

Article

Lobophytone O–T, New Biscembranoids and Cembranoid from Soft Coral *Lobophytum pauciflorum*

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Abstract: Chemical examination of a Chinese soft coral *Lobophytum pauciflorum* resulted in the isolation of five new biscembranoids named lobophytone O–S (1–5) and a new “monomeric” cembrane lobophytone T (6). The structures of the new compounds were elucidated by interpretation of 1D and 2D NMR (COSY, HSQC, HMBC, and NOESY) spectroscopic data in association with MS and IR data. Lobophytone Q showed significant inhibition against lipopolysaccharide (LPS)-induced nitric oxide (NO) release in mouse peritoneal macrophages, while lobophytone Q and T showed inhibitory activities against the bacteria *S. aureus* and *S. pneumoniae*.

Keywords: soft coral; *Lobophytum pauciflorum*; lobophytone O–T; structure elucidation; NO inhibition; antibiotic activity

1. Introduction

Biscembranes are a class of marine natural products with unusual structure patterns, which are mainly found in the marine soft coral genus of *Sarcophyton* (*S. tortuosum*, *S. glaucum*, *S. latum*, and

Table 1. ^{13}C NMR data of lobophytone O–S (1–5) ($\delta_{39.5}$, DMSO- d_6).

Position	1	2	3	4	5
1	49.4 (Cq)	49.4 (Cq)	49.3 (Cq)	48.5 (Cq)	49.9 (Cq)
2	43.9 (CH)	43.8 (CH)	43.9 (CH)	45.9 (CH)	44.0 (CH)
3	213.8 (Cq)	213.8 (Cq)	213.8 (Cq)	212.1 (Cq)	213.9 (Cq)
4	54.1 (CH ₂)	54.0 (CH ₂)	54.1 (CH ₂)	50.4 (CH ₂)	53.9 (CH ₂)
5	27.3 (CH)	27.3 (CH)	27.3 (CH)	26.9 (CH)	27.3 (CH)
6	38.0 (CH ₂)	37.9 (CH ₂)	38.0 (CH ₂)	37.0 (CH ₂)	38.0 (CH ₂)
7	26.0 (CH ₂)	26.0 (CH ₂)	26.0 (CH ₂)	24.4 (CH ₂)	26.0 (CH ₂)
8	34.1 (CH ₂)	34.0 (CH ₂)	34.1 (CH ₂)	32.7 (CH ₂)	34.1 (CH ₂)
9	47.6 (CH)	47.5 (CH)	47.7 (CH)	46.4 (CH)	47.8 (CH)
10	213.3 (Cq)	213.3 (Cq)	213.3 (Cq)	213.6 (Cq)	213.1 (Cq)
11	30.9 (CH ₂)	31.0 (CH ₂)	30.9 (CH ₂)	34.9 (CH ₂)	30.9 (CH ₂)
12	51.8 (CH)	51.8 (CH)	51.7 (CH)	51.7 (CH)	51.3 (CH)
13	210.0 (Cq)	209.9 (Cq)	209.9 (Cq)	211.5 (Cq)	210.3 (Cq)
14	45.9 (CH ₂)	45.8 (CH ₂)	45.9 (CH ₂)	46.9 (CH ₂)	46.2 (CH ₂)
15	28.7 (CH)	28.7 (CH)	28.7 (CH)	28.8 (CH)	28.9 (CH)
16	21.5 (CH ₃)	21.5 (CH ₃)	21.5 (CH ₃)	21.2 (CH ₃)	21.5 (CH ₃)
17	17.6 (CH ₃)	17.6 (CH ₃)	17.6 (CH ₃)	18.3 (CH ₃)	17.7 (CH ₃)
18	17.6 (CH ₃)	17.6 (CH ₃)	17.6 (CH ₃)	17.1 (CH ₃)	17.7 (CH ₃)
19	22.9 (CH ₃)	22.9 (CH ₃)	22.9 (CH ₃)	22.1 (CH ₃)	22.8 (CH ₃)
20	174.8 (Cq)	174.7 (Cq)	174.7 (Cq)	173.8 (Cq)	174.6 (Cq)
21	43.7 (CH)	43.5 (CH)	43.7 (CH)	50.2 (CH)	43.2 (CH)
22	128.6 (CH)	129.4 (CH)	130.0 (CH)	124.6 (CH)	124.6 (CH)
23	137.8 (Cq)	136.9 (Cq)	136.2 (Cq)	135.9 (Cq)	133.7 (Cq)
24	33.5 (CH ₂)	32.9 (CH ₂)	34.0 (CH ₂)	36.7 (CH ₂)	37.3 (CH ₂)
25	33.1 (CH ₂)	31.1 (CH ₂)	34.8 (CH ₂)	24.4 (CH ₂)	23.8 (CH ₂)
26	70.4 (CH)	73.5 (CH)	65.1 (CH)	77.8 (CH)	125.1 (CH)
27	85.1 (Cq)	83.2 (Cq)	84.8 (Cq)	74.7 (Cq)	132.8 (Cq)
28	35.9 (CH ₂)	36.1 (CH ₂)	36.8 (CH ₂)	39.7 (CH ₂)	35.6 (CH ₂)
29	28.1 (CH ₂)	28.0 (CH ₂)	28.0 (CH ₂)	27.6 (CH ₂)	25.1 (CH ₂)
30	89.2 (CH)	89.6 (CH)	90.1 (CH)	80.0 (CH)	59.8 (CH)
31	73.6 (Cq)	73.3 (Cq)	73.2 (Cq)	80.5 (Cq)	59.7 (Cq)
32	40.8 (CH ₂)	41.5 (CH ₂)	40.7 (CH ₂)	41.5 (CH ₂)	39.6 (CH ₂)
33	64.0 (CH)	64.1 (CH)	63.9 (CH)	24.4 (CH ₂)	62.6 (CH)
34	135.6 (Cq)	135.2 (Cq)	135.2 (Cq)	132.8 (Cq)	133.1 (Cq)
35	123.2 (Cq)	123.2 (Cq)	123.6 (Cq)	123.3 (Cq)	127.6 (Cq)
36	32.5 (CH ₂)	32.6 (CH ₂)	32.5 (CH ₂)	32.1 (CH ₂)	32.6 (CH ₂)
37	18.4 (CH ₃)	18.4 (CH ₃)	18.3 (CH ₃)	20.1 (CH ₃)	18.7 (CH ₃)
38	20.6 (CH ₃)	20.3 (CH ₃)	20.2 (CH ₃)	17.9 (CH ₃)	17.6 (CH ₃)
39	20.3 (CH ₃)	20.8 (CH ₃)	20.3 (CH ₃)	25.4 (CH ₃)	17.3 (CH ₃)
40	23.5 (CH ₃)	23.4 (CH ₃)	23.5 (CH ₃)	15.5 (CH ₃)	20.3 (CH ₃)
OMe	51.2 (CH ₃)	51.1 (CH ₃)	51.2 (CH ₃)	51.0 (CH ₃)	51.2 (CH ₃)
Ac		171.3 (Cq)			
		21.4 (CH ₃)			

Table 2. ^1H NMR data of lobophytone O–S (1–5) (δ 2.50, DMSO- d_6).

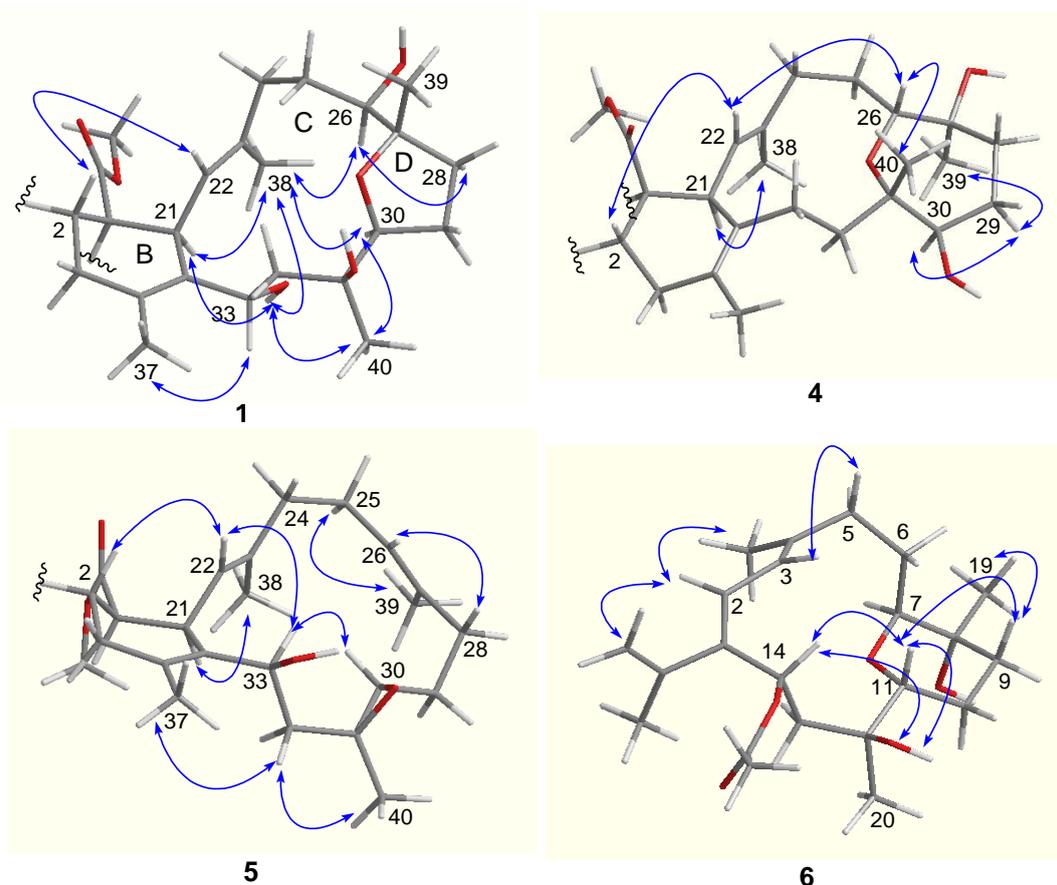
Position	1	2	3	4	5
2	3.47 t (8.3)	3.45 m	3.47 t (8.8)	3.49 m	3.59 t (8.1)
4	3.09 dd (10.3, 19.6)	3.09 dd (10.3, 19.5)	3.09 dd (10.3, 20.0)	2.61 dd (6.9, 17.8)	3.06 dd (10.3, 19.6)
	2.40 d (19.6)	2.40 d (19.5)	2.40 d (20.0)	2.32 m	2.44 d (19.6)
5	1.68 m	1.69 m	1.67 m	1.85 m	1.63 m
6	1.01 m	1.01 m	0.97 m	1.03 m	0.96 m
	1.07 m	1.06 m		0.87 m	1.00 m
7	1.10 m	1.12 m	1.08 m	0.95 m	0.94 m
	1.01 m	1.00 m	1.00 m		1.07 m
8	1.43 m	1.49 m	1.43 m	1.40 m	1.38 m
	1.36 m	1.35 m	1.35 m		1.40 m
9	2.33 m	2.33 m	2.32 m	2.38 m	2.30 m
11	2.80 dd (10.3, 16.6)	2.80 m	2.80 dd (10.5, 16.9)	2.73 dd (2.7, 16.9)	1.88 d (20.0)
	1.84 d (16.6)	1.84 m	1.83 d (16.9)	2.15 dd (2.4, 16.9)	2.82 dd (10.5, 20.0)
12	2.88 m	2.87 m	2.89 m	2.89 m	2.89 brd (10.5)
14	2.86 d (19.3)	2.88 d (19.3)	2.86 d (19.6)	2.96 m	2.94 d (19.6)
	2.72 d (19.3)	2.74 d (19.3)	2.72 d (19.6)	2.90 m	2.69 d (19.6)
15	2.33 m	2.34 m	2.32 m	2.07 m	2.22 m
16	0.95 d (6.7)	0.96 d (6.6)	0.95 d (6.9)	0.91 d (6.9)	0.92 d (6.6)
17	0.63 d (6.7)	0.63 d (6.6)	0.63 d (6.9)	0.71 d (6.9)	0.61 d (6.6)
18	1.05 d (6.9)	1.05 d (6.6)	1.05 d (7.0)	1.07 d (7.1)	1.04 d (6.9)
19	0.81 d (6.9)	0.81 d (6.6)	0.81 d (6.9)	0.81 d (6.9)	0.81 d (6.6)
21	3.38 d (10.5)	3.47 d (10.3)	3.38 d (10.3)	3.23 m	3.20 d (11.0)
22	4.78 d (10.5)	4.85 d (10.3)	4.87 d (10.3)	4.94 d (11.5)	4.70 d (11.0)
24	2.02 m	2.13 m	2.18 m	2.38 m	2.07 m
	1.91 m	1.51 m	1.96 m	1.87 m	1.92 m
25	1.65 m	1.80 m	2.08 m	1.82 m	2.22 m
	1.36 m	1.60 m	1.66 m	1.63 m	2.00 m
26	3.01 brd (10.5)	4.56 d (10.8)	3.55 d (11.7)	3.39 m	4.72 br

Table 2. Cont.

28	2.25 m	1.71 m	2.32 m	1.58 m	2.10 m
	1.38 m	1.45 m	1.62 m	1.42 m	1.91 m
29	1.67 m	1.71 m	1.75 m	1.85 m	1.76 m
	1.36 m	1.43 m	1.40 m	1.31 m	1.47 m
30	3.77 dd (5.3, 10.5)	3.80 dd (5.1, 10.5)	3.86 dd (5.4, 11.5)	2.91 m	2.30 m
32	1.65 m	1.71 m	1.62 m	2.07 m	1.96 dd (10.0, 15.4)
	1.09 m	1.13 m	1.09 m	1.04 m	1.70 dd (2.0, 15.4)
33	4.80 m	4.82 m	4.82 brt	2.10 m	4.26 dd (2.0, 10.0)
				1.83 m	
36	2.18 dd (8.3, 19.3)	2.21 dd (8.8, 19.3)	2.21 m	2.47 m	2.12 d (8.1)
	2.06 dd (8.8, 19.3)	2.03 m	2.05 m	1.98 dd (5.4, 18.3)	2.12 d (8.1)
37	1.66 s	1.67 s	1.67 s	1.54 s	1.63 s
38	1.65 s	1.64 s	1.67 s	1.69 s	1.53 s
39	0.99 s	1.09 s	1.19 s	0.98 s	1.52 s
40	0.97 s	0.99 s	0.99 s	0.92 s	1.06 s
OMe	3.42 s	3.39 s	3.42 s	3.39 s	3.40 s
Ac		2.06 s			
OH-26	4.53 br				
OH-27				4.18 s	
OH-30				4.49 br	
OH-31	3.98 s	4.14 s	4.21 s		
OH-33	4.40 br	4.45 br	4.46 br		4.63 br

The relative configurations of the stereogenic centers in **1** were determined on the basis of NOE relationships (Figure 2) and J values. The NOE relationship between H-2 (δ_{H} 3.47) and OMe suggested *cis*-conjunction of rings A and B. The exclusive β -face of the methyl ester group as found in all known biscebranones led to the biogenetical assignment of the methyl ester at C-1 of **1** to be β -oriented. The obvious NOE correlation between H-22 (δ_{H} 4.78)/H-2 suggested the spatial vicinity of the two protons, thus H-21 was α -oriented. The NOE correlations between H-21 (δ_{H} 3.38)/H₃-38 (δ_{H} 1.65), H₃-38/H-26 (δ_{H} 3.01), and H₃-38/H-30 (δ_{H} 3.77) indicated α -orientations of H-26 and H-30, as well as *22E* configuration. The β -orientation of H₃-39 (δ_{H} 0.99) was evident from the NOE relationship between H-26 and H-28a (δ_{H} 2.25), which further correlated to H-30, while the assignment of H-33 β was based on the NOE interactions from OH-33 (δ_{H} 4.40) to H-21, H₃-38, and H₃-40 (Figure 2). In addition, the NOE relationship between H-30/H₃-40 (δ_{H} 0.97) suggested H₃-40 to be α -oriented.

Figure 2. Key NOE correlations of **1**, **4–6** related to rings B–D.



Lobophytone P (**2**) has a molecular formula of $\text{C}_{43}\text{H}_{66}\text{O}_{10}$ as determined by HR-ESIMS (m/z 765.4570 $[\text{M} + \text{Na}]^+$), indicating 42 amu more than that of **1**. Comparison of NMR data revealed that the structure of **2** was closely related to that of **1**, except for the presence of an acetyl group as evident from the ^{13}C NMR signals at δ_{C} 171.3 (Cq) and 21.4 (CH_3) and the corresponding ^1H NMR signal at δ_{H} 2.06 (3H, s). The HMBC correlation from H-26 (δ_{H} 4.56, d, $J = 10.8$ Hz) to the acetyl carbonyl carbon ascertained the location of the acetoxy group at C-26. The similar NOE and NMR

data of **2** and **1** indicated that **2** shared the same configurations of the stereogenic centers of **1**. Thus, compound **2** was determined to be a C-26 acetylated analogue of **1**.

Lobophytone Q (**3**) has a molecular formula of $C_{41}H_{63}O_8Cl$ as determined by HR-ESIMS (m/z 741.4096 $[M + Na]^+$, calcd 741.4104), indicating 10 degrees of unsaturation and the presence of a chlorine atom. The 1D and 2D NMR spectroscopic analysis revealed that the structure of **3** was also closely related to that of **1**. The presence of two D_2O exchangeable protons at δ_H 4.18 (s) and 4.46 (br) was attributed to two hydroxy groups, which were deduced to be attached at C-31 and C-33, based on the HMBC interactions from OH-31 (δ_H 4.18, s) to C-30 (δ_C 90.1), C-31 (δ_C 73.2) and C-32 (δ_C 40.7), and the COSY correlation between OH-33 (δ_H 4.46, bs) and H-33 (δ_H 4.82). The chlorine atom of **3** was assumed to be substituted at C-26 according to the upfield-shifted C-26 (δ_C 65.1), which showed around $\Delta\delta$ 5 less than the same carbon of **1** linked by hydroxy group. The relative configurations of the chiral centers in **3** were in agreement with those of **1** based on the similar NMR and NOE data.

The molecular formula of lobophytone R (**4**) was determined as $C_{41}H_{64}O_8$ on the basis of HR-ESIMS (m/z 685.4670 $[M + H]^+$), implying 10 degrees of unsaturation. Comparison of NMR data indicated that **4** is a biscembranoid possessing the same partial structure, with respect to rings A and B, of **1**. However, the obvious downfield shifted C-26 (δ_C 77.8) and C-31 (δ_C 80.5), and the upfield shifted C-27 (δ_C 74.7) and C-30 (δ_C 80.0), in comparison with the corresponding carbons of **1**, suggested that **4** contains an ether bridge at C-26/C-31 in ring C rather than C-27/C-30 ether bond of **1**. The HMBC correlation from H-26 (δ_H 3.39) to C-31 confirmed this assignment. The COSY correlation between OH (δ_H 4.49, br)/H-30 (δ_H 2.91) and the HMBC relationships from OH (δ_H 4.18) to C-26 and C-27 disclosed two hydroxy groups at C-27 and C-30, respectively. The NOE relationships observed between H-2 (δ_H 3.49, m)/H-22 (δ_H 4.94, d, $J = 11.5$ Hz), H-22/H-26, and H-26/H₃-40 (δ_H 0.92) indicated H-26 and H₃-40 to be β -oriented, while the NOE correlations between OH-27 (δ_H 4.18)/H-26, H-30 (δ_H 2.91, m)/H-29a (δ_H 1.31, m), and H₃-39 (δ_H 0.98)/H-29a assigned to H₃-39 α and H-30 α .

Lobophytone S (**5**) has a molecular formula of $C_{41}H_{62}O_7$, as determined by HR-ESIMS (m/z 689.4384 $[M + Na]^+$). The NMR spectroscopic data of **5** (Tables 1 and 2) indicated that it is a biscembrane, structurally related to ximaolide A [3], a biscembrane isolated from soft coral *Sarcophyton tortuosum*. Analysis of the NMR spectroscopic data revealed an additional vinyl group (δ_C 125.1, 132.8) in ring C of **5**, in addition to the double bond located at C-22/C-23. This double bond was deduced to be located at C-26/C-27 based on the COSY and HMBC correlations. The carbon resonances of C-30 (δ_C 59.8, CH) and C-31 (δ_C 59.7, Cq) were attributed to epoxy carbons, while a hydroxy group linked to C-33 was confirmed by the HMBC correlations from H-33 (δ_H 4.26, dd, $J = 2.0, 10.0$ Hz) to C-21 and C-35 and the COSY relationship between OH (δ_H 4.63, br)/H-33. The NOE correlations between H₃-39 (δ_H 1.52)/H₂-25 (δ_H 2.00, 2.22) and H-22 (δ_H 4.70)/H₂-24 (δ_H 1.92, 2.07) were in accordance with 22*E* and 26*E* geometries (Figure 2). The NOE relationships of H-33/H-30 (δ_H 2.30, m), H-33/H-22, and H-22/H-2 led to the assignment of β -faces of H-30 and H-33 and the *trans*-configuration of the epoxide group.

Lobophytone T (**6**) has a molecular formula of $C_{22}H_{34}O_5$ as determined by HR-ESIMS (m/z 401.2292 $[M + Na]^+$), implying six degrees of unsaturation. The 1H MMR displayed five methyl singlets including two olefinic methyls at δ_H 1.65 (3H, s) and 1.80 (3H, s) and an acetyl methyl group at δ_H 1.87 (3H, s), in addition to four olefinic protons for an AB spin system at δ_H 6.02 (d, $J = 11.3$ Hz) and 6.03 (d, $J = 11.3$ Hz) and for the *exo*-methylene at δ_H 4.86 (brs) and 4.82 (brs). The COSY

correlations established the segments from C-2 to C-3, C-5 to C-7, C-9 to C-11, and C-13 to C-14, while ^{13}C NMR and HMQC data indicated the methines of C-7 (δ_{C} 85.0), C-11 (δ_{C} 70.5) and C-14 (δ_{C} 69.7) bearing oxygen atom. The HMBC relationships connected each segment to form a backbone that featured a cembrane with 14-membered ring. An isopropene was deduced to position at C-1 based on the HMBC cross-peaks from H₂-16 (δ_{H} 4.86, 4.82) and H₃-17 (δ_{H} 1.80) to C-1 (δ_{C} 143.5), while additional HMBC correlations indicated two vinyl groups to be conjugated at C-1/C-2 and C-3/C-4. The HMBC correlations from H₃-19 (δ_{H} 1.01) to C-7, C-8 (δ_{C} 69.2, Cq), and C-9 (δ_{C} 32.2), and from H₃-20 (δ_{H} 1.05) to C-11, C-12 (δ_{C} 73.7, Cq), and C-13 (δ_{C} 44.3), in addition to the HMBC relationship from H-7 (δ_{H} 3.26) to C-11 established an ether bridge which linked C-7 and C-11. The acetoxy group was deduced to position at C-14 on the basis of the HMBC correlations from H-14 (δ_{H} 6.01) to the acetyl carbonyl carbon and to C-1, C-2, and also C-15 (δ_{C} 144.2, Cq). Thus, C-8 and C-12 were hydroxylated, and the gross structure of **6** was established as shown in Figure 1. The relative configurations of the chiral centers in **6** were determined through *J* values and NOESY analysis. The coupling constants of H-11 (δ_{H} 3.71, dd, *J* = 2.7, 11.7 Hz) suggested its axial orientation in the chair-conformation of the perhydropyrano ring, while the NOE relationship between H-11/H-14 indicated the same face (β -orientation) of both protons. The NOE interactions of OH-12 (δ_{H} 4.03, s) with H-11 and H-14 assigned to OH-12 β , whereas the NOESY cross-peaks between H-11/H-9a (δ_{H} 1.50, m), H₃-19/H-9a, and H-7/OH-8 (δ_{H} 3.95, s) suggested α -orientation for H-7 and OH-8.

The “monomeric” cembrane lobophytone T (**6**) is likely a precursor (diene) to form the right part of biscembranes when it reacted with methyl tortuosoate [6]. Thus, it provided new evidence to support the plausible biogenetic pathway to generate biscembranes. It is noted that all of the isolated biscembranoids (**1–5**) maintained the same partial structure of ring A, which was considered to be derived from methyl tortuosoate as a cembranoid-dienophile. The difference was found in ring C where epoxide rearrangement, oxidation, acetylation, and halogen substitution occurred.

In primary bioassays, compound **3** showed significant inhibition against lipopolysaccharide (LPS)-induced nitric oxide (NO) production in mouse peritoneal macrophages with $\text{IC}_{50} = 2.8 \mu\text{M}$, whereas the other compounds showed weak activity ($\text{IC}_{50} > 10 \mu\text{M}$). In addition, all compounds were weakly cytotoxic toward the mouse peritoneal macrophages ($\text{IC}_{50} > 10 \mu\text{M}$). The antibiotic assay indicated that compounds **3** and **6** exhibited strong inhibition against *Staphylococcus aureus*, *S. pneumoniae*, and *Saccharomyces cerevisiae* with the inhibitory rates around 90% at 20 $\mu\text{g/mL}$, but all compounds possessed weakly inhibitory activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, and *Aspergillus fumigatus*.

3. Experimental Section

3.1. General Procedures

Optical rotations were measured on a Perkin-Elmer 243B polarimeter. IR spectra were determined on a Thermo Nicolet Nexus 470 FTIR spectrometer. ^1H and ^{13}C NMR and 2D NMR spectra were recorded on an Avance-500 FT 500 MHz NMR spectrometer using TMS as an internal standard. δ values are expressed in parts per million (ppm), and *J* values are reported in Hertz (Hz). HR-ESIMS data were obtained from Bruker APEX IV instrument. Low pressure column chromatography was

carried out with silica gel (160–200 and 200–300 mesh), and GF₂₅₄ silica gel for TLC was provided by Qingdao Marine Chemistry Co. Ltd. HPLC chromatography was performed on an Alltech instrument (426-HPLC pump, Alltech UV–vis-200 detector) equipped with Kromasil semipreparative column (10 µm, ODS, 10 mm × 250 mm) and YMC-Pack C₈ (5 µm, 10 mm × 250 mm) column.

3.2. Animal Material

Soft coral *Lobophytum pauciflorum* was collected from the inner coral reef at a depth of 10 m in Sanya Bay, Hainan Island of China, in 2008. Fresh samples were frozen immediately. The specimen was identified by Leen van Ofwegen (National Museum of Natural History Naturalis). The coral specimen (HSF-6) was deposited at State Key Laboratory of Natural and Biomimetic Drugs, Peking University, China.

3.3. Extraction and Isolation

The frozen soft coral *L. pauciflorum* (2.3 kg) was homogenized and then extracted with EtOH (twice, 5 L/each time). The concentrated extract was desalted by dissolving in MeOH to yield a MeOH soluble fraction which was concentrated to an oily residue (92.7 g). This residue was partitioned between H₂O and EtOAc, and then the water phase was extracted with *n*-BuOH. The EtOAc fraction (12.1 g) was subjected to silica gel (200–300 mesh) column chromatography, eluting with a gradient (petroleum ether–acetone, 20:1, 10:1, 3:1, 1:1) to obtain seven fractions (F1–F7). Fractions F1–F4 mainly contained lipids and steroids, as detected by ¹H NMR, while F5 and F6 showed the ¹H NMR spectral features of terpenoids. Thus, F5 and F6 were combined (1.69 g) and subsequently subjected to Sephadex LH-20 column eluting with CH₂Cl₂–MeOH (1:2) to afford 6 portions (P1–P6). P2 (180 mg) showing blue-green spots after spraying with anisaldehyde reagent was further separated on reversed-phase semi-preparative HPLC with CH₃CN–H₂O (61%) as a mobile phase to obtain **1** (12.5 mg). P3 (49.2 mg) was separated on ODS HPLC with MeOH–H₂O (82%) as a mobile phase to afford **4** (7.6 mg), while **2** (8.1 mg) was obtained from P4 (40.5 mg) by the same separation process as that for P3. Followed by the same process as for P3 and P4, **5** (7.1 mg), **3** (17.7 mg), **3** (14.5 mg), and **6** (5.4 mg) were separated and purified from P5 (112.7 mg) by reversed-phase semi-preparative HPLC (ODS) with CH₃CN–H₂O (87%) as a mobile phase.

3.3.1. Lobophytone O (**1**)

Amorphous powder; $[\alpha]_D^{25} + 140.4$ (*c* 1.25, CHCl₃); IR (KBr) ν_{\max} 3413, 2960, 2931, 1740, 1705, 1459, 1436, 1386, 1370, 1206, 1086, 1049, 1026 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; HR-ESIMS *m/z* 723.4428 [M + Na]⁺ (calcd for C₄₁H₆₄NO₉Na, 723.4442).

3.3.2. Lobophytone P (**2**)

Amorphous powder; $[\alpha]_D^{25} + 133.7$ (*c* 0.33, CHCl₃); IR (KBr) ν_{\max} 3467, 2959, 2928, 1741, 1708, 1459, 1373, 1237, 1028 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; HR-ESIMS *m/z* 765.4570 [M + Na]⁺ (calcd for C₄₃H₆₆O₁₀Na, 765.4548).

3.3.3. Lobophytone Q (3)

Amorphous powder; $[\alpha]_D^{25} + 121.2$ (*c* 1.30, CHCl₃); IR (KBr) ν_{\max} 3440, 2958, 2930, 1741, 1706, 1459, 1374, 1206, 1027 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; HR-ESIMS *m/z* 741.4096 [M + Na]⁺ (calcd for C₄₁H₆₃O₈NaCl, 741.4104).

3.3.4. Lobophytone R (4)

Amorphous powder; $[\alpha]_D^{25} + 151.1$ (*c* 0.51, CHCl₃); IR (KBr) ν_{\max} 3423, 2958, 2928, 1709, 1460, 1376, 1203, 1050 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; HR-ESIMS *m/z* 685.4670 [M + H]⁺ (calcd for C₄₁H₆₅O₈, 685.4674).

3.3.5. Lobophytone S (5)

Amorphous powder; $[\alpha]_D^{25} + 101.1$ (*c* 0.60, CHCl₃); IR (KBr) ν_{\max} 3399, 2958, 2927, 1742, 1706, 1459, 1435, 1387, 1204, 1074 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; HR-ESIMS *m/z* 689.4384 [M + Na]⁺ (calcd for C₄₁H₆₂O₇Na, 689.4388).

3.3.6. Lobophytone T (6)

Colorless oil; $[\alpha]_D^{25} + 36.5$ (*c* 0.27, CHCl₃); IR (KBr) ν_{\max} 3423, 2969, 2935, 1734, 1453, 1375, 1246, 1071, 1026 cm⁻¹; ¹H NMR δ (ppm, DMSO-*d*₆): 6.02 (1H, d, *J* = 11.3 Hz, H-2), 6.03 (1H, d, *J* = 11.3 Hz, H-3), 2.47 (1H, m, H-5a), 1.96 (1H, m, H-5b), 1.44 (1H, m, H-6a), 2.00 (1H, m, H-6b), 3.26 (1H, brd, *J* = 8.1 Hz, H-7), 1.42 (1H, m, H-9a), 1.50 (1H, m, H-9b), 1.43 (1H, m, H-10a), 1.77 (1H, m, H-10b), 3.71 (1H, dd, *J* = 2.7, 11.7 Hz, H-11), 1.85 (1H, m, H-13a), 1.97 (1H, m, H-13b), 6.01 (1H, brd, *J* = 10.0 Hz, H-14), 4.82 (1H, br, H-16a), 4.86 (1H, br, H-16b), 1.80 (3H, s, H-17), 1.65 (3H, s, H-18), 1.01 (3H, s, H-19), 1.05 (3H, s, H-20), 1.87 (3H, s, Ac); ¹³C NMR δ (ppm, DMSO-*d*₆): 143.5 (Cq, C-1), 120.5 (CH, C-2), 119.6 (CH, C-3), 137.8 (Cq, C-4), 39.4 (CH₂, C-5), 21.6 (CH₂, C-6), 85.0 (CH, C-7), 69.2 (Cq, C-8), 32.2 (CH₂, C-9), 19.9 (CH₂, C-10), 70.5 (CH, C-11), 73.7 (Cq, C-12), 44.3 (CH₂, C-13), 69.7 (CH, C-14), 144.2 (Cq, C-15), 114 (CH₂, C-16), 23.0 (CH₃, C-17), 18.1 (CH₃, C-18), 27.8 (CH₃, C-19), 25.0 (CH₃, C-20), 169.9 (Cq, Ac), 21.5 (CH₃, Ac); HR-ESIMS *m/z* 401.2292 [M + Na]⁺ (calcd for C₂₂H₃₄O₅Na, 401.2298).

3.4. Assay for Inhibition of Nitric Oxide (NO) Production

Dexamethasone (DEX, positive control, 20 mM in DMSO) and each compound (20 mM in DMSO) were diluted to 1–20 μ M range at r.t. before experiment. The final percentage of DMSO in the reaction mixture was less than 0.5% (v/v). LPS (1 μ g/mL), 4% sodium thioglycolate, RPMI1640, FBS, PBS, MTT and Griess reagents were purchased from Sigma (St. Louis, MO, U.S.). Mouse peritoneal macrophages (PEM Φ) were obtained from C57BL6J male mice, and then plated onto 48 well plates and cultured for 2 hrs in DMEM containing 5% FBS at 5% CO₂ in 37 °C. Mouse PEM Φ were incubated with compounds for 1 h at 37 °C before stimulation with 1 μ g/mL of lipopolysaccharide (LPS) for 24 h. Cells (5 \times 10⁵ cells) were preincubated at 37 °C for 24 h in serum-free medium and NO production was monitored by measuring nitrite levels in culture media using Griess reagent.

Absorbance was measured at 548 nm in incubated media with Griess reagent for 10 min. Viable adherent cells were stained with MTT (2 µg/mL) for 4 h. The medium was then removed and the produced formazan crystals were dissolved in DMSO (200 µL). Absorbance was measured at 540 nm.

One-way analysis of variance was applied for all statistical analyses by independent experiments, and data were represented as means ± standard error of the measurement. Individual values were compared by t-test and a P-value < 0.01 to evaluate the significant.

3.5. Antimicrobial and Antifungal Assays

Antimicrobial and antifungal bioassays were conducted in triplicate following the method as recommended by the National Center for Clinical Laboratory Standards (NCCLS) [10]. The bacterial strains *Staphylococcus aureus*, *S. pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* were grown on Mueller-Hinton agar. The yeasts, *Candida albicans* and *Saccharomyces cerevisiae*, were grown on Sabouraud dextrose agar, and the fungus, *Aspergillus fumigatus*, was grown on potato dextrose agar. Targeted microbes (3–4 colonies) were prepared from broth culture (bacteria: 37 °C for 24 h; fungus: 28 °C for 48 h), and the final spore suspensions of bacteria (in MHB medium), yeasts (in SDB medium), and fungus (in PDB medium) were 10⁶ and 10⁵ cells/mL and 10⁴ mycelial fragments/mL, respectively. Testing compounds (10 mg/mL as stock solution in DMSO and serial dilutions) were transferred to a 96-well clear plate in triplicate, and the suspension of the test microorganisms were added to each well (200 µL) (antimicrobial peptide AMP, streptomycin, and fluconazole were used as positive controls). After incubation, the absorbance at 595 nm was measured with a microplate reader (TECAN), and the inhibition rate was calculated and plotted versus test concentrations.

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Samples Availability: Available from the authors.

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