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A triterpene and four sesquiterpenes from the Vietnamese sea hare *Aplysia dactylomela*

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ABSTRACT

Five compounds, including thyrsiferol (1), 11-hydroxy-8-oxo-*β*-cyperon (2), (+)-elatol (3), [3(15)*E*,4*Z*,6*S*,9*S*,10*R*]-10,15-dibromochamigra-3(15),4,7(14)-trien-9-ol (4), and pacifidiene (5) were isolated from the methanol extract of the sea hare *Aplysia dactylomela*, collected at Quang Ngai province, Vietnam. Their structures were elucidated from HR-ESI-MS and NMR spectral data. Compound 2 was obtained for the first time from *A. dactylomela*, and its complete NMR spectral data were reported here. All isolated compounds were evaluated for cytotoxic activity against three human cancer cell lines (A549, MCF-7, and HepG2). Among them, compound 3 exhibited significant cytotoxicity against all tested cell lines, with IC_{50} values ranging from 5.07 ± 0.96 to 6.55 ± 0.44 µM.

Keywords: *Aplysia dactylomela*, sea hare, mollusk, terpene, cytotoxic activity.

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Introduction

Aplysia dactylomela Rang, also known as the spotted sea hare, is a herbivorous marine mollusk of the family Aplysiidae. With a broad distribution in tropical and warm-temperate marine environments, this species primarily feeds on marine macroalgae [1, 2]. Previous studies have shown that *A. dactylomela* preferentially consumes red algae of the genus *Laurencia* (Ceramiales, Rhodomelaceae), and consequently, its metabolites are often closely related to those of *Laurencia* species [3]. It has been demonstrated that terpenoids, C_{15} acetogenins, indoles, macrolides, sterols, and alkaloids are the main chemical classes of this mollusk [4, 5]. Among these, halogenated sesquiterpenes were reported to be the majority of metabolites from *A. dactylomela*, exhibiting potent cytotoxic, antimicrobial, and anti-inflammatory properties [4, 6–8].

As part of an ongoing search for new bioactive metabolites from Vietnamese mollusks, we investigated the methanol extract of *A. dactylomela* collected on Ly Son Island, Vietnam. Herein, we report the isolation and structure elucidation of five compounds, **1**–**5** (Fig. 1), as well as their cytotoxicity evaluation against the tumor cell lines A549, MCF7, and HepG2. Notably, this study provides the first complete NMR spectroscopic data for 11-hydroxy-8-oxo-β-cyperon (**2**) and reports its isolation from the sea hare *A. dactylomela* for the first time.

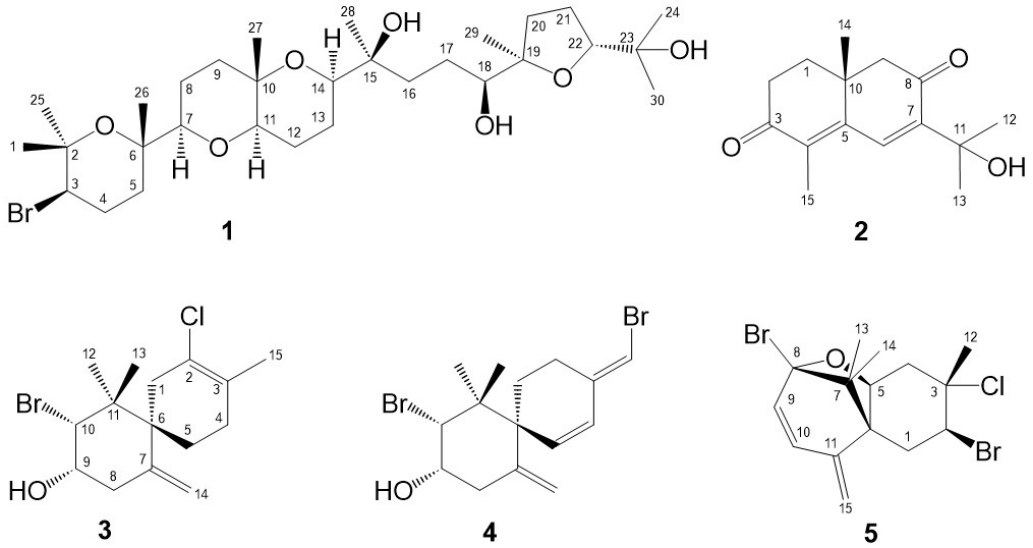

Figure 1. Chemical structures of compounds **1**–**5**

Materials and methods

General experimental procedures

The procedures and instruments used to isolate compounds, measure optical rotation, and record IR, NMR, ESI-MS, TLC, and MPLC data are similar to those described in a previous paper [9].

Biological material

Specimens of *Aplysia dactylomela* were collected on Ly Son Island, Quang Ngai Province, Vietnam, in May 2023, and identified by Dr. Nguyen Chi Mai and Dr. Tran My Linh. A voucher specimen (ĐLTE02) was deposited in the Department of Bioactive Natural Products, Institute of Marine Biochemistry, VAST, Hanoi, Vietnam.

Extraction and isolation

Frozen whole-body samples of *A. dactylomela* (1.25 kg) were cleaned, chopped into small pieces, and extracted three times with 4.0 L of methanol under ultrasonic conditions (30 min

each time). The resulting solutions were filtered, combined, and evaporated under reduced pressure to obtain a methanol residue (115 g), which was suspended in water and then sequentially extracted with *n*-hexane and CH_2Cl_2 (each 1.5 L, 3 times) to afford *n*-hexane (H, 21.6 g), CH_2Cl_2 (D, 81.5 g) extracts, and water layer.

The *n*-hexane extract was passed through a silica gel column chromatography (CC) using *n*-hexane: ethyl acetate mixtures with increasing amounts of ethyl acetate, yielding nine fractions (H1–H9). Fraction H5 (117.5 mg) was chromatographed over a silica gel column, eluting with *n*-hexane: acetone (80:1, v/v) to yield two smaller fractions (H5.1 and H5.2). Subfraction H5.1 (65.1 mg) was separated by silica gel CC using n-hexane:CH_2Cl_2 (10:1, v/v) as the mobile phase, yielding two fractions (H5.1a and H5.1b). Fraction H5.1b (28.6 mg) was isolated on RP-18 HPLC using MeOH 70% in water to get two compounds **3** (7.3 mg, t_R = 44.93) and **4** (4.2 mg, t_R = 53.60 min). Similarly, subfraction H5.2 (52.3 mg) was separated into three subfractions (H5.2a–H5.2c) by a Sephadex LH-20 column, using MeOH: H_2O (2:3, v/v). Compound **5** (3.6 mg, t_R 23.81 min) was purified from subfraction H5.2a (16.5 mg) by RP-18 HPLC column using CH_3CN: H_2O (20:80, 0.8 mL/min). Subfraction H7 (278.5 mg) was chromatographed by a silica gel column, using *n*-hexane: CH_2Cl_2:acetone (20:1:1, v/v/v) as eluent, to give three subfractions (H7.1-H7.3). Subfraction H7.2 (47.0 mg) was subjected to RP-18 HPLC using CH_3CN: H_2O (30:70, 0.6 mL/min) to yield compound **2** (6.4 mg, t_R 41.11 min). Finally, compound **1** (9.5 mg, t_R 58.11 min) was purified from subfraction H8 (96 mg) using an RP-18 HPLC column with MeOH:H_2O (85:15, 4 mL/min). All column chromatography procedures were performed at room temperature.

Table 1. ^{1}H and ^{13}C NMR data of compound **1**

Pos.	δ_C	δ_H mult. (*J* in Hz)	Pos.	δ_C	δ_H mult. (*J* in Hz)
1	23.7	1.39 (3H, s)	16	33.7	1.38 (1H, m) 1.81 (1H, m)
2	74.9	-	17	25.4	1.45 (1H, m) 1.57 (1H, m)
3	58.9	3.88 (1H, dd, 3.9, 13.2)	18	77.5	3.44 (1H, dd, 1.5. 9.9)
4	28.2	2.23 (1H, ddd, 3.6, 13.2, 13.2) 2.08 (1H, overlapped)	19	86.0	-
5	37.0	1.53 (1H, m) 1.79 (1H, m)	20	32.4	1.59 (1H, m) 2.10 (1H, overlapped)
6	74.3	-	21	26.7	1.82 (2H, m)
7	86.5	3.03 (1H, dd, 2.4, 12.0)	22	87.3	3.76 (1H, t, 7.8)
8	22.9	1.41 (1H, m) 1.74 (1H, m)	23	70.6	-
9	38.5	1.54 (1H, m) 1.74 (1H, m)	24	23.8	1.12 (3H, s)
10	71.8	-	25	31.0	1.26 (3H, s)
11	76.3	3.56 (1H, dd, 7.2, 10.8)	26	20.3	1.19 (3H, s)
12	21.1	1.49 (1H, m) 1.88 (1H, m)	27	21.4	1.17 (3H, s)
13	20.6	1.72 (1H, m) 1.82 (1H, m)	28	22.6	1.08 (3H, s)
14	76.0	3.69 (1H, dd, 3.0, 12.6)	29	23.4	1.14 (3H, s)
15	73.2	-	30	27.5	1.19 (3H, s)

Table 2. ^{1}H and ^{13}C NMR data of compounds **2**–**3**

Pos.	2		3	
	δ_C	δ_H mult. (*J* in Hz)	δ_C	δ_H mult. (*J* in Hz)
1	36.2	2.05 (1H, overlapped) 1.86 (1H, ddd, 13.2, 5.4, 2.4)	38.6	2.36 (1H, dd, 1.2, 17.4) 2.58 (1H, ddd, 1.8, 3.6, 17.4)
2	34.0	2.65 (1H, ddd, 18.0, 13.8, 4.2) 2.43 (1H, ddd, 18.0, 4.8, 2.4)	128.1	-
3	197.9	-	124.2	-
4	134.5	-	29.4	1.81 (1H, overlapped) 1.96 (1H, m)
5	152.1	-	25.6	1.62 (1H, ddd, 3.0, 12.0, 12.0) 1.83 (1H, overlapped)
6	135.2	7.77 (1H, s)	49.2	-
7	148.7	-	140.8	-
8	198.7	-	38.0	2.50 (1H, dd, 3.0, 14.4) 2.63 (1H, td, 1.8, 14.4)
9	54.5	2.33 (1H, d, 15.0) 2.61 (1H, dd, 15.0, 0.9)	72.2	4.14 (1H, br s)
10	38.2	-	70.9	4.60 (1H, d, 3.0)
11	71.7	-	43.1	-
12	30.0	1.49 (3H, s)	20.7	1.07 (3H, s)
13	29.1	1.42 (3H, s)	24.2	1.08 (3H, s)
14	23.5	1.27 (3H, s)	115.9	4.80 (1H, br s) 5.13 (1H, br s)
15	11.0	1.92 (3H, s)	19.4	1.70 (3H, s)
11-OH	-	4.22 (1H, s)	-	-

Thyrsiferol (1): colorless oil; $[\alpha]_D^{20}$ + 28 (*c* 0.6, $CHCl_3$); HR-ESI-MS *m/z* 627.2872 and 629.2870 $[M + Na]^+$ (calcd. for $[C_{30}H_{53}BrNaO_7]^+$, 627.2867 and 629.2847); ^{1}H (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) of compound **1** (Table 1).

11-hydroxy-8-oxo-*β*-cyperon (2): colorless oil; HR-ESI-MS *m/z* 271.1302 $[M + Na]^+$ (calcd. for $[C_{15}H_{20}NaO_3]^+$, 271.1305); ^{1}H (600 MHz, acetone-d_6) and ^{13}C NMR (150 MHz, acetone-d_6) of compound **2** (Table 2).

(+)-elatol (3): colorless oil; $[\alpha]_D^{24}$ + 75.3 (*c* 0.3, MeOH); mp.: 62–66°C; ^{1}H (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) of compound **3** (Table 2).

[3(15)*E*,4*Z*,6*S*,9*S*,10*R*]-10,15-dibromochamigra-3(15),4,7(14)-trien-9-ol (4): colorless oil; ^{1}H (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) of compound **4** (Table 3).

Pacifidiene (5): colorless oil; $[\alpha]_D^{24}$ + 75.3 (*c* 0.3, MeOH); ^{1}H (600 MHz, acetone-d_6) and ^{13}C NMR (150 MHz, acetone-d_6) of compound **5** (Table 3).

Bioassay

The cytotoxic assay was performed using the MTT method according to a previous protocol [9].

Results and discussion

Compound characterization

From the *n*-hexane extract of *A. dactylomela*, five compounds, thyrsiferol (**1**), 11-hydroxy-8-oxo-*β*-cyperon (**2**), elatol (**3**), [3(15)*E*,4*Z*,6*S*,9*S*,10*R*]-10,15-dibromochamigra-3(15),4,7(14)-trien-9-ol (**4**), and pacifidiene (**5**) were isolated (Fig. 1). Their structures were determined via spectral data analysis, including HR-ESI-MS and NMR data.

Compound **1** was isolated as a colorless oil. Its molecular formula was determined to be $C_{30}H_{53}BrO_7$ based on 1D NMR data and HR-ESI-MS sodium adduct molecular ion peaks at *m/z* 627.2872 and 629.2870 $[M + Na]^+$. The ^{1}H-NMR spectrum of **1** showed the presence of eight singlet methyls [δ_H 1.39 (3H, s, H-1), 1.12 (3H, s, H-24), 1.36 (3H, s, H-25), 1.19 (6H, s, H-26 and H-30), 1.17 (3H, s, H-27), 1.08 (3H, s, H-28), and 1.14 (3H, s, H-29)], one doublet-doublet brominated methines [δ_H 3.88 (1H, dd, *J* = 3.9, 13.2 Hz, H-3)], four doublet-doublet methines bearing oxygen [δ_H 3.03 (1H, dd, *J* = 2.0, 12.0 Hz, H-7), 3.56 (1H, dd, *J* = 7.2, 10.8 Hz, H-11), 3.69 (1H, dd, *J* = 3.0, 12.6 Hz, H-14), and 3.44 (1H, dd, *J* = 1.5, 9.9 Hz, H-18)], one triplet methine bearing oxygen [δ_H 3.76 (1H, t, *J* = 7.8 Hz, H-22)]. The ^{13}C-NMR and HSQC spectra of **1** exhibited the presence of 30 sp^3 carbons, including eight methyl groups, ten methylene groups, five oxymethine groups, six oxygenated tertiary carbons, and one brominated methine group [δ_C 58.9 (C-3)]. Not having any sp^2 carbons, in combination with the molecular formula above, compound **1** was predicted to be a tetracyclic triterpenoid.

Table 3. ^{1}H and ^{13}C NMR data of compounds **4**–**5**

Pos.	4		5	
	δ_C	δ_H mult. (*J* in Hz)	δ_C	δ_H mult. (*J* in Hz)
1	25.7	1.99 (1H, overlapped) 1.75 (1H, m)	33.2	2.28 (1H, dd, 3.0, 14.4) 2.33 (1H, dd, 13.2, 14.4)
2	24.5	2.65 (1H, m) 2.00 (1H, overlapped)	60.4	4.46 (1H, dd, 3.0, 13.2)
3	139.5	-	69.3	-
4	129.26	6.21 (1H, d, 10.8)	46.1	2.40 (1H, dd, 13.2, 15.0) 2.58 (1H, dd, 4.8, 15.0)
5	132.84	5.89 (1H, d, 10.8)	79.5	3.94 (1H, dd, 4.8, 13.2)
6	51.8	-	54.9	-
7	143.0	-	50.8	-
8	38.0	2.71 (1H, ddd, 1.8, 4.2, 15.0) 2.59 (1H, dd, 2.4, 15.0)	101.3	-
9	72.04	4.17 (1H, m)	133.6	6.07 (1H, dd, 0.6, 8.4)
10	70.30	4.64 (1H, d, 3.0)	131.8	6.24 (1H, d, 8.4)
11	42.7	-	148.8	-
12	26.6	1.01 (3H, s)	31.8	1.81 (3H, s)
13	21.46	1.24 (3H, s)	20.1	0.95 (3H, s)
14	117.51	5.10 (1H, t, 1.8) 4.82 (1H, dd, 1.8)	23.4	1.30 (3H, s)
15	105.6	6.10 (1H, d, 2.4)	112.3	5.25 (1H, s) 4.97 (1H, d, 1.2)

Detailed analysis of the COSY spectrum revealed the presence of five spin systems, including H-3/H-4/H-5, H-7/H-8/H-9, H-11/H-12/H-13/H-14, H-16/H-17/H-18, and H-20/H-21/H-22 (Fig. 2). These results, together with the HMBC correlations, allowed the establishment of the planar structure of **1**. Indeed, the structures of rings A, B, and C were determined by HMBC correlations from H3-1 to C-2, C-3, and C-25, from H_3-26 to C-5, C-6, and C-7, and from H3-27 to C-9, C-10, and C-11. The HMBC correlations from H3-28 to C-14, C-15, and C-16; from H3-29 to C-18, C-19, and C-20; and from H3-24 to C-22, C-23, and C-30 confirmed the structural fragment extending from C-15 to C-30. Finally, compound **1** was identified as thyrsiferol, a triterpenoid previously reported from the sea hare *Aplysia dactylomela* and the red algae *Laurencia thyrsifera* and *Laurencia viridis* [10–12], based on the close agreement of

its NMR spectral data with those of the reported compound.

Compound **2** was isolated as a colorless oil. Its positive HR-ESI-MS showed the sodium adduct molecular ion peak at *m/z* 271.1302 [M + Na]$^+$, leading to the molecular formula of $C_{15}H_{20}O_3$ and six degrees of unsaturation. A detailed assessment of the NMR data indicated that **2** is a sesquiterpene, a major components of *A. dactylomela*. The ^{13}C NMR and HSQC spectra of **2** showed 15 signals, including four methyls [δ_C 30.0 (C-12), 29.1 (C-13), 23.5 (C-14), and 11.0 (C-13)], three methylenes [δ_C 36.2 (C-1), 34.0 (C-2), and 54.5 (C-9)], one quaternary carbon [δ_C 38.2 (C-10)], one oxygenated carbon [δ_C 71.7 (C-10)], four sp^2 carbons [δ_C 134.5 (C-4), 152.1 (C-5), 135.2(C-6), and 148.7 (C-7)] and two keton groups [δ_C 197.9 (C-3) and 198.7 (C-7)]. The ^{1}H-NMR spectrum exhibited signals for four singlet methyl groups [δ_H 1.49 (H-12), 1.42 (H-13), 1.27 (H-14), and 1.92 (H-13)], one singlet hydroxy group [δ_H 4.22 (11-OH)], and one olefinic proton [δ_H 7.77 (H-6)]. Four singlet methyls at δ_H 1.49, 1.42, 1.27 and 1.92 were assigned to H-12, H-13, H-14, and H-15 of compound **2** based on HMBC correlations (Fig. 2). The HMBC correlations from proton of the hydroxy group (δ_H 4.22) to C-7 and C-11, as well as the downfield shifted of C-11 (δ_C 71.7) indicated that the hydroxy group linked to C-11. Finally, HMBC correlations from H-2 to C-1 and C-3, and from H-6 to C-4, C-8, and C-11, confirmed the structure of **2**, as shown in Figure 2. Thus, compound **2** was identified as 11-hydroxy-8-oxo-*β*-cyperon, an eudesmane derivative previously reported from the plant *Isocoma wrightii* with only ^{1}H NMR data available [13]. In this study, the complete NMR spectral data of 11-hydroxy-8-oxo-β-cyperone are reported for the first time, and the compound is also documented for the first time from the sea hare *A. dactylomela*.

Compound **3** was isolated as a colorless oil. In the ^{1}H-NMR spectrum, signals of three singlet methyls at δ_H 1.07 (H-12), 1.08 (H-13) and 1.70 (H-15), and two protons of a 1,1-disubstituted double bond [δ_H 4.80 (1H, br s) and 5.13 (1H, br s)] were observed. Additionally, two signals resonating at δ_H 4.60 (1H, d, *J* = 3.0 Hz) and 4.14 (1H, br s) were assigned for a brominated methine group and an oxymethine proton, respectively. The ^{13}C-NMR and HSQC spectra of **3** showed signals for 15 carbons, including five non-protonated carbons, five methylenes, two methines and three methyls. Among these, four were olefinic carbons [δ_C 128.1 (C-2), 124.2 (C-3), 140.8 (C-7) and 115.9 (C-14)] and two were oxygenated or halogenated methines [δ_C 72.2 (C-9) and 70.9 (C-10)]. Key HMBC correlations from protons H-12 (δ_H 1.07) and H-13 (δ_H 1.08) to C-6 (δ_C 49.2), C-10 (δ_C 70.9) and C-11 (δ_C 43.1) (Fig. 2), which indicated that compound **3** contains geminal-dimethyl groups. The structure of A ring was confirmed by HMBC correlations from H-8 to C-6, C-7, C-9, C-10 and C-14. Additionally, a methyl signal was assigned to C-3 on the basis of HMBC correlations from H-15 to C-2, C-3, and C-4. The structure of B ring as well as the spiro-type between the A and B rings, was supported by HMBC correlations from H-5 to C-1, C-4, C-6, and C-7. Finally, compound **3** was determined to be (+)-elatol by comparing its NMR data and optical rotation with those reported in the literature [14–16]. This compound has been previously isolated from the sea hare *A. dactylomela* [17, 18] and the red algae *Laurencia chondrioides* [19] and *Laurencia dendroidea* [15].

Compound **4** was isolated as a colorless oil. The ^{1}H NMR and HSQC spectra displayed the characteristic signals for one endocyclic double bond at δ_H 6.21 and 5.89 (each 1H, d, *J* = 10.8 Hz), one exocyclic double bond at δ_H 5.10 (1H, t, *J* = 1.8 Hz) and 4.82 (1H, dd, *J* = 1.8, 1.2 Hz), two singlet methyls at δ_H 1.01 and 1.24, one brominated methine [δ_H 4.64 (1H, d, *J* = 3.0 Hz)], and one oxygenated methine δ_H 4.17 (1H, m). The ^{13}C NMR and HSQC spectrum of **4** showed the resonance signals for 15 carbons, including four non-protonated carbons, five methines, four methylenes, and two methyl groups. Of these carbons, six were olefinic carbons (δ_C 143.0, 139.5, 132.8, 129.3, 117.5, and 105.6), and two were linked to a bromine or oxygen atom (δ_C 72.0 and 70.3). Structure of the A ring of **4** was the same as that of **3**, which was demonstrated by the HMBC correlations from H-12 to C-6, C-10, C-11 and C-13, and from H-8 to C-6, C-7, C-9, C-10 and C-14 (Fig. 2).

Additionally, other HMBC correlations from H-5 (δ_H 5.89) to C-1 (δ_C 25.7), C-3 (δ_C 139.5) and C-11 (δ_C 42.7), from H_a-14 (δ_H 4.82) and H_b-14 (δ_H 5.10) to C-6 (δ_C 51.8) and C-8 (δ_C 38.0), and from H-15 (δ_H 6.10) to C-2 (δ_C 24.5), C-3 (δ_C 139.5), and C-4 (δ_C 129.3), clearly confirmed the positions of the three double bonds at C-4/C-5, C-7/C-14, and C-3/C-15. With the above evidence, the structure of **4** was determined to be [3(15)*E*,4*Z*,6*S*,9*S*,10*R*]-10,15-dibromochamigra-3(15),4,7(14)-trien-9-ol [19]. This sesquiterpene has previously been reported from the sea hare *A. dactylomela* of Puerto Rico [20], as well as from the red algae *Laurencia dendroidea* [21], *Laurencia chondrioides* [19], and *Laurencia majuscula* [22].

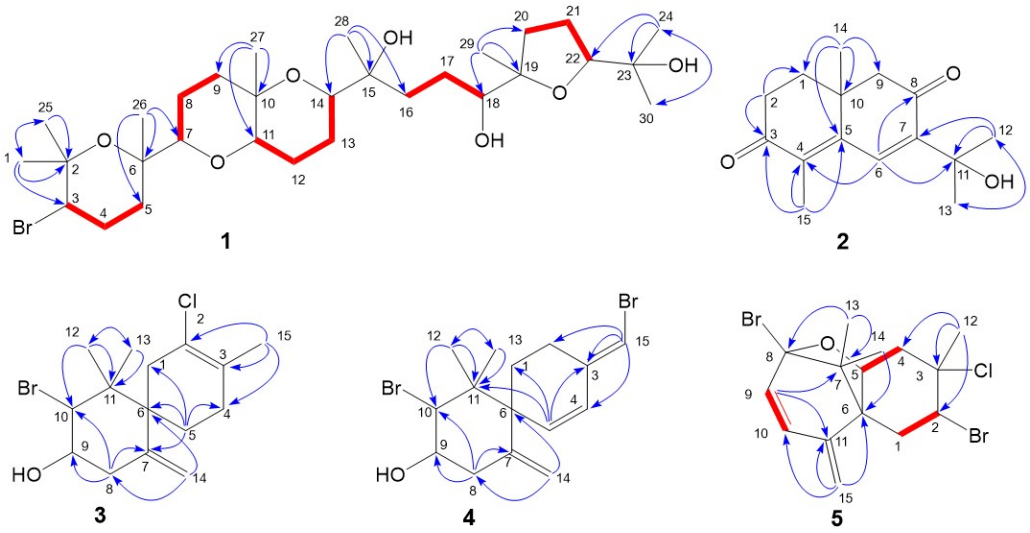

Figure 2. Key COSY (▬) and HMBC (⟶) correlations of compounds **1–5**

Compound **5** was obtained as a colorless oil. The ^{1}H-NMR spectrum exhibited three singlet methyls at δ_H 1.81, 0.95 and 1.30 (each, 3H, s), four olefinic protons at δ_H 6.07 (1H, dd, *J* = 0.6, 8.4 Hz), 6.24 (1H, d, *J* = 8.4 Hz), 5.25 (1H, s), and 4.97 (1H, d, *J* = 1.2 Hz), one oxymethine proton at δ_H 3.94 (1H, dd, *J* = 4.8, 13.2 Hz), one halogenated methine at δ_H 4.46 (1H, dd, *J* = 3.0, 13.2 Hz). The ^{13}C NMR and HSQC data showed three methyl groups [δ_C 31.8 (C-12), 20.1 (C-13), and 23.4 (C-14)], four olefinic carbons [δ_C 133.6 (C-9), 131.8 (C-10), 148.8 (C-11), and 112.3 (C-15)], one brominated methine [δ_C 60.4 (C-2)], one chlorinated carbon [δ_C 69.3 (C-3)], one oxygenated methine [δ_C 79.5 (C-5)], and one brominated-oxygenated carbon [δ_C 101.3 (C-8)]. In the COSY spectrum, the cross-peaks were observed between H_b-1~~b~~/H-2, H_a-4~~a~~/H-5, and H-9/H-10 (Fig. 2). The positions of the brominated methine group and chlorinated carbon of **5** were located at C-2 and C-3, respectively, which were confirmed by the HMBC correlations from H-1a (δ_H 2.28) to C-2 and C-3, from H_3-12 (δ_H 1.81) to C-2, C-3, and C-4. Additionally, other HMBC correlations from H-13 (δ_H 0.95) and H-14 (δ_H 0.95) to C-6, C-7, C-8; from H_b-4 (δ_H 2.58) to C-5, C-6; from H_a-15 (δ_H 4.97) to C-6, C-10, and C-11, clearly confirmed the positions of the oxymethine group at C-5, the brominated- oxygenated carbon at C-8, and two double bonds at C-9/C-10 and C-11/C-15. From the above evidence, compound **5** was identified as pacifidiene, a halogenated sesquiterpene previously isolated from the sea hare *A. dactylomela* from Brazil [23].

Bioassay

An MTT-based bioassay was used to evaluate the in vitro cytotoxicity of compounds **1**–**5**. Among them, compound **3** exhibited significant inhibitory activity against three cancer cell lines, with IC_{50} values ranging from 5.07 ± 0.96 to 6.55 ±

0.44 µM, consistent with previously reported data [24, 25]. Notably, compound **3**, the most active, was also the most abundant constituent isolated. Compound **4** exhibited lower cytotoxic activity than compound **3**, with IC_{50} values ranging from 49.89 ± 1.78 to 63.10 ± 1.73 µM. In contrast, compounds **1**, **2**, and **5** were considered inactive at the initial tested concentration of 30 µM, as they inhibited less than 70% of the cell population (Table 4).

Table 4. Cytotoxic activities of selected compounds against three cancer cell lines

Compounds	The IC_{50} values (µM)[a]		
	A549	HepG2	MCF-7
3	5.07 ± 0.96	6.55 ± 0.44	6.22 ± 0.65
4	49.89 ± 1.78	63.10 ± 1.73	54.20 ± 1.21
Camptothecin[b]	1.58 ± 0.12	1.09 ± 0.12	2.24 ± 0.15

Notes: [a]The remaining compounds did not show cytotoxic activity (IC_{50} > 80 µM). Data are expressed as the mean of at least three independent experiments; [b]Camptothecin was used as a positive control.

Conclusion

In this study, five metabolites were successfully isolated from the sea hare *Aplysia dactylomela* collected from Ly Son Island, Quang Ngai Province, Vietnam. These compounds were identified as thyrsiferol (**1**), and four sesquiterpenoids, 11-hydroxy-8-oxo-*β*-cyperon (**2**), (+)-elatol (**3**), [3(15)*E*,4*Z*,6*S*,9*S*,10*R*]-10,15-dibromochamigra-3(15),4,7(14)-trien-9-ol (**4**), and pacifidiene (**5**) by HR-ESI-MS and NMR spectroscopic analyses. This study reports, for the first time, the isolation of compound **2** from the sea hare *A. dactylomela*, together with its complete NMR data. Evaluation of cytotoxic activity revealed that compound **3** exhibited the most potent effects against A549, MCF-7, and HepG2 cell lines, while the other isolates were weakly active or inactive under the tested conditions. These findings expand the chemical knowledge of *A. dactylomela* metabolites and advance the field of marine natural products chemistry.

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