



Simplexolides A–E and plakorfuran A, six butyrate derived polyketides from the marine sponge *Plakortis simplex*

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ABSTRACT

Six new polyketides, simplexolides A–E (**1–5**) and a furan ester, plakorfuran A (**6**), together with four known furanylidenic methyl esters (**7–10**) were isolated from the marine sponge *Plakortis simplex*. Compounds **1–5** feature a tetrahydrofuran ring opened *seco*-plakortone skeleton. These new structures, including relative configurations, were determined on the basis of extensive analysis of spectroscopic data. The absolute configurations of **1–6** were established by the modified Mosher's method, and the CD exciton chirality method. However, configurations of the remote stereocenters at C-8 in compounds **1–5** were not determined. Antifungal, cytotoxicity, antileishmanial, and antimalarial activities of these polyketides were evaluated.

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1. Introduction

Sponges of the genera *Plakortis* and *Plakinastrella* have been widely investigated for their biologically active polyketides.^{1–8} Apart from a large number of cyclic peroxides they also contain a group of γ -lactones, including bicyclic peroxy lactones (plakortolides),^{9,10} bicyclic furanolactones (plakortones),^{8,11} *N*-alkylated lactones (amphiasterins),¹² α,β -unsaturated lactones (butenolides),¹³ and some *seco* derivatives (*seco*-plakortolides).⁸ Plakortones A–F, featuring bicyclic furanolactones, are a class of ethyl branched (butyrate derived) polyketides from the genus *Plakortis*. They show interesting biological activities that have been the focus of extensive chemical and pharmaceutical studies. Plakortones A–D are cardiac sarcoplasmic reticulum Ca^{2+} -pumping ATPase activators, being active at micromolar concentrations;¹¹ plakortones B–F exhibited moderate cytotoxicity against the murine fibro sarcoma cell line WEHI 164;¹⁴ plakortone G, a closely related analog of the γ -lactone isolated by Stierle and Faulkner,¹⁵ without a tetrahydrofuran moiety, showed antimalarial activity against both the D6 and

W2 clones of *Plasmodium falciparum*.¹⁶ The absolute configurations of these plakortones were not confirmed during the initial isolation. The total syntheses of plakortones B, D, and E helped to establish their absolute configurations later.^{17–19} However, the absolute configurations of the other four plakortones A, C, F, and G still remain unknown. Recently, plakortones L, N, and P were isolated from the sponge of *Plakinastrella clathrata*.⁸ They have bicyclic furanolactone fragments like plakortones A–F, but with methyl rather than ethyl substituents.

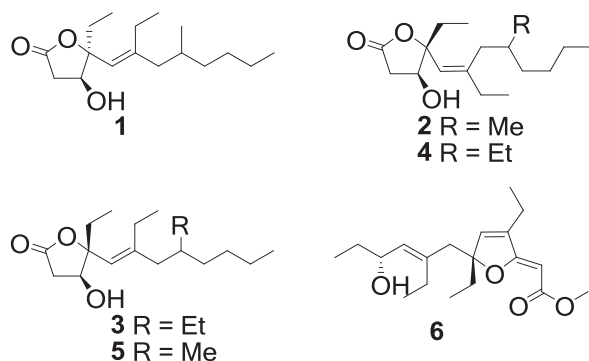
During the course of our continuing search for new drug leads from marine sponges collected off the Xisha Islands in the South China Sea, besides the previously reported two unusual polyketides simplextones A and B,²⁰ we have recently isolated and identified six new polyketides, simplexolides A–E (**1–5**) and plakorfuran A (**6**), together with four known furanylidenic methyl esters (2Z,6R,8R,9E) [3-ethyl-5-(2-ethyl-hex-3-enyl)-6-ethyl-5H-furan-2-ylidene]-acetic acid methyl ester (**7**),¹⁵ methyl (2Z,6R,8S)-4,6-diethyl-3,6-epoxy-8-methyldeca-2,4-dienoate (**8**),²¹ methyl (2Z,6R,8S)-3,6-epoxy-4,6,8-triethyldodeca-2,4-dienoate (**9**),²¹ and (2Z,6R,8R,9E)[3-ethyl-5-(2-ethyl-hex-3-enyl)-6-methyl-5H-furan-2-ylidene]-acetic acid methyl ester (**10**)²² from the marine sponge *Plakortis simplex*. The primary difference between compounds **1–5** and the previously isolated plakortones A–F from *Plakortis* spp. is the opening of the

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tetrahydrofuran ring in **1–5**. Herein, we describe the isolation, structure elucidation, and initial biological evaluation of compounds **1–6**.

2. Results and discussion

A sample of the sponge *P. simplex*, collected off the Xisha Islands in the South China Sea, was exhaustively extracted with MeOH. The extract was suspended in water and successively extracted with *n*-hexane, CH₂Cl₂, EtOAc, and *n*-BuOH. The CH₂Cl₂-soluble extract was subjected to repeated column chromatography followed by reversed-phase preparative HPLC to afford compounds **1–10** as colorless oils.



The molecular formula of compound **1** was assigned as C₁₇H₃₀O₃ by its HRESIMS (*m/z* 305.2094, [M+Na]⁺) and NMR data (Tables 1 and 2), displaying three degrees of unsaturation. The IR absorptions indicated the presence of hydroxyl (3451 cm⁻¹), double bond (1655 cm⁻¹), and ester carbonyl (1763 cm⁻¹) groups. Analysis of the ¹H NMR data (Table 1) and HSQC spectrum revealed the presence of four methyls, seven methylenes, two sp³ methines, one of which was oxygenated, one sp² methine, one oxygenated sp³ quaternary carbon, and two sp² quaternary carbons. By interpretation of ¹H–¹H COSY correlations, it was possible to establish five partial structures of consecutive proton systems: H-2/H-3, H-7/H-8/Me-17,

Table 2

¹³C NMR data of compounds **1–6** in CDCl₃

No.	1 ^a	2 ^b	3 ^b	4 ^b	5 ^b	6 ^b
1	174.9 qC	175.7 qC	175.1 qC	175.0 qC	175.6 qC	166.8 qC
2	37.6 CH ₂	38.8 CH ₂	38.8 CH ₂	38.8 CH ₂	38.9 CH ₂	84.0 CH
3	72.4 CH	74.4 CH	74.6 CH	74.6 CH	74.5 CH	171.4 qC
4	93.3 qC	92.8 qC	92.3 qC	92.2 qC	92.7 qC	140.3 qC
5	120.1 CH	123.4 CH	124.0 CH	123.5 CH	124.0 CH	139.4 CH
6	148.6 qC	147.3 qC	147.3 qC	147.7 qC	147.0 qC	97.4 qC
7	44.9 CH ₂	37.5 CH ₂	41.4 CH ₂	35.1 CH ₂	44.7 CH ₂	43.6 CH ₂
8	30.8 CH	30.6 CH	32.6 CH	37.0 CH	30.9 CH	139.0 qC
9	36.5 CH ₂	37.1 CH ₂	36.8 CH ₂	33.1 CH ₂	36.8 CH ₂	132.9 CH
10	29.2 CH ₂	29.2 CH ₂	28.8 CH ₂	29.2 CH ₂	29.3 CH ₂	69.6 CH
11	22.9 CH ₂	22.9 CH ₂	23.1 CH ₂	23.1 CH ₂	23.0 CH ₂	30.4 CH ₂
12	14.0 CH ₃	14.1 CH ₃	14.1 CH ₃	14.1 CH ₃	14.1 CH ₃	9.7 CH ₃
13	31.8 CH ₂	27.4 CH ₂	27.2 CH ₂	27.4 CH ₂	27.1 CH ₂	18.5 CH ₂
14	8.1 CH ₃	8.3 CH ₃	8.4 CH ₃	8.4 CH ₃	8.4 CH ₃	12.1 CH ₃
15	23.9 CH ₂	29.8 CH ₂	23.4 CH ₂	30.0 CH ₂	23.5 CH ₂	30.9 CH ₂
16	12.9 CH ₃	13.0 CH ₃	12.8 CH ₃	13.1 CH ₃	12.8 CH ₃	8.1 CH ₃
17	19.6 CH ₃	19.1 CH ₃	25.5 CH ₂	26.1 CH ₂	19.5 CH ₃	24.5 CH ₂
18			10.6 CH ₃	11.6 CH ₃		13.6 CH ₃
19						50.6 CH ₃

^a Recorded at 150 MHz.

^b Recorded at 125 MHz.

H-11/Me-12, H-13/Me-14, and H-15/Me-16 (Fig. 1). The HMBC correlations from Me-14 (δ_{H} 1.01) to C-4 (δ_{C} 93.3), from H-13a (δ_{H} 1.64) to C-3 (δ_{C} 72.4) and C-5 (δ_{C} 120.1), and from H-3 (δ_{H} 4.22) to C-1 (δ_{C} 174.9) indicated the attachment of an ethyl group at C-4. Moreover, the HMBC correlations from Me-16 (δ_{H} 1.03) to C-6 (δ_{C} 93.3) and from H-15 (δ_{H} 2.13) to C-5 (δ_{C} 120.1), C-6 (δ_{C} 148.6), and C-7 (δ_{C} 44.9) demonstrated the linkage of C-5, C-6, and C-15 via C-6. The HMBC correlations from Me-17 (δ_{H} 0.85) to C-9 (δ_{C} 36.5), from H-9a (δ_{H} 1.11) to C-11 (δ_{C} 22.9), and from Me-12 (δ_{H} 0.89) to C-10 (δ_{C} 29.2) defined the structure of the C-1 to C-12 portion of the molecule. The C-1 ester carbonyl was confirmed to form a γ -butyrolactone with the oxygenated C-4, which was severely downfield shifted at δ_{C} 93.3, to consume the remaining one degree of unsaturation.

The relative configuration of **1** was established on the basis of NOESY data (Fig. 1). The crucial NOE correlations between H-3 (δ_{H} 4.22) and H-13a (δ_{H} 1.64) suggested that these protons were oriented on the same face of the γ -butyrolactone moiety. The *E*-

Table 1

¹H NMR data of compounds **1–6** in CDCl₃

No.	1 ^a	2 ^b	3 ^b	4 ^b	5 ^b	6 ^b
2	2.59, dd (18.0, 1.2) 2.85, dd (18.0, 6.0) 4.22, d (5.4) 5.09, s	2.42, d (17.5) 2.80, dd (17.5, 5.5) 4.30, d (5.0) 5.14, s	2.43, d (17.5) 2.83, dd (17.5, 5.5) 4.32, t (4.0) 5.04 s	2.42, d (17.5) 2.82, dd (17.5, 5.5) 4.34, t (4.0) 5.15 s	2.46, d (17.5) 2.83, dd (17.5, 5.5) 4.31, d (5.5) 5.03, s	4.80, s
5	1.87, dd (13.8, 8.4) 2.17, m	2.11, dd (14.0, 9.5) 2.21, dd (13.5, 7.0)	1.95, m	2.15, dd (14.5, 7.5) 2.23, dd (14.5, 7.5)	1.70, dd (13.5, 9.0) 2.13, dd (13.5, 5.5)	6.22, s 2.53 d (14.0) 2.36 d (14.0)
8	1.62, m	1.65, m	1.21, m	1.45, m	1.58, m	
9	1.11, m 1.23, m	1.14, m 1.28, m	1.41, m	1.17, m 1.27, m	1.10, m 1.27, m	5.17, d (9.0)
10	1.32, m	1.30, m	1.23, m	1.28, m	1.27, m	4.24, dt (9.0, 6.5)
11	1.30, m	1.27, m	1.24, m	1.28, m	1.27, m	1.55, m 1.39, m
12	0.89, t (7.2)	0.90, t (7.5)	0.84, t (7.5)	0.99, t (7.5)	0.89, t (7.5)	0.86, t (7.5)
13	1.64, dq (7.2, 7.2) 1.72, dq (7.2, 7.2)	1.89, m 1.99, m	1.88, m 2.02, m	1.92, m 2.02, m	1.89, dq (15.0, 7.5) 2.03, dq (15.0, 7.5)	2.15, m
14	1.01, t (7.2)	0.95, t (7.5)	0.99, t (7.5)	0.98, t (7.5)	1.01, t (7.5)	1.15, t (7.5)
15	2.13, m	2.04, m	2.10, dq (7.5, 7.5) 2.27, dq (7.5, 7.5)	2.03, m	2.03, dq (14.0, 7.0) 2.33, dq (14.0, 7.0)	1.88, m 1.76, m
16	1.03, t (7.2)	0.99, t (7.5)	1.00, t (7.5)	1.00, t (7.5)	0.98, t (7.5)	0.81, t (7.5)
17	0.85, d (6.6)	0.84, d (6.5)	1.25, m	1.20, m	0.81, d (6.5)	2.09, m
18			0.89, t (7.5)	0.85, t (7.5)		0.97, t (7.5)
19						3.68, s

^a Recorded at 600 MHz.

^b Recorded at 500 MHz.

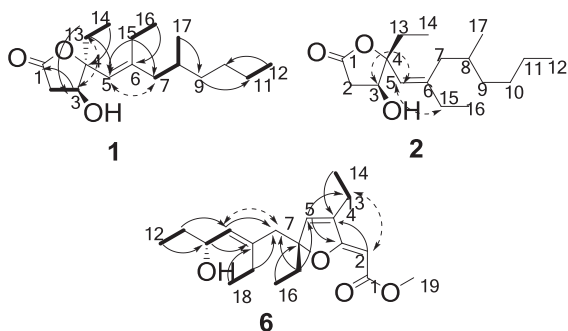


Fig. 1. COSY (—), key HMBC (---), and selected NOE (····) correlations of **1**, **2**, and **6**.

geometry of the $\Delta^{5,6}$ double bond was deduced from a NOESY correlation between H-5 (δ_{H} 5.09) and H-7a (δ_{H} 1.87). The absolute configuration of C-3 was determined by applying the modified Mosher's method to the secondary hydroxyl group.^{23,24} Compound **1** was reacted with (R)-(–)- and (S)-(+)- α -methoxy- α -trifluoromethylphenylacetic acid (MTPA) chlorides to give MTPA esters **1a** and **1b**, respectively. A consistent distribution of positive and negative $\Delta\delta$ values around C-3 allowed the assignment of S-configuration for C-3 (Fig. 2). On the basis of the previously determined relative configuration, a 3*S*,4*S* configuration was assigned to simplexolide A (**1**). However, configuration of the remote stereocenter at C-8 was not determined.

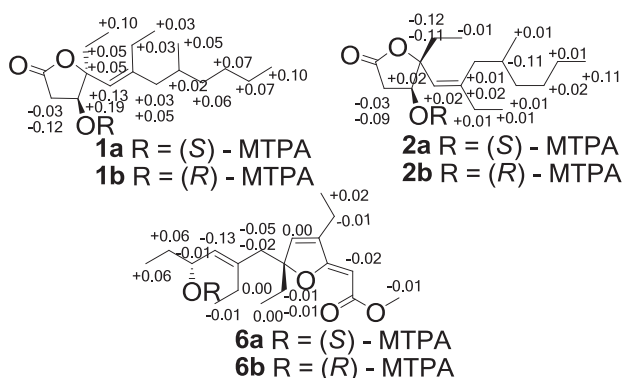


Fig. 2. $\Delta\delta_{\text{S-R}}$ values (ppm) for the MTPA derivatives of **1**, **2**, and **6** in CDCl_3 .

Simplexolide B (**2**) was clearly an isomer of compound **1** on the basis of the identical molecular formula of $\text{C}_{17}\text{H}_{30}\text{O}_3$ obtained by HRESIMS (m/z 305.2095 $[\text{M}+\text{Na}]^+$). Comparison of the NMR data between **2** and **1** (Table 1) suggested that they had the same planar structures except for the configuration of the double bond at C-5–C-6, which was assigned as *Z* geometry on the basis of a NOESY correlation between H-5 and H-15 (Fig. 1). The NOE correlation between H-3 and H-5 indicated the same orientation of these two protons. The absolute configuration at C-3 was determined to be *S* by the modified Mosher's method (Fig. 2), implying that **1** and **2** were epimeric at C-4. On the basis of above-mentioned analysis, a 3*S*,4*R* configuration was assigned to simplexolide B (**2**).

The molecular formula of compound **3** was established as $\text{C}_{18}\text{H}_{32}\text{O}_3$ on the basis of HRESIMS (m/z 319.2247, $[\text{M}+\text{Na}]^+$) and NMR data. The ^1H and ^{13}C NMR data indicated that **3** is a homolog of **2**. The ^1H NMR spectrum of **3** was almost identical to that of **2** except that a methyl group was replaced by an ethyl group at C-8 (Table 2). The geometry of the $\Delta^{5,6}$ double bond was assigned as *E* based on the NOESY correlation between H-5 and H-7. The crucial NOE correlation between H-3 and H-5 indicated that the relative

configuration was assumed to be the same as **2**. The CD spectrum of **3** displayed a positive Cotton effect at 196 nm, which was similar to that of **2** (Fig. 3), suggesting that the absolute configuration of **3** was the same as that of **2**. Thus, a 3*S*,4*R* configuration was assigned to simplexolide C (**3**).

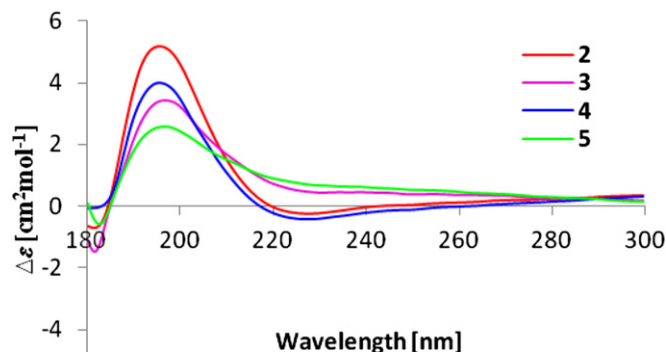


Fig. 3. CD curves of compounds **2**–**5**.

Compound **4** exhibited a quasi-molecular ion peak at m/z 319.2251 ($[\text{M}+\text{Na}]^+$, calcd 319.2249), consistent with the molecular formula of $\text{C}_{18}\text{H}_{32}\text{O}_3$. The ^1H and ^{13}C NMR data of **4** (Tables 1 and 2) were similar to those of **3**. The geometry of the double bond at C-5 and H-15. The CD spectrum of **4** revealed a pronounced positive Cotton effect with maxima observed at 196 nm (Fig. 3), and this closely matched the CD spectrum of **3**. Therefore, the absolute configuration of simplexolide D (**4**) was assigned to be 3*S*,4*R*, consistent with those of **3**.

The positive HRESIMS spectrum of simplexolide E (**5**) exhibited a pseudomolecular ion peak at m/z 305.2091 $[\text{M}+\text{Na}]^+$, consistent with the molecular formula of $\text{C}_{17}\text{H}_{30}\text{O}_3$, implying three degrees of unsaturation. Analysis of its NMR spectroscopic data revealed nearly identical structural features to those of **2**, except for the geometry of the double bond at C-5, which was assigned as *E* on the basis of an NOE correlation between H-5 and H-7. The CD spectra of **5** showed a positive Cotton effect at 196 nm (Fig. 3), similar to that of **2**, suggesting that the absolute configuration of **5** was 3*S*,4*R*.

The HRESIMS of compound **6** exhibited a pseudomolecular ion peak at m/z 345.2040 $[\text{M}+\text{Na}]^+$ and established a molecular formula of $\text{C}_{19}\text{H}_{30}\text{O}_4$, indicating five degrees of unsaturation. The ^{13}C NMR (Table 2) displayed 19 carbon signals, which were identified by the assistance of the DEPT spectrum as 5 methyls, 5 methylenes, 3 sp^2 methines, 1 oxygenated sp^3 methine, and 5 quaternary carbons. The carbon resonances at δ_{C} 84.0 (C-6) and 171.4 (C-3) are identical as those for a furano α,β -unsaturated ester.^{14,15} Furthermore, an ester carbonyl was recognized as being present in **6** from its ^{13}C NMR signal at δ_{C} 166.8 (qC, C-1) and strong IR absorptions at 1752 cm^{-1} . By interpretation of COSY correlations (Fig. 1), it was possible to establish the proton connections between H-15 and H-16, H-17 and H-18, H-9 and H-10, H-13 and H-14, and between H-11 and H-12. The connectivities of these partial structures were further established by the HMBC correlations (Fig. 1). The conjunction of C-10 and C-11 was elucidated on the basis of the HMBC correlation from H₃-12 to C-10. Moreover, the HMBC correlations from H₃-18 and H-10 to C-8 indicated the attachment between C-9 and C-17 via C-8. The HMBC correlations observed from H₃-16 to C-6, and from H-15 and H-17 to C-7 indicated the connection of C-8 and C-15 through C-6 and C-7. The HMBC correlations of H₃-19/C-1, H₃-14 and H-2/C-4, H-5/C-3 and C-13, and H-15/C-5 further confirmed

the existence of an unsaturated furan ester. With this assignment secured, the final oxymethine at C-10 had to be substituted with a hydroxyl group to satisfy the molecular formula. Finally, the *E*-geometry of the $\Delta^{8,9}$ double bond was deduced from a NOESY correlation between H-7 and H-9. The crucial NOE correlation between H-2 and H-13 indicated that the geometry of the $\Delta^{2,3}$ double bond is assumed to be *Z* (Fig. 1). This completed the assignment of the planar structure of plakorfuran A (**6**).

The absolute configuration at C-10 was determined to be *R* by applying a modified Mosher's ester method to the secondary hydroxyl group (Fig. 2). The absolute configuration at C-6 was determined by applying the CD exciton chirality method.²⁵ The CD spectrum of **6** revealed a negative Cotton effect at λ_{\max} 285 nm ($\Delta\epsilon$ –5.49) and a positive Cotton effect at λ_{\max} 205 nm ($\Delta\epsilon$ 5.85) due to the transition interaction between two different chromophores of the unsaturated furan ester and the $\Delta^{8,9}$ double bond (Fig. 4), indicating a negative chirality for **6**, thus concluding the absolute configuration as 6*R*,10*R*.

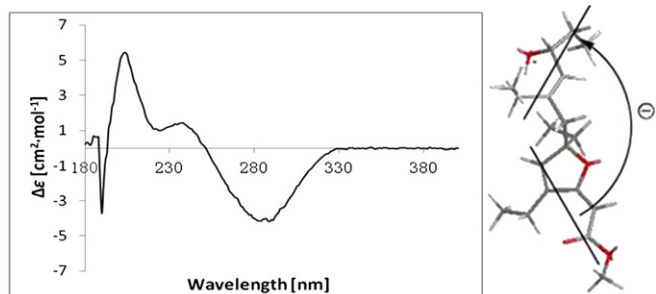


Fig. 4. Experimental CD spectrum of **6**. Bold lines denote the electric transition dipole of the chromophores for **6**.

Simplexolides A–E (**1–5**) feature a previously unknown tetrahydrofuran opened plakortone skeleton. A possible biogenetic pathway is proposed as shown in Scheme 1. Acetate, propionate, and butyrate units are required to assemble the polyketide skeleton.²⁶ The double bond at $\Delta^{3,4}$ is oxidized to an epoxide. With acyl

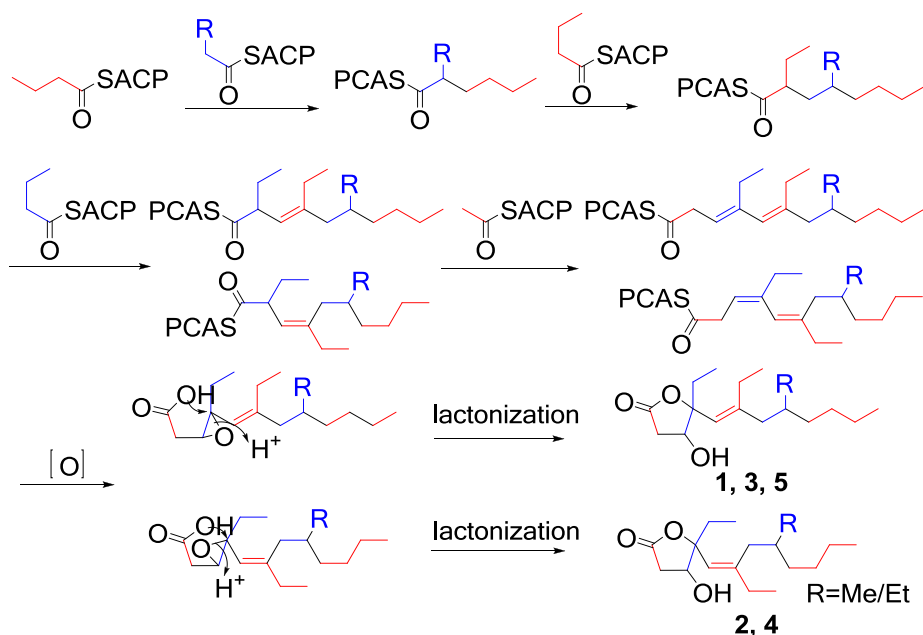
carrier protein (ACP) domain releasing, the carboxylic acid cyclizes onto the opening epoxide to generate a γ -lactone with the insertion of a hydroxyl.²⁷ In this lactonization step, a pair of stereoisomers are formed at C-4 due to the nucleophilic attack of the hydroperoxy group onto C-4 of the epoxide group from both of the side.

Compounds **1**, **2**, and **5–10** were tested for antifungal, cytotoxicity, antileishmanial, and antimalarial activities (Table 3). Compounds **2**, **5**, and **7–10** showed weak to moderate antifungal activity against the fungi *Cryptococcus neoformans*. The cytotoxic activity of compounds **1** and **2** against five human cancer cell lines, HCT-116 (colon cancer), HeLa (cervical cancer), SW480 (colon cancer), QGY-7703 (hepatocarcinoma), and A549 (lung carcinoma) was also assayed. The results showed that compound **1** exhibited much stronger cytotoxicity against the five cancer cell lines, which was due to the stereochemistry influenced. Compounds **2** and **10** showed moderate antileishmanial activity against *Leishmania donovani*, while compounds **7–9** were a little weaker, and compounds **1**, **5** and **6** were inactive. Furthermore, the antimalarial activity against chloroquine sensitive (D6, Sierra Leone) and resistant (W2, Indo China) strains of *P. falciparum* was tested. In this assay, only compound **10** displayed antimalarial activity against D6 and W2 with IC_{50} values of 2.0 and 2.0 μ g/mL, respectively, and both with SI [IC_{50} (fibroblast)/ IC_{50} (parasite)] of 2.4, while the other compounds were inactive.

3. Experimental section

3.1. General experimental procedures

Optical rotations were determined with a Perkin–Elmer 341 polarimeter equipped with a 1 mm cell. The CD spectra were obtained with a JASCO J-715 spectropolarimeter. IR spectra were recorded on a Bruker Vector 22 spectrometer using KBr pellets. The NMR experiments were conducted on Bruker AVANCE-600 and Bruker AMX-500 MHz instruments in $CDCl_3$ with TMS as an internal standard. HRESIMS and ESIMS were obtained on a Q-ToF micro YA019 mass spectrometer. Reversed-phase HPLC was performed using Sunfire C_{18} (5 μ m) and YMC-Pack Pro C_{18} RS (5 μ m) columns with a Waters 1525/2998 liquid chromatograph. Column



Scheme 1. Plausible biogenetic pathway for simplexolides A–E (**1–5**).

Table 3
Biological activities of **1**, **2** and **5–10**

Compound	Antifungal IC ₅₀ (μg/ml)/MIC (μg/ml)	Cytotoxicity IC ₅₀ (μg/ml)					Antileishmanial IC ₅₀ (μg/ml)	Antimalarial IC ₅₀ (μg/ml)	
		HCT-116	HeLa	SW480	QGY-7703	A549		D6	W2
1	—	9.23	27.43	17.08	26.53	16.79	>40	>50	>50
2	10.49/20	4.34	6.51	11.28	17.98	10.77	13.82	>50	>50
5	3.66	>50	—	—	—	—	>40	>50	>50
6	—	>50	—	—	—	—	>40	>50	>50
7	20.00	—	—	—	—	—	38.56	>50	>50
8	15.30	—	—	—	—	—	31.24	>50	>50
9	20.00	—	—	—	—	—	20.20	>50	>50
10	11.72	—	—	—	—	—	7.11	2.0	2.0
Amphotericin B	0.317	—	—	—	—	—	0.34	—	—
Camptothecin	—	3.22	2.43	8.26	1.41	0.81	—	—	—
Pentamidine	—	—	—	—	—	—	1.62	—	—
Chloroquine	—	—	—	—	—	—	—	0.06	0.83

chromatography (CC) was carried out on silica gel 60 (200–300 mesh; Yantai, China), and Sephadex LH-20 (Pharmacia). TLC was carried out using HSGF 254 plates and visualized by spraying with anisaldehyde/H₂SO₄ reagent. Samples were weighed on a Shushi analytical balance.

3.2. Animal material

The sponge specimen was collected around Yongxing Island and seven connected islets in the South China Sea in June 2007, and were identified by Prof. Jin-He Li (Institute of Oceanology, Chinese Academy of Sciences, China). A voucher sample (No. B-3) was deposited in the Laboratory of Marine Drugs, Department of Pharmacy, Changzheng Hospital, Second Military Medical University, China.

3.3. Extraction and isolation

The air-dried and powdered sponge (2.0 kg, dry weight) was extracted with MeOH, and the crude extract was concentrated under reduced pressure at 45 °C to yield 500 g of residue. The residue was then extracted successively with *n*-hexane, CH₂Cl₂, EtOAc, and *n*-BuOH. The CH₂Cl₂ extract (41 g) was separated by vacuum liquid chromatography (VLC) on silica gel using CH₂Cl₂/MeOH as the eluent to give three fractions (Fr. A–C). The lower polarity part fraction A (CH₂Cl₂/MeOH 25:1) was subjected to VLC eluting with petroleum ether/EtOAc (PE) to give four subfractions (Fr. A1–A4). Fraction A3 was purified by reversed-phase preparative HPLC (Sunfire C₁₈, 5 μm, 10×250 mm) to yield 31.7 mg of compound **1** (CH₃OH/H₂O 95:5, 2.0 mL/min, UV detection at 210 nm, *t*_R=17.0 min), 22.1 mg of compound **6** (CH₃CN/H₂O 50:50, 2.0 mL/min, UV detection at 282 nm, *t*_R=17.2 min), a mixture of compounds **3** and **4** (CH₃CN/H₂O 70:30, 2.0 mL/min, UV detection at 200 nm, *t*_R=29.0 min), and a mixture of compounds **2** and **5** (CH₃CN/H₂O 70:30, 2.0 mL/min, UV detection at 200 nm, *t*_R=24.4 min). The mixture of compounds **3** and **4** and the mixture of compounds **2** and **5** were further purified by reversed-phase preparative HPLC (YMC-Pack Pro C₁₈ RS, 5 μm, 10×250 mm) to yield 21.3 mg of compound **3** (CH₃CN/H₂O 85:15, 2.0 mL/min, UV detection at 200 nm, *t*_R=18.4 min), 1.8 mg of compound **5** (CH₃CN/H₂O 85:15, 2.0 mL/min, UV detection at 200 nm, *t*_R=17.4 min), 8.2 mg of compound **2** (CH₃CN/H₂O 85:15, 2.0 mL/min, UV detection at 200 nm, *t*_R=66.9 min), and 52.1 mg of compound **5** (H₃CN/H₂O 85:15, 2.0 mL/min, UV detection at 200 nm, *t*_R=62.6 min). Similarly, the four known compounds **7** (22.0 mg), **8** (15.1 mg), **9** (8.3 mg), and **10** (2.2 mg) were obtained from fraction A1.

3.3.1. Simplexolide A (1). Colorless oil; [α]_D²³ +1 (c 0.215, MeOH); IR (KBr) ν_{\max} 3451, 2960, 2927, 2873, 1763, 1655, 1464, 1412, 1378,

1343, 1290, 1248, 1210, 1182, 1119, 1103, 1090, 1071, 1014, 980, 958, 926, 885, 800 cm⁻¹; ¹H NMR (CDCl₃, 600 MHz) and ¹³C NMR (CDCl₃, 150 MHz) data, see Tables 1 and 2; HRESIMS *m/z* 305.2094 [M+Na]⁺ (calcd for C₁₇H₃₀O₃Na, 305.2093). CD spectrum (c 1.1 mg/mL, CH₃CN), 193 nm ($\Delta\epsilon$ -0.65), 222 nm ($\Delta\epsilon$ 0.84).

3.3.2. Simplexolide B (2). Colorless oil; [α]_D²³ +2 (c 0.120, MeOH); IR (KBr) ν_{\max} 3439, 2962, 2930, 2874, 1758, 1655, 1460, 1400, 1379, 1341, 1280, 1250, 1208, 1165, 1111, 1063, 1023, 972, 954, 916, 879, 799 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) and ¹³C NMR (CDCl₃, 125 MHz) data, see Tables 1 and 2; HRESIMS *m/z* 305.2095 [M+Na]⁺ (calcd for C₁₇H₃₀O₃Na, 305.2093). CD spectrum (c 0.5 mg/mL, CH₃CN), 196 nm ($\Delta\epsilon$ +5.16).

3.3.3. Simplexolide C (3). Colorless oil; [α]_D²³ -9 (c 0.090, MeOH); IR (KBr) ν_{\max} 3444, 2962, 2928, 2874, 1758, 1655, 1464, 1399, 1379, 1341, 1287, 1251, 1203, 1164, 1113, 1099, 1022, 975, 951, 915, 879, 800 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) and ¹³C NMR (CDCl₃, 125 MHz) data, see Tables 1 and 2; HRESIMS *m/z* 319.2247 [M+Na]⁺ (calcd for C₁₈H₃₂O₃Na, 319.2249). CD spectrum (c 0.5 mg/mL, CH₃CN), 196 nm ($\Delta\epsilon$ +3.43).

3.3.4. Simplexolide D (4). Colorless oil; [α]_D²³ +14 (c 0.130, MeOH); IR (KBr) ν_{\max} 3448, 2962, 2930, 2874, 1759, 1655, 1460, 1400, 1379, 1340, 1280, 1250, 1205, 1163, 1111, 1036, 1024, 975, 955, 914, 798 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) and ¹³C NMR (CDCl₃, 125 MHz) data, see Tables 1 and 2; HRESIMS *m/z* 319.2251 [M+Na]⁺ (calcd for C₁₈H₃₂O₃Na, 319.2249). CD spectrum (c 0.9 mg/mL, CH₃CN), 196 nm ($\Delta\epsilon$ +3.99).

3.3.5. Simplexolide E (5). Colorless oil; [α]_D²³ -18 (c 0.170, MeOH); IR (KBr) ν_{\max} 3446, 2961, 2927, 2874, 1758, 1655, 1465, 1400, 1379, 1342, 1288, 1252, 1202, 1166, 1112, 1063, 1021, 974, 951, 916, 879, 800 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) and ¹³C NMR (CDCl₃, 125 MHz) data, see Tables 1 and 2; HRESIMS *m/z* 305.2091 [M+Na]⁺ (calcd for C₁₇H₃₀O₃Na, 305.2093). CD spectrum (c 0.7 mg/mL, CH₃CN), 196 nm ($\Delta\epsilon$ +2.57).

3.3.6. Plakorforan A (6). Colorless oil; [α]_D²³ -96 (c 0.085, MeOH); ¹H NMR (CDCl₃, 500 MHz) and ¹³C NMR (CDCl₃, 125 MHz) data, see Tables 1 and 2; HRESIMS *m/z* 345.2040 [M+Na]⁺ (C₁₉H₃₀O₄Na, calcd 345.2042). CD spectrum (c 0.85 mg/mL, CH₃CN), 205 nm ($\Delta\epsilon$ 5.85), 239 nm ($\Delta\epsilon$ 1.70), 285 nm ($\Delta\epsilon$ -5.49).

3.4. Preparation of MTPA esters 1a and 1b

Simplexolide A (**1**; 1.0 mg and 0.8 mg, respectively) was reacted with R-(−)- or S-(+)-MTPACl (15 μL) in freshly distilled dry pyridine (500 μL) and stirred under N₂ at room temperature for 18 h, respectively, and the solvent was removed in vacuo. The products

were purified by mini-column chromatography on silica gel (200 mesh, petroleum ether/EtOAc, 1:1) to afford *S*-(–)- and *R*-(+)-MTPA esters **1a** and **1b**, respectively.

3.5. Preparation of MTPA esters **2a** and **2b**

Simplexolide C (**3**; 1 mg each) was similarly processed to give *S*-(–)- and *R*-(+)-MTPA esters **3a** and **3b**, respectively.

3.6. Preparation of MTPA esters **6a** and **6b**

Plakorfulan A (**6**; 1 mg each) was similarly processed to give *S*-(–)- and *R*-(+)-MTPA esters **6a** and **6b**, respectively.

3.7. Biological tests

Antifungal assay against *C. neoformans* was performed as described by Ikhlas A. Khan et al.²⁸ Amphotericin B was used as the positive control. Cytotoxicity was determined against human cancer cell lines HCT-116 (colon cancer), HeLa (cervical cancer), SW480 (colon cancer), QGY-7703 (hepatocarcinoma), and A549 (lung carcinoma) using the MTT assay method. Camptothecin was used as the positive control. The experimental details of this assay were carried out according to a previously described procedure.²⁹ Antimalarial activity was determined in vitro against chloroquine sensitive (D6, Sierra Leone) and resistant (W2, Indo China) strains of *P. falciparum* by measuring plasmodial LDH activity.³⁰ Chloroquine was used as the positive control. In vitro antileishmanial activity was tested on a culture of *L. donovani* promastigotes. In a 96-well microplate assay, test compounds were diluted to an appropriate concentration and added to the *Leishmania* promastigotes culture (2×10^6 cell/mL). The plates were incubated at 26 °C for 72 h and growth of *Leishmania* promastigotes was determined by Alamar blue assay.³¹ Pentamidine and Amphotericin B were used as the standard antileishmanial agents. The growth inhibition curve was used to compute the IC₅₀ value for each compound.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2012.04.025. These data include MOL files and InChIKeys of the most important compounds described in this article.

References and notes

- Higgs, M. D.; Faulkner, D. J. *J. Org. Chem.* **1978**, *43*, 3454–3457.
- Braekman, J. C.; Daloze, D.; De Groote, S.; Fernandes, J. B.; Van Soest, R. W. M. *J. Nat. Prod.* **1998**, *61*, 1038–1042.
- Hu, J. F.; Gao, H. F.; Kelly, M.; Hamann, M. T. *Tetrahedron* **2001**, *57*, 9379–9383.
- Kossuga, M. H.; Nascimento, A. M.; Reimao, J. Q.; Tempone, A. G.; Taniwaki, N. N.; Veloso, K.; Ferreira, A. G.; Cavalcanti, B. C.; Pessoa, C.; Moraes, M. O.; Mayer, A. M. S.; Hajdu, E.; Berlinck, R. G. S. *J. Nat. Prod.* **2008**, *71*, 334–339.
- Feng, Y.; Davis, R. A.; Sykes, M.; Avery, V. M.; Camp, D.; Quinn, R. J. *J. Nat. Prod.* **2010**, *73*, 716–719.
- Rahm, F.; Hayes, P.; Kitching, W. *Heterocycles* **2004**, *64*, 523–575.
- Qureshi, A.; Salvá, J.; Harper, M. K.; Faulkner, D. J. *J. Nat. Prod.* **1998**, *61*, 1539–1542.
- Yong, K. W. L.; De Voss, J. J.; Hooper, J. N. A.; Garson, M. J. *J. Nat. Prod.* **2011**, *74*, 194–207.
- Rudi, A.; Afanir, R.; Gravalos, L. G.; Akinin, M.; Gaydou, E.; Vacelet, J.; Kashman, Y. *J. Nat. Prod.* **2003**, *66*, 682–685.
- Perry, T. L.; Dickerson, A.; Khan, A. A.; Kondru, R. K.; Beratan, D. N.; Wipf, P.; Kelly, M.; Hamann, M. T. *Tetrahedron* **2001**, *57*, 1483–1487.
- Patil, A. D.; Freyer, A. J.; Bean, M. F.; Carte, B. K.; Westley, J. W.; Johnson, R. K.; Lahouratate, P. *Tetrahedron* **1996**, *52*, 377–394.
- Zampella, A.; Giannini, C.; Debitus, C.; D'Auria, M. V. *Tetrahedron* **2001**, *57*, 257–263.
- De Guzman, F. S.; Schmitz, F. J. *J. Nat. Prod.* **1990**, *53*, 926–931.
- Cafieri, F.; Fattorusso, E.; Tagliatela-Scafati, O.; Ianaro, A. *Tetrahedron* **1999**, *55*, 13831–13840.
- Stierle, D. B.; Faulkner, D. J. *J. Org. Chem.* **1980**, *45*, 3396–3401.
- Gochfeld, D. J.; Hamann, M. T. *J. Nat. Prod.* **2001**, *64*, 1477–1479.
- Xie, X. G.; Wu, X. W.; Lee, H. K.; Peng, X. S.; Wong, H. N. *Chem.—Eur. J.* **2010**, *16*, 6933–6941.
- Hayes, P. Y.; Kitching, W. J. *Am. Chem. Soc.* **2002**, *124*, 9718–9719.
- Akiyama, M.; Isoda, Y.; Nishimoto, M.; Narazaki, M.; Oka, H.; Kuboki, A.; Ohira, S. *Tetrahedron Lett.* **2006**, *47*, 2287–2290.
- Liu, X. F.; Song, Y. L.; Zhang, H. J.; Yang, F.; Yu, H. B.; Jiao, W. H.; Piao, S. J.; Chen, W. S.; Lin, H. W. *Org. Lett.* **2011**, *13*, 3154–3157.
- Compagnone, R. S.; Pina, I. C.; Rangel, H. R.; Dagger, F.; Suarez, A. I.; Venkata Rami Reddy, M.; John Faulkner, D. *Tetrahedron* **1998**, *54*, 3057–3068.
- Epifanio, R.; Pinheiro, L.; Alves, N. J. *Braz. Chem. Soc.* **2005**, *16*, 1367–1371.
- Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
- Hoye, T. R.; Jeffrey, C. S.; Shao, F. *Nat. Protoc.* **2007**, *2*, 2451–2458.
- Cai, G.; Bozhkova, N.; Odingo, J.; Berova, N.; Nakanishi, K. *J. Am. Chem. Soc.* **1993**, *115*, 7192–7198.
- Huang, X. H.; van Soest, R.; Roberge, M.; Andersen, R. J. *Org. Lett.* **2003**, *6*, 75–78.
- Richard, G. B.; Mary, J. G.; James, S. J. *Chem. Soc., Chem. Commun.* **1980**, 1165–1167.
- Ma, G.; Khan, S. I.; Jacob, M. R.; Tekwani, B. L.; Li, Z.; Pasco, D. S.; Walker, L. A.; Khan, I. A. *Antimicrob. Agents Chemother.* **2004**, *48*, 4450–4452.
- Carmichael, J.; De Graff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936–942.
- Muhammad, I.; Bedir, E.; Khan, S. I.; Tekwani, B. L.; Khan, I. A.; Takamatsu, S.; Pelletier, J.; Walker, L. A. *J. Nat. Prod.* **2004**, *67*, 772–777.
- Mikus, J.; Steverding, D. *Parasitol. Int.* **2000**, *48*, 265–269.