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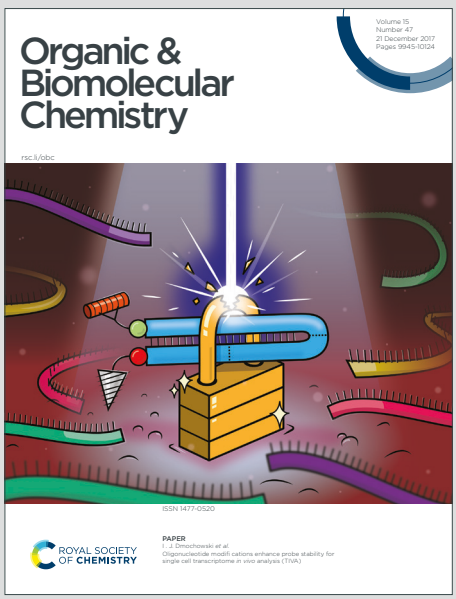

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A novel diterpene and six new sesquiterpenes from the sea hare *Aplysia dactylomela*

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Aplydactylonin D (**1**), a diterpene possessing an unprecedented carbon skeleton with a 3-(p-tolyl)-7,7a-dihydrocyclopenta[b]pyran-6(2H)-one core, along with six new brominated sesquiterpenes, aplydactylonins E–K (**2**–**7**), were isolated from the sea hare *Aplysia dactylomela*. Their structures and absolute configurations were elucidated by interpretion of spectroscopic data, quantum chemical calculations, and electronic circular dichroism (ECD) analyses. All of the isolated compounds were evaluated for their cytotoxicity against HepG2, A549 and MCF7 cells. Aplydactylonin G (**4**) exhibited cytotoxicity against A549 cell line with an IC_{50} value of 8.15 ± 0.96 μM.

Introduction

Within the field of natural product chemistry, marine organisms, particularly sea slugs, have been recognized as abundant sources of structurally diverse and biologically active secondary metabolites. These soft-bodied gastropods, often employing chemical defenses against predators, produce an array of unique compounds with significant pharmaceutical potential.[1, 2] *Aplysia dactylomela*, a shell-less marine mollusk belonging to the family Aplysiidae and commonly referred to as the sea hare, has a cosmopolitan distribution in tropical and warm temperate marine environments. Its habitat is typically in the intertidal zone, extending to depths of approximately 20 meters. This herbivorous mollusk primarily feeds on red algae of the genus *Laurencia*, from which it acquires and sequesters a diverse array of secondary metabolites in its digestive gland as a chemical defense mechanism. The major classes of secondary metabolites isolated from *A. dactylomela* and its dietary algal source, *Laurencia* species, include sesquiterpenes, diterpenes, triterpenes, and C15 acetogenins, some of which exhibit new carbon skeletons. Notably, a large number of these compounds are halogenated and possess a broad spectrum of of significant biological activities, including cytotoxic, antibacterial, antifungal, antibiotic, and anti-inflammatory properties.[3-5]

In our ongoing search for natural products from Vietnamese marine mollusk,[6-8] we investigated the chemical constituents of *Aplysia dactylomela* collected from Ly Son island, Quang Ngai, Vietnam. Our phytochemical studies has led to the isolation and characterization of a previously undescribed diterpene featuring a new bicyclic carbon skeleton (**1**) and six new brominated sesquiterpenes (**2–7**) (Fig. 1). To our knowledge, over 20 diterpene carbon frameworks have been identified from *A. dactylomela* and *Laurencia* algae.[5] Among these, only the obtusane,[9-11] 15,14-friedo-obtusane,[12, 13] and prevezols A–C skeletons (Fig. 1) are structurally closely related to that of **1**.[14, 15] Herein, we describe the isolation, structural elucidation, and cytotoxic evaluation of compounds **1–7**.

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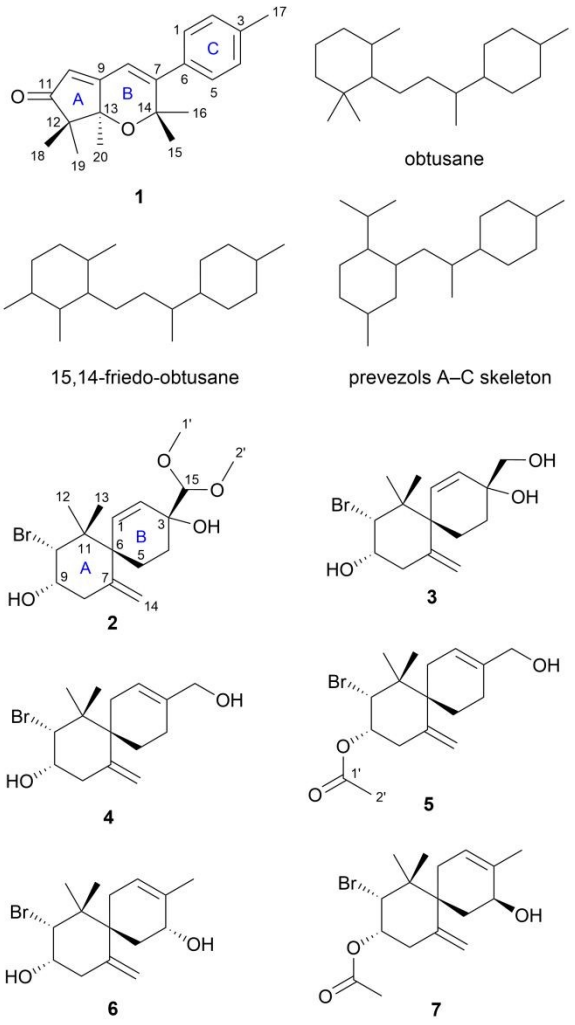

Fig. 1 Chemical structures of compounds **1**-**7** and three bicyclic carbon skeletons of diterpenes isolated from *A. dactylomela* and *Laurencia* species.

Results and discussion

Aplydactylonin D (**1**) was isolated as a pale yellow oil. Its molecular formula was established as $C_{20}H_{24}O_2$ by the HR-ESI-MS ion peaks at *m/z* 297.1855 $[M + H]^+$ (calcd for $C_{20}H_{25}O_2^+$, 297.1849), 319.1679 $[M + Na]^+$ (calcd for $C_{20}H_{24}O_2Na^+$, 319.1669), requiring nine degrees of unsaturation. Examination of the ^{13}C NMR and HSQC spectra (in $CDCl_3$) of **1** revealed the presence of 20 carbon resonances (Table 1), which corresponded to one ketone group (δ_C 210.5), ten sp^2 carbons, three nonprotonated sp^3 carbons including two oxygenated (δ_C 76.2 and 79.9), and six methyls. The existence of one ketone and ten olefinic carbons accounted for 6 out of 9 double bond equivalents, thus implying that **1** was a tricyclic structure. The ^1H NMR spectrum showed signals for one *para*-substituted benzene ring [δ_H 7.16 (2H, d, J = 9.0 Hz) and 7.17 (2H, d, J = 9.0 Hz)], two olefinic methine [δ_H 5.73 (1H, s) and 6.44 (1H, s)], and six singlet methyls (δ_H 1.12, 1.16, 1.37, 1.43, 1.47, and 2.38). In the HMBC spectrum, the correlations from H_3-17 to C-2, C-3 and C-4 placed the Me-17 at C-3, whereas the correlations from aromatic proton H-1 to C-3, C-5 and C-7 suggested the direct connection of benzene ring and C-7 through C-6 (Fig. 2). The structure of a 5,5-dimethyl-4-methyl-2-cyclopenten-1-one ring (ring A) was established by the HMBC correlations from H-10 to C-11, C-12 and C-13, from H_3-18 to C-11, C-12, C-13 and C-19, and from H_3-20 to C-12, C-13 and C-9. This five-membered ring was fused to a 6,6-dimethyl-3,6-dihydro-2*H*-pyran ring (ring B) at C-9 and C-13, which was demonstrated by the HMBC correlations from H-8 to C-9, C-10, C-13, C-14, C-7 and C-6, from H_3-16 to C-7, C-14 and C-15, and a weak ^4J long-range HMBC cross-peak from H_3-15 to C-13. Further evidence for the ether linkage between C-13 and C-14 was provided by the infrared spectrum, which showed no characteristic absorption band for a hydroxyl group in the 3200–3600 cm^{-1} region.[16] Thus, the planar structure of **1** was determined and the new carbon skeleton was named aplydactylonane.

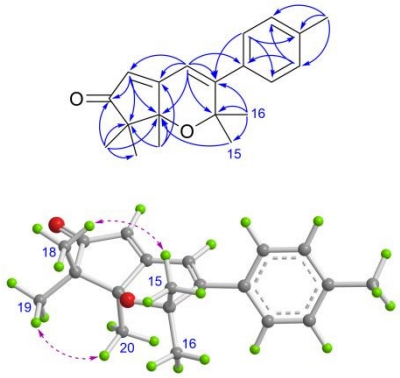

Fig. 2 Key HMBC and NOESY correlations of compound **1**.

In the NOESY spectrum (recorded in DMSO-d_6), the observed correlations of H_3-19/H_3-20 and H_3-18/H_3-15 indicated that Me-19 and Me-20 groups were in cofacial of ring A, whereas H_3-18 and H_3-15 located on the opposite side (Fig 2). Moreover, to verify the structure of **1** as wel as its carbon framework, the ^1H and ^{13}C NMR chemical shifts of isomer 13*R**-**1** were calculated at mPW1PW91/6–31+G(d,p)/IEFPCM-$CHCl_3$ level.[17] As a result (Fig. 3A, Tables S2 and S3), the correlation coefficient (R^2) obtained from the linear regression analysis, the corrected mean absolute error (CMAE), and the root mean square deviation (RMSD) for ^{13}C NMR data were 0.9990, 1.28 ppm, and 1.79 ppm, respectively, and the R^2, CMAE, and RMSD values for proton data were 0.9996, 0.037 ppm, and 0.052 ppm, respectively. These results indicated that the calculated chemical shifts agreed well with the experimental values and provided additional evidence supporting the structure of **1**.[18] Finally, to determine the absolute configuration of **1**, two possible enantiomers (13*R*)-**1** and (13*S*)-**1** were subjected to ECD calculations using TD-DFT method at B3LYP/cc-PVDZ/IEFPCM-MeOH level. As shown in Fig. 3B, the computed ECD spectrum of isomer (13*R*)-**1** was consistent with the experimental data, which assigned the absolute configuration of **1** as 13*R*.

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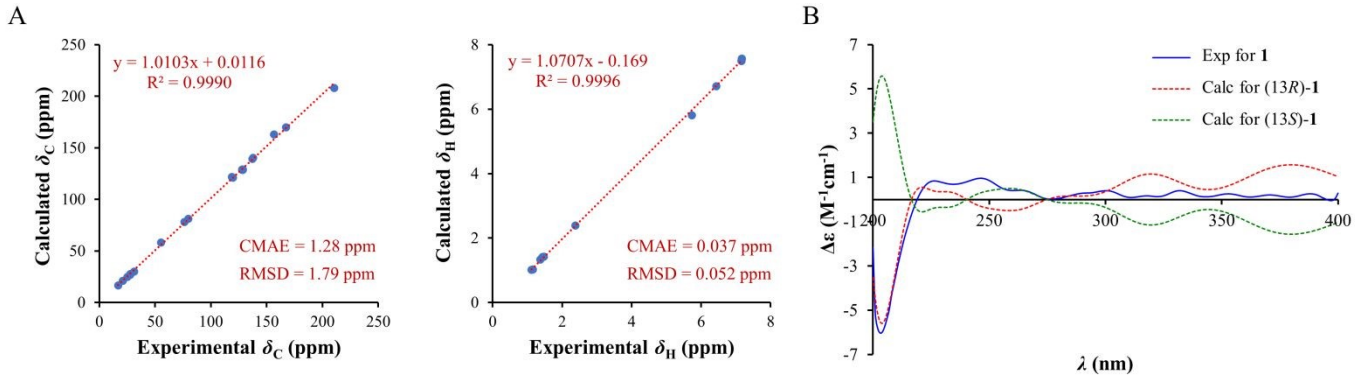

Fig. 3 (A) Linear regression analysis of the experimental versus calculated chemical shifts of **1**. (B) Experimental and calculated ECD spectra of **1**.

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

No.	**1** (in $CDCl_3$)		**1** (in DMSO-d_6)		No.	**2** (in $CDCl_3$)		**3** (in $CDCl_3$)	
	δ_C[a]	δ_H[b] mult. (*J* in Hz)	δ_C[a]	δ_H[b] mult. (*J* in Hz)		δ_C[a]	δ_H[b] mult. (*J* in Hz)	δ_C[c]	δ_H[d] mult. (*J* in Hz)
1, 5	128.1	7.16 d (9.0)	127.9	7.25 d (8.4)	1	134.0	5.93 dd (10.5, 0.6)	134.0	5.88 brd (10.5)
2, 4	128.8	7.17 d (9.0)	128.6	7.22 d (8.4)	2	131.7	5.77 dd (10.5, 0.6)	132.8	5.75 dd (10.5, 1.5)
3	137.9	-	137.4	-	3	72.1	-	71.6	-
6	137.2	-	136.7	-	4a	28.6	1.93 br d (13.2)	29.0	1.97 br d (13.0)
					4b		1.54 ddd (13.2, 13.2, 3.6)		1.48 ddd (13.0, 10.5, 6.5)
7	156.4	-	155.6	-	5a	24.3	2.03 ddd (13.2, 13.2, 3.0)	24.6	1.80 m
					5b		1.75 dddd (13.2, 3.6, 3.6, 1.8)		
8	118.8	6.44 s	118.3	6.53 s	6	50.7	-	51.2	-
9	167.3	-	166.8	-	7	144.3	-	144.3	-
10	119.7	5.73 s	119.2	5.83 s	8a	38.2	2.73 ddd (15.0, 2.4, 1.8)	38.1	2.74 ddd (15.0, 2.0, 2.0)
					8b		2.59 dd (15.0, 2.4)		2.60 dd (15.0, 2.5)
11	210.5	-	208.5	-	9	72.1	4.15 m	71.9	4.15 q (2.5)
12	55.4	-	54.4	-	10	70.6	4.60 d (3.0)	70.1	4.57 d (3.0)
13	79.9	-	79.2	-	11	42.9	-	42.7	-
14	76.2	-	75.6	-	12	21.7	1.19 s	21.6	1.19 s
15	31.2	1.37 s	31.0	1.31 s	13	26.7	1.02 s	26.6	0.99 s
16	27.8	1.47 s	27.4	1.42 s	14a	116.8	5.09 br s	117.1	5.13 t (1.5)
					14b		4.92 br s		4.90 t (1.5)
17	21.2	2.38 s	20.7	2.33 s	15	109.3	4.13 s	67.0	3.51 d (11.0)
									3.49 d (11.0)
18	25.1	1.12 s	24.7	1.00 s	1′	57.8	3.53 s		
19	16.8	1.16 s	16.8	1.03 s	2′	57.6	3.54 s		
20	27.9	1.43 s	27.4	1.38 s					

[a] 600 MHz. [b] 150 MHz. [c] 500 MHz. [d] 125 MHz.

Aplydactylonin E (**2**) was obtained as a pale yellow oil. Its HR-ESI-MS displayed ion peaks at *m/z* 397.0996 and 399.0975 $[M + Na]^+$ (calcd for $C_{17}H_{27}BrO_4Na^+$, 397.0985 and 399.0965) with isotopic intensities of 1:1, corresponding to molecular formula $C_{17}H_{27}BrO_4$ (four degrees of unsaturation). Analysis of the ^{1}H, ^{13}C NMR and HSQC spectra of **2** (Table 1) revealed the presence of one 1,1-disubstituted

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double bond [δ_H 5.09 and 4.92 (each 1H, br s)], one endocyclic double bond [δ_H 5.93 (1H, dd, J = 10.5, 0.6 Hz) and 5.77 (1H, dd, J = 10.5, 0.6 Hz)], one acetal group (δ_C 109.3, δ_H 4.13), one bromomethine (δ_C 70.6, δ_H 4.60),[6] one oxymethine (δ_C 72.1, δ_H 4.15), one oxygenated quaternary carbon (δ_C 72.1), two methoxy groups (δ_C 57.8 and 57.6), along with other sp^3 carbon signals, including two nonprotonated carbons (δ_C 50.7 and 42.9), three methylenes (δ_C 24.3, 28.6 and 38.2) and two tertiary methyls (δ_C 26.7 and 21.7). Since the aforementioned functional groups accounted for two degrees of unsaturation, compound **2** was a bicyclic sesquiterpenoid.

Further analysis of the COSY and HMBC spectra enabled the determination of the planar structure of compound **2** which was similar to that of ma'iliohydrin,[19] except for the replacement of two bromine atoms at C-15 in ma'iliohydrin by two oxymethyl groups in **2**. Specifically, the HMBC cross signals from H_2-14 to C-8, and C-6, from H-8 to C-14, and from H_3-12 to C-6, C-11, C-10 and C-13, in combination with the COSY cross-peaks between H-10/H-9 and H-9/H_2-8, established the six-membered ring A (Fig. 4). The correlations between H-1/H-2 and H_2-4/H_2-5 displayed in the COSY spectrum, in conjunction with the HMBC correlations from H-15 to C-2, and C-4, from H-1 to C-3 and C-5, and from H-2 to C-4 and C-6, verified the structure of ring B. Furthermore, the linkage between ring B and ring A via the C-6 spiro carbon was suggested by the HMBC correlation from H-5 to C-7. The attachment of two methoxy groups to C-15 was confirmed by the HMBC correlations from both H_3-1' and H_3-2' to C-15.

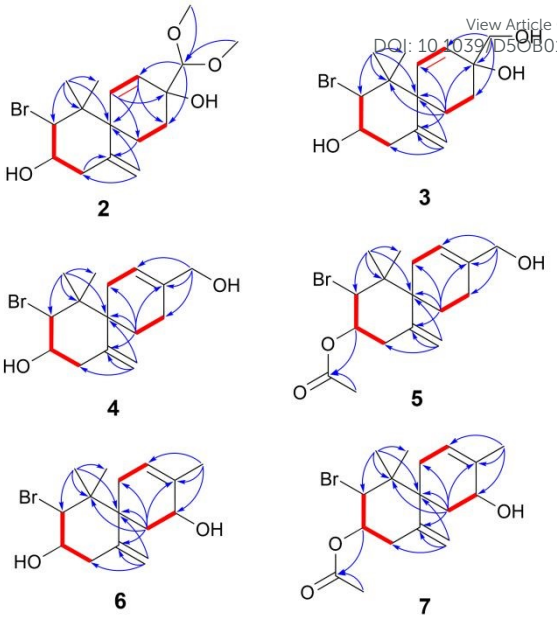

Fig. 4 Key COSY and HMBC correlations of compounds **2–7**.

The relative configuration of compound **2** was determined as 3R^*,6R^*,9S^*,10R^* by considering the coupling constants and NOESY experiments. Concretely, the low-energy conformation of **2** revealed that ring A existed in the chair conformation while ring B possessed the half-chair conformation (Fig. 5). The observed NOESY cross-peak of H-10/H-8a indicated their 1,3-diaxial relationship on the A ring. The small coupling constant of H-10 (J = 3.0 Hz) suggested the equatorial orientation of H-9, which was corroborated by the NOESY correlation of H-9/H-10. In the B ring, the large coupling constant (J = 13.2 Hz) between H-5a and H-4b implied that these protons had a *trans* pseudo-diaxial orientation. Additionally, the NOESY correlation of H-5a/H-15 revealed that they were pseudo 1,3-diaxial and the configuration of C-3 was R^*. The stereochemistry at C-6 of spiro ring system was deduced on the basis of the NOESY cross-peaks of H-5a/H_3-13, H-5b/H-8a, and H-1/H_3-12. Finally, the calculated ECD spectrum of isomer (3R,6R,9S,10R)-**2** was in accordance with the experimental spectrum (Fig 6), indicating the 3R,6R,9S,10R absolute configuration of **2**. Notably, compound **2** possessesed a dimethyl acetal moiety, which may have formed spontaneously from a corresponding aldehyde during the extraction and chromatographic processes involving methanol.[20-23]

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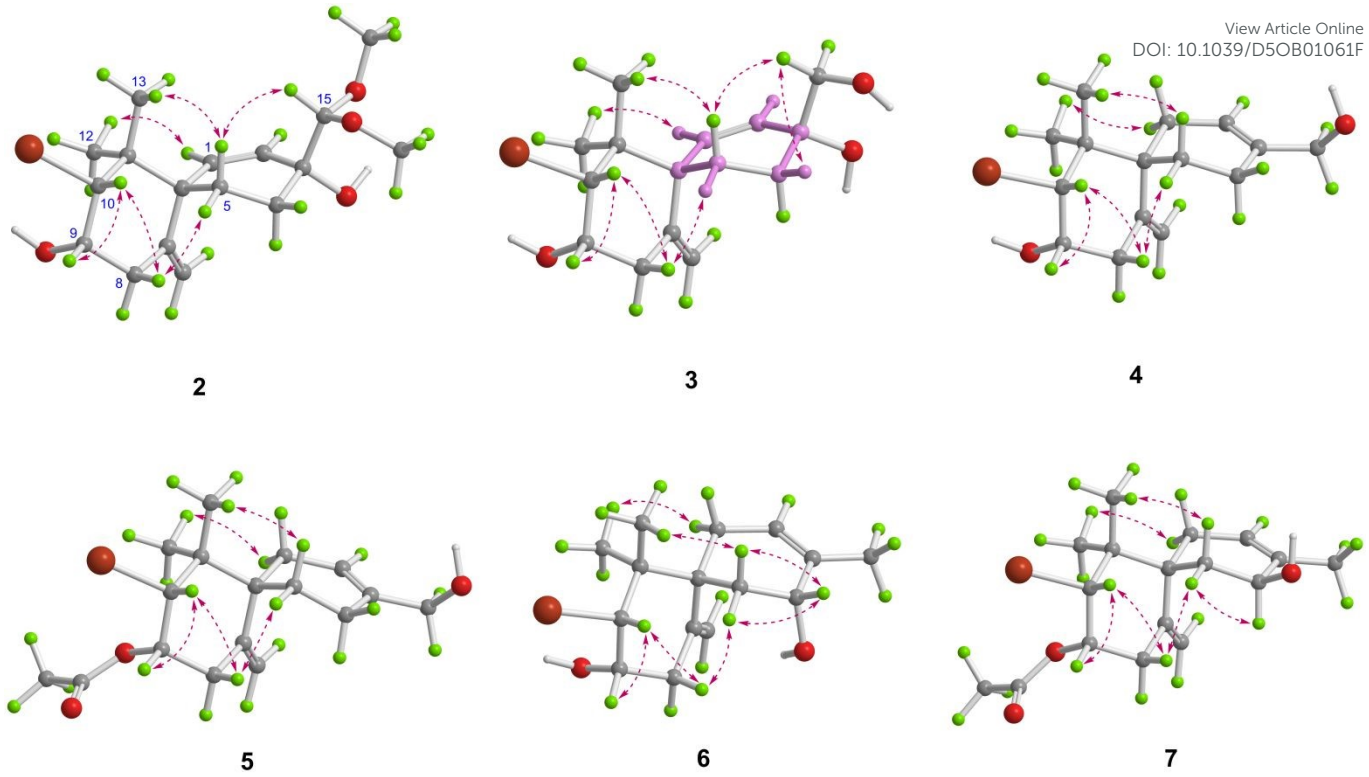

Fig. 5 Key NOESY correlations of compounds **2–7** and the long-range W-couplings observed for compound **3** (highlighted with purple bonds).

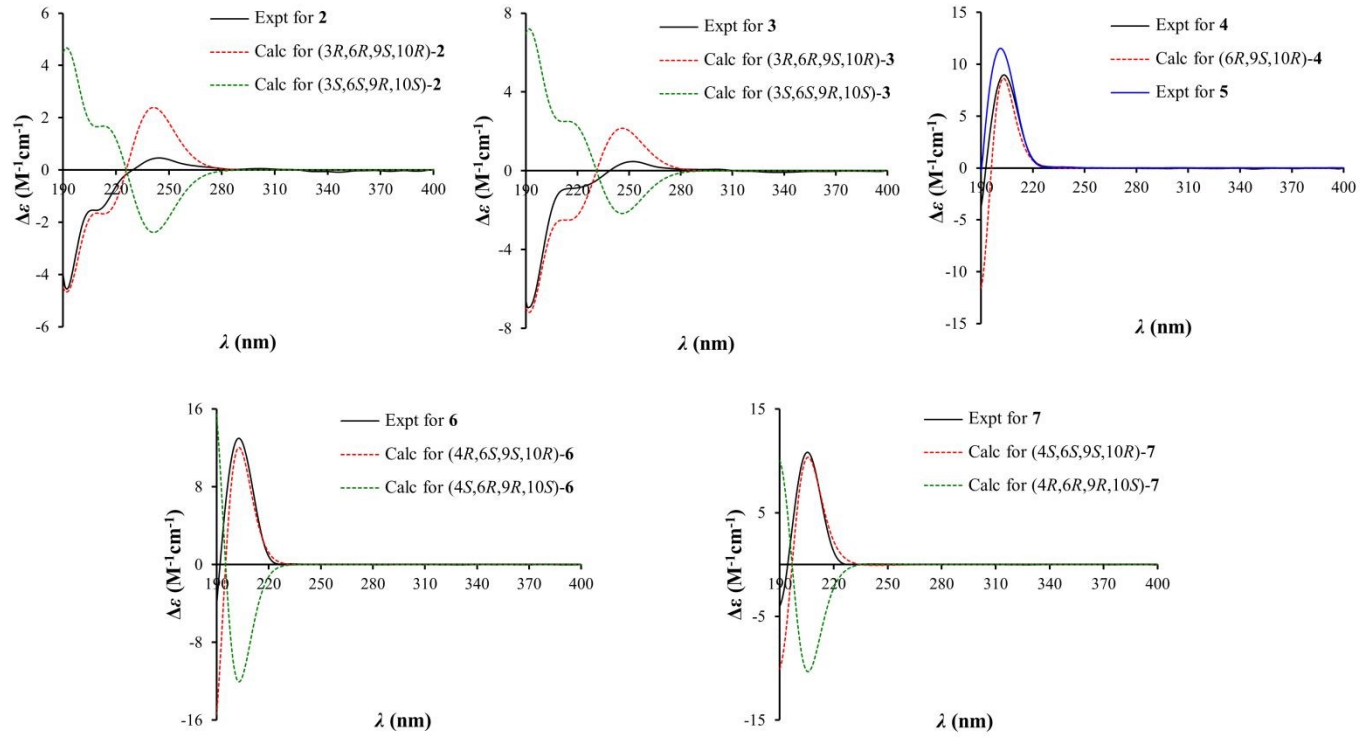

Fig. 6 Experimental and calculated ECD spectra of compounds **2–7**.

Aplydactylonin F (**3**) was isolated as a pale yellow powder. Its molecular formula was deduced as $C_{15}H_{23}BrO_3$ based on the HR-ESI-MS ion peaks at *m/z* 365.0517 and 367.0491 $[M + Cl]^-$ (calcd for $C_{15}H_{23}BrO_3Cl^-$, 365.0525 and 367.0504). Inspection of the 1D and 2 D NMR data (Fig. 4) revealed that compound **3** share the same planar structure with tristichol B,[24] as well as its reassigned structure, epi-3-tristichol B.[25] The only difference between two compounds was the different configuration of C-3, which was supported by the upfield

shifts ($\Delta\delta_C$ 2.3) and downfield shifts ($\Delta\delta_C$ −3.0) observed for C-3 and C-15 of compound **3**, respectively. Further analysis of coupling constant and NOESY spectrum showed that the relative configuration of **3** was identical to that of **2** (Fig. 5). The large vicinal coupling of H-4b (J = 10.5 Hz), along with the long-range W-coupling observed between H-4a and H-2 (J = 1.5 Hz) indicated a pseudo-axial orientation for H-4b and thus a pseudo-equatorial position for H-4a. Although the signals of the two methylene protons of C-5 were overlapped, the NOESY correlations of H_2-15/H-5, H_2-15/H-4a and the lack of NOE cross-peak between H-15 and H-4b confirmed that the oxymethylene group had a pseudo-axial orientation. Finally, through a comparison of the calculated and recorded ECD spectra, the absolute configuration of **3** was determined as 3*R*,6*R*,9*S*,10*R* (Fig. 6).

Table 2 ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of **4**–**7** in $CDCl_3$

No.	**4**		**5**		**6**		**7**	
	δ_C	δ_H mult. (J in Hz)	δ_C	δ_H mult. (J in Hz)	δ_C	δ_H mult. (J in Hz)	δ_C	δ_H mult. (J in Hz)
1	29.9	2.27 m 2.21 br d (18.0)	29.9	2.29 m 2.19 m	30.6	2.32 m 2.24 br d (18.0)	30.4	2.28 dddd (18.0, 2.5, 2.5, 2.5) 2.12 br d (18.0)
2	121.6	5.61 dd (2.5, 1.5)	121.4	5.60 m	123.3	5.53 m	122.5	5.41 m
3	136.3	-	136.3	-	133.4	-	134.9	-
4a	23.4	2.00 m	23.4	2.00 m	69.3	3.87 br s	69.2	3.73 m
4b		1.73 overlapped		1.67 m				
5a	25.7	1.91 m	25.7	1.89 m	34.5	2.41 dt (14.0, 2.0)	36.1	2.22 dddd (12.5, 2.5, 2.5, 2.5)
5b		1.61 ddd (12.5, 12.5, 4.0)		1.61 m		1.84 dd (14.0, 4.5)		1.61 dd (12.5, 11.0)
6	47.4	-	47.2	-	45.4	-	48.6	-
7	141.1	-	140.9	-	145.9	-	140.7	-
8a	38.0	2.70 ddd (14.5, 2.0, 2.0)	36.7	2.66 ddd (15.5, 3.5, 2.0)	39.5	3.08 dt (14.5, 2.0)	36.7	2.74 dt (15.0, 1.5)
8b		2.49 dd (14.5, 2.5)		2.40 dd (15.5, 3.0)		2.58 dd (14.5, 2.5)		2.42 dd (15.0, 3.0)
9	72.4	4.16 q (3.0)	73.9	5.27 q (3.5)	72.5	4.20 q (3.0)	73.8	5.27 q (3.0)
10	71.5	4.69 d (3.0)	63.7	4.57 d (3.5)	71.1	4.69 d (3.0)	63.2	4.57 d (3.0)
11	43.2	-	43.5	-	43.7	-	43.5	-
12	20.8	1.07 s	20.2	1.05 s	20.2	1.03 s	19.6	1.01 s
13	24.3	1.07 s	24.3	1.08 s	24.9	1.06 s	24.4	1.10 s
14a	115.9	5.09 br s	115.7	4.95 br s	116.6	5.15 br s	115.6	4.92 br s
14b		4.78 br s		4.74 br s		4.92 br s		4.72 br s
15	66.9	3.96 br d (12.5) 3.92 br d (12.5)	66.9	3.95 br d (12.5) 3.91 br d (12.5)	20.4	1.75 br s	18.4	1.69 br s
1′			170.2	-			170.1	-
2′			21.1	2.06 s			21.0	2.06 s

Aplydactylonin G (**4**), a white, amorphous powder, had the molecular formula of $C_{15}H_{23}BrO_2$ as deduced from the HR-ESI-MS ion peaks at m/z 332.1208 and 334.1182 $[M + NH_4]^+$ (calcd for $C_{15}H_{27}BrO_2N^+$, 332.1220 and 334.1200). The ^{1}H, and ^{13}C NMR and HSQC spectra displayed resonances due to one trisubstituted double bond (δ_C 121.6, δ_H 5.61 and δ_C 136.3), one exocyclic double bond (δ_C 141.1, 115.9, and δ_H 5.09, 4.78), one bromomethine (δ_C 71.5, δ_H 4.69), one oxymethine (δ_C 72.4, δ_H 4.16), one oxymethylene (δ_C 66.9, δ_H 3.96, 3.92), and two singlet methyls (δ_H 1.07). Examination of the 2D NMR data suggested that the planar structure of **4** (Fig. 4) was closely resembled that of the known compound aplydactylonin B.[6] The only structural difference was the replacement of the methoxy group at C-15 of aplydactylonin B (δ_C 76.6, C-15) by a hydroxy group in **4** (δ_C 66.9, C-15). This was supported by HMBC correlations from H_2-15 to C-2, C-3 and C-4. The relative configurations at C-6, C-9 and C-10 of **4** were identical to those of **2**, which was confirmed by NOE correlations as shown in Fig. 5. The experimental ECD spectrum of **4** showed a positive Cotton effect at 203 nm, consistent with the calculated spectrum for the (6*R*,9*S*,10*R*)-**4** isomer, thereby indicating the 6*R*,9*S*,10*R* absolute configuration of **4** (Fig. 6).

Aplydactylonin H (**5**) was also obtained as a white amorphous powder. The molecular formula of $C_{17}H_{25}BrO_3$ was determined from the HR-ESI-MS ion peaks at m/z 379.0871 and 381.0836 $[M + Na]^+$ (calcd for $C_{17}H_{25}BrO_3Na^+$, 379.0880 and 381.0859). The ^{1}H and ^{13}C NMR data of **5** (Table 2) were nearly identical to those of **4**, except for the presence of an additional acetyl group (δ_C 170.2, 21.1, and δ_H 2.06) in **5**. The downfield shifted of H-9 (δ_H 5.27) along with the HMBC correlation from H-9 to C-1' confirmed the position of the acetoxy group at C-9 (Fig. 4). The relative configuration of **5** was established on the basis of NOESY experiments (Fig. 5), and the absolute configuration was determined as 6*R*,9*S*,10*R* by comparison of the ECD spectrum of **5** with that of **4** (Fig. 6).

Aplydactylonin I (**6**), a white amorphous powder, exhibited the same molecular formula as **4**, as deduced from the HR-ESI-MS and

^{13}C NMR spectrum. Comparison of the 1D NMR data of **6** with those of **4** revealed that these two isomers had the same bromochamigrane framework. The notable differences were related to the structure of the B ring, where the hydroxy group was linked at C-4 in **6** instead of at C-15 in **4**. Indeed, the COSY correlations of H-1/H-2, H-4/H-5, together with the HMBC cross-peaks from H_2-5 to C-1, C-3 and C-6, and from H_3-15 to C-2, C-3 and C-4 confirmed the structure of the B ring of **6**, in which a methyl group (δ_C 20.4 and δ_H 1.75) and an oxymthine group (δ_C 69.3 and δ_H 3.87) were assigned to C-15 and C-4, respectively. Further analysis of 2D NMR data allowed the complete assignment for planar structure of **6** (Fig. 4). The small vicinal coupling constants of H-4 ($J_{4,5a}$ = 2.0 Hz and $J_{4,5b}$ = 4.5 Hz), as well as the NOESY correlations of H-4/H-5a and H-4/H-5b indicated that H-4 was pseudo-equatorial. The relative configurations of the other chiral centers of **6** was established on the basis of the NOESY data (Fig. 5). Finally, the calculated ECD curve of isomer (4*R*,6*S*,9*S*,10*R*)-**6** showed similar Cotton effect to that of the experimental ECD spectrum, allowing the assignment of the absolute configuration of **6** (Fig. 6).

Aplydactylonin K (**7**) was isolated as a white amorphous powder. The molecular formula of $C_{17}H_{25}BrO_3$ was determined by the HR-ESIMS ion peaks at *m/z* 391.0669 and 393.0648 $[M + Cl]^-$ (calcd for $C_{17}H_{25}BrO_3Cl^-$, 391.0681 and 393.0661). The planar structure of **7** were very similar to that of **6** except that the hydroxy group at C-9 in **6** was replaced by an acetyl group in **7**. This was supported by the HMBC correlations from H-9 (δ_H 5.27) and H-2' (δ_H 2.06) to C-1' (δ_C 170.1) (Fig. 4). The orientation of H-4 was demonstrated as pseudo-axial based on the large coupling constant between H-4 and H-5b (*J* = 11.0 Hz) and the NOE crosss-peak of H-4/H-5a (Fig. 5). The relative configurations of **7** at C-6, C-9 and C-10 were assigned analogously to those of compound **6** by the correlations observed in the NOESY spectrum. The calculated ECD spectrum for the (4*S*,6*S*,9*S*,10*R*)-**7** isomer displayed a positive Cotton effect at 205 nm, which fit well with that of the experimental one, confirming the (4*S*,6*S*,9*S*,10*R*) absolute configuration of **7** (Fig. 6).

All isolated compounds were evaluated for their in vitro cytotoxic activities against HepG2, A549, and MCF-7 cancer cell lines by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay,[26] in which camptothecin was used as the positive control. As shown in Table 3, compound **4** displayed selective and significant cytotoxicity against the A549 cells with the IC_{50} value of 8.15 ± 0.96 μM, while the remaining compounds showed weak or no activity.

Table 3 Cytotoxicity of compound **1–7**

Compound	IC_{50} (μM)		
	HepG2	A549	MCF7
1	44.87 ± 1.44	30.83 ± 1.52	> 50
2	> 50	> 50	> 50
3	37.27 ± 0.73	> 50	> 50
4	> 50	8.15 ± 0.96	> 50
5	45.39 ± 1.72	34.51 ± 1.45	49.20 ± 1.61
6	> 50	> 50	> 50
7	29.65 ± 1.06	25.23 ± 0.97	31.19 ± 1.33
Camptothecin [a]	2.24 ± 0.15	1.58 ± 0.12	1.09 ± 0.12

[a] Positive control

Experimental

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General experimental procedures

Optical rotations were obtained using a JASCO P-2000 polarimeter (JASCO, Tokyo, Japan). IR spectra were measured on a Perkin-Elmer FT-IR Spectrum Two. HR-ESI-MS spectra were recorded on a 6530 Accurate-Mass Q-TOF LC/MS system (Agilent, CA, USA). Nuclear magnetic resonance (NMR) spectra were recorded with Ascend 500/AVANCE III HD and AVANCE NEO 600 FT-NMR spectrometers (Bruker, Billerica, MA, USA) at a temperature of 303 K. NMR chemical shifts (δ) were referenced to tetramethylsilane (TMS) at 0.00 ppm. The ECD spectrum was measured on a Chirascan spectropolarimeter (Applied Photophysics, Leatherhead, UK). Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70–230, and 230–400 mesh, Merck, Darmstadt, Germany) and gel resins (ODS-A, 12 nm S-150 µm; YMC, Kyoto, Japan). Analytical thin-layer chromatography (TLC) was performed on precoated silica gel 60 F_{254} (1.05554.0001; Merck) and RP-18 F_{254S} plates (1.15685.0001; Merck) and the isolated compounds were visualized by spraying with 10% H_2SO_4 in water and then heating for 1.5–2 minutes. All procedures were conducted with solvents that were purchased from commercial sources and used without further purification.

Biological material

The sample of *Aplysia dactylomela* were collected at Ly Son island, Quang Ngai province, Vietnam, in May 2023, and identified by Dr. Tran My Linh and Dr. Nguyen Chi Mai. A voucher specimen (ĐLTE02) has been deposited in the Department of Marine Biochemical Resources, Institute of Chemistry, VAST, Hanoi, Vietnam.

Extraction and isolation

Frozen sea hares (1.25 kg) were cut into small pieces and then exhaustively extracted three times with methanol (4L, each) in an ultrasonic bath at room temperature. The concentrated extract (115 g), after evaporation of the solvent, was further suspended in water and partitioned successively with *n*-hexane (3 × 1.5 L) and CH_2Cl_2 (3 × 1.5 L). The CH_2Cl_2-soluble portion (81.5 g) was subjected to a silica gel column eluting with a gradient of *n*-hexane/acetone (100:0 to 0:100, v/v) to give ten fractions (C1–C10). Fraction C1 was applied to a silica gel column eluted with *n*-hexane/acetone (60:1, v/v), affording two fractions (C1A and C1B). Fraction C1B was chromatographed on a silica gel column using *n*-hexane/EtOAc/CH_2Cl_2 (28:1:0.1. v/v/v) as the mobile phase, to yield compound **1** (9.9 mg). Fraction C6 was separated by a silica gel column using dichloromethane with increasing amounts of MeOH as the eluent to afford four fractions (C6A–C6D). Subfraction C6B was chromatographed on a silica gel column eluted with mixture of *n*-hexane/EtOAc (7:2, v/v), and then purified by a reversed-phase C_{18} silica gel column with MeOH/H_2O (1:1) to give compound **2** (4.2 mg). Fraction C6C was fractionated by a silica gel column eluted with *n*-hexane/EtOAc/CH_2Cl_2 mixtures (8:9:1. v/v/v) to yield three subfractions (C6C1–C6C3). Subfraction C6C2 was separated by a reversed-phase C_{18} silica gel column using MeOH/H_2O (1:1) as mobile phase to afford three smaller subfractions (C6C2A–C6C2C). Compounds **6** (6.3 mg) and **7** (8.3 mg) were obtain from subfraction C6C2B by a silica gel column eluting with *n*-hexane/EtOAc (7:2, v/v). Subfraction C6C2C was further purified by a silica gel column eluted with a miture of *n*-hexane/EtOAc (3:1, v/v) to give compound **5** (9.8

mg). Fraction C8 was chromatographed on a silica gel column with *n*-hexane/EtOAc mixture (2:1, v/v) to yield two fractions (C8A and C8B). Fraction C8A was further fractionated by a reversed-phase C_{18} silica gel column using acetone/H_2O (1:1, v/v), and then purified by a silica gel column with *n*-hexane/CH_2Cl_2/acetone (7:2:1, v/v/v) to give compound **4** (10.0 mg). Fraction C8B was loaded into a reversed-phase C_{18} silica gel column (acetone/H_2O, 1:1, v/v) to yielded subfraction C8B1, which was then purified utilizing a silica gel column (hexane/acetone, 3:1, v/v) to afford compound **3** (5.0 mg).

Aplydactylonin D (1). Yellow oil; $[\alpha]^{25}_D$ +3.4 (c 0.1, MeOH); IR (KBr) ν_{max} 2928, 1728, 1600, 1462, 1379 cm^{-1}; UV (MeOH) λ_{max} (log ε) 210 (shoulder, 3.68), 290 (3.93) nm; ECD (*c* 0.17 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 203 (–6.02), 226 (0.84), 246 (0.97), 300 (2.40), 332 (0.41); ^{1}H and ^{13}C NMR data, see Table 1; HR-ESIMS *m/z* 297.1855 [M + H]$^+$ (calcd for $C_{20}H_{25}O_2^+$, 297.1849), *m/z* 319.1679 [M + Na]$^+$ (calcd for $C_{20}H_{24}O_2Na^+$, 319.1669).

Aplydactylonin E (2). Yellow oil; $[\alpha]^{25}_D$ –7.3 (c 0.1, MeOH); IR (KBr) ν_{max} 3442, 2966, 1659, 1371 cm^{-1}; UV (ACN) λ_{max} (log ε) 217 (shoulder, 2.27) nm; ECD (*c* 0.49 mM, ACN) λ_{max} ($\Delta\varepsilon$) 192 (–4.55), 244 (0.46); ^{1}H and ^{13}C NMR data, see Table 1; HR-ESIMS *m/z* 397.0996 and 399.0975 [M + Na]$^+$ (calcd for $C_{17}H_{27}BrO_4Na^+$, 397.0985 and 399.0965).

Aplydactylonin F (3). Yellow powder; $[\alpha]^{25}_D$ –16.7 (c 0.1, MeOH); IR (KBr) ν_{max} 3401, 2924, 1681, 1399 cm^{-1}; UV (ACN) λ_{max} (log ε) 220 (shoulder, 3.06) nm; ECD (*c* 0.73 mM, ACN) λ_{max} ($\Delta\varepsilon$) 192 (–6.92); ^{1}H and ^{13}C NMR data, see Table 1; HR-ESIMS *m/z* 365.0517 and 367.0491 [M + Cl]$^-$ (calcd for $C_{15}H_{23}BrO_3Cl^-$, 365.0525 and 367.0504).

Aplydactylonin G (4). White amorphous powder; $[\alpha]^{25}_D$ +21.0 (c 0.1, MeOH); IR (KBr) ν_{max} 3385, 2925, 1678, 1371 cm^{-1}; UV (ACN) λ_{max} (log ε) 217 (shoulder, 2.46) nm; ECD (*c* 0.7 mM, ACN) λ_{max} ($\Delta\varepsilon$) 203 (8.98); ^{1}H and ^{13}C NMR data, see Table 2; HR-ESIMS *m/z* 332.1208 and 334.1182 [M + NH_4]$^+$ (calcd for $C_{15}H_{27}BrO_2N^+$, 332.1220 and 334.1200).

Aplydactylonin H (5). White amorphous powder; $[\alpha]^{25}_D$ +69.1 (c 0.1, MeOH); IR (KBr) ν_{max} 3450, 2926, 1740, 1679, 1376 cm^{-1}; UV (ACN) λ_{max} (log ε) 217 (shoulder, 2.15) nm; ECD (*c* 1.68 mM, ACN) λ_{max} ($\Delta\varepsilon$) 201 (11.54); ^{1}H and ^{13}C NMR data, see Table 2; HR-ESIMS *m/z* 379.0871 and 381.0836 [M + Na]$^+$ (calcd for $C_{17}H_{25}BrO_3Na^+$, 379.0880 and 381.0859).

Aplydactylonin I (6). White amorphous powder; $[\alpha]^{25}_D$ +70.4 (c 0.1, MeOH); IR (KBr) ν_{max} 3364, 2968, 1637, 1391 cm^{-1}; UV (ACN) λ_{max} (log ε) 217 (shoulder, 2.24) nm; ECD (*c* 1.27 mM, ACN) λ_{max} ($\Delta\varepsilon$) 203 (13.00); ^{1}H and ^{13}C NMR data, see Table 2; HR-ESIMS *m/z* 349.0576 and 351.0554 [M + Cl]$^-$ (calcd for $C_{15}H_{23}BrO_2Cl^-$, 349.0575 and 351.0554).

Aplydactylonin K (7). White amorphous powder; $[\alpha]^{25}_D$ +24.4 (c 0.1, MeOH); IR (KBr) ν_{max} 3443, 2950, 1741, 1641, 1376 cm^{-1}; UV (ACN) λ_{max} (log ε) 217 (shoulder, 1.95) nm; ECD (*c* 2.23 mM, ACN) λ_{max} ($\Delta\varepsilon$) 205 (10.82); ^{1}H and ^{13}C NMR data, see Table 2; HR-ESIMS *m/z* 391.0669 and 393.0648 [M + Cl]$^-$ (calcd for $C_{17}H_{25}BrO_3Cl^-$, 391.0681 and 393.0661).

Computational methods

The methods and details for NMR and ECD calculations are provided in the ESI.†

Cell culture

The cancer cell lines HepG2 (human hepatocellular carcinoma cells), A549 (human lung carcinoma cells), and MCF-7 (breast cancer) were kindly provided by Prof. Jeong-Hyung Lee, Department of Biochemistry, College of Natural Sciences, Kangwon National University, Korea. The cells were cultured in DMEM medium (Gibco, Grand Island, NY, USA) supplemented with 10% sterile-filtered fetal bovine serum (FBS) (Gibco) and 1% antibiotic solution (100 U/mL penicillin and 100 µg/mL streptomycin) (Gibco) at 37 ºC in a 5% CO2 incubator.

Cytotoxicity assay

The cytotoxic effect on cancer cells was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method.[26] The cells were seeded in 96- well plates at a concentration of 1 × 10^5 cells/well and treated with various concentrations of compoundss (0–100 µM) and incubated in a humidified 5% CO2 atmosphere at 37°C. After 48 h incubation, 20 µL of MTT was added to each well and incubated for another 4 h. After removing the supernatant, formazan crystals were dissolved in 200 µL DMSO. The absorbance was measured at 570 nm using an ELISA reader (Epoch, BioTek, Winooski, VT, USA). Camptothecin was used as a positive control.

Conclusions

In summary, our phytochemical investigation of the sea hare *Aplysia dactylomela* had led to the isolation of a diterpene with new carbon skeleton (**1**) and six new brominated sesquiterpenes (**2**–**7**). Detailed spectroscopic analyses, supported by quantum chemical calculations and ECD data, enabled the unambiguous elucidation of their structures. Among the isolated compounds, aplydactylonin G (**4**) demonstrated notable cytotoxic activity against A549 human lung cancer cells with an IC_{50} value of 8.15 ± 0.96 µM.

Author contributions

Pham Thanh Binh: Investigation and Formal analysis. Duong Thu Trang: Investigation. Kieu Thi Phuong Linh: Investigation. Nguyen Viet Phong: Investigation. Nguyen Phuong Thao: Investigation. Nguyen Chi Mai: Investigation. Tran My Linh: Formal analysis. Dang Vu Luong: Investigation. Nguyen Hoai Nam: Formal analysis, Validation, Supervision. Nguyen Van Thanh: Formal analysis, Validation, Writing – original draft, Supervision.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the Supplementary Information.

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Data availability

The data supporting this article have been included as part of the Supplementary Information.