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Woodylides A–C, New Cytotoxic Linear Polyketides from the South China Sea Sponge *Plakortis simplex*

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Abstract: Three new polyketides, woodylides A–C (**1**–**3**), were isolated from the ethanol extract of the South China Sea sponge *Plakortis simplex*. The structures were elucidated by spectroscopic data (IR, 1D and 2D NMR, and HRESIMS). The absolute configurations at C-3 of **1** and **3** were determined by the modified Mosher's method. Antifungal, cytotoxic, and PTP1B inhibitory activities of these polyketides were evaluated. Compounds **1** and **3** showed antifungal activity against fungi *Cryptococcus neoformans* with IC_{50} values of 3.67 and 10.85 μg/mL, respectively. In the cytotoxicity test, compound **1** exhibited a moderate effect against the HeLa cell line with an IC_{50} value of 11.2 μg/mL, and compound **3** showed cytotoxic activity against the HCT-116 human colon tumor cell line and PTP1B inhibitory activity with IC_{50} values of 9.4 and 4.7 μg/mL, respectively.

Keywords: *Plakortis simplex*; woodylides; cytotoxicity; PTP1B inhibitory activity

1. Introduction

Polyketides are a structurally diverse family of natural products with various biological activities and pharmacological properties, biogenetically derived from acetate, propionate and butyrate units [1–3].

Marine sponges provide a wide range of polyketides with antibacterial [4], antiviral [5], antitumor [6], antimalarial [7], and taxol-like microtubule-stabilizing activities [8]. The sponge-derived polyketides often contain cyclic peroxides and lactone functionalities, linear and bicyclic carbon frameworks [1,9], and macrolide and aromatic groups in some cases [8,10]. A prolific source of new and bioactive polyketides derived from sponges of the genus *Plakortis* attracted our attention. As part of our ongoing search for new pharmacologically active lead compounds from the marine sponges collected off Xisha Islands in the South China Sea [11,12], we investigated polyketides from the marine sponge *Plakortis simplex*. A preliminary study led to the isolation of two new polyketides named simplextones A and B with an unusual cyclopentane skeleton [6]. The interesting chemical and bioactive significance of *P. simplex* prompted us to continue the study of this sponge, which has led to the isolation of three new linear polyketides, named as woodylide A (**1**), B (**2**) and C (**3**) (Figure 1) [13], which are the acyclic diol analogues of the cyclic polyketide peroxides isolated from the genus of *Plakortis* [14,15]. This article describes the isolation, identification and bioactivity of the new compounds.

Figure 1. Structures of woodylides A (**1**), B (**2**) and C (**3**).
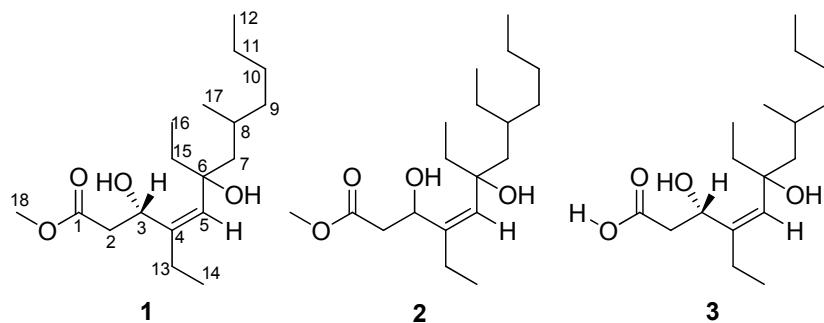
2. Results and Discussion

Compound **1** was obtained as a colorless oil. The positive HRESIMS exhibited a pseudomolecular ion peak at *m/z* 337.2354, [M + Na]$^+$ (calcd 337.2355 [16]), consistent with a molecular formula of $C_{18}H_{34}O_4$, indicating two double bond equivalents. The IR absorption bands supported the existence of hydroxyl (3275 cm^{-1}), carbonyl (1742 cm^{-1}), and olefinic (1650 cm^{-1}) functional groups. The ^{13}C NMR and DEPT spectra indicated the presence of 18 carbon atoms, corresponding to a total of one carbonyl (δ_C 173.6), one olefinic quaternary carbon (δ_C 140.5), one olefinic methine (δ_C 132.4), one oxygenated quaternary carbon (δ_C 77.0), one oxymethine (δ_C 68.8), one methoxyl (δ_C 51.8), one aliphatic methine (δ_C 29.2), seven aliphatic methylenes (δ_C 23.0, 29.4, 29.4, 36.5, 38.5, 40.7, and 48.9), and four methyl carbons (δ_C 8.3, 13.7, 14.2, and 22.2) (Table 1). The ^1H NMR spectrum displayed resonances for one methyl group attached to a tertiary carbon at δ_H 0.98 (3H, d, *J* = 6.5 Hz), three methyl groups attached to secondary carbons at δ_H 0.88 (3H, t, *J* = 7.0 Hz), δ_H 0.88 (3H, t, *J* = 7.0 Hz overlapped), and δ_H 1.05 (3H, t, *J* = 7.0 Hz), one methoxyl group at δ_H 3.71 (3H, s), and one olefinic proton at δ_H 5.11 (1H, s). The two double bond equivalents of **1** were accounted for one double bond and one carbonyl group, revealing the linearity of its carbon scaffold. Analysis of the COSY and HSQC spectra revealed the presence of five spin systems in the structure: H$_2$-2/H-3, H-8/H$_3$-17, H$_2$-11/H$_3$-12, H$_2$-13/H$_3$-14, and H$_2$-15/H$_3$-16 (Figure 2). The HMBC correlation from H$_3$-18 (δ_H 3.71) to C-1 (δ_C 173.6) positioned the methoxyl group at C-1. The olefinic proton H-5 (δ_H 5.11) afforded

HMBC correlations to C-3 (δ_C 68.8), C-4 (δ_C 140.5), and C-6 (δ_C 77.0), whereas H-7a (δ_H 1.34) showed HMBC correlations to C-5 (δ_C 132.4) and C-6, which established the connectivity of the partial structure C-3 to C-7 (δ_C 48.9). Obviously, the double bond was located between C-4 and C-5 on the linear carbon scaffold based on the carbon resonances of C-4 and C-5. Accordingly, the methyl acetate group was tethered to C-4 via C-3 by HMBC correlations from H-2b (δ_H 2.92) to C-1 and C-4, from H_2-13 (δ_H 2.02) to C-3, and from H-5 to C-3. The HMBC correlations from H_3-14 (δ_H 1.05) to C-4, and from H_3-16 (δ_H 0.88) to C-6, unambiguously assigned the ethyl groups to C-4 and C-6, respectively. Moreover, the HMBC correlations from H_3-17 (δ_H 0.98) to C-7 and C-9 (δ_C 38.5), and from H-7b (δ_H 1.55) to C-9 demonstrated the linkage of C-7, C-9 and C-17 (δ_C 22.2) via C-8. Even though no COSY correlation was observed between H_2-10 (δ_H 1.25) and H_2-11 (δ_H 1.25), the connectivity of the partial structure C-9 to C-12 (δ_C 14.2) was secured by the HMBC correlations of H-9b (δ_H 1.30)/C-10 (δ_C 29.4), and H_3-12 (δ_H 0.88)/C-10, and by comparison of the NMR date with the known derivatives [17]. With this assignment secured, the final methine (C-3) and the oxygenated quaternary carbon (C-6) had to be substituted with hydroxyl groups to satisfy the molecular formula and shifts.

Table 1. NMR data for woodylides A–C (**1–3**) in CDCl$_3$.

| Position	1 [a]		2 [b]		3 [b]	
	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
1	173.6 qC		173.6 qC		176.4 qC	
2a	40.7 CH$_2$	2.57, dd (16.5, 3.0)	40.6 CH$_2$	2.57, dd (16.8, 3.0)	41.0 CH$_2$	2.53, d (15.6)
2b		2.92, dd (16.5, 10.0)		2.99, dd (16.8, 10.2)		2.90, dd (15.6, 4.8)
3	68.8 CH	4.83, dd (10.0, 3.0)	68.8 CH	4.81, dd (10.2, 3.0)	68.9 CH	4.67,d (9.6)
4	140.5 qC		140.4 qC		140.9 qC	
5	132.4 CH	5.11, s	132.5 CH	5.11, s	131.3 CH	5.05, s
6	77.0 qC		77.0 qC		77.8 qC	
7a	48.9 CH$_2$	1.34, dd (14.0, 7.0)	45.8 CH$_2$	1.44, m	49.0 CH$_2$	1.35, dd (13.8, 6.6)
7b		1.55, dd (15.0, 7.0)		1.47, m		1.56, dd (12.0, 4.8)
8	29.2 CH	1.61, m	35.0 CH	1.55, m	29.1 CH	1.60, m
9a	38.5 CH$_2$	1.14, m	34.3 CH$_2$	1.26, m	38.5 CH$_2$	1.13, m
9b		1.30, m		1.33, m		1.30, m
10	29.4 CH$_2$	1.25, m	29.0 CH$_2$	1.22, m	29.3 CH$_2$	1.25, m
11	23.0 CH$_2$	1.25, m	23.2 CH$_2$	1.26, m	23.0 CH$_2$	1.25,m
12	14.2 CH$_3$	0.88, t (7.0)	14.2 CH$_3$	0.88, t (7.2)	14.1 CH$_3$	0.86, t (7.2)
13	29.4 CH$_2$	2.02, m	29.6 CH$_2$	2.04, m	29.8 CH$_2$	2.02, m
14	13.7 CH$_3$	1.05, t (7.0)	13.6 CH$_3$	1.05, t (7.8)	13.5 CH$_3$	1.04, t (7.2)
15	36.5 CH$_2$	1.55, m	36.6 CH$_2$	1.55, m	36.5 CH$_2$	1.56, m
16	8.3 CH$_3$	0.88, t (7.0)	8.4 CH$_3$	0.88, t (7.2)	8.3 CH$_3$	0.86, t (7.2)
17	22.2 CH$_3$	0.98, d (6.5)	27.7 CH$_2$	1.45, m	22.1 CH$_3$	0.96, d (6.6)
18	51.8 -OCH$_3$	3.71, s	10.7 CH$_3$	0.85, t (7.2)		
19			51.8-OCH$_3$	3.71, s		

[a] Measured at 500 MHz (^{1}H) and 125 MHz (^{13}C); [b] Measured at 600 MHz (^{1}H) and 150 MHz (^{13}C).

Figure 2. COSY (──), Key HMBC (→), and selected NOE ( ) correlations of **1**, **2**, and **3**.
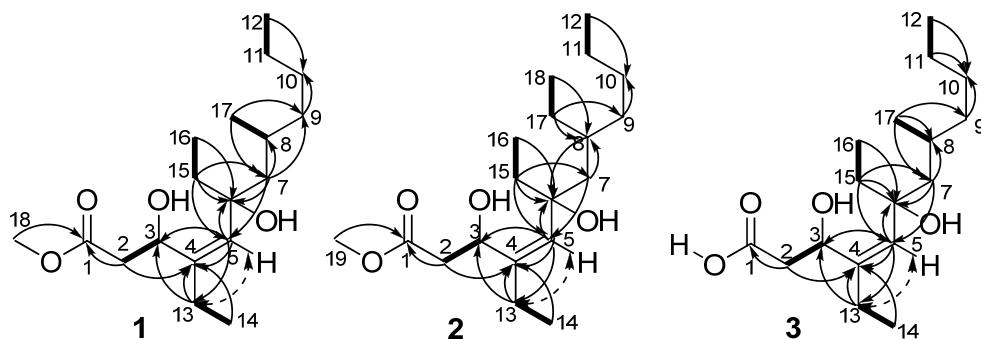
The configuration of double bond in **1** was established on the basis of NOESY data. The *Z*-geometry of the $\Delta^{4,5}$ double bond was deduced from a NOESY correlation between H-5 and H_2-13, as well as derived from devoid of NOESY correlation between H-5 and H-3 (δ_H 4.83) (Figure 2). The absolute configuration of C-3 was determined by applying the modified Mosher's method to the secondary hydroxyl group [18]. The (*S*)- and (*R*)-MTPA esters of **1** were prepared by reaction with (*R*)- and (*S*)-MTPA chlorides, respectively. The $\Delta\delta_{S-R}$ values observed for the protons near the secondary C-3 hydroxyl group for the esters indicated the *S*-configuration for the secondary alcohol stereogenic center in **1** (Figure 3).

Figure 3. $\Delta\delta_{S-R}$ values for the MTPA derivatives of **1** and **3** in CDCl$_3$.

1a R = (*S*) - MTPA
1b R = (*R*) - MTPA

3a R = (*S*) - MTPA
3b R = (*R*) - MTPA
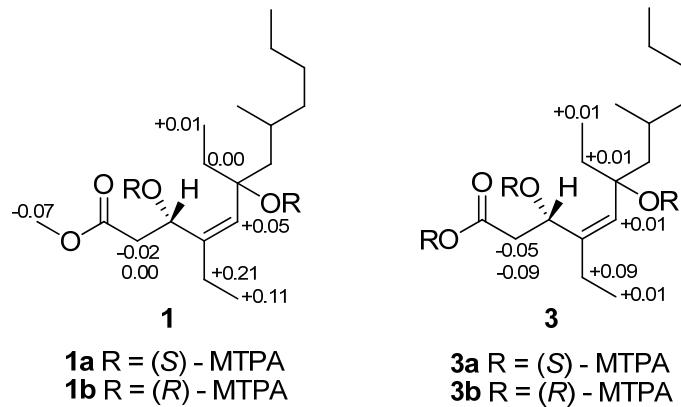
Compound **2** was also isolated as a colorless oil, with a molecular formula of $C_{19}H_{36}O_4$ as determined by HRESIMS (*m/z* 351.2513, [M + Na]$^+$, calcd 351.2511). Comparison of the ^{1}H NMR data of **2** with those of **1**, the obvious differences were the presence of an additional methyl triplet (δ_H 0.85, t, *J* = 7.2 Hz) and a methylene multiplet (δ_H 1.45, m), as well as the absence of the methyl doublet (δ_H 0.98, d, *J* = 6.5 Hz), indicating an overall structure similar to **1** except for an ethyl group C-17 (δ_C 27.7)/C-18 (δ_C 10.7) attached to C-8 (δ_C 35.0) in **2** (Table 1). This was also supported by the HMBC correlations from both H_3-18 (δ_H 0.85) and H_2-17 (δ_H 1.45) to C-8, and ^{1}H–^{1}H COSY correlations between H_3-18 and H_2-17. The geometry of the trisubstituted double bond was assigned as *Z* based on the NOESY correlation between H_2-13 (δ_H 2.04, m) and H-5 (δ_H 5.11, m) (Figure 2).

Compound **3** was assigned a molecular formula of $C_{17}H_{32}O_4$, implying two double bond equivalents, as deduced from the HRESIMS (*m/z* 323.2200, [M + Na]$^+$, calcd 323.2198) and NMR data. The

^{13}C NMR and DEPT spectra exhibited 17 carbon resonances corresponding to four methyl, seven methylene, three methine, and three quaternary carbons (Table 1). The overall appearance of the NMR spectrum showed close structural similarity between **3** and **1**, except for the absence of a methoxyl resonance in **3** instead of H$_3$-18 (δ_H 3.71)/C-18 (δ_C 51.8) in **1**, indicating **3** was a free carboxylic acid. This was also confirmed by the observation of a $\Delta\delta$ ~3 downfield shift of the C-1 from δ_C 173.6 to δ_C 176.4. The NOESY correlations observed between H$_2$-13 (δ_H 2.02) and H-5 (δ_H 5.05), confirmed the *Z* geometry of the double bond at C-4 (δ_C 140.9)/C-5 (δ_C 131.3). The absolute configuration of C-3 was determined by the modified Mosher's method [18]. Analysis of the $\Delta\delta_{S-R}$ values (Figure 3) according to Mosher's model pointed to an *S*-configuration for C-3 in **3**.

To confirm if compound **1** could be an artifact formed from **3** during the isolation processes, a solution of **3** was kept at room temperature for three days in the presence of Si-60 or RP-18 gel in MeOH, respectively. The formation of **1** was not observed, thus suggesting that compound **1** may be a natural product and not an artifact.

The three new polyketides **1**–**3** were evaluated for antifungal activity against *Cryptococcus neoformans* (ATCC 90113), *Candida albicans* (Y0109), *Trichophyton rubrum* (Cmccftla) and *Microsporum gypseum* (Cmccfmza) (Table 2), for *in vitro* cytotoxic activity against human cancer cell lines, HCT-116 (colon cancer), A549 (lung carcinoma), HeLa (cervical cancer), QGY-7703 (hepatocarcinoma), and MDA231 (breast adenocarcinoma) (Table 3), and protein tyrosine phosphatase 1B (PTP1B) inhibitory activity (Table 3). Compounds **1** and **3** showed moderate antifungal activity against the fungus *C. neoformans* with IC$_{50}$ values of 3.67 and 10.85 µg/mL, respectively, while compound **2**, bearing an ethyl group at C-8, was inactive even tested at a higher concentration. Compounds **1** and **3** showed weaker antifungal activity towards all the remaining assayed indicators. Furthermore, compound **1** showed moderate cytotoxicity (IC$_{50}$, 11.22 µg/mL) against HeLa cell line, and compound **3** exhibited cytotoxic activity (IC$_{50}$, 9.4 µg/mL) against HCT-116 cell line. Cytotoxicity of compound **3** against A549, HeLa, QGY-7703, and MDA231 cell lines was weaker when compared to that of **1**. In addition, compound **3** was tested for PTP1B inhibitory activity *in vitro*, with an IC$_{50}$ value of 4.7 µg/mL. The PTP1B inhibitors are recognized as potential therapeutic agents for the treatment of type II diabetes and obesity [19].

Table 2. Antifungal activity of woodylides A–C (**1**–**3**).

Compound	Antifungal Activity			
	C. neoformans [a]	*C. albicans* [b]	*T. rubrum* [b]	*M. gypseum* [b]
1	3.67	32	32	32
2	NA	NT	NT	NT
3	10.85	NA	32	32
Amphotericin B	0.35	NT	NT	NT
Fluconazole	NT	0.25	2	8

[a] Exhibited with IC$_{50}$ value (µg/mL); [b] Exhibited with MIC (µg/mL); NT = Not tested; NA = Not active.

Table 3. Cytotoxic and PTP1B inhibitory activitives of woodylides A–C (**1–3**).

Compound	Cytotoxicity (IC$_{50}$, μg/mL)					PTP1B Inhibitory Activity (IC$_{50}$, μg/mL)
	HCT-116	**A549**	**HeLa**	**QGY-7703**	**MDA231**	
1	NT	37.83	11.22	25.80	NA	NT
2	NT	NT	NT	NT	NT	NT
3	9.4	NA	NA	NA	NT	4.7
Sodium orthovanadate	NT	NT	NT	NT	NT	88.46

NT = Not tested; NA = Not active.

3. Experimental Section

3.1. General Experimental Procedures

Optical rotations were determined with a Perkin-Elmer 341 polarimeter with 1 mm cell. IR spectra were recorded on a Bruker vector 22 spectrometer with KBr pellets. The NMR experiments were conducted on Bruker AVANCE-600 and Bruker AMX-500 instruments. HRESIMS and ESIMS were obtained on a Q-Tof micro YA019 mass spectrometer. In antifungal evaluation, IC$_{50}$ values were calculated on XL*fit* 4.2 software (IDBS: Alameda, CA, USA, 2005). Reversed-phase HPLC was performed on YMC-Pack Pro C$_{18}$ RS (5 μm) columns with a Waters 1525/2998 liquid chromatograph. Column chromatographies were carried out on silica gel 60 (200–300 mesh; Yantai, China), Sephadex LH-20 (Amersham Biosciences). TLC was carried out using HSGF 254 plates and visualized by spraying with anisaldehyde-H$_2$SO$_4$ reagent.

3.2. Animal Material

The sponge, identified by Jin-He Li (Institute of Oceanology, Chinese Academy of Sciences, China), was collected off Woody (Yongxing) Island and seven connected islets in the South China Sea in June 2007. 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 (1.0 kg, dry weight) was extracted with 95% aqueous EtOH, and the combined extracts were concentrated under reduced pressure at 45 °C to yield the crude extract (100 g). This extract was suspended in H$_2$O and extracted with EtOAc and *n*-BuOH to afford the EtOAc- and *n*-BuOH-soluble extracts. The EtOAc-soluble extract (80 g) was partitioned between 90% aqueous MeOH and *n*-hexane to afford the *n*-hexane-soluble extract (21 g), which was subjected to Vacuum Liquid Chromatography (VLC) on silica gel by gradient elution using *n*-hexane/acetone (100:1, 50:1, 20:1, 15:1, 10:1, 5:1, 1:1, 0:1) as solvents to give seven subfractions (A–G). Subfraction G was subjected to CC on Sephadex LH-20, ODS and further purified by reversed-phase preparative HPLC (YMC-Pack Pro C$_{18}$ *RS*, 5 μm, 10 × 250 mm, 2.0 mL/min), to yield compound **1** (CH$_3$OH/H$_2$O 80:20, 2.0 mL/min, 208 nm, t_R = 44.03 min, 10.2 mg), compound **2** (CH$_3$OH/H$_2$O 80:20, 2.0 mL/min, 201 nm, t_R = 59.65 min, 2.5 mg), and compound **3** (CH$_3$OH:H$_2$O 80:20, 2.0 mL/min, 208 nm, t_R = 33.08 min, 22.3 mg).

Preparation of MTPA esters **1a** and **1b**: Woodylide A (**1**; 1.2 mg (3.8 µmol) and 1.0 mg (3.2 µmol), respectively) was reacted with *R*-(−)- or *S*-(+)-MTPACl (59.4 µmol) in freshly distilled dry pyridine (500 µL) and stirred under N_2 at room temperature for 18 h, respectively, and then the solvent was removed. The products were purified by mini-CC on silica gel (200 mesh, *n*-hexane:EtOAc, 3:1) to afford *S*-(−)- and *R*-(+)-MTPA esters **1a** and **1b**, respectively.

Preparation of MTPA esters **3a** and **3b**: Woodylide C (**3**; 1.2 mg (4.0 µmol) and 1.1 mg (3.7 µmol), respectively) was similarly processed to give *S*-(−)- and *R*-(+)- MTPA esters **3a** and **3b**, respectively.

Woodylide A (**1**): Colorless oil; $[\alpha]_D^{22}$ −15.0 (*c* 0.06, MeOH); IR (KBr) ν_{max} 3275, 2961, 2928, 2874, 2858, 1742, 1650, 1460, 1438, 1412, 1376, 1356, 1286, 1252, 1213, 1169, 1108, 1066, 1036, 1016, 992, 966, 933, 870, 852, 806, 781, 706 cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz) and ^{13}C NMR (CDCl$_3$, 125 MHz) data, see Table 1; HRESIMS *m/z* 337.2354 [M + Na]$^+$ (calcd for $C_{18}H_{34}O_4Na$, 337.2355). CD spectrum (*c* 1.91×10^{-3} M, CH$_3$CN), 197 nm ($\Delta\varepsilon$ 3.27), 200 nm ($\Delta\varepsilon$ 3.54).

Woodylide B (**2**): Colorless oil; $[\alpha]_D^{22}$ +5.5 (*c* 0.06, MeOH); IR (KBr) ν_{max} 3301, 2961, 2928, 2874, 2857, 1742, 1667, 1462, 1438, 1410, 1378, 1358, 1286, 1169, 1108, 1067, 1035, 1018, 994, 872, 852, 806, 781, 706 cm^{-1}; ^{1}H NMR (CDCl$_3$, 600 MHz) and ^{13}C NMR (CDCl$_3$, 150 MHz) data, see Table 1; HRESIMS *m/z* 351.2513 [M + Na]$^+$ (calcd for $C_{19}H_{36}O_4Na$, 351.2511). CD spectrum (*c* 2.13×10^{-3} M, CH$_3$CN), 196 nm ($\Delta\varepsilon$ 3.02), 203 nm ($\Delta\varepsilon$ 2.36).

Woodylide C (**3**): Light yellow oil; $[\alpha]_D^{22}$ −11.4 (*c* 0.14, MeOH); IR (KBr) ν_{max} 3422, 2961, 2928, 2874, 2858, 1757, 1655, 1462, 1401, 1379, 1342, 1285, 1252, 1209, 1169, 1109, 1063, 1021, 954, 915, 879, 801, 729, 666 cm^{-1}; ^{1}H NMR (CDCl$_3$, 600 MHz) and ^{13}C NMR (CDCl$_3$, 150 MHz) data, see Table 1; HRESIMS *m/z* 323.2200 [M + Na]$^+$ (calcd for $C_{17}H_{32}O_4Na$, 323.2198). CD spectrum (*c* 2.32×10^{-3} M, CH$_3$CN), 191 nm ($\Delta\varepsilon$ 3.23), 196 nm ($\Delta\varepsilon$ 4.72), 202 nm ($\Delta\varepsilon$ 3.61).

^{1}H NMR data of **1a** (CDCl$_3$, 600 MHz): δ 2.59 (1H, dd, H-2a), 2.91 (1H, dd, H-2b), 5.27 (1H, s, H-5), 1.47 (1H, dd, H-7a), 1.53 (1H, dd, H-7b), 1.60 (1H, m, H-8), 1.14 (2H, m, H-9), 1.26 (2H, m, H-10), 1.26 (2H, m, H-11), 0.88 (3H, t, H-12), 2.05 (2H, m, H-13), 1.01 (3H, t, H-14), 1.56 (2H, m, H-15), 0.86 (3H, t, H-16), 0.94 (3H, d, H-17), 3.60 (3H, s, H-18).

^{1}H NMR data of **1b** (CDCl$_3$, 600 MHz): δ 2.59 (1H, dd, H-2a), 2.93 (1H, dd, H-2b), 5.22 (1H, s, H-5), 1.49 (1H, dd, H-7a), 1.53 (1H, dd, H-7b), 1.61 (1H, m, H-8), 1.19 (2H, m, H-9), 1.26 (2H, m, H-10), 1.26 (2H, m, H-11), 0.88 (3H, t, H-12), 1.84 (2H, m, H-13), 0.90 (3H, t, H-14), 1.56 (2H, m, H-15), 0.85 (3H, t, H-16), 0.99 (3H, d, H-17), 3.67 (3H, s, H-18).

^{1}H NMR data of **3a** (CDCl$_3$, 600 MHz): δ 2.41 (1H, dd, H-2a), 2.95 (1H, dd, H-2b), 5.04 (1H, s, H-5), 1.29 (1H, dd, H-7a), 1.73 (1H, dd, H-7b), 1.80 (1H, m, H-8), 1.11 (2H, m, H-9), 1.73 (2H, m, H-10), 1.27 (2H, m, H-11), 0.81 (3H, t, H-12), 2.04 (2H, m, H-13), 0.98 (3H, t, H-14), 1.27 (2H, m, H-15), 0.79 (3H, t, H-16), 0.90 (3H, d, H-17).

^{1}H NMR data of **3b** (CDCl$_3$, 600 MHz): δ 2.50 (1H, dd, H-2a), 3.00 (1H, dd, H-2b), 5.03 (1H, s, H-5), 1.28 (1H, dd, H-7a), 1.76 (1H, dd, H-7b), 1.76 (1H, m, H-8), 1.11 (2H, m, H-9), 1.71 (2H, m, H-10), 1.26 (2H, m, H-11), 0.81 (3H, t, H-12), 1.95 (2H, m, H-13), 0.97 (3H, t, H-14), 1.26 (2H, m, H-15), 0.78 (3H, t, H-16), 0.89 (3H, d, H-17).

3.4. Antifungal Evaluation

Antifungal IC_{50} values of woodylides A–C against *C. neoformans* were calculated as described by Ikhlas A. Khan *et al.* [20]. Amphotericin B was used as the positive control. Minimal Inhibition Concentration (MIC) values of woodylides A–C were determined against three indicators (*C. albicans*, *T. rubrum*, and *M. gypseum*), following the National Center for Clinical Laboratory Standards (NCCLS) methods [21,22]. Fluconazole was used as the positive control. Briefly, samples (dissolved in DMSO) were serially diluted in 20% DMSO/saline and transferred (10 µL) in duplicate to 96 well flat bottom microplates. Bacterial strains were grown aerobically at 30 °C in SDA for 16–20 h. A set of different concentrations of compounds **1–3** prepared in RPMI 1640 were next inoculated with the microorganisms and incubated 70–74 h for *C. neoformans* at 35 °C, 46 h for *C. albicans* at 35 °C, and 4–7 days for *T. rubrum* and *M. gypseum* at 30 °C. The IC_{50} values were calculated by using the fit model 201 of XL*fit* 4.2 software. The MIC values were evaluated in triplicate for each compound (within the range 1.25–640 µg/mL).

3.5. Cytotoxicity Assay

The cytotoxicity of compounds **1–3** against HCT-116, A549, HeLa, QGY-7703, and MDA231 cell lines was evaluated by the MTT assay as described in a previous report [23]. Briefly, compounds were solubilized in DMSO with the working concentration of test substances ranging from 1 to 100 µg/mL. Cells at the exponential growth phase were harvested and seeded into 96-well plates. After incubation for 24 h, the cells were treated with various concentrations of test substances for 48 h and then incubated with 1 mg/mL MTT at 37 °C for 4 h, followed by dissolving in DMSO. The produced formazan was measured by the absorbance at 570 nm on a microplate reader. The IC_{50} values were calculated on the basis of percentage inhibition using the linear regression method.

3.6. PTP1B Inhibitory Assay

PTP1B inhibitory activity was determined using a PTP1B inhibitory assay as described previously [24]. The enzymatic activities of the PTP1B catalytic domain were determined at 30 °C by monitoring the hydrolysis of *p*NPP. Dephosphorylation of *p*NPP generated the product *p*NP, which was monitored at an absorbance of 405 nm. In a typical 100 µL assay mixture containing 50 mmol/L 3-[*N*-morpholino]propanesulfonic acid (MOPs), pH 6.5, 2 mmol/L *p*NPP, and 30 nmol/L recombinant PTP1B, activities were continuously monitored and the initial rate of the hydrolysis was determined using the early linear region of the enzymatic reaction kinetic curve.

4. Conclusions

In this paper we report the isolation and the structural determination of three new linear polyketides, woodylides A–C, endowed with antifungal, antineoplastic, and PTP1B inhibitory activities, from the South China Sea marine sponge *P. simplex*. Unfortunately, due to the lack of compound **2**, the absolute configuration at C-3 as well as the bioactivity of woodylide B could not be determined. Woodylide C exhibited a good PTP1B inhibitory activity, and deserves further study for its therapeutic potential against type II diabetes and obesity diseases.

Acknowledgments

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