Cytospyrone and Cytospomarin: Two New Polyketides Isolated from Mangrove Endophytic Fungus, *Cytospora* sp. †

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[†] Dedicated to Prof. Dr. Peter Proksch on His Research Career in Marine Natural Product Chemistry.

Abstract: Two new polyketides, cytospyrone (**1**), cytospomarin (**2**), together with three known metabolites dimethoxyphtalide (**3**), integracin A (**4**) and integracin B (**5**), were isolated from the culture of *Cytospora* sp. from the Chinese mangrove *Ceriops tagal*. Their structures were elucidated by extensive spectroscopic analyses and time dependent density functional theory (TDDFT), calculation of electronic circular dichroism (ECD) and optical rotation (OR) data. Compound **2** displayed weak inhibitory activity against *Escherichia coli* GIM1.201 (minimum inhibitory concentration (MIC) value of 0.35 mM). Compounds **4** and **5** displayed significant cytotoxicity against human cancer cell line HepG2 (IC$_{50}$ values of 5.98 ± 0.12 μM and 9.97 ± 0.06 μM, respectively), more potent than the positive control 5-fluorouracil (IC$_{50}$ value of 43.50 ± 3.69 μM).

Keywords: *Cytospora* sp.; polyketides; structure identification; antimicrobial activity; cytotoxicity

1. Introduction

Mangrove endophytic fungi are attracting considerable attention from natural product chemists and biologists alike and a great deal of structurally diverse natural products with unusual biological activity have been discovered recently [1–3]. Fungal genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Paecilomyces*, *Penicillium*, *Pestalotiopsis*, *Phoma*, *Phomopsis*, *Phyllosticta*, and *Trichoderma* are considered as the predominant producer of the most notable bioactive secondary metabolites [4–6]. However, due to the high rediscovery of previously known compounds and scaffolds from these well-investigated organisms, neglected fungal species that are poorly studied might be an alternative source for the discovery of new bioactive compounds.

During our ongoing screening for biologically active secondary metabolites from mangrove endophytic fungi [7–10], we recently investigated *Cytospora* sp., an endophytic fungus derived from the hypocotyls of Chinese mangrove *Ceriops tagal*, resulting in the isolation of a new antimicrobial biscyclic sesquiterpene, seiricardine D, together with eight known metabolites [11]. Further study of the aforementioned *Cytospora* sp. afforded two new polyketides, cytospyrone (**1**), cytospomarin (**2**), together with three known metabolites dimethoxyphtalide (**3**) [12], integracin A (**4**) [13], and integracin B (**5**) (Figure 1) [13].
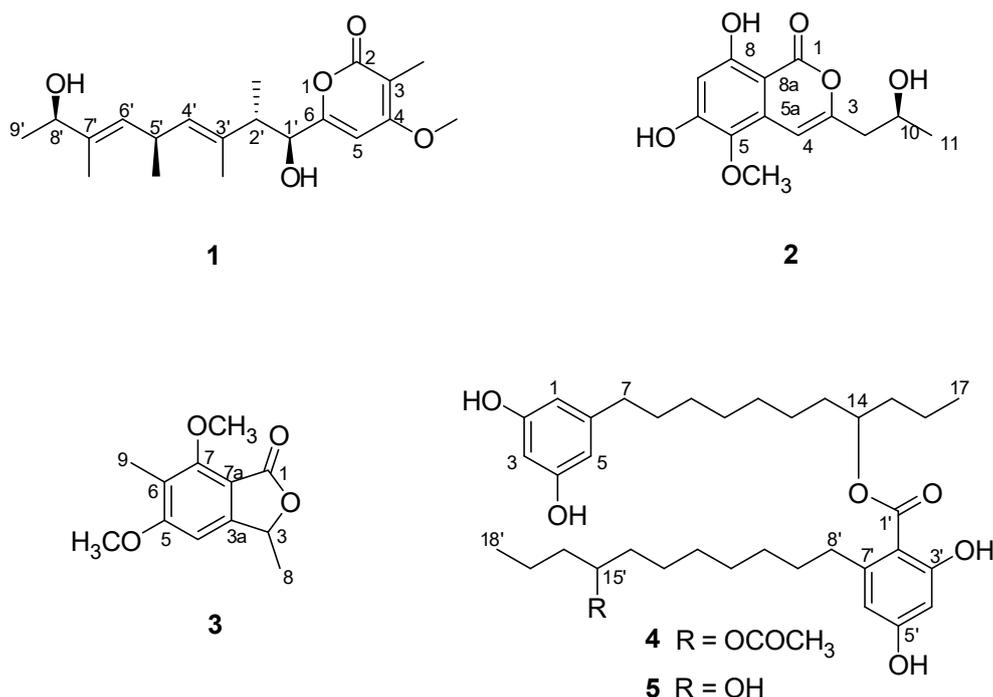
Figure 1. Structures of compounds **1–5**.

2. Results and Discussion

2.1. Isolation and Structure Elucidation

Cytospyrone A (**1**) was isolated as a white amorphous powder, and has the molecular formula $C_{20}H_{30}O_5$ established by high resolution electrospray ionization mass spectroscopy (HR-ESI-MS) at *m/z* 373.1988 [M + Na]$^+$ (calculated for 373.1991), indicating six degrees of unsaturation. The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) data of **1** in association with distortionless enhancement by polarization transfer (DEPT) and heteronuclear single quantum coherence (HSQC) spectrum suggested the presence of six upfield methyls [δ_H 1.03, (d, *J* = 6.7), δ_C 21.7, q, 5′-CH$_3$; 1.07, (d, *J* = 7.0), δ_C 16.2, q, 2′-CH$_3$; 1.18, (d, *J* = 6.4), δ_C 21.9, q, 9′-CH$_3$; 1.65, s, δ_C 11.8, q, 7′-CH$_3$; 1.67, s, δ_C 10.9, q, 3′-CH$_3$; 1.85, s, δ_C 8.4, q, 2-CH$_3$], a methoxy group (δ_H 3.94, s, δ_C 57.3, q, 4-OCH$_3$), three olefinic signals [δ_H 5.26, (d, *J* = 9.0), δ_C 130.0, d, CH-6′; δ_H 5.32, (d, *J* = 9.1), δ_C 130.0, d, CH-4′; δ_H 6.47, s, δ_C 97.5, d, CH-5], four methines [2.79, m, δ_C 44.1, d, 2′-CH; 3.33-3.42, m, δ_C 32.3, d, 5′-CH; 4.08, (d, *J* = 9.6), δ_C 80.7, d, 1′-CH; 4.10, (q, *J* = 6.4), δ_C 73.9, d, 8′-CH], and six quaternary carbons (δ_C 101.5, 134.2, 138.1, 167.8 168.5, 169.1). Two substructures, an α-pyrone moiety and a linear aliphatic chain, were assigned by analyses of detailed interpretation of ^{1}H-^{1}H correlation spectroscopy (COSY) and heteronuclear multiple bond correlation (HMBC) correlations (Figure 2). The α-pyrone moiety was established by HMBC correlations from 3-CH$_3$ to carbons C-2, C-3 and C-4, and from H-5 to C-4 and C-6. Presence of the methoxy group at C-4 was corroborated from the HMBC correlation of its protons (δ_H 3.94, s) with C-4 (δ_C 169.1) and nuclear overhauser effect spectroscopy (NOESY) correlations between 3-CH$_3$/4-OCH$_3$ and H-5/4-OCH$_3$. A linear aliphatic chain with a thirteen carbon unit was determined by the interpretation of ^{1}H–^{1}H COSY and HMBC spectroscopic data. The COSY cross-peaks [H-1′/H-2′/2′-CH$_3$, H-3′/H-4′/4′-CH$_3$/H-5′, H-8′/H-9′] and HMBC correlations from 2′-CH$_3$ to carbons C-1′, C-3′, from 3′-CH$_3$ to carbons C-2′, C-3′ and C-4′, from 7′-CH$_3$ to carbons C-6′, C-7′and C-8′, and from H-9′ to C-7′ permitted the construction of a linear aliphatic chain. The above two structural fragments were finally connected to construct the planar structure of compound **1** from the key HMBC correlations from H-1′ and H-2′ to C-6, from H-5 to C-2′. The relative configuration of **1** was deduced by analysis of the NOESY data. The NOESY correlations (Figure 3) between H-1′/2′-CH$_3$, 2′-CH$_3$/ H-4′, H-2′/3′-CH$_3$, H-4′/ H-5′, H-5′/H-6′, 7′-CH$_3$/H-8′ and H-6′/H-8′

suggested that they were positioned on the same face and both $\Delta^{4'}$ and $\Delta^{6'}$ double bonds were assigned to the *E*-configuration. In addition, the correlations of H-1′/3′-CH$_3$, 3′-CH$_3$/5′-CH$_3$ and 5′-CH$_3$/ 7′-CH$_3$ revealed they were positioned on the other face. To define its absolute configuration, the time dependent density functional theory—(TDDFT)-calculated electronic circular dichroism (ECD) spectrum at the B3LYP/6-31+g (d, p) level was compared with the experimental circular dichroism (CD) spectrum (Figure 4). Therefore, the structure of **1** was established as 6-((1*S*, 2*S*, 3*E*, 5*R*, 6*E*, 8*R*)-1,8-dihydroxy-2,3,5,7-tetramethylnona-3,6-dienyl)-4-methoxy-3-methyl-2*H*-pyran-2-one.
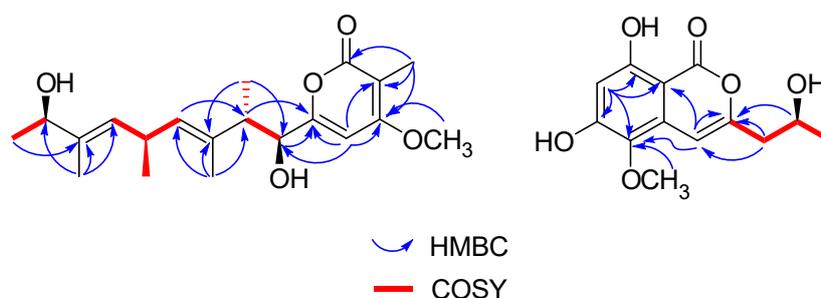
Figure 2. Key HMBC, ^{1}H-^{1}H COSY correlations of **1** and **2**.
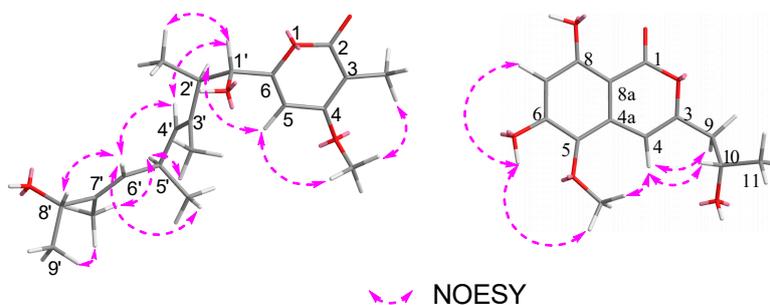
Figure 3. Key NOESY correlations of **1** and **2**.
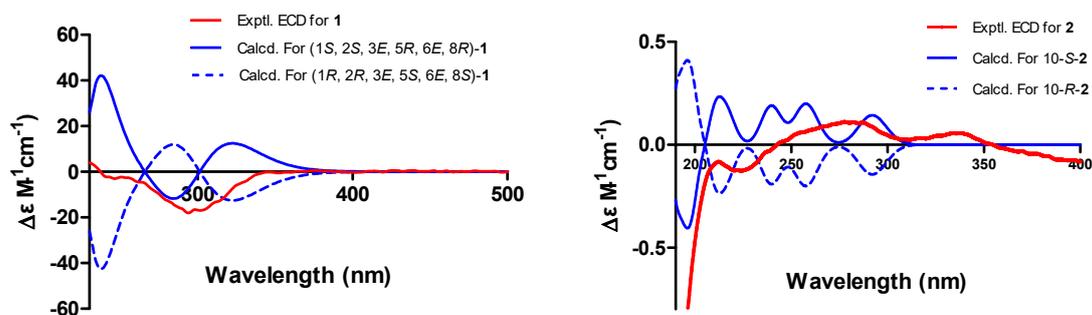
Figure 4. Experimental and calculated electronic circular dichroism (ECD) spectra of **1** and **2**.

Cytospomarin (**2**), a yellow amorphous solid, was found to have the molecular formula $C_{13}H_{14}O_6$, established by HR-ESI-MS (*m/z* 289.0674, calculated for [M + Na]$^+$ 289.0688), implying seven degrees of unsaturation. The ^{1}H and ^{13}C NMR data of **2** indicated that six of the seven units of unsaturation could be due to an aromatic ring, a double bond, and one carbonyl group. Thus, the remaining unit of unsaturation was attributed to a ring formation. The ultraviolet (UV) absorption maxima at 247 and 318 nm suggested **2** is an isocoumarin derivative. The ^{1}H and ^{13}C NMR data of **2** and the DEPT and HSQC spectra revealed the presence of two methyl groups, including a methoxy group (δ_H 3.68, s, δ_C 60.4, q, 5-OCH$_3$) and a terminal alkyl methyl [δ_H 1.12, (d, *J* = 6.2), δ_C 23.3, q, 11-CH$_3$], a methylene (δ_H 2.50–2.57, m, δ_C 42.8, t, CH$_2$-9), two olefinic methines [δ_H 6.33, s, δ_C 102.6, d, CH-7; δ_H 6.53, s, δ_C 99.9, d, CH-4], an oxymethine (δ_H 3.92–4.00, m, δ_C 64.0, d, CH-10) and seven quaternary carbons, including a carbonyl (δ_C 165.6, s, C-1). The ^{1}H and ^{13}C NMR and HSQC

spectra of compound **2** closely resembled those of an isocoumarin compound peniisocoumarin G [14], recently isolated from another endophytic fungus, *Penicillium commune* QQF-3, obtained from fresh fruit of the mangrove plant *Kandelia candel*, indicating that **2** might have the same basic molecular framework as peniisocoumarin G. The only difference between these was replacement of the hydroxyl substituent of peniisocoumarin G by a methoxy group in **2**. The HMBC and NOESY correlations (Figures 2 and 3) supported the assignments of the methoxy group 5-OCH$_3$ (δ_H 3.68, s) at C-5 (δ_C 134.3). The configuration of C-10 was determined by comparison of optical rotation with the known compounds peniisocoumarin G [14] and botryosphaerin A [15]. The result indicated that compound **2** has the same positive value as those two isocoumarins. It was confirmed by comparing experimental and calculating ECD spectra and optical rotation ($[\alpha]^{20}_D = +11.7°$ (c 0.3, MeOH); calculated for $[\alpha]^{20}_D = +89.6°$ (MeOH)) of **2** using TDDFT (Figure 4). Accordingly, compound **2** was determined as (*S*)-6,8-dihydroxy-3-(2-hydroxypropyl)-5-methoxy-1*H*-isochromen-1-one and named as cytospomarin.

2.2. Bioactivities

Compounds **1**−**5** were testing the in vitro antimicrobial activity against four human pathogens *Escherichia coli* GIM1.201, methicillin-resistant *Staphylococcus aureus* GIM1.771 (MRSA), *Pseudomonas aeruginosa* GIM1.200, *Candida albicans* GIM2.169, together with three plant pathogens including *Bacillus subtilis*, *Colletotrichum gloeosporioides* and *Magnaporthe oryze*, and the results are summarized in Table 1. Compound **1**−**5** were also tested in vitro against the human cancer cell line HepG2 with 5-fluorouracil as the positive control. Among them, compound **2** exhibited weak inhibitory activity against *E. coli* GIM1.201 (minimum inhibitory concentration (MIC) = 0.35 mM) and *M. oryzae* (MIC = 1.41 mM). Compounds **4** and **5** displayed significant cytotoxicity against human cancer cell line HepG2 with IC$_{50}$ values of 5.98 ± 0.12 μM and 9.97 ± 0.06 μM, respectively, more potent than the positive control 5-fluorouracil (IC$_{50}$ = 43.50 ± 3.69 μM) (Table 2).

Table 1. Antimicrobial activities of compounds **1**–**5**.

Compound	MIC (mM)						
	E. coli GIM1.201	MRSA GIM1.771	*P. aeruginosa* GIM1.200	*C. albicans* GIM2.169	*B. subtilis*	*C. gloeosporioides*	*M. oryzae*
1	-	-	-	-	-	-	-
2	0.35	-	-	-	-	-	1.41
3	-	1.94	-	-	-	-	-
4	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-
amphotericin	-	-	-	45.7 μM	-	7.6 μM	7.6 μM
ciprofloxacin	0.07	0.14	0.07	-	0.14	-	-

-: not detectable.

Table 2. Cytotoxicity data of compounds **1**–**5**. [a]

Cell Line	Compound				
	1–2	**3** [a]	**4** [a]	**5** [a]	**5-FU** [a]
HepG2(IC$_{50}$)	-	297.97 ± 17.54	5.98 ± 0.12	9.97 ± 0.06	43.50 ± 3.69

[a] Results are expressed as IC$_{50}$ values of mean ± SD (*n* = 3) in μM.

Mangrove endophytic fungi have been proved to be particularly productive with regard to the accumulation of diverse biological natural products and yielded more than 800 natural products [16]. In our previous study, over 120 different compounds, with thirty of them being new polyketides, were discovered from mangrove endophytic fungi, including pyrones [17], isocoumarins [9,17], coumarins [18], cytosporones [18] and chromones [19], etc. In the current study, two new polyketides, cytospyrone (**1**), cytospomarin (**2**), together with three known metabolites (**3**–**5**) were isolated from the rice culture of *Cytospora* sp. from Chinese mangrove *Ceriops tagal*. Structurally, **1** and pestalotiopyrones A–H [17] are pyrone derivatives, having both a methoxy group positioned at C-4 and a substituted alkyl side chain at C-6, which all proved to be devoid of significant activity in the antimicrobial and

cytotoxic bioassays. Many naturally occurring isocoumarin and 3,4-dihydroisocoumarin derivatives are known to have substituents at C-3 and have been shown to possess an impressive array of biological activities [20]. Five dihydroisocoumarins isolated from *Cytospora eucalypticola* KC1636 [21] exhibited mildly antifungal and antibacterial activities towards Gram positive bacteria, which is similar to our biological finding on **2**. Compound **3** is closely related to 5-dehydroxycytosporone E previously reported from another *Cytospora* sp. TT-10, both featuring a phthalan-1-one skeleton [22]. Previously other fungal cytosporone derivatives that bear structural similarities to **3** isolated in our study had been described to be cytotoxic against several cancer cell lines in vitro [23], in contrast to the previous report, **3** showed no activity towards the bioassays we investigated. Integracins A (**4**) and B (**5**), exhibited potent (human immunodeficiency virus Type 1) HIV-1 integrase inhibitors, and were dimeric alkyl aromatic compounds isolated for the first time from *Cytonaema* sp. living in twigs of *Quercus ilex* [13]. They were later found to be produced by various organisms, such as endophytic fungi *Cytospora* sp. [22], Chinese mangrove plant *Sonneratia hainanensis* [24] and *Laguncularia racemose* [25]. Moreover, compound **4** was also reported to possess potent cytotoxicity against the tumor cell lines HepG2 and NCI-H460 with both 100% inhibitions at 25 mg/mL [24]. In this work, we found not only **4** but **5** strongly inhibited the proliferation of human cancer cell line HepG2, which was in accordance with previous reports. This highlighted the high potential of bioprospecting of polyketides from mangrove endophytic fungi *Cytospora*.

3. Materials and Methods

3.1. General Experimental Procedures

Optical rotations were measured on a WYA-2S digital Abbe refractometer (Shanghai Physico-optical Instrument Factory, Shanghai, China). UV spectra were obtained on a Shimadzu UV-2401 PC spectrophotometer (Shimadzu Corporation, Tokyo, Japan). Then, 1D and 2D NMR spectra were recorded on Bruker AV 400 NMR apparatus (Bruker Biospin Group, Karlsruhe, Germany) in CD_3OD, DMSO-d_6 or $CDCl_3$ (Guangzhou Chemical Reagent Factory, Guangzhou, China). Mass spectra were obtained on a LTQ Orbitrap XL instrument (Thermo Fisher Scientific, Bremen, Germany) using peak matching. Column chromatography was performed with silica gel (200−300 mesh, Qingdao Haiyang Chemical Co., Qingdao, China) and Sephadex LH-20 (18−110 μm, Merck, Darmstadt, Germany). Semi-preparative HPLC was achieved on an Agilent 1100.

3.2. Fungal Material and Identification

Following standard procedures [26], endophytic fungi were isolated from fresh, healthy hypocotyls of *Ceriops tagal* (Rhizophoraceae), which were collected in October 2015 in Dong Zhai Gang-Mangrove Garden on Hainan Island, China. The fungus (strain no. JGM-9) was identified as *Cytospora* sp. (GenBank accession no. MG948459) according to DNA amplification and sequencing of the internal transcribed spacer (ITS) region. A voucher strain was deposited at one of the authors' laboratory (J.X.).

3.3. Fermentation, Extraction and Isolation

The fungus was grown on solid rice medium at room temperature in 1000 mL-Erlenmeyer flasks containing 100 g rice (Jiangxi COFCO Rice Industry Co., Nanchang, China) and 100 mL-seawater. After 39 days of cultivation, the mycelia and solid rice medium were extracted with ethyl acetate (EtOAc). The extract was evaporated under reduced pressure to yield 90.0 g residue. This residue was subjected to silica gel column chromatography (CC) employing a step gradient of petroleum ether/ethyl-acetate gradient (100:0 to 0:100) to obtain four fractions (Fr. 1–Fr. 7). Promising Fr. 5 was separated by CC (silica gel; petroleum ether/ethyl acetate/acetone 5:2:2) to give four sub-fractions. Sub-Fr. 5–2 was purified with silica gel by $CHCl_3$/MeOH 20:1 and reversed-phase-HPLC (MeOH/H_2O 80:20) to yield compound **2** (15.0 mg). Sub-Fr. 5–3 was subjected to CC (Sephadex LH-20, MeOH/$CHCl_3$ 1:1) and reversed-phase-HPLC (MeOH/H_2O 70:30 to 100:0) to furnish **1** (8.1 mg), **4** (5.7 mg) and **5** (3.8 mg).

Fr. 7 was subjected to CC (silica gel; gradient petroleum ether / acetone) and reversed-phase-HPLC (MeOH/H$_2$O 80:20 to 100:0) to afford **3** (3.8 mg).

Cytospyrone (**1**): white amorphous powder (MeOH); [α]^{20}$_D$ = −23.1° (c0.05, MeOH); UV(MeOH) λ_{max} 212 nm; ^{1}H NMR(400 MHz, CD$_3$OD) δ_H 6.47 (1H, s, H-5), 5.32 (1H, d, *J* = 9.1 Hz, H-4′), 5.29 (1H, d, *J* = 9.0, 6′), 4.10 (1H, q, *J* = 6.4, H-8′), 4.08 (1H, d, *J* = 9.6, H-1′), 3.94 (3H, s, 4-OCH$_3$), 3.33–3.42 (1H, m, H-5′), 2.79 (1H, m, H-2′), 1.85 (3H, s, 3-CH$_3$), 1.67 (3H, s, 3′-CH$_3$), 1.65 (3H, s, 7′-CH$_3$), 1.18 (3H, d, *J* = 6.4, H-9′), 1.07 (3H, d, *J* = 7.0, 2′-CH$_3$), 1.03 (3H, d, *J* = 6.7, 5′-CH$_3$); ^{13}C-NMR (100 MHz, CD$_3$OD) δ_C 169.1 (C-4), 168.5 (C-2), 167.8 (C-6), 138.1 (C-7′), 135.3 (C-4′), 134.2 (C-3′), 130.1 (C-6′), 101.5 (C-3), 97.5 (C-5), 80.7 (C-1′), 73.9 (C-8′), 57.3 (4-OCH$_3$), 44.1 (C-2′), 32.3 (C-5′), 21.9 (C-9′), 21.7 (5′-CH$_3$), 16.2 (3′-CH$_3$), 11.8 (7′-CH$_3$), 10.8 (3′-CH$_3$), 8.4 (3-CH$_3$); HR-ESI-MS *m/z* 373.1991 [M + Na]$^+$ (calculated for C$_{20}$H$_{30}$O$_5$Na, 373.1991) (Supplementary Materials).

Cytospomarin (**2**): a yellow amorphous solid (MeOH); [α]^{20}$_D$ = +11.7° (c 0.3, MeOH); UV(MeOH)λ_{max} 318 nm, 247 nm; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 10.8 (1H, br s, 6-OH), 6.53 (1H, s, H-4), 6.33 (1H, s, H-7), 3.92–4.00 (1H, m, H-10) 3.68 (3H, s, 5-OCH$_3$), 2.50–2.57 (2H, m, H-9), 1.12 (3H, d, *J* = 6.2, H-11); ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C 165.6 (C-1), 161.1 (C-8), 158.6 (C-6), 154.7 (C-3), 134.3 (C-5), 130.3 (C-5a), 102.6 (C-7), 99.9 (C-4), 95.5 (C-8a), 64.0 (C-10), 60.4 (5-OCH$_3$), 42.8 (C-9), 23.3 (C-11); HRESIMS *m/z* 289.0674 [M + Na]$^+$ (calculated for C$_{13}$H$_{14}$O$_6$Na, 289.0610).

Dimethoxyphtalide (**3**): white amorphous powder (MeOH); [α]^{20}$_D$ = +123° (c 0.1, MeOH), UV(MeOH)λ_{max} 216 nm, ^{1}H NMR(400 MHz, CDCl$_3$) δ_H 6.55 (1H, s, H-4), 5.40 (1H, d, *J* = 6.6, H-3), 4.05 (3H, s, 7-OCH$_3$), 3.92 (3H, s, 5-OCH$_3$), 2.14 (3H, s, 6-CH$_3$), 1.59 (3H, d, *J* = 6.6, 3-CH$_3$); ^{13}C-NMR (100 MHz, CDCl$_3$) δ_C 168.3 (C-1), 164.2 (C-5), 157.6 (C-7), 152.9 (C-3a), 120.5 (C-6), 109.7 (C-7a), 97.9 (C-4), 76.4 (C-3), 62.1 (7-OCH$_3$), 56.1 (5-OCH$_3$), 29.7 (C-9), 20.7 (C-8); HRESIMS *m/z* 233.0969 [M + H]$^+$ (calculated for C$_{12}$H$_{15}$O$_4$, 233.0969). The data of ^{1}H NMR and ^{13}C NMR are basically consistent with those reported in literature [12].

Integracin A (**4**): yellow oil (MeOH); [α]^{20}$_D$ = +97.0° (c 0.1, MeOH); UV (MeOH) max 280,259,218 nm; ^{1}H NMR(500 MHz, CD3OD) 6.23 (H, d, *J* = 1.8, H-6′), 6.19 (H, d, *J* = 1.8, H-4′), 6.14 (2H, d, *J* = 1.4, H-1, H-5), 6.10 (H, br s, H-3), 5.28 (H, pent, *J* = 5.6 Hz, H-14), 4.82 (H, overlapped, H-15′), 2.81–2.85 (2H, m, H-8′), 2.45 (H, t, *J* = 8.0 Hz, H-7), 2.03 (3H, s, Me-2′′), 1.66–1.76 (4H, m, H-13, H-15), 1.54–1.57 (4H, m, H-8, H-9′), 1.40–1.42 (4H, m, H-14′, H-16′), 1.31–1.35 (20H, m, 9-H to 12-H, H-16, H-10′ to H-13′, H-17′), 0.99 (3H, t, *J* = 7.3 Hz, Me-18′), 0.94 (3H, t, *J* = 7.3, Me-17); 13C-NMR (125 MHz, CD3OD) δ 173.1 (C-1′), 172.7 (C-1′′),165.9 (C-3′), 163.5 (C-5′), 159.2(C-2, C-4), 149.0 (C-7′), 146.3 (C-6), 111.9 (CH-6′), 107.9 (CH-1, CH-5), 105.8 (C-2′), 101.9 (CH-4′), 101.0 (CH-3), 76.7 (CH-14), 75.5 (CH-15′), 37.7 a (CH2-8′), 37.4 a (CH2-15), 37.4 a (CH2-16′), 36.9 (CH2-7), 35.2 (CH2-13), 35.2 (CH2-14′), 33.6 (CH2-9′), 32.3 (CH2-8), 31.0 a (CH2-10′), 30.7 a (CH2-11′), 30.5 a (CH2-12′), 30.5 a (CH2-11), 30.4 a (CH2-10), 30.2 (CH2-9), 26.6 (CH2-13′), 26.4 (CH2-12), 21.2 (CH3-2′′), 19.9 (CH2-16), 19.6 (CH2-17′), 14.3 (CH3-18′), 14.2 (CH3-17), chemical shifts could be interchanged; ESI-MS [M + H]+ *m/z* 629.4 (C37H57O8). The data of 1H NMR and 13C NMR are basically consistent with those reported in literature [13].

Integracin B (**5**): yellow oil (MeOH); [α]20D = +37.8° (c 0.30, MeOH); UV (MeOH) λ_{max} 300, 264, 230 nm; 1H NMR(500 MHz, CD3OD) δ 11.98 (H, s, 3′-OH), 7.61 (H, br s, 3×-OH), 6.21 (H, d, *J* = 2.4, H-6′), 6.19 (H, d, *J* = 2.4 Hz, H-4′), 6.14 (2H, d, *J* = 2.1, H-1, H-5), 6.10 (H, t, *J* = 2.1, H-3), 5.20 (H, pent, *J* = 5.7, H-14), 3.48–3.52 (H, m, H-15′), 2.80 (2H, td, *J* = 7.0, 2.5, H-8′), 2.40 (H, t, *J* = 7.5, H-7), 1.63–1.67 (2H, m, H-13), 1.59–1.63 (2H, m, H-15), 1.48–1.55 (4H, m, H-8, H-9′), 1.36–1.39 (4H, m, H-14′, H-16′), 1.34–1.36 (2H, m, H-17′), 1.28–1.30 (2H, m, H-16), 1.23–1.28 (20H, m, 9-H to 12-H, H-16, H-10′ to H-13′, H-17′), 0.91 (3H, t, *J* = 7.3, Me-18′), 0.88 (3H, t, *J* = 6.8, Me-17); 13C-NMR (125 MHz, CD3OD) δ 172.3 (C-1′), 165.5 (C-3′), 162.8 (C-5′), 158.4(C-2, C-4), 149.1 (C-7′), 146.1 (C-6), 111.8 (CH-6′), 107.8 (CH-1, CH-5), 105.1(C-2′), 101.6 (CH-4′), 100.6 (CH-3), 76.3 (CH-14), 71.9 (CH-15′), 40.0 (CH2-16′), 37.9 (CH2-8′), 37.6 (CH2-15), 37.2 (CH2-7), 36.6 (CH2-13), 35.0 (CH2-14′), 33.1 (CH2-9′), 31.9 (CH2-8), 30.7 a (CH2-10′), 30.5 a (CH2-11′), 30.4 a (CH2-12′), 30.1 a (CH2-11), 30.0 a (CH2-10), 29.9 (CH2-9), 26.4 (CH2-13′), 26.2 (CH2-12), 19.5 (CH2-16), 19.4 (CH2-17′), 14.4 (CH3-18′), 14.3 (CH3-17), a chemical

shifts could be interchanged; ESIMS [M + H]+ *m/z* 587.4 (C35H55O7). The data of 1H NMR and 13C NMR are basically consistent with those reported in literature [13].

3.4. ECD Calculations

Monte Carlo conformational searches were carried out by means of Spartan 14 software using Merck molecular force field (MMFF). The conformers with Boltzmann population of over 1% were chosen for ECD calculations, and then the conformers were initially optimized at B3LYP/6-31g level in gas. The theoretical calculation of ECD was conducted in MeOH using time dependent density functional theory (TDDFT) at the B3LYP/6-31+g (d, p) level for all conformers of compounds **1** and **2**. ECD spectra were generated using the program SpecDis 1.6 (University of Würzburg, Würzburg, Germany) and GraphPad Prism 5 (University of California, San Diego, CA, USA) from dipole-length rotational strengths by applying Gaussian band shapes with sigma = 0.3 eV.

3.5. Optical Rotation (OR) Calculations

Monte Carlo conformational searches were carried out by means of Spartan 10 software using Merck molecular force field (MMFF). The conformers with Boltzmann population of over 1% were chosen for OR calculations, and then the conformers were initially optimized at B3LYP/6-31+g (d, p) level in MeOH using the conductor-like polarizable continuum model (CPCM) polarizable conductor calculation model. The theoretical calculation of OR was conducted in MeOH using time dependent density functional theory (TDDFT) at the B3LYP/6-31+g (d, p) level for all conformers of compound **2**.

3.6. Antimicrobial Activity

The antimicrobial activity was determined against human pathogens *Escherichia coli* GIM1.201, methicillin-resistant *Staphylococcus aureus* GIM1.771 (MRSA), *Pseudomonas aeruginosa* GIM1.200, *Candida albicans* GIM2.169 and plant pathogenic fungi *Bacillus subtilis*, *Colletotrichum gloeosporioides* and *Magnaporthe oryze* (Guangdong Microbial Culture Collection Center, China, CDMCC). The bacteria was cultured in Luria-Bertani (LB) medium (10 g of peptone, 15 g of sodium chloride, 3 g of yeast extract, and 1000 mL of distilled H_2O) at 37 °C (160 rpm) for 24 h, and the fungi was cultured in potato dextrose broth (PDB) medium (200 g of potatoes, 20 g of dextrose, and 1000 mL of distilled H_2O) at 28 °C (160 rpm) for 48 h. All isolated compounds were subjected to antimicrobial assays using a serial dilution technique, as previously described [26]. The minimum inhibitory concentration (MIC) was assigned to the lowest concentration that completely inhibited the growth of the indicator microorganisms. Ciprofloxacin (Guangzhou Lianghua Chemical Co., Ltd., 99.9%, Guangzhou, China) and amphotericin (American AMRESCO, 99.9%, Branded Products Group, Cochran, USA) were used as a positive control.

3.7. Cytotoxicity Assay

The human hepatoma cell (HepG2) cells (Cell Resource Center, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences) were grown in RPMI-1640 culture medium. Cytotoxicity against HepG2 cells was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich, Missouri, St. Louis, MO, USA) method, as described previously [8] and 5-Fluorouracil (5-FU) (Beijing Solarbio Science & Technology Co., Ltd., 99.8%) (Beijing, China) was used as the positive control.

4. Conclusions

In the present work, two new polyketides cytospyrone (**1**), cytospomarin (**2**) and three known metabolites (**3**−**5**) were obtained from the ethyl acetate extract of the fermented cultures of the fungus *Cytospora* sp., which was isolated from the Chinese mangrove *Ceriops tagal*. Compound **2** exhibited

weak inhibitory activity against *Escherichiacoli* GIM1.201 and compounds **4** and **5** displayed potent cytotoxicity against human cancer cell line HepG2.

Supplementary Materials: The following are available online, Figure S1. ^{1}H-NMR of cytospyrone (**1**); Figure S2. ^{13}C-NMR of cytospyrone (**1**); Figure S3. DEPT of cytospyrone (**1**); Figure S4. ^{1}H-^{1}H COSY of cytospyrone (**1**); Figure S5. HMQC of cytospyrone (**1**); Figure S6. HMBC of cytospyrone (**1**); Figure S7. ROESY of cytospyrone (**1**); Figure S8. HR-ESI-MS of cytospyrone (**1**); Figure S9 CD spectrum of cytospyrone (**1**); Figure S10. ^{1}H-NMR of cytospomarin (**2**); Figure S11. ^{13}C-NMR of cytospomarin (**2**); Figure S12. DEPT of cytospomarin (**2**); Figure S13. ^{1}H-^{1}H COSY of cytospomarin (**2**); Figure S14. HMQC of cytospomarin (**2**); Figure S15. HMBC of cytospomarin (**2**); Figure S16. ROESY of cytospomarin (**2**); Figure S17. HR-ESI-MS of cytospomarin (**2**); Figure S18. CD spectrum of cytospomarin (**2**). **Table S1.** Gibbs free energies[a] and equilibrium populations[b] of low-energy conformers of cytospyrone; Table S2. Cartesian coordinates for the low-energy reoptimized MMFF conformers of cytospyrone (**1**) at B3LYP/6-31G(d,p) level of theory in gas; **Table S3**. Gibbs free energies[a] and equilibrium populations[b] of low-energy conformers of cytospomarin (**2**); Table S4. Cartesian coordinates for the low-energy reoptimized MMFF conformers of cytospomarin (**2**) at B3LYP/6-31G(d,p) level of theory in gas; Table S5. Gibbs free energiesa and equilibrium populationsb of low-energy conformers of cytospomarin (**2**); Table S6. Cartesian coordinates for the low-energy reoptimized MMFF conformers of cytospomarin (**2**) at B3LYP/6-31+G(d,p) level of theory in CH_3OH.

Author Contributions: C.W. and Q.D. isolated the compounds. Q.D. carried out the large-scale fermentation and extraction. M.S. performed antimicrobial and cytotoxic activity evaluation. J.X. designed and supervised this research, structure elucidation and manuscript preparation. J.X. designed and supervised this research. The final revision of the manuscript was revised by all the authors. All authors have read and agreed to the published version of the manuscript.

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