n*-BuOH fractions. Seven new diterpenoid lactones, icacinlactones A-G (**1**-**7**), were purified from the EtOAc and *n*-BuOH fractions.



1, R = H; **2**, R = OCH_3



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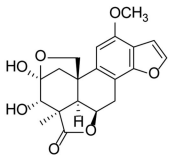

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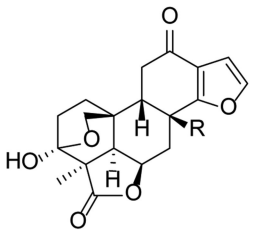
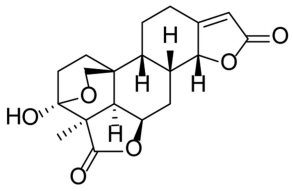


5, R = H; **6**, R = OH



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Icacinlactone A (**1**) was obtained as white powder. HRESIMS showed a quasi-molecular ion at *m/z* 327.1206 $[M + H]^+$ (cacld for $C_{19}H_{19}O_5$, 327.1232), suggesting a molecular formula of $C_{19}H_{18}O_5$ with 11 indices of hydrogen deficiency when the ^{13}C NMR spectroscopic data are taken into consideration. The IR absorption at 1750 cm^{-1} indicated the presence of a *γ*-lactone moiety. The ^1H NMR spectrum displayed four olefinic protons at δ_H 7.75 (d, J = 2.2 Hz, H-16), 7.49 (dd, J = 0.9, 8.1 Hz, H-12), 7.23 (d, J = 8.2 Hz, H-11), and 6.81 (d, J = 2.2 Hz, H-15), and one methyl group at δ_H 1.44 (s, CH_3-18) (Table 1). The ^{13}C NMR spectrum exhibited 19 carbon signals corresponding to a methyl, four methylenes (including an oxygenated one), six methines (four olefinic and two aliphatic), a dioxygenated secondary carbon, an oxygenated tertiary carbon, a carbonyl carbon, and five quaternary carbons. (Table 3). All proton signals were assignable to their attached carbons through an HSQC experiment (Table 1). Based on the ^1H-^1H COSY data, four coupled spin systems corresponding to H-1 (δ_H 2.75, 1.90) / H-2 (δ_H 2.28, 2.05), H-5 (δ_H 2.37) / H-6 (δ_H 5.19) / H-7 (δ_H 4.22, 3.03), H-11 (δ_H 7.23) / H-12 (δ_H 7.49), and H-15 (δ_H 6.81) / H-16 (δ_H 7.75) were unambiguously established, allowing the assignment of the connectivities of these fragments (Figure 1). Interpretation of the HMBC data then led to the proposed structure of **1**. It is noteworthy that the deshielded dioxygenated secondary carbon at δ_C 98.2 (C-3) displayed long-range correlations with both H-1 and H-2. On the other hand, the methylene protons (δ_H 4.12, 3.77) exhibited HMBC correlations with C-1 (δ_C 30.9), C-5 (δ_C 52.1), and C-10 (δ_C 36.2). At the same time, H-20a (δ_H 4.12) exhibited HMBC correlation with C-3 (δ_C 98.2). All the above evidence indicated the presence of a 3,20-epoxy bridge. Indeed, the chemical shift of C-3 (δ_C 98.2) was in agreement with a hemiketal structure. The other parts

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of the molecule were established as follows. The connection between the A- and B-rings was confirmed by HMBC correlations observed for C-10 and H-1a, H-2, H-5, and H-6. The γ-lactone moiety was established first by assigning the quaternary carbon at δ_C 50.9 to C-4 based on its HMBC correlations with H-2b and H-5. Other carbons in this part of the molecule, including C-3(δ_C 98.2), C-4 (δ_C 50.9), C-5 (δ_C 52.1), and C-19 (δ_C 181.7), all showed long-range correlations with CH_3-18 (δ_H 1.44). Furthermore, the benzofuran part was proposed based on the observations of the following HMBC cross signals: H-11 with C-8 / C-13, H-12 with C-14 / C-15, and H-16 with C-13 / C-14. All available evidence thus led to the determination of the structure of **1** as depicted. The relative configuration of **1** was then assigned on the basis of NOESY analysis, in which the following key correlations were clearly observed: H-5 (δ_H 2.37) with H-6 (δ_H 5.19) and H-18 (δ_H 1.44), H-6 with H-7a (δ_H 4.22) and H-18, between H-18 and H-2a (δ_H 2.28), between H-2b (δ_H 2.05) and H-20b (δ_H 3.77), as well as between H-7b (δ_H 3.03) and H-20a (δ_H 4.12) (Figure 2). Consequently, the new 17-norpimarane (**1**) was elucidated to be 3*β*,20:14,16-diepoxy-3*α*-hydroxy-17-norpimar-8(9),11,13(14),15-tetraen-19,6*β*-olide, and given the trivial name icacinlactone A.

Icacinlactone B (**2**) was obtained as white powder. The molecular formula $C_{20}H_{20}O_6$ was deduced from ^{13}C NMR spectroscopic data and HRESIMS data (*m/z* 357.1309 $[M+H]^+$, calcd for $C_{20}H_{21}O_6$, 357.1338), implying 11 indices of hydrogen deficiency. Similar to **1**, the IR absorption at 1752 cm^{-1} suggested the presence of a γ-lactone moiety. A comparison of the ^1H and ^{13}C NMR data revealed that **2** was a methoxy derivative of **1** (Tables 1 and 3). The location of the OCH_3 group (δ_H 3.92, δ_C 55.7) at C-12 (δ_C 152.3) was determined by the HMBC correlation between the methoxy protons and C-12 (Figure 1). Indeed, in comparison with **1**, significant shielding of C-8 (−8.6 ppm), C-11 (−20.3 ppm), and C-13 (−11.2 ppm), with a concomitant deshielding of C-12 (+31.9 ppm), supported the assignment based upon the resonance and inductive effects of the 12-OCH_3 subsituent. The relative configuration of **2** was then proposed on the basis of NOESY analysis (Figure 2). Finally, crystals of **2** were available from a mixture of MeOH and EtOAc, and its absolute configuration was determined by means of X-ray diffraction analysis assisted by a CCD area detector using a CuK_α X-ray source (Figure 3). The structure of the new 17-norpimarane (**2**) was thus elucidated to be (3*S*, 4*R*, 5*R*, 6*R*, 10*R*)-3*β*,20:14,16-diepoxy-3*α*-hydroxy-12-methoxy-17-norpimar-8(9),11,13(14),15-tetraen-19,6*β*-olide, and given the trivial name icacinlactone B.

Icacinlactone C (**3**) was obtained as white powder. It has a molecular formula of $C_{20}H_{20}O_7$ as determined by ^{13}C NMR spectroscopic data and HRESIMS data (*m/z* 395.1088 $[M+Na]^+$; calcd for $C_{20}H_{20}O_7Na$, 395.1107). The presence of a γ-lactone moiety was indicated by the IR absorption at 1750 cm^{-1}. The ^1H NMR spectrum displayed two olefinic doublets at δ_H 7.52 (J = 2.2 Hz, H-16), and 6.84 (J = 2.2 Hz, H-15), an olefinic singlet at δ_H 6.52 (H-11), a methoxy singlet at δ_H 3.91, and a methyl singlet at δ_H 1.60 (CH_3-18). The corresponding carbons were identified by the HSQC analysis (Table 3). Among the proton signals, two D_2O-exchangable signals at δ_H 4.29 (s) and δ_H 5.01 (d, J = 10.6 Hz) were assigned to 2-OH and 3-OH, respectively, on the basis of HMBC correlation data. A comparison between the ^{13}C NMR data of **2** and **3** revealed high similarity, the major difference being detected in the A-ring (Table 3). In contrast to **1** and **2**, in which the

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CH_3-18 protons had long-range coupling with the C-3 hemiketal carbon (δ_C > 96), the CH_3-18 of **3** correlated to an oxygenated methine (δ_C 81.3), that was assigned to C-3. Instead, H-1a (δ_H 2.92) and H-20a (δ_H 4.25) displayed long-range correlations with the hemiketal carbon at δ_C 103.9 (C-2), suggesting the presence of an oxygen bridge between C-2 and C-20. The relative configuration of **3** was proposed on the basis of NOESY analysis. Thus, correlations from CH_3-18 (δ_H 1.60) to H-5 (δ_H 2.24) and H-6 (δ_H 5.17), from H-6 to H-5, H-7a (δ_H 4.05), and CH_3-18, as well as between H-20a (δ_H 4.25) and H-7b (δ_H 2.98), indicated the α-orientation of H-5, H-6, and 4-CH_3, and the β-orientation of the 2,20-epoxy bridge (Figure 2). Owning to partial overlapping of the H-3 (δ_H 3.75) and H-20b (δ_H 3.76) signals in $CDCl_3$, unambiguous assignment of NOESY cross peaks was not possible. By changing to the methanol-d_4 solvent, however, the H-3 signal was well resolved at δ_H 3.78. Irradiation of this signal resulted in signal enhancement of 5α-H (δ_H 2.35) and 4α-CH_3 (δ_H 1.58), indicating an α-orientation of H-3. The above evidence led to the proposed structure of the new 17-norpimarane (**3**) as 2β,20:14,16-diepoxy-3β-hydroxy-12-methoxy-17-norpimar-8(9),11,13(14),15-tetraen-19,6β-olide (icacinlactone C).

Icacinlactone D (**4**) was obtained as white powder. It has the same molecular formula ($C_{20}H_{20}O_7$) as that of **3** according to the ^{13}C NMR spectroscopic data and HRESIMS data (395.1082 $[M + Na]^+$ (calcd for $C_{20}H_{20}O_7Na$, 395.1107). Its ^{13}C NMR data (Table 3) were similar to those of **3**, with major difference observed for the ^{13}C NMR chemical shifts of C-2 (+4.1 ppm) and C-3 (−6.0 ppm), indicating a possible configurational variation. The NOESY experiment failed when methanol-d_4 was used as solvent due to partial overlapping of the H-3 (δ_H 3.95, d, J = 1.0 Hz) and OCH_3 (δ_H 3.94) signals. In a mixed solvent containing $CDCl_3$-methanol-d_4 (100:1), however, the H-3 signal could be observed at δ_H 4.14 and the following NOESY correlations were recorded: H-5 (δ_H 2.20) with H-1b (δ_H 2.39) and H-18 (δ_H 1.41); H-6 (δ_H 5.12) with H-5, H-7a (δ_H 4.03), and H-18; H-3 with H-20a (δ_H 4.23); H-20a with H-7b (δ_H 2.93); as well as H-20b with H-1a (δ_H 2.46). These NOESY results supported the assignment of the relative configuration of **4** as shown, differing from **3** only at C-3. The structure of compound **4** was thus elucidated as the new 17-norpimarane, 2β,20:14,16-diepoxy-3α-hydroxy-12-methoxy-17-norpimar-8(9),11,13(14),15-tetraen-19,6β-olide, and given a trivial name icacinlactone D.

Icacinlactone E (**5**) was obtained as white powder. A molecular formula of $C_{19}H_{20}O_6$ possessing 10 indices of hydrogen deficiency was deduced from the ^{13}C NMR spectroscopic data and the quasi-molecular ion peak at *m/z* 345.1302 $[M + H]^+$ (calcd for $C_{19}H_{21}O_6$, 345.1338) in its HRESIMS. The IR spectrum displayed two strong absorptions at 1761 cm^{-1} and 1660 cm^{-1}, assignable to γ-lactone and conjugated carbonyl moieties, respectively. The ^{1}H NMR spectrum revealed two olefinic doublets at δ_H 7.53 (J = 2.0 Hz, H-16) and 6.65 (J = 2.0 Hz, H-15), and a methyl singlet at δ_H 1.36 (CH_3-18) (Table 2). The ^{13}C NMR and DEPT spectra contained nineteen carbon signals, including a methyl, five methlyenes, six methines, a dioxygenated secondary carbon, an oxygenated tertiary carbon, two carbonyl carbons, and three quaternary carbons. The ^{1}H-^{1}H COSY spectrum (Figure 1) displayed three spin systems as evidenced by the following correlations: between H-1b (δ_H 1.65) and H-2a (δ_H 2.10); between H-5 (δ_H 2.32) and H-6 (δ_H 4.79); between H-6 and H-7b (δ_H 1.94); between H-7 (δ_H 2.54, 1.94) and H-8 (δ_H 3.26), between H-9 (δ_H 2.20) and H-11 (δ_H 2.59,

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2.40), as well as between H-15 (δ_H 6.65) and H-16 (δ_H 7.53). The connectivities of C-1/C-2, C-5/C-6/C-7/C-8/C-9/C-11, and C-15/C-16 were established with the aid of HSQC results. In the ^{13}C NMR spectrum, the presence of a carbonyl carbon at δ_C 180.8 (C-19), a dioxygenated secondary carbon at δ_C 97.8 (C-3), an oxygenated methine carbon at δ_C 75.8 (C-6), an oxygenated methylene carbon at δ_C 73.9 (C-20), as well as a methyl at δ_C 17.4 (C-18), indicated **5** possessed a 19,6-γ-lactone and a 3,20-epoxy bridge as in **1** and **2** (Figure 1). The carbonyl carbon at δ_C 195.2 was assigned to C-12 since both H-9 (δ_H 2.20) and H-11 (δ_H 2.59 and 2.40) correlated to it in the HMBC spectrum. The assignments of C-13 (δ_C 120.9) and C-14 (δ_C 172.5) were supported by the following HMBC correlations: between H-7a (δ_H 2.54) and C-14, between H-11b (δ_H 2.40) and C-13, and between H-8 (δ_H 3.26) and C-13 / C-14. On the other hand, the proton signals at δ_H 6.65 and 7.53 correlated with each other in the ^{1}H-^{1}H COSY, and both exhibited HMBC correlations with C-14, leading to the assignments of H-15 (δ_H 6.65) / C-15 (δ_C 107.1) and H-16 (δ_H 7.53) / C-16 (δ_C 145.3), as well as confirming the connectivity of C-14 and C-16 via an oxygen atom. The structure of **5** was therefore determined as shown, and the relative configuration could be deduced from the NOESY data (Figure 2). Consequently, the structure of compound **5** was elucidated as the new (9βH)-17-norpimarane, 3β,20:14,16-diepoxy-3α-hydroxy-12-oxo-(9βH)-17-norpimar-13(14),15-dien-19,6β-olide, and given a trivial name icacinlactone E.

Icacinlactone F (**6**) was obtained as white powder. The ^{13}C NMR spectroscopic data and the HRESIMS result (*m/z* 361.1278 $[M + H]^+$; calcd for $C_{19}H_{21}O_7$, 361.1287) indicated a molecular formula of $C_{19}H_{20}O_7$ with 10 indices of hydrogen deficiency. The spectroscopic data (Tables 2 and 3, and Figure 1) of **6** were similar to those of **5** except for the presence of an additional hydroxy group, which could be assigned to C-8 based on ^{1}H-^{1}H COSY, HSQC, and HMBC data. Furthermore, when compared to **5**, the chemical shifts of C-8 (δ_C 69.2), C-7 (δ_C 35.4), and C-9 (δ_C 47.6) in **6** were deshielded by +40.4 ppm, +7.4 ppm, and +8.2 ppm, respectively. Such chemical shift differences were consistent with the α- and β-substituent effects of the 8-OH group. To determine the relative configuration of compound **6**, a selective 1D-NOESY experiment in $CDCl_3$ was performed. Thus, selective irradiation of H-9 (δ_H 2.04) and H-18 (δ_H 1.23) resulted in the signal enhancement of H-20 (δ_H 4.40 and 3.78) and H-5 (δ_H 1.72) / H-6 (δ_H 4.59), respectively, revealing the same configuration as **5**, i.e. 3α-OH, 4α-CH_3, 5α-H, 6α-H, 9β-H, and 3β,20-epxoy. On the other hand, selective 1D-NOE irradiation (in DMSO-d_6) on 8-OH (δ_H 5.93) enhanced the signal of 9β-H (δ_H 1.96), indicating an 8β-OH orientation. The structure of compound **6** was therefore determined as the new (9βH)-17-norpimarane, 3β,20:14,16-diepoxy-3α,8β-dihydroxy-12-oxo-(9βH)-17-norpimar-13(14),15-dien-19,6β-olide, and given a trivial name icacinlactone F.

Icacinlactone G (**7**) was obtained as white powder. The molecular formula of $C_{19}H_{22}O_6$ was established from ^{13}C NMR spectroscopic data and HRESIMS data (*m/z* 347.1472 $[M +H]^+$, calcd for $C_{19}H_{23}O_6$, 347.1495), indicating nine indices of hydrogen deficiency. The presence of a γ-lactone moiety was indicated by the strong IR absorption at 1739 cm^{-1}. Its NMR data (Tables 2, and 3) resembled those of **5** and **6**. In the ^{13}C NMR spectrum, the presence of a carbonyl carbon at δ_C 179.6 (C-19), a dioxygenated secondary carbon at δ_C 96.7 (C-3), an oxygenated methine carbon at δ_C 74.8 (C-6), an oxygenated methylene

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carbon at δ_C 73.0 (C-20), as well as a methyl at δ_C 17.6 (C-18), indicated the presence of the 19,6-γ-lactone moiety and the 3,20-epoxy bridge, which could be confirmed by HMBC analysis. Through ^{1}H-^{1}H COSY analysis, the connection from C-1 (δ_C 30.4) through C-12 (δ_C 26.4), and between C-8 (δ_C 32.8) and C-14 (δ_C 82.4), could be readily established with the aid of HSQC and further confirmed by the HMBC results (Figure 1). The rest of the unassigned groups, i.e. an olefinic methine at δ_C 114.6, an olefinic quaternary carbon at δ_C 167.1, and a carbonyl carbon at δ_C 173.0, were analyzed by the HMBC data. Thus, both H-12 (δ_H 2.89 and 2.28-2.35) and H-8 (δ_H 2.73-2.80) correlated to the olefinic quaternary carbon at δ_C 167.1 (C-13); the olefinic proton (δ_H 5.81, which was attached to C-15 at δ_C 114.6) correlated to both C-14 (δ_C 82.4) and the carbonyl carbon at δ_C 173.0 (C-16). These findings led to the assignment of an α,β-unsaturated γ-lactone moiety. Compound **7** is therefore a (9βH)-17-norpimarane dilactone. The relative configuration was then assigned on the basis of the NOESY analysis which revealed the following correlations: H-18 with both H-5 and H-6; H-5 with H-6; H-6 with H-7b; H-8 with H-7a, H-9, H-14, and H-20a; as well as H-9 with H-14. Thus, the structure of **7** was elucidated as the new (9βH)-17-norpimarane, 3β,20-epoxy-3α-hydroxy-(9βH)-17-norpimar-13(15)-en-19,6β:16,14α-diolide, and given a trivial name icacinlactone G.

Icacinlactons A-G (**1**-**7**), together with icacinol, humirianthol, humirianthenolide C, and icacenone, were evaluated for cytotoxic activity in MDA-MB-435 (human melanoma cancer), MDA-MB-231 (human breast cancer), and OVCAR3 (human ovarian cancer) cell lines (Table 4). Besides icacinol, humirianthol, humirianthenolide C, and icacenone, whose cytotoxic activity in MDA-MB-435 cells has been previously reported,[6] icacinlactone F (**6**) displayed moderate cytotoxic activity in the MDA-MB-435 cells (IC_{50} 6.16 μM). In the MDA-MB-231 cells, icacinol (IC_{50} 7.30 μM), humirianthol (IC_{50} 3.74 μM), humirianthenolide C (IC_{50} 0.67 μM), and icacinlactone F (**6**) (IC50 8.94 μM) were active. On the other hand, icacinol (IC_{50} 7.55 μM), humirianthol (IC_{50} 4.12 μM), and humirianthenolide C (IC_{50} 1.05 μM) were also active against OVCAR3 cells. Among the tested compounds, humirianthenolide C was most potent against all three cancer cell lines.

A few (9βH)-17-norpimaranes and (9βH)-pimaranes have been found to occur in the genera of *Icacina* (*I. trichantha*, *I. claessensis*, *I. mannii*, and *I. guesfeldtii*)[6-9] and *Humirianthera* (syn. *Casimirella*) (*H. rupestris*, and *H. ampla*)[10-12], both belonging to the Icacinaceae family. These icacinaceae (9βH)-pimarane derivatives are characterized by the presence of a 19,6β-γ-lactone moiety and a 3β,20-epoxy bridge. Compounds **3** and **4** are the first examples of this type of structure bearing a 2β,20-epoxy group. On the other hand, **1**-**4** are structurally unique with the absence of both 9-H and C-17, and the formation of benzofuran rings in the molecules. Biologically, several (9βH)-pimarane lactone isolated from *Casimirella* spp. have been reported to show cytotoxic activity against the A2780 human ovarian cancer cell line (IC_{50} 1.7 - 6.1 μM).[12]

In summary, icacinlactones A-G (**1**-**7**) are new pimarane-type diterpenoids obtained from the tuber of *I. trichantha*. They belong to the small subclasses of 17-norpimarane and (9βH)-17-norpimarane. While the known structures of icacinol, humirianthol, humirianthenolide C, and icacenone exhibited cytotoxicity, the new icacinlactone F (**6**) was moderately active against MDA-MB-435, MDA-MB-231, and OVCAR3 cancer cell lines.

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Among all test compounds, humirianthenolide C was most active, showing an IC_{50} of 0.7 μM in MDA-MB-435 and MDA-MB-231 cells.

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/100mL. IR spectra were measured on a Jasco Fourier Transform IR Spectrometer (FT-IR model 410) loaded with an OMNIC software. NMR spectra were recorded on a Bruker DPX-400 spectrometer. All chemical shifts were quoted on the δ scale in ppm using residual solvent as the internal standard (DMSO-d_6: 2.49 ppm for ^{1}H NMR, 39.5 ppm for ^{13}C NMR; $CDCl_3$: 7.24 ppm for ^{1}H NMR, 77.0 ppm for ^{13}C NMR; methanol-d_4: 3.30 ppm for ^{1}H NMR, 49.90 ppm for ^{13}C NMR). Coupling constants (*J*) are reported in Hz. For HPLC purification, a C_{18} semi-preparative HPLC column (Phenomenex C_{18} column, 250 × 10 mm, 5 μm) and a Shimadzu UFLC system were used. HRESIMS were measured on a Shimadzu LCMS-IT-TOF Mass Spectrometry. X-ray diffraction experiment was carried out on a Bruker Kappa APEXII DUO diffractometer with a CCD area detector using Cu$K\alpha$ X-ray source. The absolute configuration of icacinlactone B (**2**) was determined with a Flack parameter of 0.00(3). The space group was $P2_12_12_1$ (No. 19) with four molecules in the unit cell. The data have been deposited at the Cambridge Crystallographic Data Centre. CCDC 1039297 contains 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; deposit@ccdc.cam.ac.uk). 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 Pr12.0 using MM2 force field calculation.

Plant Material

Fresh 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 powdered tuber of *I. trichantha* (1.5 kg) was extracted with 80% aqueous MeOH by percolation to yield 166 g of dry crude extract. The crude extract was partitioned into petroleum ether-soluble (11 g), EtOAc-soluble (17 g), *n*-BuOH-soluble (15 g), and H_2O-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 mixtures of petroleum ether and EtOAc (from 100:0 to 0:100 v/v; 600 mL each). 17-Hydroxyicacinol, icacinol, humirianthol, and

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humirianthenolide C were previously isolated from sub-fractions 19, 47-53, and 58.[6] In the present study, sub-fractions 17-18 were combined and applied to a semi-preparative HPLC, eluted with MeOH-H_2O (75:25 v/v; 3.5 mL/min) to afford icacinlactone A (**1**, 12 mg, t_R = 7.10 min, soluble in MeOH). Combination of sub-fractions 24-25 was further separated by HPLC (MeOH-H_2O, 75:25 v/v; 3.5 mL/min) to yield icacinlactone B (**2**, 50 mg, t_R = 8.41 min, soluble in $CHCl_3$ but sparingly soluble in MeOH). Sub-fractions 36-42 were further separated into five fractions by a Sephadex LH-20 chromatography (10 mm × 1 m, bed volume of 80 mL, MeOH). The 4th and 5th fractions were further purified by a semi-preparative HPLC (MeOH-H_2O, 60:40 v/v; 3.5 mL/min) to afford icacinlactone C (**3**, 3.27 mg, t_R = 14.17 min, soluble in both $CHCl_3$ and MeOH). Subfraction 43 was applied to a semi-preparative HPLC, eluted with MeOH-H_2O (60:40 v/v; 3.5 mL/min), to afford icacinlactone D (**4**, 3.6 mg, t_R = 12.10 min, soluble in MeOH). A whiter precipitate obtained from subfractions 47-53, containing mostly icacinol, was further purified by a semi-preparative HPLC (MeOH-H_2O, 35:65 v/v, 3.5 mL/min) to yield a crop of icacinlactone E (**5**, 9 mg, t_R = 12.03 min, soluble in MeOH). Icacinlactone F (**6**, 1.71 mg, t_R = 6.57 min, soluble in MeOH and $CHCl_3$) was purified from a precipitate from subfractions 47-53, by HPLC separation (MeOH-H_2O, 35:65 v/v; 3.5 mL/min). The white precipitate was shown to be a mixture of icacinol, humirianthol, and **6**. The *n*-butanol-soluble fraction (19 g) was fractionated on a macroporous resin column (4 × 30 cm) eluted with aqueous MeOH (from 5% to 80%, 800 mL each) to obtain a crop of icacenone. From a precipitate containing mostly icacenone, icacinlactone G (**7**, 1.2 mg, t_R = 8.19 min, soluble in $CHCl_3$) was purified by semi-preparative HPLC (MeOH-H_2O, 35:65 v/v, 3.5 mL/min).

Icacinlactone A (1)—white powder; mp 185 - 186 °C; $[\alpha]_D^{20} = +5.9 \quad (c \quad 0.2, \quad MeOH)$; IR (film) ν_{max} 3495, 2930, 2874, 1750, 1303, 1229, 1155, 1125, 1110, 1050, 1017, 991, 934, 896, 873, 815, 741 cm^{-1}; ^1H NMR (methanol-d_4, 400 MHz), see Table 1; ^{13}C NMR (methanol-d_4, 100 MHz), see Table 3; (+)-HRESIMS *m/z* 327.1206 $[M + H]^+$ (calcd for $C_{19}H_{19}O_5$, 327.1232).

Icacinlactone B (2)—white powder; mp 207 - 208 °C; $[\alpha]_D^{20} = +5.6 \quad (c \quad 0.2, \quad DCM)$; IR (film) ν_{max} 3494, 2937, 2875, 1752, 1623, 1604, 1497, 1465, 1401, 1375, 1332, 1178, 1151, 1110, 1101, 1041, 1017, 994, 951, 935, 897, 876, 831, 782, 768, 736, 695 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 3; (+)-HRESIMS *m/z* 357.1309 $[M + H]^+$ (calcd for $C_{20}H_{21}O_6$, 357.1338).

Icacinlactone C (3)—white powder; mp 235 - 236 °C;

$[\alpha]_D^{20} = -6.6 \quad (c \quad 0.2, \quad MeOH)$; IR (film) ν_{max} 3448, 2969, 2875, 1750, 1625, 1604, 1499, 1454, 1403, 1375, 1316, 1284, 1185, 1152, 1122, 1088, 1029, 1006, 976, 903, 827, 806, 774, 759, 688 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 3; ^1H NMR (methanol-d_4, 400 MHz) δ7.65 (1H, d, *J* = 2.2 Hz, H-16), 6.84 (1H, d, *J* = 2.2 Hz, H-15), 6.66 (1H, s, H-11), 5.27 (1H, ddd/br quintet-like, *J* = 5.0, 5.0, 10.2 Hz, H-6), 4.22 (1H, dd, *J* = 1.5, 8.8 Hz, H-20a), 4.04 (1H, dd, *J* = 9.9, 17.3 Hz, H-7a), 3.94 (3H, s, OC*H*$_3$), 3.78 (1H, s, H-3), 3.66 (1H, dd, *J* = 1.5, 8.8 Hz, H-20b), 2.94 (1H, dd, *J* = 4.7, 17.3 Hz, H-7b), 2.81 (1H, d, *J* = 11.8 Hz, H-1a), 2.35 (1H, dd, *J* = 1.4, 5.8 Hz, H-5),

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1.99 (1H, dd, *J* = 1.5, 11.7 Hz, H-1b), 1.58 (3H, s, H-18). (+)-HRESIMS *m/z* 395.1088 [M + Na]$^+$ (calcd for $C_{20}H_{20}O_7Na$, 395.1107).

Icacinlactone D (4)—white powder; mp 240 - 241 °C; $[\alpha]_D^{20} = -9.4 \quad (c \quad 0.3, \quad MeOH)$; IR (film) ν_{max} 3411, 2916, 1767, 1625, 1604, 1452, 1402, 1374, 1336, 1297, 1279, 1186, 1151, 1123, 1088, 1025, 999, 960, 903, 835, 767, 740, 681 cm^{-1}; ^{1}H NMR (methanol-*d*$_4$, 400 MHz), see Table 1; ^{13}C NMR (methanol-*d*$_4$, 100 MHz), see Table 3; ^{1}H NMR ($CDCl_3$- methanol-*d*$_4$ 100 : 1, 400 MHz) δ_H 7.51 (1H, d, *J* = 2.1 Hz, H-16), 6.82 (1H, d, *J* = 2.2 Hz, H-15), 6.53 (1H, s, H-11), 5.12 (1H, ddd/br quintet-like, *J* = 5.2, 5.3, 10.2 Hz, H-6), 4.23 (1H, br d, *J* = 8.8 Hz, H-20a), 4.14 (1H, s, H-3), 4.03 (1H, dd, *J* = 9.9, 17.4 Hz, H-7a), 3.91 (3H, s, OC*H*$_3$), 3.65 (1H, br d, *J* = 8.3 Hz, H-20b), 2.93 (1H, dd, *J* = 4.8, 17.3 Hz, H-7b), 2.46 (1H, d, *J* = 11.6 Hz, H-1a), 2.39 (1H, d, *J* = 11.5 Hz, H-1b), 2.20 (1H, br d, *J* = 5.7 Hz, H-5), 1.41 (3H, s, H-18). (+)-HRESIMS *m/z* 395.1082 [M + Na]$^+$ (calcd for $C_{20}H_{20}O_7Na$, 395.1107).

Icacinlactone E(5)—white powder; mp 231 - 232 °C; $[\alpha]_D^{20} = +6.0 \quad (c \quad 0.4, \quad MeOH)$; IR (film) ν_{max} 3495, 2936, 2879, 1761, 1670, 1454, 1290, 1215, 1197, 1125, 1098, 1074, 1040, 1024, 1003, 981, 942, 904, 741 cm^{-1}; ^{1}H NMR (methanol-*d*$_4$, 400 MHz), see Table 2; ^{13}C NMR (methanol-*d*$_4$, 100 MHz), see Table 3; (+)-HRESIMS *m/z* 345.1302 [M + H]$^+$ (calcd for $C_{19}H_{21}O_6$, 345.1338).

Icacinlactone F(6)—white powder; $[\alpha]_D^{20} = -0.9 \quad (c \quad 0.1, \quad DCM)$; IR (film) ν_{max} 3406, 2932, 1756, 1676, 1451, 1305, 1263, 1232, 1199, 1128, 1070, 1039, 946, 923, 873, 742 cm^{-1}; ^{1}H NMR (methanol-*d*$_4$, 400 MHz), see Table 2; ^{13}C NMR (methanol-*d*$_4$, 100 MHz), see Table 3; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 7.47 (1H, d, *J* = 2.0 Hz, H-16), 6.69 (1H, d, *J* = 2.0 Hz, H-15), 4.59 (1H, ddd, *J* = 1.0, 7.1, 15.0 Hz, H-6), 4.40 (1H, ddd, *J* = 3.9, 5.6, 9.8 Hz, H-20a), 3.78 (1H, br d, *J* = 10.0 Hz, H-20b), 3.00 (1H, dd, *J* = 5.4, 16.7 Hz, H-11a), 2.97 (1H, dd, *J* = 7.1, 14.8 Hz, H-7a), 2.56 (1H, dd, *J* = 5.4, 17.0 Hz, H-11b), 2.47 (1H, ddd, *J* = 3.1, 7.0, 14.5 Hz, H-7b), 2.04 (1H, dd, *J* = 1.2, 5.6 Hz, H-9), 1.72 (1H, ddd/dt-like, *J* = 2.4, 2.7, 6.7 Hz, H-5), 1.23 (3H, s, H-18). ^{1}H NMR (DMSO-*d*$_6$, 400 MHz, multiplicities of some signals were not clear due to poor peak shape) δ_H 7.81 (1H, d, *J* = 1.9 Hz, H-16), 6.65 (1H, d, *J* = 1.9 Hz, H-15), 5.93 (1H, d, *J* = 2.1 Hz, 8-OH), 5.57 (1H, s, 3-OH), 4.63 (1H, m, H-6), 4.56 (1H, m, H-20a), 3.49 (1H, br d, *J* = 10.1 Hz, H-20b), 1.96 (1H, br d, *J* = 5.7 Hz, H-9), 1.91 (1H, m, H-5), 1.65 (2H, m, H-1a and 2b), 1.52 (1H, m, H-1b), 1.16 (3H, s, H-18). (+)-HRESIMS *m/z* 361.1278 [M + H]$^+$ (calcd for $C_{19}H_{21}O_7$, 361.1287).

Icacinlactone G(7)—white powder; $[\alpha]_D^{20} = -3.3 \quad (c \quad 0.1, \quad DCM)$; IR (film) ν_{max} 3436, 2918, 2850, 1739, 1648, 1463, 1367, 1303, 1237, 1197, 1178, 1120, 1077, 1038, 996, 919, 884, 733, 684, 651 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 3; (+)-HRESIMS *m/z* 347.1472 [M + H]$^+$ (calcd for $C_{19}H_{23}O_6$, 347.1495).

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Cytotoxicity Assays

The cell line was propagated at 37°C in 5% CO_2 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% CO_2). Samples dissolved in DMSO were then diluted and added to the appropriate wells (concentrations: 20 μM, 4 μM, 0.8 μM, 0.16 μM, 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® AQ_{ueous} One Solution Cell Proliferation Assay, Promega Corp, Madison, WI) that measured viable cells. IC_{50} 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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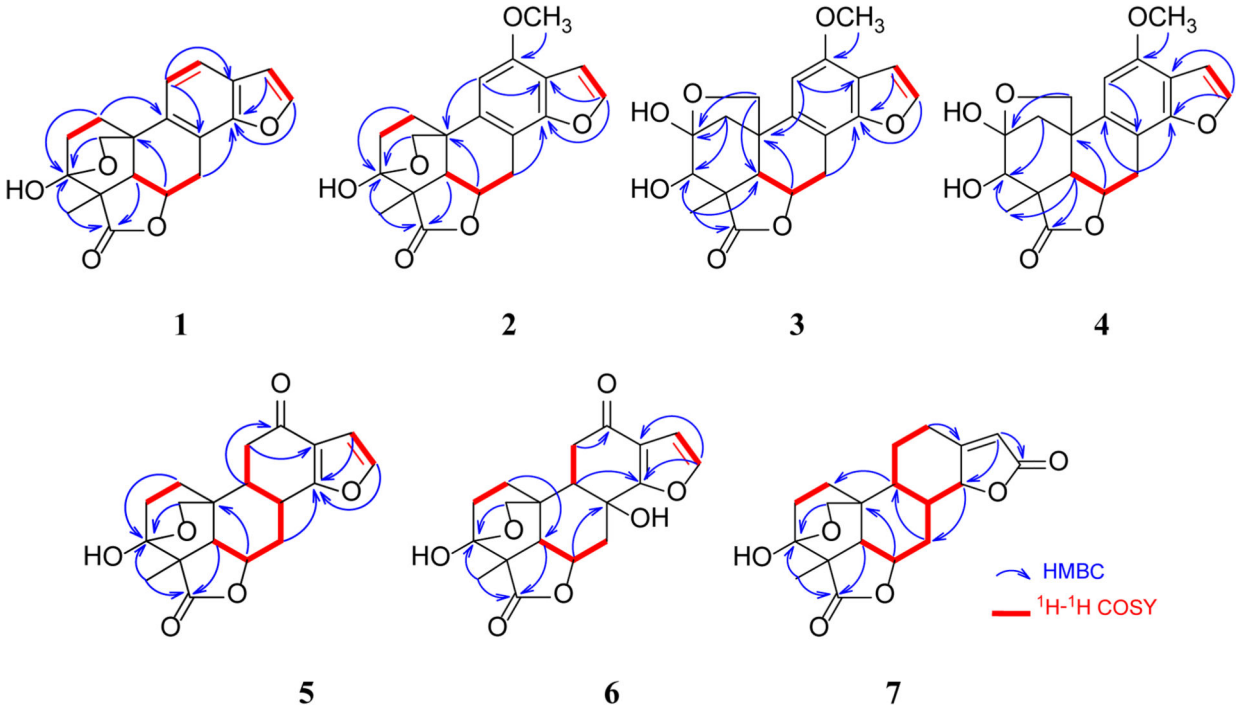

Figure 1.
^{1}H-^{1}H COSY and selected HMBC correlations for **1-7**

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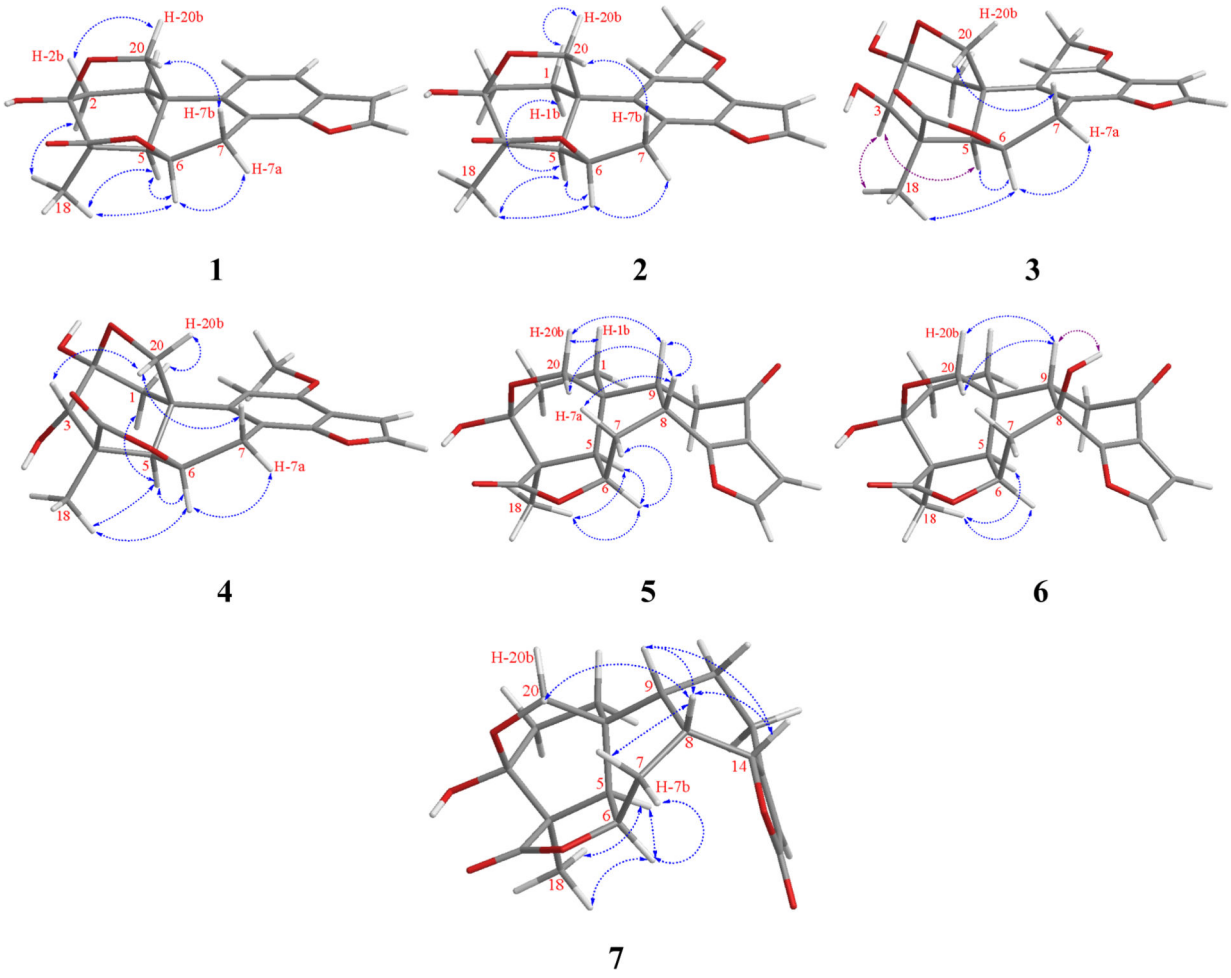

Figure 2.
Selected NOESY correlations for **1-7**

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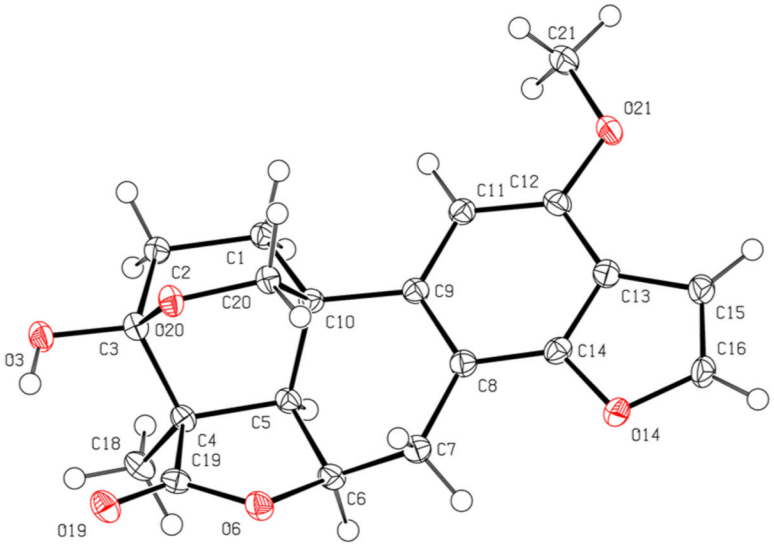

Figure 3.
ORTEP representation of **2**

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Table 1

^{1}H (400 MHz) NMR Spectroscopic Data for Compounds 1-4 (δ in ppm, J in Hz)[a]

	1[b]	2[c]	3[c]	4[b]
position	**δ_H (J in Hz)**	**δ_H (J in Hz)**	**δ_H (J in Hz)**	**δ_H (J in Hz)**
1	2.75, ddd (4.5, 12.4, 12.4)	2.68, ddd (4.8, 12.1, 12.18)	2.92, d (11.8)	2.45, dd (0.8, 11.5)
	1.90, dddd (4.1, 4.2, 12.2, 12.4)	1.88, dddd (4.2, 4.4, 12.2, 12.5)	1.86, dd (1.5, 11.8)	2.31, dd (1.6, 11.5)
2	2.28, ddd (4.5, 12.0, 14.0)	2.21, m[d]		
	2.05, ddd (4.5, 12.1, 14.0)		-	-
3	-	-	3.75, d (11.0)	3.95, d (1.0)
5	2.37, dd (2.2, 8.0)	2.26, dd (2.2, 8.0)[d]	2.24, dd (1.3, 5.8)	2.19, dd (1.1, 5.8)
6	5.19, ddd (5.7, 8.0, 9.7)	5.11, ddd (6.0, 8.1, 9.7)	5.17, ddd/br quintet-like (5.1, 5.1, 10.2)	5.21, ddd/br quintet-like (4.9, 5.7, 10.2)
7	4.22, dd (9.7, 17.3)	4.15, dd (9.9, 17.2)	4.05, dd (9.9, 17.4)	4.03, dd (9.9, 17.3)
	3.03, dd (5.6, 17.2)	2.98, dd (5.9, 17.1)	2.98, dd (4.8, 17.4)	2.93, dd (4.7, 17.3)
11	7.23, d (8.2)	6.57, s	6.52, s	6.65, s
12	7.49, dd (0.9, 8.1)	-	-	-
15	6.81, d (2.2)	6.83, d (2.2)	6.84, d (2.2)	6.84, d (2.2)
16	7.75, d (2.2)	7.53, d (2.2)	7.52, d (2.2)	7.64, d (2.2)
18	1.44, s	1.44, s	1.60, s	2.14, s
20	4.12, dd (3.7, 9.6)	4.14, dd (3.7, 9.7)	4.25, dd (1.5, 9.0)	4.18, dd (1.6, 8.6)
	3.77, dd (2.2, 9.6)	3.82, dd (2.2, 9.7)	3.76, dd (1.5, 9.0)	3.54, dd (1.5, 8.6)
OCH_3	-	3.92, s	3.91, s	3.94, s
2-OH	-	-	4.29, s	-
3-OH	-	-	5.01, d (10.6)	-

[a] For CH_2, the deshielded signal was assigned as H_a, and the shielded signal as H_b.

[b] Data measured in methanol-d_4.

[c] Data measured in $CDCl_3$.

[d] Signal was partially obscured.

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Table 2

^{1}H (400 MHz) NMR Spectroscopic Data for Compounds 5-7 (δ in ppm, J in Hz)[a]

	5[b]	6[b]	7[c]
position	**δ_H (J in Hz)**	**δ_H (J in Hz)**	**δ_H (J in Hz)**
1	1.81-1.88, m[d]	1.82, br d (8.6)[d]	1.75, dddd (3.3, 3.3, 10.3, 10.3)
	1.65, ddd/dt-like (6.6, 11.7, 11.7)	1.67, ddd (3.5, 9.5, 12.8)	1.60-1.67, m[d]
2	2.10, ddd (6.2, 12.0, 12.8)	2.03-2.08, m[d]	1.95-2.01, m[d]
	1.81-1.88, m[d]	1.82, br d (8.6)[d]	
5	2.32, dd (1.3, 5.4)	2.03-2.04, m[d]	1.98-2.01, m[d]
6	4.79, ddd/br t-like (0.8, 5.3, 5.4)	4.71-4.77, m[d]	4.67, ddd/br t-like (0.9, 5.0, 5.2)
7	2.54, ddd (1.1, 5.2, 16.1)	2.56-2.64, m[d]	2.20-2.25, m
	1.94, ddd (4.1, 12.9, 16.2)		1.23-1.29, m
8	3.26, ddd/dt-like (4.8, 4.8, 12.8)[d]	-	2.73-2.80, m
9	2.20, ddd/dt-like (4.2, 4.2, 13.9)	2.04-2.08, m[d]	1.57-1.62, m[d]
11	2.59, dd (13.9, 17.0)	2.81, dd (4.8, 16.9)	1.82-1.83, m
	2.40, dd (4.1, 17.0)	2.52-2.62, m[d]	1.32-1.40, m[d]
12			2.89, ddd (1.5, 4.6, 14.4)
	-	-	2.27-2.35, m
14	-	-	4.88, dd (1.0, 6.3)
15	6.65, d (2.0)	6.66, d (2.0)	5.81, s
16	7.53, d (2.0)	7.63, d (2.0)	-
18	1.36, s	1.29, s	1.33, s
20	4.39, dd (3.4, 9.6)	4.71-4.77, m[d]	4.42, dd (3.6, 9.6)
	3.65, dd (1.7, 9.5)	3.67, dd (1.9, 10.1)	3.65, dd (1.6, 9.5)

[a] For CH_2, the deshielded signal was assigned as H_a, and the shielded signal as H_b.

[b] Data measured in methanol-*d4*.

[c] Data measured in $CDCl_3$.

[d] Signal was partially obscured.

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Table 3

^{13}C (100 MHz) NMR Spectroscopic Data for Compounds 1-7 (δ in ppm)

	1[a]	2[b]	3[b]	4[a]	5[a]	6[a]	7[b]
position	**δ_C, type**	**δ_C, type**	**δ_C, type**	**δ_C, type**	**δ_C, type**	**δ_C, type**	**δ_C, type**
1	30.9, CH_2	29.7, CH_2	40.3, CH_2	37.9, CH_2	31.0, CH_2	31.4, CH_2	30.4, CH_2
2	28.4, CH_2	26.2, CH_2	103.9, C	108.0, C	29.4, CH_2	29.5, CH_2	27.1, CH_2
3	98.2, C	96.9, C	81.3, CH	75.3, CH	97.8, C	97.3, C	96.7, C
4	50.9, C	49.1, C	44.2, C	48.8, C	53.3, C	52.3, C	51.5, C
5	52.1, CH	51.4, CH	54.5, CH	52.8, CH	45.5, CH	47.0, CH	45.7, CH
6	75.9, CH	75.0, CH	74.7, CH	76.4, CH	75.8, CH	75.1, CH	74.8, CH
7	26.9, CH_2	25.7, CH_2	24.7, CH_2	25.7, CH_2	28.0, CH_2	35.4, CH_2	19.4, CH_2
8	118.2, C	109.6, C	109.2, C	110.7, C	28.8, CH	69.2, C	32.8, CH
9	135.0, C	134.3, C	133.8, C	136.5, C	39.4, CH	47.6, CH	37.2, CH
10	36.2, C	35.5, C	48.0, C	48.8, C	33.5, C	34.6, C	33.1, C
11	120.4, CH	100.1, CH	98.7, CH	99.9, CH	35.2, CH_2	38.8, CH_2	20.9, CH_2
12	120.4, CH	152.3, C	152.5, C	153.8, C	195.2, C	194.9, C	26.4, CH_2
13	127.9, C	116.7, C	117.0, C	117.9, C	120.9, C	121.0, C	167.1, C
14	154.4, C	154.2, C	154.1, C	155.6, C	172.5, C	169.9, C	82.4, CH
15	107.7, CH	104.3, CH	104.4, CH	105.2, CH	107.1, CH	106.9, CH	114.6, CH
16	146.8, CH	144.0, CH	143.9, CH	145.2, CH	145.3, CH	146.1, CH	173.0, C
18	20.0, CH_3	20.0, CH_3	22.7, CH_3	20.9, CH_3	17.4, CH_3	18.2, CH_3	17.6, CH_3
19	181.7, C	180.6, C	180.1, C	181.9, C	180.8, C	180.8, C	179.6, C
20	72.6, CH_2	71.2, CH_2	72.2, CH_2	72.2, CH_2	73.9, CH_2	75.3, CH_2	73.0, CH_2
OCH_3	-	55.7, CH_3	55.7, CH_3	56.2, CH_3	-	-	-

[a] Data measured in methanol-*d4*.

[b] Data measured in $CDCl_3$.

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Table 4

Cytotoxic Activity (IC_{50} μM) in Cancer Cell Lines

	IC_{50} (μM)		
compound	**MDA-MB-435**	**MDA-MB-231**	**OVCAR3**
Icacinol	1.25*	7.30	7.55
Humirianthol	1.65*	3.74	4.12
Humirianthenolide C	0.66*	0.67	1.05
Icacenone	6.44*	10.85	18.71
Icacinlactone A (**1**)	> 20	> 20	17.76
Icacinlactone B (**2**)	> 20	> 20	> 20
Icacinlactone C (**3**)	> 20	> 20	> 20
Icacinlactone D (**4**)	> 20	> 20	> 20
Icacinlactone E (**5**)	> 20	> 20	> 20
Icacinlactone F (**6**)	6.16	8.94	10.50
Icacinlactone G (**7**)	> 20	> 20	> 20
Vinblastine	0.49 *n*M	8.78 *n*M	1.82 *n*M

*cited for comparison[6]