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Germacrane-type sesquiterpenoids with cytotoxic activity from *Sigesbeckia orientalis*

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ABSTRACT

Eleven new highly oxygenated germacrane-type sesquiterpenoids (**1–11**) and 16 known analogues (**12–27**) were isolated from the aerial parts of *Sigesbeckia orientalis*. Their structures, including absolute configurations, were determined by comprehensive spectroscopic methods especially NMR and ECD analyses. Compounds **13**, **21** and **23** possessing an 8-methacryloxy group showed stronger *in vitro* cytotoxicity against human A549 and MDA-MB-231 cancer cell lines than other co-metabolites, with IC_{50} values ranging from 6.02 to 10.77 μM comparable to the positive control adriamycin.

1. Introduction

Sigesbeckia L. is a small genus of the plant family Compositae (also known as Asteraceae) but distributed all over the Northern Hemisphere. Three species, *S. orientalis* L., *S. pubescens* (Makino) Makino and *S. glabrescens* (Makino) Makino, widely grow in most provinces of china [1]. The aerial parts of all three Chinese species, known as 'Xixiancao' in Traditional Chinese Medicine, have been used for centuries to treat snakebites, cutaneous disorders, inflammatory diseases and rheumatic arthritis [2,3]. A series of *ent*-kaurane and *ent*-pimarane type diterpenoids, as well as sesquiterpenoids and flavonoids, have previously been reported from plants of this genus, exhibiting anti-inflammatory, antiproliferative, antithrombotic, and antihistamine-release properties [4–7]. Among them, the sesquiterpenoids have attracted our attention due to not only their structural diversity but also their potential bioactivities [8,9].

One of the major types of sesquiterpene lactones found in *Sigesbeckia* plants are germacranolides, with over 20 structures reported to date [4,9–11]. The typical structural features of a germacranolide from *Sigesbeckia* species contain a 5-membered α-methylene-γ-lactone ring fused with a 10-membered carbocycle, which is usually oxidized at C-8, C-9 C-14 or C-15 [11]. Previous phytochemical investigations of the plants from this genus reported that germacranolides possessing an α-methylene-γ-lactone group in the structure showed potent cytotoxic activity [12]. For example, four germacranolides from *Siegesbeckia glabrescens* and one from *Siegesbeckia orientalis* exhibited cytotoxicity against human cancer cells with IC_{50} values ranging from 0.9 to 33.3 μM [8,9]. However, to the best of our knowledge, most of the germacranolides isolated previously have not been biologically tested. In our continuing search for new bioactive molecules from medicinal plants, 11 undescribed germacrane-type sesquiterpenoids and 16 known analogues were isolated and identified from the ethanolic extract of *S. orientalis* L. (Fig. 1). In this report, the separation, structural elucidation and cytotoxic evaluation of these compounds are presented.

2. Experimental section

2.1. General experimental procedures

Optical rotations were measured on a Rudolph VI polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). ECD spectra were recorded on a Chirascan CD spectropolarimeter (Applied Photophysics Ltd., Surrey, UK). UV data were acquired on a Shimadzu UV-2600 spectrophotometer (Shimadzu, Kyoto, Japan). IR spectra were recorded on a VERTEX70 spectrometer (Bruker Optics Inc., Billerica, USA) with KBr disks. NMR experiments were performed on a Bruker Avance DRX-600 spectrometer (Bruker BioSpin AG, Fällanden, Switzerland) and referenced to residual solvent signals ($CDCl_3$: δ_H 7.26, δ_C 77.16). ESIMS and HRESIMS analyses were carried out on Agilent 6460 Triple Quad LC/MS and Agilent 6545 Q-TOF LC/MS spectrometers (Agilent Technologies Inc., Waldbronn, Germany), respectively. Column chromatography (CC) was performed on D101-macroporous

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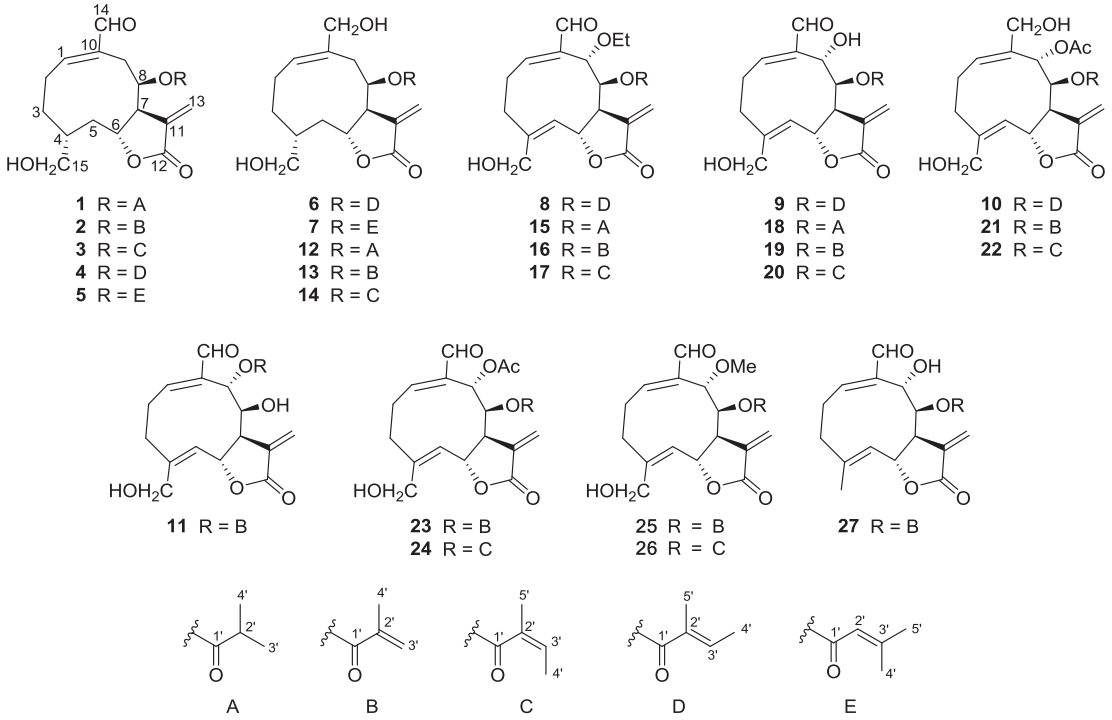

Fig. 1. Structures of compounds **1–27**.

absorption resin (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China), Silica gel (200–300 mesh, Qingdao Marine Chemical Plant Ltd., Qingdao, China), Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden), MCI gel (CHP20P, Mitsubishi Chemical Corporation, Tokyo, Japan) and reversed phase C18 silica gel (Merck KGaA, Darmstadt, Germany). Pre-coated silica gel GF_{254} plates (Yantai Jiangyou Silica Gel Development Co. Yantai, China) were used for TLC. Semipreparative HPLC was performed on an Agilent 1260 series LC instrument (Agilent Technologies Inc., Waldbronn, Germany) coupled with an ODS column (YMC-pack ODS-A, 10 × 250 mm, 5 μm). All solvents used for CC were of analytical grade (Tianjin Fuyu Fine Chemical Co. Ltd., Tianjin, China) and solvents used for HPLC were of HPLC grade (Oceanpak Alexative Chemical Ltd., Goteborg, Sweden).

2.2. Plant material

The aerial parts of *S. orientalis* L. were collected in July 2017 at Mount Kunyu, Shandong Province, and were authenticated by Prof. Jie Zhou from University of Jinan. A voucher specimen has been deposited at School of Biological Science and Technology, University of Jinan (Accession number: npmc-011).

2.3. Extraction and isolation

The air-dried and powdered plant materials (15 kg) were extracted exhaustively with 95% EtOH at room temperature (3 × 120 L, each for one week). The crude extract (1.08 kg) was suspended in H_2O (2.5 L) and then partitioned with EtOAc (4 × 2.5 L). The EtOAc partition (750 g) was separated by CC over D101-macroporous absorption resin, eluted with 50% MeOH-H_2O to afford a brown gum (146 g) and was then applied to a silica gel (200–300 mesh) column using petroleum ether-acetone (8:1, 4:1, 2:1, 1:1) as eluent to afford seven fractions (Fr. 1–Fr. 7).

Fr. 3 (40.6 g) was subjected to silica gel CC eluted with $CHCl_3$-MeOH (1:0 to 1:1) to give five subfractions (Fr. 3.1–Fr. 3.5) and Fr. 3.2 (6.7 g) was further chromatographed on RP-C18 silica gel to afford eight subfractions (Fr. 3.2.1–Fr. 3.2.8). Fr. 3.2.1 (1.1 g) was separated by silica gel CC, eluted with petroleum ether-EtOAc (15:1 to 1:1), followed by purification on HPLC (58% MeOH-H_2O, 2.0 mL/min) to afford **25** (1.6 mg, t_R = 9.0 min) and **26** (1.4 mg, t_R = 14.0 min). Fr. 3.2.2 (824 mg) was chromatographed on Sephadex LH-20 using $CHCl_2$-MeOH (1:1) and then purified by semipreparative HPLC (50% MeOH-H_2O, 2.0 mL/min) to yield **27** (0.5 mg, t_R = 20.0 min). Fr. 3.2.4 (2.6 g) was separated on a column of RP-18 silica gel (MeOH-H_2O, 40:60 to 80:20, v/v) to give six fractions (Fr. 3.2.4.1–Fr. 3.2.4.6). Fr. 3.2.4.1 (61.3 mg) was further purified by semipreparative HPLC (60% MeOH-H_2O, 2.0 mL/min) to yield compound **23** (30.3 mg, t_R = 13.8 min). Fr. 3.2.4.2 (729 mg) was also separated using semipreparative HPLC (50% MeOH-H_2O, 2.0 mL/min) to afford compounds **15** (1.3 mg, t_R = 13.0 min), **24** (0.5 mg, t_R = 14.0 min), **8** (2.8 mg, t_R = 14.9 min) and **17** (20.4 mg, t_R = 16.2 min). Fr. 3.2.4.3 (151 mg) was purified by silica gel CC (petroleum ether-Me_2CO, 5:1) to yield compound **16** (70.7 mg).

Fr. 3.3 (11.8 g) was first separated on a MCI gel column (MeOH-H_2O, 30:70 to 80:20, v/v) to give three subfractions (Fr. 3.3.1–Fr. 3.3.2). Fr. 3.3.1 (94 mg) was further chromatographed on silica gel CC using petroleum ether-Me_2CO (8:1 to 1:1) as eluent and then purified by semipreparative HPLC (45% MeOH-H_2O, 2.0 mL/min) to yield **21** (12.2 mg, t_R = 24.1 min), **10** (1.8 mg, t_R = 31.3 min) and **22** (4.3 mg, t_R = 33.6 min). Separation of Fr. 3.3.2 (169 mg) following the similar procedure as for Fr. 3.3.1 yielded **2** (14.2 mg, t_R = 17.2 min), **1** (2.0 mg, t_R = 20.0 min), **4** (0.6 mg, t_R = 26.1 min) and a mixture (4.0 mg) with 49% MeOH-H_2O as HPLC eluent (2.0 mL/min). The mixture was repeatedly purified via semipreparative HPLC (45% MeOH-H_2O, 2.0 mL/min) to yield compounds **3** (2.0 mg, t_R = 26.5 min) and **5** (0.5 mg, t_R = 28.0 min). Fraction 3.3.3 (85 mg) was applied to silica gel CC using petroleum ether-Me_2CO (6:1 to 1:1), and then purified by semipreparative HPLC (36% MeOH-H_2O, 2.0 mL/min) to give **11** (2.0 mg).

Fr. 3.4 (7.5 g) was subjected to RP-C18 silica gel CC with step gradient MeOH-H_2O (40:60 to 90:10, v/v) and then to a Sephadex LH-20 column ($CHCl_2$-MeOH, 1:1) to give five subfractions (Fr. 3.4.1–Fr. 3.4.5). Fr. 3.4.1 (1.1 g) was purified by semipreparative HPLC using MeOH-H_2O (41%, 2.0 mL/min) to yield compounds **19** (24.3 mg, t_R = 16.3 min) and **18** (1.5 mg, t_R = 17.7 min). Compounds **9** (0.8 mg,

Table 1
^{1}H NMR Data of compounds **1–6** (600 MHz) in $CDCl_3$ (δ in ppm and J in Hz).

Position	1	2	3	4	5	6
1	6.72 t (8.2)	6.74 t (8.2)	6.72 t (8.2)	6.72 t (8.2)	6.72 t (8.2)	5.69 m[m]
2a	2.69 m[a]	2.68 m[d]	2.69 m[g]	2.68 m[i]	2.67 m[k]	2.35 m
2b	2.69 m[a]	2.68 m[d]	2.69 m[g]	2.68 m[i]	2.67 m[k]	2.28 m
3a	2.23 m	2.22 m	2.23 m	2.23 m	2.22 m	1.15 m
3b	1.45 m[b]	1.43 m[e]	1.43 m	1.40 m	1.42 m	2.03 m[n]
4	1.96 m	1.96 m[f]	1.95 m[h]	1.96 m[j]	1.94 m[l]	1.90 m
5a	1.45 m[b]	1.46 m[e]	1.48 m	1.47 m	1.46 m	1.55 m
5b	1.91 m	1.95 m[f]	1.95 m[f]	1.96 m[j]	1.93 m[l]	2.03 m[n]
6	4.72 m	4.78 m	4.73 m	4.78 m	4.72 m	4.95 m
7	2.75 br s	2.79 br s	2.77 br s	2.76 br s	2.71 br s	3.16 m
8	5.55 br s	5.52 m	5.61 m	5.55 m	5.60 m	5.33 m
9a	2.85 m	2.95 m	2.97 m	2.96 m	2.94 m	2.61 dd (5.2, 13.8)
9b	2.69 m[a]	2.68 m[d]	2.69 m[g]	2.68 m[i]	2.68 m[k]	2.74 dd (10.2, 13.8)
13a	6.29 d (2.2)	6.28 d (2.1)	6.30 d (2.2)	6.27 d (2.1)	6.28 d (2.2)	6.28 d (2.0)
13b	5.28 d (1.8)	5.70 d (1.7)	5.70 d (1.8)	5.68 d (1.8)	5.67d (1.8)	5.68 m[m]
14	9.50 s	9.50 s	9.51 s	9.50 s	9.50 s	4.16 d (3.2)
15	3.50 m	3.50 m	3.50 m	3.51 m	3.50 m	3.46 m
2′	2.45 m				5.54 s	
3′	1.11 d (7.0)	6.00 s 5.55 s	6.07 q (7.2)	6.76 m		6.80 m
4′	1.07 d (7.0)	1.87 s	1.96 d (7.2)	1.76 d (7.2)	1.87 s	1.78 d (7.0)
5′			1.78 s	1.75 s	2.13 s	1.77 s

[a–n] Overlapping signals.

t_R = 21.3 min), **20** (5.1 mg, t_R = 22.6 min) and **28** (3.5 mg, t_R = 20.0 min) were separated from Fr. 3.4.2 by semipreparative HPLC using MeOH-H_2O (45%, 2.0 mL/min). Fr.3.4.3 (56.8 mg) was also fractionated by HPLC eluted with MeOH-H_2O (47%, 2.0 mL/min) to yield **13** (51.2 mg, t_R = 21.8 min) and **14** (5.2 mg, t_R = 37.4 min), while Fr.3.4.4 (9.3 mg) was purified by HPLC with MeOH-H_2O (47%, 2.0 mL/min) as eluent to yield **12** (6.4 mg, t_R = 24.4 min), **6** (2.2 mg, t_R = 34.5 min) and **7** (2.0 mg, t_R = 35.7 min).

(4*β*,10*E*)-6α,15-dihydroxy-8*β*-(isobutyryloxy)-14-oxogermacra-1(10),11(13)-diene-12-oic acid 12,6-lactone (1): Colorless oil; $[\alpha]_D^{20}$ − 98.2 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 224 (3.97) nm; IR (KBr) ν_{max} 3450, 2929, 1766, 1742, 1685, 1272, 1158, 1069 cm^{-1}; ECD (MeOH) λ(Δε) 220 (−1.02), 261 (+0.06); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; (+)-ESIMS *m/z* 373.1 [M + Na]$^+$; (+)-HRESIMS *m/z* 373.1620 [M + Na]$^+$ (calcd for $C_{19}H_{26}O_6Na$, 373.1622).

(4*β*,10*E*)-6α,15-dihydroxy-8*β*-(methacryloxy)-14-oxogermacra-1(10),11(13)-diene-12-oic acid 12,6-lactone (2): Colorless oil; (MeOH); $[\alpha]_D^{20}$ − 155.3 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.08) nm; IR (KBr) ν_{max} 3447, 2929, 1760, 1718, 1679, 1641, 1171 cm^{-1}; ECD (MeOH) λ(Δε) 218 (−1.62), 261 (+0.09) nm; ^{13}C NMR and ^{1}H NMR data, see Tables 1 and 2; (+)-ESIMS *m/z* 371.1 [M + Na]$^+$; (+)-HRESIMS *m/z* 371.1464 [M + Na]$^+$ (calcd for $C_{19}H_{24}O_6Na$, 371.1465).

(4*β*,10*E*)-6α,15-dihydroxy-8*β*-(angeloyloxy)-14-oxogermacra-1(10),11(13)-diene-12-oic acid 12,6-lactone (3): Colorless oil; $[\alpha]_D^{20}$ − 161.3 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.27) nm; IR (KBr) ν_{max} 3453, 2932, 1763, 1721, 1679, 1230, 1156,1046 cm^{-1}; ECD (MeOH) λ (Δε) 228 (−1.85) nm; ^{13}C NMR and ^{1}H NMR data, see Tables 1 and 2; (+)-ESIMS *m/z* 385.1 [M + Na]$^+$; (+)-HRESIMS *m/z* 385.1616 [M + Na]$^+$ (calcd for $C_{20}H_{26}O_6Na$, 385.1622).

Table 2
^{13}C NMR Data of compounds **1–11** (150 MHz) in $CDCl_3$ (δ in ppm).

Position	1	2	3	4	5	6	7	8	9	10	11
1	157.3	157.3	157.2	157.1	157.1	131.4	131.4	155.5	155.3	134.1	158.9
2	27.9	27.9	28.0	28.0	28.0	26.6	26.6	27.7	27.6	26.5	28.0
3	30.4	30.3	30.4	30.4	30.4	30.3	30.4	32.9	32.7	33.3	33.0
4	42.1	42.1	42.2	42.2	42.3	42.3	42.3	139.7	140.4	142.1	140.6
5	40.6	40.5	40.7	40.6	40.6	40.6	40.6	129.8	129.3	128.2	129.5
6	79.2	79.2	79.3	79.2	79.2	79.2	79.3	73.9	73.9	74.2	73.0
7	48.7	48.6	48.7	48.7	48.6	48.0	48.0	51.3	51.8	51.0	52.0
8	73.8	75.1	74.1	74.7	73.2	77.2	75.4	69.8	72.0	69.5	68.5
9	27.6	27.5	27.8	27.7	27.9	31.0	31.2	76.3	70.7	72.3	73.2
10	138.9	138.8	139.0	139.1	139.1	133.6	133.6	141.9	144.6	136.2	141.4
11	136.0	135.9	136.0	136.0	133.4	136.9	136.9	133.9	134.3	134.5	135.9
12	169.5	169.6	169.6	169.6	169.7	169.9	170.0	169.7	169.3	169.7	169.6
13	124.5	124.5	124.5	124.5	124.3	123.9	123.7	123.0	122.2	121.6	120.8
14	194.5	194.5	194.5	194.5	194.6	68.1	68.1	194.5	195.6	64.2	194.4
15	67.8	67.7	67.8	67.8	67.8	67.9	67.9	61.2	61.0	61.2	61.3
1′	175.9	166.2	166.5	166.8	165.3	167.3	165.9	166.8	168.2	167.0	167.2
2′	34.2	135.7	127.2	128.1	115.3	128.1	115.3	128.5	128.0	127.7	135.6
3′	18.7	126.6	139.9	138.6	158.8	138.9	159.0	137.9	139.4	139.3	127.2
4′	19.1	18.3	15.9	14.7	27.6	14.7	27.7	14.7	14.8	14.8	18.4
5′			20.5	12.1	20.5	12.0	20.5	12.5	12.4	12.2	
7-OEt								64.7 15.2			
9-OAc										170.3 21.0	

(4*β*,10*E*)-6α,15-dihydroxy-8*β*-(tigloyloxy)-14-oxogermacra-1(10),11(13)-diene-12-oic acid 12,6-lactone (4): Colorless oil; $[\alpha]_D^{20}$ − 107.2 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 217 (3.99) nm; IR (KBr) ν_{max} 3438, 2932, 1757, 1715, 1679, 1640, 1266,1138,1075 cm^{-1}; ECD (MeOH) λ (Δε) 225 (−1.22), 258 (+0.03); ^1H and ^{13}C NMR data, see Tables 1 and 2; (+)-ESIMS *m/z* 385.1 $[M+Na]^+$; (+)-HRESIMS *m/z* 385.1623 $[M+Na]^+$ (calcd for $C_{20}H_{26}O_6Na$, 385.1622).

(4*β*,10*E*)-6α,15-dihydroxy-8*β*-(senecioyloxy)-14-oxogermacra-1(10),11(13)-diene-12-oic acid 12,6-lactone (5): Colorless oil; $[\alpha]_D^{20}$ − 112.2 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 221 (4.41) nm; IR (KBr) ν_{max} 3450,2932, 1762, 1724, 1682, 1650, 1272, 1233, 1144, 1078 cm^{-1}; ECD (MeOH) λ (Δε) 231 (−2.47), 266 (+0.04) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; (+)-ESIMS *m/z* 385.1 $[M+Na]^+$; (+)-HR-ESIMS *m/z* 385.1619 $[M+Na]^+$ (calcd for $C_{20}H_{26}O_6Na$, 385.1622).

(4*β*,10*E*)-6α,14,15-trihydroxy-8*β*-(tigloyloxy)-germacra-1(10),11(13)-diene-12-oic acid 12,6-lactone (6): Colorless oil; $[\alpha]_D^{20}$ − 96.2 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.47) nm; IR (KBr) ν_{max} 3441, 2929, 1755, 1710, 1388, 1263, 1139, 1079, 1027 cm^{-1}; ECD (MeOH) λ (Δε) 213 (−1.41), 259 (+0.08); ^1H and ^{13}C NMR data, see Tables 1 and 2; (+)-ESIMS *m/z* 387.1 $[M+Na]^+$; (+)-HR-ESIMS *m/z* 387.1788 $[M+Na]^+$ (calcd for $C_{20}H_{28}O_6Na$, 387.1778).

(4*β*,10*E*)-6α,14,15-trihydroxy-8*β*-(senecioyloxy)-germacra-1(10),11(13)-diene-12-oic acid 12,6-lactone (7): Colorless oil; $[\alpha]_D^{20}$ − 116.2 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 201 (4.33) nm; IR (KBr) ν_{max} 3432, 2938, 1757, 1712, 1648, 1271, 1225, 1143, 1074, 1003 cm^{-1}; ECD (MeOH) λ(Δε) 216 (−2.39), 258 (+0.04) nm; ^{13}C and ^1H NMR data, see Tables 2 and 3; (+)-ESIMS *m/z* 387.1 $[M+Na]^+$; (+)-HRESIMS *m/z* 387.1775 $[M+Na]^+$ (calcd for $C_{20}H_{28}O_6Na$, 387.1778).

(1(10)*E*,4*Z*)-9α-ethoxy-6α,15-dihydroxy-8*β*-(tigloyloxy)-14-oxogermacra-1(10),4,11(13)-triene-12-oic acid 12,6-lactone (8): Colorless oil; $[\alpha]_D^{20}$ − 22.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 200 (3.63) nm; IR (KBr) ν_{max} 3456, 2929, 1769, 1685, 1257, 1138, 1081, 1034, 983 cm^{-1}; ECD (MeOH) λ (Δε) 198 (+1.56), 235 (−1.17) nm; ^{13}C and ^1H NMR data, see Tables 2 and 3; (+)-ESIMS *m/z* 405.1 $[M+H]^+$; (+)-HRESIMS *m/z* 405.1913 $[M+H]^+$ (calcd for $C_{22}H_{29}O_7$, 405.1908).

(1(10)*E*,4*Z*)-6α,9α,15-trihydroxy-8*β*-(tigloyloxy)-14-oxogermacra-1(10),4,11(13)-triene-12-oic acid 12,6-lactone (9): Colorless oil; $[\alpha]_D^{20}$ − 11.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 201 (4.34) nm; IR (KBr) ν_{max} 3436, 2965, 2927, 1760, 1261, 1096, 1033 cm^{-1}; ECD (MeOH) λ (Δε) 198 (+1.36), 228 (−0.81) nm; ^{13}C and ^1H NMR data, see Tables 2 and 3; (+)-ESIMS *m/z* 399.1 $[M+Na]^+$; (+)-HR-ESIMS *m/z* 399.1414 $[M+Na]^+$ (calcd for $C_{20}H_{24}O_7Na$, 399.1414).

(1(10)*E*,4*Z*)-9α-acetyloxy-6α,14,15-trihydroxy-8*β*-(tigloyloxy)-germacra-1(10),4,11(13)-triene-12-oic acid 12,6-lactone (10): Colorless oil; $[\alpha]_D^{20}$ + 4.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 200 (4.61) nm; IR (KBr) ν_{max} 3449, 2929, 1742, 1628, 1384, 1255 cm^{-1}; ECD (MeOH) λ (Δε) 204 (−1.69), 226 (+0.29) nm; ^{13}C and ^1H NMR data, see Tables 2 and 3; (+)-ESIMS *m/z* 443.1 $[M+Na]^+$; (+)-HR-ESIMS *m/z* 443.1684 $[M+Na]^+$ (calcd for $C_{22}H_{28}O_8Na$, 443.1676).

(1(10)*E*,4*Z*)-6α,8*β*,15-trihydroxy-9α-(methacryloxy)-14-oxogermacra-1(10),4,11(13)-triene-12-oic acid 12,6-lactone (11): Colorless oil; $[\alpha]_D^{20}$ − 13.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 200 (3.93) nm; IR (KBr) ν_{max} 3441, 2933, 1762, 1714, 1686, 1302, 1255, 1031 cm^{-1}; ECD (MeOH) λ (Δε) 194 (+1.50), 222 (−0.90) nm; ^{13}C and ^1H NMR data, see Tables 2 and 3; (+)-ESIMS *m/z* 385.0 $[M+Na]^+$; (+)-HR-ESIMS *m/z* 385.1254 $[M+Na]^+$ (calcd for $C_{19}H_{22}O_7Na$, 385.1258).

2.4. Cytotoxicity assay

Cytotoxicity against human A549 and MDA-MB-231 cell lines was evaluated using CCK-8 method as described previously [13], and adriamycin was used as a positive control. Cells were treated with different concentrations of tested samples in the growth medium for 24 h.

Table 3
^1H NMR Data of compounds **7–11** (600 MHz) in $CDCl_3$ (δ in ppm and *J* in Hz).

Position	7	8	9	10	11
1	5.67 m[a]	6.76 dd (10.2, 7.5)	6.68 dd (10.2, 7.4)	5.80 t (8.2)	6.77 dd (10.0, 7.6)
2a	2.33 m	2.72 m	2.70 m	2.33 m	2.69 m
2b	2.27 m	2.58 m	2.55 m	2.43 m	3.01 m
3a	1.16 m	2.03 m	2.04 m	1.92 m[c]	2.03 t (12.1)
3b	2.00 m[b]	2.85 m	2.85 m	2.69 m	2.82 m
4	1.90 m				
5	1.55 m 2.00 m[b]	5.04 d (10.6)	5.05 d (10.6)	5.16 d (10.6)	5.03 d (10.6)
6	4.87 m	5.23 t (10.2)	5.31 t (10.2)	5.39 t (10.1)	5.43 t (10.2)
7	3.12 br s	2.64 m	2.63 m	3.32 m	2.48 d (9.5)
8	5.37 m	6.58 dd (1.6, 8.3)	6.44 dd (1.6, 8.3)	6.11dd (1.8, 9.5)	5.31 d (8.2)
9	2.57 dd (5.2, 13.8) 2.71 dd (10.2, 13.8)	3.95 dd (2.1, 8.3)	4.18 d (8.3)	5.56 d (9.5)	5.37 dd (1.6, 8.3)
13a	6.27 d (2.0)	6.27 d (3.1)	6.26 d (3.1)	6.22 d (3.5)	6.36 (3.1)
13b	5.67 m[a]	5.91 d (3.1)	5.70 d (3.1)	5.68 d (3.1)	5.67 (3.1)
14	4.16 s	9.51 d (2.1)	9.51 d (2.1)	4.39 d (12.1) 4.23 d (12.1)	9.46 s
15	3.45 m	4.41 d (12.5) 4.51 d (12.5)	4.35 d (12.8) 4.51 d (12.8)	4.53 s	4.49 m
2′	5.58 s				
3′		6.83 dq (1.4, 7.0)	6.88 dq (1.4, 7.0)	6.79 q (7.2)	6.13 s 5.62 s
4′	1.87	1.82 dd (1.0, 7.0)	1.83 dd (1.0, 7.0)	1.80 d (7.2)	1.92 s
5′	2.12 s	1.84 t (1.1)	1.85 t (1.1)	1.78 s	
7-OEt		3.38 m 3.10 m 1.03 t (7.0)			
9-OAc				1.91 s[c]	

[a–c] Overlapping signals.

3. Results and discussion

Compound **1** was assigned the molecular formula of $C_{19}H_{26}O_6$ by HR-ESIMS analysis at *m/z* 373.1620 ([M + Na]$^+$ calcd 373.1622), requiring seven indices of hydrogen deficiency. The ^{1}H NMR data (Table 1) displayed signals characteristic of an aldehyde group with a singlet at δ_H 9.50 and an α-methylene-γ-lactone moiety with a pair of doublets at δ_H 6.29 (d, J = 2.2 Hz, H-13a) and 5.28 (d, J = 1.8 Hz, H-13b). One olefinic proton (δ_H 6.72, t, J = 8.2 Hz, H-1), two oxymethine protons (δ_H 4.72, m, H-6 and 5.55, br s, H-8), two oxymethylene protons (δ_H 3.50, m, H_2-15) and two methyl protons (δ_H 1.11, d, J = 7.0 Hz, H-3′ and 1.07, d, J = 7.0 Hz, H-4′) were also observed. The ^{13}C NMR data (Table 2) displayed 19 signals categorized by DEPT experiment into two methyls, six methylenes (one oxygenated and one olefinic), seven methines (one aldehydic, two oxygenated and one olefinic) and four quaternary carbons (two carbonyl and two olefinic). The aforementioned spectroscopic data of **1** were similar to those of (4*β*,10*E*)-6α,14,15-trihydroxy-8*β*-(isobutyryloxy)-germacra-10,11(13)-diene-12-oic acid 12,6-lactone (**12**) [14], a major germacrane-type sesquiterpenoid isolated in this study, except for the resonances of an aldehyde group (δ_H 9.50, δ_C 194.5) in **1** replacing those for an oxymethylene group (δ_H 3.50, δ_C 67.8) in **14** at C-10. This assignment was further confirmed by the HMBC correlations (Fig. 2) from H-14 (δ_H 9.50, *s*) to C-1 (δ_C 157.3), C-10 (δ_C 138.9) and C-9 (δ_C 27.6). The other correlations in the HMBC and ^{1}H–^{1}H COSY spectra as depicted in Fig. 2 also confirmed the planar structure of **1** as shown.

The relative configuration of **1** was assigned to be identical with that of **12** on the basis of excellent NMR comparisons at all chiral centers, as well as analysis of NOESY data described below. The *E*-geometry for the C-1(10) double bond was first established by the NOESY correlations of H-1/H-14. Then the cross-peaks of H-5a/H-7, H-7/H-8 and H-8/H-9a, as well as H-9b/H-6 and H-6/H-4, suggested that H-7, H-8 and H-9a were located at the same side of the ring, while H-4, H-6 and H-9b were on the other side (Fig. 3). The absolute configuration of **1** was eventually determined by computational approach of quantum chemistry using Time-Dependent Density Functional Theory (TD-DFT) method. We randomly selected 4*R*,6*R*,7*S*,8*R*-configuration as the model to carry out the conformational search using MMFFs force field with an energy window of 3.01 kcal/mol. Geometry optimizations were carried out at the B3LYP/6-311 G (d, p) level using Gaussian 09 program. Then the 30 lowest electronic transitions for the obtained conformers *in vacuum* were calculated using time-dependent density functional theory (TD-DFT) methods at the CAM-B3LYP/6-311G (d, p) level. After Boltzmann averaging, the computed ECD spectrum for the 4*R*,6*R*,7*S*,8*R* stereoisomer of **1** was in close agreement with the experimental ECD spectrum (Fig. 4). Accordingly, compound **1** was unambiguously elucidated as a new germacranolide named (4*β*,10*E*)-6α,15-dihydroxy-8*β*-(isobutyryloxy)-14-oxogermacra-1(10),11(13)-diene-12-oic acid 12,6-lactone.

Comparison of the NMR data of **1** with those of compounds **2–5** disclosed that the only structural differences between these cometabolites occurred at the substituent at C-8. Compound **2** gave a molecular formula of $C_{19}H_{24}O_6$, as assigned by the HR-ESIMS ion at *m/z* 371.1464 [M + Na]$^+$ (calcd for 371.1465), being two mass units less than that of **1**. The ^{1}H NMR data of **2** (Table 1) showed high similarity to those of **1**, except for the presence of a $\Delta^{2'}$ double bond with the signals of two vinyl proton singlets at δ_H 6.00 and 5.55 (H_2-3′) and a vinyl methyl proton singlet at δ_H 1.87 (H-4′). These data as well as the ^{13}C and DEPT NMR signals at δ_C 135.7 (C, C-2′), 126.6 (CH_2, C-3′) and 14.7 (CH_3, C-4′) suggested the presence of a methacrylate ester at C-8, which was supported by the HMBC correlations of H-3′/C-1′, H-3′/C-2′, H-3′/C-4′ and H-8/C-1′ (Fig. 2). The relative configuration of **2**, established by examination of NOESY spectrum and NMR comparison with **1**, was the same as that of **1**. Thus, compound **2** was established as (4*β*,10*E*)-6α,15-dihydroxy-8*β*-(methacryloxy)-14-oxogermacra-1(10),11(13)-diene-12-oic acid 12,6-lactone.

Compounds **3**, **4** and **5** were assigned the same molecular formula of $C_{20}H_{26}O_6$, based on HRESIMS analysis (*m/z* [M + Na]$^+$, 385.1616, 385.1623 and 385.1619, respectively). As mentioned above, they only differed from **1** at the C-8 substituent. The NMR data of **3** (Tables 1 and 2) showed characteristic signals of an angeloyloxy group at δ_H 6.07 (1H, q, J = 7.2 Hz, H-3′), 1.96 (3H, d, J = 7.2 Hz, H-4′) and 1.78 (3H, s, H-5′), as well as signals at δ_C 166.4 (C-1′), 127.5 (C-2′), 137.9 (C-3′), 15.7 (C-4′) and 20.5 (C-5′). All of these data, as well as the HMBC correlations (Supporting Information), indicated that compound **3** had an angeloyloxy moiety at C-8 instead of the isobutyryloxy group in **1**. Examination of ROESY data further confirmed that **3** possessed the same relative configuration as **1**. Similarly, analysis of the NMR data (Tables 1 and 2) for **4** revealed that it had a tigloyloxy group [δ_H 6.76 (1H, m, H-3′), 1.76 (3H, d, J = 7.2 Hz, H-4′) and 1.75 (3H, s, H-5′); δ_C 166.8 (C-1′), 128.1 (C-2′), 138.6 (C-3′), 14.7 (C-4′) and 12.1 (C-5′)] at C-8 in place of the angeloyloxy in **3**, while compound **5** possessed a senecioyloxy residue at C-8 as evidenced by the NMR resonances at δ_H 5.54 (1H, s, H-2′), 1.87 (3H, s, H-4′) and 2.13 (3H, s, H-5′), as well as δ_C 165.3 (C-1′), 115.3 (C-2′), 158.8 (C-3′), 27.6 (C-4′) and 20.5 (C-5′). The structures of **4** and **5** were further corroborated by inspection of 2D NMR data (Supporting Information) with relative configurations being identical to those of **1–3** as supported by NMR comparison (Tables 1 and 2) and ROESY data.

Both compounds **6** and **7** were isolated as colorless oils and had the same molecular formula of $C_{20}H_{28}O_6$ deduced from the HRESIMS ions at *m/z* [M + Na]$^+$ 387.1788 and 387.1775, respectively. The NMR data of **6** (Tables 1 and 2) were highly comparable to those of **4**, except for the presence of an oxymethylene moiety (δ_H 4.16, d, J = 3.2 Hz, 2H; δ_C 68.1) and the absence of an aldehydic group at C-10. Compared with **6**, compound **7** was also a structural analogue that differed in the ester group attached to C-8, as could be seen from the NMR data given in Tables 2 and 3. The carbon signals at δ_C 165.9 (C-1′), 115.3 (C-2′), 159.0 (C-3′), 27.7 (C-4′) and 20.5 (C-5′), which were very close to those of **5**, indicated the presence of a senecioyloxy residue at C-8. Their relative configurations were determined by comparison of NMR data especially key coupling constants and also analysis of ROESY data (Supporting information). Based on the similar ECD spectra and a biogenetic consideration, the absolute configurations of **2–7** were assumed to be identical to that of **1**.

Compound **8** displayed a [M + H]$^+$ ion at *m/z* 405.1913 in the

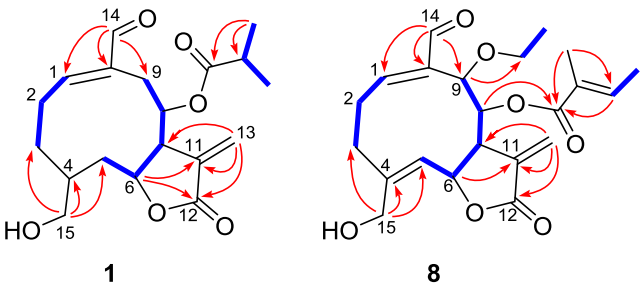

Fig. 2. Key ^{1}H − ^{1}H COSY (▬) and HMBC (H ⟶ C) correlations of **1**, **8** and **11**.

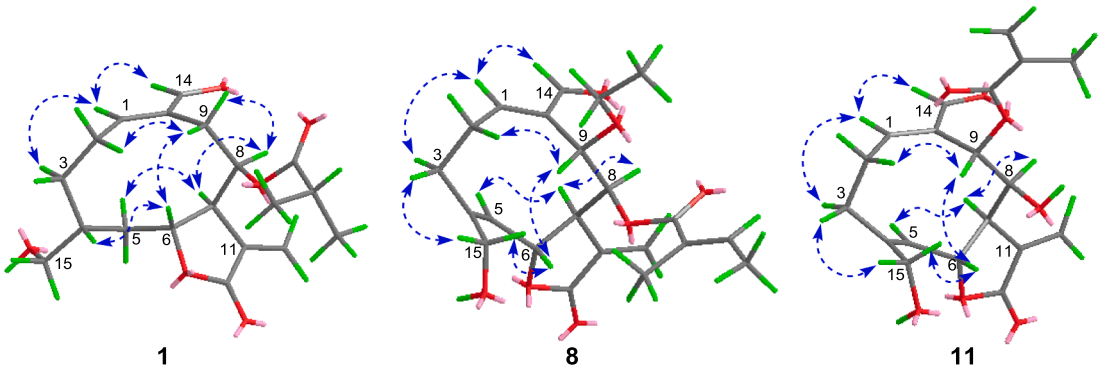

Fig. 3. NOSEY correlations of compounds **1**, **8** and **11**.

HRESIMS analysis, consistent with a molecular formula of $C_{22}H_{28}O_7$. Both ^{13}C and ^{1}H NMR data of **8** (Tables 2 and 3) indicated the presence of the same skeleton as the isolated known compound **17**, with the only difference being the ester moiety at C-8. The ^{13}C NMR resonances at δ_C 166.8 (C-1′), 128.5 (C-2′), 137.9 (C-3′), 14.7 (C-4′) and 12.5 (C-5′) undoubtedly supported the presence of a tigloyloxy residue. The NOESY correlations of H_3-15/H-6 and H-5/H-3α indicated a *Z*-configuration of Δ^4, while those of H-6/H-9 and H-9/H-2*β* led to the assignment of *β*-orientation for H-9, which was also consistent with the coupling constant of $J_{8,9}$ (8.3 Hz) [15]. The relative configurations of other chiral centers were also the same as those of **17**. The absolute configuration of **8** was finally determined to be (6*R*,7*S*,8*S*,9*S*) via comparison of its experimental ECD spectrum with the calculated one (Fig. 3). Thus, compound **8** was characterized as (1(10)*E*,4*Z*)-9α-ethoxy-6α,15-dihydroxy-8*β*-(tigloyloxy)-14-oxogermacra-1(10),4,11(13)-triene-12-oic acid 12,6-lactone. The ethoxyl group at C-9 in **8** was suggestive of a possible artifact due to the use of ethanol as extracting solvent.

The molecular formulae of **9** and **10** were assigned as $C_{20}H_{24}O_7$ and $C_{22}H_{28}O_8$, by (+)-HRESIMS analysis at *m/z* $[M+Na]^+$ 399.1414 and 443.1684, respectively. As with the case of **8** and **17**, compound **9** was different from the known cometabolite **20** only at the C-8 ester moiety, with signals for a tigloyloxy group [δ_H 6.88 (1H, dq, J = 1.4, 7.0 Hz, H-3′), 1.83 (3H, dd, J = 1.0, 7.0 Hz, H-4′) and 1.85 (3H, t, J = 1.1 Hz, H-5′); δ_C 168.2 (C-1′), 128.0 (C-2′), 139.4 (C-3′), 14.8 (C-4′) and 12.4 (C-5′)] rather than the angeloyloxy group in **20**. Similarly, the difference between **10** and **22** was also attributed to the presence of a C-8 tigloyloxy residue [δ_H 6.79 (1H, q, J = 7.2 Hz, H-3′), 1.80 (3H, d, J = 7.2 Hz, H-4′) and 1.78 (3H, s, H-5′); δ_C 167.0 (C-1′), 127.7 (C-2′),

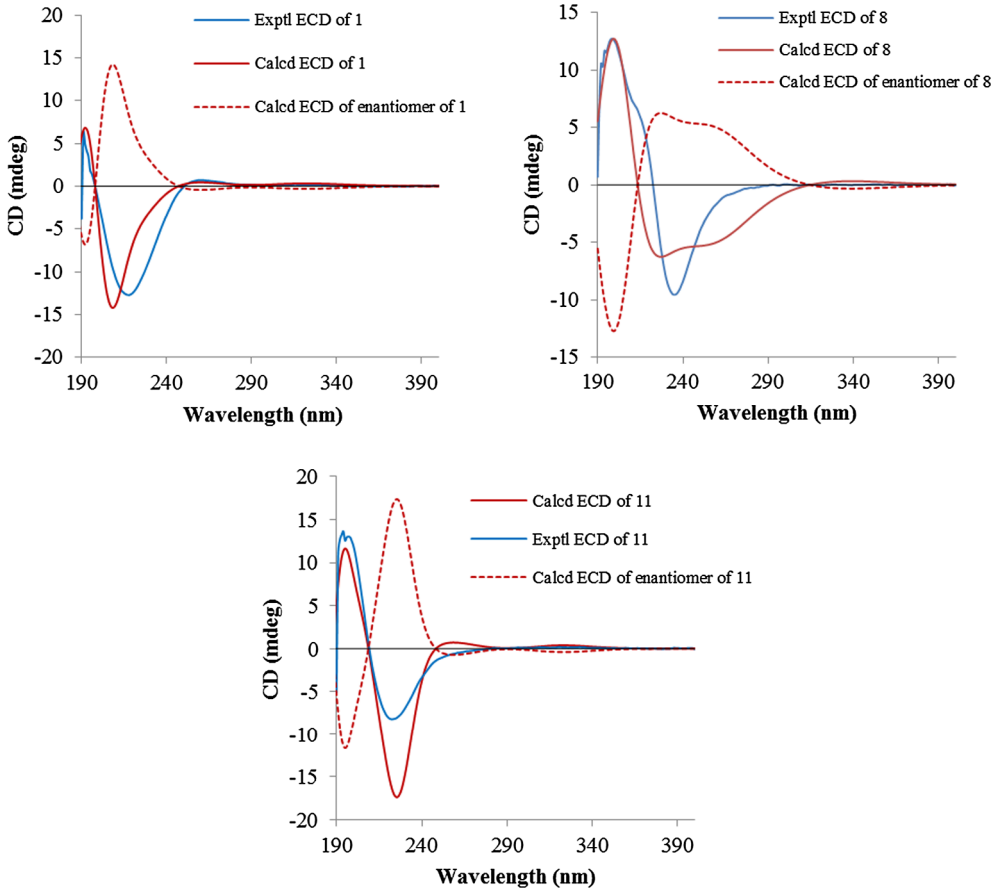

Fig. 4. Experimental ECD (blue) and simulated Boltzmann-averaged ECD (red) spectra of **1**, **8** and **11**.

Table 4
Cytotoxic activities of isolated compounds (IC_{50} in μM).

	13	16	21	23	25	Adriamycin
A549	10.77 ± 1.64	> 20	9.31 ± 2.26	6.02 ± 2.71	> 20	3.16 ± 2.94
MDA-MB-231	8.92 ± 1.87	18.61 ± 3.89	7.65 ± 2.01	9.31 ± 2.39	19.73 ± 3.87	7.35 ± 2.18

The results are means ± standard deviation from three independent experiments.

139.3 (C-3′), 14.8 (C-4′) and 12.2 (C-5′)] replacing the angeloyloxy residue in the latter. Such assignments were further corroborated by analysis of 2D NMR data (Supporting information) and particularly the relative configurations of **9** and **10** were confirmed as shown by excellent NMR comparisons with **20** and **22** and analysis of ROESY spectra (Supporting information).

Compound **11** had the molecular formula of $C_{19}H_{22}O_7$ as established by HRESIMS analysis at *m/z* 385.1254 ($[M+Na]^+$, calcd 385.1258). The ^{1}H NMR data (Table 3) of **11** was very similar to those of **19**, with the only difference being attributable to the chemical shifts of H-8 and H-9. The ^{1}H–^{1}H COSY correlations of H-7/H-8/H-9 as well as the HMBC correlations of H-14/C-9, H-9/C-7 and H-9/C-1′ (Fig. 2) supported that the methacrylate ester was located at C-9, while the hydroxyl was located at C-8. Examination of the NOESY data (Fig. 3) confirmed that it had the same relative configuration as **19**. The calculated ECD spectrum of **11** displayed similar cotton effects to the experimental ECD curve (Fig. 4), allowing the assignment of the absolute configuration as 6*R*,7*R*,8*S*,9*S*.

Compounds **12–28** were identified as (4*β*,10*E*)-6α,14,15-trihydroxy-8*β*-(isobutyryloxy)germacra-10,11(13)-diene-12-oic acid 12,6-lactone (**12**) [14], 2-propenoic acid, 2-methyl-2,3,3a,4,5,8,9,10,11,11a-decahydro-6,10-bis(hydroxymethyl)-3-methylene-2-oxocyclodeca[*b*]furan-4-yl ester (**13**) [16], (1(10)*E*,4*β*)-8*β*-(angeloyloxy)-6α,14,15-trihydroxygermacra-1(10),11(13)-diene-12-oic acid 12,6-lactone (**14**) [10], 9α-ethoxy-8*β*-(2-isobutyryloxy)-14-oxo-acanthospermolide (**15**) [17], (1(10)*E*,4Z,6α,8*β*,9α)-9-ethoxy-6,15-dihydroxy-8-(2-methylacryloxy)-14-oxogermacra-1(10),4,11(13)-trieno-12,6-lactone (**16**) [18], (2Z)-2-methylbut-2-enoic acid (3a*S*,4*S*,5*S*,6*E*,10*Z*,11a*R*)-5-(ethoxy)-6-formyl-2,3,3a,4,5,8,9,11a-octahydro-10-(hydroxymethyl)-3-methylene-2-oxocyclodeca[*b*]furan-4-yl ester (**17**) [10], (1(10)*E*,4Z)-9α,15-dihydroxy-8*β*-isobutyryloxy-14-oxo-melampolide (**18**) [19], (1(10)*E*,4Z,6α,8*β*,9α)-6,9,15-trihydroxy-8-(methylacryloxy)-14-oxogermacra-1(10),4,11(13)-trieno-12,6-lactone (**19**) [18], lecocarpinolide F (**20**) [20], sigesbeckialide A (**21**) [4], (3a*S*,4*S*,5*S*,6*Z*,10*Z*,11a*R*)-5-(acetyloxy)-2,3,3a,4,5,8,9,11a-octahydro-6,10-bis(hydroxymethyl)-3-methylene-2-oxo-cyclodeca[*b*]furan-4-yl ester (**22**) [21], orientalide (**23**) [22], lecocarpinolide B (**24**) [23], pubetallin (**25**) [11], (1(10)*E*,4Z)-8*β*-(angeloyloxy)-9α-methoxy-6α,15-dihydroxy-14-oxogermacra-1(10),4,11(13)-trien-12-oic acid 12,6-lactone (**26**) [24], 9α-Hydroxy-8*β*-methacryloyloxy-14-oxo-acanthospermolide (**27**) [25], and 3*β*-hydroxybalchanolide (**28**) [26], respectively, based on the comparisons of NMR and MS data with reported values.

All the compounds except **5** and **24**, which were obtained only in a limited amount, were assayed for their cytotoxicity against human MDA-MB-231 (breast) and A549 (lung) cancer cell lines with adriamycin as a positive control (Table 4). Among them, compounds **13**, **21** and **23** exhibited promising cytotoxicity against both cell lines with IC_{50} values ranging from 6.02 to 10.77 μM. Moreover, compounds **16** and **25** showed moderate cytotoxicity against MB-MDA-231 cell line with IC_{50} values of 18.61 and 19.73 μM, respectively. Other tested compounds showed weak or no effects in the same assay (IC_{50} > 20 μM). Interestingly, compounds **13**, **21** and **23** possessing a methacryloxy group at C-8 showed stronger activities, as compared with compounds which have the same skeleton but with other four substituents at C-8. This result indicated that the methacryloxy group at C-8 could be related to the enhanced cytotoxicity.

4. Conclusions

In conclusion, the phytochemical investigation of *S. orientalis* for new bioactive natural products led to the isolation and identification of 27 highly oxygenated germacranolides including 11 new and 16 known ones. Chemotaxonomically, these germacranolides showed characteristic structural features as those reported in the literature, most incorporating isobutyryl, methacryloyl, angeloyl, tigloyl, or senecioyl groups at C-8 or C-9. Moreover, cytotoxicity screening of this mini sesquiterpenoid library against two human cancer cell lines demonstrated that compounds **13**, **21** and **23**, which all contain a methacryloxy group at C-8, exhibited stronger activity than other analogues. A further study on the less polar fractions for bioactive diterpenoid constituents remains a work in due course.

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Declaration of Competing Interest

There are no conflicts of interest to declare.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103196.

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