Biosynthetically Distinct Cytotoxic Polyketides from *Setophoma terrestris*

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Abstract:

Sixteen polyketides belonging to diverse structural classes, including monomeric/dimeric tetrahydroxanthones and resorcylic acid lactones, were isolated from an organic extract of a fungal culture *Setophoma terrestris* (MSX45109) by bioactivity-directed fractionation as part of a search for anticancer leads from filamentous fungi. Of these, six were new: penicillixanthone B (**5**), blennolide H (**6**), 11-deoxyblennolide D (**7**), blennolide I (**9**), blennolide J (**10**), and pyrenomycin (**16**). The known compounds were: secalonic acid A (**1**), secalonic acid E (**2**), secalonic acid G (**3**), penicillixanthone A (**4**), paecilin B (**8**), aigialomycin A (**11**), hypothemycin (**12**), dihydrohypothemycin (**13**), pyrenochaetic acid C (**14**), and nidulalin B (**15**). The structures were elucidated by a set of spectroscopic and spectrometric techniques: the absolute configurations of compounds **1**–**10** were determined by ECD spectroscopy combined with time-dependent density functional theory (TDDFT) calculations, whereas a modified Mosher's ester method was used for compound **16**. The cytotoxic activities of compounds **1**–**15** against the MDA-MB-435 (melanoma) and SW-620 (colon) cancer cell lines were evaluated. Compounds **1**, **4**, and **12**were the most potent, with IC_{50} values ranging from 0.16 to 2.14 μM. When tested against a panel of bacteria and fungi, compounds **3** and **5** showed promising activity against the Gram-positive bacterium *Micrococcus luteus*, with MIC values of 5 and 15 $\mu g\,mL^{-1}$, respectively.

Keywords: Natural products | Polyketides | Medicinal chemistry | Cytotoxicity | Antitumor agents | Configuration determination | Structure–activity relationships

Article:

Introduction

Structurally diverse cytotoxic secondary metabolites have been isolated and identified from filamentous fungi of the Mycosynthetix library, representing over 55000 accessions, as part of ongoing bioactivity-directed studies for discovery of anticancer drug leads.[1] The organic extract of a solid-phase culture of *Setophoma terrestris* (MSX45109), isolated from plant material collected in a mangrove habitat in 1989, showed potent cytotoxic activities against the SW-620 (colon) and MDA-MB-435 (melanoma) cancer cell lines (ca. 91 % and 100 % inhibition of cell growth, respectively, when tested at 20 μg mL^{-1}). Of the hundreds of cultures that have been investigated as part of this project, *S. terrestris* was intriguing, due to the robust biosynthesis of 16 polyketides (**1**–**16**) of distinct structural classes. Over a third of the isolated compounds were new, whereas structural revisions and augmentations to the literature were provided for several others.

Compounds **1**–**10** (Figure 1, below) were identified as a series of monomeric/dimeric tetrahydroxanthones, an important class of mycotoxins produced by a variety of microorganisms displaying remarkable biological activities, including antitumor, antibacterial, and anti-HIV.[2] In this study, a series of new monomeric and homo-/heterodimeric tetrahydroxanthones were identified, thereby expanding the diversity of this class of natural products and updating the literature on structural elucidation and absolute configuration considerations of structurally related compounds. These points are also critical for accurate and comprehensive dereplication studies of fungal metabolites, an area of growing prominence in the field.[1a,3] Interestingly, a recent and comprehensive study reported a similar series of compounds, albeit with opposite absolute configurations.[4]

Compounds **11**–**13** were characterized as a series of structurally related resorcylic acid lactones (RALs), a family of benzannulated macrolides produced by a variety of fungi exhibiting a wide range of biological activities, including antitumor, antifungal, antibiotic, and antiviral.[5] Compounds **14** and **16**, biogenetically related to RALs, were also isolated and identified.

The absolute configurations of compounds **1**–**10** were determined with the aid of time-dependent density functional theory (TDDFT) calculations of ECD spectra, whereas for **16** a modified Mosher's ester method was used. The dimeric tetrahydroxanthone derivatives **1**and **4** and the RAL **12** all showed potent inhibition of MDA-MB-435 and SW-620 cancer cell lines. On the other hand, compounds **3** and **5** showed promising activity against the Gram-positive bacterium *Micrococcus luteus*.

Results and Discussion

The organic extract of a large-scale solid-substrate fungal culture of *S. terrestris* (MSX45109) showed potent cytotoxic activity against the SW-620 and MDA-MB-435 cancer cell lines, and so it was fractionated by silica gel flash chromatography to yield five fractions. The third fraction, which eluted with 10 % MeOH/$CHCl_3$, showed potent cytotoxic activity against the two cancer cell lines, and as such, it was purified by reversed-phase preparative and semipreparative HPLC to yield polyketides **1**–**16**. The purities of the isolated compounds were verified by UPLC (Figure S1 in the Supporting Information).

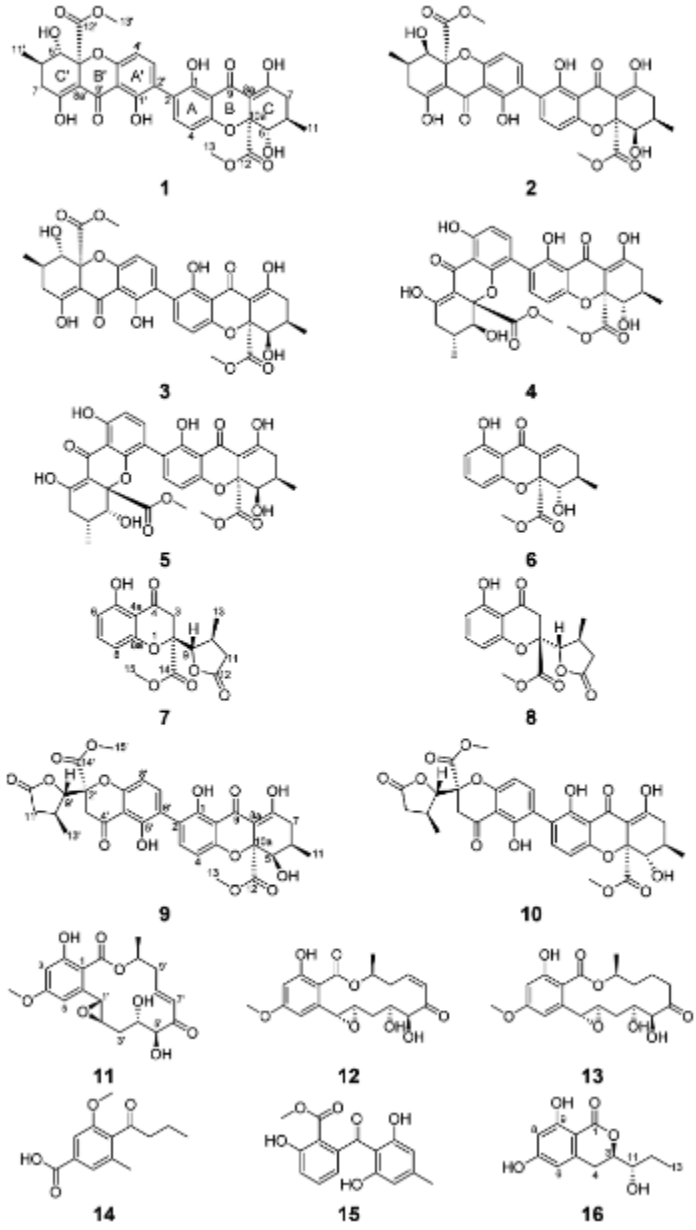


Figure 1. Compounds **1**–**16** from *S. terrestris* (MSX45109).

Monomeric/Dimeric Tetrahydroxanthones

Compounds **1**–**10** were identified as a series of monomeric (**6**–**8**), homodimeric (**1**–**5**), and heterodimeric (**9** and **10**) tetrahydroxanthones by comparison of HRMS, NMR, and ECD data with each other and with structurally related compounds (Figure 1). Stereoisomeric compounds **1** (35.1 mg), **2** (51.3 mg), and **3** (9.5 mg) were all obtained as yellow powders and had molecular formulae of $C_{32}H_{30}O_{14}$ as determined by HRMS (ESI). The ^{1}H and ^{13}C NMR spectra of compounds **1** and **2** showed the presence of only 15 H and 16 C atoms, indicating that these compounds were homodimers, whereas **3** was clearly a heterodimer (Figures S2–S4 in the

Supporting Information). A search of the "Dictionary of Natural Products"[6] for the molecular formula and a UV range of 325–345 nm resulted in twelve hits, five of which were excluded on the basis of NMR spectroscopic data. The remaining seven hits were the secalonic acids A–G, each containing a 2,2′-linkage. The fact that compounds **1** and **2** were homodimeric cut down the number of possibilities for compounds **1** and **2** to secalonic acids A, B, D, or E, whereas the heterodimeric nature of **3** suggested secalonic acid C, F, or G. Key differences between compounds **1** and **2** were the chemical shift values and splitting patterns of 5-H, 6-H, and H_2-7. A proton doublet of doublets corresponding to 5-H/5′-H (δ_H = 3.92 ppm, dd, J = 11.2, 0.5 Hz) in **1** was downfield-shifted in **2** (δ_H = 4.11 ppm, d, J = 1.2 Hz). These J values implied a pseudodiaxial *trans* orientation of 5-H/6-H in **1** and a pseudoaxial/pseudoequatorial *cis* orientation in **2** (Figures S2 and S3, Table S1 in the Supporting Information). On the other hand, the ^{1}H NMR spectrum of **3** showed similarity with the ^{1}H NMR spectra of both **1** and **2**, revealing two sets of protons corresponding to asymmetric monomers (Figure S4, Table S1 in the Supporting Information). The 2,2′-linkage in each of **1**–**3** was confirmed by diagnostic HMBC correlations of 3-H and 3′-H with C-2′ and C-2, respectively.

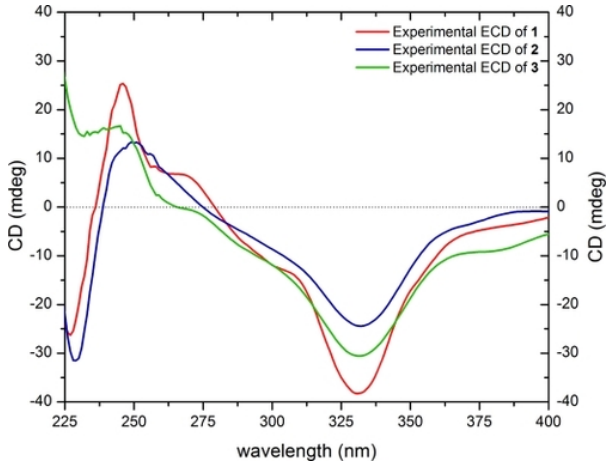


Figure 2. Comparison of the experimentally measured ECD spectra of secalonic acids A (**1**), E (**2**), and G (**3**) [0.03 mM $CHCl_3$, cell length 2 cm].

The absolute configurations of the 2,2′-secalonic acids have been determined by ECD spectroscopy.[7] The Cotton effect around 330 nm corresponds to the n–π* electronic transition, and it has been correlated with the configurations of C-10a and C-10a′.[7a,8] Negative Cotton effects at 332 nm in the ECD spectra of compounds **1**, **2**, and **3** ($\Delta\epsilon$ = –38.8, –24.6, and –31.2, respectively), indicated the *S* configuration at both C-10a and C-10a′ (Figure 2). This allowed the assignment of the *R* configuration at C-6 and C-6′ on the basis of the *trans* orientation of the C-6/6′ and C-10a/10a′ substituents deduced from the biosynthetic route for the formation of stereoisomeric tetrahydroxanthone precursors of secalonic acids, with no exception so far in the literature.[9] Combining all the data together suggested the absolute configurations as (5*S*,6*R*,10a*S*,5′*S*,6′*R*,10a′*S*), (5*R*,6*R*,10a*S*,5′*R*,6′*R*,10a′*S*), and (5*R*,6*R*,10a*S*,5′*S*,6′*R*,10a′*S*) for compounds **1**–**3**, respectively. Consequently, compounds **1**–**3** were identified as secalonic acid A,[10] secalonic acid E,[11] and secalonic acid G,[12] respectively, in accordance with published data (Table S2 in the Supporting Information). Most literature pertaining to these compounds dates back to the 1960s and 1970s, and so the ^{1}H and ^{13}C NMR spectroscopic data for **1**–**3** are provided in Figures S2–S4 and Table S1 in the Supporting Information.

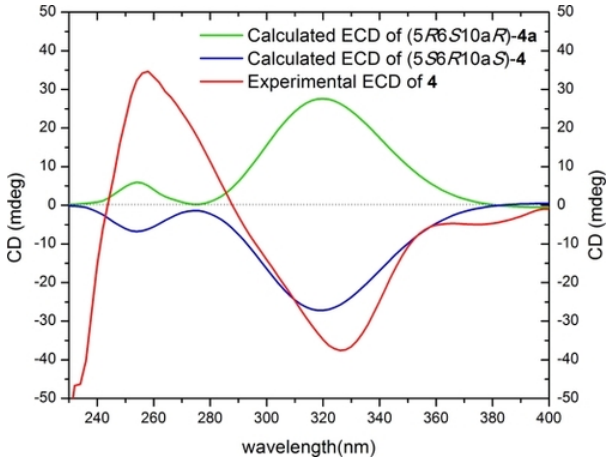


Figure 3. Experimentally measured and calculated ECD spectra for penicillixanthone A (**4**, 0.05 mM, $CHCl_3$, cell length 2 cm).

Compound **4** (5.4 mg) was obtained as a yellow powder. Its chemical formula was determined to be $C_{32}H_{30}O_{14}$ by HRMS (ESI) and analysis of ^{1}H, ^{13}C, and edited-HSQC NMR spectroscopic data (Figure S5 and Table S3 in the Supporting Information). These data suggested an asymmetric secalonic acid analogue with structural similarity to **1**. However, a key difference between **4** and **1** was the linkage of the monomeric units, this being 2,2′ in **1** versus 2,4′ in **4**, thus making **4** asymmetric, as evidenced by diagnostic HMBC correlations of 3-H and 3′-H with C-4′ and C-4, respectively (Figure S6 in the Supporting Information). The two monomeric moieties in **4** were assigned the same configuration as in **1**, as evidenced by a negative Cotton effect at 331 nm in the ECD spectrum of **4** ($\Delta\epsilon$ = –24.8), and negative values of optical rotation for both **1** and **4** (Figure 2, Figure S7 and Table S2 in the Supporting Information). The absolute configuration of **4** was established as (5*S*,6*R*,10a*S*,5′*S*,6′*R*,10a′*S*) by comparing experimentally measured and calculated ECD spectra predicted by the time-dependent density functional theory/electronic circular dichroism (TDDFT-ECD) approach (Figure 3).[13] Briefly, conformation analysis of 3D models of compound (5*S*,6*R*,10a*S*,5′*S*,6′*R*,10a′*S*)-**4** and its hypothetical enantiomer (5*R*,6*S*,10a*R*,5′*R*,6′*S*,10a′*R*)-**4a** gave 12 and 11 conformers, respectively, within a 2 kcal mol^{-1} energy window from the global minimum. All conformers were geometrically optimized at the B3LYP/DGDZVP level.[14] Relative free energies ($\Delta G°$) as well as the Boltzmann distributions for the most relevant optimized conformers are given in the Supporting Information (Figure S8). The Boltzmann-averaged ECD spectra of re-optimized conformers of **4** based on TDDFT showed an excellent fit with the experimentally measured data, with a negative Cotton effect around 320 nm (n–π* transition), whereas the **4a** enantiomer showed a positive Cotton effect at the same wavelength (Figure 3). The NMR (Figure S5 and Table S3 in the Supporting Information) and optical rotation data (Table S2 in the Supporting Information) were in agreement with those reported for penicillixanthone A, though the absolute configuration was not determined.[15] Interestingly, compound **4** was reported by Kurobane et al.[16] as a chemically rearranged analogue of **1** obtained by dissolving **1** in polar organic solvents, such as pyridine, acetone, or acetonitrile, either at room temperature or above for hours to days, depending on the solvent used. This suggests that **4** might have been an artefact of the purification scheme.

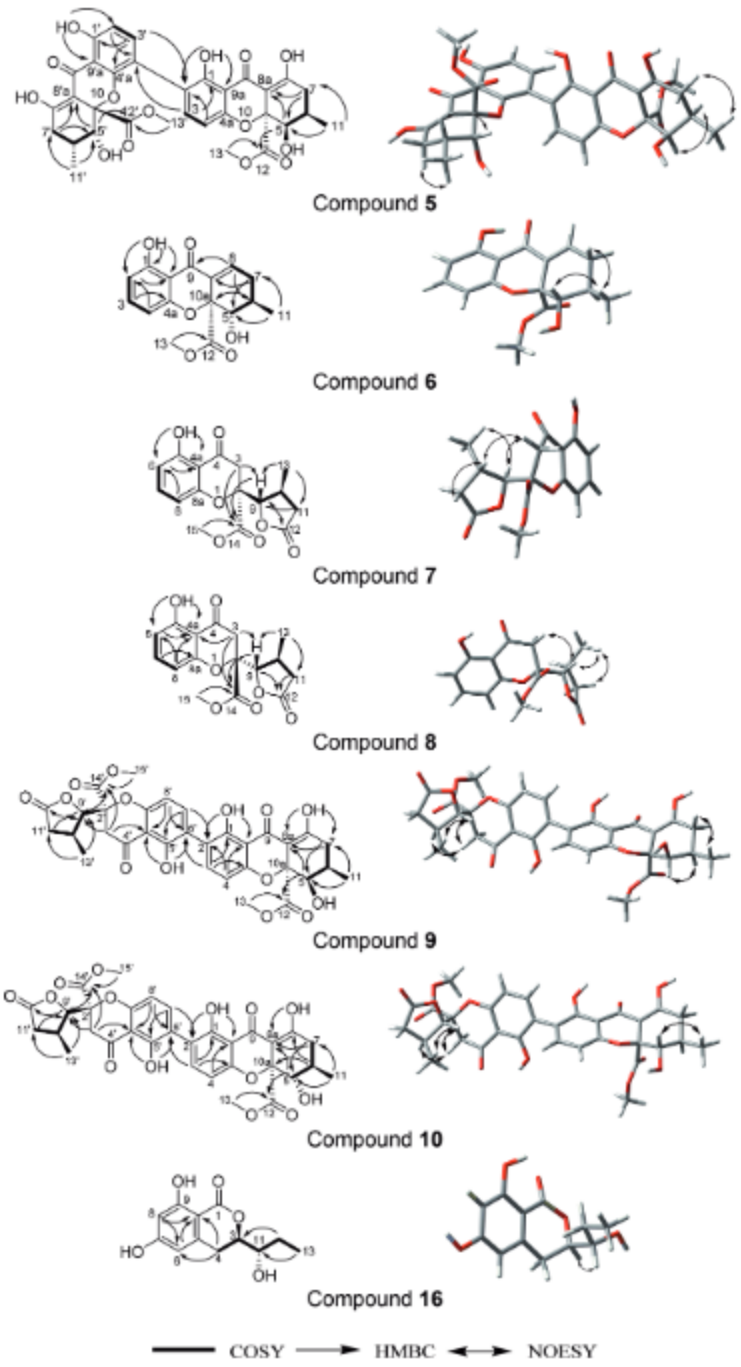


Figure 4. Key COSY, HMBC, and NOESY correlations of **5**–**10** and **16**. Gaussian 09 was used for ground-state mechanics optimization, based on the NOESY correlations, to generate the structures shown on the right.

Compound **5** (2.1 mg), obtained as a yellow powder, had a chemical formula of $C_{32}H_{30}O_{14}$ as determined by HRMS (ESI) and analysis of ^{1}H, ^{13}C, and edited-HSQC NMR spectroscopic data (Figure S9 in the Supporting Information), corresponding to an index of hydrogen deficiency of 18. The NMR spectroscopic data suggested an asymmetric secalonic acid analogue with structural similarity to **2** (Figure 4, Figure S9 in the Supporting Information, Table 1). However,

as was also observed in **1** versus **4**, the 2,2′-linkage in **2** was supplanted by a 2,4′-linkage in **5**, as evidenced by diagnostic HMBC correlations of 3-H and 3′-H with C-4′ and C-4, respectively (Figure 4). Two singlets, corresponding to 5-H/5′-H (δ_H = 4.14/3.99 ppm) in **5**, implied a pseudoaxial/pseudoequatorial *cis* orientation of 5-H/6-H and 5′-H/6′-H, similar to that in **2**; this was further supported by NOESY correlations between 5-H/6-H and 5′-H/6′-H (Figure 4). The two monomeric moieties in **5** were assigned the same configuration as in **2**, as evidenced by a negative Cotton effect at 332 nm in the ECD spectrum of **5** ($\Delta\epsilon$ = –18.9, Figure S7 in the Supporting Information) and negative values of optical rotation for both compounds (Table S2 in the Supporting Information). The absolute configuration of **5** was established as (5*R*,6*R*,10a*S*,5′*R*,6′*R*,10a′*S*) by comparing experimentally measured ECD spectra and those calculated by TDDFT by use of the same protocol as described for compound **4** (Figure 5 and Figure S10 in the Supporting Information).[13a–13d] These data suggested the structure of **5**, which was ascribed the trivial name penicillixanthone B. As noted for **4**,[10] compound **5** was potentially an artefact produced by rearrangement of **2** in polar organic solvents.

Table 1. NMR spectroscopic data for **5** (500 MHz for ^{1}H, 125 MHz for ^{13}C; chemical shifts in δ, coupling constants in Hz, $CDCl_3$).

Position	δ_C	δ_H **mult. (*J* in Hz)**	**Position**	δ_C	δ_H **mult. (*J* in Hz)**
1	159.4		1′	161.9	
2	119.4		2′	110.8	6.60, d (8.6)
3	139.6	7.47, d (8.6)	3′	140.1	7.33, d (8.6)
4	107.9	6.58, d (8.6)	4′	115.9	
4a	157.2		4a′	154.5	
5	71.5	4.14, s	5′	70.9	3.99, s
6	28.6	2.12, m	6′	28.5	2.12, m
7	32.7	2.38, m	7′	32.6	2.38, m
		2.49, m			2.49, m
8	180.2		8′	179.4	
8a	99.9		8a′	99.7	
9	187.6		9′	187.9	
9a	107.0		9a′	106.9	
10a	84.9		10a′	84.4	
11	17.6	1.17, dd (6.9, 0.6)	11′	17.6	1.13, dd (6.9, 0.6)
12	171.4		12′	170.9	
13	53.7	3.75, s	13′	53.4	3.64, s
1-OH		11.91, s	1′-OH		11.56, s
5-OH		2.55, br. s	5′-OH		2.77, br. s

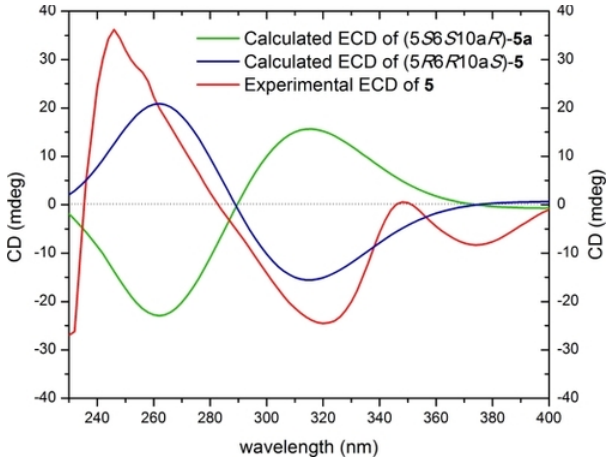


Figure 5. Experimentally measured and calculated ECD spectra for penicillixanthone B (**5**, 0.06 mM, $CHCl_3$, cell length 2 cm).

Table 2. NMR spectroscopic data for **6** (500 MHz for ^1H, 175 MHz for ^{13}C; chemical shifts in δ, coupling constants in Hz, $CDCl_3$).

Position	δ_C	δ_H **mult. (*J* in Hz)**
1	163.0	
2	110.5	6.54, dd (8.6, 0.6)
3	138.5	7.39, dd (8.6, 8.0)
4	107.7	6.56, dd (8.0, 0.6)
4a	159.5	
5	77.5	3.92, dd (11.5, 2.9)
6	30.0	2.36, m
7	33.8	2.16, ddd (20.6, 10.3, 2.9)
		2.72, dt (20.6, 5.2)
8	141.9	7.23, dd (5.2, 2.9)
8a	129.9	
9	184.7	
9a	107.7	
10a	85.8	
11	17.7	1.15, d (6.3)
12	169.1	
13	53.3	3.69, s
1-OH		12.00, s
5-OH		2.74, s

Compound **6** (0.8 mg) was obtained as a yellow gum. Its molecular formula was deduced to be $C_{16}H_{16}O_6$ by HRMS (ESI) and analysis of ^1H, ^{13}C and edited-HSQC NMR spectroscopic data (Figure S11 in the Supporting Information), indicating an index of hydrogen deficiency of 9. Inspection of the HRMS and NMR spectroscopic data suggested that **6** was a tetrahydroxanthone derivative with structural similarity to the monomeric unit of **1**. However, key differences were the replacement of the C-2 quaternary carbon in **1** (δ_C = 118.3 ppm) by an aromatic methine system in **6** (δ_H/δ_C = 6.54/110.5 ppm), and the replacement of the hydrogen-bonded phenolic proton in **1** (δ_H = 13.78 ppm) by an olefinic proton in **6** (δ_H = 7.23 ppm, Table 2, Figure S11 in the Supporting Information). COSY data identified two spin systems as 2-H/3-H/4-H and 5-H/6-

H/H_2-7/8-H (Figure 4). Further examination of the NMR spectra, including HMBC data (Figure 4), yielded a planar structure of **6**, which was ascribed the trivial name blennolide H. The relative configuration of **6** was found to be (5*S*,6*R*,10a*S*), the same as that of the monomeric units of **1**, deduced from NOESY correlations and coupling constants for both compounds (Figure 4). However, the ECD calculations suggested the opposite (5*R*,6*S*,10a*R*) configuration (Figure S12 in the Supporting Information). Because this finding was inconsistent with biosynthetic considerations, the calculations were repeated, to yield identical results. As such, we regard the absolute configuration of **6** as tentative.

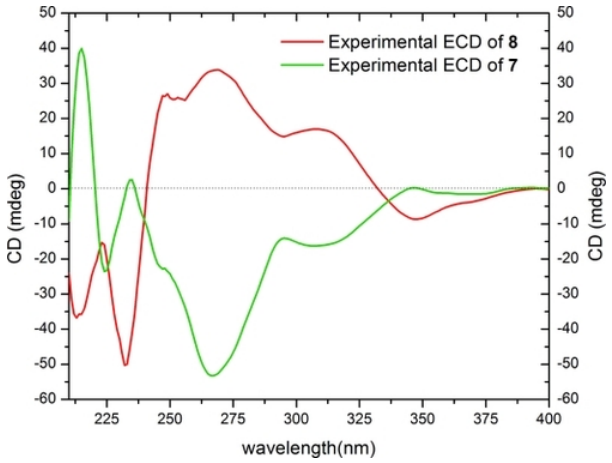


Figure 6. Experimentally measured ECD spectra of 11-deoxyblennolide D (**7**, 0.16 mM) and paecilin B (**8**, 0.56 mM, $CHCl_3$, cell length 2 cm).

Diastereoisomers **7** (5.4 mg) and **8** (1.0 mg) were obtained as colorless oils and both had molecular formulae of $C_{16}H_{16}O_7$, as determined by HRMS (ESI) and analysis of ^{1}H, ^{13}C, and edited-HSQC NMR spectroscopic data (Figures S13 and S14 in the Supporting Information), corresponding to an index of hydrogen deficiency of 9. Compounds **7** and **8** showed similar ^{1}H and ^{13}C NMR spectra (Table 3) with small differences in four chemical shift values. Interestingly, compounds **7** and **8** displayed opposite signs for optical rotation data: whereas compound **7** was levorotatory (–26.14°), compound **8** was dextrorotary (+42.05°, Table S2 in the Supporting Information). Moreover, the ECD spectra of **7** and **8** were mirror images (Figure 6). Analysis of the HRMS and NMR spectroscopic data indicated that **7** and **8** were monomers and structurally related to the monomeric units of compounds **1**–**5**. In comparison with the chromanone moiety in compounds **1**–**5**, rings A and B were preserved in **7** and **8**, as evidenced by comparable ^{13}C NMR spectroscopic data and the hydrogen-bonded phenolic proton (δ_H = 11.43 ppm), except for a quaternary aromatic carbon that was replaced by an aromatic methine system in **7** and **8**. In contrast, however, ring C had experienced significant changes, as evidenced by 1D- and 2D-NMR spectroscopic data. For example, the ^{13}C NMR spectra displayed signals characteristic of ester functionalities (δ_C = 175.3 and 175.7 ppm for **7** and **8**, respectively; Table 3). Thus, ring C was established as a γ-lactone moiety, as evidenced by the COSY spin system of 9-H/10-H/(H_3-13)/11-H and HMBC correlations from H_3-13 to C-11 and C-9, from 9-H to C-12, and from 11-H to C-9 (Figure 4). On the other hand, HMBC correlations from H_2-3 to C-9 and C-14 and from 9-H to C-14 indicated a 2,9-linkage between the chromanone and the γ-lactone moieties, establishing the planar structures of **7** and **8** (Figure 4). The relative configuration of ring C in compounds **7** and **8** was similar, on the basis of NOESY

correlations between H_3-13 and 9-H and coupling constant values of 4.0 and 3.4 ppm for 9-H in compounds **7** and **8**, respectively, implying a pseudodiaxial *trans* orientation of 9-H/10-H (Figure 4, Table 3). The spatial arrangements of **7**and **8** were different, as observed from NOESY data, particularly between 10-H to 11α-H and 3α-H, as well as 9-H to H_2-3 in **7** and from 9-H to 3α-H in **8** (Figure 4). Putting these data together, and taking into consideration the fact that compounds **7** and **8** could not be enantiomers, because they had been separated by non-enantioselective methods, compounds **7** and **8** could either be epimers at C-2 (2*R*9*S*10*S* or 2*S*9*S*10*S*) or have the same configuration at C-2 but the opposite configuration of ring C. The absolute configurations of **7** and **8** were established by comparing the experimentally measured ECD data with those obtained through molecular modeling calculations (Figures S15 and S16 in the Supporting Information). The excellent fit between the observed and calculated ECD plots (Figure 7) established the absolute configuration of **7** as (2*S*,9*S*,10*S*) and that of **8** as (2*R*,9*S*,10*S*), supporting epimerization at C-2. The NMR (Table S4 in the Supporting Information) and optical rotation data for **8** (Table S2 in the Supporting Information) indicated similarity with paecilin B (isolated from the mangrove endophytic fungus *Paecilomyces* sp.), the planar structure of which was published in 2007.[17] However, significant differences in the ^{1}H and ^{13}C NMR spectroscopic data for compound **8** and for paecilin B were observed (Table S4 in the Supporting Information). Recently, paecilin B was reported as a monomer and as a subunit of a dimer from the seagrass-derived fungus *Bipolaris* sp.[18] Our data were in agreement with this study, including the absolute configuration. With respect to compound **7**, two structurally related compounds, blennolide D and blennolide E, in which C-11 is hydroxylated, were reported previously from *Blennoria* sp.[4] Hence, the trivial name 11-deoxyblennolide D was ascribed to **7**. The planar structures of compounds **7** and **8** were reported previously, on the basis of their synthesis, but without reporting of NMR spectroscopic data.[19]

Table 3. NMR spectroscopic data for **7** and **8** (500 MHz for ^{1}H, 125 MHz for ^{13}C; chemical shifts in δ, coupling constants in Hz, $CDCl_3$).

Position	**7**		**8**	
	δ_C	δ_H **mult. (*J* in Hz)**	δ_C	δ_H **mult. (*J* in Hz)**
2	84.3		84.3	
3	39.8	3.04, dd (17.2, 0.6)	40.7	3.07, d (17.2)
		3.17, d (17.2)		3.48, d (17.2)
4	194.1		194.9	
4a	107.7		107.5	
5	162.0		161.9	
6	110.7	6.55, dd (8.6, 0.6)	110.6	6.54, dd (8.6, 0.6)
7	139.2	7.42, t (8.6)	139.0	7.40, t (8.6)
8	107.7	6.54, dd (8.6, 0.6)	107.5	6.49, dd (8.6, 0.6)
8a	159.2		159.3	
9	87.6	4.43, dd (4.0, 0.6)	86.5	4.36, dd (3.4, 0.6)
10	30.1	2.83, m	29.7	2.85, m
11	36.2	2.21, dd (17.8, 4.6)	36.4	2.21, dd (17.8, 4.6)
		2.89, dd (17.8, 9.2)		3.01, dd (17.8, 9.2)
12	175.3		175.7	
13	20.9	1.28, d (6.9)	20.7	1.18, d (6.9)
14	168.9		169.2	
15	53.8	3.72, s	53.7	3.74, s
5-OH		11.43, s		11.45, s

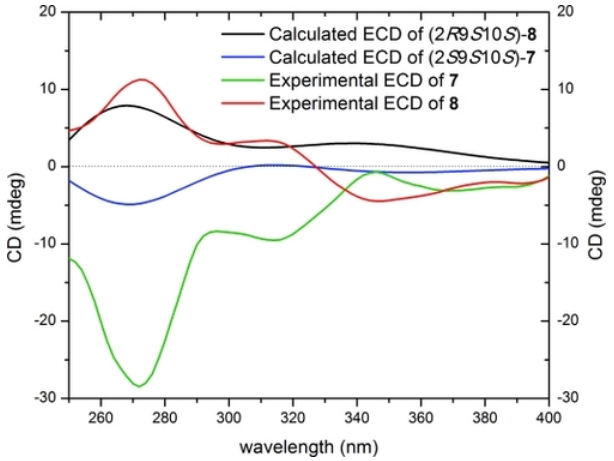


Figure 7. Experimentally measured and calculated ECD spectra for 11-deoxyblennolide D (**7**, 0.16 mM) and paecilin B (**8**, 0.56 mM, $CHCl_3$, cell length 2 cm).

Compound **9** (1.1 mg) was isolated as a yellow gum with a molecular formula of $C_{32}H_{30}O_{14}$as deduced from HRMS (ESI) and ^{1}H, ^{13}C, and edited-HSQC NMR spectroscopic data (Figure S17 in the Supporting Information), indicating an index of hydrogen deficiency of 18. Analysis of the NMR spectroscopic data, including HMBC and NOESY spectra, suggested that **9** was a heterodimer, with one monomeric moiety similar to a monomeric unit of **2** and the other similar to **7** (Table 4, Figure 4). A key difference between a monomeric moiety in **9** that was related to **7** was the replacement of the C-6 aromatic methine (δ_H/δ_C = 6.55/110.7 ppm) in **7** by a quaternary carbon in **9** ($\delta_{C\text{-}6'}$ = 118.1 ppm). A 2,6′-linkage in **9** was evident from diagnostic HMBC correlations of 3-H to C-6′ and 7′-H to C-2, establishing the planar structure of **9** (Figure 4). The absolute configuration was deduced on the basis of the monomeric constituents. The secalonic acid moiety was assigned the same configuration as in **2**, as evidenced from similar NOESY correlations and coupling constant values of 5-H and preservation of the configuration at C-10a through the series. In addition, the moiety similar to **7** was assigned the same configuration as **7** on the basis of consistencies in the ^{1}H, ^{13}C, and NOESY NMR spectroscopic data (Table 3 and Table 4, Figure 4). Hence, the absolute structure of **9** was assigned as (5*R*,6*R*,10a*S*,2′*S*,9′*S*,10′*S*) and confirmed by ECD calculations (Figure 8, Figures S18 and S19 in the Supporting Information). The NMR spectroscopic data for **9** were found to be in agreement with those for blennolide G, which was reported previously from *Blennoria* sp.[4] However, compound **9** and blennolide G showed opposite ECD and optical rotation data, and opposite absolute configuration at four chiral centers, thus indicating that **9** was a diastereoisomer of blennolide G (Table S2 in the Supporting Information, Figure 9). The trivial name blennolide I was ascribed to **9**.

Table 4. NMR spectroscopic data for **9** and **10** (700 MHz for ^{1}H, 175 MHz for ^{13}C; chemical shifts in δ, coupling constants in Hz, $CDCl_3$).

Position	**9**		**10**	
	δ_C	δ_H **mult. (*J* in Hz)**	δ_C	δ_H **mult. (*J* in Hz)**
1	159.4		159.3	
2	118.3		117.8	
3	139.6	7.42, d (8.3)	140.1	7.45, d (8.5)
4	107.5	6.57, d (8.3)	107.5	6.62, d (8.5)
4a	157.2		158.4	
5	71.3	4.12, s	78.0	3.93, dd (11.2, 2.7)
6	28.5	2.11, m	29.2	2.41, m
7	32.6	2.40, dd (18.9, 6.1)	36.2	2.32, dd (19.4, 10.6)
		2.52, dd (18.9, 11.2)		2.73, dd (19.4, 6.5)
8	179.9		177.6	
8a	99.9		101.5	
9	187.5		187.1	
9a	107.0		106.9	
10a	84.8		84.8	
11	17.5	1.17, d, (7.1)	18.0	1.17, d, (6.5)
12	171.2		170.2	
13	53.5	3.71, s	53.3	3.71, s
1-OH		11.85, s		11.72, s
5-OH		2.52, br. s		2.77, d (2.7)
8-OH		13.95, s		13.76, s
2′	84.2		84.2	
3′	39.7	3.05, d (17.0)	39.7	3.05, d (17.0)
		3.21, d (17.0)		3.21, d (17.0)
4′	194.0		194.0	
4a′	107.6		107.6	
5′	159.2		159.2	
6′	118.1		118.1	
7′	141.3	7.52, d (8.3)	141.3	7.51, d (8.5)
8′	107.3	6.61, d (8.3)	107.3	6.62, d (8.5)
8a′	158.6		158.6	
9′	87.5	4.45, d (3.8)	87.5	4.46, d (4.1)
10′	30.0	2.84, m	30.0	2.84, m
11′	36.0	2.91, dd (18.0, 9.3)	36.0	2.92, dd (17.9, 9.4)
		2.23, dd (18.0, 4.8)		2.22, dd (17.9, 4.6)
12′	175.1		175.1	
13′	20.9	1.28, d (7.1)	20.9	1.28, d (6.8)
14′	168.8		168.8	
15′	53.7	3.76, s	53.7	3.75, s
5′-OH		11.88, s		11.89, s

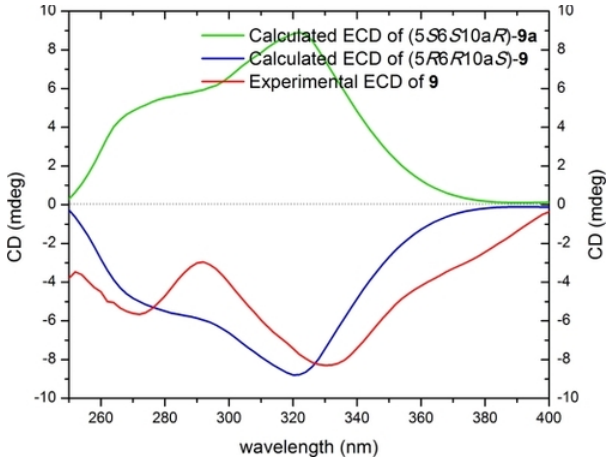


Figure 8. Experimentally measured and calculated ECD spectra for blennolide I (**9**, 0.06 mM, $CHCl_3$, cell length 2 cm).

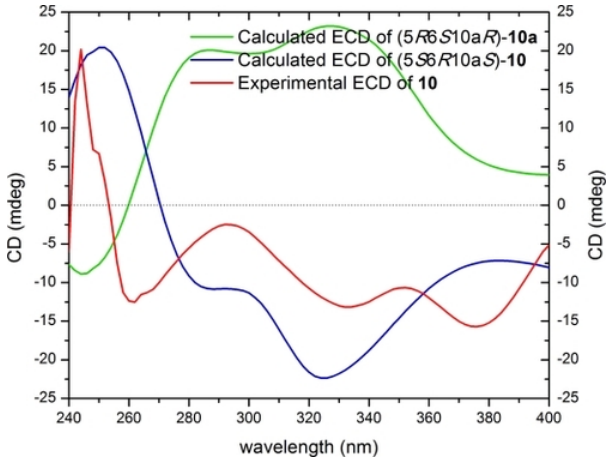


Figure 9. Experimentally measured and calculated ECD spectra for blennolide J (**10**, 0.17 mM, $CHCl_3$, cell length 2 cm).

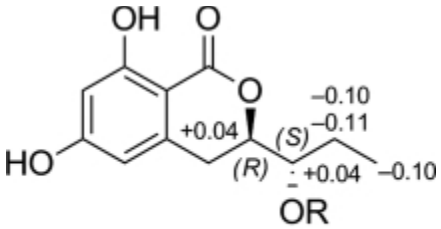
Compound **10** (0.6 mg), isolated as a yellow gum, had a molecular formula of $C_{32}H_{30}O_{14}$ as deduced from HRMS (ESI) and ^{1}H, ^{13}C, and edited-HSQC NMR spectroscopic data (Figure S20 in the Supporting Information), indicating an index of hydrogen deficiency of 18. Analysis of the NMR spectroscopic data indicated that **10** was an asymmetric heterodimer with structural similarity to **9**. Key differences between **9** and **10** were chemical shift values and splitting patterns of 5-H, 6-H, and H_2-7 (Table 4, Figures S17 and S20 in the Supporting Information). A proton singlet in **9** (δ_H = 4.12 ppm), corresponding to 5-H, was replaced by a doublet of doublets (δ_H = 3.93 ppm, dd, J = 11.2, 2.7 Hz) in **10**, suggesting a pseudodiaxial *trans* orientation of 5-H/6-H in **10**. These data indicated that the secalonic acid moiety in **10**was similar to the monomeric units of **1**, whereas the 11-deoxyblennolide D moiety was preserved, as in **9**. A 2,6′-linkage in **10**, also similar to **9**, was confirmed by diagnostic HMBC correlations of 3-H with C-6′ and 7′-H with C-2, establishing the planar structure of **10** (Figure 4). As in the case of **9**, the absolute configuration of **10** was deduced on the basis of the monomeric constituents and ECD calculations to be (5*S*,6*R*,10a*S*,2′*S*,9′*S*,10′*S*) (Figure 9, Figures S18 and S21 in the Supporting Information). The trivial name blennolide J was ascribed to compound **10**.

In addition to compounds **1**–**10**, the biogenetically related benzophenone nidulalin B (**15**, 1.7 mg) was isolated and identified by comparison of its spectroscopic and spectrometric data with those reported in the literature (Figure S26 in the Supporting Information).[20]

Resorcylic Acid Lactones

Compounds **11**–**13** were identified as the resorcylic acid lactones aigialomycin A (4.2 mg),[21] hypothemycin (19.6 mg),[22] and dihydrohypothemycin (3.7 mg),[21] respectively (Figures S22–S24 in the Supporting Information), whereas compound **14** was the biogenetically related pyrenochaetic acid C (2.7 mg, Figure S25 in the Supporting Information).[23] In all cases, the spectroscopic and spectrometric data were in agreement with those reported in the literature.

Compound **16** (0.8 mg) was isolated as a colorless oil with a molecular formula of $C_{12}H_{14}O_5$as revealed by HRMS (ESI) and ^{1}H, ^{13}C, and edited-HSQC NMR spectroscopic data (Figure S27 in the Supporting Information), indicating an index of hydrogen deficiency of six. UV absorption maxima of 301, 270, and 233 nm were indicative of an aromatic carbonyl compound.[24] Inspection of the NMR spectroscopic data showed signals characteristic of six aromatic carbons, two of which were oxygenated, two doublet aromatic protons with coupling constant values of 2.3 Hz, and two phenolic protons (Table 5, Figure S27 in the Supporting Information). These data suggested a 1,2,3,5-tetrasubstituted benzene ring with two aromatic protons *meta* to each other (Figure S27 in the Supporting Information, Table 5). Benzene ring substituents were confirmed by HMBC correlations (Figure 4). COSY data identified a 1,2,3-trisubstituted pentane moiety (H_2-4/3-H/11-H/12-H/13-H) with C-3 and C-11 being oxygenated and C-4 attached to the benzene ring (Figure 4). HMBC correlations from H_2-4 to C-6 and C-10 confirmed C-5 as the attachment point of the aliphatic side chain with the aromatic ring. Chemical shift values (δ_H/δ_C = 4.43/81.1 ppm) for 3-H/C-3 indicated esterification of C-3 to form a six-membered ring with the ester carbonyl group (δ_C = 170.2 ppm) that was attached to the benzene ring at C-2. Further examination of the NMR spectroscopic data yielded the planar dihydroisocoumarin structure of **16**, which was ascribed the trivial name pyrenomycin. The absolute configuration of **16** was assigned by a modified Mosher's ester method,[25] establishing the configuration as 3*R* and 11*S* (Figure 10). Similar dihydroisocoumarin compounds have been isolated previously from marine sponges, plants, fungi, and insects; they include hiburipyranone,[26] 3-(2-hydroxypropyl)-8-hydroxy-3,4-dihydroisocoumarin,[27] and (3*S*,1′*R*)-3-(1-hydroxyethyl)-6,8-dihydroxy-7-methyl-3,4-dihydroisocoumarin.[28]



16a: R = (*S*)-MTPA
16b: R = (*R*)-MTPA

Figure 10. $\Delta\delta_H$ values [$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$] obtained for (*S*)- and (*R*)-MTPA esters (**16a** and **16b**, respectively) of pyrenomycin (**16**) in [D_5]pyridine.

Table 5. NMR spectroscopic data for **16** (700 MHz for ^{1}H, 175 MHz for ^{13}C; chemical shifts in δ, coupling constants in Hz, CD_3OD).

Position	δ_C	δ_H mult. (*J* in Hz)
1	170.2	
3	81.1	4.43, dt (12.7, 3.3)
4	29.0	2.75, dd (16.4, 3.3)
		3.11, dd (16.4, 12.7)
5	142.2	
6	107.2	6.21, br. d (2.3)
7	164.2	
8	100.9	6.15, d (2.3)
9	168.8	
10	99.6	
11	73.1	3.58, dt (8.6, 3.3)
12	25.1	1.67, m
13	9.2	1.03, t (7.5)
9-OH		8.55, s
11-OH		1.28, br. s
7-OH		4.57, br. s

Cytotoxicities and Antimicrobial Evaluations of Isolated Compounds

Compounds **1**–**15** were tested for cytotoxicity against the MDA-MB-435 and SW-620 cancer cell lines. Of the tetrahydroxanthone derivatives, **1** and **4** were the most potent, with IC_{50}values less than 0.50 μM in both cell lines (Table 6). From the cytotoxicity data for related analogues, the importance of the configuration of C-5/C-5′ became evident (Table 6). Compounds **1** and **4**, each with (5*S*,5′*S*) configuration, and with a 2,2′- and a 2,4′-linkage for **1**and **4**, respectively, were the most potent. A (5*S*,5′*R*) configuration, as in **3**, resulted in a compound approximately 20 times less active than **1**, whereas a (5*R*,5′*R*) configuration rendered **2** inactive; both had a 2,2′-linkage of the monomeric units. For those with a 2,4′-linkage, compound **5**, with (5*R*,5′*R*) configuration, was approximately 30 times less active than **4**. Heterodimeric **10**, composed of the monomeric units of **1** and compound **7**, was approximately 25 times less active than **1**. Compound **9**, composed of the monomeric units of **2** and compound **7**, was inactive. The difference in the potencies of **10** and **9** further supported the importance of the (5*S*,5′*S*) configuration, and all three monomeric compounds (**6**–**8**) were inactive. Consistent with the cytotoxicity data obtained in this study, **1** and its chemically rearranged 2,4′-dimer **4** were reported to have equipotent activity against cultured mouse leukemia L1210 cells.[2d] Moreover, **1** has been reported to have a protecting effect for the dopaminergic neurons from cell death induced by 1-methyl-4-phenylpyridinium (MPP+) salts[29] and to attenuate colchicine-induced apoptosis of the cortical neurons.[30]

In agreement with the literature,[5] cytotoxicity data for the structurally related RALs **11**–**13** indicated the importance of the (*Z*)-enone for activity, as in hypothemycin (**12**, IC_{50} value of 0.58 μM, MDA-MB-435). Alternatively, aigialomycin A (**11**), with an (*E*)-enone, and dihydrohypothemycin (**13**), with a reduced enone, were both inactive (IC_{50} value > 10 μM). RALs containing (*Z*)-enone systems have been reported as potent inhibitors of several ATPases and kinases, including TAK1.[31]

Table 6. Cytotoxicities of compounds **1**–**15** against two human tumor cell lines.

Compound[a]	IC_{50} values in μM[b]	
	MDA-MB-435[c]	SW-620[c]
1	0.16	0.41
2	n.a.	19.12
3	3.27	3.67
4	0.18	0.21
5	5.20	5.55
10	4.06	6.14
12	0.58	2.14

[a] Compounds **6**–**9**, **11**, and **13**–**15** were inactive, with IC_{50} values > 20 μM.
[b] IC_{50} values were determined as the concentrations required to inhibit growth to 50 % of control with 72 h incubation.
[c] Positive control was vinblastine, tested at concentrations of 1 nM in MDA-MB-435 cells and 10 nM in SW620 cells, which had 23 % and 76 % viable cells, respectively.

The antimicrobial activities of the isolated compounds **1**–**15** were evaluated against a panel of bacteria and fungi (Table 7 and Figure S5 in the Supporting Information). Compounds **3** and **5** showed promising activity against the Gram-positive bacterium *Micrococcus luteus*, with MIC values of 5 and 15 μg mL^{-1}, respectively. Secalonic acid A (**1**) was reported to have activity against *Bacillus subtilis* and *Piriculaia oryzae*[2b] and a phlogistic activity,[32] whereas penicillixanthone A (**4**) was reported to have medium antibacterial activity against *M. luteus*, *Pseudoalteromonas nigrifaciens*, and *B. subtilis*.[33] Interestingly, compounds **3** and **5**, which were both inactive in cytotoxicity assays, were the most promising in the antimicrobial assays. This finding demonstrates the importance of testing isolated natural compounds in a variety of assays.

Table 7. Antimicrobial activities of compounds **1**–**15**.

Compound[a]	Antimicrobial activity MIC [μg mL^{-1}]	
	M. luteus	***S. aureus***
1	38	75
2	36	n.a.[b]
3	5	39
4	46	93
5	15	59
10	43	43
vancomycin[c]	–	0.25

[a] See Table S5 in the Supporting Information for compounds **6**–**9**, **11**, and **12**–**15**.
[b] n.a.: not active, with MIC > 145 μg mL^{-1}.
[c] Positive control.

Conclusions

A total of 16 polyketides **1**–**16**, of which six were new, were isolated from a fungal culture of *S. terrestris* (MSX45109). Cytotoxicity assays suggested compounds **1**, **4**, and **12** as the most potent. When evaluated for antimicrobial activity, compounds **3** and **5** showed promising *M. luteus* activity. This fungus was a prolific producer of polyketides, affording the opportunity to examine many analogues in a series of bioassays concomitantly. Moreover, because a few of the

known compounds were first described decades ago, this study also served to update the literature with spectroscopic and spectrometric data obtained with modern instruments.

Experimental Section

General Procedures: UV and ECD spectra were obtained with a Varian Cary 100 Bio UV/Vis spectrophotometer (Varian Inc., Walnut Creek, CA, USA) and an Olis DSM 17 ECD spectrophotometer (Olis, Inc. Bogart, GA, USA), respectively. NMR spectroscopic data were collected variously with a JEOL ECA-500 NMR spectrometer, operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C, a JEOL ECS-400 NMR spectrometer, operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C and equipped with a high-sensitivity JEOL Royal probe and a 24-slot autosampler (both from JEOL Ltd., Tokyo, Japan), or an Agilent 700 MHz NMR spectrometer (Agilent Technologies, Inc., Santa Clara, CA, USA), equipped with a cryoprope, operating at 700 MHz for ^{1}H and 175 MHz for ^{13}C. Residual solvent signals were utilized for referencing. High-resolution mass spectra (HRMS) were obtained with a Thermo LTQ Orbitrap XL mass spectrometer equipped with an electrospray ionization source (Thermo Fisher Scientific, San Jose, CA, USA). A Waters Acquity UPLC system (Waters Corp., Milford, MA, USA) utilizing a Waters BEH C_{18} column (1.7 µm; 50 × 2.1 mm) was used to check the purities of the isolated compounds, with data collected and analyzed with the aid of Empower software. Phenomenex Gemini-NX C_{18} analytical (5 µm, 250 × 4.6 mm), preparative (5 µm, 250 × 21.2 mm), and semipreparative (5 µm, 250 × 10.0 mm) columns (all from Phenomenex, Torrance, CA, USA) were used with a Varian Prostar HPLC system equipped with ProStar 210 pumps and a Prostar 335 photodiode array detector (PDA), with data collected and analyzed with the aid of Galaxie Chromatography Workstation software (version 1.9.3.2, Varian Inc.). Flash chromatography was performed with a Teledyne ISCO CombiFlash R_f 200 and Silica Gold columns (both from Teledyne Isco, Lincoln, NE, USA) and monitored by UV and evaporative light-scattering detectors.

Fungal Strain Isolation and Identification: Mycosynthetix fungal strain MSX45109 was isolated from leaf litter collected in a mangrove habitat in 1989. Molecular techniques were used to identify MSX45109 by sequencing the internal transcribed spacer (ITS) regions 1 & 2 and 5.8S nrDNA.[34] DNA extraction, PCR amplification, sequencing, and phylogenetic analyses were performed as described previously.[1b,35] BLAST search in GenBank and the curated BOLD systems (http://www.boldsystems.org/index.php/IDS_OpenIdEngine) database, as well as Q-bank through the use of ITS rDNA sequences suggested that MSX45109 shared high sequence similarity with several CBS (Centraalbureau voor Schimmelcultures) strains of *Setophoma terrestris* (Pleosporales, Ascomycota) with ≥80 % query coverage and 99–100 % sequence identity. The top BLAST matches were downloaded with Seaview v4.4.2., and Maximum Likelihood analysis was performed by methods outlined earlier.[36] From the results of the BLAST search and Maximum Likelihood phylogeny of the ITS region (Figure S28 in the Supporting Information), the strain MSX45109 was identified as *Setophoma terrestris* (H. N. Hansen) Gorenz J. C. Currently, three species of *Setophoma* have been described, with *S. terrestris* as the type species.[37] The ITS sequences of four isolates of MSX45109 were deposited in the GenBank (accession numbers KM203887, KM203888, KM203889, and KM203890).

Fermentation, Extraction, and Isolation: Storage, fermentation, and extraction procedures for fungal strain MSX45109 were as described previously.[1b,1d,1h] In brief, a seed culture of strain MSX45109 grown in YESD medium was used to inoculate a Fernbach flask (2.8 L, Corning, Inc., Corning, NY, USA) containing rice (150 g) and H_2O (300 mL). The solid culture was incubated at room temp. for 14 days and then extracted by addition of a MeOH/$CHCl_3$ mixture (1:1, 500 mL). The culture was chopped into small pieces and left to shake at 125 rpm at room temp., followed by vacuum filtration. The solid phase was washed with MeOH/$CHCl_3$ (1:1, 100 mL). $CHCl_3$ (900 mL) and H_2O (1500 mL) were added to the filtrate so that the final $CHCl_3$/MeOH/H_2O ratio was 4:1:5. The mixture was stirred for 0.5 h and then transferred into a separatory funnel. The organic bottom layer was drawn off, and the solvents were evaporated to dryness and then reconstituted in MeOH/CH_3CN (1:1, 100 mL) and hexanes (100 mL). The biphasic solution was stirred for 15 min and then transferred to a separatory funnel. The MeOH/CH_3CN layer was drawn off and the solvents were evaporated to dryness under vacuum. The defatted material (1.5 g) was dissolved in a mixture of $CHCl_3$/MeOH, adsorbed onto Celite 545, and fractionated by flash chromatography with use of a gradient solvent system of hexane/$CHCl_3$/MeOH at a 40 mL min^{-1} flow rate and 53.3 column volumes over 63.9 min to afford five fractions. Fraction 3 (627 mg) was subjected to preparative reversed-phase HPLC over a Phenomenex Gemini-NX C_{18} preparative column with use of a gradient system of 40:60 to 60:40 CH_3CN/H_2O (acidified with 0.1 % formic acid) over 30 min at a flow rate of 21.24 mL min^{-1} to yield thirteen subfractions. Subfractions 4, 5, 10, and 12 yielded compounds **11** (4.2 mg), **12** (19.6 mg), **1**(35.1), and **2** (48.4 mg), which eluted at 7.8, 8.7, 25.1, and 29.0 min, respectively.

The other subfractions were subjected to further purifications as follows:

Subfraction 1 (2.3 mg) was subjected to semipreparative HPLC purification over a Phenomenex Gemini-NX C_{18} column with use of a gradient system of 50:50 to 70:30 MeOH/H_2O (0.1 % formic acid) over 15 min at a flow rate of 4.72 mL min^{-1} to yield compound **16** (0.8 mg), which eluted at 13.1 min.

Subfraction 6 (8.5 mg) was subjected to preparative HPLC purification (Phenomenex Gemini-NX C_{18}) with a gradient system of 50:50 to 70:30 MeOH/H_2O (0.1 % formic acid) over 15 min at a flow rate of 21.24 mL min^{-1} to yield compounds **15** (1.7 mg) and **13** (3.7 mg), which eluted at 8.0 and 18.9 min, respectively.

Subfraction 7 (1.5 mg) was subjected to semipreparative HPLC purification over a Phenomenex Gemini-NX C_{18} column with use of a gradient system of 50:50 to 70:30 MeOH/H_2O (0.1 % formic acid) over 15 min at a flow rate of 4.72 mL min^{-1} to yield compound **6** (0.8 mg), which eluted at 17.5 min.

Subfraction 9 (18.2 mg) was subjected to preparative HPLC purification (Phenomenex Gemini-NX C_{18}) with a gradient system of 50:50 to 70:30 MeOH/H_2O (0.1 % formic acid) over 15 min at a flow rate of 21.24 mL min^{-1} to yield compounds **7** (5.4 mg), **8** (1.0 mg), and **14** (2.7 mg), which eluted at 11.6, 13.5, and 19.5 min, respectively.

An aliquot of subfraction 10 (20 mg) was further purified by preparative HPLC (Phenomenex Gemini-NX C_{18}) with use of a gradient solvent system of 70:30 to 90:10 MeOH/H_2O (0.1 % formic acid) over 15 min at a flow rate of 21.24 mL min^{-1} to yield compounds **10** (0.6 mg) and **1** (3.1 mg), which eluted at 9.5 and 14.8 min, respectively.

An aliquot of subfraction 11 (20 mg) was subjected to semi-preparative HPLC purification over a Phenomenex Gemini-NX C_{18} column with use of a gradient system of 70:30 to 90:10 MeOH/H_2O (0.1 % formic acid) over 15 min at a flow rate of 4.72 mL min^{-1} to yield compound **3** (9.5 mg), which eluted at 12.3 min, and compound **9** (1.1 mg), which was obtained by yet another round of similar purification.

Subfraction 13 (21.5 mg) was subjected to preparative HPLC purification (Phenomenex Gemini-NX C_{18}) with a gradient system of 70:30 to 90:10 MeOH/H_2O (0.1 % formic acid) over 15 min at a flow rate of 21.24 mL min^{-1} to yield compounds **2** (2.9 mg) and **4** (5.4 mg), which eluted at 10.5 and 19.9 min, respectively, and compound **5** (2.1 mg), which was obtained by another round of HPLC purification.

Penicillixanthone B (5): Yellow powder. $[\alpha]_D^{20} = -112$ ($c = 0.03$ in acetone). CD ($c = 6.26 \times 10^{-5}$ M, $CHCl_3$): λ ($\Delta\epsilon$) = 225 (–55.3) nm, 243 (+48.4) nm, 332 (–18.9) nm, 347 (+2.6) nm, 376 (–8.8) nm. ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz): see Table 1 and Figure S9 in the Supporting Information. UV (MeOH): λ_{max} [log (ϵ/M^{-1} cm^{-1})] = 349 (3.64), 336 (3.63), 250 (3.58) nm. HRMS (ESI) calcd. for $C_{32}H_{31}O_{14}$ 639.1708 $[M + H]^+$; found 639.1691.

Blennolide H (6): Yellow gum. $[\alpha]_D^{20} = -25.3$ ($c = 0.01$ in chloroform). CD ($c = 4.27 \times 10^{-4}$ M, MeOH): λ ($\Delta\epsilon$) = 231 (+11.9) nm, 269 (–20.9) nm, 298 (–19.8) nm. ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 175 MHz): see Table 2 and Figure S11 in the Supporting Information. UV (MeOH): λ_{max} [log (ϵ/M^{-1} cm^{-1})] = 283 (3.48), 193 (3.68) nm. HRMS (ESI) calcd. for $C_{16}H_{17}O_6$305.1020 $[M + H]^+$; found 305.1012.

11-Deoxyblennolide D (7): Colorless oil. $[\alpha]_D^{20} = -26.14$ ($c = 0.03$ in chloroform). CD ($c = 1.56 \times 10^{-4}$ M, $CHCl_3$): λ ($\Delta\epsilon$) = 235 (+6.2) nm, 267 (–53.8) nm, 307 (–16.4) nm; 346 (+0.5) nm. ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz): see Table 3 and Figure S13 in the Supporting Information. UV (MeOH): λ_{max} [log (ϵ/M^{-1} cm^{-1})] = 349 (3.21), 270 (3.45), 224 (3.37) nm. HRMS (ESI) calcd. for $C_{16}H_{17}O_7$ 321.0969 $[M + H]^+$; found 321.0963.

Paecilin B (8): Colorless oil. $[\alpha]_D^{20} = +42.05$ ($c = 0.09$ in chloroform). CD ($c = 5.62 \times 10^{-4}$ M, $CHCl_3$): λ ($\Delta\epsilon$) = 235 (–44.7) nm, 267 (+35.2) nm, 307 (+17.0) nm; 346 (–8.7) nm. ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz): see Table 3 and Figure S14 in the Supporting Information. UV (MeOH): λ_{max} [log (ϵ/M^{-1} cm^{-1})] = 346 (3.20), 271 (3.49), 193 (3.70) nm. HRMS (ESI) calcd. for $C_{16}H_{17}O_7$ 321.0969 $[M + H]^+$; found 321.0961.

Blennolide I (9): Yellow powder. $[\alpha]_D^{20} = -102.30$ ($c = 0.09$ in chloroform). CD ($c = 6.26 \times 10^{-5}$M, $CHCl_3$): λ ($\Delta\epsilon$) = 241 (+13.9), 275 (–6.3) nm, 334 (–8.7) nm. ^{1}H NMR ($CDCl_3$, 700 MHz) and ^{13}C NMR ($CDCl_3$, 175 MHz): see Table 4 and Figure S17 in the Supporting Information. UV (MeOH): λ_{max} [log (ϵ/M^{-1} cm^{-1})] = 349 (2.81), 258 (3.69), 224 (3.69) nm. HRMS (ESI) calcd. for $C_{32}H_{31}O_{14}$ 639.1708 $[M + H]^+$; found 639.1682.

Blennolide J (10): Yellow powder. $[\alpha]_D^{20} = -93.5$ ($c = 0.08$ in chloroform). CD ($c = 1.72 \times 10^{-4}$M, $CHCl_3$): λ ($\Delta\epsilon$) = 245 (+38.9), 262 (–14.9) nm, 337 (–13.9) nm, 377 (–16.7) nm. ^{1}H NMR ($CDCl_3$, 700 MHz) and ^{13}C NMR ($CDCl_3$, 175 MHz): see Table 4 and Figure S20 in the Supporting Information. UV (MeOH): λ_{max} [log (ϵ/M^{-1} cm^{-1})] = 349 (3.37), 336 (3.37), 237 (3.4) nm. HRMS (ESI) calcd. for $C_{32}H_{31}O_{14}$ 639.1708 $[M + H]^+$; found 639.1683.

Pyrenomycin (16): Colorless oil. $[\alpha]_D^{20} = -5.7$ ($c = 0.05$ in chloroform). ^{1}H NMR (CD_3OD, 700 MHz) and ^{13}C NMR (CD_3OD, 175 MHz): see Table 5 and Figure S27 in the Supporting Information. UV (MeOH): λ_{max} [log (ϵ/M^{-1} cm^{-1})] = 301 (3.09), 270 (3.12), 233 (3.07) nm. HRMS (ESI) calcd. for $C_{12}H_{16}O_5$ 239.0914 $[M + H]^+$; found 239.0909.

Preparation of the (*R*)- And (*S*)-MTPA Ester Derivatives of Pyrenomycin (16): [D_5]Pyridine (400 µL) was added to compound **16** (0.1 mg), and the solution was transferred into an NMR tube. To initiate the reaction, *S*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride (20 µL) was added with careful shaking and then monitored immediately by ^{1}H NMR at the following time points: 0, 5, 10, 15, 30, 60, and 120 min. The reaction was found to be complete after 2 h, yielding the mono (*R*)-MTPA ester derivative **16b** of **16**. ^{1}H NMR spectroscopic data for **16b** (500 MHz, [D_5]pyridine): δ_H = 1.85 (d, J = 7.5 Hz, 3 H, H_3-13), 1.81 (m, 1 H, 12a-H), 1.90 (m, 1 H, 12b-H), 4.81 (m, 1 H, 11-H), 5.61 (m, 1 H, 3-H) ppm. In an analogous manner, compound **16** (0.1 mg), dissolved in [D_5]pyridine (400 µL), was treated in a second NMR tube with (*R*)-(–)-α-MTPA chloride (20 µL) for 1 h to afford the mono (*S*)-MTPA ester **16a**. ^{1}H NMR spectroscopic data for **16a** (500 MHz, [D_5]pyridine): δ_H = 1.75 (d, J = 6.9 Hz, 3 H, H_3-13), 1.70 (m, 1 H, 12a-H), 1.80 (m, 1 H, 12b-H), 4.85 (m, 1 H, 11-H), 5.65 (m, 1 H, 3-H) ppm.

Molecular Modeling Calculations: Theoretical calculations of ECD spectra for compounds **4**, **5**, and **7**–**10**, and their corresponding enantiomers or epimers, were performed with the Gaussian 09 (Gaussian Inc., Wallingford CT, USA) program package as described previously[13e] (for details see the Supporting Information).[38]

Cytotoxicity Assay: The cytotoxicities of compounds **1**–**15** were tested against the MDA-MB-435[39] human melanoma (HTB-129, ATCC) and the SW-620[40] human colorectal adenocarcinoma (CCL-227, ATCC) cell lines as described previously.[41]

Antimicrobial Assay: Minimal inhibitory concentrations (MICs) of compounds **1**–**15** were measured against a panel of bacteria and fungi as described previously.[41a] All measurements were made in duplicate.

Supporting Information: Molecular modeling calculations, UPLC chromatograms of compounds **1**–**16**, ^{1}H and ^{13}C NMR spectra for compounds **1**–**16**, key COSY, HMBC, and NOESY correlations of **4**, comparison of the experimentally measured ECD spectra of penicillixanthone A (**4**) and penicillixanthone B (**5**), experimentally measured and calculated ECD spectra for blennolide H (**6**), comparison of the experimentally measured ECD spectra of blennolide I (**9**) and blennolide J (**10**), DFT B3LYP/DGDZVP global minimum energy models, relative Gibbs free energies (ΔG_{rel}) and equilibrium population (p) values for the most relevant conformations of compounds **4**, **5**, and **7**–**10**, and their corresponding enantiomers **4a**, **5a**,

and **7a**–**10a**, phylogram of the most likely tree; ECD and optical rotation data, experimentally measured versus literature, for compounds **1**–**4**, **6**, **8**–**9**, and blennolide G, ^{1}H NMR spectroscopic data for **1**–**3**, NMR spectroscopic data for **4**, and NMR spectroscopic data for **8** and paecilin B.

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