Stereochemical Assignment of the Fungal Metabolites
Pestalotiopsones D and E through Enantiopure Synthesis

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Supporting Information

ABSTRACT: The pestalotiopsones are fungal metabolites isolated from an endophytic fungus *Pestalotiopsis* sp. found in the mangrove *Rhizophora mucronata*, used in traditional Chinese medicine to treat symptoms of dysentery. The absolute configurations of pestalotiopsones D (4) and E (5) were elucidated through total synthesis of both the *R* and *S* enantiomers, allowing for the assignment of the stereochemistry of the natural compounds as the (+)-*S* enantiomers. The key steps include homologation of a substituted benzoic acid to the appropriate phenylacetate derivative using Birch reductive alkylation, an oxa-Michael cyclization induced by microwave irradiation to form the chromanone substructure, and an IBX-mediated dehydrogenation yielding the chromone skeleton. Assessment of the synthetic compounds against clinical pathogens was performed.

In the search for new pharmaceuticals ethnopharmacology is a resource that has overwhelming potential. With a large array of prospective structures for medicinal testing the information provided by ethnopharmacology gives research a significant head start. The compounds isolated from traditional medicines often display the traits of proven efficacy and low toxicity, two significant hurdles that face a new drug candidate.

The role that mushrooms play in ethnopharmacology is well documented in traditional Chinese and Ayurvedic medicine,¹ and the biodiversity of fungi has resulted in a plethora of bioactive molecules with pharmaceutical potential. Additionally, studies have suggested that the activity attributed to some traditional medicinal plants is in fact due to endophytic fungi.²

In 2009, Proksch and co-workers isolated a number of chromone derivatives from an endophytic fungal species of the genus *Pestalotiopsis* cultured from the mangrove *Rhizophora mucronata*, used as a traditional medicinal to treat symptoms of dysentery, which they named pestalotiopsones A–F (1–6) (Figure 1).³ Chromones have been shown to possess a wide range of bioactivity,⁴ and the pestalotiopsones have been shown to exhibit cytotoxic and antibiotic activity.^{3,5} While pestalotiopsones D (4) and E (5) were isolated as optically active compounds ($[\alpha]_D^{25} +16$ and $+37$, respectively), the absolute configuration of the compounds was not determined.

The syntheses of pestalotiopsones A, B, C, and F were reported by us recently.⁵ We report here the first synthesis of pestalotiopsones D and E as well as *ent*-D and *ent*-E, leading to the determination of the absolute configuration of the natural products.

RESULTS AND DISCUSSION

Pestalotiopsones D (4) and E (5) differ from pestalotiopsones A (1) and B (2) by the existence of a hydroxy group at position

Figure 1. Pestalotiopsones A–F (1–6) as reported by Proksch and co-workers.³

6". Therefore, it was envisaged that 4 and 5 could be synthesized in an analogous manner to the other pestalotiopsones (Scheme 1).⁵ The chromones could be afforded through dehydrogenation from the appropriately protected chromanone, which itself could be obtained by a microwave-mediated condensation of hydroxyacetophenone (7) and the necessary enantiopure aldehyde.

Utilizing this synthetic route required the use of an appropriate protecting group for the 6" hydroxy group. Our previous syntheses had shown that an ester moiety could

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Scheme 1. Retrosynthesis of Pestalotiopsone D (4)

withstand the microwave conditions as well as chromanone oxidation, and so the acetate protecting group was chosen for this task. As such, it was envisaged that aldehyde 8 could be afforded from the ring-opening of enantiopure propylene oxide by the Grignard reagent prepared from 6-bromohex-1-ene, followed by acetate protection and subsequent ozonolysis.

Synthetic work began with the preparation of alcohol (S)-9 from enantiopure (S)-propylene oxide (Scheme 2), with yields

Scheme 2. Synthesis of Aldehyde (S)-8

of the desired alcohol significantly increased by addition of cuprous iodide to the preformed Grignard reagent. Protection using pyridine and acetic anhydride yielded acetoxynonene (S)-10 in excellent yield. Conversion to the aldehyde (S)-8 was achieved using ozonolysis conditions with reduction of the secondary ozonide being achieved with triphenylphosphine.

With aldehyde (S)-8 in hand the one-pot microwave-mediated aldol condensation, dehydration, and oxa-Michael cyclization of the resulting enone could be undertaken (Scheme 3). Addition of acetophenone 7, synthesized as described by us previously,⁵ to aldehyde (S)-8 and diisopropylamine in methanol heated to 140 °C for 60 min yielded chromanone (6''S)-11 in good yield. Despite (6''S)-11 being a diastereomeric mixture, both the ¹H and ¹³C NMR data presented as a single compound. Dehydrogenation to chromone (S)-12 was achieved with IBX,⁶ and subsequent deprotection with aluminum chloride in refluxing toluene gave (S)-4 in excellent yield.

The spectroscopic data of (S)-4 corresponded closely to that of the natural 4,³ while the specific rotation, $[\alpha]_D^{20} +12.6$ (c

0.93, MeOH), was identical in sign and of comparable magnitude to that of natural 4 isolated by Proksch et al., $[\alpha]_D^{20} +16$ (c 0.02, MeOH). Additionally, the enantiomer (R)-4 synthesized from (R)-propylene oxide using the same synthetic procedure returned a specific rotation of $[\alpha]_D^{20} -10.8$ (c 0.81, MeOH), opposite in sign of that of the natural 4. Synthesis of (S)-4 and (R)-4 has led to the determination of the absolute configuration of pestalotiopsone D as S.

Subsequently, both (S)-4 and (R)-4 were subjected to transesterification conditions with sulfuric acid in ethanol to yield (S)-5 and (R)-5 in excellent yield (Scheme 3). The spectroscopic data matched those of the natural 5, while the specific rotation of (S)-5, $[\alpha]_D^{20} +14.1$ (c 0.73, MeOH), was again identical in sign to that of natural 5, $[\alpha]_D^{20} +37$ (c 0.02, MeOH), leading to the assignment of the absolute configuration of pestalotiopsone E as S also. The difference in magnitude in the specific rotation values between the synthetic (S)-5 and the natural 5 may be attributed to the small amount of product isolated from the natural source (0.26 mg).³

The synthetic pestalotiopsones ((S)-4, (S)-5) as well as their enantiomers ((R)-4, (R)-5), along with a number of synthetic intermediates ((6''S)-11, (6''R)-11, (S)-12, (R)-12), were tested against clinical strains of *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Mycobacterium smegmatis*. While active, the MIC values for the evaluated compounds against these strains were all greater than 500 µg/mL. The IC₅₀ values for the natural enantiomers and their precursors are displayed in Table 1. While both enantiomers were tested, there was not a significant difference in activity between them, and as such only the IC₅₀ values for the natural isomers are presented. Kanamycin was used in the assay as a positive control and for comparison. Pestalotiopsones D (4) and E (5) show comparable activity when compared to pestalotiopsones A, B, C, and F (1–3, 6),⁵ but possess weak activity when compared to the clinical antibiotic kanamycin.

In conclusion, the first syntheses of pestalotiopsones D and E were achieved in seven and eight linear steps, respectively, from commercially available 3,5-dimethoxybenzoic acid, in addition to *ent*-pestalotiopsone D and *ent*-E, allowing for the structural elucidation of natural pestalotiopsones D and E as the S enantiomers. Biological testing of the synthetic compounds showed they possessed weak antimicrobial activity against a panel of human pathogens.

EXPERIMENTAL SECTION

General Experimental Procedures. Reagents were purchased from commercial suppliers and used without further purification unless otherwise stated. All solvents were dried and distilled immediately prior to use or stored as appropriate. Etheral solvents Et₂O and THF were refluxed over sodium and benzophenone. Toluene was refluxed over sodium, CH₂Cl₂ was refluxed with and distilled from CaH₂, and acetone was refluxed with and distilled from Na₂SO₄. Petroleum ether used is the 60–80 °C fraction. Optical data were measured on a Perkin-Elmer 341 polarimeter. Concentrations are expressed as g/100 mL, measured at the sodium D-line (589 nm) at 20 °C in the solvent indicated. UV spectra were measured on a Cary 4G UV–visible spectrophotometer using a 1 cm solution cell in the solvent indicated. Spectra maxima are recorded in nm (log ε). IR spectra were recorded on a Bruker Alpha-P ATR spectrophotometer as neat solid or film. Characteristic peaks are recorded in wavenumbers. ¹H and ¹³C NMR spectra were recorded at 298 K, at 300 MHz or 400 MHz and 75, 100 or 125 MHz, on an Inova 300, Varian Mercury 300, Varian MR-400, or Inova 500 instrument. Chemical shifts are reported in ppm (δ). ¹H NMR spectra are referenced to the resonance from residual CHCl₃ at 7.26 ppm or the central peak in the resonance from residual

Scheme 3. Synthesis of (S)-Pestalotiopsone D (4) and (S)-Pestalotiopsone E (5)

Table 1. Antibiotic Activity of (S)-4, (S)-5, (6''S)-11, and (S)-12 Showing 50% Inhibition of Selected Strains

strain ^a	IC ₅₀ (μg/mL)				
	kan ^b	(S)-4	(S)-5	(6''S)-11	(S)-12
<i>S. epidermidis</i>	90	>500	>500	>500	>500
<i>E. coli</i>	<1	>500	>500	>500	>500
<i>P. aeruginosa</i>	209	>500	440	445	465
<i>M. smegmatis</i>	<1	>500	484	492	>500

^aClinical strains obtained from The Canberra Hospital. ^bKanamycin.

CHD₂COCD₃ at 2.05 ppm. ¹³C NMR spectra are referenced to the central peak in the signal from CDCl₃ at 77.0 ppm or the central peak in the resonance from (CD₃)₂CO at 29.8 ppm. The appearance and multiplicities of ¹H resonances are expressed by the abbreviations s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet) and combinations thereof for more highly coupled systems. ¹³C NMR experiments were run as proton-decoupled spectra. ¹H signals and ¹³C signals where appropriate are described by chemical shift δ (integration, multiplicity, |J| (Hz), assignment). When ambiguous, assignments were made based on appropriate 2D NMR data, including HSQC, HMBC, and NOESY. EIMS and HREIMS were recorded on a VG autospec mass spectrometer or Waters Autospec Premier mass spectrometer, operating at 70 eV. ESIMS and HRESIMS were recorded on a Bruker Apex 3. Positive ionization was detected unless otherwise indicated. Mass/charge ratios (*m/z*) are reported and relative abundance of the ions as percentage of base peak intensity. Thin layer chromatography (TLC) was run on Merck silica gel 60 F₂₅₄ aluminum-backed plates and was run in the eluting system described for each plate. Flash chromatography was performed under pressure using silica gel (230–400 mesh Scharlau 60) as solid support and HPLC-grade solvents as eluent. Analytical HPLC was performed using an Agilent 1100 system, utilizing a Phenomenex LUX cellulose-4 5 μ m column and an Agilent 1100 series diode array detector for eluate detection. Elution was carried out using prefiltered (0.45 μ m nylon membrane filter) ethanol and *n*-hexane in the ratio indicated, at 0.5 mL/min with detection at 243 nm. Microwave reactions were carried out using an Explorer CEM Automated Microwave Workstation in 10 mL reaction vessels at the wattage (W) and pressure stated.

Methyl 2-(2-Acetyl-3-hydroxy-5-methoxyphenyl)acetate (7). To a solution of 3,5-dimethoxybenzoic acid (1.82 g, 10.0 mmol) in THF (20 mL) was condensed NH₃ (50 mL), and lithium (153 mg, 21.9 mmol) was added in portions at –33 °C. Methyl bromoacetate (1.14

mL, 1.84 g, 12.0 mmol) was added dropwise, causing the deep blue solution to become a pale yellow color. The NH₃ was evaporated under a stream of nitrogen, and the resulting solid residues were dissolved in water and Et₂O. The layers were separated, and the aqueous layer was cooled to 0 °C and acidified to pH 4 with careful addition of aqueous 1 M HCl. The acidic solution was then extracted with EtOAc, dried with MgSO₄, filtered, and concentrated *in vacuo* to give 3,5-dimethoxy-1-(2-methoxy-2-oxoethyl)cyclohexa-2,5-dienecarboxylic acid as a colorless solid (93%, 2.38 g, 9.30 mmol). IR (neat): $\nu_{\max}$ 2982, 2651, 1703, 1649, 1695 cm^{–1}; ¹H NMR (300 MHz, CDCl₃) δ 4.77 (2H, s, 2,6-H), 3.67 (3H, s, 2'-OCH₃), 3.58 (6H, s, 3,5-OCH₃), 2.7–2.9 (4H, m, 4-H_A, 4-H_B, 1'-H); ¹³C NMR (75 MHz, CDCl₃) δ 180.9 (COOH), 171.4 (2'-C), 154.2 (3,5-C), 93.9 (2,6-C), 54.5 (3,5-OCH₃), 51.6 (2'-OCH₃), 47.8 (1-C), 45.6 (1'-C), 31.0 (4-C); ESIMS 257 (100, [M + H]⁺); HRESIMS *m/z* 257.1024 [M + H]⁺ (calcd for C₁₂H₁₇O₆, 257.1025).

To a solution of 3,5-dimethoxy-1-(2-methoxy-2-oxoethyl)cyclohexa-2,5-dienecarboxylic acid (1.85 g, 7.20 mmol) in toluene (150 mL) was added lead tetraacetate (4.15 g, 9.40 mmol). After 40 min water was added and the mixture was filtered through a pad of silica. The organic phase was separated, washed with saturated aqueous NaHCO₃, dried with MgSO₄, filtered, and concentrated *in vacuo* to give an orange oil. Purification was achieved by flash column chromatography on silica with 33% EtOAc in petroleum ether as the eluent (*R_f* = 0.6) to yield methyl 2-(3,5-dimethoxyphenyl)acetate as a pale yellow oil (91%, 1.38 g, 6.55 mmol): IR (neat) $\nu_{\max}$ 1723, 1602 cm^{–1}; ¹H NMR (300 MHz, CDCl₃) δ 6.44 (2H, d, ⁴J = 2.3 Hz, 2',6'-H), 6.37 (1H, t, ⁴J = 2.3 Hz, 4'-H), 3.78 (6H, s, 3',5'-OCH₃), 3.69 (3H, s, 1-OCH₃), 3.56 (2H, s, 2-H); ¹³C NMR (75 MHz, CDCl₃) δ 171.8 (1-C), 160.8 (3',5'-C), 136.0 (1'-C), 107.3 (2',6'-C), 99.2 (4'-C), 55.3 (3',5'-OCH₃), 52.1 (1-OCH₃), 41.4 (2-C); EIMS 210 (100, [M]⁺), 151 (95), 121 (30), 91 (20); HREIMS *m/z* 210.0890 [M]⁺ (calcd for C₁₁H₁₄O₄, 210.0892).

To a solution of methyl 2-(3,5-dimethoxyphenyl)acetate (210 mg, 1.00 mmol) and acetic anhydride (0.950 mL, 1.02 g, 10.0 mmol) in acetic acid (20 mL) at 35 °C was added perchloric acid (70%, 1.44 mL, 10.0 mmol) dropwise. The solution was stirred for 5 min and then poured into ice water. Et₂O and saturated aqueous NaHCO₃ were added, and the organic layer was separated. The organic layer was washed with saturated aqueous NaHCO₃, dried with MgSO₄, filtered, and concentrated *in vacuo* to give a yellow oil. Purification was achieved by flash column chromatography on silica with 33% EtOAc in petroleum ether as eluent (*R_f* = 0.4), yielding methyl 2-(2-acetyl-3,5-dimethoxyphenyl)acetate as a pale yellow, amorphous solid (89%, 224 mg, 0.890 mmol): IR (neat) $\nu_{\max}$ 1745, 1686 cm^{–1}; ¹H NMR (300

MHz, CDCl₃) δ 6.40 (1H, d, 4J = 2.2 Hz, 6'-H), 6.34 (1H, d, 4J = 2.2 Hz, 4'-H), 3.82 (3H, s, 5'-OCH₃), 3.80 (3H, s, 3'-OCH₃), 3.68 (2H, s, 2-H), 3.66 (3H, s, 1-OCH₃), 2.49 (3H, s, C(O)CH₃); ¹³C NMR (75 MHz, CDCl₃) δ 203.7 (C(O)CH₃), 171.7 (1-C), 161.5 (5'-C), 159.4 (3'-C), 134.9 (1'-C), 123.6 (6'-C), 108.2 (2'-C), 97.4 (4'-C), 55.6 (5'-OCH₃), 55.3 (3'-OCH₃), 51.9 (1-OCH₃), 39.0 (2-C), 32.2 (C(O)CH₃); EIMS 252 (55, [M]⁺⁺), 237 (60), 221 (35), 209 (100), 192 (65), 178 (50); HREIMS m/z 252.0996 [M]⁺⁺ (calcd for C₁₃H₁₆O₅, 252.0998).

To a solution of methyl 2-(2-acetyl-3,5-dimethoxyphenyl)acetate (913 mg, 3.62 mmol) in CH₂Cl₂ (15 mL) at -78 °C under an atmosphere of argon was added boron trichloride (1 M *n*-hexane solution, 5.43 mL, 5.43 mmol) dropwise. The solution was allowed to slowly warm to room temperature and stirred for 16 h, at which time ice (5 g) was added. The solution was partitioned between water and CH₂Cl₂, and the organic layer separated, dried with MgSO₄, filtered, and concentrated under reduced pressure to give the title compound as a colorless solid (93%, 801 mg, 3.37 mmol): IR (neat) $\nu_{\max}$ 3268, 1701, 1598 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 13.06 (1H, s, 3'-OH), 6.38 (1H, d, 4J = 1.7 Hz, 6'-H), 6.32 (1H, d, 4J = 1.7 Hz, 4'-H), 3.90 (2H, s, 2-H), 3.81 (3H, s, 5'-OCH₃), 3.72 (3H, s, 1-OCH₃), 2.60 (3H, s, C(O)CH₃); ¹³C NMR (75 MHz, CDCl₃) δ 203.3 (C(O)CH₃), 171.3 (1-C), 166.2 (5'-C), 164.0 (3'-C), 136.7 (1'-C), 115.4 (2'-C), 112.8 (6'-C), 100.4 (4'-C), 55.4 (5'-OCH₃), 52.4 (1-OCH₃), 41.7 (2-C), 31.9 (C(O)CH₃); EIMS 238 (50, [M]⁺⁺), 195 (100), 178 (60), 164 (50); HREIMS m/z 238.0843 [M]⁺⁺ (calcd for C₁₂H₁₄O₅, 238.0841).

(S)-Non-8-en-2-ol ((S)-9). To a solution of copper iodide (286 mg, 1.5 mmol) in THF (10 mL) at -40 °C was added freshly prepared hex-5-en-1-ylmagnesium bromide (from 0.530 g, 22.0 mmol of magnesium and 2.68 mL, 3.26 g, 20.0 mmol of 6-bromohex-1-ene in 20 mL of THF), and the mixture was stirred for 15 min. (S)-Propylene oxide (1.40 mL, 1.16 g, 20.0 mmol) was then added dropwise, and the solution was allowed to stir at -40 °C for 4 h. Aqueous NH₄Cl was then added, and the solution extracted with Et₂O. The organic layer was separated, dried with MgSO₄, filtered, and concentrated to give the title compound as a colorless oil (89%, 2.53 g, 17.8 mmol): [α]_D²⁰ +8.2 (c 1.23, MeOH); IR (neat) $\nu_{\max}$ 3398, 3081, 1639 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 5.81 (1H, ddt, 3J = 16.9, 10.3, 6.6 Hz, 8-H), 5.0–4.9 (2H, m, 9-H), 3.9–3.7 (1H, ddt, 2-H), 2.1–2.0 (2H, m, 7-H), 1.5–1.2 (8H, m, 3–6-H), 1.18 (3H, d, 3J = 6.2 Hz, 1-H); ¹³C NMR (75 MHz, CDCl₃) δ 139.0 (8-C), 114.2 (9-C), 68.1 (2-C), 39.3 (3-C), 33.7 (7-C), 29.1, 28.9, 25.6 (4-C), 23.5 (1-C); EIMS 142 (60, [M]⁺⁺), 127(100); HREIMS m/z 142.1356 [M]⁺⁺ (calcd for C₉H₁₈O, 142.1358).

(R)-Non-8-en-2-ol ((R)-9). The title compound was synthesized in the same manner as (S)-9 from (R)-propylene oxide: [α]_D²⁰ -8.9 (c 1.16, MeOH). All remaining spectroscopic data were identical to (S)-non-8-en-2-ol.

(S)-Non-8-en-2-yl Acetate ((S)-10). To a solution of (S)-9 (2.42 g, 17.0 mmol) in pyridine (3.00 mL, 2.95 g, 37.2 mmol) was added acetic anhydride (3.00 mL, 3.25 g, 31.8 mmol), and the solution was stirred at room temperature for 3 h. Aqueous NH₄Cl was added, and the solution was extracted with Et₂O. The organic layer was then washed with aqueous NH₄Cl and saturated aqueous NaHCO₃, dried with MgSO₄, filtered, and concentrated to give a colorless oil (98%, 3.07 g, 16.7 mmol): [α]_D²⁰ +8.3 (c 10.6, MeOH); IR (neat) $\nu_{\max}$ 1742, 1235 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 5.80 (1H, ddt, 3J = 17.0, 10.2, 6.7 Hz, 8-H), 5.1–4.8 (3H, m, 2,9-H), 2.1–2.0 (2H, m, 7-H), 2.02 (3H, s, C(O)CH₃), 1.8–1.2 (8H, m, 3–6-H), 1.20 (3H, d, 3J = 6.3 Hz, 1-H); ¹³C NMR (100 MHz, CDCl₃) δ 170.8 (C(O)CH₃), 140.1 (8-C), 114.3 (9-C), 71.0 (2-C), 35.9 (3-C), 33.7 (7-C), 28.9, 28.8, 25.2 (4-C), 21.4 (C(O)CH₃), 20.0 (1-C); EIMS 184 (40, [M]⁺⁺), 137(100); HREIMS m/z 184.1466 [M]⁺⁺ (calcd for C₁₁H₂₀O₂, 184.1463).

(R)-Non-8-en-2-yl Acetate ((R)-10). The title compound was synthesized in the same manner as (S)-10 from (R)-9: [α]_D²⁰ -7.8 (c 10.2, MeOH). All remaining spectroscopic data were identical to those of (S)-non-8-en-2-yl acetate.

(S)-8-Oxoocetan-2-yl Acetate ((S)-8). (S)-10 (2.76 g, 15.0 mmol) was dissolved in CH₂Cl₂ (40 mL) and cooled to -78 °C. Ozone was then bubbled through the solution until a blue color persisted, at which time oxygen was bubbled through the solution for 15 min. Triphenylphosphine (7.87 g, 30.0 mmol) was then added to the solution, and the reaction mixture was allowed to slowly warm to room temperature. The solvent was removed *in vacuo*, and the crude product was purified via flash column chromatography on silica using 10% EtOAc in petroleum ether as eluent (*R*_f = 0.2), yielding a colorless oil (78%, 2.8 g, 11.7 mmol): [α]_D²⁰ +2.0 (c 10.3, MeOH); IR (neat) $\nu_{\max}$ 2981, 2733, 1750, 1728 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 9.76 (1H, t, 3J = 1.8 Hz, 8-H), 5.00–4.75 (1H, m, 2-H), 2.42 (2H, dt, 3J = 1.8, 7.3 Hz, 7-H), 2.02 (3H, s, C(O)CH₃), 1.7–1.2 (8H, m, 3–6-H), 1.19 (3H, d, 3J = 6.2 Hz, 1-H); ¹³C NMR (100 MHz, CDCl₃) δ 202.4 (8-C), 170.6 (C(O)CH₃), 70.7 (2-C), 43.6 (7-C), 35.5 (3-C), 28.7, 25.0, 21.7, 21.2 (C(O)CH₃), 19.8 (1-C); ESIMS 209 (100, [M + Na]⁺); HRESIMS m/z 209.1155 [M + Na]⁺ (calcd for C₁₀H₁₈O₃⁺Na, 209.1154).

(R)-8-Oxoocetan-2-yl Acetate ((R)-8). The title compound was synthesized in the same manner as (S)-8 from (R)-10: [α]_D²⁰ -3.1 (c 10.6, MeOH). All remaining spectroscopic data were identical to those of (S)-8-oxoocetan-2-yl acetate.

(2'R,6'S) and (2'S,6'S)-Methyl 2-(2-(6-Acetoxiheptyl)-7-methoxy-4-oxochroman-5-yl)acetate ((6'S)-11). Diisopropylamine (140.0 μ L, 101.0 mg, 1.00 mmol) and (S)-8 (186.0 mg, 1.00 mmol) were added to a solution of 7 (238 mg, 1.00 mmol) in anhydrous methanol (2 mL), and the mixture was heated to 140 °C in a microwave reactor (200 W, 200 psi) and stirred for 1 h. The resulting dark red solution was diluted with CH₂Cl₂ and was subsequently washed with saturated aqueous NaHCO₃, water, and brine. The organic layer was then dried with MgSO₄, filtered, and concentrated *in vacuo* to yield an orange oil. Purification was achieved via flash column chromatography on silica with 33% EtOAc in petroleum ether as eluent (*R*_f = 0.3) to yield the title compounds as an amorphous solid (59%, 238 mg, 0.587 mmol): [α]_D²⁰ +26.7 (c 4.20, MeOH); IR (neat) $\nu_{\max}$ 2916, 1742, 1730, 1600 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 6.39 (1H, d, 4J = 2.3 Hz, 6'-H), 6.34 (1H, d, 4J = 2.3 Hz, 8'-H), 4.88 (1H, tq, 3J = 6.2, 6.2 Hz, 6''-H) 4.39 (1H, dddd, 3J = 4.0, 4.8, 7.3, 11.7 Hz, 2'-H), 3.96 (1H, d, 2J = 16.7 Hz, 2-H_A), 3.88 (1H, d, 2J = 16.7 Hz, 2-H_B), 3.81 (3H, s, 7'-OCH₃), 3.70 (3H, s, 1-OCH₃), 2.62 (1H, dd, 2J = 12.5 Hz, 3J = 11.7 Hz, 3'-H_A), 2.54 (1H, dd, 2J = 12.5 Hz, 3J = 4.0 Hz, 3'-H_B), 2.02 (3H, s, C(O)CH₃), 1.9–1.2 (10H, m, 1''–5''-H), 1.20 (3H, d, 3J = 6.2 Hz, 7''-H); ¹³C NMR (75 MHz, CDCl₃) δ 192.4 (4'-C), 171.8 (1-C), 170.9 (C(O)CH₃), 165.1 (7'-C), 164.5 (8'-a-C), 138.4 (5'-C), 113.9 (6'-C), 113.2 (4'-a-C), 100.3 (8'-C), 77.4 (2'-C), 70.9 (6''-C), 55.4 (7'-OCH₃), 51.8 (1-OCH₃), 43.5 (2-H), 41.2 (3'-H), 35.8 (5''-C) 34.7, 29.2, 25.2, 24.7, 21.4 (C(O)CH₃), 19.9; EIMS 406 (30, [M]⁺⁺), 217(100); HREIMS m/z 406.1987 [M]⁺⁺ (calcd for C₂₂H₃₀O₇, 406.1992).

(2'R,6'R) and (2'S,6'R)-Methyl 2-(2-(6-Acetoxiheptyl)-7-methoxy-4-oxochroman-5-yl)acetate ((6'R)-11). The title compounds were synthesized in the same manner as (S)-11 from (R)-8: [α]_D²⁰ -23.4 (c 3.60, MeOH). All remaining spectroscopic data were identical to those of (2'R,6'S) and (2'S,6'S)-methyl 2-(2-(6-acetoxiheptyl)-7-methoxy-4-oxochroman-5-yl)acetate.

(S)-Methyl 2-(2-(6-Acetoxiheptyl)-7-methoxy-4-oxo-4H-chroman-5-yl)acetate ((S)-12). (S)-11 (100 mg, 0.246 mmol) was dissolved in dimethylsulfoxide (1 mL), and the mixture was heated to 90 °C under an atmosphere of argon. 2-Iodoxybenzoic acid (230 mg, 0.810 mmol) was added, and the solution was stirred until all starting material had been consumed according to TLC (48 h). The reaction was then cooled to room temperature and diluted with EtOAc (10 mL). The solution was then washed with saturated aqueous NaHCO₃, water, and brine. The organic layer was separated, dried with MgSO₄, filtered, and concentrated to give a yellow oil. Purification was achieved via flash column chromatography on silica using 33% EtOAc in petroleum ether as eluent (*R*_f = 0.1) to give the title compound as a colorless, amorphous solid (71%, 70.6 mg, 0.175 mmol): [α]_D²⁰ +9.0 (c 1.00, MeOH); IR (neat) $\nu_{\max}$ 1751, 1649, 1601 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 6.78 (1H, d, 4J = 2.5 Hz, 8'-H),

6.69 (1H, d, $^4J = 2.5$ Hz, 6'-H), 5.99 (1H, s, 3'-H), 4.89 (1H, tq, $^3J = 6.2$, 6.2 Hz, 6''-H), 4.15 (2H, s, 2-H), 3.88 (3H, s, 7'-OCH₃), 3.72 (3H, s, 1-OCH₃), 2.53 (2H, t, $^3J = 7.7$ Hz, 1''-H), 2.03 (3H, s, C(O)CH₃), 1.75–1.25 (8H, m, 2''–5''-H), 1.21 (3H, d, $^3J = 6.2$ Hz, 7''-H); ¹³C NMR (75 MHz, CDCl₃) δ 179.1 (4'-C), 171.8 (1-C), 170.8 (C(O)CH₃), 167.5 (2'-C), 162.5 (7'-C), 159.6 (8'a-C), 137.1 (5'-C), 117.5 (6'-C), 116.0 (4'a-C), 110.7 (3'-C), 99.9 (8'-C), 70.8 (6''-C), 55.7 (7'-OCH₃), 51.8 (1-OCH₃), 40.9 (2-H), 35.7, 33.6, 28.8, 26.6, 25.1, 21.4 (C(O)CH₃), 20.0; ESIMS 427 (100, [M + Na]⁺), 405 (10, [M + H]⁺); HRESIMS m/z 405.1914 [M + H]⁺ (calcd for C₂₂H₂₉O₇, 405.1913).

(R)-Methyl 2-(2-(6-Acetoxyheptyl)-7-methoxy-4-oxo-4H-chromen-5-yl)acetate ((R)-12). The title compound was synthesized in the same manner as (S)-12 from (R)-11: [α]_D²⁰ –7.1 (c 1.20, MeOH). All remaining spectroscopic data were identical to those of (S)-methyl 2-(2-(6-acetoxyheptyl)-7-methoxy-4-oxo-4H-chromen-5-yl)acetate.

Pestalotiopsone D: (S)-Methyl 2-(7-Hydroxy-2-(6-hydroxyheptyl)-4-oxo-4H-chromen-5-yl)acetate ((S)-4). To a solution of (S)-12 (40.0 mg, 0.0985 mmol) in toluene (10 mL) was added aluminum chloride (30.0 mg, 0.225 mmol), and the solution was stirred at reflux for 16 h under an atmosphere of nitrogen. The reaction was then cooled to room temperature, and ice was added followed by aqueous 1 M HCl. The solution was extracted with EtOAc, and the organic layer was separated, dried with MgSO₄, filtered, and concentrated to give a brown oil. Purification via flash column chromatography on silica with 50% EtOAc in petroleum ether as eluent ($R_f = 0.3$) yielded the title compound as a colorless, amorphous solid (96%, 32.9 mg, 0.0946 mmol). Purity was confirmed via HPLC employing a Phenomenex LUX cellulose-4 5 μ m enantioselective column (250 mm $\times$ 4.6 mm) using 25% ethanol in *n*-hexane as solvent, resulting in a retention time of 7.65 min: [α]_D²⁰ +12.6 (c 0.93, MeOH); UV (CH₃OH) $\lambda_{\max}$ (log ϵ) 210 (4.19), 242 (4.08), 250 (4.11), 290 (3.91) nm; IR (neat) $\nu_{\max}$ 3492, 1712, 1600 cm^{–1}; ¹H NMR (300 MHz, acetone-*d*₆) δ 6.81 (1H, d, $^4J = 2.4$ Hz, 8'-H), 6.74 (1H, d, $^4J = 2.4$ Hz, 6'-H), 5.93 (1H, s, 3'-H), 4.11 (2H, s, 2-H), 3.71 (1H, m, 6''-H), 3.60 (3H, s, 1-OCH₃), 2.59 (2H, t, $^3J = 7.5$ Hz, 1''-H), 1.72 (2H, m, 2''-H), 1.45–1.35 (6H, m, 3''–5''-H), 1.12 (3H, d, $^3J = 6.2$ Hz, 7''-H); ¹³C NMR (125 MHz, acetone-*d*₆) δ 181.0 (4'-C), 173.8 (1-C), 170.5 (2'-C), 165.5 (7'-C), 162.4 (8'a-C), 140.4 (5'-C), 120.3 (6'-C), 118.8 (4'a-C), 113.1 (3'-C), 102.6 (8'-C), 69.4 (6'-C), 53.6 (1-OCH₃), 42.9 (2-C), 42.1 (5''-C), 36.1, 29.5, 28.3, 26.1, 23.5 (7''-C); ESIMS 349 (80, [M + H]⁺), 334 (100); HRESIMS m/z 349.1648 [M + H]⁺ (calcd for C₁₉H₂₅O₆, 349.1651).

(R)-Methyl 2-(7-Hydroxy-2-(6-hydroxyheptyl)-4-oxo-4H-chromen-5-yl)acetate ((R)-4). The title compound was synthesized in the same manner as (S)-4 from (R)-12. Purity was confirmed via HPLC employing a Phenomenex LUX cellulose-4 5 μ m enantioselective column (250 mm $\times$ 4.6 mm) using 25% ethanol in *n*-hexane as solvent, resulting in a retention time of 12.72 min: [α]_D²⁰ –10.8 (c 0.81, MeOH). All remaining spectroscopic data were identical to those of (S)-methyl 2-(7-hydroxy-2-(6-hydroxyheptyl)-4-oxo-4H-chromen-5-yl)acetate.

Pestalotiopsone E: (S)-Ethyl 2-(7-Hydroxy-2-(6-hydroxyheptyl)-4-oxo-4H-chromen-5-yl)acetate ((S)-5). To a solution of (S)-4 (10.0 mg, 28.6 μ mol) in ethanol (5 mL) was added 2 drops of sulfuric acid. The reaction was stirred at 50 °C for 24 h, at which time water was added and the solution was extracted with ethyl acetate. The combined organic layers were washed with aqueous NaHCO₃, dried with MgSO₄, filtered, and concentrated to give the title compound as a colorless, amorphous solid (99%, 10.3 mg, 28.4 μ mol). Purity was confirmed via HPLC employing a Phenomenex LUX cellulose-4 5 μ m enantioselective column (250 mm $\times$ 4.6 mm) using 25% ethanol in *n*-hexane as solvent, resulting in a retention time of 15.91 min: [α]_D²⁰ +14.1 (c 0.73, MeOH); UV (EtOH) $\lambda_{\max}$ (log ϵ) 208 (4.19), 242 (4.16), 250 (4.20), 292 (4.03) nm; IR (neat) $\nu_{\max}$ 3339, 1617, 1599 cm^{–1}; ¹H NMR (300 MHz, acetone-*d*₆) δ 6.80 (1H, d, $^4J = 2.3$ Hz, 8'-H), 6.74 (1H, d, $^4J = 2.3$ Hz, 6'-H), 5.93 (1H, s, 3'-H), 4.10 (2H, s, 2-H), 4.07 (2H, q, $^3J = 7.1$ Hz, 1-OCH₂CH₃), 3.70 (1H, m, 6''-H), 2.59 (2H, t, $^3J = 7.4$ Hz, 1''-H), 1.72 (2H, m, 2''-H), 1.45–1.35 (6H, m, 3''–5''-H), 1.20 (3H, t, $^3J = 7.1$ Hz, 1-OCH₂CH₃), 1.12 (3H, d, $^3J = 6.2$

Hz, 7''-H); ¹³C NMR (125 MHz, acetone-*d*₆) δ 181.1 (4'-C), 174.0 (1-C), 170.6 (2'-C), 165.7 (7'-C), 162.7 (8'a-C), 140.3 (5'-C), 120.3 (6'-C), 118.7 (4'a-C), 113.4 (3'-C), 102.6 (8'-C), 69.4 (6'-C), 61.0 (1-OCH₂CH₃), 42.9 (2-C), 42.1 (5''-C), 36.1, 29.6, 28.3, 26.1, 23.5 (7''-C), 14.5 (1-OCH₂CH₃); ESIMS 363 (20, [M + H]⁺), 334 (100); HRESIMS m/z 363.1804 [M + H]⁺ (calcd for C₂₀H₂₇O₆, 363.1808).

(R)-Ethyl 2-(7-Hydroxy-2-(6-hydroxyheptyl)-4-oxo-4H-chromen-5-yl)acetate ((R)-5). The title compound was synthesized in the same manner as (S)-5 from (R)-4. Purity was confirmed via HPLC employing a Phenomenex LUX cellulose-4 5 μ m enantioselective column (250 mm $\times$ 4.6 mm) using 25% ethanol in *n*-hexane as solvent, resulting in a retention time of 18.07 min: [α]_D²⁰ –15.8 (c 0.62, MeOH). All remaining spectroscopic data were identical to those of (S)-ethyl 2-(7-hydroxy-2-(6-hydroxyheptyl)-4-oxo-4H-chromen-5-yl)acetate.

Determination of the Sensitivity of Selected Bacterial Strains against Selected Compounds. The sensitivity of four clinical bacterial strains (*Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Mycobacterium smegmatis*), was tested by a turbidity assay (OD₆₀₀, optical density at $\lambda = 600$ nm).⁷ To prepare the inoculum, the bacterial suspension adjusted to equal the density of 0.5 McFarland standard (OD₆₀₀ = 0.08) was diluted 1:100 with Mueller Hinton broth (MHB), and 150 μ L was used as an inoculum. Then 40 μ L of MHB was added to wells 1B to 1H of sterile 96-well plates. A 80 μ L portion of the unfiltered test sample (3 mg/mL) or appropriate antibiotic control (1 mg/mL) was dispensed into well 1A, 40 μ L was removed, and serial 2-fold dilutions of the test sample were prepared directly on the plate. The plate was read at A₆₀₀ to control for pre-existing turbidity of the samples before incubation. The plate was incubated at 37 °C for 18 h on a shaker incubator. After incubation, the plate was read at A₆₀₀ to assess the relative turbidity (i.e., growth) of the treated cultures; 50% inhibition was determined based on a comparison with the average turbidity readings of the untreated control. Inhibition was calculated as

$$IC_{50} = 10[\log(A/B) \times (50 - C) / (D - C) + \log(B)]$$

where A is the higher concentration of test compound of the two points on the graph that bracket 50% inhibition, B is the lower concentration of test compound of the two points on the graph that bracket 50% inhibition, C is the inhibitory activity (%) at the concentration B, and D is the inhibitory activity (%) at the concentration A.

■ ASSOCIATED CONTENT

● Supporting Information

¹H and ¹³C NMR spectra for all synthetic compounds and analytical data for the synthesized natural products. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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