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Six new derivatives from *Alternaria sp* MCCC 3A00467

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Abstract: Terrestrial plant resources are becoming increasingly scarce; moreover, the development of science and technology has facilitated the exploration of marine ecosystems. We isolated and identified six novel (**1–6**) and six (**7–12**) known secondary metabolites of *Alternaria sp* MCCC 3A00467 by chromatographic and spectroscopic techniques and investigated their antitumor activities in human myeloma (U266), liver cancer (HepG2), and lung cancer (A549) cells by the MTT assay. Among these compounds, the new compounds **2, 3** and **4** exhibited excellent anticancer activities with IC_{50} values of 21.98, 24.99 and 14.78 μg/mL, respectively. These compounds obtained from a marine source have the potential to be developed into novel anticancer drugs.

Keywords: antitumor; *Alternaria sp*

1. Introduction

A wide range of bioactive natural products from numerous new species have been discovered in the past [1]; however, terrestrial plant resources are becoming increasingly scarce [2]. In recent years, the development of science and technology has facilitated the exploration of marine ecosystems [3]. Over the past few decades, more than 6,000 kinds of marine natural products have been identified, including more than 200 novel kinds of compounds with important bioactivities and patented applications [4]. Continued progress in research has made possible the identification of marine compounds having novel structures and better biological activities, especially antibacterial and antitumor activities, than compounds from terrestrial plants [5]. It is reported that *Alternaria sp* metabolites have various biological activities [6]. They can also cause toxic pathological effects in plants [7], such as sickle leaf spot disease in several plants [8] and brown spot disease in the tobacco plant [9]. In addition, these metabolites also have good antibacterial activity [10]. In this study, we explored the secondary metabolites of *A. alternate sp* (Marine Culture Collection of China [MCCC] 3A00467) to identify additional active compounds from these fungi. We found that the six new and six known compounds exhibit good cytotoxic activities. Therefore, our results might provide a reference for the development of novel anticancer compounds based on marine sources.

2. Results and Discussion

2.1 Identification of new metabolites from A. alternata 3A00467

A. alternata sp MCCC 3A00467 was incubated in a solid medium for one month and extracted with EtOAc to afford a crude extract. The crude extract (28.7 g) was separated by extensive chromatography, including silica gel, Sephadex LH-20, and preparative HPLC, to give compounds **1–12** (Figure 1), including six new compounds: **1**, **2**, **3**, **4**, **5**, and **6**, and six known ones, including 7-hydroxy-8-(2-hydroxy-ethyl)-5-methoxy-2-methyl-chroman-4-one (**7**) [11], 2-methyl-8-ethyl-7-hydroxy-5-methoxy-chroman-4-one (**8**) [12],

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7-hydroxy-5-methoxy-2-methyl-8-ethoxyacetyl-chroman-4-one (**9**) [12], phomalone (**10**) [11], 1-(3-ethyl-2,4-dihydroxy-6-methoxy-phenyl)-butan-1-one (**11**) [11], and 5-methoxy-2-methyl-tetrahydrofuro[7,8-h]-l-benzopyran-4-one (**12**) [13].

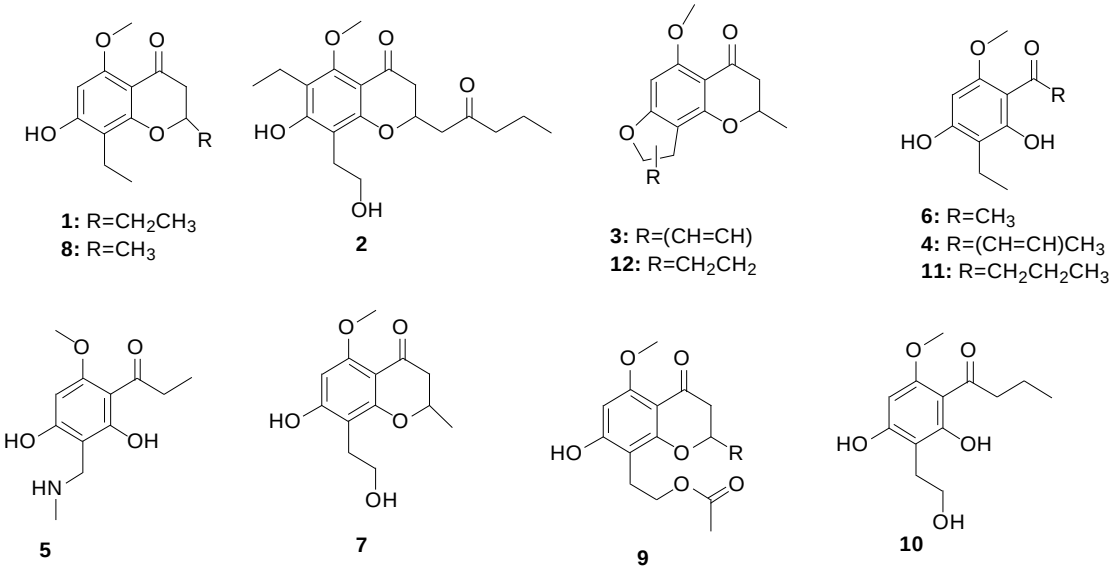

Figure 1. Chemical structures of the metabolites (**1**–**12**) from *Alternaria alternata* 3A00467

Compound **1** was obtained as pale-yellow crystals from the $CHCl_3$ fraction. Its molecular formula was determined as $C_{14}H_{18}O_4$ based on a quasimolecular ion $[M+Na]^+$ at *m/z* 274.2737 in its positive HR-ESI-MS. The ^1H NMR spectroscopic data (400 MHz, $CDCl_3$) (Table 1) showed signals for three methyl groups at δ 3.71 (s, 3H, O-CH_3), 1.45 (d, J = 6.2 Hz, 3H), and 1.07 (t, J = 7.4 Hz, 3H), and one methoxy group δ 3.71 (s, 3H). The ^{13}C NMR spectroscopic data (400 MHz, $CDCl_3$) of **2** presented 14 carbon signals, including one carbonyl [δ_C 191.19(C-4)], three methyl groups [13.7(C-12, 20.65(C-14), 55.68(O-CH_3)], one benzene ring [92.79(C-6), 105.30(C-10), 110(C-8), 160.10(C-9), 161.45(C-5), 162.28(C-7)], and one pyran ring. The NMR data of **1** were very similar to those of **8** (2-methyl-8-ethyl-7-hydroxy-5-methoxy-chroman-4-one). The main difference between them was that compound **1** had one more carbon than compound **8**, at C-2 (δ_C 73.52, δ_H 4.45), suggesting that it is attached to the oxygen atom and contains a branch. In combination with 2D NMR, COSY correlations connected H-2 to H-3, 3′. HMBC peaks from H3 to C-2,4 concluded a pyran ring (Figure 2). A side chain was determined by COSY correlation (H-2/H-4′ and H3′-H4′) and HMBC peaks (H-3′/C-4′, H-4′/C-2, H4′-C3). Based on ^{13}C NMR chemical shifts, compound **1** was determined to have one benzene ring, a hydroxyl group attached to C-7 (162.28), and a methoxy group attached to C-5. Another side chain could also be determined by COSY correlation (H-1′/H-2′) and HMBC peaks (H-1′/C-2′, H-1′/C-8, H-2′/C-1′, H-2′/C-8).

Table 1. ^1H and ^{13}C NMR Spectroscopic Data (400 MHz, $CDCl_3$) for compounds **1, 2,** and **3**

position	**1**		**2**		**3**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
2	73.5	4.45, m	75.2	4.48, m	74.5	4.71–4.62, m
3	45.5	2.56, dd (8.5,5.3)	44.1	2.64, d (12.1)	45.8	2.69, d (4.0)
4	191.1		198.1		190.4	
5	161.4		163.0		159.5	
6	92.7	6.11, s	111.6		88.7	6.64, s
7	162.2		165.8		159.7	
8	110.5		105.7		110.5	

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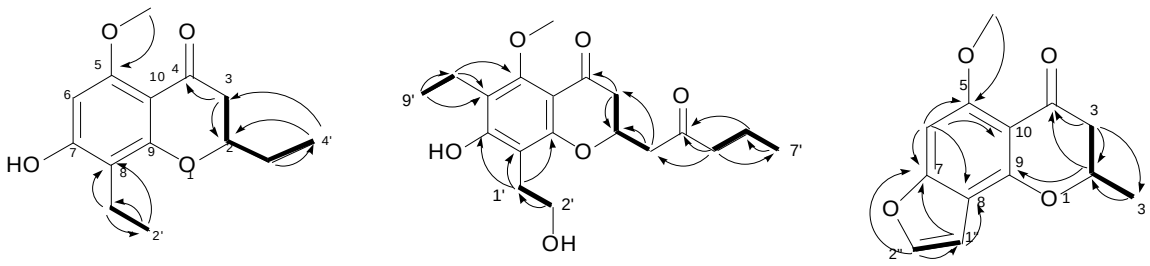
9	160.1		162.4		157.5	
10	105.3		102.9		107.3	
1'	14.0	2.56, dd (8.5,5.3)	26.6	2.83, t (7.5)		
2'	13.7	1.07, t (7.4)	62.0	3.62, t (7.5)		
3'	15.9	1.60, m	21.0	1.44, d (6.2)	20.7	
4'	20.4	1.45, t (6.2)	207.1			
5'			47.1	2.99–2.92, m		
6'			19.5	1.68, m		
7'			13.7	0.98, t (7.4)		
8'			16.0	2.56–2.52, m		
9'			14.3	1.05, t (7.4)		
1''					104.5	6.80, d (2.2)
2''					143.6	7.47, d (2.3)
O-CH3	55.6	3.74, s	55.9	3.85, s	56.4	3.93, s



1 **2** **3**

Figure 2 Major COSY correlations (▬) and HMBC peaks (→)of **1**, **2**, and **3**

Compound **2** was obtained as pale-yellow crystals from the $CHCl_3$ fraction. Its molecular formula was determined as $C_{19}H_{26}O_6$ based on a quasimolecular ion $[M+H]^+$ at *m/z* 351.1481 in its positive HR-ESI-MS, suggesting seven degrees of unsaturation. The ^1H and ^{13}C spectra of **2** were similar to **1**. The 2D NMR interpretations (Figure 2) showed that both the compounds had the same carbon skeleton. The ^1H NMR spectroscopic data (400 MHz, $CDCl_3$) (Table 1) showed signals for three methyl groups at δ_H 0.98 (t, *J* = 7.4 Hz, 3H), 1.05(t, *J* = 7.4 Hz, 3H), and 3.85 (s, 3H), and seven methylene groups at 3.62 (t, *J* = 7.5 Hz, 2H), 2.99–2.92 (m, 2H), 2.83 (t, *J* = 7.5 Hz, 2H), 2.64 (d, *J* = 12.1 Hz, 2H), 2.56–2.52 (m, 2H), 1.68 (p, *J* = 7.4 Hz, 2H), and 1.44 (d, *J* = 6.2 Hz, 2H). The ^{13}C NMR spectroscopic data (400 MHz, $CDCl_3$) showed the presence of one benzene ring and two carbonyl groups (Table 1). An ethyl group attached to C-6 was determined by COSY correlation (H-8'/ H-9') and HMBC peaks (H-8'/C-5, 6, 7, 9', H-9'/C-6, 8'). Another side chain was determined by COSY (H-2/H-3', H-5'/H-6',7', H-6'/H-5',7') and HMBC peaks (H-3'/C-2, 3, 5', H-5'/C-3', 4', 6', 7').

Compound **3** was obtained as pale-yellow crystals from the $CHCl_3$ fraction. Its molecular formula was determined as $C_{13}H_{12}O_4$ by $[M+Na]^+$ at *m/z* 255.0745 in its positive HR-ESI-MS. The ^1H and ^{13}C NMR spectra of **3** were similar to those of **12** [13]. The 2D NMR interpretations showed that both the compounds had the same carbon skeleton. However, ^{13}C chemical shifts changes were observed (C-1'': δ 25.54 in compound **8**, δ 104.58 in compound **11**, C-12: δ 72.99 in **12**, δ 143.61 in **11**). The molecular formula and molecular weight suggest that compound **11** have eight degrees of unsaturation, one more unsaturation than **12** [13]. ^{13}C NMR spectroscopic data (400 MHz, $CDCl_3$) showed the presence of one double bond, suggesting a bicyclic structure (Table 3). COSY correlations connected H-1'' to H-2'', H-2 to H-3, and H-3'. HMBC peaks from H-1'' to C-7, 8, 2'' and from H-2'' to C-7,8,1'' concluded a furan ring (Figure 2).

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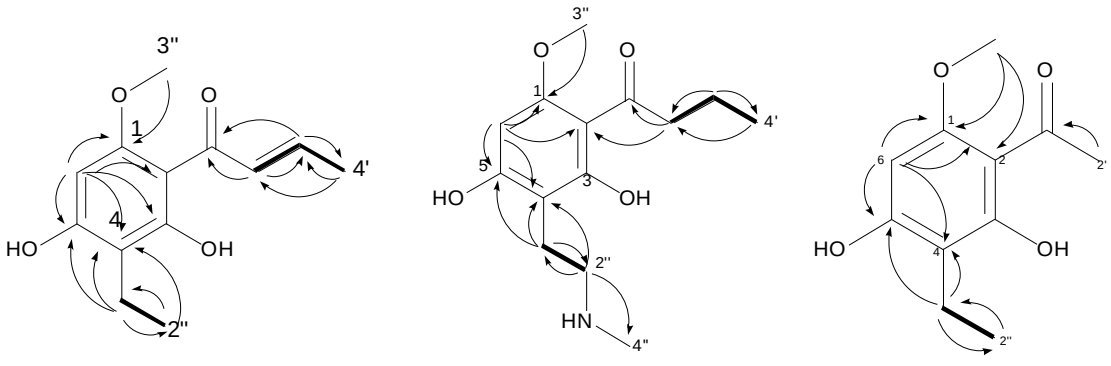
Compound **4** was obtained as pale-yellow crystals from the $CHCl_3$ fraction. Its molecular formula was determined as $C_{13}H_{16}O_4$ based on a quasimolecular ion at *m/z* 236.1 in its positive HR-ESI-MS, suggesting six degrees of unsaturation. The ^1H and ^{13}C spectra of **4** were similar to those of **11**. The 2D NMR interpretations (Figure 3) showed that both compounds had the same carbon skeleton. However, ^{13}C chemical shift changes were observed (C-2': δ 45.72 in **9**, δ 132.18 in **8** and C-3': δ 12.98 in **9**, δ 142.59 in **8**). In conclusion, C-2',3' were considered to be a pair of double bonds.

Table 2. ^1H and ^{13}C NMR Spectroscopic Data (400 MHz, $CDCl_3$) for compounds **4, 5,** and **6**

position	**4**		**5**		**6**	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	162.4		162.5		161.3	
2	104.4		105.0		105.9	
3	160.9		162.1		160.2	
4	110.		105.2		109.6	
5	164.7		165.4		164.82	
6	90.1	6.01, s	92.2	6.05, s	90.2	5.9, s
1'	192.9		206.0		203.4	
2'	132.2	7.28, d (15.1)	46.22	2.99, t (7.4)	33.08	
3'	140.8	7.11–6.92, m	18.2	1.73, m		
4'	15.0	1.96, d (6.9)	14.1	1.01, t (7.3)		
1''	17.1	3.62, t (7.5)	15.3	3.12, t (6.2)	15.5	2.59–2.54
2''	12.2	1.44, d (6.2)	50.9	3.25, dd (13.1, 5.9)	13.4	1.10, t (7.5)
3''	54.5	3.86, s	55.5	3.87, s	55.4	3.85, s
4''			37.5	2.60, s		



4 **5** **6**

Figure 3 Major COSY correlations (▬) and HMBC (→) peaks of **4**, **5**, and **6**

Compound **5** was obtained as pale-yellow crystals from the $CHCl_3$ fraction. Its molecular formula was determined as $C_{14}H_{21}NO_4$, based on a quasimolecular ion of $[M+H]^+$ at *m/z* 268.1010 in its positive HR-ESI-MS, suggesting five degrees of unsaturation. The ^1H NMR (400 MHz, $CDCl_3$) spectroscopic data (Table 4) showed signals for three methyl groups at δ_H 1.01 (t, *J* = 7.4 Hz, 3H), 2.60 (s, 3H), and 3.87 (s, 3H), and four methylene groups at 2.99 (t, *J* = 7.4 Hz, 2H), 1.73 (m, *J* = 7.4 Hz, 2H),

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3.12 (t, *J* = 6.2 Hz, 2H), and 3.25 (dd, *J* = 13.1, 5.9 Hz, 2H). The ^{13}C NMR (400 MHz, $CDCl_3$) spectroscopic data (Table 4) showed the presence of one benzene ring and one carbonyl group. HMBC peaks from H-6 to C-1, 2, 3, 4, 5 concluded the benzene ring (Figure 2). Based on ^{13}C NMR chemical shifts and 2D NMR, a methoxy group was found to be attached to C-1 and a hydroxy group connected to C-5, another hydroxyl group connected to C-3. COSY correlations connected H-3'' to H-6 and HMBC peaks were observed from H-3'' to C-1, 2, 6 (Figure 3). A side chain was determined by COSY correlations (H-2'/H-3', 4' and H-3'/H-2', 4', and H-4'/H-2', 3') and HMBC peaks (H-2'/C-2,1', 3', 4', H-3'/C-1', 2', 4', and H-4'/C-2', 3'). Based on ^{13}C NMR chemical shifts, a nitrogen atom was found to be attached to C-2'' and C-4''. Another side chain was determined by COSY correlation (H-1''/H-2'') and HMBC peaks (H-1''/C-3, 4, 5, 2'', H-2''/C-3, 4, 5, 1'', 4'', and H-4''/C-1'', 2'').

Compound **6** was obtained as pale-yellow crystals from the $CHCl_3$ fraction. Its molecular formula was determined as $C_{11}H_{14}O_4$, determined by $[M+Na]^+$ at *m/z* 233.0816 in its positive HR-ESI-MS. The ^{1}H NMR spectroscopic data (Table 2) showed signals for three methyl groups at δ 3.82 (s, 3H), 2.60 (s, 3H), and 1.10 (t, *J* = 7.5 Hz, 3H), and one methoxy group δ 3.82 (s, 3H and one methylene δ 2.59–2.54 (m, 2H). The ^{13}C NMR spectrum of **6** presented 11 carbon signals, including one carbonyl [δ_C 203.41(C-1')], three methyl groups [13.46(C-2'', 33.08(C-2'), and 55.45(O-CH_3)], one benzene ring [90.22(C-6), 105.92(C-2), 109.64(C-4), 160.20(C-3), 161.34(C-1), and 164.82(C-5)]. ^{13}C NMR chemical shifts clearly indicate signals of a benzene ring and a hydroxyl group. In combination with 2D NMR, a side chain was determined by COSY correlation (H-1''/H-2'') and HMBC peaks (H-1''/C-5, 4, 2'', H-2''/C-4, 1'') (Figure 3). Another side chain was also determined by HMBC peaks (H-6/C-1', H-2'/C-1').

2.2 Antitumor activities of metabolites from A. alternata

The compounds were evaluated for their cytotoxicities against U266, HepG2, and A549 cells (Table 3) by means of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-3H-tetrazolium bromide (MTT) assay [14]. IC_{50} values were used to determine growth inhibition in the presence of test compounds.

Table 3. Antitumor activities (IC_{50} values) of metabolites from *Alternaria alternata* (μg/ml)

Compound	U266	HepG2	A549
1	>100	>100	>100
2	21.98	25.77	>100
3	24.99	33.16	80.25
4	14.78	15.79	20.41
5	97	>100	>100
6	>100	>100	>100
7	15.48	33.52	>100
8	>100	>100	>100
9	70.4	80.6	78.5
10	23.49	15.6	61.15
11	13.26	14.69	24.39
12	>100	>100	>100

Results of the cytotoxicity assays indicated that compounds **2**, **3, 4, 10**, and **11**, which contain several hydroxyls, were active against the three human tumor cells. In particular, compound **11** was the most active against all three human tumor cells, with IC_{50} values ranging from 13.26 to 24.39 μg/ml. Based on the IC_{50} values of compounds **2**, **3**, **8**, and **12**, we speculated that the number of rings negatively correlates with cytotoxicity. Compounds **2** and **7**, with two hydroxyl groups, showed moderate inhibitory effects against these tumor cells, with IC_{50} values ranging from 15.45 to 33.52 μg/ml. This indicated that the hydroxyl group at C-2' was responsible for their enhanced antitumor activities compared with those of **1** and **8**. Due to the lack of a hydroxyl group at C-2', compounds **1**

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and **8** showed no cytotoxicity against these tumor cells (IC_{50} >100 μg/ml). The results suggested that the hydroxyl group at C-2′ plays a significant effect on antitumor activity.

3. Materials and Methods

3.1 General procedures

^{1}H NMR and ^{13}C NMR were recorded in $CDCl_3$ by using a BRUKER Avance II 400 MHz spectrometer (Bruker BioSpin, Faellanden, Switzerland). HR-ESI-MS was performed on G2 QToF Waters Xevo (Waters Corporation, Milford, MA, USA) with the MassLynx 4.1 software. Sephadex LH-20 (SE-751) was purchased from GE Healthcare Life Sciences (Uppsala, Sweden). SiliaSphere C18 (50 μm, 120 Å) was obtained from SiliCycle Inc. (Quebec City, Canada). MeOH (AR grade), EtOAc (AR grade), $CHCl_3$ (AR grade), and petroleum ether (AR grade) was purchased from Xilong Scientific Co., Ltd. (Shantou, China). Chromatographic grade acetonitrile and MeOH were obtained from Tedia Company Inc. (Fairfield, OH, USA).

3.2 Fungi

The fungal strain (*A. alternate sp* was obtained from the Marine Culture Collection of China (MCCC 3A00467) used in this study was collected from the Pacific Ocean and a voucher specimen was deposited.

3.3 Culture method

The strain was cultured on a sterilized seed medium for 72 h on a rotary shaker at 28°C and 130 rpm in a 1000-ml Erlenmeyer flask. The components of the seed medium were as follows: maltose (2%), monosodium glutamate (1%), KH_2PO_4 (0.05%), $MgSO_4$-$7H_2O$ (0.03%), glucose (1%), yeast extract (0.3%), corn steep liquor (0.1%), mannitol (2%), and seed salt (3.3%); the pH was adjusted to 6.5. After achieving logarithmic growth, the strain was transferred to a solid medium, containing glucose (2%), yeast extract (1%), peptone (2%), rice (70%), and millet (30%), and cultured for 30 days at 28°C.

3.4 Separation and purification of metabolites

The fungal strain was soaked in MeOH for three days, after which the MeOH solution was concentrated in vacuum, and the residue was partitioned between EtOAc and water. Then, the EtOAc fraction was separated on an ODS column and the monomers were separated by preparative HPLC.

3.5 Cytotoxicity activity evaluation

The cytotoxicity activities of each compound were measured against human myeloma (U266), liver cancer (HepG2), and lung cancer (A549) cells by the MTT assay [15]. Test cells at logarithmic growth phase (4×10^4) were added to each well in 96-well plates and incubated with each compound (0.1–100 μg/mL) in triplicate for 24 h in 5% CO_2 and at 37°C. Then, 20 μl MTT solution (5 mg/ml) was added to each well and further incubated for 4 h. Next, DMSO was added to each well, and after standing for 15 min at room temperature, the OD of each well was measured on a microplate reader at 570 nm. IC_{50} values were obtained by a linear regression analysis of percentage absorbance versus log [drug concentration].

4. Conclusions

In summary, six new compounds and six known ones were isolated from *A. alternata* 3A00467 and their cytotoxic activities were evaluated against U266, HepG2, and A549 cells. Compound **11** showed the strongest activity against all three human cell lines. Among the novel compounds,

compounds **2**, **3**, and **4** exhibited good cytotoxic effects. These compounds obtained from a marine source have the potential to be developed into novel anticancer drugs.

Supplementary material:
Supplementary material relating to this article is available online, including 1D NMR data of compounds 1-12, 2D NMR correlations of compounds 1-6, HRESIMS and NMR spectra for compounds 1-12.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Katz, L.; Baltz, R.H. Natural product discovery: past, present and future. *J. Ind Microbiol Biot* **2016**, *43*, 155–176, 10.1007/s10295-015-1723-5.
2. Chen, S.L.; Yu, H.; Luo, H.M.; Wu, Q.; Li, C.F.; Steinmetz, A. Conservation and sustainable use of medicinal plants: problems, progress, and prospects. *Chinese Med*. **2016**, *11*, 37, 10.1186/s13020-016-0108-7.
3. Garrison, T.S.; Ellis, R. *Oceanography: an invitation to marine science*, 9th ed.; Cengage Learning: Boston, MA, USA, 2015, ISBN-13: 978-1-305-10516-4.
4. Guo, Y.W. The Status of the art of marine products research in European and the perspective for the tesearch and development of Chinese marine drugs. Chin J New Drugs **2001**,28,81-84.
5. Mann, J. Natural products in cancer chemotherapy: past, present and future. Nat. Rev. Cancer **2002**, 2, 143 , 10.1038/nrc723.
6. Lou, J.; Fu, L.; Peng, Y.; Zhou, L. Metabolites from Alternaria fungi and their bioactivities. *Molecules* **2013**, *18*, 5891–5935, 10.3390/molecules18055891.
7. Hu, Y.; Chen, J.; Hu, G. Statistical research on the bioactivity of new marine natural products discovered during the 28 years from 1985 to 2012. *Mar. Drugs* **2015**, *13*, 202–221, 10.3390/md13010202.
8. Gerwick, W.H.; Moore, B.S. Lessons from the past and charting the future of marine natural products drug discovery and chemical biology. *Chem. Biol.* **2012**, *19*, 85–98, 10.1016/j.chembiol.2011.12.014.
9. Choudhary, S. Incidence and management of Alternaria sp. associated with blond psyllium (Plantago ovata L.) seeds. Master of Science in Agriculture (Plant Pathology), Sri Karan Narendra Agriculture University, Jobner, Rajasthan, India, 2015.
10. Lou, J.; Fu, L.; Peng, Y. Metabolites from Alternaria fungi and their bioactivities. *Molecules* **2013**, *18*, 5891–5935, 10.3390/molecules18055891.
11. Ayer, W.A.; Jimenez, L.D. Phomalone, an antifungal metabolite of Phoma etheridgei. *Can. J. Chem.* **1994**, *72*, 2326–2332, 10.1139/v94-296.
12. Michaelá, C.I.; Rupertá, M.C. Structural revision and synthesis of LL-D253α and related chromanone fungal metabolites. *J. Chem. Soc.* **1992**, *18*, 2271–2284, 10.1039/P19920002271.
13. Michaelá, C.I.; Rupertá, M.C. Biosynthesis of LL-D253α, a polyketide chromanone metabolite of Phoma pigmentivora: incorporation of 13 C, 2 H and 18 O labelled precursors. *J. Chem. Soc.* **1992**, *18*, 2285–2293, 10.1039/P19920002285.
14. Tang, M.; Shen, D.; Hu, Y.; Gao, S.; Yu, S. Cytotoxic triterpenoid saponins from Symplocos chinensis. *J. Nat. Prod.* **2004**, *67*: 1969–1974, 10.1021/np040013g.
15. Ma, X.H.; Wang, Z.B.; Zhang, L.; Li, W.; Deng, C.M.; Zhong, T.H.; Li, G.Y.; Zheng, W.M., Zhang, Y.H. Diterpenoids from Wedelia prostrata and their derivatives and cytotoxic activities. *Chem. Biodivers*. **2017**, *14*, 10.1002/cbdv.201600423.