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Dendronephthols A–C, new sesquiterpenoids from the Red Sea soft coral *Dendronephthya* sp.

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ABSTRACT

Three new ylangene-type sesquiterpenoids: dendronephthol A (7,13-dihydroxy-3,4-dihydro-α-ylang-5-one) (**1**), dendronephthol B (6,7,13-trihydroxy-3,4-dihydro-α-ylangene) (**2**), and dendronephthol C (6,7,13-trihydroxy-α-ylang-5-one) (**3**), together with two known compounds: dendronesterone A and cholesterol were isolated from the $CHCl_3$ fraction of Red Sea soft coral *Dendronephthya* sp. (Nephtheidae). The structures of the new compounds were established on the basis of one- and two-dimensional NMR spectroscopic studies (^1H, ^{13}C, DEPT, COSY, HSQC, HMBC, and NOESY) as well as MS spectroscopy and by comparison of the spectral data with those of related known compounds. Compounds **1** and **3** showed cytotoxic activity against the murine lymphoma L5187Y cancer cell line with ED_{50} values of 8.4 and 6.8 μg/mL, respectively.

1. Introduction

Soft corals of the genus *Dendronephthya* are widely distributed throughout tropical coastal water of the Indo-Pacific Ocean to the Red Sea. The genus *Dendronephthya* is highly prolific and is represented by about 248 species.[1] Many secondary metabolites have been isolated from the genus *Dendronephthya* including structurally unique steroids[2–7] and sesquiterpenes.[2,4] They exhibit cytotoxic,[2,5] anti-inflammatory,[8] and antifouling activities.[3,6]

In the present study, freeze-dried coral material was exhaustively extracted with acetone and MeOH. The total extract was dissolved in water and fractionated with *n*-hexane, $CHCl_3$, and EtOAc. The $CHCl_3$ fraction showed 80% mortality in the brine shrimp toxicity assay at a concentration of 5 μg/mL, then was subjected to repeated silica gel column chromatography and compounds were purified by semi-preparative HPLC to yield three new ylangene-type sesquiterpenoids: dendronephthol A (7,13-dihydroxy-3,4-dihydroylangen-5-one) (**1**), dendronephthol B (6,7,13-trihydroxy-3,4-dihydroylangene) (**2**), and dendronephthol C (6,7,13-trihydroxy-α-ylang-5-one) (**3**), along with dendronesterone A (**4**) and cholesterol (**5**) (Fig. 1). Herein, we report the isolation and structure elucidation as well as cytotoxic activity of the new compounds.

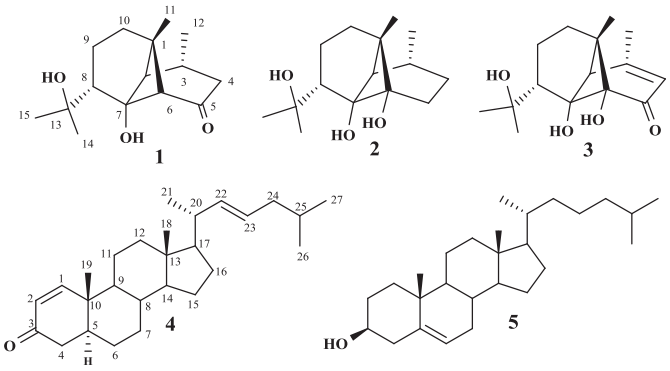

Fig. 1. Structures of the isolated compounds.

2. Results and discussion

Compound **1** was isolated as a yellow oily residue, showing an ESIMS quasimolecular ion peak at *m/z* 253 $[M+H]^+$, with a base peak at *m/z* 59, suggesting the presence of an oxygenated isopropyl moiety $Me_2C(OH)$.[9] Through HRESIMS, the molecular formula of **1** was assigned as $C_{15}H_{24}O_3$, requiring four degrees of unsaturation. The IR spectrum showed absorption bands at 3495 (OH), 1710 (carbonyl ketone), and 1056 (C–O) cm^{-1}. The ^1H NMR spectrum (Table 1) displayed signals for 22 protons including 4 methyls, 3 methylenes,

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Table 1
NMR data of **1** and **2** (400 and 100 MHz, $CDCl_3$)

No.	**1**				**2**			
	δ_H, m, (*J* in Hz)	δ_C, m	HMBC H→C	NOESY	δ_H, m, (*J* in Hz)	δ_C, m	HMBC H→C	NOESY
1	—	43.7, C	—	—	—	35.1, C	—	—
2	1. 32 d (5.8)	38.4, CH	1, 7, 10, 11	6	3.32 brs	51.1, CH	3, 6, 7, 13	12, 15
3	2.03 m	30.0, CH	1, 5, 10	11	2.08 m	30.0, CH	4	11
4	1.69 ddt (15.2, 6.6, 3.0); 1.88 ddt (15.2, 6.6, 3.0)	33.4, CH_2	1, 5, 11	—	1.61 m; 1.22 m	33.4, CH_2	1, 3, 6, 11	12
5	—	204.1, C	—	—	1.59 m	21.7, CH_2	1, 3, 5	—
6	3.04 d (5.8)	51.1, CH	1, 3, 7	2, 12, 14	—	65.0, C	—	—
7	—	65.0, C	—	—	—	62.1, C	—	—
8	3.17 t (6.2)	49.7, CH	7, 10, 13	11, 15	3.20 t (7.2)	49.7, CH	1, 7, 9, 13	11, 14
9	1.56 m	21.9, CH_2	7, 13		1.54 m	36.7, CH_2	1, 6, 7, 8, 10, 11	—
10	1.31 m; 1.46 m	36.0, CH_2	7, 11	—	1.38 m	38.4, CH_2	1, 6, 11	—
11	1.00 s	21.7, CH_3	2, 5, 6, 7, 10	3, 8	1.05 s	21.4, CH_3	1, 2, 6, 9, 10	3, 8
12	0.67 d (6.2)	14.3, CH_3	3, 4, 7	2, 4, 6	0.73 d (6.7)	14.3, CH_3	3, 4, 6	2, 4
13	—	69.5, C	—	—	—	69.5, C	—	—
14	1.35 s	26.0, CH_3	7, 13, 15	8	1.42 s	26.0, CH_3	7, 13, 15	8
15	1.20 s	25.0, CH_3	7, 13, 14	6, 2	1.24 s	25.0, CH_3	7, 13, 14	2

and 4 methines. The ^{13}C NMR data displayed resonances for 15 carbons, including 1 carbonyl ketone at δ_C 204.1 (C-5) and 2 oxygenated carbons at δ_C 69.5 (C-13) and 65.0 (C-7). The HSQC spectrum correlated the protons at δ_H 1.32 (d, *J*=5.8 Hz, H-2), 2.03 (m, H-3), 3.04 (d, *J*=5.8 Hz, H-6), and 3.17 (t, *J*=6.2 Hz, H-8) to the carbons at δ_C 38.4, 30.0, 51.1, and 49.7, respectively. Furthermore, the methylenes at δ_H 1.88 (1H, ddt, *J*=15.2, 6.6, 3.0 Hz, H-4A), 1.68–1.70 (1H, m, H-4B), 1.55–1.57 (2H, m, H-9), and 1.30–1.32, 1.45–1.47 (2H, m, H-10) were assigned to the carbons at δ_C 33.4, 21.9, and 36.0, respectively. The COSY experiment revealed the presence of two spin systems. The first extended from H-2 to H-4 with branching methyl at C-3 to give the structural fragment CH–CH(CH_3)–CH_2 (Fig. 2). The second system consists of two coupled methylene groups at δ_H 1.55–1.57 (2H, m, H-9) and 1.30–1.32, 1.45–1.47 (2H, each m, H-10), which further coupled with the methine proton at δ_H 3.17 (t, *J*=6.2 Hz, H-8) to give the fragment CH_2–CH_2–CH–. These two spin systems were confirmed by the HMBC experiment (Fig. 2). A large long range coupling constant ($^4J_{2,6}$=5.8 Hz) observed between H-2 and H-6, due to the W-type coupling indicated the presence of a bridged cyclobutane system.[2] The HMBC correlations of H_3-14 and H_3-15 to C-13 (δ_C 69.5) confirmed the presence of an oxygenated isopropyl moiety (Fig. 2). Its attachment at C-8 was established by the HMBC cross peaks of H-8 and H-9 to C-13. The presence of an oxygenated carbon at C-7 was established by HMBC correlations of H-2, H-6, H-8, H-9, H-10, and H_3-11 to C-7. The location of the carbonyl (δ_C 204.1) at C-5 was secured based on the HMBC cross peaks of H-3, H-4, and H-6 to C-5. Moreover, HMBC correlations of the tertiary methyl at δ_H 1.00 (H_3-11) with C-2, C-6, and C-10, and H-8 with C-1, C-2, C-7, and C-9 clarified ylangene-type sesquiterpene, which was previously isolated from several soft corals[2,4,10] and *Scapania* genus liverwort.[11] The relative configuration of **1** was assigned by the NOESY experiment. The NOESY correlations of H_3-11 to H-3 and H-8 showed that these protons occurred on the same side of the molecule. NOESY correlations of H-2 with H-6 and H-12 positioned these protons on the other side of the molecule (Fig. 3). The NOESY correlation between H-2 and H-15 and H-8 with H-14 confirmed the α-orientation of the isopropyl group. Accordingly, **1** was identified as 7,13-dihydroxy-3,4-dihydro-α-ylang-5-one, which we assigned the name dendronephthol A.

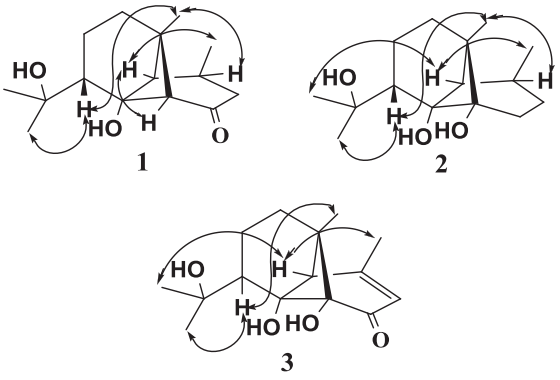

Fig. 3. Some key NOESY correlations of **1**–**3**.

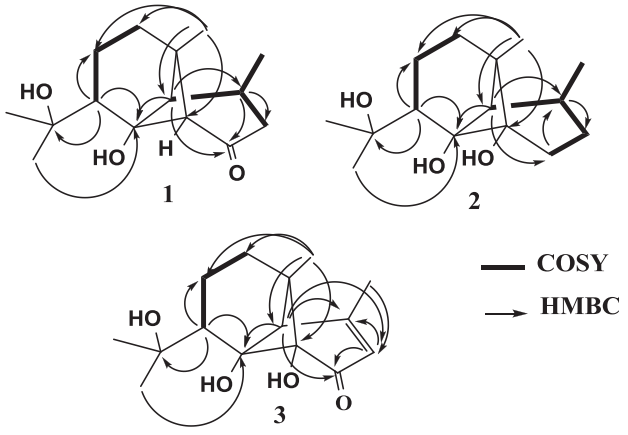

Fig. 2. Some key COSY and HMBC correlations of **1**–**3**.

Compound **2** was isolated as a yellowish brown oily residue, which showed an ESIMS quasimolecular ion peak at *m/z* 255 $[M+H]^+$ and a base peak fragment at *m/z* 59 due to the oxygenated isopropyl residue as in **1**. The HRESIMS established the molecular formula of **2** to be $C_{15}H_{26}O_3$, requiring three degrees of unsaturation. Compound **2** showed an increase of two mass units and a decrease of one degree of unsaturation compared to **1**. The ^1H and ^{13}C NMR spectra of **2** and **1** were quite similar but the signals associated with the methine group at δ_H 3.04 (1H, d, *J*=5.8 Hz, H-6)/δ_C 51.1 (C-6) and carbonyl carbon at δ_C 204.1 (C-5) found in **1** were not present (Table 1). Instead, new signals for one methylene at δ_H 1.57–1.60 (2H, m, H-5)/δ_C 21.7 (C-5) and an additional oxygenated carbon resonating at δ_C 65.0 (C-6) were observed. Two spin systems were evident from the COSY experiment. The first one extends from H-8 to H-10 and the second from H-2 to H-5 with methyl group at C-3. These two spins were confirmed by HMBC experiment (Fig. 2).

HMBC correlations of H-4, H-8, H-10, and H-11 with C-6 (δ_C 65.0), and H-2, H-5, H-9 with C-7 (δ_C 62.1) indicated the presence of oxygenated carbons at C-6 and C-7, respectively. The oxygenated isopropyl moiety was attached at C-8 based on the HMBC correlations of H-8 (δ_H 3.20) with the oxygenated quaternary carbon at δ_C 69.5 (C-13). The ylangene structure was confirmed by the HMBC correlations of the tertiary methyl at δ_H 1.05 (H_3-11) with C-2, C-6, and C-10 and the triplet methine at δ_H 3.20 (H-8) with C-1, C-7, C-9, and C-13. The NOESY experiment assigned the relative configuration of **2** (Fig. 3). Based on the data obtained from COSY, HMQC, HMBC, and NOESY experiments, the structure of **2** was unambiguously elucidated as 6,7,13-trihydroxy-3,4-dihydro-α-ylangene and named dendronephthol B.

Compound **3** was isolated as a brown oily residue with an ESIMS quasimolecular ion peak at *m/z* 267 $[M+H]^+$ and a base peak fragment at *m/z* 59 due to the oxygenated isopropyl residue as in **1**. Through HRESIMS, it was evident that **3** has the molecular formula $C_{15}H_{22}O_4$, which requires five degrees of unsaturation. Compound **3** was 14 mass units and one degree of unsaturation more than **1**. The IR spectrum indicated the presence of a hydroxyl group (3546 cm^{-1}), ketone carbonyl (1715 cm^{-1}), and double bond (1615 cm^{-1}) absorption bands. The ^1H and ^{13}C NMR spectra were very comparable to those of **1** (Table 2). However, the signals associated with the methine at δ_H 3.04 (1H, d, *J*=5.8 Hz, H-6)/δ_C 51.1 (C-6), methylene at 1.69, 1.88 (ddt, *J*=3.0, 6.6, 15.2 Hz, H_2-4), and secondary methyl at δ_H 0.67 (d, *J*=6.2 Hz, H_3-12), which were encountered in **1** were not present. Instead, new signals for a trisubstituted double bond at δ_H 5.88 (1H, s, H-4)/δ_C 124.2 (C-4), 165.7 (C-3), and a tertiary methyl substituent at δ_H 1.27 (H_3-12)/δ_C 25.0 were observed, together with an additional signal for an oxygenated carbon at δ_C 77.2 (C-6). The COSY spectrum revealed one spin system (CH_2–CH_2–CH) assigned to H-8 to H-10 (Fig. 2). The HMBC correlations of the secondary methyl at δ_H 1.27 with carbons at δ_C 165.7 (C-3) and 124.2 (C-4) confirmed the presence of a trisubstituted double bond. HMBC correlations of H-2 (δ_H 2.35) with C-3 and C-4, and correlations of the secondary methyl at δ_H 1.27 (H_3-12) with C-2 have attached the tri-substituted double bond at C-3 and C-4, respectively. The attachment of a carbonyl group at C-5 was established by the HMBC correlations of H-2, the olefinic proton at δ_H 5.88 (H-4) and H_3-12 with the carbonyl at δ_C 208.2 (C-5). Moreover, the HMBC correlations of the olefinic proton (H-4) to the carbon resonating at δ_C 77.2, H-8 and H-9 with the carbon signal at δ_C 62.1 allowed the oxygenated carbons to be located at C-6 and C-7, respectively. The key HMBC correlations of H_3-11 to C-2 and C-6, and H-8 to C-2, C-7, and C-13 have clarified α-ylangene structure.[12] The relative configuration of **3** was established based on the observed NOESY correlations (Fig. 3). This unambiguously led to the elucidation of **3** as 6,7,13-trihydroxy-α-ylang-5-one and named dendronephthol C.

Table 2
NMR data of **3** (400 and 100 MHz, $CDCl_3$)

No.	δ_H, m, (*J* in Hz)	δ_C, m	HMBC H→C	NOESY
1	—	35.1, C	—	—
2	2.35 brs	44.1, CH	3, 4, 5, 9, 10	12
3	—	165.7, C	—	—
4	5.88 s	124.2, CH	2, 6, 11	12
5	—	208.2, C	—	—
6	—	77.2, C	—	—
7	—	62.1, C	—	—
8	3.12 t (6.2)	49.7, CH	2, 7, 10, 13	11, 14
9	1.47 m	18.4, CH_2	1, 7, 8	—
10	1.38 m	36.7, CH_2	1, 8, 11	—
11	1.05 s	21.4, CH_3	1, 2, 6, 10	8
12	1.27 d (2.7)	25.0, CH_3	2, 3, 4, 6	2, 4
13	—	69.5, C	—	—
14	1.35 s	26.0, CH_3	7, 13, 15	8
15	1.20 s	25.0, CH_3	7, 13, 14	2

Compound **4** was identified by comparison of its spectroscopic data (1D, 2D NMR, and MS) with those of dendronesterone A, previously isolated from *Dendronephthya gigantea* collected at Green Island.[4]

Compound **5** was identified as cholesterol based on its spectroscopic data (^1H and ^{13}C NMR, and MS) and co-TLC with authentic sample.

The isolated sesquiterpenoids were evaluated for their cytotoxicity towards murine lymphoma (L5178Y), rat brain tumor (PC12), and human cervix carcinoma (Hela) cancer cell lines. The tested compounds showed no activity towards PC12 and Hela cell lines. However, **1** and **3** were found to be active against L5178Y cells with ED_{50} values of 8.4 and 6.8 μg/mL, respectively.

3. Conclusion

Three new ylangene-type sesquiterpenoids dendronephthols A–C together with two known compounds were isolated from the Red Sea soft coral *Dendronephthya* sp. The structures of these compounds were established on the basis of their spectroscopic data. The newly isolated metabolites dendronephthol A (**1**) and dendronephthol C (**3**) exhibited cytotoxicity against murine lymphoma L5187Y cancer cell line.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured with a Perkin–Elmer 241 automatic polarimeter (Perkin–Elmer Inc, Massachusetts, USA). UV spectra were recorded in MeOH on Shimadzu 1601 UV/VIS spectrophotometer (Kyoto, Japan). The IR spectra were measured on a Shimadzu Infrared-400 spectrophotometer (Kyoto, Japan).1D (^1H and ^{13}C) and 2D (COSY, HSQC, HMBC, and NOESY) NMR spectra were recorded on Bruker ARX 400 NMR spectrometers (Bruker BioSpin, Massachusetts, USA). ESIMS spectra were obtained with a LCQ DECA mass spectrometer (Thermo Finnigan, Bremen, Germany) coupled to an Agilent 1100 HPLC system equipped with a photodiode array detector. EIMS spectra were recorded on JEOL JMS-SX/SX 102A mass spectrometer. HRESIMS was recorded on a LTQ Orbitrap (Thermo-Finnigan, Bremen, Germany). Solvents were distilled prior to use and spectral grade solvents were used for spectroscopic measurements. Column chromatographic separations were performed on silica gel 60 (0.04–0.063 mm; Merck, Darmstadt, Germany). TLC was performed on plates precoated with silica gel F_{254} (Merck, Darmstadt, Germany). Semi-preparative HPLC, a HPLC system (Merck, Darmstadt, Germany) coupled with UV detector L7400 (UV detection at 280 nm) was used. Separation column (250–8 mm, i.d.) pre-packed with Eurosphere 100-C_{18} (Knauer, Berlin, Germany). Separation was achieved by applying a linear gradient from 80% H_2O (pH 2.0) and 20% MeOH to 100% MeOH over 45 min. The compounds were eluted with mixtures of MeOH and H_2O at a flow rate of 5 mL/min.

4.2. Animal material

The soft coral *Dendronephthya* sp. (Class Cnidaria, subclass Octocorallia Haeckel, 1866, order Alcyonacea, family Nephtheidae) was collected near the coast of Hurghada, Egypt, in August 2006 at a depth of 12–16 ft. A voucher specimen was kept in the Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt, under the registration no. DE 875-1.

4.3. Extraction and isolation

The coral material was stored in EtOH upon collection. Prior to extraction, it was freeze-dried and the EtOH macerate was

concentrated. The lyophilized coral (118.4 g) was extracted exhaustively with acetone and then with MeOH. The total extract (15.5 g), which included the EtOH concentrate, was dissolved in water and sequentially fractionated with *n*-hexane, $CHCl_3$, and EtOAc. The biological activity of the extracts was tested for brine shrimp lethality. The $CHCl_3$ fraction was chosen for further isolation work, as it displayed the highest lethal activity in the brine shrimps assay. The $CHCl_3$ fraction (3.79 g) was subjected to normal-phase silica gel column chromatography and eluted with *n*-hexane and *n*-hexane/EtOAc gradient. Elution by *n*-hexane/EtOAc (90:10) afforded **5** (4.3 mg) and **4** (2.9 mg). Elution by *n*-hexane/EtOAc (80:20) afforded **2** (2.1 mg). Elution by *n*-hexane/EtOAc (77:23) afforded fractions containing mixture of compounds **1** and **3** (6.3 mg), which were further purified by semi-preparative HPLC eluted with MeOH/H_2O to yield **1** (1.8 mg) and **3** (1.6 mg). Other fractions afforded mixture of compounds still under investigations.

*4.3.1. Dendronephthol A (**1**).* A yellow oily residue; $[\alpha]_D$ −15.3 (*c* 0.9, $CHCl_3$); UV λ_{max} (log ε) (MeOH): 225 (3.74) nm; IR (KBr) ν_{max} 3490, 1725, 1291, 1056 cm^{-1}; NMR data: see Table 1; HRESIMS *m*/*z* 253.1712 (calcd for $C_{15}H_{25}O_3$, $[M+H]^+$, 253.1725).

*4.3.2. Dendronephthol B (**2**).* A yellowish brown oily residue; $[\alpha]_D$ −24.5 (*c* 0.9, $CHCl_3$); UV λ_{max} (log ε) (MeOH): 218 (3.51) nm; IR (KBr) ν_{max} 3560, 2950, 1053 cm^{-1}; NMR data: see Table 1; HRESIMS *m*/*z* 255.1878 (calcd for $C_{15}H_{27}O_3$, $[M+H]^+$, 255.1881).

*4.3.3. Dendronephthol C (**3**).* A brown oily residue; $[\alpha]_D$ −42.1 (*c* 0.9, $CHCl_3$); UV λ_{max} (log ε) (MeOH): 232 (3.83) nm; IR (KBr) ν_{max} 3546, 1715, 1061 cm^{-1}; NMR data: see Table 2; HRESIMS *m*/*z* 267.1521 (calcd for $C_{15}H_{23}O_4$, $[M+H]^+$, 267.1518).

*4.3.4. Dendronesterone A (**4**).* A white amorphous solid; IR (KBr) ν_{max} 2965, 1683, 1617 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ_H 7.14 (1H, d, *J*=9.7 Hz, H-1), 5.85 (1H, d, *J*=9.8 Hz, H-2), 2.21 (1H, dd, *J*=18.1, 4.5 Hz, H-4α), 2.38 (1H, d, *J*=18.1, 14.0 Hz, H-4β), 1.93–1.95 (1H, m, H-5), 1.27–1.29 (1H, m, H-6α), 1.70–1.72 (1H, m, H-6β), 1.73–1.75 (2H, m, H-7), 1.49–1.51 (1H, m, H-8), 1.01–1.03 (1H, m, H-9), 1.77–1.79 (1H, m, H-11α), 1.51–1.53 (1H, m, H-11β), 1.17–1.19 (1H, m, H-12α), 2.04–2.06 (1H, m, H-12β), 1.16–1.18 (1H, m, H-14), 1.59–1.61 (2H, m, H-15), 1.44–1.46 (2H, m, H-16), 1.20–1.21 (1H, m, H-17), 0.72 (3H, s, H-18), 1.01 (3H, s, H-19), 2.05–2.08 (1H, m, H-20), 1.03 (3H, d, *J*=6.6 Hz, H-21), 5.20 (1H, dd, *J*=15.3, 7.5 Hz, H-22), 5.25–5.27 (1H, m, H-23), 1.83–1.84 (2H, m, H-24), 1.54–1.56 (1H, m, H-25), 0.86 (3H, d, *J*=6.6 Hz, H-26), 0.87 (3H, d, *J*=6.6 Hz, H-27); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 158.5 (C-1), 127.3 (C-2), 200.2 (C-3), 41.2 (C-4), 44.4 (C-5), 28.7 (C-6), 31.4 (C-7), 35.6 (C-8), 50.2 (C-9), 39.0 (C-10), 21.3 (C-11), 39.7 (C-12), 42.6 (C-13), 56.5 (C-14), 24.2 (C-15), 27.7 (C-16), 56.1 (C-17), 12.4 (C-18), 13.0 (C-19), 40.3 (C-20), 21.0 (C-21), 138.1 (C-22), 126.5 (C-23), 42.0 (C-24), 28.6 (C-25), 22.4 (C-26), 22.2 (C-27); EIMS *m*/*z* 382 $[M]^+$.

4.4. Brine shrimp lethality test

Eggs of *Artemia salina* (Dohse, Aquaristik, GMBH, Bonn, Germany) were hatched in small tanks filled with artificial sea water. After 48 h, 20 napulii were transferred to each sample vial and artificial sea water was added to obtain final volume of 5 mL. The percent mortality was determined after 24 h.[13]

4.5. Cell proliferation assay

Anti-proliferative activity was tested against murine lymphoma (L5178Y), rat brain (PC12), and human cervix (Hela) cancer cell lines using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay as described previously.[14,15]

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