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Communication

Six New Tetraprenylated Alkaloids from the South China Sea Gorgonian *Echinogorgia pseudossapo*

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Abstract: Six new tetraprenylated alkaloids, designated as malonganenones L–Q (**1**–**6**), were isolated from the gorgonian *Echinogorgia pseudossapo*, collected in Daya Bay of Guangdong Province, China. The structures of **1**–**6** featuring a methyl group at N-3 and a tetraprenyl chain at N-7 in the hypoxanthine core were established by extensive spectroscopic analyses. Compounds **1**–**6** were tested for their inhibitory activity against the phosphodiesterases (PDEs)-4D, 5A, and 9A, and compounds **1** and **6** exhibited moderate inhibitory activity against PDE4D with IC_{50} values of 8.5 and 20.3 μM, respectively.

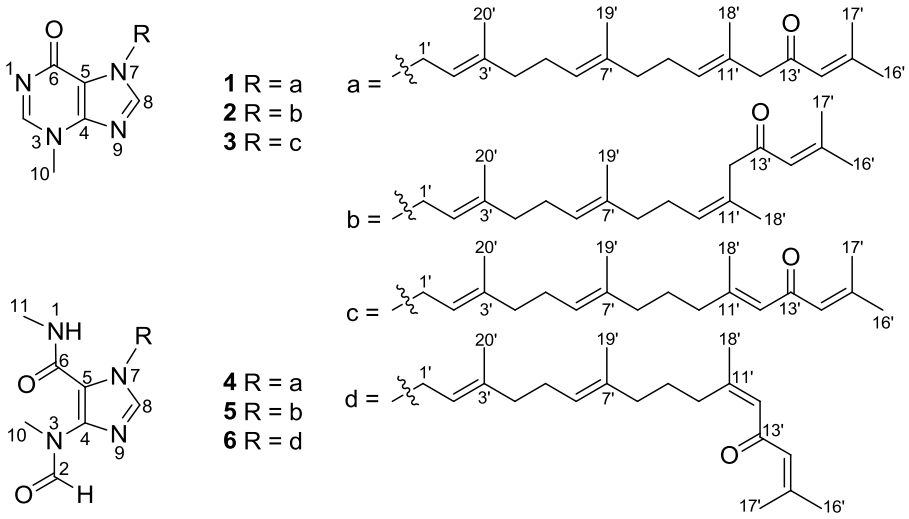
Keywords: gorgonian; *Echinogorgia pseudossapo*; tetraprenylated alkaloids; phosphodiesterases

1. Introduction

Tetraprenylated purine alkaloids and their derivatives are relatively uncommon in nature [1,2]. They are structurally characterized by a methyl group at N-3 and a tetraprenyl chain at N-7 in the hypoxanthine core. Malonganenone A [3], the first typical representative of this series, was isolated from the gorgonian *Leptogorgia gilchristi*, collected in Ponto Malongane, Mozambique in 2006. Until

now, only 14 analogues have been reported from marine organisms [3–5], some of which exhibited antitumor activity [4,5]. Although the genus *Echinogorgia* is highly prolific in the South China Sea, only a few species of *Echinogorgia* have been chemically investigated, which led to the isolation of a series of metabolites including sterols [6–12], alkaloids [11–14], sesquiterpenes [14–16], ceramides [17], and coumarins [18]. In our screening program aimed at discovering new biologically active natural products from marine organisms of the South China Sea [19], a fraction of the CH_2Cl_2/MeOH extract of *E. pseudosassapo* showed inhibitory activity towards phosphodiesterases (PDEs)-4D, 5A, and 9A. Subsequent chemical investigation resulted in the purification of six new tetraprenylated alkaloids, malonganenones L–Q (**1**–**6**, Figure 1, Supplementary Figures S1–S31). The resulting inhibitory activity screening against PDE4D, PDE5A, and PDE9A showed that compounds **1** and **6** exhibited moderate activities against PDE4D with IC_{50} values of 8.5 and 20.3 μM, respectively. The present report describes the isolation, structure elucidation, and PDEs inhibitory activities of these tetraprenylated alkaloids.

Figure 1. Structures of malonganenones L–Q (**1**–**6**).



2. Results and Discussion

2.1. Structural Elucidation of New Compounds

The CH_2Cl_2/MeOH (v/v, 1:1) extract of the gorgonian was subjected to chromatography using Sephadex LH-20 followed by silica gel and HPLC separations to yield compounds **1**–**6**.

Compound **1**, a colorless oil, exhibited a molecular formula of $C_{26}H_{36}N_4O_2$ as determined by HRESIMS ($[M + Na]^+$, 459.2721, calcd. 459.2736), implying 11 double bond equivalents (DBE). The IR absorption bands at 1709 and 1610 cm^{-1} indicated the presence of two carbonyls. The ^1H NMR spectrum of **1** (Table 1) showed signals for two aromatic singlets [δ_H 8.26 (H-2) and 7.69 (H-8)], four olefinic protons [δ_H 6.07 (H-14′), 5.45 (H-2′), 5.19 (H-10′), and 5.05 (H-6′)], five vinylic methyls [δ_H 2.10 (H-17′), 1.84 (H-16′), 1.77 (H-20′), 1.58 (H-18′), and 1.56 (H-19′)], one heteroatom-functionalized methyl [δ_H 3.86 (H-10)], and a series of aliphatic methylene multiplets. The ^{13}C NMR spectrum of **1** (Table 2) resolved 26 resonances attributable to five double bonds (δ_C 155.5 C, 122.9 CH; 147.3 C, 115.0 C; 143.4 C, 117.6 CH; 135.5 C, 123.5 CH; and 129.6 C, 129.0 CH), two carbonyls (δ_C 199.3 and 162.0), two imines (δ_C 147.7 and 140.3), five vinylic methyls

(δ_C 27.7, 20.6, 16.5, 16.4, and 16.0), a *N*-methyl (δ_C 35.0), and six sp^3 methylenes (δ_C 55.3, 44.5, 39.4, 39.3, 26.7, and 26.1). As nine of the eleven DBE were accounted for by abovementioned unsaturated functional groups, the remaining two DBE required that **1** was bicyclic. The collective spectroscopic information pointed clearly to a fused diterpene-*N*-methylhypoxanthine structure, which bore a high similarity to that of malonganenone D [4]. The *N*-methylhypoxanthine moiety of **1** was readily identified by comparison of its NMR data with that of malonganenone D, which gave almost identical ^{13}C NMR data regarding to this portion. The tetraprenyl side-chain of **1** was deduced from detailed analysis of COSY and HMBC data (Figure 2). ^1H–^1H COSY correlations revealed four spin systems: (**a**) H-1′/H-2′/H-3′/H_3-20′; (**b**) H-4′/H-5′/H-6′/H-7′/H_3-19′; (**c**) H-8′/H-9′/H-10′/H-11′/H_3-18′; and (**d**) H-14′/H-15′/H-16′ or H-17′. The connections from **a** to **c** were achieved by HMBC correlations of H-20′/C-4′ and H-19′/C-8′. The fragments **c** and **d** were linked via a methylene and a ketone (C-13) by HMBC correlations of H-18′/C-12′, H-12′/C-13′, and H-14′/C-13′. The geometries of the three olefins in the tetraprenyl side-chain were established from analysis of both ^{13}C chemical shift and NOE data. The ^{13}C chemical shifts of the vinylic methyls of C-18′, C-19′, and C-20′ (δ_C 16.4, 16.0, and 16.5, respectively) suggested the *E* geometries for $\Delta^{10'}$, $\Delta^{6'}$, and $\Delta^{2'}$ as the vinyl methyl corresponding to *Z* geometry are known to resonate at around 25 ppm [4]. This was further supported by NOE correlations (Figure 2) of H-9′/H-18′ and H-10′/H-12′, H-5′/H-19′ and H-6′/H-8′, and H-1′/H-20′ and H-2′/H-4′, respectively. Finally, the tetraprenyl side-chain was attached to N-7 by HMBC correlations from H-1′ to C-5 and C-8. Thus, compound **1** was determined as depicted and given the trivial name malonganenone L.

Table 1. ^1H NMR spectroscopic data for malonganenones L–Q (**1**–**6**) (δ in ppm, *J* in Hz).

No.	1 [a]	2 [a]	3 [a]	4 [b]	5 [b]	6 [b]
1				7.23, brs		7.22, brs
2	8.26, br s	8.57, br s	8.62, br s	8.23, s	8.23, s	8.23, s
8	7.69, s	7.73, s	7.73, s	7.59, s	7.59, s	7.57, s
10	3.86, s	3.94, s	3.95, s	3.13, s	3.13, s	3.13, s
11				2.84, s	2.84, s	2.84, d (4.7)
1′	5.07, d (7.3)	5.09, d (7.2)	5.08, d (7.4)	4.90, d (7.0)	4.90, d (7.0)	4.89, d (7.1)
2′	5.45, t (7.0)	5.47, t (6.9)	5.47, t (6.9)	5.40, t (7.1)	5.41, t (7.0)	5.39, t (7.0)
4′	2.08, m	2.11, m	2.11, m	2.07, m	2.08, m	2.09, m
5′	2.09, m	2.11, m	2.12, m	2.12, m	2.13, m	2.13, m
6′	5.05, m	5.08, m	5.07, m	5.14, t (6.2)	5.14, t (6.1)	5.14, t (6.3)
8′	1.98, m	1.99, m	1.97, m	2.01, m	2.01, m	2.02, m
9′	2.04, m	2.07, m	1.55, m	2.12, m	2.12, m	1.56, m
10′	5.19, t (6.4)	5.32, t (6.6)	2.06, m	5.26, t (6.8)	5.30, t (6.3)	2.55, br t, (7.9)
12′	2.99, s	3.09, s	6.00, br s	3.01, s	3.12, s	6.08, br s
14′	6.07, br s	6.06, br s	6.04, br s	6.17, br s	6.15, br s	6.10, br s
16′	1.84, s	1.87, s	1.87, d (0.9)	1.86, d (1.1)	1.87, d (1.1)	1.86, d (1.0)
17′	2.10, s	2.13, s	2.13, d (1.0)	2.09, d (1.1)	2.09, d (1.0)	2.12, d (1.0)
18′	1.58, s	1.69, s	2.15, d (1.2)	1.61, s	1.66, d (1.2)	1.87, d (1.3)
19′	1.56, s	1.58, s	1.58, s	1.59, s	1.60, s	1.62, s
20′	1.77, s	1.79, s	1.80, s	1.79, s	1.79, s	1.79, s

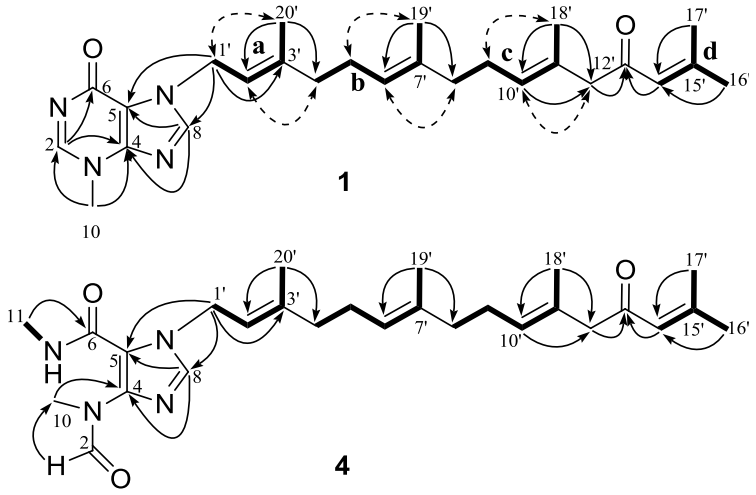
[a] Measured at 400 MHz in $CDCl_3$; [b] Measured at 400 MHz in Acetone-d_6.

Table 2. ^{13}C NMR spectroscopic data for malonganenones L–Q (**1**–**6**) (δ in ppm).

No.	1 [a]	2 [a]	3 [a]	4 [b]	5 [b]	6 [b]
2	147.7	148.1	148.1	163.3	163.3	163.3
4	147.3	147.2	147.2	141.8	141.8	141.8
5	115.0	115.2	115.2	118.2	118.2	118.2
6	162.0	160.4	160.3	160.9	160.9	161.0
8	140.3	140.7	140.8	137.6	137.6	137.6
10	35.0	35.5	35.5	31.9	31.9	31.9
11				26.1	26.1	26.3
1′	44.5	44.6	44.7	45.2	45.2	45.2
2′	117.6	117.3	117.4	120.5	120.5	120.4
3′	143.4	143.7	143.7	142.0	142.0	142.0
4′	39.4	39.5	39.4	40.1	40.2	40.2
5′	26.1	26.1	26.1	27.0	26.9	26.9
6′	123.5	123.6	123.9	124.8	124.8	124.8
7′	135.5	135.5	135.4	135.8	135.8	136.0
8′	39.3	39.5	39.1	40.1	40.3	40.6
9′	26.7	26.9	25.8	27.4	27.7	27.2
10′	129.0	128.2	40.8	129.4	128.7	33.8
11′	129.6	129.2	157.8	130.7	130.2	158.6
12′	55.3	47.9	125.7	55.8	48.3	126.9
13′	199.3	198.6	191.7	198.8	198.1	191.0
14′	122.9	123.0	126.3	123.7	124.0	127.0
15′	155.5	155.8	154.2	155.0	155.3	154.2
16′	27.7	27.7	27.7	27.5	27.5	27.6
17′	20.6	20.7	20.5	20.5	20.5	20.4
18′	16.4	24.1	19.1	16.5	24.4	25.5
19′	16.0	16.0	15.9	16.1	16.1	16.0
20′	16.5	16.6	16.6	16.5	16.5	16.5

[a] Measured at 100 MHz in $CDCl_3$; [b] Measured at 100 MHz in Acetone-d_6.

Figure 2. Key ^{1}H–^{1}H COSY (–), HMBC (→) and NOESY (dashed arrows) correlations for **1** and **4**.



Compound **2** exhibited the same molecular formula of $C_{26}H_{36}N_4O_2$ as **1** on the basis of the HRESIMS data ($[M + Na]^+$, 459.2725, calcd. 459.2736). The NMR spectroscopic data of **2** (Tables 1 and 2) was very similar to that of **1**. In comparison with **1**, the ^{13}C NMR spectroscopic data for **2** differed significantly about $\Delta^{10'}$ moiety, with the upfield-shifted carbon at C-12′ and the downfield-shifted vinyl methyl at C-18′ (δ_C 55.3 and 16.3 in **1**; δ_C 47.9 and 24.1 in **2**, respectively). This indicated that $\Delta^{10'}$ in **2** adopts a *Z* configuration. Similar ^{13}C NMR changes were also reported in malonganenone I [5], which possessed the same *Z* configuration of $\Delta^{10'}$ as **2**. Thus, compound **2** was determined as depicted and named malonganenone M.

Compound **3** had a molecular formula of $C_{26}H_{36}N_4O_2$ as established by HRESIMS data. The ^1H and ^{13}C NMR data of **3** (Tables 1 and 2) showed high similarity to those of **1** except that the $\Delta^{10'}$ double bond in **1** was migrated to $\Delta^{11'}$, forming a conjugated system with the C-13′ carbonyl. This was suggested by the significant downfield-shifted carbon at C-11′ and the upfield-shifted carbon at C-13′ as compared with those of **1** (δ_C 129.6 and 199.3 in **1**; δ_C 157.8 and 191.7 in **3**, respectively), and by the presence of a singlet olefinic signal (δ_H 6.00, H-12′) in the ^1H NMR spectra of **3** instead of a triplet olefinic signal (δ_H 5.19, t, J = 6.4 Hz, H-10′) in **1**. The configuration of $\Delta^{11'}$ in **3** was established to be *E* by the characteristic chemical shift of the vinylic methyl at C-18′ (δ_C 19.1) and by comparison of its NMR data with those of reported. Therefore, the structure of compound **3** was determined as depicted and given the trivial name malonganenone N.

Compound **4** exhibited an $[M - H]^-$ ion at *m/z* 467.3021 (calcd. for $C_{27}H_{39}N_4O_3$, 467.3022), suggesting the molecular formula $C_{27}H_{40}N_4O_3$ (ten DBE). The ^1H and ^{13}C NMR spectra of **4** (Tables 1 and 2) bore a resemblance to those of **1**, with the notable differences occurring in the hypoxanthine core. The NMR spectra of **4** showed the presence of an *N*-methylamide (δ_H 2.84, H-11; δ_C 26.1, C-11) and an *N*-methylformamide (δ_H 8.23, H-2 and 3.13, H-10; δ_C 163.3, C-2 and 31.9, C-10) groups, which were identical to those previously reported in malonganenones B, F, and G, indicating that **4** possessed the same trisubstituted imidazole ring. This was further supported by HMBC correlations (Figure 2) of H_3-11/C-6, H_3-10/C-4, and H-2/C-10. Thus, the structure of compound **4** was determined as depicted and given the trivial name malonganenone O.

Compound **5** had a molecular formula $C_{27}H_{40}N_4O_3$ by analysis of the HRESIMS data. Comparing the NMR data (Tables 1 and 2) of **5** and **4**, it appeared that the former had a *Z* configuration of $\Delta^{10'}$ instead of an *E* configuration of $\Delta^{10'}$ in **4**. This was suggested by the upfield-shifted carbon at C-12′ and the downfield-shifted vinyl methyl at C-18′ (δ_C 48.3 and 24.4 in **5**; δ_C 55.8 and 16.5 in **4**, respectively). Thus, the structure of compound **5** was determined as depicted and given the trivial name malonganenone P.

The molecular formula of compound **6** was established as $C_{27}H_{40}N_4O_3$ by HRESIMS. The NMR data of **6** (Tables 1 and 2) showed high similarity to that of **4** except that the $\Delta^{10'}$ double bond in **6** was migrated to $\Delta^{11'}$, forming a conjugated system with the C-13′ carbonyl. This was further suggested by the significant downfield-shifted carbon at C-11′ and the upfield-shifted carbon at C-13′ as compared with those of **4** (δ_C 130.7 and 198.8 in **4**; δ_C 158.6 and 191.0 in **6**, respectively), and by the presence of a singlet olefinic signal (δ_H 6.08, H-12′) in the ^1H NMR spectra of **6** instead of a triplet olefinic signal (δ_H 5.26, t, J = 6.8 Hz, H-10′) in **4**. The characteristic chemical shift of the vinylic methyl at C-18′ (δ_C 25.5) indicated the *Z* configuration of $\Delta^{11'}$ in **6**. Thus, the structure of **6** was determined as depicted and given the trivial name malonganenone Q.

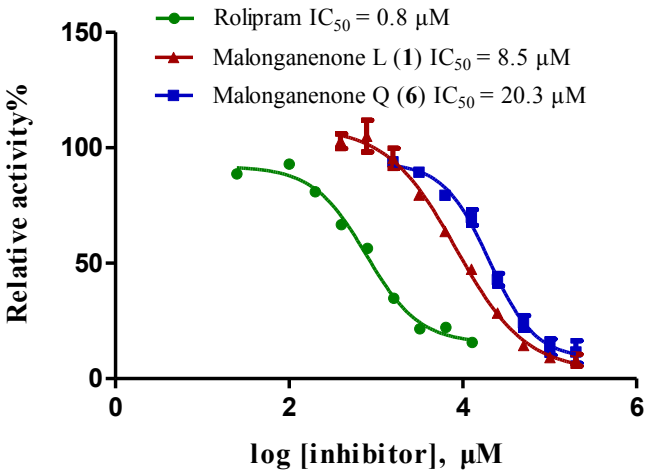
2.2. In Vitro *Inhibitory Activity Screening against PDEs*

Compounds **1**–**6** were screened for their inhibitory activities against PDE4D, PDE5A, and PDE9A by using our previously reported methods [20–23]. As shown in Table 3, all compounds exhibited inhibition at 50 μM against PDE4D with degree of inhibition from 72% to 85%, while displaying weaker activities against PDE5A and PDE9A. The two most active compounds, **1** and **6**, were selected to test for the half maximal inhibitory concentration (IC_{50}), which gave IC_{50} values of 8.5 and 20.3 μM, respectively (Figure 3).

Table 3. Inhibitory activities of compounds **1**–**6** at 5 μM and 50 μM towards PDE4D, PDE5A and PDE9A.

Compound	Inhibition (%) of Compounds at 50 μM			Inhibition (%) of Compounds at 5 μM		
	PDE4D	PDE5A	PDE9A	PDE4D	PDE5A	PDE9A
1	85	53	18	17	11	<10
2	72	32	15	<10	<10	<10
3	81	35	27	17	<10	10
4	79	38	<10	18	<10	<10
5	75	36	11	14	<10	<10
6	85	38	15	18	<10	<10

Figure 3. Inhibition of phosphodiesterase-4D by compounds **1** and **6** (rolipram as positive control).



3. Experimental Section

3.1. General Experimental Procedures

UV spectra were recorded on a Shimadzu UV-2450 spectrophotometer. IR spectra were determined on a Bruker Tensor 37 infrared spectrophotometer with KBr disks. NMR spectra were measured on a Bruker AM-400 spectrometer at 25 °C. ESIMS and HRESIMS were carried out on a Finnigan LC Q^{DECA} instrument. A Shimadzu LC-20 AT equipped with an SPD-M20A PDA detector was used for HPLC, a YMC-pack ODS-A column (250 × 10 mm, 5 μm, 12 nm) and a chiral column (Phenomenex Lux, cellulose-2, 250 × 10 mm, 5 μm) was used for semipreparative HPLC separation.

Silica gel (300–400 mesh, Qingdao Marine Chemical Co., Ltd., Qingdao, Shandong, China), C_{18} reversed-phase (Rp-C_{18}) silica gel (12 nm, 50 μm, YMC Co., Ltd., Kyoto, Japan), Sephadex LH-20 gel (Amersham Biosciences, Piscataway, NJ, USA), used for column chromatography (CC). All solvents used were of analytical grade (Guangzhou Chemical Reagents Company, Ltd., Guangzhou, Guangdong, China).

3.2. Animal Material

The gorgonian *E. pseudosassapo* was collected at a depth of 18–25 m in 29 July 2012 in Daya Bay of Guangdong Province, China and frozen immediately after collection, and were identified by one of the authors (Cheng-Qi Fan). A voucher specimen (accession number: LSH201207) has been deposited at the School of Pharmaceutical Sciences, Sun Yat-sen University, China.

3.3. Extraction and Isolation

Specimens of *E. pseudosassapo* (550 g, wet weight) were extracted with CH_2Cl_2/MeOH (1:1, 3 × 1 L) at room temperature (rt) to give 13.7 g of crude extract. The crude extract was subjected to silica gel column chromatography eluted with a CH_2Cl_2/MeOH gradient (9:1→1:9) to afford five fractions (Fr. I–V). Fr. III (1.4 g) was chromatographed over Sephadex LH-20 (CH_2Cl_2/MeOH, v/v, 1:1), followed by Rp-C_{18} silica gel eluted with a CH_3CN/H_2O gradient (5:5→10:0) to obtain four sub-fractions (Fr. IIIa–IIId). Fr. IIIb was further separated by HPLC equipped with a chiral column (CH_3CN, 3 mL/min) to afford **1** (17 mg, t_R 17 min) and **2** (4.9 mg, t_R 20 min). Fr. IIId was purified by repeating the HPLC conditions described above to yield **4** (11 mg, t_R 23 min) and **5** (3.7 mg, t_R 27 min). Fr. IIIc was chromatographed by HPLC equipped with an ODS-A column (CH_3CN/H_2O, 90:10, 3 mL/min) to afford **3** (5.2 mg, t_R 15 min) and **6** (5.1 mg, t_R 20min).

Malonganenone L (**1**): colorless oil; UV (MeOH) λ_{max} (log ε) 211 (4.42), 225 (4.38), 253 (4.31) nm; IR ν_{max} 3145, 1709, 1610, 1464, 1250, 1128, 1060 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS $[M + Na]^+$ *m*/*z* 459.2721 (calcd. for $C_{26}H_{36}N_4O_2Na$, 459.2736).

Malonganenone M (**2**): colorless oil; UV (MeOH) λ_{max} (log ε) 209 (4.22), 225 (4.17), 254 (4.06) nm; IR ν_{max} 3011, 1714, 1607, 1458, 1240, 1123, 1046 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS $[M + Na]^+$ *m*/*z* 459.2725 (calcd. for $C_{26}H_{36}N_4O_2Na$, 459.2736).

Malonganenone N (**3**): colorless oil; UV (MeOH) λ_{max} (log ε) 208 (3.68), 223 (3.60), 254 (3.16) nm; IR ν_{max} 2937, 1732, 1627, 1439, 1379, 1215, 1136, 1039 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS $[M + Na]^+$ *m*/*z* 459.2725 (calcd. for $C_{26}H_{36}N_4O_2Na$, 459.2736).

Malonganenone O (**4**): colorless oil; UV (MeOH) λ_{max} (log ε) 211 (4.39), 249 (4.22) nm; IR ν_{max} 2932, 1744, 1654, 1514, 1445, 1218, 1127, 1033 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS $[M - H]^-$ *m*/*z* 467.3021 (calcd. for $C_{27}H_{39}N_4O_3$, 467.3022).

Malonganenone P (**5**): colorless oil; UV (MeOH) λ_{max} (log ε) 210 (4.27), 254 (4.02) nm; IR ν_{max} 2933, 1674, 1621, 1437, 1220, 1128, 1018 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS $[M + Na]^+$ *m*/*z* 491.2994 (calcd. for $C_{27}H_{40}N_4O_3Na$, 491.2998).

Malonganenone Q (**6**): colorless oil; UV (MeOH) λ_{max} (log ε) 212 (4.52), 257 (4.46) nm; IR ν_{max} 2932, 1664, 1623, 1532, 1443, 1224, 1057 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRESIMS $[M + Na]^+$ *m/z* 491.2994 (calcd. for $C_{27}H_{40}N_4O_3Na$, 491.2998).

4. Conclusions

In our continuing investigation on the chemical constituents of marine invertebrates collected from the South China Sea, six new tetraprenylated alkaloids, designated as malonganenones L–Q (**1**–**6**), were isolated from the gorgonian *Echinogorgia pseudossapo*. The structures of **1**–**6** featuring a methyl group at N-3 and a tetraprenyl chain at N-7 in the hypoxanthine core were established by extensive spectroscopic analyses. Compounds **1**–**6** were tested for their inhibitory activity against the phosphodiesterases (PDEs)-4D, 5A, and 9A, and compounds **1** and **6** exhibited moderate inhibitory activity against PDE4D with IC_{50} values of 8.5 and 20.3 μM, respectively. Phosphodiesterase-4 (PDE4), which specifically catalyzes the hydrolysis of cyclic adenosine monophosphate (cAMP), is a therapeutic target of high interest for central nervous system (CNS), inflammatory, and respiratory diseases [24]. Natural PDE4 inhibitors are very rare. To the best of our knowledge, this is the first investigation of this group of compounds on the inhibitory activity of the phosphodiesterases.

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Conflicts of Interest

The authors declare no conflict of interest.

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