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# Dichotellides A–E, five new iodine-containing briarane type diterpenoids from *Dichotella gemmacea*

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ABSTRACT

Five new briarane type diterpenoids, dichotellides A–E (**1**–**5**), were isolated from the South China Sea gorgonian *Dichotella gemmacea* together with four known analogues (**6**–**9**). Compounds **1**–**5** represent the first examples of iodine-containing briarane type diterpenoids from nature. The structures of these diterpenoids were elucidated by spectroscopic analysis, including 1D, 2D-NMR, and HRESIMS, and the absolute configuration of **1** was further confirmed by single crystal X-ray diffraction analysis. All the isolates were evaluated for cytotoxicity activity against four human tumor cell lines, and only **3** exhibited marginal activity against SW1990 (human pancreatic cancer).



## 1. Introduction

The marine environments contain high concentrations of halogenated metabolites, with a great variety of structural arrangements and biological activities. Among them, the chlorinated and brominated secondary metabolites are the most abundant in marine organisms, while iodinated marine natural products are less common, with only few examples involved volatiles, nucleosides, tyrosine and tryptophan derivatives, fatty acids, and terpenoids.[1–8] To some extent, this may be considered as a consequence of the relatively low abundance of iodine in seawater (almost one thousand times less than bromine).[1,2] Thus, discovery of iodinated metabolites will be of considerable interest.

The briarane diterpenoids constitutes an important class of the halogenated metabolites from the marine source. These diterpenoids are characterized by a bicycle [8.4.0] ring system fused by a γ-lactone group, with high oxidization and esterification by all kinds of acyls, i.e., acetyl, isovalerate. These compounds exhibit a variety of biological activities, such as cytotoxicity,[9] anti-inflammatory,[10] antivirus,[11] insecticide,[12] immunomodulation,[13] and antifouling.[14] Because of their unique structures and interesting biological activities, significant efforts have gone into the discovery of new briarane diterpenoids, and these efforts have culminated in the discovery of more than five hundred briarane family members[15–17] since the first briarane-type diterpenoid, briarane A, was isolated from the West Indian gorgonian *Briareum asbestinum* by Burks et al. in 1977.[18] Most of these briaranes possess chlorine atom, epoxy group, and double bond as substituents.

As part of our continuous efforts towards discovering new and bioactive metabolites from the South China Sea gorgonian *Dichotella gemmacea*, we recently identified five new iodine-bearing briarane type diterpenoids, dichotellides A–E (**1**–**5**), together with four known congeners (**6**–**9**). Herein, the isolation, structure elucidation, and biological activity of these compounds are described.

## 2. Results and discussion

Dichotellide A (**1**) was obtained as colorless crystals (in MeOH). The molecular formula was established as $C_{31}H_{40}ClIO_{12}$ on the basis of HRESIMS, suggesting 11° of unsaturation. The ESIMS spectrum of **1** showed a cluster of isotopic $[M+Na]^+$ ion peaks at *m*/*z* 789/791 in approximate ratio of 3:1, indicating the presence of a chlorine atom in the molecule. The IR absorptions implied the presence of

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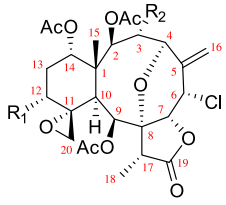
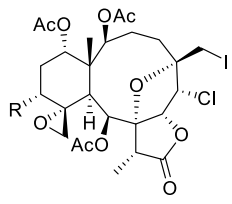
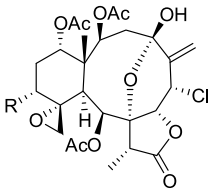


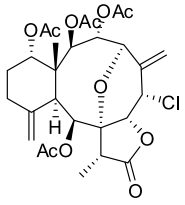
**1** $R_1$= $OC(O)CH_2CH(CH_3)_2$, $R_2$= I
**2** $R_1$= OAc, $R_2$= I; **3** $R_1$= H, $R_2$= I
**6** $R_1$= H, $R_2$= OAc



**4** R= $OC(O)CH_2CH(CH_3)_2$
**5** R= OAc



**7** R= OAc; **8** R= H



**9**

γ-lactone (1793 $cm^{-1}$), and ester carbonyl group (1745, 1730 $cm^{-1}$). The $^{13}C$ NMR and DEPT spectra showed 31 carbon signals (Table 1), including seven methyls, four methylenes (one olefinic and one oxygenated), eleven methines (two halogenated and six oxygenated), and nine quaternary carbons (five ester carbonyls, two oxygenated, and one olefinic). The $^1H$ and $^{13}C$ NMR spectra (Tables 1 and 2) presented signals for three acetates and an isovalerate moiety ($\delta_C$ 22.3, q; 22.4, q; 25.7, d; 43.4, t; 171.7, s), a γ-lactone ($\delta_C$ 173.8, s), and an exocyclic olefinic bond ($\delta_H$ 5.69, 1H, d, $J$=2.0 Hz; 5.48, 1H, d, $J$=2.0 Hz; $\delta_C$ 122.0, t; 133.1, s), which accounted for 6° of unsaturation. Since the total degrees of unsaturation are eleven, the remaining 5° of unsaturation led us to conclude that **1** must be pentacyclic. In addition, the carbonyl at $\delta_C$ 173.8 (C-19), along with the oxygenated methine ($\delta_H$ 4.41, 1H, d, $J$=2.5 Hz, H-7; $\delta_C$ 79.2, d, C-7) and the oxygenated quaternary carbon ($\delta_C$ 82.5, s, C-8), was attributed to a γ-lactone ring, and this assignment was supported by HMBC correlations observed from $H_3$-18 ($\delta_H$ 1.40) to C-8 and C-19, from H-6 ($\delta_H$ 4.62) to C-8. Moreover, an exocyclic epoxy group (spirocyclic oxirane ring) was revealed by the two *gem*-protons at $\delta_H$ 2.82 (d, 1H, $J$=3.5 Hz, $H_a$-20) and 2.42 (d, 1H, $J$=3.5 Hz, $H_b$-20), together with the corresponding $CH_2$-20 ($\delta_C$ 50.3, t) and quaternary carbon ($\delta_C$ 56.6, s, C-11).[19] The $^1H$ NMR spectrum (Table 2) also showed the presence of a secondary methyl ($H_3$-18), a tertiary methyl ($H_3$-15), two aliphatic methine protons (H-17, C-3′), two halogenated methine (H-3, 6), six oxymethine protons (H-2, 4, 7, 9, 12, 14), and one aliphatic methylene ($H_2$-13).

The above NMR data implied that dichotellide A (**1**) was a briarane diterpenoid with a γ-lactone ring and an exocyclic C-11/C-20 epoxide group. As the $^1H$ and $^{13}C$ NMR spectral patterns appeared to be similar to those of praelolide (**6**).[20], it was evident that both **1** and **6** shared the same carbon framework. A minor difference between them lies on the appearance of an additional signal for an isovaleryloxyl attached at C-12 in **1**, which was supported by the HMBC correlations from H-12 ($\delta_H$ 4.57) to the carbonyl carbon ($\delta_C$ 171.7, s), from $H_2$-20 to C-12 and C-11, and from H-14 to C-12. Perhaps the most interesting and surprising finding was that a methine proton at $\delta_H$ 5.51 (dd, $J$=6.0, 12.0 Hz) was correlated to the carbon $\delta_C$ 28.7 in the HSQC spectrum. In other words, the carbon chemical shift is only five times the corresponding proton chemical shift, much less than 10–20 fold differences (between proton chemical shifts and carbon chemical shifts), which are routinely observed. This relative high field carbon chemical shift usually appears with the substituent iodine due to the well known heavy atom effect.[6] To this end, we assigned the iodine substituted methine as C-3, and this assignment was supported by the HMBC correlations of H-2/C-1, C-3; H-3/C-1, C-2, C-4, C-5; and H-4/C-2, C-3, and by the $^1H$–$^1H$ COSY connectivities of H-2/H-3/H-4. Hence, the major difference between **1** and praelolide (**6**) is that the former bears an iodine-substituted methine, while an oxygenated methine ($\delta_C$ 63.8, d; $\delta_H$ 6.15, dd, $J$=7.0, 11.0 Hz) exists in praelolide (**6**). The presence of an iodine atom in **1** agreed with the HRESIMS data. Thus, the gross structure of **1** and its relative stereochemistry were established by spectroscopic means (Fig. 1).

The existence of an iodine atom in **1** was confirmed by a single crystal X-ray diffraction analysis, which also established the absolute configuration of **1** (Fig. 2). The absolute configuration of C-3 was assigned as 3*S*, and all other chiral centers were unambiguously determined. Of note is that the absolute stereochemistry at C-1, C-10, and C-7 agrees with that of known briaranes reported so far. Consequently, **1** represents the first natural iodinated briarane diterpenoid.

The molecular formula of dichotellide B (**2**) was determined as $C_{28}H_{34}ClIO_{12}$ by analysis of the molecular ion peak at *m*/*z* 747/749 (3:1) $[M+Na]^+$ in the ESIMS and ion peak at *m*/*z* 747.0715 $[M+Na]^+$ (calcd for 747.0740) in the HRESIMS, in combination of the $^1H$ and $^{13}C$ NMR spectra. Like compound **1**, compound **2** showed IR absorptions typical of γ-lactone and ester carbonyl. The $^1H$ and $^{13}C$ NMR spectroscopic data (Tables 1 and 2) of **2** are similar to those of **1**, indicating that both beared identical substituents, such as C4/8 ether bridge, exocyclic methylene at C-5, secondary acetates (C-2, 9, 14), and the same C-11/C-20 exocyclic epoxy. The only difference is the presence of an acetate at C-12 in **2** instead of an isovalerate at C-12 in **1**. These NMR data suggested that compound **2** belonged to be the same 6-chlorinated and iodinated briarane diterpenoid family. Thus the structure of compound **2** was assigned and named as dichotellide B. The relative stereochemistry of **2** was assigned to be the same as that of **1** by comparison of NMR data and analysis of the NOESY spectrum (Fig. 3). However, the absolute stereochemistry of **2** remains to be established.

**Table 1**
$^{13}C$ NMR (125 MHz) spectral data of compounds **1**–**5** in $CDCl_3$

| Position | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | 47.4 s | 47.4 s | 47.5 s | 48.8 s | 48.8 s |
| 2 | 71.3 d | 71.3 d | 71.5 d | 78.1 d | 78.1 d |
| 3 | 28.7 d | 28.7 d | 29.3 d | 26.3 t | 26.3 t |
| 4 | 82.4 d | 82.4 d | 82.3 d | 34.5 t | 34.6 t |
| 5 | 133.1 s | 133.1 s | 133.3 s | 85.1 s | 85.1 s |
| 6 | 53.3 d | 53.3 d | 53.4 d | 58.7 d | 58.7 d |
| 7 | 79.2 d | 79.1 d | 79.1 d | 82.5 d | 82.5 d |
| 8 | 82.5 s | 82.5 s | 82.4 s | 90.3 s | 90.3 s |
| 9 | 71.7 d | 71.7 d | 71.7 d | 72.5 d | 72.5 d |
| 10 | 36.8 d | 36.7 d | 41.5 d | 37.7 d | 37.7 d |
| 11 | 56.6 s | 56.7 s | 56.4 s | 56.6 s | 56.7 s |
| 12 | 73.0 d | 73.5 d | 29.8 t | 73.0 d | 73.6 d |
| 13 | 29.0 t | 28.8 t | 24.6 t | 28.9 t | 28.7 t |
| 14 | 73.9 d | 73.9 d | 74.7 d | 73.5 d | 73.6 d |
| 15 | 14.2 q | 14.2 q | 14.9 q | 13.6 q | 13.7 q |
| 16 | 122.0 t | 122.0 t | 121.8 t | 14.5 t | 14.5 t |
| 17 | 49.7 d | 49.7 d | 49.7 d | 46.9 d | 47.0 d |
| 18 | 7.1 q | 6.9 q | 7.3 q | 9.7 q | 9.5 q |
| 19 | 173.8 s | 173.8 s | 173.8 s | 174.7 s | 174.6 s |
| 20 | 50.3 t | 50.1 t | 50.1 t | 50.1 t | 49.9 t |
| Acetate | 170.3 s | 170.3 s | 170.3 s | 170.3 s | 170.3 s |
| | 21.6 q | 21.6 q | 21.7 q | 21.6 q | 21.5 q |
| | 168.8 s | 168.8 s | 170.0 s | 169.0 s | 169.0 s |
| | 21.4 q | 21.4 q | 21.4 q | 21.3 q | 21.3 q |
| | 169.7 s | 169.0 s | 169.0 s | 170.1 s | 170.0 s |
| | 20.9 q | 21.0 q | 21.0 q | 21.2 q | 21.1 q |
| | | 169.6 s | | | 169.4 s |
| | | 20.9 q | | | 21.0 q |
| Isovalerate | 171.7 s | | | 171.7 s | |
| | 43.4 t | | | 43.4 t | |
| | 25.7 d | | | 25.6 d | |
| | 22.3 q | | | 22.3 q | |
| | 22.4 q | | | 22.4 q | |

**Table 2**
$^1$H NMR (500 MHz) data of compounds **1**–**5** (*J* in Hz within parentheses)

| Position | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 2 | 5.28 d (5.5) | 5.28 d (5.5) | 5.16 d (5.5) | 5.51 m | 5.51 s |
| 3 | 5.51dd (12.0,5.5) | 5.51 dd (12.0, 5.5) | 5.50 dd (12.0, 5.5) | 2.83 m | 2.83 m |
| | | | | 1.36 dt (15.0, 4.0) | 1.36 dt (16.0, 4.0) |
| 4 | 4.79 d (12.0) | 4.79 d (12.0) | 4.77 d (12.0) | 2.84 m | 2.84 m |
| | | | | 1.71 dd (14.0, 6.0) | 1.71 dd (14.5, 7.0) |
| 6 | 4.62 d (2.5) | 4.62 d (2.5) | 4.62 d (2.0) | 4.29 d (4.0) | 4.30 d (4.0) |
| 7 | 4.41 d (2.5) | 4.41 d (2.5) | 4.40 d (2.0) | 4.57 d (4.0) | 4.57 d (4.0) |
| 9 | 5.75 s | 5.77 s | 5.72 s | 5.51 s | 5.51 s |
| 10 | 3.33 s | 3.35 s | 2.84 s | 3.42 s | 3.44 s |
| 12 | 4.57 br s | 4.53 br s | 2.17 m | 4.58 br s | 4.54 br s |
| | | | 1.25 m | | |
| 13 | 2.22 t (3.0) | 2.30 t (3.0) | 1.90 m | 2.01 m | 1.97 m |
| | 2.03 t (3.5) | 1.98 t (3.5) | 1.84 m | 2.27 m | 2.29 m |
| 14 | 4.95 t (3.0) | 4.96 t (3.0) | 4.98 s | 4.97 t (2.5) | 4.98 br s |
| 15 | 1.25 s | 1.26 s | 1.23 s | 1.17 s | 1.25 s |
| 16 | 5.69 d (2.0) | 5.69 d (2.0) | 5.67 d (2.0) | 3.24 d (11.0) | 3.24 d (11.0) |
| | 5.48 d (2.0) | 5.48 d (2.0) | 5.46 d (2.0) | 3.57 d (11.0) | 3.56 d (11.0) |
| 17 | 2.82 q (7.0) | 2.82 q (7.0) | 2.82 q (7.0) | 2.86 q (7.0) | 2.86 q (7.0) |
| 18 | 1.40 d (7.0) | 1.39 d (7.0) | 1.32 d (7.0) | 1.63 d (3H, 7.0) | 1.63 d (3H, 7.0) |
| 20 | 2.42 d (3.5) | 2.44 d (3.5) | 2.35 d (1.5) | 2.44 d (3.5) | 2.45 d (3.5) |
| | 2.82 d (3.5) | 2.82 d (3.5) | 2.65 d (1.5) | 2.82 d (3.5) | 2.83 d (3.5) |
| Acetate methyls | 2.10 s | 2.11 s | 2.12 s | 2.03 s | 2.03 s |
| | 2.26 s | 2.26 s | 2.25 s | 2.20 s | 2.22 s |
| | 2.00 s | 2.05 s | 2.05 s | 2.00 s | 2.06 s |
| | | 2.01 s | | | 2.00 s |
| Isovalerate | | | | | |
| 2′ | 2.13 m (2H) | | | 2.15 m (2H) | |
| 3′ | 2.08 m (1H) | | | 2.07 m (1H) | |
| 4′ | 0.94 d (3H, 6.5) | | | 0.94 d (3H, 6.5) | |
| 5′ | 0.95 d (3H, 6.5) | | | 0.96 d (3H, 6.5) | |

Dichotellide C (**3**) was isolated as white powder. The molecular formula was established as $C_{26}H_{32}ClIO_{10}$ on the basis of HRESIMS data (*m*/*z*, 689.0651 $[M+Na]^+$, calcd for 689.0626). The IR absorptions and NMR spectra (Tables 1 and 2) were very similar to those of **2** except that the C-12 acetate disappeared and 12-oxymethine was replaced by an aliphatic methylene. Thus, on the basis of comprehensive NMR studies, the structure of **3** was elucidated and the relative configuration was assigned to be same as that of **1**. As in the case of 2, the absolute stereochemistry of **3** has not been determined.

Dichotellide D (**4**) was obtained as white powder. The HRESIMS spectrum showed an ion peak at *m*/*z* 791.1326 $[M+Na]^+$ (calcd for 791.1307) for the monosodium salt ($C_{31}H_{42}ClIO_{12}Na$) of the molecule, with 10° of unsaturation. The IR spectrum indicated absorptions due to γ-lactone (1793 $cm^{-1}$) and ester carbonyl groups (1740, 1735 $cm^{-1}$). The $^1$H and $^{13}$C NMR spectra (Tables 1 and 2) were remarkably similar to those of **1**, suggesting that **4** belonged to the same 6-chlorinated and iodinated briarane. The only differences between them were the replacement of an iodinated C-3 methine and an oxygenated C-4 methine in **1** by two aliphatic methylenes in **4**, the disappearance of C-5/C-16 double bond, and the appearance of oxygenated quaternary carbon ($\delta_C$ 85.1, C-5), and an aliphatic methylene (C-16). Again, the unusually high field of the signal at $\delta_C$ 14.5 clearly distinguished the methylene (C-16) carbon atom linked to the iodine atom. The uncommon HSQC correlation of H-16 ($\delta_H$ 3.24 and 3.57) with $\delta_C$ 14.5, together with the iodine atom present in the molecule, suggested that the iodine atom is located at position C-16. The remaining different structure features between **4** and **1**, especially the oxygen bridge linkage of C5/8, were evidenced by comparing their NMR data including by $^1$H–$^1$H COSY and HMBC correlations (Fig. 4a).

According to known literature data on briarane diterpenoids,[21] when the chemical shifts of C-11 and C-20 of exocyclic C-11/C-20-epoxy groups resonated at $\delta_C$ 55–61 and 47–52, respectively, in

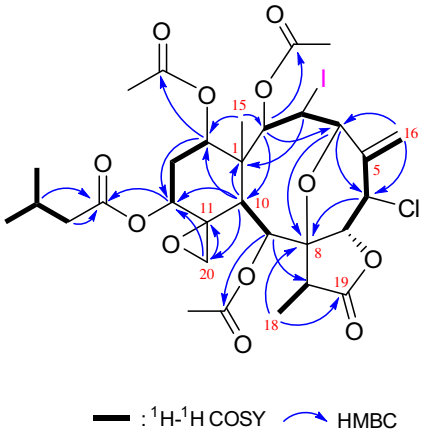



**Fig. 1.** The $^1$H–$^1$H COSY and selective HMBC correlations of **1**.

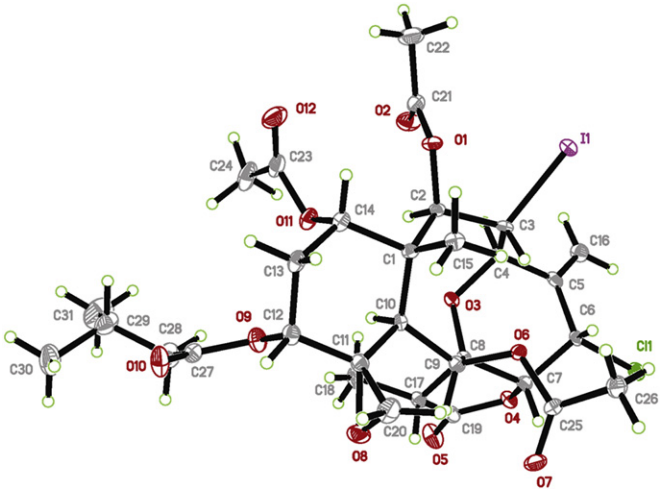



**Fig. 2.** Computer-generated ORTEP plot of **1**.

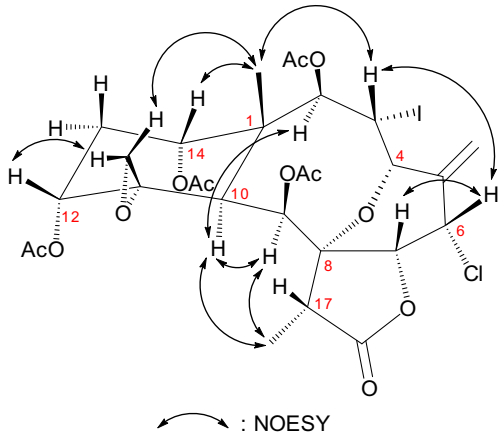



**Fig. 3.** Selective NOESY correlations of **2**.

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 **4**, we assigned the configuration of C-11/C-20-epoxide ($\delta_C$ 56.6, s, C-11; 50.1, t, C-20) in **4** as 11*R*. As shown in the NOESY spectrum of **4**, the NOE correlations of H-6 with H-4$\beta$, H-4$\beta$ with $H_2$-16 suggested the $\beta$-orientation of $CH_2$-16, and the configuration of C-5 was deduced to be 5*S*. The other chiral centers of 4 were assigned to be same as those of **1** on the basis of the NMR data comparison with **1** including NOESY correlations (Fig. 4b). Thus, the relative configuration of the all 12 chiral centers in **4** were assigned as 1*S*, 2*S*, 5*S*, 6*R*, 7*R*, 8*R*, 9*S*, 10*S*, 11*R*, 12*R*, 14*S*, and 17*R*. Again, the absolute stereochemistry of compound **4** has not been established.

The HRESIMS data of **5** indicated this molecule possessed an iodine atom and a chlorine-containing of molecular formula of $C_{28}H_{36}ClIO_{12}$ for dichotellide E (**5**). The $^1H$ and $^{13}C$ NMR spectroscopic data (Tables 1 and 2) showed high similarity to those of **4**, except that the signals corresponding to a 12α-isovalerate in **4** was replaced by a 12α-acetate in **5**. This can be deduced by a downfield shift of 0.6 ppm for the C-12 in the $^{13}C$ NMR spectrum of **5** and confirmed by the HMBC spectrum. Full assignments of the $^1H$ and $^{13}C$ NMR spectra (Tables 1 and 2) were carried out on the basis of the analysis of COSY, HSQC, and HMBC data. The relative configuration of **5** was shown to be the same as that of **4** by analysis of NOESY correlations and comparison of the spectroscopic data with **4**.

The four known compounds were identified as praelolide (**6**),[20] juncin P (**7**),[22] juncin ZI (**8**),[14] and junceellin A (**9**)[23] by comparison of their NMR data with those reported.

Inspired by the reported cytotoxicity of briarane diterpenoids,[24,25] we evaluated the newly isolated iodine-bearing briarane compounds in the cytotoxicity studies against four tumor cell lines (MCF-7, SW1990, HepG2, and H460). However, only **3** showed marginal activity against SW1990 cells ($IC_{50}$=45.0 μM), with fluorouracil as positive control ($IC_{50}$=121.0 μM against SW1990).

In summary, we have isolated the first five iodine-containing briarane type diterpenoids dichotellides A–E (**1**–**5**) together with four known ones from the South China Sea gorgonian *Dichotella gemmacea*. Their gross structures and relative stereochemistry were elucidated based on extensive NMR studies, and the absolute configuration of dichotellide A was determined by X-ray diffraction studies. Preliminary cytotoxicity studies against four tumor cell lines were carried out. Even though only **3** showed marginal activity, it could serve as new lead compound for further medicinal chemistry optimization. In addition, the discovery of these new dichotellides A–E opens up the opportunity of studying their ecological role in the gorgonian.

## 3. Experimental

### 3.1. General experimental procedures

Optical rotation values were measured with a Perkin–Elmer 341 polarimeter. IR spectra were measured on JASCO FT/IR-480 plus spectrometers. NMR spectra were recorded using Bruker 500 MHz NMR spectrometers. HRESIMS 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 gorgonian *D. gemmacea* was 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. A voucher specimen (No. M090405) was 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 (ca. 4.0 kg) was exhaustively extracted with 95% EtOH twice, and $CHCl_3$/MeOH (1:1) for one time at room temperature. After the solvent was evaporated *in vacuum*, the combined residue was suspended in $H_2O$ and partitioned with

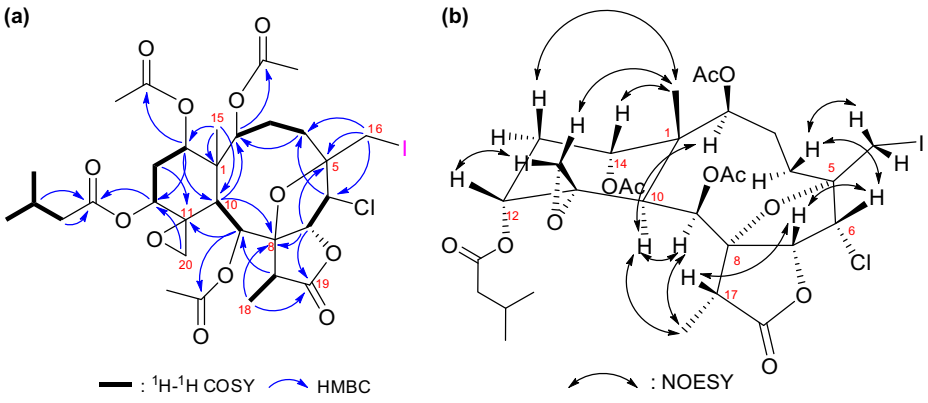



**Fig. 4.** (a) $^1H$–$^1H$ COSY, selective HMBC and (b) NOESY correlations of **4**.

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 yield 9 fractions (Frs.A–H). Fr.D (1.2 g) was subjected on Sephadex LH-20 (MeOH), followed by silica gel CC, eluting with PE/EtOAc (5:3), to get 5 subfractions (Frs.Da–De). Fr.Db was further applied on silica gel CC, eluted using hexane/EtOAc, to afford compounds **1** (9.6 mg, 3:1) and **3** (4.0 mg, 2:1). Fr.Dc was subjected to silica gel CC, eluting with $CHCl_3$/$Me_2CO$ (10:1), to afford **4** (5.6 mg). A further isolation of Fr. De yielded **5** (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 **6** (25 mg), **7** (11 mg), and **8** (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 **2** (5.8 mg) and **9** (7.2 mg).

### 3.4. Characteristics of compounds

*3.4.1. Dichotellide A (**1**).* Colorless crystals (MeOH); $[\alpha]_D^{20}$ −39.4 (*c* 0.7, acetone); IR (KBr) $\upsilon_{max}$ $cm^{-1}$: 2338, 2360, 1793, 1745, 1626, 1595; $^1H$ and $^{13}C$ NMR ($CDCl_3$, 500/125 MHz), see Tables 1 and 2; ESIMS *m*/*z* 789 $[M+Na]^+$; HRESIMS *m*/*z* 789.1148 $[M+Na]^+$ (calcd for 789.1151).

*3.4.2. Dichotellide B (**2**).* White powder; $[\alpha]_D^{20}$ −35.1 (*c* 0.6, acetone); IR (KBr) $\upsilon_{max}$ $cm^{-1}$: 2364, 2338, 1793, 1740, 1626, 1595; $^1H$ and $^{13}C$ NMR ($CDCl_3$, 500/125 MHz), see Tables 1 and 2; ESIMS *m*/*z* 747 $[M+Na]^+$; HRESIMS *m*/*z* 747.0715 $[M+Na]^+$ (calcd for 747.0740).

*3.4.3. Dichotellide C (**3**).* White powder; $[\alpha]_D^{20}$ −15.0 (*c* 0.2, acetone); IR (KBr) $\upsilon_{max}$ $cm^{-1}$: 2938, 2329, 1793, 1733, 1371; $^1H$ and $^{13}C$ NMR ($CDCl_3$, 500/125 MHz), see Tables 1 and 2; ESIMS *m*/*z* 689 $[M+Na]^+$; HRESIMS *m*/*z* 689.0651 $[M+Na]^+$ (calcd for 689.0626).

*3.4.4. Dichotellide D (**4**).* White powder; $[\alpha]_D^{20}$ −10.2 (*c* 0.5, acetone); IR (KBr) $\upsilon_{max}$ $cm^{-1}$: 2953, 2914, 1793, 1740, 1648; $^1H$ and $^{13}C$ NMR ($CDCl_3$, 500/125 MHz), see Tables 1 and 2; ESIMS *m*/*z* 791 $[M+Na]^+$; HRESIMS *m*/*z* 791.1326 $[M+Na]^+$ (calcd for 791.1307).

*3.4.5. Dichotellide E (**5**).* White powder; $[\alpha]_D^{20}$ −18.3 (*c* 0.4, acetone); IR (KBr) $\upsilon_{max}$ $cm^{-1}$: 2958, 1783, 1743, 1641; $^1H$ and $^{13}C$ NMR ($CDCl_3$, 500/125 MHz), see Tables 1 and 2; ESIMS *m*/*z* 749 $[M+Na]^+$; HRESIMS *m*/*z* 749.0842 $[M+Na]^+$ (calcd for 749.0838).

### 3.5. Single crystal X-ray crystallography of 1

Colorless crystal of $C_{31}H_{40}ClIO_{12}$, *M*=766.98. P2(1)2(1)2(1), *a*=10.0332(4) Å, *b*=13.1440(5) Å, *c*=25.5549(10) Å, *V*=3370.1(2) $Å^3$, *Z*=4; crystal size 0.47×0.33×0.22 $mm^3$. A total of 17 200 unique reflections ($\theta$=1.74–27.06°) were collected using graphite monochromated Mo Kα radiation ($\lambda$=0.71073 Å) on a Bruker Smart 1000 CCD diffractometer at 273 K. 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 7303 data, 0 restraints, and 413 variable parameters. Final *R* indicates *R*1=0.0276, *wR*2=0.0574 [$I>2\sigma(I)$]. Crystallographic data for the structure of **1** in this paper have been deposited in the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 784232. 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. Cytotoxicity

MCF-7 (human breast carcinoma), SW1990 (human pancreatic cancer), HepG2 (human hepatocellular carcinoma), and H460 (human non-small lung cancer) cells in the logarithmic growth phase were trypsinized and diluted with medium to $2\times10^5$ cells/ml, and then 100 μl of these cell suspension were added to each well of a 96-well plate, respectively. Thereafter, the plate was incubated for 24 h and the supernatant was removed. Then the test compounds were diluted with test medium to different concentrations (200–3.125 mg/ml), and 100 μl of this diluted solution was placed in the 96-well plate. After the plate was incubated for 48 h, the toxicity concentration of test compounds were investigated and recorded. The inhibition effects of test compounds on proliferation of four cells were determined by MTT method as described previously,[26] and using Fluorouracil as the positive control with $IC_{50}$ values of 30.0, 121.0, 110.0, and 0.008 μM, respectively.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## Supplementary data

This data include the $^1H$ and $^{13}C$ NMR, DEPT, COSY, HSQC, and HMBC data of compounds **1**–**5** (500\125 MHz in $CD_3OD$). Supplementary data related to this article can be found online version, at doi:10.1016/j.tet.2010.11.087.

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