Tetrahedron 69 (2013) 871–880

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Antifouling briarane type diterpenoids from South China Sea gorgonians *Dichotella gemmacea*

Jian-Fan Sun [a,b], Zhuang Han [c], Xue-Feng Zhou [a], Bin Yang [a], Xiuping Lin [a], Juan Liu [a], Yan Peng [a], Xian-Wen Yang [a], Yonghong Liu [a,*]

[a] *Key Laboratory of Marine Bio-resources Sustainable Utilization/Guangdong Key Laboratory of Marine Materia Medica/RNAM Center for Marine Microbiology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, People's Republic of China*
[b] *School of Environment, Jiangsu University, Zhenjiang 212013, People's Republic of China*
[c] *KAUST Global Academic Partnership Program, Section of Marine Ecology and Biotechnology, Division of Life Science, The Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong SAR, People's Republic of China*

ARTICLE INFO

Article history:
Received 23 July 2012
Received in revised form 25 September 2012
Accepted 9 October 2012
Available online 15 November 2012

Keywords:
Dichotella gemmacea
Antifouling agents
Nontoxic
Natural products
Diterpenoids
Structure elucidation

ABSTRACT

Our continued investigation on the South China Sea gorgonian *Dichotella gemmacea* led to the isolation of 16 new briarane-type diterpenoids, dichotellides F–U (**1**–**16**), along with 18 known analogues (**17**–**34**). Their structures were determined by MS, 1D and 2D NMR spectra analyses and by comparison with those reported in literature. The absolute configuration of **15** was confirmed by single-crystal X-ray diffraction data. The antifouling test showed that compounds **3**, **4**, **6**–**11**, **16**, and **23** had potent antifouling activities at nontoxic concentrations with EC_{50} values of 4.1, 1.82, 6.3, 7.6, 4.6, 1.2, 5.6, 0.79, 2.0, and 0.2 μg/mL, respectively.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Biofouling, the undesirable build-up of sessile marine organisms (such as barnacles, mussels, tubeworms, and seaweeds) onto man-made surfaces, is one of the most serious problems currently facing worldwide maritime domains,[1,2] and can cause substantial technical and economical problems for many marine-related operations. Due to new prohibition on toxic antifouling paints, such as tributyltin (TBT)-based compounds, alternative agents that are effective against biofouling and are environmentally benign are urgently needed.[3,4]

Gorgonians, as well as some other marine invertebrates, such as sponges, soft corals, ascidians, and seaweeds, are relatively free from fouling organisms because they produce a diverse array of secondary metabolites with the antifouling activity, which could be explored for potential sources of antifouling agents and development of antifouling paints.[5–8] The interest in the antifouling natural substances from marine organisms prompted us to investigate gorgonians *Dichotella gemmacea* and *Junceella fragilis*. Previously, five iodine-containing briarane type diterpenoids and other thirteen analogues were isolated from *D. gemmacea*.[9–11] Our continued studies on this species led to the isolation of sixteen new briarane type diterpenoids, dichotellides F–U (**1**–**16**), along with eighteen known analogues (**17**–**34**). The structures of these diterpenoids were determined by extensive spectroscopic analysis (^{1}H and ^{13}C NMR, DEPT, HSQC, HMBC, NOESY, and HRESIMS) and by comparison of the spectroscopic data with those of the related compounds known in the literature. A natural product library of total thirty four briarane type diterpenoids was evaluated against the larval settlement of barnacle *Balanus amphitrite* with the aim of discovering new potential natural antifoulants. Herein we report the isolation, structure elucidation, and biological activities of these compounds, and the structure–activity relationship of these compounds was discussed.

2. Results and discussion

The EtOH and $CHCl_3$/MeOH (1:1) extract of fresh gorgonian *D. gemmacea* was separated into an EtOAc-soluble fraction and *n*-BuOH fraction. The EtOAc-soluble fraction were subjected to

* Corresponding author. Tel./fax: +86 20 8902 3244; e-mail addresses: yonghongliu@scsio.ac.cn, yhliu6@yahoo.com.cn (Y. Liu).

0040-4020/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tet.2012.10.102

normal phase Si gel column, Sephadex LH-20 column followed by reversed-phase HPLC to afford 16 new briarane-type diterpenoids, dichotellides F–U (**1**–**16**), along with eighteen known analogues (**17**–**34**).

Dichotellide F (**1**) was obtained as white, amorphous powder. The molecular formula was established as $C_{36}H_{48}O_{17}$ on the basis of positive HRESIMS (*m*/*z*, 775.2805 $[M+Na]^+$, calcd for 775.2789) and NMR data. Its IR and UV spectra indicated the presence of hydroxyl (3433 cm^{-1}), γ-lactone (1793 cm^{-1}), ester carbonyls (1745, 1730 cm^{-1}), and a conjugated diene system (274 nm). The ^1H and ^{13}C NMR spectra (Tables 1 and 2) presented signals for four acetates and a 2-(3-methylbutanoyloxy) acetate moiety (δ_C 166.5, s; 60.8, t; 172.3, s; 42.7, t; 25.6, d; 22.3, q; 22.3, q), a tertiary methyl (δ_H 1.12, s), a secondary methyl (δ_H 1.14, d, *J*=7.0 Hz), an oxygenated methyl (δ_C 58.4, q; δ_H 3.43, s), a γ-lactone (δ_C 175.3, s), an exocyclic C-11/C-20 epoxide (δ_H 3.58, 2.90, each br s; δ_C 58.1, s, 49.0, t), a conjugated diene (δ_C 130.4, d; 129.4, d; 141.5, s; 122.5, d δ_H 5.58, t, *J*=10.0 Hz; 6.32, d, *J*=10.5 Hz; 5.87, d, *J*=8.5 Hz), an oxymethylene (δ_C 71.9, t; δ_H 4.53, 4.13, each d, *J*=16.0 Hz), an oxygenated quaternary carbon, and six oxygenated methines. These data showed that **1** was a briarane type diterpenoid with a 3, 5 (6)-conjugated diene and an exocyclic C-11/C-20 epoxide. As the ^1H and ^{13}C NMR spectral pat-

Table 1
^1H NMR (500 MHz) data of compounds **1**–**8** (*J* in Hz within parentheses)

Position	1	2	3	4	5	6	7	8
2	5.62 d (9.5)	5.63 d (9.5)	5.58 d 10.0)	5.68 d 10.0)	5.61 d (9.0)	5.61 d (9.0)	5.59 d (10.0)	5.58 d (8.5)
3	5.58 t (10.0)	5.58 t (10.0)	5.64 t (10.0)	5.61 t (10.0)	5.57 t (9.0)	5.60 t (9.0)	5.55 t (10.0)	5.56 t (8.5)
4	6.32 d (10.5)	6.32 d (10.0)	6.40 d (10.0)	6.33 d (10.0)	6.30 d (8.5)	6.28 d (8.5)	6.28 d (10.0)	6.27 d (7.5)
6	5.87 d (8.5)	5.88 d (8.5)	6.06 d (9.0)	5.66 d (9.0)	5.71 d (9.0)	5.70 d (9.0)	5.72 d (8.5)	5.87 d (8.5)
7	4.98 d (8.5)	4.98 d (8.5)	4.95 d (9.0)	4.96 d (9.0)	4.97 d (9.0)	4.97 d (9.0)	4.97 d (8.5)	4.99 d (8.5)
9	4.74 d (4.5)	4.75 d (4.5)	4.74 d (5.0)	4.72 d (4.5)	4.74 d (5.0)	4.73 d (5.0)	4.73 d (4.5)	4.74 d (4.5)
10	3.61 d (4.5)	3.61 d (4.5)	3.59 d (5.0)	3.61 d (4.5)	3.61 d (5.0)	3.60 d (5.0)	3.60 d (4.5)	3.58 d (4.5)
12	4.86 d (2.0)	4.88 d (3.5)	4.91 d (3.0)	4.84 d (3.0)	4.85 d (2.0)	4.87 d (2.5)	4.87 d (2.5)	4.91 d (2.5)
13	5.06 t (2.0)	5.08 t (3.5)	5.07 t (3.0)	5.05 t (3.0)	5.06 t (3.0)	5.08 t (3.0)	5.08 t (3.0)	5.10 t (3.0)
14	5.16 d (2.0)	5.16 d (3.0)	5.17 d (2.5)	5.18 d (3.0)	5.21 d (2.5)	5.21 d (2.5)	5.26 d (2.5)	5.20 d (2.5)
15	1.12 s	1.12 s	1.12 s	1.12 s	1.13 s	1.13 s	1.13 s	1.13 s
16	4.53 d (16.0)	4.53 d (15.0)	4.68 d (14.5)	5.45 d (16.0)	5.39 d (15.5)	5.45 d (16.0)	5.40 d (16.0)	4.48 d (15.0)
	4.13 d (16.0)	4.10 d (15.0)	4.45 d (14.5)	4.55 d (16.0)	4.64 d (15.5)	4.60 d (16.0)	4.63 d (16.0)	4.22 d (15.0)
17	2.27 q (7.0)	2.29 q (7.0)	2.29 q (7.0)	2.28 q (7.0)	2.30 q (7.0)	2.28 q (7.0)	2.29 q (7.0)	2.29 q (7.0)
18	1.14 d (7.0)	1.14 d (7.0)	1.13 d (7.0)	1.13 d (7.0)	1.14 d (7.0)	1.14 d (7.0)	1.14 d (7.0)	1.12 d (7.0)
20	3.58 br s	3.58 d (2.5)	3.58 d (3.0)	3.59 d (2.0)	3.59 d (2.0)	3.60 d (2.0)	3.60 br s	3.58 d (2.5)
	2.90 br s	2.91 d (2.5)	2.93 d (3.0)	2.91 d (2.0)	2.91 d (2.0)	2.93 d (2.0)	2.92 br s	2.92 d (2.5)
Acetate methyls	2.17 s	2.17 s	2.18 s	2.17 s	2.17 s	2.18 s	2.18 s	2.17 s
	2.12 s	2.14 s	2.07 s	2.16 s	2.16 s	2.15 s	2.16 s	2.06 s
	2.05 s	2.05 s	1.94 s	2.10 s	2.13 s	2.09 s	1.95 s	1.94 s
	1.93 s			1.94 s	2.09 s	1.94 s	1.94 s	
					1.95 s			
					1.94 s			
Isovalerate								
a		2.22 m (2H)	2.14 m (2H)	2.13 m (2H)		2.12 m (2H)	2.31 m (2H)	2.32 m (2H)
		2.01 m (1H)	1.99 m (1H)	1.97 m (1H)		1.98 m (1H)	1.99 m (1H)	1.99 m (1H)
		0.92 d (3H, 6.5)	0.97 d (3H, 6.5)	0.97 d (3H, 6.5)		0.97 d (3H, 6.5)	0.99 d (3H, 6.5)	0.99 d (3H, 7.0)
		0.90 d (3H, 6.5)	0.98 d (3H, 6.5)	0.98 d (3H, 6.5)		0.98 d (3H, 6.5)	0.98 d (3H, 6.5)	0.98 d (3H, 7.0)
b						2.14 m (2H)	2.14 m (2H)	2.14 m (2H)
						1.96 m (1H)	1.96 m (1H)	1.96 m (1H)
						0.91 d (3H, 6.5)	0.97 d (3H, 6.5)	0.90 d (3H, 6.5)
						0.90 d (3H, 6.5)	0.96 d (3H, 6.5)	0.89 d (3H, 6.5)
c								
2′	4.51 d (16.0)	4.53 d (16.0)	4.53 d (16.0)	4.52 d (16.0)				
	4.43 d (16.0)	4.44 d (16.0)	4.44 d (16.0)	4.42 d (16.0)				
4′	2.29 m (2H)	2.30 m (2H)	2.30 m (2H)	2.30 m (2H)				
5′	2.12 m (1H)	2.18 m (1H)	2.18 m (1H)	2.17 m (1H)				
6′	0.98 d (3H, 6.5)	0.98 d (3H, 6.5)	0.99 d (3H, 6.5)	0.98 d (3H, 6.5)				
7′	0.96 d (3H, 6.5)	0.97 d (3H, 6.5)	0.98 d (3H, 6.5)	0.96 d (3H, 6.5)				
OCH_3	3.43 s (3H)	3.43 s (3H)						3.44 s (3H)

Table 2
^{13}C NMR (125 MHz) spectral data of compounds **1**–**8** in $CDCl_3$

Position	1	2	3	4	5	6	7	8
1	46.4, C	46.5, C	46.4, C	46.3, C	46.4, C	46.4, C	46.4, C	46.5, C
2	74.3, CH	75.3, CH	75.2, CH	75.5, CH	74.1, CH	74.2, CH	74.1, CH	74.1, CH
3	130.4, CH	130.5, CH	131.0, CH	131.3, CH	132.1, CH	132.1, CH	132.1, CH	131.3, CH
4	129.4, CH	129.4, CH	129.0, CH	128.5, CH	127.8, CH	127.8, CH	127.8, CH	128.6, CH
5	141.5, C	141.5, C	139.9, C	139.6, C	139.7, C	139.8, C	139.9, C	141.5, C
6	122.5, CH	122.5, CH	125.9, CH	122.3, CH	122.6, CH	122.4, CH	122.3, CH	122.7, CH
7	79.0, CH	79.0, CH	78.5, CH	78.7, CH	78.7, CH	78.7, CH	78.8, CH	78.9, CH
8	81.0, C	81.0, CH	80.9, C	81.0, C	81.1, C	81.1, C	81.1, C	80.9, C
9	63.7, CH	63.8, CH	63.6, CH	63.7, CH	63.7, CH	63.8, CH	63.8, CH	63.8, CH
10	32.6, CH	32.6, CH	32.6, CH	32.6, CH	32.7, CH	32.6, CH	32.6, CH	32.7, CH
11	58.1, C	58.1, C	58.2, C	58.4, C	58.4, C	58.4, C	58.4, C	58.4, C
12	73.2, CH	73.2, CH	72.7, CH	73.1, CH	73.3, CH	73.3, CH	73.3, CH	72.8, CH
13	66.4, CH	66.2, CH	66.3, CH	66.5, CH	66.6, CH	66.4, CH	66.6, CH	66.2, CH
14	73.8, CH	73.8, CH	73.6, CH	73.3, CH	73.7, CH	73.8, CH	73.3, CH	73.8, CH

Table 2 (*continued*)

Position	1	2	3	4	5	6	7	8
15	14.3, CH_3	14.3, CH_3	14.4, CH_3	14.5, CH_3	14.4, CH_3	14.4, CH_3	14.5, CH_3	14.4, CH_3
16	71.9, CH_2	71.9, CH_2	44.2, CH_2	62.8, CH_2	63.1, CH_2	62.8, CH_2	62.7, CH_2	72.1, CH_2
17	44.1, CH	44.1, CH	44.0, CH	4.1, CH	44.1, CH	44.1, CH	44.1, CH	44.1, CH
18	6.30, CH_3	6.34, CH_3	6.26, CH_3	6.35, CH_3	6.30, CH_3	6.33, CH_3	6.33, CH_3	6.29, CH_3
19	175.3, C	175.3, C	175.0, C	175.2, C	175.2, C	175.2, C	175.3, C	175.4, CH
20	49.0, CH_2	48.9, CH_2	49.0, CH_2	48.9, CH_2	48.8, CH_2	48.8, CH_2	49.0, CH_2	49.0, CH_2
Acetate methyls	21.5, CH_3	21.5, CH_3	21.5, CH_3	21.5, CH_3	21.5, CH_3	21.5, CH_3	21.5, CH_3	21.5, CH_3
	20.9, CH_3	20.9, CH_3	20.8, CH_3	20.7, CH_3	1.3, CH_3	21.3, CH_3	21.3, CH_3	21.4, CH_3
	20.7, CH_3	20.8, CH_3	20.5, CH_3	20.6, CH_3	20.8, CH_3	20.8, CH_3	20.9, CH_3	20.9, CH_3
	20.5, CH_3			20.5, CH_3	20.8, CH_3	20.7, CH_3	20.6, CH_3	
					20.7, CH_3			
					20.5, CH_3			
Acetate carbonyls	170.2, C	170.2, C	170.2, C	170.6, C	170.5, C	170.4, C	170.2, C	170.2, C
	170.1, C	170.0, C	170.1, C	170.2, C	170.2, C	170.2, C	69.9, C	170.0, C
	169.9, C	169.6, C	169.6, C	169.7, C	170.1, C	169.7, C	169.8, C	169.6, C
	169.6, C			169.6, C	169.8, C	169.6, C	169.4, C	
					169.8, C			
					169.6, C			
Isovalerate								
a		171.7, C	171.7, C	172.0, C		172.1, C	172.6, C	171.7, C
		42.6, CH_2	42.7, CH_2	42.7, CH_2		43.2, CH_2	43.3, CH_2	43.5, CH_2
		25.0, CH	25.6, CH	25.6, CH		25.7, CH	25.7, CH	25.7, CH
		22.3, CH_3	22.3, CH_3	22.3, CH_3		22.3, CH_3	22.4, CH_3	22.4, CH_3
		22.4, CH_3	22.4, CH_3	22.4, CH_3		22.4, CH_3	22.5, CH_3	22.3, CH_3
b						171.8, C	172.0, C	171.7, C
						42.6, CH_2	43.0, CH_2	42.6, CH_2
						25.0, CH	25.1, CH	25.0, CH
						22.3, CH_3	22.3, CH_3	22.3, CH_3
						22.4, CH_3	22.4, CH_3	22.4, CH_3
1′	166.5, C	166.6, C	167.0, C	166.6, C				
2′	60.8, CH_2	60.8, CH_2	60.8, CH_2	60.8, CH_2				
3′	172.3, C	172.4, C	172.4, C	172.4, C				
4′	42.7, CH2	42.7, CH_2	43.6, CH_2	43.0, CH_2				
5′	25.6, CH	25.6, CH	25.7, CH	25.6, CH				
6′	22.3, CH_3	22.3, CH_3	22.3, CH_3	22.3, CH_3				
7′	22.3, CH_3	22.3, CH_3	22.3, CH_3	22.4, CH_3				
OCH_3	58.4, CH_3	58.5, CH_3						58.4, CH_3

terns appeared to be similar to those of juncenolide D (**17**), it was evident that both **1** and **17** shared the same carbon framework. The ^1H and ^{13}C NMR spectra of **1** showed signals for four acetate groups assigned to C-9, C-12, C-13, and C-14 because their carbonyl carbons were correlated with the corresponding oxymethine protons in the HMBC spectrum of **1**. A minor difference between them lies on the appearance of an additional ester signal in **1**. The additional signals were assigned to be an $-OCOCH_2OCOCH_2CH(CH_3)_2$ unit, which was supported by the HMBC correlations of δ_H 4.51, 4.43 (each d, J=16.0 Hz, H-2′) with δ_C 166.5 (s, C-1′) and 172.3 (s, C-3′), of δ_H 2.29 (2H, m, H-4′) and 2.12 (1H, m, H-5′) with 172.3 (s, C-3′), and $^1H-^1H$ COSY spectrum show correlations of H-4′/H-5′/H-6′(H-7′). The additional ester was assigned to C-2 because of HMBC correlation of H-2 with δ_C 166.5 (s, C-1′). Accordingly, the planar structure of **1** was assigned and further supported by the $^1H-^1H$ COSY and HMBC spectra (Fig. 1).

According to the literature data on briarane diterpenoids,[12] when the chemical shifts of C-11 and C-20 of exocyclic C-11/C-20-epoxy groups resonated at δ_C 55–61 and 47–52, respectively, in briarane derivatives, the epoxy group is α-oriented (11*R*), thus leading to a chair conformation for the cyclohexane ring. Based on the carbon chemical shifts of C-11 and C-20 in **1**, we assigned the configuration of C-11/C-20 epoxide (δ_C 58.1, s, C-11; 49.0, t, C-20) in **1** as α-oriented. The *Z* configuration of the disubstituted double bond at C-3 was determined by the proton coupling constant (J=10.5 Hz) between the olefinic protons H-3 and H-4. The *E* configuration of the trisubstituted double bond at C-5 was demonstrated by the NOE correlation between H-6 and H-16 (Fig. 2). In the NOESY spectrum of **1**, NOE correlations between Me-15 with H-13/14/20, and H-20 with H-12 indicated that H-20, H-13, H-12, H-14, and Me-15 were all in the β-orientation; meanwhile, correlations of

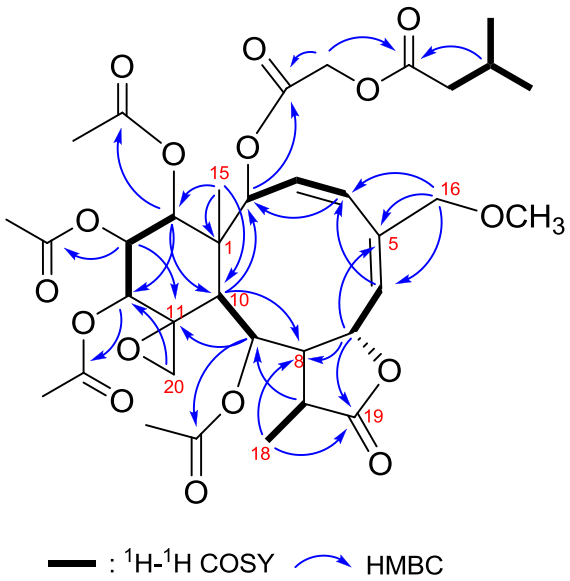


Fig. 1. The $^1H-^1H$ COSY and selective HMBC correlations of dichotellide F (**1**).

H-2 with H-10, H-9 with H-10, and Me-18 with H-9 and H-10 suggested that H-2, H-9, H-10, and Me-18 were all in the α-orientation, and the correlation of H-17 with H-7 showed the β-orientation of the H-17 and H-7 (Fig. 2). Taken together, these data led us to assign the relative configuration of dichotellide F (**1**) as 1*R**, 2*R**, 3*Z*, 5*E*, 7*S**, 8*R**, 9*S**, 10*S**, 11*R**, 12*R**, 13*R**, 14*R**, and 17*R**.

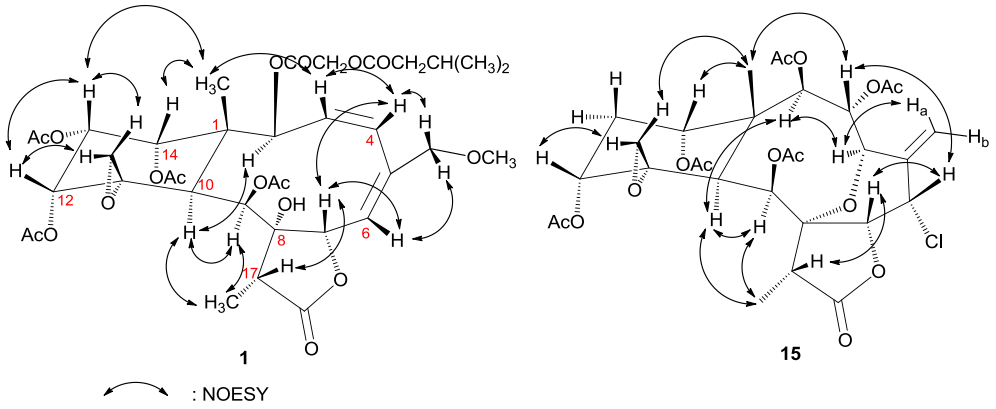


Fig. 2. Key NOESY correlations of dichotellides F (**1**) and T (**15**).

Dichotellides G–U (**2**–**14**) were analogues of dichotellide F (**1**). Close inspection of their NMR data revealed a general similarity: a 3, 5 (6)-conjugated diene and an exocyclic C-11/C-20 epoxide. All of them contained similar oxygenation patterns and they all had the same relative configuration based on the NOESY spectroscopic data. Their configurational assignments were made by comparison of their spectroscopic data with those of **1** and other known briarane type diterpenoids. Detailed NMR studies (including HSQC, HMBC, $^{1}H-^{1}H$ COSY, and NOESY spectra) on these new compounds were performed in order to unambiguously determine their structures and to assign all the proton and carbon resonances, and some of these data are described below.

Dichotellide G (**2**) possessed a molecular formula $C_{39}H_{54}O_{17}$ as determined from its positive HRESIMS at *m*/*z*, 817.3297 $[M+Na]^+$ (calcd for 817.3259). Its IR and UV spectra indicated hydroxyl (3450 cm^{-1}), γ-lactone (1783 cm^{-1}), ester carbonyls (1743, 1730 cm^{-1}), and a conjugated diene system (274 nm) in the structure. The ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2) were similar to those of **1**, with the only difference being the presence of an isovalerate at C-13 in **2** instead of an acetate in **1**, which was supported by the HMBC correlations of H-13/2′/3′ with C-1′ (δ_C 171.7, s). The relative configuration of **2** was assigned to be the same as that of **1** by comparison of NMR data and analysis of the NOESY spectrum.

Dichotellide H (**3**) had a molecular formula of $C_{38}H_{51}ClO_{16}$ by positive HRESIMS at *m*/*z*, 821.2786 $[M+Na]^+$ (calcd for 821.2673). The ESIMS spectrum of **3** showed a cluster of isotopic $[M+Na]^+$ ion peaks at *m*/*z* 821/823 in approximate ratio of 3:1, indicating the presence of a chlorine atom. The ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) were similar to those of **1**. The only difference between them was the replacement of an oxygenated C-16 methylene (δ_C 71.9, t; δ_H 4.53, 4.13, each d, *J*=16.0 Hz) and an acetate at C-12 in **1** by a chlorinated methylene (δ_C 44.2, t; δ_H 4.68, 4.45, each d, *J*=14.5 Hz) and an isovalerate at C-12, respectively, which were proved by the HMBC correlations of H-16 with C-4/C-5/C-6, and H-12/2′/3′ with the carbonyl (δ_C 171.7, C-1′) of isovalerate group.

Dichotellide I (**4**) possessed a molecular formula of $C_{40}H_{54}O_{18}$ by its HRESIMS at *m*/*z*, 845.3189 $[M+Na]^+$ (calcd for 845.3208), and it exhibited ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) very similar to those of **1** except that the methoxyl at C-16 was replaced by an isovalerate, which was supported by the HMBC correlations of H-16/2′/3′ with the carbonyl (δ_C 172.0, C-1′) of isovalerate group.

Dichotellide J (**5**) showed a molecular ion peak at *m*/*z* 703.2201 $[M+Na]^+$ (calcd for 703.2214) in its positive HRESIMS, corresponding to the molecular formula of $C_{32}H_{40}O_{16}$. Its ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2) matched with those of **17** with the only difference of an acetate group at C-16 in **5** instead of a methoxy group. This was confirmed by the HMBC spectrum with correlations of δ_H 5.39, 4.64 (each d, *J*=15.5 Hz) and 2.13 (3H, s) with δ_C 170.1 (s).

Dichotellide K (**6**) was assigned the molecular formula $C_{38}H_{52}O_{16}$ by its HRESIMS at *m*/*z* 787.3145 $[M+Na]^+$. Its NMR spectroscopic data (Tables 1 and 2) were similar to those of **5** with the exception that the two acetate groups instead of two isovalerate groups. Careful inspection of the ^{13}C NMR data of **6** revealed the difference from those of **5**: the oxygenated methine at C-13 and C-16 position in **6** was shifted upfield by 0.4 ppm and 0.6 ppm, respectively, which supported the replacement of two acetates with two isovalerate groups. This assignment was confirmed by the HMBC correlations of H-13/H-16 to the corresponding carbonyl group (δ_C 172.1, 171.8) of the isovalerate group, respectively.

The HRESIMS and NMR data of dichotellide (**7**) suggested the same molecular formula $C_{38}H_{52}O_{16}$ as **6**. In addition, the two compounds exhibited virtually the same IR, ^{1}H, and ^{13}C NMR spectroscopic data. However, detailed comparison of the HMBC spectrum of **7** with that of **6** revealed that the subtle difference between them were the substituent positions of an isovalerate group and an acetate group. In compound **7**, an isovalerate group was at C-14 instead of at C-13, and an acetate group was at C-13 rather than C-14. These were deduced from HMBC correlations of δ_H 5.26 (d, *J*=2.5 Hz, H-14), 2.31 (2H, m, H-2′), 2.99 (1H, m, H-3′) with δ_C 172.6 (s, C-1′), and correlations of δ_H 5.08 (t, *J*=3.0 Hz, H-13) with δ_C 170.2 (s). Based on these data, the structure of dichotellide L (**7**) was established.

Dichotellide M (**8**) had a molecular formula of $C_{37}H_{52}O_{15}$ as suggested by its positive HRESIMS at *m*/*z*, 759.3213 $[M+Na]^+$ (calcd for 759.3204). Its ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) showed high similarity to those of **17**, except that two acetate groups at C-12 and C-13 in **17** were replaced by two isovalerate groups in **8**, respectively. This can be deduced by an upfield shift of 0.5 ppm for C-12 and 0.3 ppm for C-13 in the ^{13}C NMR spectrum of **8** relative to **17** (δ_C 73.3, C-12; 66.5, C-13), and confirmed by the HMBC correlations of H-12/H-13 to the corresponding carbonyl group of the isovalerate group, respectively.

Dichotellide N (**9**) was isolated as a white amorphous powder with the molecular formula $C_{32}H_{44}O_{13}$ as deduced by its HRESIMS at *m*/*z* 659.2691 $[M+Na]^+$. The ^{1}H and ^{13}C NMR spectral data (Tables 3 and 4) of **9** were nearly identical with those of **18** with the only difference due to the presence of an isovalerate group at C-14, instead of an acetate group found in **18**. This assignment was confirmed by the HMBC correlations of H-14/H-2′/H-3′ to the carbonyl group of the isovalerate.

Dichotellide O (**10**) showed the same molecular formula of $C_{32}H_{44}O_{13}$ as **9**, which indicated by its positive HRESIMS. In addition, the two compounds shared almost the same IR, ^{1}H, and ^{13}C NMR spectroscopic data. However, close comparison of the overall

Table 3
^{1}H NMR (500 MHz) data of compounds **9**–**16** (*J* in Hz within parentheses)

Position	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
2	5.64 d (9.5)	5.70 d (9.5)	5.66 d 10.0)	5.88 d (10.0)	5.96 d (10.0)	5.95 d (10.0)	5.51 d (7.0)	4.82 d (4.5)
3	5.57 t (10.0)	5.59 t (10.0)	5.61 t (10.0)	5.71 t (10.0)	5.72 t (10.0)	5.68 t (10.0)	6.21 dd (10.5, 7.0)	2.79 t (14.0) 1.73 m
4	6.26 d (10.0)	6.27 d (10.0)	6.27 d (10.0)	6.39 d (10.0)	6.27 d (10.0)	6.27 d (10.0)	4.98 d (10.5)	5.13 dd (13.0, 5.5)
6	5.88 d (9.5)	5.89 d (8.5)	5.74 d (9.0)	5.93 d (8.0)	5.75 d (8.5)	5.75 d (8.5)	4.95 d (2.5)	5.77 d (10.0)
7	5.01 d (9.5)	5.01 d (8.5)	4.98 d (9.0)	4.96 d (8.5)	4.97 d (8.5)	4.97 d (8.5)	4.41 d (2.5)	5.62 d (10.0)
9	4.80 d (4.5)	4.80 d (4.5)	4.80 d (5.0)	4.79 d (5.0)	4.79 d (4.5)	4.80 d (4.5)	5.63 s	5.22 d (5.5)
10	3.66 d (4.5)	3.67 d (4.5)	3.61 d (5.0)	3.55 d (5.0)	3.59 d (4.5)	3.57 d (4.5)	3.32 s	3.23 d (5.5)
12	4.54 br s	4.57 br s	4.52 br s	3.62 br s	3.59 br s	3.58 br s	4.53 s	2.26 m 1.75 m
13	2.24 m 2.05 m	2.24 m 2.05 m	2.26 m 1.96 m	4.84 t(3.0)	4.83 t (2.5)	4.82 t (3.0)	2.28 m 1.99 m	2.04 m 1.72 m
14	4.94 t (3.0)	4.89 t (3.0)	4.97 d (3.0)	3.77 d (2.5)	3.75 d (2.5)	3.77 br s	4.97 s	4.74 d (5.0)
15	1.04 s	1.04 s	1.04 s	1.25 s	1.28 s	1.25 s	1.26 s	1.11 s
16	4.48 d (15.0) 4.23 d (15.0)	4.48 d (15.0) 4.25 d (15.0)	5.36 d (16.0) 4.65 d (16.0)	4.62 d (12.0) 4.38 d (12.0)	5.05 d (15.0) 4.73 d (15.0)	5.05 d (15.0) 4.74 d (15.0)	5.57 s 5.36 s	5.35 dd (16.0, 2.0) 4.75 dd (16.0, 2.0)
17	2.32 q (7.0)	2.32 q (7.0)	2.28 q (7.0)	2.31 q (7.0)	2.30 q (7.0)	2.30 q (7.0)	2.79 q (7.0)	2.46 q (7.0)
18	1.14 d (7.0)	1.14 d (7.0)	1.14 d (7.0)	1.16 d (7.0)	1.15 d (7.0)	1.16 d (7.0)	1.39 d (7.0)	1.12 d (7.0)
20	3.53 d (2.5) 2.79 d (2.5)	3.54 d (3.0) 2.79 d (3.0)	3.52 d (2.0) 2.79 d (2.0)	3.53 d (2.5) 2.78 d (2.5)	3.50 d (2.5) 2.77 d (2.5)	3.49 d (2.0) 2.76 d (2.0)	2.82 d (3.0) 2.52 d (3.0)	4.98 s 4.86 s
Acetate methyls	2.18 s 2.09 s 1.95 s	2.19 s 2.02 s 1.96 s	2.18 s 2.11 s 1.94 s	2.19 s 2.06 s	2.18 s 2.06 s	2.18 s 2.16 s 2.05 s	2.32 s 2.05 s 2.03 s 2.03 s 2.00 s	2.25 s 2.05 s 1.98 s 1.95 s
Isovalerate								
a	2.24 m (2H) 2.11 m (1H) 0.97 d (3H, 6.5) 0.94 d (3H, 6.5)	2.25 m (2H) 2.12 m (1H) 0.98 d (3H, 6.5) 0.96 d (3H, 6.5)	2.23 m (2H) 2.11 m (1H) 0.98 d (3H, 6.5) 0.96 d (3H, 6.5)	2.31 m (2H) 2.11 m (1H) 0.98 d (3H, 6.5) 0.96 d (3H, 6.5)	2.28 m (2H) 2.10 m (1H) 0.97 d (3H, 6.5) 0.96 d (3H, 6.5)	2.26 m (2H) 2.11 m (1H) 0.98 d (3H, 6.5) 0.96 d (3H, 6.5)		2.27 m (2H) 2.12 m (1H) 0.98 d (3H, 7.0) 0.97 d (3H, 7.0)
b			2.15 m (2H) 2.10 m (1H) 0.90 d (3H, 6.5) 0.88 d (3H, 6.5)		2.29 m (2H) 2.11 m (1H) 0.97 d (3H, 6.5) 0.96 d (3H, 6.5)			
OCH_3	3.45 s (3H)	3.45 s (3H)						

Table 4
^{13}C NMR (125 MHz) spectral data of compounds **9**–**16** in $CDCl_3$

Position	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	47.2, C	47.2, C	47.1, C	47.3, C	47.5, C	47.4, C	46.7, C	47.4, C
2	74.4, CH	74.3, CH	74.5, CH	75.1, CH	75.1, CH	75.2, CH	72.6, CH	72.0, CH
3	131.7, CH	131.7, CH	132.5, CH	131.8, CH	132.4, CH	132.4, CH	63.7, CH	38.2, C
4	128.2, CH	128.3, CH	127.5, CH	127.8, CH	127.2, CH	127.2, CH	78.9, CH	69.2, CH
5	141.6, C	141.5, C	140.0, C	139.9, C	139.4, C	139.4, C	134.1, C	142.0, C
6	122.7, CH	122.8, CH	122.5, CH	126.7, CH	123.2, CH	122.3, CH	53.8, CH	121.9, CH
7	79.1, CH	79.0, CH	78.7, CH	78.8, CH	78.9, CH	79.0, CH	79.0, CH	76.4, CH
8	81.2, C	81.0, C	81.2, C	81.3, C	81.2, C	81.2, C	82.9, C	83.2, C
9	64.1, CH	64.1, CH	64.1, CH	64.0, CH	64.2, CH	64.2, CH	70.7, CH	70.9, CH
10	32.9, CH	32.9, CH	32.8, CH	30.7, CH	30.6, CH	30.6, CH	36.3, CH	42.3, CH
11	59.1, C	59.1, C	59.2, C	60.4, C	60.4, C	60.4, C	56.4, C	151.0, C
12	73.0, CH	72.5, CH	73.0, CH	76.0, CH	75.9, CH	75.9, CH	73.6, CH	25.6, CH_2
13	29.0, CH_2	28.8, CH_2	29.0, CH_2	67.8, CH	67.9, CH	68.2, CH	28.8, CH_2	27.6, CH_2
14	73.2, CH	73.7, CH	73.1, CH	77.1, CH	76.9, CH	77.1, CH	73.2, CH	73.2, CH
15	14.3, CH_3	14.1, CH_3	14.1, CH_3	13.7, CH_3	13.7, CH_3	13.7, CH_3	15.2, CH_3	15.2, CH_3
16	72.3, CH_2	72.4, CH_2	62.9, CH_2	46.1, CH_2	63.5, CH_2	63.5, CH_2	119.7, CH_2	65.5, CH_2
17	44.2, CH	44.2, CH	44.2, CH	44.2, CH	44.2, CH	44.3, CH	49.5, CH	42.3, CH
18	6.38, CH_3	6.33, CH_3	6.30, CH_3	6.36, CH_3	6.36, CH_3	6.36, CH_3	7.01, CH_3	6.48, CH_3
19	175.6, C	175.5, C	175.4, C	175.2, C	175.5, C	175.5, C	174.1, C	175.8, C
20	49.2, CH_2	49.1, CH_2	49.0, CH_2	48.6, CH_2	48.5, CH_2	48.4, CH_2	50.1, CH_2	112.9, CH_2
Acetate methyls	21.6, CH_3 21.3, CH_3 21.2, CH_3	21.6, CH_3 21.2, CH_3 21.1, CH_3	21.5, CH_3 21.2, CH_3 21.1, CH_3	21.5, CH_3 21.3, CH_3	21.5, CH_3 21.3, CH_3	21.5, CH_3 21.4, CH_3 21.2, CH_3	21.1, CH_3 21.0, CH_3 20.9, CH_3 20.4, CH_3 20.3, CH_3	21.9, CH_3 21.1, CH_3 21.0, CH_3 20.8, CH_3
Acetate carbonyls	170.3, C 170.0, C 169.1, C	170.3, C 170.0, C 169.3, C	170.2, C 170.1, C 169.1, C	170.2, C 170.2, C	170.5, C 170.2, C	170.5, C 170.3, C 170.2, C	170.2, C 169.9, C 169.7, C 169.4, C 169.4, C	171.0, C 170.8, C 170.1, C 169.4, C

(*continued on next page*)

Table 4 (*continued*)

Position	9	10	11	12	13	14	15	16
Isovalerate								
a	172.0, C	172.0, C	172.3, C	172.5, C	172.6, C	172.5, C		171.9, C
	43.1, CH_2	43.6, CH_2	43.3, CH_2	43.2, CH_2	43.3, CH_2	43.3, CH_2		43.1, CH_2
	24.8, CH	25.6, CH	25.6, CH	25.7, CH	25.8, CH	25.7, CH		25.6, CH
	22.6, CH_3	22.5, CH_3	22.3, CH_3	22.3, CH_3	22.3, CH_3	22.5, CH_3		22.5, CH_3
	22.4, CH_3	22.4, CH_3	22.4, CH_3	22.2, CH_3	22.4, CH_3	22.4, CH3		22.4, CH_3
b			172.0, C		172.5, C			
			42.9, CH_2		43.2, CH_2			
			24.8, CH		25.7, CH			
			22.3, CH_3		22.3, CH_3			
			22.4, CH_3		22.4, CH_3			
OCH_3	58.4, CH_3	58.4, CH_3						

NMR data indicated that **9** and **10** were isomers and exhibited significant variations in the ^{13}C NMR chemical shifts for C-12 and C-14. Compared to **9**, C-12 was shifted upfield 0.5 ppm, while C-14 was shifted downfield 0.5 ppm, which supported that the isovalerate group at C-14 in **9** was located to C-12 in **10**. This assignment was confirmed by the HMBC correlations of H-12/H-2′/H-3′ with the carbonyl group of the isovalerate, and correlation of H-20 with the C-12 (δ_C 72.4, d).

Dichotellide P (**11**) was obtained as a white amorphous powder, its molecular formula was $C_{36}H_{50}O_{14}$ based on its HRESIMS coupled with the NMR spectroscopic data. The NMR features of **11** were analogous to those of **9** with the exception that the methoxy group at C-16 in **9** was replaced by an isovalerate group, which was supported by the HMBC correlations of H-16 with the corresponding carbonyl group.

The molecular formula of dichotellide Q (**12**), $C_{29}H_{39}ClO_{12}$, was determined by its HRESIMS at *m*/*z*, 637.2035 $[M+Na]^+$. The structure of **12** was closely related to **19** due to the close similarity of the NMR data. The distinction was ascribed to the substitutions of C-13 and C-16, namely, the replacement of a hydroxyl at C-16 and an acetate at C-13 in **19** by a chlorine atom and an isovalerate at C-13, respectively. This assignment were proved by the HMBC correlations of H-6/H-4 with C-16 (δ_C 46.1, t), and H-13/2′/3′ with the carbonyl (δ_C 172.5, C-1′) of an isovalerate group.

The HRESIMS and NMR data gave the molecular formula of dichotellide R (**13**) as $C_{34}H_{48}O_{14}$. A comparison of the NMR, UV, and IR data revealed that **13** differed from **12** only in the substitution at C-16, where an isovalerate group of **13** replaced a chlorine atom of the latter, which was supported by the additional signals for an isovalerate group and the HMBC correlations of H-16/2′/3′ with the corresponding carbonyl carbon.

Dichotellide S (**14**) showed a molecular ion peak at *m*/*z*, 661.2430 $[M+Na]^+$ in its HRESIMS, corresponding to the molecular formula of $C_{31}H_{42}O_{14}$. Its ^1H and ^{13}C NMR spectroscopic data (Tables 3 and 4) were resembled those of **19** with the only difference of an additional isovalerate group at C-16 because of the HMBC correlation of δ_H 5.05, 4.74 (each d, *J*=15.0 Hz, H-16) with the carbonyl group (δ_C 172.5, s).

Dichotellide T (**15**) was assigned the molecular formula of $C_{30}H_{37}ClO_{14}$ on the basis of its HRESIMS (*m*/*z*, 679.1877 $[M+Na]^+$). The ESIMS spectrum of **15** showed a pair of peaks at *m*/*z*, 679/681 $[M+Na]^+$ in approximate ratio of 3:1, indicating the presence of a chlorine atom in the molecule. The IR spectrum suggested the presence of hydroxyl (3480 cm^{-1}), a γ-lactone (1782 cm^{-1}), ester carbonyls (1744 cm^{-1}). The ^1H and ^{13}C NMR spectroscopic data (Tables 3 and 4) of **15** were similar to those of **20**, indicating that both bearing identical substituent, such as C4/8 ether bridge, exocyclic methylene at C-5, secondary acetates (C-2, C-3, C-9, and C-14), the same 6-chlorinated methine, and the same C-11/C-20 exocyclic epoxy. A minor difference between them lies on the appearance of an additional signal for an acetate group attached at C-12 in **15**, which was supported by the HMBC correlations from H-12 (δ_H 4.57, s) to the carbonyl carbon (δ_C 169.9, s), C-10 (δ_C 36.3, d), C-11 (δ_C 56.4, s), and C-14 (δ_C 73.2, d), from H-20 to C-12 and C-11. Thus, the planar structure of **15** was established.

Comparison of ^{13}C NMR chemical shifts of C-11 and C-20 of **15** with those of **1** (δ_C 58.1, s, C-11; 49.0, t, C-20) showed that the stereochemistry of C-11/C-20 exocyclic epoxy in **15** should be α-oriented, leading to the configuration of cyclohexane ring in a chair form. The relative configuration of the 13 chiral centers of **15** was deduced from its NOESY spectrum (Fig. 2). In the NOESY spectrum, NOE correlations between H-2 with H-10, H-9 with H-10, and Me-18 with H-9 and H-10 suggested α-configuration of H-2, H-9, H-10, and Me-18, while correlations of Me-15 with H-14/H-20/H-3, H-20 with H-12, and H-3 with H-6 indicated the β-orientation of H-6, H-20, H-12, H-14, and Me-15, with a corresponding correlation of H-17 with H-7 suggesting the β-orientation of the H-17 and H-7. Moreover, H-4 showed correlations with H-2 and one proton of C-16 methylene (δ_H 5.36, s, Ha-16), and a large coupling constant was found between H-4 and H-3 (*J*=10.5 Hz), implying that the dihedral angle between H-3 and H-4 is approximately 180° and H-4 has an α-orientation at C-4.

The absolute configuration of **15** was deduced by a single-crystal X-ray diffraction analysis (Fig. 3). The absolute configuration of C-6 was assigned as 6*S*, and all other chiral centers were unambiguously determined.

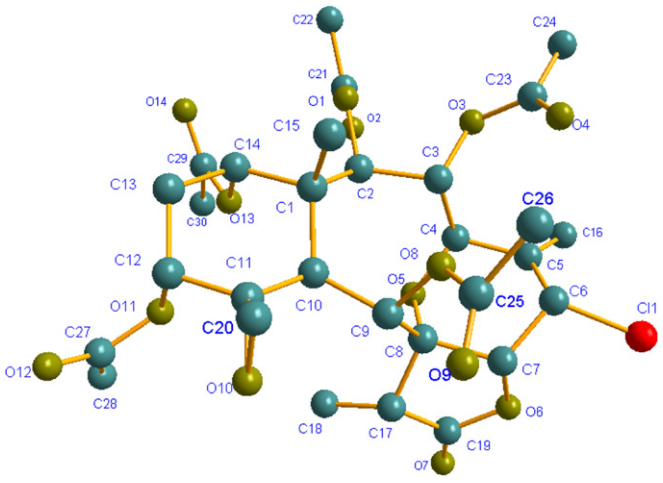


Fig. 3. Perspective drawing of the X-ray structure of dichotellide T (**15**).

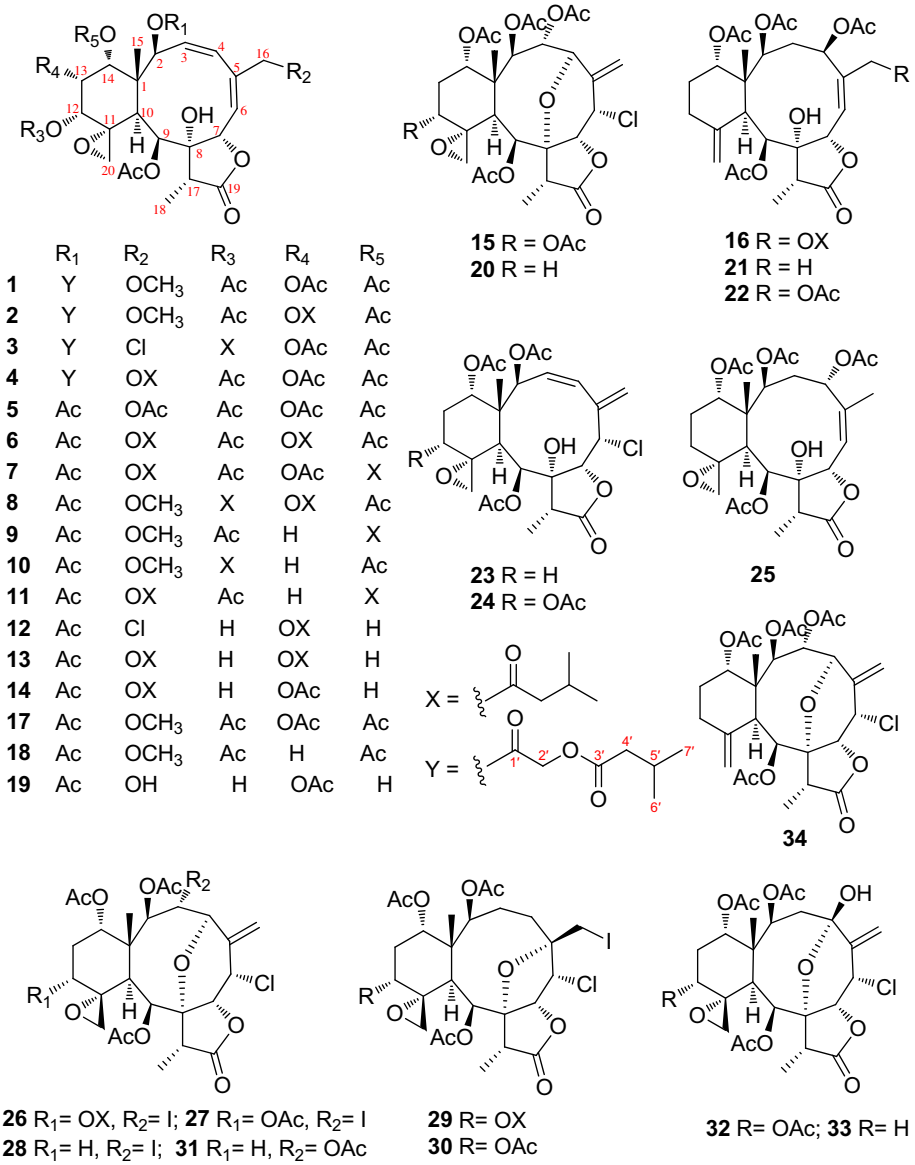
Dichotellide U (**16**) was obtained as a colorless powder with the molecular formula of $C_{33}H_{46}O_{13}$ as determined by its positive HRESIMS (*m*/*z*, 673.2820 $[M+Na]^+$). The IR spectrum suggested hydroxyl (3460 cm^{-1}), a γ-lactone (1776 cm^{-1}), ester carbonyls (1726 cm^{-1}) in the structure. The ^1H and ^{13}C NMR spectroscopic

data (Tables 3 and 4) of **16** were closely related to those of **21**, and both bearing identical substituents, such as exocyclic methylene at C-11, secondary acetates (C-2, 4, 9, 14), and the same trisubstituted double bond at C-5/C-6. A small distinction was ascribed to the presence of an additional signal for an isovalerate group attached at C-16 in **16**, as indicated by the ^{13}C NMR resonance at δ_C 171.9 (s), 43.1 (t), 25.6 (d), 22.5 (q), 22.4 (q). This assignment was confirmed by the HMBC correlations from H-16 (δ_H 5.35, 4.75, each dd, J=16.0, 2.0 Hz) to the carbonyl carbon (δ_C 171.9, s), C-4 (δ_C 69.2, d), and C-6 (δ_C 121.9, d).

The relative stereochemistry of **16** was assigned by NOESY spectrum, in which NOE correlation between H-6 and H-16 led the assignment of *E* configuration of the trisubstituted double bond at C-5. The NOE correlations of H-2 with H-10/H-4, H-9 with H-10, and Me-18 with H-9 and H-10 indicated that H-2, H-4, H-9, H-10, and Me-18 were all in the α-configuration, while, correlations of Me-15 with H-14/H-17, and H-17 with H-7 suggested the β-orientation of H-7, H-14, and Me-15. Based on these data, the relative configuration of **16** was assigned as 1*R**, 2*S**, 4*R**, 5*E*, 7*S**, 8*R**, 9*S**, 10*S**, 14*S**, and 17*R**, and its structure was elucidated as shown in Fig. 4.

The structure of eighteen known compounds were determined to be juncenolide D (**17**),[13] gemmacolide N (**18**),[11] juncin Q (**19**),[14] praelolide (**20**),[15] junceellolide D (**21**),[16] juncin Y (**22**),[17] junceellolide C (**23**),[16] juncin D (**24**),[18] (+)-11α, 20α-epoxyjunceellolide D (**25**),[19] dichotellides A–E (**26**–**30**),[9] praelolide (**31**),[15] juncin P (**32**),[14] juncin ZI (**33**),[17] and junceellin A (**34**)[19] by a comparison of the NMR spectroscopic data with those reported in the literature.

All the isolated briarane type diterpenoids (**1**–**34**) were evaluated for antifouling activity against the larval settlement of barnacle *B. amphitrite* according to literature procedures.[20] Ten of the thirty-four compounds tested completely inhibited the larval settlement of *B. amphitrite* at a concentration of 25.0 μg/mL (nontoxic concentration). Compound **23** showed a significant inhibitory effect on larval settlement even at a concentration of 5.0 μg/mL (none of the larvae settled). Further investigation revealed that compound **3**, **4**, **6**–**11**, **16**, and **23** had potent antifouling activities at nontoxic



	R_1	R_2	R_3	R_4	R_5
1	Y	OCH_3	Ac	OAc	Ac
2	Y	OCH_3	Ac	OX	Ac
3	Y	Cl	X	OAc	Ac
4	Y	OX	Ac	OAc	Ac
5	Ac	OAc	Ac	OAc	Ac
6	Ac	OX	Ac	OX	Ac
7	Ac	OX	Ac	OAc	X
8	Ac	OCH_3	X	OX	Ac
9	Ac	OCH_3	Ac	H	X
10	Ac	OCH_3	X	H	Ac
11	Ac	OX	Ac	H	X
12	Ac	Cl	H	OX	H
13	Ac	OX	H	OX	H
14	Ac	OX	H	OAc	H
17	Ac	OCH_3	Ac	OAc	Ac
18	Ac	OCH_3	Ac	H	Ac
19	Ac	OH	H	OAc	H

Fig. 4. The chemical structures of compounds **1**–**34**.

concentrations with EC_{50} values of 4.1, 1.82, 6.3, 7.6, 4.6, 1.2, 5.6, 0.79, 2.0, and 0.2 μg/mL, respectively (Table 5), which were lower than the standard requirement of an EC_{50} of 25 μg/mL established by the U.S. Navy program as an efficacy level for natural antifoulants, indicating that these compounds are potential natural nontoxic antifouling agents.

Table 5
Antifouling activity

Compounds	EC_{50} (μg/mL)	LC_{50}/EC_{50}
3	4.1	>24
4	1.82	>54.9
6	6.3	>16
7	7.6	>13
8	4.6	>11
9	1.2	>88
10	5.6	>18
11	0.79	>126.6
16	2.0	>48
23	0.2	>500

The above results indicated that compound **4**, **9**, **11**, **16**, and **23** showed better potent antifouling activity against the larval settlement of barnacle *B. amphitrite* with EC_{50} values equal to or less than 2.0 μg/mL, especially for compound **23**, which was the most potent molecule, with small EC_{50} values of 0.2 μg/mL, and present in relatively high amounts in the crude extract. Furthermore, the therapeutic ratio (LC_{50}/EC_{50}) is a way of expressing the effectiveness of the compound in relation to its toxicity, and the desired target ratio should be much greater than 15 for use in an antifouling coating. Compounds **4**, **9**, **11**, **16**, and **23** all have high therapeutic ratios (Table 5), suggesting that they might be useful as environmentally benign antifouling agents.

We also evaluated the newly isolated briaranes (**1**–**34**) in the cytotoxicity studies against four tumor cell lines. Only **28** showed marginal activity against SW1990 cells (IC_{50}=45.0 μM), while the rest of briaranes were not cytotoxic toward MCF-7, SW1990, HepG2, and H460 cells.

To the best of our knowledge, there have been only three reports on the briarane type analogues with potent antifouling activity against larval settlement: renillafoulins A–C (EC_{50}: 0.02–0.2 μg/mL),[21] juncins R–ZI (EC_{50}: 0.004, 0.34, 2.65, 1.61, 3.77, 21.06, 0.004, 0.14, 1.47, and 0.51 μg/mL),[17] juncin ZII, gemmacolide B, gemmacolide A, and junceellolide D (EC_{50}: 0.004, 0.005, 2.82, and 0.447 μg/mL).[22] So far no clear structure–activity relationships have been established.[3] This report describes additional briarane type diterpenoids with significant activity against barnacle *B. amphitrite* larvae (Table 5) and preliminary structure–activity relationship findings. In general, the derivatives with the diene moiety (**3**, **4**, **6**–**11**, and **23**) appear to be more potent than those with one double bond. There are some exceptions as the olefin function may not be the only factor to determine the activity. Based on our studies, we tentatively conclude the following structure–activity relationships: (1) lactone and diene are essential for the activity (one double bond derivative can also be potent, is diene essential for activity?). (2) The activity also depends on the number of the substitutes on the ten-membered ring. (3) Both the number and the location of double bonds on the lactone ring affect the potency. For example, exocyclic double bond increases the activity. (4) Esterification of the hydroxy on the cyclohexane ring enhances the activity, and the number of the substitutes of on the cyclohexane ring also influences the activity. (5) The iodinated and chlorinated derivatives have little impact on the activity. These observations should be valuable in the discovery of novel briarane derivatives with potent antifouling activity.

3. Experimental

3.1. General procedures

Optical rotation values were measured with a Perkin–Elmer 341 polarimeter. UV spectra were recorded on a Shimadzu UV-2401PC spectrometer with KBr pellets. IR spectra were measured on JASCO FT/IR-480 plus spectrometers. NMR spectra were collected using Bruker 500 MHz NMR spectrometers with TMS as an internal standard at room temperature (δ in parts per million, J in Hertz). HRESIMS (including ESIMS) spectra were recorded on an Applied Biosystems Mariner 5140 spectrometer. The column chromatography was applied on the Büchi Sepacore (C-615/605) system. All solvents used were of analytical grade (Tianjin Fuyu Chemical and Industry Factory). Silica gel and preparative TLC plates (20×20×0.04 cm) (Qingdao Mar. Chem. Ind. Co. Ltd.), Sephadex LH-20 gel (Pharmacia), and C_{18} reverse-phased silica gel (150–200 mesh, Merck) were used for chromatography.

3.2. Animal material

The gorgonians *D. gemmacea* and *J. fragilis* were collected from Meishan Island, Hainan province of China in April 2009 (7–10 m depth) and identified by Professor Hui Huang, South China Sea Institute of Oceanology, Chinese Academy of Sciences. Voucher specimen (No. M090405 and M090408) have been deposited in the Key Laboratory of Marine Bio-resources Sustainable Utilization, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, China.

3.3. Extraction and isolation

The fresh gorgonian *D. gemmacea* (ca. 4.0 kg) was exhaustively extracted with 95% EtOH twice, and $CHCl_3$/MeOH (1:1) for one time at room temperature. After evaporation of the solvent in vacuum, the combined residue was suspended in H_2O and partitioned with EtOAc and *n*-BuOH to provide the EtOAc extract (18.0 g) and the *n*-BuOH extract (5.0 g). The EtOAc extract was chromatographed by silica gel column (CC) (300–400 mesh), eluting with a gradient of petroleum ether (PE)/Me_2CO (50:1–0:100), to obtain 15 fractions (F_1–F_{15}). Fraction F_8 (1.4 g) was divided into six subfractions ($F_{8\text{-}1}$–$F_{8\text{-}6}$) by medium pressure liquid chromatography (MPLC) eluting with PE/EtOAc (90:10–0:100). Compounds **5** (4.6 mg), **16** (6.0 mg), and **18** (13.0 mg) were obtained after CC over LH-20 ($CHCl_3$/MeOH, 1:1) followed by repeated Pre. TLC using $CHCl_3$/Me_2CO (10:1) or *n*-hexane/EtOAc (2:1) from subfractions $F_{8\text{-}2}$, $F_{8\text{-}5}$, and $F_{8\text{-}6}$, respectively. From fraction F_9 (1.0 g), **21** (16.0 mg), **25** (13.0 mg), and **15** (5.6 mg) were isolated after CC over LH-20 ($CHCl_3$/MeOH, 1:1) and Pre. TLC using $CHCl_3$/Me_2CO (9:1) or PE/EtOAc (3:2). Fraction F_{10} (1.3 g) was subjected to CC over ODS (MeOH/H_2O, 30:70–100:0) and LH-20 ($CHCl_3$/MeOH, 1:1), followed by repeated Pre. TLC using $CHCl_3$/MeOH (20:1) and/or *n*-hexane/EtOAc (1:1) led to the isolation of **10** (7.0 mg), **4** (6.4 mg), **23** (49.0 mg), and **24** (11.0 mg). Fraction F_{11} (1.6 g) was subjected to CC on silica, eluted with $CHCl_3$/Me_2CO (15:1–9:1) to give three subfractions ($F_{11\text{-}1}$–$F_{11\text{-}3}$), subfractions $F_{11\text{-}1}$ was subjected to CC on silica gel, eluting with *n*-hexane–EtOAc (2:1) to give **6** (7.5 mg); subfractions $F_{11\text{-}2}$ and $F_{11\text{-}3}$ were chromatographed over LH-20 eluted with $CHCl_3$/MeOH (1:1) then purified with semi-preparative reversed-phase HPLC using MeOH/H_2O (65:35) as a mobile phase to obtain **9** (6.7 mg), **12** (3.8 mg), and **3** (7.2 mg), and using MeOH/H_2O (60:40) as eluent to yield **1** (14.0 mg), **7** (5.6 mg), and **8** (6.0 mg). Similarly, **2** (5.5 mg), and **13** (3.8 mg) were isolated from Fraction F_{12}. Fraction F_{13} (1.8 g) was subjected on LH-20 (MeOH), followed by silica gel CC and eluted with PE/EtOAc (5:3–1:1) to get five

subfractions ($F_{13\text{-}1}$–$F_{13\text{-}5}$). $F_{13\text{-}2}$ was further applied on silica gel CC and eluted using hexane/EtOAc (2:1) to afford **20** (21 mg) and **11** (5.0 mg). $F_{13\text{-}4}$ was subjected to silica gel CC, eluting with $CHCl_3$/Me_2CO (12:1) afforded **17** (8.0 mg). A further isolation of $F_{13\text{-}5}$ yielded **22** (10.5 mg) by Pre. TLC with $CHCl_3$/Me_2CO (10:1) as developer. Fraction F_{14} (900 mg) was fractionalized into three subfractions ($F_{14\text{-}1}$–$F_{14\text{-}3}$), by silica gel CC, and eluted with a solvent system of $CHCl_3$/Me_2CO (10:1–8:1). $F_{14\text{-}1}$ was subjected to silica gel CC and eluted with *n*-hexane/EtOAc (3:2), to yield **14** (9.0 mg). $F_{14\text{-}3}$ was subjected to silica gel CC, eluted with PE/EtOAc (3:1), followed by Pre. TLC ($CHCl_3$/MeOH (20:1)) provided **19** (8.6 mg). $Fr._{15}$ (1.2 g) was subjected on Sephadex LH-20 (MeOH), followed by silica gel CC, eluting with PE/EtOAc (5:3), to get five subfractions ($Frs._{15a-15e}$). Fr. Db was further applied on silica gel CC, eluted using hexane/EtOAc, to afford compounds **26** (9.6 mg, 3:1) and **28** (4.0 mg, 2:1). Fr. Dc was subjected to silica gel CC, eluting with $CHCl_3$/Me_2CO (10:1), to afford **29** (5.6 mg). A further isolation of Fr. De yielded **30** (6.3 mg) by preparative thin layer chromatography (Pre. TLC, one plate, 20×20×0.05 cm) with PE/EtOAc (1:1, R_f 0.6) as developer. Fr. E (900 mg) was subjected to silica gel CC, eluting with $CHCl_3$/Me_2CO (from 10:1 to 8:1), to give four subfractions (E_1–E_4), Frs. E_2 and E_4 were chromatographed over Sephadex LH-20, eluting with $CHCl_3$/MeOH (1:1), respectively, followed by silica gel CC and eluted with *n*-hexane/EtOAc (2:1), to yield **31** (25 mg), **32** (11 mg) and **33** (7.8 mg), respectively. Fr. E_3 (83 mg) was subjected to silica gel CC, eluted with PE/EtOAc (3:2), followed by Pre. TLC (*n*-hexane/EtOAc 5:4, two plate, R_f 0.5) provided **27** (5.8 mg) and **34** (7.2 mg).

3.4. Characteristics of compounds

*3.4.1. Dichotellide F (**1**).* Colorless crystals (in acetone); $[\alpha]_D^{20}$ −20.0 (*c* 0.6, acetone); IR (KBr) ν_{max} 3433, 1793, 1745, 1730, 1595 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 500/125 MHz), see Tables 1 and 2; ESIMS *m*/*z* 775 $[M+Na]^+$; HRESIMS *m*/*z* 775.2805 $[M+Na]^+$ (calcd for $C_{36}H_{48}O_{17}Na$, 775.2789).

*3.4.2. Dichotellide G (**2**).* White amorphous powder; $[\alpha]_D^{20}$ −18.9 (*c* 0.56, acetone); IR (KBr) ν_{max} 3453, 1790, 1753, 1736, 1457 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 500/125 MHz), see Tables 1 and 2; ESIMS *m*/*z* 817 $[M+Na]^+$; HRESIMS *m*/*z* 817.3297 $[M+Na]^+$ (calcd for $C_{39}H_{54}O_{17}$ Na, 817.3259).

*3.4.3. Dichotellide H (**3**).* White amorphous powder; $[\alpha]_D^{20}$ −15.7 (*c* 0.6, acetone); IR (KBr) ν_{max} 3538, 1771, 1743, 1377 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 500/125 MHz), see Tables 1 and 2; ESIMS *m*/*z* 821.3 $[M+Na]^+$; HRESIMS *m*/*z* 821.2786 $[M+Na]^+$ (calcd for $C_{38}H_{51}ClO_{16}Na$, 821.2673).

*3.4.4. Dichotellide I (**4**).* White amorphous powder; $[\alpha]_D^{20}$ −13.8 (*c* 0.7, acetone); IR (KBr) ν_{max} 3446, 1784, 1739, 1377 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 500/125 MHz), see Tables 1 and 2; HRESIMS *m*/*z* 845.3189 $[M+Na]^+$ (calcd for $C_{40}H_{54}O_{18}Na$, 845.3208).

*3.4.5. Dichotellide J (**5**).* White amorphous powder; $[\alpha]_D^{20}$ −22.5 (*c* 0.8, acetone); IR (KBr) ν_{max} 3378, 1792, 1746, 1456 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 500/125 MHz), see Tables 1 and 2; HRESIMS *m*/*z* 703.2201 $[M+Na]^+$ (calcd for $C_{32}H_{40}O_{16}Na$, 703.2214).

*3.4.6. Dichotellide K (**6**).* White amorphous powder; $[\alpha]_D^{20}$ −68.0 (*c* 0.5, acetone); IR (KBr) ν_{max} 3339, 1782, 1738, 1375 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 500/125 MHz), see Tables 1 and 2; HRESIMS *m*/*z* 787.3145 $[M+Na]^+$ (calcd for $C_{38}H_{52}O_{16}Na$, 787.3153).

*3.4.7. Dichotellide L (**7**).* White amorphous powder; $[\alpha]_D^{20}$ −68.0 (*c* 0.5, acetone); IR (KBr) ν_{max} 3339, 1782, 1738, 1375 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 500/125 MHz), see Tables 1 and 2; HRESIMS *m*/*z* 764.3263 $[M+Na]^+$ (calcd for $C_{38}H_{52}O_{16}Na$, 764.3255).

*3.4.8. Dichotellide M (**8**).* White amorphous powder; $[\alpha]_D^{20}$ −19.8 (*c* 0.9, acetone); IR (KBr) ν_{max} 3358, 1775, 1738, 1256 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 500/125 MHz), see Tables 1 and 2; HRESIMS *m*/*z* 759.3213 $[M+Na]^+$ (calcd for $C_{37}H_{52}O_{15}Na$, 759.3204).

*3.4.9. Dichotellide N (**9**).* White amorphous powder; $[\alpha]_D^{20}$ −10.0 (*c* 0.5, acetone); IR (KBr) ν_{max} 3418, 1780, 1734, 1252 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 500/125 MHz), see Tables 3 and 4; ESIMS *m*/*z* 659.4 $[M+Na]^+$; HRESIMS *m*/*z* 659.2691 $[M+Na]^+$ (calcd for $C_{32}H_{44}O_{13}Na$, 659.2680).

*3.4.10. Dichotellide O (**10**).* White amorphous powder; $[\alpha]_D^{20}$ −20.5 (*c* 0.6, acetone); IR (KBr) ν_{max} 3452, 1783, 1740, 1720 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 500/125 MHz), see Tables 3 and 4; ESIMS *m*/*z* 659.4 $[M+Na]^+$; HRESIMS *m*/*z* 659.2693 $[M+Na]^+$ (calcd for $C_{32}H_{44}O_{13}Na$659.2680).

*3.4.11. Dichotellide P (**11**).* White amorphous powder; $[\alpha]_D^{20}$ −32.6 (*c* 0.8, acetone); IR (KBr) ν_{max} 3564, 1779, 1748, 1738, 1252 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 500/125 MHz), see Tables 3 and 4; HRESIMS *m*/*z* 729.3118 $[M+Na]^+$ (calcd for $C_{36}H_{50}O_{14}Na$, 729.3098).

*3.4.12. Dichotellide Q (**12**).* White amorphous powder; $[\alpha]_D^{20}$ −9.3 (*c* 1.2, acetone); IR (KBr) ν_{max} 3533, 1788, 1736, 1252 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 500/125 MHz), see Tables 3 and 4; ESIMS *m*/*z* 637 $[M+Na]^+$; HRESIMS *m*/*z* 637.2035 $[M+Na]^+$ (calcd for $C_{29}H_{39}ClO_{12}Na$, 637.2028).

*3.4.13. Dichotellide R (**13**).* White amorphous powder; $[\alpha]_D^{20}$ −22.4 (*c* 0.9, acetone); IR (KBr) ν_{max} 3530, 1789, 1748, 1718, 1231 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 500/125 MHz), see Tables 3 and 4; HRESIMS *m*/*z* 703.3041 $[M+Na]^+$ (calcd for $C_{34}H_{48}O_{14}Na$, 703.2942).

*3.4.14. Dichotellide S (**14**).* White amorphous powder; $[\alpha]_D^{20}$ −38.9 (*c* 0.35, acetone); IR (KBr) ν_{max} 3447, 1785, 1766, 1728, 1225 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 500/125 MHz), Tables 3 and 4; HRESIMS *m*/*z* 661.2430 $[M+Na]^+$ (calcd for $C_{31}H_{42}O_{14}Na$, 661.2472).

*3.4.15. Dichotellide T (**15**).* White amorphous powder; $[\alpha]_D^{20}$ −31.3 (*c* 0.76, acetone); IR (KBr) ν_{max} 3480, 1782, 1744, 1738, 1214 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 500/125 MHz), see Tables 3 and 4; HRESIMS *m*/*z* 679.1877 $[M+Na]^+$ (calcd for $C_{30}H_{37}ClO_{14}Na$, 679.1770).

*3.4.16. Dichotellide U (**16**).* White amorphous powder; $[\alpha]_D^{20}$ −16.0 (*c* 0.2, acetone); IR (KBr) ν_{max} 3460, 1776, 1726, 1216 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 500/125 MHz), see Tables 3 and 4; HRESIMS *m*/*z* 673.2820 $[M+Na]^+$ (calcd for $C_{33}H_{46}O_{13}Na$, 673.2836).

3.5. X-ray crystallographic analysis of dichotellide T (15·(0.5-C_2H_5OH))

Colorless crystal of $C_{30}H_{37}ClO_{14}$·(0.5 C_2H_5OH) with the Flack parameter of −0.11 (7), *M*=680.08. *P212121*, *a*=12.2117(7) Å, *b*=12.3115(7) Å, *c*=22.5748(12) Å, *V*=3394.0(3) $Å^3$, *Z*=4; crystal size 0.45×0.18×0.15 mm^3. A total of 17,169 unique reflections (θ=1.80–27.14°) were collected using graphite monochromated Mo *K*α radiation (λ=0.71073 Å) on a Bruker Smart 1000 CCD diffractometer at −110 °C. Absorption corrections were done by semi-empirical from equivalents. The structure was solved using direct methods (SHELXL-97) and refined with Full-matrix least-squares on 5406 data, 0 restraints and 441 variable parameters. Final *R* indicates R_1=0.0475, wR_2=0.1020 [$I>2\sigma(I)$]. Crystallographic data for the structure of **7** in this paper have been deposited in the Cambridge

Crystallographic Data Centre as supplementary publication numbers CCDC 821961. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK [fax: +44(0) 1223 336033 or e-mail:deposit@ccdc.cam.ac.uk].

3.6. Antifouling bioassay

The antilarval-attachment activity was determined using cyprids of the barnacle *B. amphitrite* Darwin. Adults of *B. amphitrite* were collected from the intertidal zone in Hong Kong, After 12 h of exposed to air, hundreds of adults were placed in a container filled with 0.22 μm of filtered seawater (FSW) to induce the release of nauplii. The collected nauplii were reared to cyprid stage according to the method described by Thiyagarajan et al.[20] When kept at 26–28 °C and fed with *Chaetoceros gracilis*, larvae developed to cyprids on the fourth day. Fresh cyprids were used in the tests. Larval settlement assays were performed using 24-well polystyrene plates. The tested samples were first dissolved in a small amount of DMSO and then diluted with filtered FSW to achieve final concentrations at 25, 10, 5, 2.5, 1.25, 0.625, 0.31, 0.15 μg/mL. About 15–20 competent larvae were added to each well with 1 mL of testing solution in three replicates, and wells containing larvae in FSW with DMSO only served as a positive control. Then the plates were incubated for 24–48 h at 25 °C. The effects of the test samples against biofouling were determined by examining the plates under a dissecting microscope to check for (1) attached larvae and (2) unattached larvae, and (3) any possible toxic effects of the treatments, such as death or paralysis of larvae, which were also recorded. The number of settled or metamorphosed larvae was expressed as a percentage of the total number of larvae added into each well. The EC_{50} (inhibits 50% of settlement of *B. amphitrite* larvae in comparison with the control) was calculated by using the Probit software program with the mean of three repeated experiments using different batches of larvae.

3.7. Cytotoxicity

The inhibition effects of test compounds on proliferation of MCF-7 (human breast carcinoma), SW1990 (human pancreatic cancer), HepG2 (human hepatocellular carcinoma), and H460 (human nonsmall lung cancer) cells were determined by MTT method as described previously.[9]

Acknowledgements

This study was supported by the National Basic Research Program of China (973 Program, Nos. 2010CB833800 and 2011CB915503), the National High Technology Research and Development Program (863 Program, 2012AA092104), Knowledge Innovation Program of Chinese Academy of Science (KSCX2-YW-G-073 and KSCX2-EW-G-12B), and National Natural Science Foundation of China (Nos. 30973679, 20902094, 21172230, 41176148, 21002110).

Guangdong Province-CAS Joint Research Program (2011B0 90300023), Guangdong Province Marine and Fishery Special Project for Science and Technology Popularization (A201101F03), the start-up grant from Jiangsu University (11JDG1147).

Supplementary data

This data include the ^{1}H and ^{13}C NMR data of compounds **1**–**16**. Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.tet.2012.10.102.

References and notes

1. Dobretsov, S.; Dahms, H. U.; Qian, P. Y. *Biofouling* **2006**, *22*, 43–54.
2. Li, X.; Dobretsov, S.; Xu, Y.; Xiao, X.; Hung, O. S.; Qian, P. Y. *Biofouling* **2006**, *22*, 201–208.
3. Fusetani, N. *Nat. Prod. Rep.* **2011**, 400–410.
4. Fusetani, N. *Nat. Prod. Rep.* **2004**, *21*, 94–104.
5. Qiu, Y.; Deng, Z.; Xu, M.; Li, Q.; Lin, W. *Steroids* **2008**, *73*, 1500–1504.
6. Bianco, M.; Rogers, R.; Teixeira, V.; Pereira, R. *J. Appl. Phycol.* **2009**, *21*, 341–346.
7. Viano, Y.; Bonhomme, D.; Camps, M.; Briand, J.; Ortalo-Magne, A.; Blache, Y.; Piovetti, L.; Culioli, G. *J. Nat. Prod.* **2009**, *72*, 1299–1304.
8. Culioli, G.; Ortalo-Magne, A.; Valls, R.; Hellio, C.; Clare, A.; Piovetti, L. *J. Nat. Prod.* **2008**, *71*, 1121–1126.
9. Sun, J. F.; Huang, H.; Chai, X. Y.; Yang, X. W.; Meng, L.; Huang, C. G.; Zhou, X. F.; Yang, B.; Hu, J.; Chen, X. Q.; Lei, H.; Wang, L. S.; Liu, Y. H. *Tetrahedron* **2011**, *67*, 1245–1250.
10. Li, C.; La, M. P.; Li, L.; Li, X. B.; Tang, H.; Liu, B. S.; Krohn, K.; Sun, P.; Yi, Y. H.; Zhang, W. *J. Nat. Prod.* **2011**, *74*, 1658–1662.
11. Li, C.; La, M.-P.; Sun, P.; Kurtan, T.; Mandi, A.; Tang, H.; Liu, B.-S.; Yi, Y.-H.; Li, L.; Zhang, W. *Mar. Drugs* **2011**, *9*, 1403–1418.
12. Sheu, J. H.; Chen, Y. P.; Hwang, T. L.; Chiang, M. Y.; Fang, L. S.; Sung, P. J. *J. Nat. Prod.* **2006**, *69*, 269–273.
13. Shen, Y. C.; Lin, Y. C.; Ko, C. L.; Wang, L. T. *J. Nat. Prod.* **2003**, *66*, 302–305.
14. Qi, S. H.; Zhang, S.; Huang, H.; Xiao, Z. H.; Huang, J. S.; Li, Q. X. *J. Nat. Prod.* **2004**, *67*, 1907–1910.
15. Sung, P. J.; Fan, T. Y.; Fang, L. S.; Wu, S. L.; Li, J. J.; Chen, M. C.; Cheng, Y. M.; Wang, G. H. *Chem. Pharm. Bull.* **2003**, *51*, 1429–1431.
16. Shin, J.; Park, M.; Fenical, W. *Tetrahedron* **1989**, *45*, 1633–1638.
17. Qi, S. H.; Zhang, S.; Qian, P. Y.; Xiao, Z. H.; Li, M. Y. *Tetrahedron* **2006**, *62*, 9123–9130.
18. Isaacs, S.; Carmely, S.; Kashman, Y. *J. Nat. Prod.* **1990**, *53*, 596–602.
19. Garcia, M.; Rodriguez, J.; Jimenez, C. *J. Nat. Prod.* **1999**, *62*, 257–260.
20. Thiyagarajan, V.; Harder, T.; Qiu, J.-W.; Qian, P.-Y. *Mar. Biol.* **2003**, *143*, 543–554.
21. Keifer, P. A.; Rinehart, K. L.; Hooper, I. R. *J. Org. Chem.* **1986**, *51*, 4450–4454.
22. Qi, S. H.; Zhang, S.; Qian, P. Y.; Xu, H. H. *Chem. Nat. Compd.* **2009**, *45*, 49–54.