Article

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Abstract: Fifteen new diterpenoids, namely xishaklyanes A-O (**1-15**), along with three known related ones (**16-18**), were isolated from the soft coral *Klyxum molle* collected from Xisha Islands, South China Sea. The stereochemistry of the new compounds were elucidated by a combination of detailed spectroscopic analyses, chemical syntheses, quantum chemical calculations and comparison with the reported data. The absolute configuration of compound **18** was established by the modified Mosher's method for the first time. In bioassay, some of these compounds exhibited considerable antibacterial activities on fish pathogenic bacteria, and compound **4** showed the most effective activity with MIC of 0.225 µg/mL against *Lactococcus garvieae*.

Keywords: soft coral; *Klyxum molle*; diterpenoids; stereochemistry; antibacterial activity

1. Introduction

Soft corals of the genus *Klyxum* (order Alcyonacea, family Alcyoniidae) were broadly distributed over the tropical Indo-Pacific, including the South China Sea [1]. Different from the commonly chemically investigated *Sinularia* [2] and *Sarcophyton* [3] soft corals, only three species from the genus *Klyxum* have been chemically studied, including *Klyxum simplex* [4-7], *Klyxum molle* [8-11], and *Klyxum flaccidum* [12-15]. Besides, diverse secondary metabolites were discovered, including novel skeleton (Klyflaccilides A and B) and eunicellin-type diterpenoids [8-12]. These compounds exhibited widespread biological activities, such as antibacterial, cytotoxic, and anti-inflammatory effects [4-12,14,15].

As part of our continuous research project aiming for the discovery of bioactive metabolites from Chinese marine *Cnidaria* [12,16-18], *Klyxum molle* collected off the Xisha Islands were conducted systematic research, yielding fifteen new diterpenoids **1−15** and three known related diterpenoids **16−18** (Figure 1). Herein, the isolation, structural elucidation, and biological evaluations of these compounds are reported.
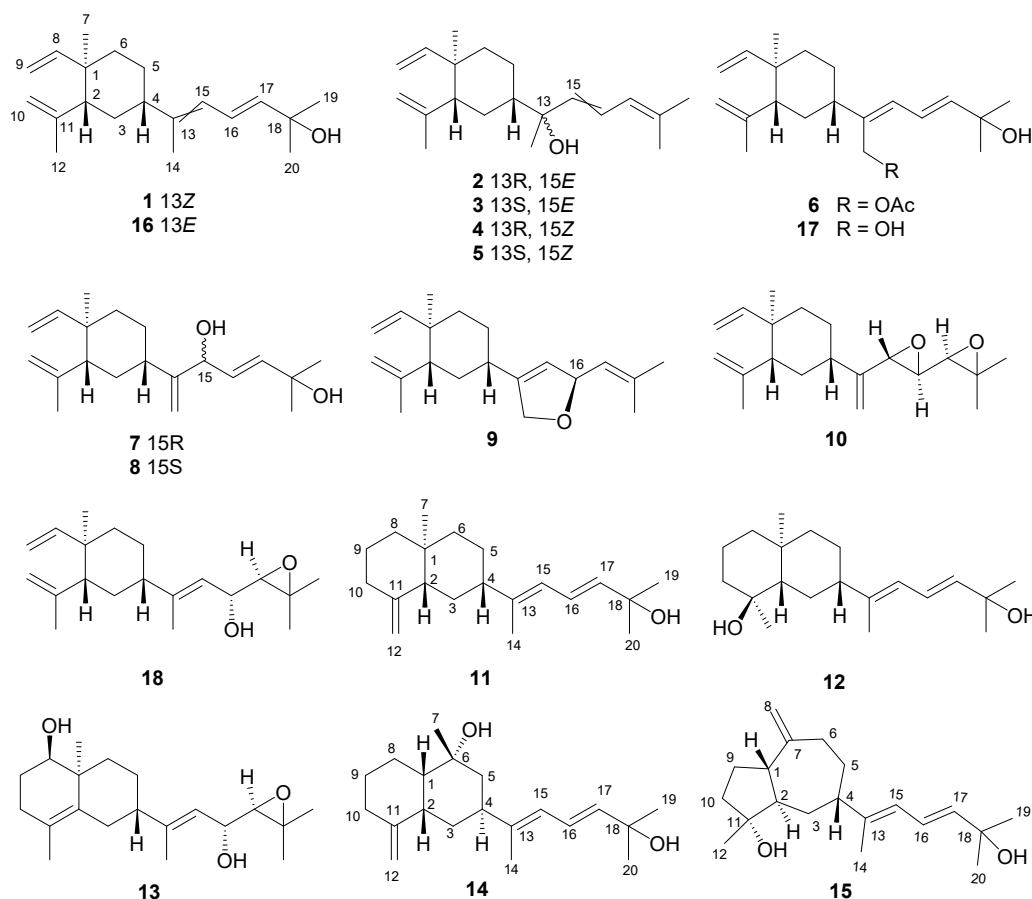
Figure 1. This is a figure. Schemes follow the same formatting.

2. Results and Discussion

By a series of column chromatography in combination with HPLC, the acetone extract of *K. molle* resulted in the purification of fifteen new diterpenoids, namely xishaklyanes A-O (**1**-**15**), along with three known related ones (**16-18**) (Figure 1). Those known diterpenoids were unambiguously identified as fuscol (**16**) [19], lobovarol H (**17**) [20], and 17,18-epoxyloba-8,10,13(15)-trien-16-ol (**18**) [21], respectively, by comparing their NMR data and specific rotation values with those reported in the literature.

Xishaklyane A (**1**) was obtained as an optically active colorless oil. From the molecular ion peak at *m*/*z* 289.2527 ([M + H]⁺, calcd. 289.2526) in the HRESIMS spectrum, a molecular formula of $C_{20}H_{32}O$ was established, indicating five degrees of unsaturation. The ¹H and ¹³C NMR data of **1**   (Tables 1 and 3) highly resembled those of co-occurring **16**, with the only differences of signals on C-4 (δ_C 47.5 in **16** and 40.5 in **1**), C-14 (δ_C 15.3 in **16** and 20.2 in **1**), and C-15 (δ_C 122.9 in **16** and 124.6 in **1**), suggesting that **1** was the analogue of **16**, with the opposite geometry on $\Delta^{13,15}$. Besides, the characteristic NOE correlation between H₃-14 and H-15 also confirmed the *Z* geometry of $\Delta^{13,15}$ in **1** (Figure 2). The structure of **1** was further confirmed by 2D NMR analysis including HMBC and NOESY correlations. Thus, compound **1** was determined to be 13Z-fuscol, namely xishaklyane A.

Table 1. The ¹H NMR Data (600 MHz, δ_H in ppm, *J* in Hz) for compounds **1-5** in CDCl₃.

No.	1	2	3	4	5
	δ_H mult. (*J* Hz)	δ_H mult. (*J* Hz)	δ_H mult. (*J* Hz)	δ_H mult. (*J* Hz)	δ_H mult. (*J* Hz)
2	2.08, m	1.94, m	1.94, m	1.97, m	1.97, m
3a	1.68, m	1.43, m	1.43, m	1.50, m	1.50, m
3b	1.74, m	1.59, m	1.59, m	1.62, m	1.62, m
4	2.70, m	1.43, m	1.43, m	1.50, m	1.50, m

No.					
5a	1.38, m	1.44, m	1.44, m	1.33, m	1.33, m
5b	1.59, m	1.63, m	1.63, m	1.45, m	1.45, m
6a	1.44, m	1.44, m	1.44, m	1.46, m	1.46, m
6b	1.55, m				
7	1.02, s	0.97, s	0.97, s	0.97, s	0.97, s
8	5.83, dd (17.2, 11.3)	5.80, dd (15.3, 10.9)	5.80, dd (15.3, 10.9)	5.80, dd (15.3, 10.9)	5.80, dd (15.3, 10.9)
9a	4.90, d (11.3)	4.88, d (10.9)	4.88, d (10.9)	4.88, d (10.9)	4.88, d (10.9)
9b	4.90, d (17.2)	4.89, d (15.3)	4.89, d (15.3)	4.89, d (15.3)	4.89, d (15.3)
10a	4.59, s	4.58, s	4.58, s	4.58, s	4.58, s
10b	4.81, s	4.81, s	4.81, s	4.81, s	4.81, s
12	1.71, s	1.70, s	1.70, s	1.70, s	1.70, s
14	1.76, s	1.29, s	1.29, s	1.35, s	1.35, s
15	5.77, d (10.9)	5.65, d (15.3)	5.65, d (15.3)	5.31, d (11.9)	5.31, d (11.9)
16	6.50, dd (15.2, 10.9)	6.43, dd (15.3, 10.9)	6.43, dd (15.3, 10.9)	6.19, t (11.9)	6.19, t (11.9)
17	5.70, d (15.2)	5.84, d (10.9)	5.84, d (10.9)	6.63, d (11.9)	6.63, d (11.9)
19	1.34, s	1.78, s	1.78, s	1.74, s	1.74, s
20	1.34, s	1.78, s	1.78, s	1.81, s	1.81, s

* Chemical shifts (ppm) refer to CHCl$_3$ (δH 7.26). Assignments were deduced by analysis of 1D and 2D NMR spectra.

Table 2. The ^{1}H NMR Data (600 MHz, δ_H in ppm, J in Hz) for compounds **6-10** in CDCl$_3$.

No.	6	7	8	9	10
	δ_H mult. (J Hz)	δ_H mult. (J Hz)	δ_H mult. (J Hz)	δ_H mult. (J Hz)	δ_H mult. (J Hz)
2	2.02, m	1.99, m	1.99, m	2.01, m	2.02, m
3a	1.57, m	1.57, m	1.57, m	1.61, m	1.53, m
3b					1.60, m
4	2.11, m	1.99, m	1.99, m	2.10, m	1.99, m
5a	1.48, m	1.47, m	1.47, m	1.44, m	1.50, m
5b	1.63, m	1.62, m	1.62, m	1.68, m	1.62, m
6a	1.48, m	1.45, m	1.45, m	1.46, m	1.48, m
6b	1.51, m	1.51, m	1.51, m	1.51, m	1.51, m
7	1.01, s	1.02, s	1.02, s	1.01, s	1.01, s
8	5.82, dd (17.6, 10.6)	5.81, dd (17.8, 10.5)	5.81, dd (17.8, 10.5)	5.81, dd (17.8, 10.5)	5.81, dd (17.8, 10.5)
9a	4.90, d (10.6)	4.90, d (10.5)	4.90, d (10.5)	4.90, d (10.5)	4.90, d (10.5)
9b	4.90, d (17.6)	4.91, d (17.8)	4.91, d (17.8)	4.90, d (17.8)	4.92, d (17.8)
10a	4.58, s	4.58, s	4.58, s	4.58, s	4.57, s
10b	4.82, s	4.82, s	4.82, s	4.83, s	4.83, s
12	1.70, s	1.70, s	1.70, s	1.71, s	1.71, s
14a	4.75, d (3.3)	4.99, s	4.99, s	4.54, m	4.95, s
14b		5.15, s	5.15, s	4.64, m	5.09, s
15	6.10, d (11.0)	4.62, m	4.62, m	5.31, m	3.31, d (2.2)
16	6.55, dd (15.2, 11.0)	5.66, dd (15.6, 6.5)	5.66, dd (15.6, 6.5)	5.50, m	2.73, ss (5.9, 2.2)
17	5.89, d (15.2)	5.90, d (15.6)	5.90, d (15.6)	5.15, m	2.63, d (5.9)
19	1.35, s	1.33, s	1.33, s	1.73, s	1.35, s
20	1.35, s	1.33, s	1.33, s	1.73, s	1.39, s
22	2.07 s				

* Chemical shifts (ppm) refer to CHCl$_3$ (δH 7.26). Assignments were deduced by analysis of 1D and 2D NMR spectra.

Table 3. The ^{13}C NMR Data (125 MHz, δ_C in ppm) for compounds **1-10** in CDCl$_3$.

No.	1 δ_C mult.	2 δ_C mult.	3 δ_C mult.	4 δ_C mult.	5 δ_C mult.	6 δ_C mult.	7 δ_C mult.	8 δ_C mult.	9 δ_C mult.	10 δ_C mult.
1	39.7, C	39.9, C	39.9, C	39.9, C	39.9, C	39.9, C	39.9, C	39.9, C	39.9, C	39.8, C
2	52.6, CH	52.8, CH	52.8, CH	52.7, CH	52.8, CH	52.9, CH	53.0, CH	53.0, CH	52.6, CH	52.8, CH
3	31.9, CH$_2$	28.2, CH$_2$	28.5, CH$_2$	28.1, CH$_2$	28.5, CH$_2$	33.3, CH$_2$	34.3, CH$_2$	34.7, CH$_2$	33.1, CH$_2$	33.9, CH$_2$
4	40.5, CH	48.8, CH	48.9, CH	49.5, CH	49.6, CH	41.1, CH	40.9, CH	41.0, CH	37.2, CH	41.1, CH
5	26.0, CH$_2$	22.7, CH$_2$	22.4, CH$_2$	22.6, CH$_2$	22.2, CH$_2$	27.2, CH$_2$	28.7, CH$_2$	28.2, CH$_2$	27.2, CH$_2$	27.1, CH$_2$
6	39.8, CH$_2$	40.0, CH$_2$	39.9, CH$_2$	40.0, CH$_2$	40.0, CH$_2$	40.0, CH$_2$	40.2, CH$_2$	40.1, CH$_2$	39.7, CH$_2$	39.8, CH$_2$
7	16.8, CH$_3$	16.7, CH$_3$	16.7, CH$_3$	16.7, CH$_3$	16.7, CH$_3$	16.8, CH$_3$	16.8, CH$_3$	16.8, CH$_3$	16.7, CH$_3$	16.7, CH$_3$
8	150.4, CH	150.4, CH	150.4, CH	150.5, CH	150.5, CH	150.2, CH	150.3, CH	150.3, CH	150.1, CH	150.1, CH
9	110.1, CH$_2$	110.0, CH$_2$	110.0, CH$_2$	110.0, CH$_2$	110.0, CH$_2$	110.2, CH$_2$	110.1, CH$_2$	110.1, CH$_2$	110.2, CH$_2$	110.3, CH$_2$
10	112.3, CH$_2$	112.2, CH$_2$	112.2, CH$_2$	112.2, CH$_2$	112.2, CH$_2$	112.4, CH$_2$	112.3, CH$_2$	112.4, CH$_2$	112.4, CH$_2$	112.4, CH$_2$
11	147.7, C	148.1, C	148.0, C	148.1, C	148.0, C	147.6, C	147.6, C	147.6, C	147.5, C	147.4, C
12	25.0, CH$_3$	24.9, CH$_3$	24.9, CH$_3$	24.8, CH$_3$	24.9, CH$_3$	24.9, CH$_3$	24.9, CH$_3$	24.9, CH$_3$	24.9, CH$_3$	25.0, CH$_3$
13	143.1, C	75.0, C	75.1, C	76.7, C	76.7, C	140.2, C	156.0, C	156.0, C	145.7, C	149.2, C
14	20.2, CH$_3$	26.2, CH$_3$	26.2, CH$_3$	27.4, CH$_3$	27.5, CH$_3$	61.6, CH$_2$	109.1, CH$_2$	108.9, CH$_2$	75.3, CH$_2$	109.4, CH$_2$
15	124.6, CH	135.3, CH	135.2, CH	133.5, CH	133.4, CH	128.4, CH	75.0, CH	74.9, CH	120.9, CH	56.0, CH
16	122.0, CH	124.4, CH	124.4, CH	125.5, CH	125.5, CH	122.0, CH	128.1, CH	128.2, CH	83.1, CH	58.5, CH
17	139.3, CH	124.8, CH	124.8, CH	121.4, CH	121.5, CH	143.0, CH	139.6, CH	139.6, CH	125.7, CH	63.0, CH
18	71.1, C	137.0, C	137.0, C	137.3, C	137.3, C	71.1, C	70.8, C	70.8, C	135.5, C	58.6, C
19	30.2, CH$_3$	18.5, CH$_3$	18.5, CH$_3$	17.8, CH$_3$	17.8, CH$_3$	29.9, CH$_3$	29.9, CH$_3$	29.9, CH$_3$	18.2, CH$_3$	19.7, CH$_3$
20	30.2, CH$_3$	26.0, CH$_3$	26.1, CH$_3$	26.7, CH$_3$	26.7, CH$_3$	29.9, CH$_3$	30.0, CH$_3$	29.9, CH$_3$	26.0, CH$_3$	24.7, CH$_3$
21						171.3, C				
22						21.2, CH$_3$				

* Chemical shifts (ppm) refer to CHCl$_3$ (δ_C 77.2). Assignments were deduced by analysis of 1D and 2D NMR spectra.
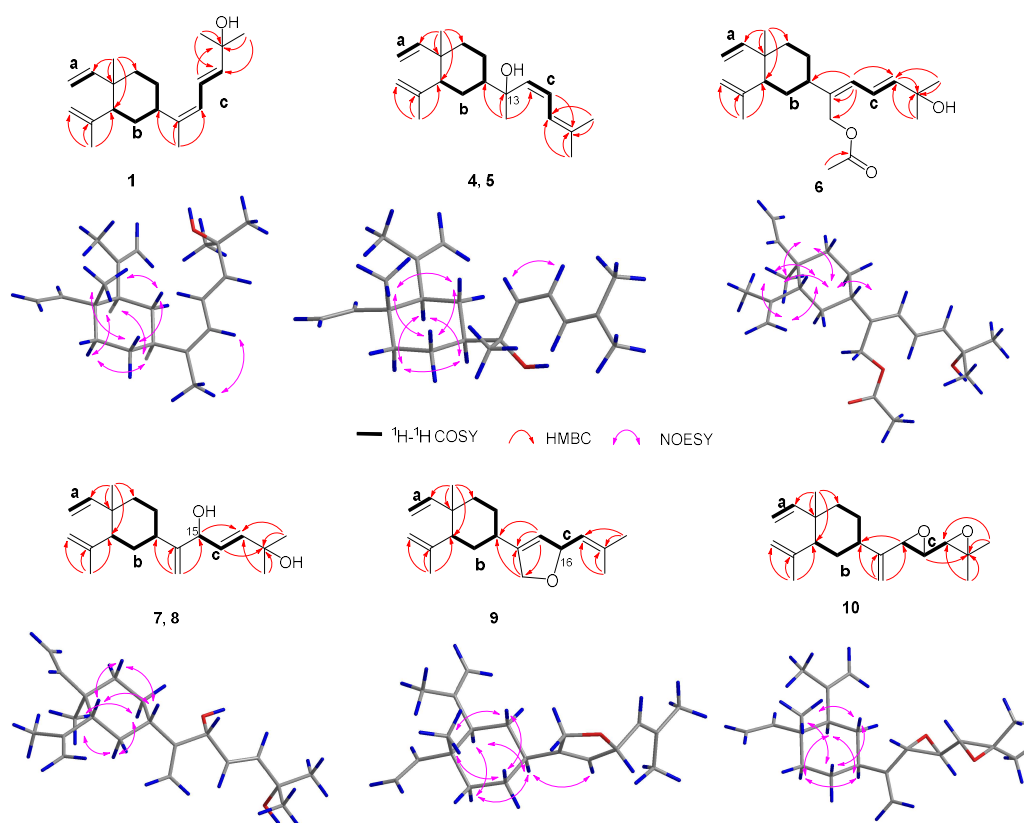
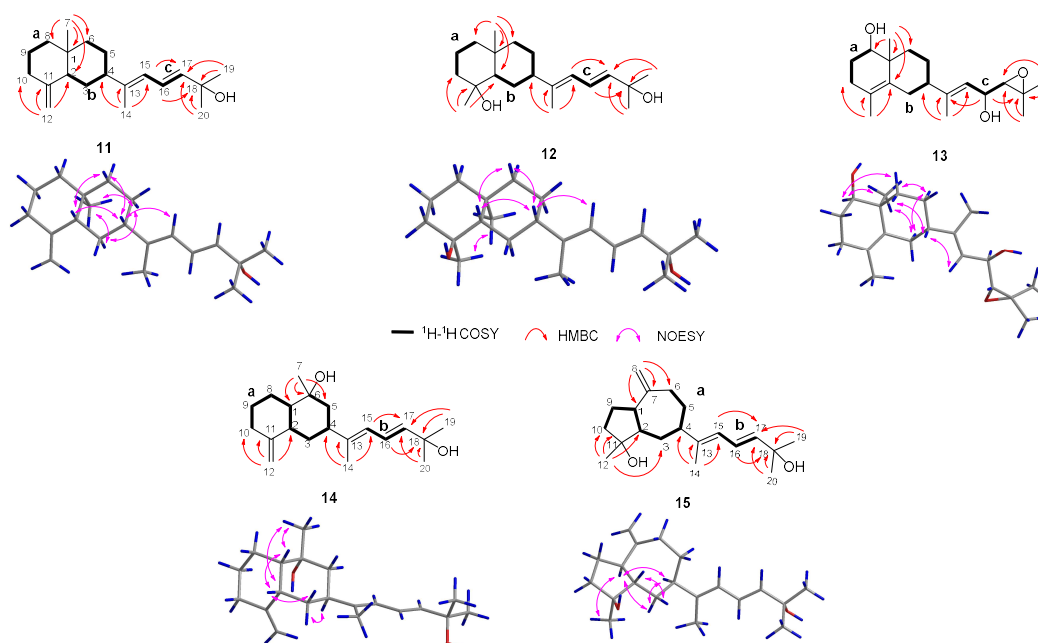**Figure 2.** ¹H-¹H COSY, key HMBC and NOESY correlations of compounds **1**, **4-10**.

Figure 3. ¹H-¹H COSY, key HMBC and NOESY correlations of compounds **1**, **4-10**.

Xishaklyane B (**2**) and xishaklyane C (**3**) were initially isolated as a mixture [22], displaying two sets of carbon signals in the ¹³C NMR spectrum. Normal Phase-High Performance Liquid Chromatography (NP-HPLC) CHIRALPAK® IC (250 mm × 4.6 mm, 5 μm, Daicel Corporation, Japan) [n-hexane/isopropyl (99.7:0.3), 1.0 mL/min] (**2**: t_R= 4.6 min; **3**: t_R= 5.5 min) was used to successfully separate the mixture into **2** and **3**. The absolute configurations (ACs) of **2** and **3** were further established by TDDFT-ECD calculations, a reliable approach to determine the ACs of natural products with chiral carbons near the chromophore groups [23-26]. As shown in Figure 4, the

Boltzmann-averaged ECD spectrum of (1*R*,2*R*,4*S*,13*R*,15*E*)-**2** matched to the experimental ECD spectrum of **2**, while the Boltzmann-averaged ECD spectrum of (1*R*,2*R*,4*S*,13*S*,15*E*)-**3** matched to the experimental ECD spectrum of **3**. Consequently, the ACs of **2** and **3** were determined to be 1*R*, 2*R*, 4*S*, 13*R*, 15*E*, and 1*R*, 2*R*, 4*S*, 13*S*, 15*E*, respectively. Thus, xishaklyanes B (**2**) and C (**3**) were deduced to be 13*R*, 15*E*-isofuscol and 13*S*, 15*E*-isofusol, respectively.
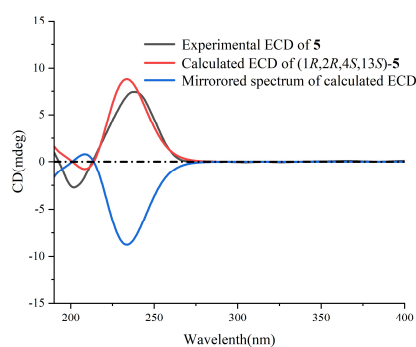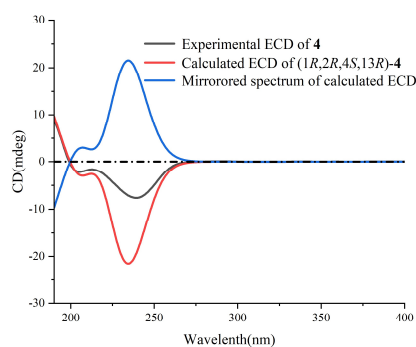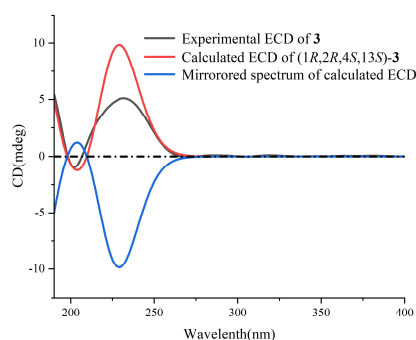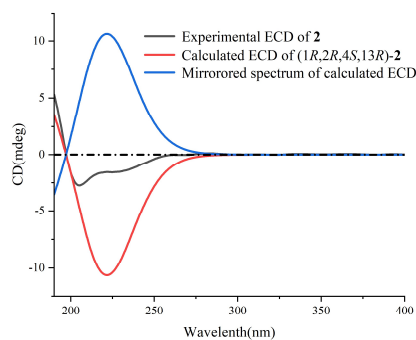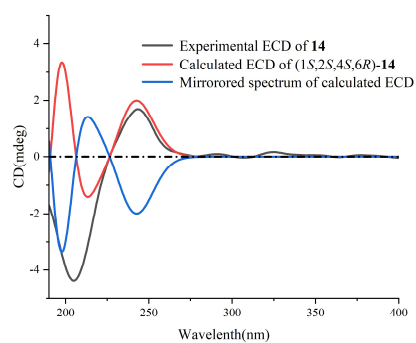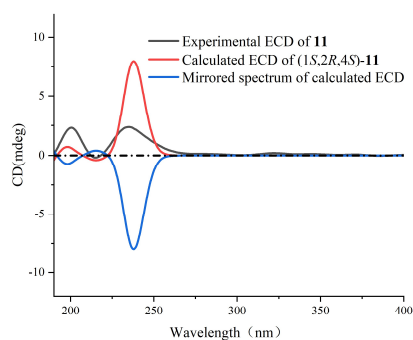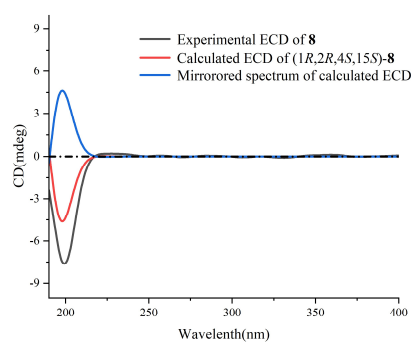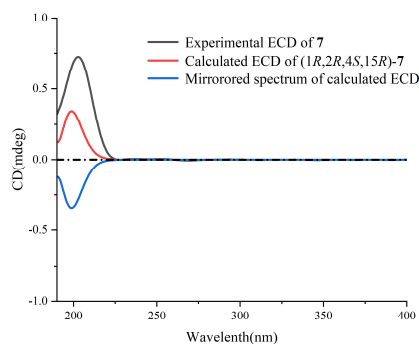
Figure 4. Comparison of experimental ECD spectrum (black) and DFT-predicted ECD spectra of **2-5**. ECD spectra were predicted by means of time-dependent DFT calculations at the MPW1PW91/6-31G(d, p) level.

Xishaklyane D (**4**) and xishaklyane E (**5**) were also isolated as a pair of epimers which were separated by NP-HPLC [n-hexane/isopropyl (99.2:0.8), 1.0 mL/min] (**5**: t_R= 6.5 min; **6**: t_R= 5.6 min). The 1D and 2D NMR spectra of the mixture (Tables 1 and 3) showed great similarity to those of the mixture of **2** and **3**, with the only differences of the chemical shifts between C-13 and C-17, indicating the opposite geometry of $\Delta^{15,16}$. The *Z* geometry of $\Delta^{15,16}$ was deduced by the ^1H–^1H coupling constants (*J* = 12.0 Hz). The ACs of **4** and **5** were also determined by TDDFT-ECD calculation. As shown in Figure 4, the Boltzmann-averaged ECD spectrum of (1*R*,2*R*,4*S*,13*R*,15*Z*)-**4** matched to the experimental ECD spectrum of **4**, while the Boltzmann-averaged ECD spectrum of (1*R*,2*R*,4*S*,13*S*,15*Z*)-**5** matched to the experimental ECD spectrum of **5**. Consequently, the ACs of **4** and **5** were determined to be 1*R*,2*R*,4*S*,13*R*,15*Z*. and 1*R*,2*R*,4*S*,13*S*,15*Z*, respectively. Finally, xishaklyane D (**4**) was determined as 13*R*,15*Z*-isofuscol, while xishaklyane E (**5**) was determined to be 13*S*,15*Z*-isofusol.

Xishaklyane F (**6**) was obtained as an optically active colorless oil. Its molecular formula was deduced to be $C_{22}H_{34}O_3$ on the basis of HRESIMS [sodiated ion peak at *m/z* 369.2408 ([M + Na]$^+$, calcd. 369.2400)], indicating six degrees of unsaturation. The ^1H and ^{13}C NMR data of **6** were reminiscent of those of the known compound **17** (Tables 2 and 3), with the only difference on C-14 (δ_H 4.29, 4.31, δ_C 60.0 in **17** and δ_H 4.75, 4.75, δ_C 61.6 in **6**), as well as an additional acetyl group in **6** (δ_H 2.07 s, δ_C 21.2, 171.3), indicating the acetylation of 14-OH of **17** towards **6**. To confirm our deduction, the acetylation of **17** (3 mg) was carried out using pyridine and Ac_2O at room temperature for 24 h. yielding the acetate **6** (2 mg). Thus, compound **6** was determined as the 14-acetate of the known compound lobovarol H (**17**), namely xishaklyane F.

Xishaklyanes G and H (**7** and **8**) were initially obtained as a mixture, which were further separated by NP-HPLC [n-hexane/isopropyl (90:10), 0.9 mL/min] (**7**: t_R= 6.4 min; **8**: t_R= 5.6 min). They showed the same molecular ion peak at *m/z* 303.2329 ([M - H]$^-$, calcd. 303.2330) in the HRESIMS spectrum, and owned the same molecular formula of $C_{20}H_{32}O_2$, indicating compound **7** to be isomeric with **8**. Detailed analysis of their NMR data suggested an epimeric relationship between **7** and **8**. The ^1H and ^{13}C NMR data of **7** and **8** (Tables 2 and 3) showed difference at C-15 and its neighboring carbons (e.g., C-3, C-5 and C-14), indicating **7** and **8** may have the opposite configuration of 15-OH. For the planar structure of both compounds, taking **7** for an example, its ^1H and ^{13}C NMR and HSQC resonances as well as coupling constants of the connected protons, indicated the presence of one monosubstituted terminal double bond [δ_H 5.81 (dd, *J* = 17.8, 10.5 Hz), 4.91 (d, *J* = 17.8 Hz), 4.90 (d, *J* = 10.5 Hz), δ_C 110.1 (t), δ_C 150.3 (s)], two disubstituted terminal olefinic bond [δ_H 4.82 (s), δ_H 4.58 (s), δ_C 112.4 (t), δ_C 147.7 (s); δ_H 4.99 (s), δ_H 5.15 (s), δ_C 109.0 (t), δ_C 156.0 (s)], and two monosubstituted double bond [δ_H 5.66 (dd, *J* = 15.6, 6.5 Hz), δ_H 5.90 (d, *J* = 15.6 Hz), δ_C 128.2 (d), δ_C 139.7 (d)]. The above olefinic bonds accounted for four degrees of unsaturation, so the remaining one degree should be ascribed to a ring in the molecule. Further analysis of ^1H–^1H COSY spectrum of compound **7** revealed three structural fragments **a–c**. These fragments were connected with well resolved HMBC correlations from H$_3$-7 to C-1/C-2/C-6/C-8, from H$_3$-12 to C-2/C-10/C-11, from H$_3$-19/H$_3$-20 to C-17/C-18, and from H$_2$-14 to C-4/C-13/C-15 (Figure 2). Consequently, the planar structure of **7** was identified as shown in Figure 1, featuring a lobane-type diterpenoid skeleton. The determination of the relative configurations (RCs) of **7** and **8** were highly challenging, because chiral carbon on side chain whose RCs cannot be elucidated by only a NOESY experiment. The *E* geometry of $\Delta^{16,17}$ was deduced by the ^1H–^1H coupling constants (*J* = 15.6 Hz). NOE correlations of H-4/H-3β/H-2 revealed that these protons were disposed at the same side of the molecule, and randomly assigned as β-oriented. Besides, the correlation of H$_3$-7/H-3α revealed that these protons and proton-bearing groups were positioned at the other side of the molecule, thus α-directed (Figure 2). The ACs of **7** and **8** were determined by the TDDFT-ECD calculation. As shown in Figure 5, the Boltzmann-averaged ECD spectrum of (1*R*,2*R*,4*S*,15*R*,16*E*)-**7** matched to the experimental ECD spectrum of **7**, while the Boltzmann-averaged ECD spectrum of (1*R*,2*R*,4*S*,15*S*,16*E*)-**8** matched to the experimental ECD spectrum of **8**.

Consequently, the absolute configuration of **7** was determined to be 1*R*,2*R*,4*S*,15*R*,16*E*. The absolute configuration of **8** was determined to be 1*R*,2*R*,4*S*,15*S*,16*E*.
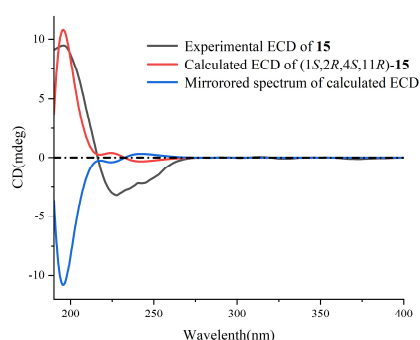
Figure 5. Comparison of experimental ECD spectrum (black) and DFT-predicted ECD spectra of **7, 8, 11, 14, 15**. ECD spectra were predicted by means of time-dependent DFT calculations at the MPW1PW91/6-31G(d, p) level.

Xishaklyane I (**9**) was obtained as an optically active colorless oil. Its molecular formula of $C_{20}H_{30}O$ was determined from the molecular ion peak at m/z 286.2288 ([M]$^+$, calcd. 286.2291) in the HREIMS spectrum, indicating six degrees of unsaturation. Under detailed diagnostic 2D NMR spectra, as well as coupling constants of the connected protons (Tables 2 and 3), compound **9** own the same skeleton as the previously mentioned compounds. The major differences between them mainly happened at the C-14 position. An ether bridge between C-14 and C-16 formed a furan ring, which can further verificated by HMBC correlation from H-16 to C-14. As for the relative stereochemistry of **9**, the relative configurations at C-1, C-2 and C-4 were suggested to be the same as that of **1-8**, which was supported by the similar NOE relationships observed in the NOESY spectrum (Figure 2). Therefore, there are only two possibilities for the RC of **9** [(1*R**, 2*R**, 4*S**, 16*R**)-**9** and (1*R**, 2*R**, 4*S**, 16*S**)-**9**]. Thus, the QM-NMR calculation and DP4+ analyses [27-29] of ^{13}C NMR parameter on the two possible candidate diastereoisomers were performed. Finally, the experimentally observed ^{13}C NMR data for **9** gave its best match for the 1*R**, 2*R**, 4*S**, 16*R** isomer (**9a**, see the details in SI), with a 99.83% probability. Like the AC of fuscol, consequently, the absolute configuration of **9** was determined to be 1*R*,2*R*,4*S*,13*Z*,16*R*.

The molecular formula $C_{20}H_{30}O_2$ of Xishaklyane J (**10**) was deduced by the HRESIMS molecular ion peak at m/z 303.2315, implying six degrees of unsaturation. The ^{1}H and ^{13}C NMR data of **10** (Tables 2 and 3) was closely reminiscent of those of the co-occurrent 17,18-epoxyloba-8,10,13(15)-trien-16-ol (**18**). The only difference between them was the presence of a terminal alkene (C-13/C-14) and an epoxide (C-15/C-16) in **10** instead of a trisubstituted double bond (C-13/C-15) and a hydroxyl (C-16) in **18**. The detailed 2D NMR analysis shown in Figure 2 confirmed its planar structure. To further confirm the structure and RC of **10**, the QM-NMR calculation and DP4+ analyses were used. Finally, the experimentally observed NMR data for **10** gave its best match for the 1*R**, 2*R**, 4*S**, 16*S**, 17*R**, 18*R** isomer (**10d**, see the details in SI), with a 100.00% probability. Like the AC of fuscol, consequently, the absolute configuration of **10** was determined to be 1*R*,2*R*,4*S*,16*S*,17*R*,18*R*.

Biogenetically, **10** was believed to be derived from compound **18** by an acid induced electron delivery from 16-OH to first generate the 15,16-epoxyl, and then promote the double bond migration towards the terminal olefin. The AC of the known compound 17,18-epoxyloba-8,10,13(15)-trien-16-ol (**18**) has not been defined. To obtain its absolute configuration at C-16, two aliquots of compound **18** were treated with (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenyl acetyl (MTPA) chlorides to obtain the (*S*)- and (*R*)-esters, respectively. Analysis of $\Delta\delta^{SR}$ values (δ_S - δ_R) observed for the signals of the protons close to 16-OH indicated the *R* configuration at this carbon (Figure 6).

Xishaklyane K (**11**) was obtained as an optically active colorless oil. Its molecular formula was deduced to be $C_{20}H_{32}O$ on the basis of the HREIMS [molecular ion peak at m/z 288.2443 ([M]$^+$, calcd. 288.2448)]. Careful analysis of the 1D NMR spectra of **11** (Tables 4 and 5) showed a close similarity with those of co-occurring **16**, indicating **11** also being a same side carbon chain at C-4. Further

analysis of its 1D and 2D NMR spectra revealed that the main difference was between C-8 and C-12. The ^1H–^1H COSY correlation of H_2-9/H_2-10 formed a six-membered ring, which can also deduce from the HMBC correlation from H_2-10 to C-8. Therefore, the planar structure was identified as shown in Figure 1, a prenyleudesmane type diterpene. Now, the remaining task is to determine the structure and RC of **11**. The *E* geometry of $\Delta^{16,17}$ was deduced by the ^1H–^1H coupling constants (J = 15.3 Hz) and the *E* geometry of $\Delta^{13,15}$ was deduced by the NOE correlations between H-15 and H-4 (Figure 3). The relative configuration of chiral centers C-1, C-2 and C-4 in compound **11** was as the same as those compounds mentioned above. To determine the AC of **11**, TDDFT-ECD calculation was performed. As shown in Figure 5, the Boltzmann-averaged ECD spectrum of (1*S*,2*R*,4*S*)-**11** matched to the experimental ECD spectrum of **11**. Consequently, the AC of **11** was determined to be 1*S*,2*R*,4*S*,13*E*,16*E*.

Table 4. The ^1H NMR Data (600 MHz, δ_H in ppm, J in Hz) for compounds **11-15** in CDCl$_3$.

No.	11 δ_H mult. (J Hz)	12 δ_H mult. (J Hz)	13 δ_H mult. (J Hz)	14 δ_H mult. (J Hz)	15 δ_H mult. (J Hz)
1				2.02, m	2.27, m
2	1.82, m	1.25, m		3.10, m	1.79, m
3a	1.34, m	1.50, m	1.84, m	1.71, m	1.52, m
3b	1.52, m	1.50, m	2.52, m	1.86, m	1.83, m
4	2.00, m	1.99, m	1.84, m	2.30, m	2.44, m
5a	1.50, m	1.24, m	1.53, m	1.72, m	1.64, m
5b	1.51, m	1.82, m	1.70, m		1.66, m
6a	1.28, m	1.21, m	1.21, m		2.12, m
6b	1.51, m	1.44, m	2.04, m		2.49, m
7	0.73, s	0.89, s	1.03, s	1.15, s	
8a	1.28, m	1.10, m	3.49, m	1.52, m	4.70, s
8b	1.44, m	1.39, m		1.57, m	4.71. s
9a	1.59, m	1.55, m	1.53, m	1.55, m	1.72, m
9b	1.64, m		1.70, m	1.82, m	1.83, m
10a	2.00, m	1.37, m	1.97, m	2.30, m	1.72, m
10b	2.31, m	1.79, m	2.17, m	2.39, m	1.76, m
12a	4.42 s	1.11, s	1.59, s	4.75, s	1.18, s
12b	4.70, s			4.87, s	
14	1.80, s	1.79, s	1.73, s	1.77, s	1.75, s
15	5.88, d (10.8)	5.87, d (10.8)	5.33, d (8.8)	5.87, d (10.7)	5.90, d (10.7)
16	6.50, dd (15.3, 10.8)	6.48, dd (15.3, 10.8)	4.26, dd (8.8, 7.8)	6.46, dd (15.3, 10.7)	6.45, dd (15.3, 10.7)
17	5.75, d (15.3)	5.75, d (15.3)	2.84, d (7.8)	5.75, d (15.3)	5.75, d (15.3)
19	1.36, s	1.35, s	1.33, s	1.36, s	1.35, s
20	1.36, s	1.35, s	1.35, s	1.36, s	1.35, s

* Chemical shifts (ppm) refer to CHCl$_3$ (δH 7.26). Assignments were deduced by analysis of 1D and 2D NMR spectra.

Table 5. The ^{13}C NMR Data (125 MHz, δ_C in ppm) for compounds **11-15** in CDCl$_3$.

No.	11 δ_C mult.	12 δ_C mult.	13 δ_C mult.	14 δ_C mult.	15 δ_C mult.
1	36.1, C	34.7, C	39.6, C	48.6, CH	48.4, CH
2	50.0, CH	55.0, CH	133.4, C	45.3, CH	52.7, CH
3	29.4, CH$_2$	26.7, CH$_2$	30.8, CH$_2$	26.4, CH$_2$	31.5, CH$_2$
4	48.0, CH	48.5, CH	47.7, CH	44.0, CH	45.6, CH
5	26.7, CH$_2$	26.0, CH$_2$	27.3, CH$_2$	40.7, CH$_2$	31.2, CH$_2$
6	41.3, CH$_2$	44.7, CH$_2$	38.9, CH$_2$	81.8, C	37.0, CH$_2$
7	16.5, CH$_3$	18.9, CH$_3$	17.5, CH$_3$	24.1, CH$_3$	153.4, C

8	42.1, CH_2	41.2, CH_2	78.5, CH	29.0, CH_2	107.2, CH_2
9	23.6, CH_2	20.3, CH_2	27.3, CH_2	30.0, CH_2	26.4, CH_2
10	37.0, CH_2	43.5, CH_2	32.1, CH_2	34.8, CH_2	40.8, CH_2
11	151.1, C	72.4, C	124.4, C	151.5, C	81.1, C
12	105.6, CH_2	22.9, CH_3	19.2, CH_3	109.3, CH_2	24.0, CH_3
13	144.1, C	143.8, C	145.9, C	143.2, C	143.9, C
14	15.4, CH_3	15.5, CH_3	15.9, CH_3	16.1, CH_3	14.7, CH_3
15	122.4, CH	122.5, CH	120.9, CH	122.8, CH	123.0, CH
16	123.4, CH	123.3, CH	67.9, CH	123.4, CH	123.2, CH
17	139.3, CH	139.3, CH	67.6, CH	139.5, CH	139.5, CH
18	71.1, C	71.1, C	60.0, C	71.1, C	71.1, C
19	30.1, CH_3	30.1, CH_3	19.7, CH_3	30.1, CH_3	30.1, CH_3
20	30.1, CH_3	30.1, CH_3	25.1, CH_3	30.1, CH_3	30.1, CH_3

* Chemical shifts (ppm) refer to $CHCl_3$ (δ_C 77.2). Assignments were deduced by analysis of 1D and 2D NMR spectra.

Xishaklyane L (**12**) was obtained as an optically active colorless oil. From the molecular ion peak at *m/z* 306.2553 ([M]+, calcd. 306.2553) in the HREIMS spectrum, a molecular formula of $C_{20}H_{32}O_2$ was established. The structural features of **12** were reminiscent of the known compound lobovarol K[20]. Compound **12** was methylated at the hydroxyl of C-18 to form lobovarol K. Besides, the same NOE correlations (Figure 3) meant that the structure of **12** was tentatively determined to be the same as lobovarol K.

Xishaklyane M (**13**) has a molecular formula $C_{20}H_{32}O_3$, as displayed from the ion peak in the HREIMS (*m/z* 320.2347 [M]+). The 1D and 2D NMR data of **13** (Tables 4 and 5) further revealed that it is a diterpene possessing the same double rings with identical substitutions as that of the eudesmane derivative. The NOE correlations between H_3-7 and H-3α/H-5α, H-3α and H-5α, H-8 and H-6α, and between H-4 and H-6β, suggested the β-orientation of H-4, and the α-orientation of H_3-7 and H-8, respectively (Figure 3). The side chain was established by detailed 1D and 2D NMR data of **13**, indicating a hydroxyl at C-16 and an epoxide at C-17/C-18, which is same to the known compound **18**. Comparing their NMR data, the absolute configuration of **13** was tentatively determined to be 1*S*,2*Z*,4*S*,8*R*,13*E*,16*R*,17*R*.

Xishaklyane N (**14**) displayed a molecular formula of $C_{20}H_{32}O_2$ as established by the HREIMS ion peak at *m/z* 304.2400 ([M]+ calcd for 304.2397). The ^{1}H and ^{13}C NMR data of **14** (Tables 4 and 5) were very similar with **11**. Detailed analysis of those spectra of **14** and comparing with those of **11** recealed that the main differences between them happened at C-6 to C-8 segments. The HMBC correlations from H_3-7 to three carbons (C-1/C-5/C-6) insteated of four carbons (C-1/C-2/C-5/C-6) in **11**, indicating a methyl was displaced at C-6. As for the stereochemistry of compound **14**, the chemical shifts of C-14 (δ_C 15.4, CH_3) and *J* value of H-16 and H-17 (15.3 Hz) indicated the *E*-geometry for the 13,15- and 16,17-double bonds, respectively. The NOE correlations between H_3-7 and H-1/H-2, H-1 and H-2, H-2 and H-3β, and between H-4 and H-3α, suggested the β-orientation of H-1, H-2 and H_3-7, and the α-orientation of H-4, respectively (Figure 3). RC of **14**: 1*S**, 2*S**, 4*S**, 6*R**. As shown in Figure 5, the Boltzmann-averaged ECD spectrum of (1*S*,2*S*,4*S*,6*R*)-**14** matched to the experimental ECD spectrum of **14**. Consequently, the AC of **14** was determined to be 1*S*,2*S*,4*S*,6*R*,13*E*,16*E*.

Xishaklyane O (**15**) was also obtained as an optically active colorless oil. From the molecular ion peak at *m/z* 304.2402 ([M]+, calcd. 304.2397) in the HREIMS spectrum, a molecular formula of $C_{20}H_{32}O_2$ was established, indicating five degrees of unsaturation. Detailed analysis of 1D and 2D NMR spectra (Tables 4 and 5) revealed that the same side chain as compounds **11**, **12**, **14** and **16**. Further analysis of ^{1}H–^{1}H COSY and HMBC spectrum of **15** revealed a 5/7-fused carbon ring system (Figure 3). As for the stereochemistry of compound **15**, the *E* geometry of $\Delta^{16,17}$ was deduced by the [1]H–[1]H coupling constants (*J* = 15.3 Hz) and the *E* geometry of $\Delta^{13,15}$ was deduced by the chemical shifts of C-14 (δ_C 14.9, CH_3). The NOE correlations between H-3β and H-1/H-4, H-3α and H-2, H-1 and H-4, and between H_3-12 and H-1, suggested the α-orientation of H-1, H-2 and H_3-12, and the β-orientation of H-4, respectively. RC of **15**: 1*S**, 2*R**, 4*S**, 11*R**. As shown in Figure 5, the Boltzmann-averaged ECD

spectrum of (1*S*,2*R*,4*S*,11*R*)-**15** matched to the experimental ECD spectrum of **15**. Consequently, the absolute configuration of **15** was determined to be 1*S*,2*R*,4*S*,11*R*,13*E*,16*E*.

All compounds were screened for antibacterial activities on fish pathogenic bacteria. As shown in Table 6, some of them exhibited considerable antibacterial activities. Among them, compound **5** was the most effective one with MIC of 0.225 μg/mL against *Lactococcus garvieae*, whereas compound **11** showed the best antibacterial activity against *Streptococcus parauberis* with MIC of 0.9 μg/mL.

Table 6. Antibacterial activities on fish pathogenic bacteria of compounds **1-6**, **10-11**, **16-18**.

Compd.	MIC (μg/mL)				
	Streptococcus parauberis	*Lactococcus garvieae*	*Streptococcus parauberis*	*Phoyobacterium damselae*	*Aeromonas salmonicida*
	FP KSP28	MP5245	SP0F3K	FP2244	AS42
1	14.4	28.8	7.2	7.2	NA
2	7.2	NA	28.8	14.4	NA
3	7.2	NA	NA	14.4	28.8
4	7.2	0.225	NA	28.8	NA
5	7.2	28.8	NA	28.8	NA
6	17.3	NA	17.3	17.3	NA
10	15.1	NA	NA	NA	NA
11	0.9	14.4	NA	7.2	28.8
16	7.2	14.4	3.6	7.2	NA
17	NA	NA	NA	NA	NA
18	7.6	NA	NA	15.2	NA
Tetr	3.01	0.38	>24.05	0.02	6.11
Oxy	1.55	0.19	12.42	0.02	0.39
Lev	1.24	0.62	1.24	0.02	0.31
AMP	4.64	0.58	0.58	0.02	>18.57

* Tetr, Oxy, Lev and AMP were used as positive control; NA = not active at 30 μg/mL.

3. Materials and Methods

3.1. General Experimental Procedures

Optical rotations were measured on a Perkin-Elmer 241MC polarimeter (PerkinElmer, Fremont, CA, USA). UV and CD spectra recorded on a Jasco J-815 spectropolarimeter (JASCO, Japan) at ambient temperature using chromatographic grade CH_3OH and CH_3CN as solvents. IR spectra were recorded on a Nicolet 6700 spectrometer (Thermo Scientific, Waltham, MA, USA); peaks are reported in cm^{-1}. The NMR spectra were measured at 300K on Bruker Avance III 400, 500, 600 or 800 MHz NMR spectrometers (Bruker Biospin AG, Fällanden, Germany). Chemical shifts are reported in parts per million (δ) in $CDCl_3$ ($δ_H$ reported referred to $CHCl_3$ at 7.26 ppm; $δ_C$ reported referred to $CDCl_3$ at 77.16 ppm) and coupling constants (*J*) in Hz; assignments were supported by ^{1}H-^{1}H COSY, HSQC, HMBC, and NOESY experiments. HREIMS data were recorded on a Finnigan-MAT-95 mass spectrometer (Finnigan-MAT, San Jose, CA, USA). HRESIMS spectra was recorded on an Agilent G6520 Q-TOF mass spectrometer (Agilent, Santa Clara, CA, USA). Semi-preparative RP-HPLC was performed on an Agilent-1260 system (Agilent, Santa Clara, CA, USA) equipped with a DAD G1315D detector at 210 and 254 nm using XDB-C18 column (250 mm × 9.4 mm, 5 μm) by eluting with CH_3OH-

H$_2$O or CH$_3$CN-H$_2$O system at 3 mL/min. NP-HPLC was performed on a Shimadzu LC-6A system (Shimadzu, Japan) equipped with a DAD SPD-M20A detector using CHIRALPAK® IA or CHIRALPAK® IC (250 mm × 4.6 mm, 5 μm, Daicel Corporation, Japan) by eluting with n-hexane-isopropyl system at 1 mL/min. Commercial silica gel (100-200, 200-300 and 300-400 mesh; Qingdao Haiyang Chemical Group Co., Ltd., Qingdao, China) was used for column chromatography (CC). Precoated silica gel GF254 plates (Sinopharm Chemical Reagent Co., Shanghai, China) were used for analytical TLC. Spots were detected on TLC under UV light or by heating after spraying with anisaldehyde H$_2$SO$_4$ reagent. Sephadex LH-20 (Amersham Biosciences) was also used for CC. All solvents used for column chromatography and HPLC were of analytical grade (Shanghai Chemical Reagents Co., Ltd., Shanghai, China) and chromatographic grade (Dikma Technologies Inc., CA, USA), respectively.

3.2. Animal Material

Specimens of the soft coral *Klyxum molle* were collected by scuba diving at a depth of -20 m in Xisha Islands, Hainan Province, China, in 2019, and identified by Professor Xiu-Bao Li (Hainan University, Hainan, China). The biological material was frozen immediately after collection. A voucher specimen (19-XS-41) is available for inspection at Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

3.3. Extraction and Isolation

The frozen animals (868 g, dry weight) of *K. molle* were cut into pieces and extracted exhaustively with acetone at room temperature (3 × 3.0 L). The organic extract was evaporated to give a brown residue, which was then partitioned between Et$_2$O and H$_2$O. The upper layer was concentrated under reduced pressure to give a brown residue (43.3 g), which was fractioned by gradient silica gel (200–300 mesh) column chromatography (CC) (0~100% Et$_2$O in petroleum ether (PE)), yielding 11 fractions (A–K). Fr. D was fractioned by Sephadex LH-20 (PE/CH$_2$Cl$_2$/MeOH, 2:1:1) to obtain three sub-fractions Fr. Da, Db, and Dc. The subfraction Dc was separated on a column of silica gel (10~20% Et$_2$O in PE) to afford **16** (1.31 g), **1** (0.26 g). Fr. C was fractioned by Sephadex LH-20 (PE/CH$_2$Cl$_2$/MeOH, 2:1:1) to obtain four sub-fractions Fr. Ca, Cb, Cc and Cd. The subfraction Cd was separated on a column of silica gel (5~20% Et$_2$O in PE) to afford mixtures Cd2 and Cd6. Cd2 was further purified by RP-HPLC [MeOH/H$_2$O (90:10), 3.0 mL/min] to give **10** (3.9 mg, t_R = 11.0 min), Cd2c (0.7 mg, t_R = 11.8 min). Cd6 was further purified by RP-HPLC [MeOH/H$_2$O (95:5), 3.0 mL/min] to give **11** (1.1 mg, t_R = 8.0 min), Cd6a (3.1 mg, t_R = 5.3 min). Cd2c was purified by NP-HPLC [n-hexane/isopropyl (99.2:0.8), 1.0 mL/min] to give **4** (0.5 mg, t_R = 6.5 min), **5** (0.2 mg, t_R = 5.6 min). Cd6a was purified by NP-HPLC [n-hexane/isopropyl (99.7:0.3), 1.0 mL/min] to give **2** (1.4 mg, t_R = 4.6 min), **3** (1.4 mg, t_R = 5.5 min). Fr. G was fractioned by Sephadex LH-20 (PE/CH$_2$Cl$_2$/MeOH, 2:1:1) to obtain three sub-fractions Fr. Ga, Gb, and Gc. Gb was further purified by RP-HPLC [MeOH/H$_2$O (85:15), 3.0 mL/min] to give **17** (0.9 mg, t_R = 8.3 min), **18** (3.7 mg, t_R = 13.7 min). Gc was further purified by RP-HPLC [MeCN/H$_2$O (60:40), 3.0 mL/min] to give **9** (0.8 mg, t_R = 10.4 min), **13** (0.6 mg, t_R = 5.3 min). Fr. H was fractioned by Sephadex LH-20 (PE/CH$_2$Cl$_2$/MeOH, 2:1:1) to obtain three sub-fractions Fr. Ha, Hb, and Hc. The subfraction Hc was separated on a column of silica gel (50~75% Et$_2$O in PE) to afford **6** (15.0 mg), and mixtures Hc1 and Hc2. Hc2 was further purified by RP-HPLC [MeCN/H$_2$O (70:30), 3.0 mL/min] to give **14** (2.0 mg, t_R = 9.3 min), Hc1 was further purified by RP-HPLC [MeCN/H$_2$O (55:45), 2.0 mL/min] to give **12** (7.0 mg, t_R = 21.3 min), **15** (1.0 mg, t_R = 18.2 min), and Hc1d (1.3 mg, t_R = 23.6 min). Hc1d was purified by NP-HPLC [n-hexane/isopropyl (90:10), 0.9 mL/min] to give **7** (0.5 mg, t_R = 6.4 min), **8** (0.7 mg, t_R = 5.6 min).

3.4. Spectroscopic Data of Compounds

Xishaklyane A (**1**): Colorless oil; $[\alpha]_D^{20}$ −43.1 (c 1.95, CHCl$_3$); UV (MeCN) λ_{max} (log ε) 240 (3.31) nm; ECD (CH$_3$CN) λ_{max} ($\Delta\varepsilon$) 215 (−5.2) nm; IR (KBr) ν_{max} 3382, 2969, 2928, 2860, 1637, 1440, 1376, 1148,1005 cm^{-1}; HRESIMS [M+H]$^+$ m/z 289.2527 (calcd. for 289.2526, C$_{20}$H$_{33}$O).

Xishaklyane B (**2**): Colorless oil; $[\alpha]_D^{20}$ −7.4 (c 0.14, CHCl₃); UV (MeCN) λ_{max} (log ε) 238 (3.23) nm; ECD (CH₃CN) λ_{max} ($\Delta\varepsilon$) 201 (−1.6) nm.

Xishaklyane C (**3**): Colorless oil; $[\alpha]_D^{20}$ +30.2 (c 0.14, CHCl₃); UV (MeCN) λ_{max} (log ε) 238 (3.27) nm; ECD (CH₃CN) λ_{max} ($\Delta\varepsilon$) 232 (+2.3) nm.

Xishaklyane D (**4**) and Xishaklyane E (**5**): For **4**, Colorless oil; $[\alpha]_D^{20}$ −35.0 (c 0.05, CHCl₃); UV (MeCN) λ_{max} (log ε) 240 (3.29) nm; ECD (CH₃CN) λ_{max} ($\Delta\varepsilon$) 240 (−3.4) nm; For **5**, Colorless oil; $[\alpha]_D^{20}$ +75.8 (c 0.02, CHCl₃); UV (MeCN) λ_{max} (log ε) 240 (3.35) nm; ECD (CH₃CN) λ_{max} ($\Delta\varepsilon$) 244 (+3.8) nm; For mixture of **4** and **5**, IR (KBr) ν_{max} 3455, 2966, 2925, 2854, 1438, 1376, 1180, 1142, 1099, 1075,1029 cm⁻¹; HREIMS [M]⁺ m/z 288.2454 (calcd. for 288.2448, C₂₀H₃₂O).

Xishaklyane F (**6**): Colorless oil; $[\alpha]_D^{20}$ +33.5 (c 0.09, CHCl₃); UV (MeCN) λ_{max} (log ε) 239 (3.30) nm; ECD (CH₃CN) λ_{max} ($\Delta\varepsilon$) 234 (+2.9) nm; IR (KBr) ν_{max} 3451, 2968, 2925, 2854, 1735, 1377, 1260, 1230, 1075,1027 cm⁻¹; HRESIMS [M + Na]⁺ m/z 369.2408 (calcd. for 369.2400, C₂₂H₃₄NaO₃).

Xishaklyane G (**7**) and Xishaklyane H (**8**): For **7**, Colorless oil; $[\alpha]_D^{20}$ +25.3 (c 0.05, CHCl₃); ECD (CH₃CN) λ_{max} ($\Delta\varepsilon$) 196 (−4.4) nm; For **8**, White solid; $[\alpha]_D^{20}$ +24.0 (c 0.07, CHCl₃); ECD (CH₃CN) λ_{max} ($\Delta\varepsilon$) 204 (+5.6) nm; For mixture of **7** and **8**, IR (KBr) ν_{max} 3443, 2968, 2924, 2853, 1384, 1180, 1143, 1095, 1076, 1029 cm⁻¹; HRESIMS [M - H]⁻ m/z 303.2329 (calcd. for 303.2330, C₂₀H₃₁O₂).

Xishaklyane I (**9**): Colorless oil; $[\alpha]_D^{20}$ +67.9 (c 0.08, CHCl₃); IR (KBr) ν_{max} 3451, 2967, 2925, 2854, 1444, 1374, 1180, 1059, 1029 cm⁻¹; HREIMS [M]⁺ m/z 286.2288 (calcd. for 286.2291, C₂₀H₃₀O).

Xishaklyane J (**10**): Colorless oil; $[\alpha]_D^{20}$ +2.8 (c 0.09, CHCl₃); IR (KBr) ν_{max} 3454, 2965, 2926, 2856, 1643, 1456,1379, 1075, 1029 cm⁻¹; HRESIMS [M + H]⁺ m/z 303.2315 (calcd. for 303.2319, C₂₀H₃₁O₂).

Xishaklyane K (**11**): Colorless oil; $[\alpha]_D^{20}$ -20.0 (c 0.11, CHCl₃); UV (MeCN) λ_{max} (log ε) 240 (3.34) nm; ECD (CH₃CN) λ_{max} ($\Delta\varepsilon$) 200 (+2.0) nm; IR (KBr) ν_{max} 3451, 2869, 2925, 2852, 1442, 1386, 1180, 1143, 1075, 1030 cm⁻¹; HREIMS [M]⁺ m/z 288.2443 (calcd. for 288.2448, C₂₀H₃₂O).

Xishaklyane L (**12**): Colorless oil; $[\alpha]_D^{20}$ +25.7 (c 0.70, CHCl₃); UV (MeCN) λ_{max} (log ε) 240 (3.33) nm; ECD (CH₃CN) λ_{max} ($\Delta\varepsilon$) 242 (+2.1) nm; IR (KBr) ν_{max} 3385, 2970, 2926, 2864, 1456, 1384, 1143, 1105 cm⁻¹; HREIMS [M]⁺ m/z 306.2553 (calcd. for 306.2553, C₂₀H₃₄O₂).

Xishaklyane M (**13**): Colorless oil; $[\alpha]_D^{20}$ −13.3 (c 0.06, CHCl₃); IR (KBr) ν_{max} 3450, 2963, 2925, 2854, 1436, 1378, 1180, 1075, 1028 cm⁻¹; HREIMS [M]⁺ m/z 320.2347 (calcd. for 320.2346, C₂₀H₃₂O₃).

Xishaklyane N (**14**): Colorless oil; $[\alpha]_D^{20}$ −15.5 (c 0.20, CHCl₃); UV (MeCN) λ_{max} (log ε) 239 (3.34) nm; ECD (CH₃CN) λ_{max} ($\Delta\varepsilon$) 210 (−1.9) nm; IR (KBr) ν_{max} 3450, 2963, 2925, 2857, 1386, 1180, 1143, 1095, 1075, 1028 cm⁻¹; HREIMS [M]⁺ m/z 304.2400 (calcd. for 304.2397, C₂₀H₃₂O₂).

Xishaklyane O (**15**): Colorless oil; $[\alpha]_D^{20}$ −16.8 (c 0.10, CHCl₃); UV (MeCN) λ_{max} (log ε) 240 (3.38) nm; ECD (CH₃CN) λ_{max} ($\Delta\varepsilon$) 197 (+4.2) nm; IR (KBr) ν_{max} 3450, 2959, 2923, 2852, 1384, 1180, 1143, 1129, 1099, 1075, 1029 cm⁻¹; HREIMS [M]⁺ m/z 304.2402 (calcd. for 304.2397, C₂₀H₃₂O₂).

3.5. Esterification of 3 and 5 with MTPA chlorides

Compound **18** (2.0 mg) was dissolved in dry pyridine (1000 μL), and the solution was transferred into two NMR tubes (500 μL each), treated with (*R*)-(-)-2-methoxy-2-(trifluoromethyl) phenylacetyl chloride ((*R*)-(-)-MTPA-Cl) (20 μL) and (*S*)-(+)-2-methoxy-2-(trifluoromethyl) phenylacetyl chloride ((*S*)-(+)-MTPA-Cl) (20 μL), respectively. Carefully shaking and then monitored immediately by ¹H NMR. The reaction was found to be completed in 30 min. Then the solutions were evaporated in vacuo and the residue was purified by silica gel CC (10% Et₂O in PE) to obtain the *S*-MTPA ester **18s**, and *R*-MTPA ester **18r**, respectively. For (*S*)-MTPA ester of **18** (**18s**), Selected ¹H NMR (CDCl₃, 400 MHz): δ_H 5.812 (1H, dd, J = 17.6, 10.7 Hz, H-8), 5.626 (1H, t, J = 9.2 Hz, H-16), 5.359 (1H, d, J = 9.8 Hz, H-15), 4.917 (1H, d, J = 15.9 Hz, H-9α), 4.912 (1H, d, J = 12.4 Hz, H-9β), 4.842 (1H, s, H-11α), 4.585 (1H, s, H-11β), 2.997 (1H, d, J = 8.4 Hz, H-17), 2.009 (1H, d, J = 12.2 Hz, H-4), 1.827 (3H, s, Me-14), 1.713 (3H, s, Me-12), 1.338 (3H, s, Me-20), 1.323 (3H, s, Me-19), 1.013 (3H, s, Me-7). For (*R*)-MTPA ester of **18** (**18r**), Selected ¹H NMR (CDCl₃, 400 MHz): δ_H 5.807 (1H, dd, J = 17.6, 10.6 Hz, H-8), 5.591 (1H, t, J = 9.2 Hz, H-16), 5.148 (1H, d, J = 10.1 Hz, H-15), 4.911 (1H, d, J = 16.6 Hz, H-9α), 4.910 (1H, d, J = 11.5 Hz, H-9β), 4.848 (1H, s, H-11α), 4.579 (1H, s, H-11β), 2.996 (1H, d, J = 8.6 Hz, H-17), 1.995 (1H, d, J = 11.9 Hz, H-4), 1.856 (3H, s, Me-14), 1.713 (3H, s, Me-12), 1.358 (3H, s, Me-20), 1.331 (3H, s, Me-19), 0.997 (3H, s, Me-7).

3.6. QM-NMR Calculational Section

For the QM-NMR calculations of compounds, torsional sampling (MCMM) conformational searches using OPLS_2005 force field were carried out by the means of conformational search module in Macro model 9.9.223 software (Schrodinger, http://www.schrodinger. com/MacroModel), applying an energy window of 21 kJ/mol (5.02 kcal/mol) for saving structures. The following DFT calculations were performed using Gaussian 09, and Conformers above 1% population were reoptimized at B3LYP/6-311G(d,p) level of theory. Magnetic shielding constants (σ) were calculated by means of the gauge including atomic orbitals (GIAO) method at mPW1PW91/6-31+G(d,p) level of theory as recommended for DP4+ analysis.

3.7. TDDFT-ECD Calculational Section

For the time-dependent density functional theory/electronic circular dichroism (TDDFT-ECD) calculations of compounds, conformational searches were done following the general protocols previously described for QM-NMR calculation. Conformers above 1% population were used for re-optimizations and the following TDDFT-ECD calculations, which were performed using Gaussian 09 at the B3LYP/6-311G(d,p) level of theory with IEFPCM solvent model for acetonitrile. Finally, the SpecDis 1.62 software was used to obtain the calculated ECD spectrum and visualize the results.

3.8. Antibacterial assays

Five pathogenic bacteria, namely Streptococcus parauberis KSP28, Streptococcus parauberis SPOF3K, Lactococcus garvieae MP5245, Aeromonas salmonicida AS42, and Photobacterium damselae FP2244, were provided by National Fisheries Research & Development Institute, Korea. MIC values of test compounds were determined by the modified 0.5 Mcfarland standard method.

4. Conclusions

This detailed chemical investigation on the South China Sea soft coral *K. molle* yielded fifteen new diterpenes, namely xishaklyanes A-O (**1**-**15**), as well as three related known analogues (**16**-**18**). Among them, the absolute configuration of compound **18** was determined by the modified Mosher's method. And the stereochemistry of the new compounds was determined by QM-NMR and TDDFT-ECD, or chemical connections. In antibacterial activities on fish pathogenic bacteria, compound **4** exhibited the best activity with MIC of 0.225 µg/mL against *Lactococcus garvieae*. Further study should be conducted on the accumulation of the most effective compounds for in-depth antibacterial research.

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