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First syntheses of the biologically active fungal metabolites pestalotiopsones A, B, C and F†

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A synthetic approach accessing the pestalotiopsones, fungal chromones possessing a rare skeletal subtype, is reported for the first time. The synthesis of pestalotiopsone A (**1**) has been achieved in 7 linear steps (28%), from commercially available 3,5-dimethoxybenzoic acid and subsequently the first syntheses of pestalotiopsone B (**2**), C (**3**) and F (**4**) were performed utilising this chemistry. The key steps include a newly described homologation of a substituted benzoic acid to afford phenylacetate derivatives utilising Birch reductive alkylation conditions, a microwave mediated chromanone formation proceeding through an oxa-Michael cyclisation, and an IBX induced dehydrogenation to the desired chromone skeleton. The synthetic natural products were completely characterised for the first time, confirming their structures and their biological activities evaluated against a panel of bacterial pathogens.

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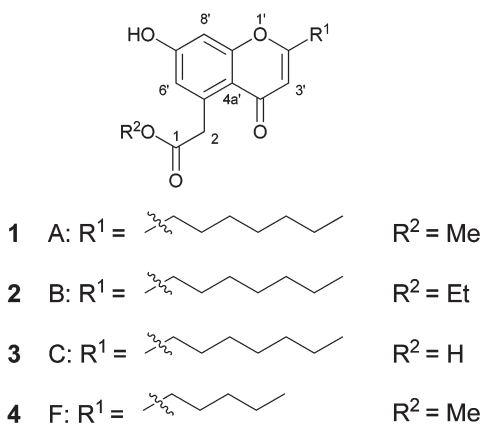
Introduction

The vital role of natural products as new pharmaceutical leads is undeniable. Over half of all new drugs approved between January 1981 and December 2010 were natural products or derived from natural products.[1] The structural diversity of natural products as well as their high biospecificity makes them very attractive leads for researchers. However, investigating this wide-ranging chemical space efficiently and effectively is an overwhelming challenge for researchers.[2] In this regard, the knowledge base of ethnopharmacology is an amazing resource. The characterisation of the bioactive compounds in traditional medicines offers the possibility for new pharmaceuticals with proven efficacy and inherently low-toxicity.

The use of fungi as traditional medicines is widespread and well documented, perhaps best known in traditional Chinese and Ayurvedic medicine. In addition there is widespread contemporary, albeit poorly documented, use of fungi in communities such as those in the highlands of Papua New Guinea. The effectiveness of fungal metabolites as pharmaceuticals is exemplified by the β-lactams, *e.g.* the penicillins. However, their bioactivity ranges greatly, and their presence is known across all areas of pharmaceuticals including antifungal, antitumour and immunosuppressive among others.[1,3]

During an investigation of the endophytic fungi of the genus *Pestalotiopsis*, obtained from the traditional Chinese medicinal plant *Rhizophora mucronata*, Proksch and co-workers isolated the bioactive chromones pestalotiopsones A, B, C and F (**1–4**).[4] Due to the small quantities isolated full characterisations were not achieved for all compounds. The isolated chromones were evaluated for their cytotoxicity against the murine cancer cell line L5178Y. Interestingly, only pestalotiopsone F (**4**) demonstrated any activity, despite its moderate structural differences from **1–3**.



The pestalotiopsones are of polyketide origin but possess an unusual folding pattern in which an F-type pattern appears to have been interrupted by a heterocyclisation to generate a rare subtype of chromones, reported only three times previously.[5–7]

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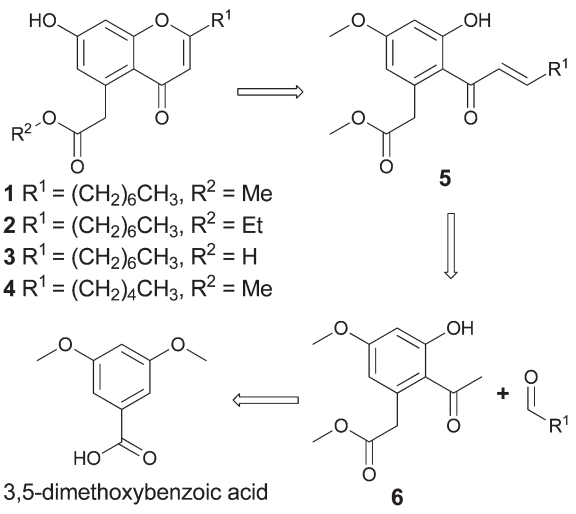
†Electronic supplementary information (ESI) available: General experimental conditions and copies of NMR data for all synthesised compounds, along with analytical data for the synthesised natural products are available online. See DOI: 10.1039/c2ob26904j

We report here the first syntheses of pestalotiopsones A, B, C and F and confirm the natural product structures through complete characterisations.

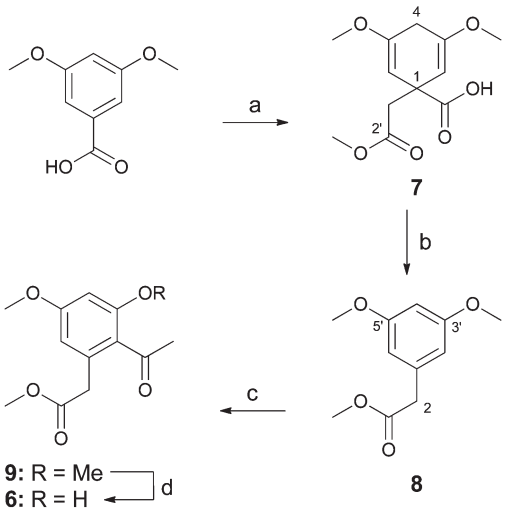
Results and discussion

The literature offers a plethora of ways to access the chromone skeleton. We required a pathway which was tolerant of an ester functionality, as well as offering alkyl substitution at the 2′-position, which could be introduced at a late stage to allow divergence. Retrosynthetically we envisaged the pestalotiopsones could be afforded *via* a cyclisation and subsequent oxidation from the appropriate α,β-unsaturated ketone intermediate **5**. This could be derived through an aldol coupling of the requisite aldehyde and acetophenone **6** which could be obtained *via* homologation and Friedel–Crafts acylation of 3,5-dimethoxybenzoic acid (Scheme 1). In this direction acetophenone **6** was obtained *via* a previously unreported homologation utilising a Birch reductive alkylation pathway. Current literature on the homologation of benzoic acids utilises a five-step process requiring reaction over multiple days (61–75%).[8–10] We report here a two-step homologation of 3,5-dimethoxybenzoic acid to methyl 3,5-dimethoxyphenylacetate (Scheme 2). Treatment of the benzoic acid derivative with lithium in liquid ammonia, followed by addition of methyl bromoacetate yielded cyclohexadiene **7**. Rearomatisation was achieved upon treatment of **7** with lead tetraacetate in toluene resulting in loss of carbon dioxide to give the desired phenylacetate **8** in a yield of 85% over two steps, with preparation time of approximately five hours. Friedel–Crafts acylation with acetic anhydride and perchloric acid provided acetophenone **9**, which upon exposure to boron trichloride resulted in a chelation controlled selective deprotection of the methyl ether *ortho* to the acetyl group yielding the desired *o*-hydroxy acetophenone **6**.

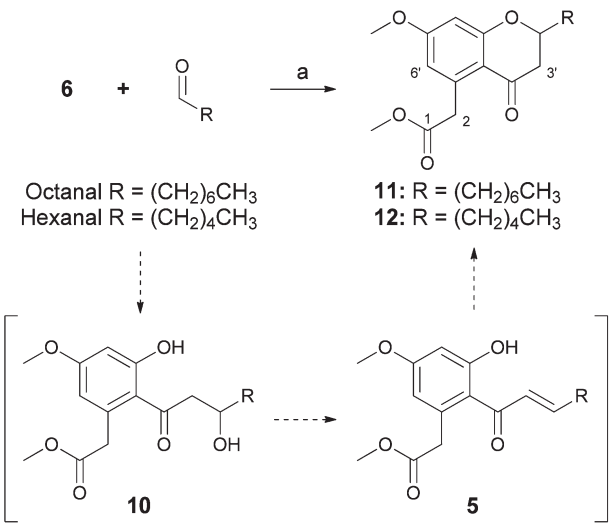
It was at this point that the synthesis diverged for the five carbon or seven carbon side chain lengths present in pestalotiopsones A, B and C or pestalotiopsone F respectively. It was envisaged that an aldol condensation to **10**, followed by dehydration to **5** and subsequent oxa-Michael mediated cyclisation would yield chromanones **11** and **12** (Scheme 3). Traditional methods for performing these steps require forcing conditions involving strongly basic and acidic reaction media, which are intolerable to the ester moiety. Work performed by Luthman and co-workers demonstrated an efficient method towards a diverse range of 2-alkyl-substituted chromanones utilising mild reagents under microwave heating.[11,12] In this manner **11** and **12** were prepared by the treatment of hydroxyacetophenone **6** with the appropriate aldehyde and diisopropylamine in anhydrous methanol heated to 140 °C (200 W, 200 psi), in good yields (68–72%) in a single step.



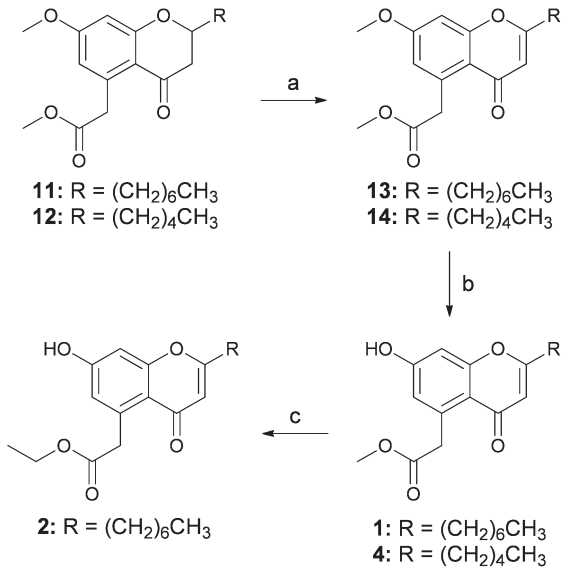
Scheme 1 The retrosynthetic analysis of the pestalotiopsones.



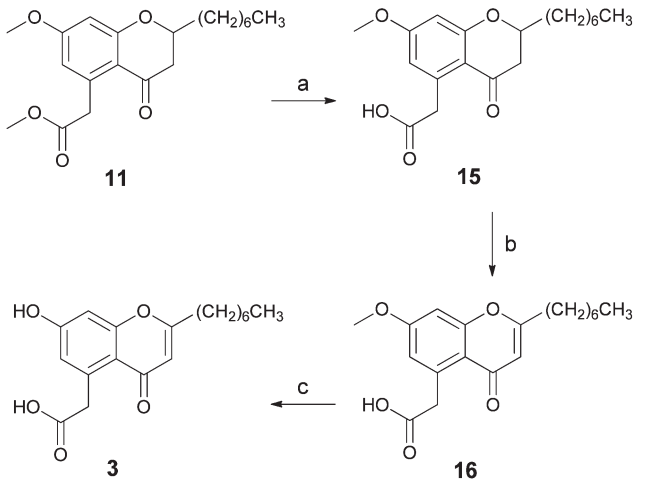
Scheme 2 Homologation of 3,5-dimethoxybenzoic acid. (a) (i) Li/NH_3, THF, −33 °C; (ii) methyl bromoacetate, THF, −33 °C to r.t., 93%; (b) $Pb(OAc)_4$, toluene, r.t., 91%; (c) Ac_2O, $HClO_4$, AcOH, 35 °C, 89%; (d) BCl_3, CH_2Cl_2, −78 °C to r.t., 93%.



Scheme 3 One-step synthesis of chromanones **11** and **12**. (a) DIPA, MeOH, μwave (200 W, 200 psi), 140 °C, 68–72%.



Scheme 4 First syntheses of pestalotiopsones A (**1**), B (**2**), and F (**3**). (a) IBX, DMSO, 90 °C, 62–89%; (b) $AlCl_3$, CH_2Cl_2, 40 °C, 96%; (c) EtOH, H_2SO_4, 78 °C, 85–92%.



Scheme 5 Completion of the first synthesis of pestalotiopsone C **3**. (a) 1 N NaOH, MeOH, 65 °C, 96%; (b) IBX, DMSO, 90 °C, 73%; (c) $AlCl_3$, CH_2Cl_2, 40 °C, 62%.

Diagnostic of chromanone formation was the appearance of an AB spin system in the ^{1}H NMR spectrum for the protons attached at C-2, whereas previously these resonances had always been isochronous.

With chromanones **11** and **12** in hand our attention was turned towards their oxidation to afford the analogous chromones (Scheme 4). Application of conditions described by Nicolaou and co-workers employing 2-iodoxybenzoic acid (IBX) at 85 °C for 48 hours resulted in formation of chromones **13** and **14** in good yields (62–89%).[13] Diagnostically, the protons attached at C-2 were returned to equivalence in the ^{1}H NMR spectrum. Deprotection of the methyl ether in the presence of aluminium chloride at 40 °C returned pestalotiopsones A (**1**) and F (**4**) in excellent yields (85–92%). Pestalotiopsone B (**2**) was obtained by subjecting **1** to transesterification conditions with sulphuric acid in ethanol.

We reasoned that pestalotiopsone C (**3**) would be easily obtained by the saponification of **1**. However, when **1** was treated with a hydroxide source, such as lithium hydroxide, complex mixtures were consistently returned under a variety of conditions. We suggest that the electrophilicity of the α,β-unsaturated chromone was greater than that of the ester carbonyl, and therefore was reacting with the hydroxide preferentially. With this in mind it was proposed that the carboxylic acid be introduced at an earlier stage by saponification of the chromanone ester **11** (Scheme 5). Gratifyingly, treatment of **11** with sodium hydroxide proceeded as predicted to return the chromanone acid **15**, which could then be oxidised, as before, in the presence of IBX. Removal of the IBA was achieved by reversed phase flash chromatography yielding the desired chromone **16** (73%). Finally, deprotection with aluminium chloride provided pestalotiopsone C (**3**).

While the synthesis of **3** had been achieved, it was felt that a more elegant solution could be developed. It was apparent

Table 1 Antibiotic activity of pestalotiopsones A, B, C and F (**1–4**) and intermediates **11–12** showing 50% inhibition of selected strains

	IC_{50} (μg mL^{-1})						
Strain[a]	Cam[b]	**1**	**2**	**3**	**4**	**11**	**12**
S. epidermidis	23	345	421	421	323	62.5	125
E. coli	8	>500	>500	>500	421	>500	>500
P. aeruginosa	33	>500	>500	>500	>500	>500	>500
M. smegmatis	33	>500	>500	>500	421	>500	>500

[a] Clinical strains obtained from The Canberra Hospital. [b] Chloramphenicol.

that the chromone moiety was unstable to the basic conditions required for saponification, but displayed high stability to acidic conditions. When we subjected **1** to the acidic hydrolysis conditions of water and sulphuric acid, utilising THF to aid in solubility, **3** was returned quantitatively.

The pestalotiopsones A, B, C and F (**1–4**), in addition to a number of the synthetic intermediates **11–16** were subject to biological assay to evaluate their antibiotic activity against clinical strains of *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Mycobacterium smegmatis.* Compounds that displayed an IC_{50} < 500 μg mL^{-1} against at least one of the test pathogens are displayed in Table 1. Chloramphenicol was employed in the assay as a positive control and for comparison. The pestalotiopsones A, B, C and F (**1–4**) showed activity against all clinical strains tested, as did two of the synthetic intermediates tested **11** and **12**. All compounds showed moderate activity towards *S. epidermidis*, with **1–4** exhibiting IC_{50} values of 345 μg mL^{-1}, 421 μg mL^{-1}, 421 μg mL^{-1} and 323 μg mL^{-1} respectively. Furthermore two chromanones **11** and **12**, representing synthetic intermediates enroute to the pestalotiopsones, were considerably more active than the corresponding chromones. Of particular interest to us, it was apparent that pestalotiopsone F (**4**), which was the only active compound identified by Proksch and co-workers in the

Table 2 Antibiotic activity of pestalotiopsones A, B, C and F (**1–4**) showing percentage of inhibition of selected strains up to 50%

	1		**2**		**3**		**4**	
Strain[a]	μg mL^{-1}	%	μg mL^{-1}	%	μg mL^{-1}	%	μg mL^{-1}	%
S. epidermidis	345	50	421	50	421	50	323	50
E. coli	421	13	421	33	421	43	421	50
P. aeruginosa	421	29	421	36	421	33	421	17
M. smegmatis	421	18	421	30	421	41	421	50

[a] Clinical strains obtained from The Canberra Hospital.

cytotoxicity assays they employed,[4] showed the greatest spectrum of activity, amongst the pestalotiopsones tested, in the antibiotic assays employed here (Tables 1 and 2).

Conclusions

An efficient route has been established resulting in the first total synthesis of pestalotiopsone A (**1**) in 7 linear steps with an overall yield of 28%, *via* a microwave promoted aldol condensation and oxa-Michael cyclisation followed by an IBX induced dehydrogenation. Pestalotiopsones B (**2**) and C (**3**) were synthesised from **1** resulting in an overall synthesis of 8 linear steps (26% and 28% yield respectively). Pestalotiopsone F (**4**) was synthesised in an analogous manner as **1** in 7 linear steps (38%). The synthetic products showed identical characteristic data to that reported for the natural products.[4] In addition complete characterisation of all natural products is provided for the first time. Biological testing of the synthetic compounds showed they exhibited antimicrobial activity for all compounds against clinical strains of human pathogens and indicated that chromanones were more active than the corresponding chromones.

Experimental

3,5-Dimethoxy-1-(2-methoxy-2-oxoethyl)cyclohexa-2,5-dienecarboxylic acid (7)

To a solution of 3,5-dimethoxybenzoic acid (1.82 g, 10.0 mmol) in THF (20 mL) was condensed NH_3 (50 mL). Lithium (153 mg, 21.9 mmol) was added in portions at −33 °C whereon a deep blue colour persisted. Methyl bromoacetate (1.14 mL, 1.84 g, 12.0 mmol) was then added dropwise, causing the solution to become a pale yellow colour. The NH_3 was evaporated under a stream of nitrogen and the resulting solid residue was dissolved in water and Et_2O. The layers were separated and the aqueous layer was cooled to 0 °C and acidified to pH 4 with careful addition of 1 M HCl. The acidic solution was extracted with EtOAc, dried with $MgSO_4$, filtered and concentrated *in vacuo* to give the title compound as a colourless solid (93%, 2.38 g). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.77 (2H, s, 2,4-H), 3.67 (3H, s, 2′-OCH_3), 3.58 (6H, s, 3,5-OCH_3), 2.7–2.9 (4H, m, 4-H_A, 4-H_B, 1′-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 180.9 (COOH), 171.4 (2′C), 154.2 (3,5-C), 93.9 (2,6-C), 54.5 (3,5-OCH_3), 51.6 (2′-OCH_3), 47.8 (1-C), 45.6 (1′-C), 31.0 (4-C). ESI-MS: 279 (100, $[M + Na]^+$), 257 (40, $[M + H]^+$). HRESI-MS: Found *m*/*z* of 295.0584 (calculated for $C_{12}H_{16}O_6{}^{39}K$ $[M + K]^+$ 295.0584). Found *m*/*z* of 279.0845 (calculated for $C_{12}H_{16}O_6{}^{23}Na$ $[M + Na]^+$ 279.0845). Found *m*/*z* of 257.1024 (calculated for $C_{12}H_{17}O_6$ $[M + H]^+$ 257.1025). IR (ν_{max}): 2982, 2651, 1703, 1649, 1695.

Methyl 2-(3,5-dimethoxyphenyl)acetate (8)

To a solution of **7** (1.85 g, 7.20 mmol) in CH_2Cl_2 (150 mL) was added lead tetraacetate (4.15 g, 9.40 mmol). The reaction was monitored by TLC and after 40 min water was added. The mixture was filtered through a pad of silica. The organic phase was separated, washed with saturated $NaHCO_3$, dried with $MgSO_4$, filtered and concentrated *in vacuo* to give an orange oil. Purification was achieved by flash column chromatography on silica with 33% EtOAc in petroleum spirits (60–80 fraction) as the eluent (R_f = 0.6) to yield the title compound as a yellow oil (91%, 1.38 g). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.44 (2H, d, ^4J = 2.3, 2′,6′-H), 6.37 (1H, t, ^4J = 2.3, 4′-H), 3.78 (6H, 3′,5′-OCH_3), 3.69 (3H, s, 1-OCH_3), 3.56 (2H, s, 2-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): 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′-OCH_3), 52.1 (1-OCH_3), 41.4 (2-CH_2). EI-MS: 210 (100, $[M]^{+\cdot}$), 151 (95), 121 (30), 91 (20). HREI-MS: Found *m*/*z* of 210.0890 (calculated for $C_{11}H_{14}O_4$ $[M]^{+\cdot}$ 210.0892). IR (ν_{max}) 1723, 1602.

Methyl 2-(2-acetyl-3,5-dimethoxyphenyl)acetate (9)

To a solution of **8** (1.44 g, 5.7 mmol) and acetic anhydride (5.42 mL, 5.81 g, 57.0 mmol) in acetic acid (50 mL) at 35 °C was added perchloric acid solution (70%, 8.21 mL, 57.0 mmol) dropwise. The solution was stirred for 5 min and then poured into ice water. Et_2O and saturated $NaHCO_3$ were added and the organic layer was separated, washed with saturated $NaHCO_3$, dried with $MgSO_4$, filtered and concentrated *in vacuo* to give a yellow oil. Purification was achieved by flash column chromatography on silica (60–80 fraction) with 33% EtOAc in petroleum spirits as eluent (R_f = 0.4) yielding the title compound as a pale yellow amorphous solid (89%, 22.4 mg). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.40 (1H, d, ^4J = 2.2, 6′-H), 6.34 (1H, d, ^4J = 2.2, 4′-H), 3.82 (3H, s, 5′-OCH_3), 3.80 (3H, s, 3′-OCH_3), 3.68 (2H, s, 2-CH_2), 3.66 (3H, s, 1-OCH_3), 2.49 (3H, s, 2′-C(O)CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 203.7 (2′-*C*(O)CH_3), 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 (2′-C), 55.6 (5′-OCH_3), 55.3 (3′-OCH_3), 51.9 (1-OCH_3), 39.0 (2-CH_2), 32.2 (2′-C(O)*C*H_3). EI-MS: 252 (55, $[M]^{+\cdot}$), 237 (60), 221 (35), 209 (100), 192 (65), 178 (50). HREI-MS: Found *m*/*z* of 252.0996 (calculated for $C_{13}H_{16}O_5$ $[M]^{+\cdot}$ 252.0998). IR (ν_{max}) 1745, 1686.

Methyl 2-(2-acetyl-3-hydroxy-5-methoxyphenyl)acetate (6)

To a solution of **9** (913 mg, 3.62 mmol) in CH_2Cl_2 (15 mL) at −78 °C under an atmosphere of argon was added dropwise a boron trichloride solution (1 M, 5.43 mL, 5.43 mmol). The solution was allowed to slowly warm to room temperature and stirred for 16 hours at which time ice was added. The organic

layer was separated, dried with $MgSO_4$, filtered and concentrated under reduced pressure to give the title compound as a colourless solid (93%, 801 mg). ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.06 (1H, s, 3′-OH), 6.38 (1H, d, ^4J = 1.7, 6′-H), 6.32 (1H, d, ^4J = 1.7, 4′-H), 3.90 (2H, s, 2-CH_2), 3.81 (3H, s, 5′-OCH_3), 3.72 (3H, s, 1-OCH_3), 2.60 (3H, s, C(O)CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 203.3 (CO), 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′OCH_3), 52.4 (1-OCH_3), 41.7 (2-CH_2), 31.9 (C(O)CH_3) EI-MS: 238 (50, $[M]^{+\cdot}$), 195 (100), 178 (60), 164 (50). HREI-MS: Found m/z of 238.0843 (calculated for $C_{12}H_{14}O_5$ $[M]^{+\cdot}$ 238.0841). IR (ν_{max}) 3268, 1701, 1598.

Methyl 2-(2-heptyl-7-methoxy-4-oxochroman-5-yl)acetate (11)

Diisopropylamine (97.0 μL, 70.0 mg, 0.690 mmol) and octanal (108 μL, 89.0 mg, 0.690 mmol) were added to a solution of **6** (149 mg, 0.630 mmol) in anhydrous methanol (2 mL) and was heated to 140 °C in a microwave reactor (200 W, 200 psi) and stirred for 1 hour. The resulting dark red solution was diluted with CH_2Cl_2 and was subsequently washed with saturated $NaHCO_3$ solution, water and brine. The organic layer was then dried with $MgSO_4$, filtered and concentrated *in vacuo* to yield an orange oil. Purification was achieved *via* flash column chromatography on silica with 25% EtOAc in petroleum spirits (60–80 fraction) as eluent (R_f = 0.4) to yield the title compound as an amorphous solid (68%, 149 mg). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.39 (1H, d, ^4J = 2.5, 6′-H), 6.34 (1H, d, ^4J = 2.5, 8′-H), 4.39 (1H, dddd, ^3J = 4.0, 4.8, 7.3, 11.7, 2′-H), 3.96 (1H, d, ^2J = 16.7, 2-H_A), 3.88 (1H, d, ^2J = 16.7, 2-H_B), 3.81 (3H, s, 5′-OCH_3), 3.70 (3H, s, 1-OCH_3), 2.62 (1H, dd, ^2J = 12.5, ^3J = 11.7, 3′-H_A), 2.54 (1H, dd, ^2J = 12.5, ^3J = 4.0, 3′-H_B), 1.79 (1H, m, 1″-H_A), 1.65 (1H, m, 1″-H_B), 1.47 (2H, m, 2″-CH_2), 1.4–1.2 (8H, m, 3″-6″-CH_2), 0.88 (3H, t, ^3J = 6.5, 7″-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 192.4 (4′-C), 171.8 (1-C), 165.1 (7′-C), 164.5 (8a′-C), 138.4 (5′-C), 113.9 (6′-C), 113.3 (4′-C), 100.4 (8′-C), 77.6 (2′-C), 55.5 (7′-OCH_3), 51.8 (1-OCH_3), 43.6 (2-CH_2), 41.1 (3′-CH_2), 34.9, 29.4, 29.2, 24.9, 22.7, 14.1 (7″-CH_3). EI-MS: 348 (30, $[M]^{+\cdot}$), 316 (100), 282 (45), 217 (40). HREI-MS: Found m/z of 348.1937 (calculated for $C_{20}H_{28}O_5$ $[M]^{+\cdot}$ 348.1937). IR (ν_{max}) 2927, 1727, 1603.

Methyl 2-(2-heptyl-7-methoxy-4-oxo-4*H*-chromen-5-yl)acetate (13)

11 (93.0 mg, 0.270 mmol) was dissolved in dimethylsulfoxide (1 mL) and 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 hours). The reaction was then cooled to room temperature and diluted with EtOAc. The solution was then washed with saturated $NaHCO_3$, water and brine. The organic layer was separated, dried with $MgSO_4$, filtered and concentrated to give a yellow oil. Purification was achieved *via* flash column chromatography on silica using 25% EtOAc in petroleum spirits (60–80 fraction) as eluent (R_f = 0.1) to give a colourless amorphous solid (62%, 60.0 mg). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.77 (1H, d, ^4J = 2.5, 8′-H), 6.68 (1H, d, ^4J = 2.5, 6′-H), 6.00 (1H, s, 3′-H), 4.14 (2H, s, 2-CH_2), 3.87 (3H, s, 7′-OCH_3), 3.72 (3h, s, 1-OCH_3), 2.53 (2H, t, ^3J = 7.4, 1″-CH_2), 1.69 (2H, m, 2″-CH_2), 1.45–1.20 (8H, m, 3″-6″-CH_2), 0.88 (3H, t, ^3J = 6.7, 7″-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 179.1 (4′-C), 171.8 (1-C), 167.8 (2′-C), 162.5 (7′-C), 159.6 (8a′-C), 137.1 (5′-C), 117.5 (6′-C), 116.0 (4a′-C), 110.6 (3′-C), 99.9 (8′-C), 55.7 (7′-OCH_3), 51.8 (1-OCH_3), 40.1 (2-CH_2), 33.7 (1″-CH_2), 31.7, 29.0, 28.9, 26.7, 22.6, 14.1 (7″-CH_3). ESI-MS: 347 (40, $[M + H]^+$), 315 (100). HRESI-MS: Found m/z of 369.1677 (calculated for $C_{20}H_{26}O_5{}^{23}Na$ $[M + Na]^+$ 369.1678). IR (ν_{max}): 1645, 1604.

Pestalotiopsone A: methyl 2-(2-heptyl-7-hydroxy-4-oxo-4*H*-chromen-5-yl)acetate (1)

To a solution of **13** (40.0 mg, 0.115 mmol) in CH_2Cl_2 (10 mL) was added aluminium chloride (30.7 mg, 0.230 mmol) and the solution was stirred at reflux for 16 hours under an atmosphere of nitrogen. The reaction was then cooled to room temperature and ice was added followed by 1 M HCl. The solution was extracted with EtOAc and the organic layer was separated, dried with $MgSO_4$, filtered and concentrated to give brown oil. Purified *via* flash column chromatography on silica with 25% EtOAc in petroleum spirits (60–80 fraction) as eluent (R_f = 0.2) yielding a colourless amorphous solid (96%, 36.5 mg). Purity was confirmed *via* HPLC on a phenomenex LUNA 5μ C18 analytical column using 70% methanol in water as solvent with a flow rate of 1.5 mL min^{-1} resulting in a retention time of 10.34 min. ^{1}H NMR (300 MHz, acetone-d_6): δ 6.81 (1H, d, ^4J = 2.4, 8′-H), 6.75 (1H, d, ^4J = 2.4, 6′-H), 5.93 (1H, s, 3′-H), 4.11 (2H, s, 2-CH_2), 3.60 (3H, s, 1-OCH_3), 2.59 (2H, t, ^3J = 7.4, 1″-CH_2), 1.72 (2H, m, 2″-CH_2), 1.45–1.35 (4H, m, 5″-6″-CH_2), 1.35–1.25 (4H, m, 3″-4″-CH_2), 0.88 (3H, t, ^3J = 6.5, 7″-CH_3). ^{13}C NMR (75 MHz, acetone-d_6): δ 179.1 (4′-C), 171.8 (1-C), 168.4 (2′-C), 161.7 (7′-C), 160.4 (8a′-C), 138.8 (5′-C), 118.6 (6′-C), 116.2 (4a′-C), 110.8 (3′-C), 102.8 (8′C), 51.6 (1-OCH_3), 40.1 (2-CH_2), 34.1 (1″-CH_2), 32.4, 29.7, 27.4, 23.3, 14.3 (7″-CH_3). ESI-MS: 333 (80, $[M + H]^+$), 319 (70), 301 (90), 287 (100), 273 (50). HRESI-MS: Found m/z of 333.1695 (calculated for $C_{19}H_{25}O_5$ $[M + H]^+$ 333.1702). IR (ν_{max}): 3497, 1708, 1591. UV (CH_3OH) λ_{max} (log ε): 217 (4.37), 242 (4.26), 250 (4.29), 291 (4.14).

Pestalotiopsone B: ethyl 2-(2-heptyl-7-hydroxy-4-oxo-4*H*-chromen-5-yl)acetate (2)

To a solution of **1** (25.0 mg, 75.3 μmol) in ethanol (5 mL) was added 2 drops of sulphuric acid. The reaction was stirred at 50 °C for 24 hours, at which time water was added and the solution was extracted with ethyl acetate. The combined organic layers were washed with sat. $NaHCO_3$ dried with $MgSO_4$, filtered and concentrated to give the title compound as a colourless amorphous solid (92%, 24.0 mg). Purity was confirmed *via* HPLC on a phenomenex LUNA 5μ C18 analytical column using 70% methanol in water as solvent with a flow rate of 1.5 mL min^{-1} resulting in a retention time of 24.43 min. ^{1}H NMR (300 MHz, acetone-d_6): δ 6.80 (1H, d, ^4J = 2.4, 8′-H), 6.74 (1H, d, ^4J = 2.4, 6′-H), 5.93 (1H, s, 3′-H), 4.10 (2H, s, 2-CH_2), 4.07 (2H, q, ^3J = 7.1, 1-OCH_2CH_3), 2.59 (2H, t, ^3J = 7.4, 2-CH_2), 1.72 (2H, m, 1″-CH_2), 1.45–1.35 (4H, m, 5″-6″-CH_2), 1.35–1.25 (4H, m, 3″-4″-CH_2), 1.20 (3H, t, ^3J = 7.1, 1-OCH_2CH_3), 0.88 (3H, t,

^3J = 6.8, 7″-CH_3). ^{13}C NMR (75 MHz, acetone-d_6): δ 178.1 (4′-C), 170.4 (1-C), 167.4 (2′-C), 160.8 (7′-C), 159.4 (8a′-C), 138.0 (5′-C), 117.6 (6′-C), 115.3 (4a′-C), 109.9 (3′-C), 101.8 (8′-C), 59.6 (1-OCH_2CH_3), 40.3 (2-CH_2), 33.2 (1″-CH_2), 31.5, 28.7, 26.5, 22.4, 13.7, 13.4. ESI-MS: 347 (20, $[M + H]^+$), 301 (100), 273 (25). HRESI-MS: Found *m*/*z* of 347.1856 (calculated for $C_{20}H_{27}O_5$ $[M + H]^+$ 347.1858). IR (ν_{max}): 3334, 1623, 1594. UV (CH_3CH_2OH) λ_{max} (log ε): 220 (4.23), 242 (4.19), 250 (4.21), 291 (4.06).

Methyl 2-(2-pentyl-7-methoxy-4-oxochroman-5-yl)acetate (12)

Prepared in an analogous manner to **11** substituting hexanal for octanal. The title compound was obtained as an amorphous solid (72%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.39 (1H, d, ^4J = 2.5, 6′-H), 6.34 (1H, d, ^4J = 2.5, 8′-H), 4.39 (1H, dddd, ^3J = 4.1, 4.7, 7.3, 11.6, 2′-H), 3.96 (1H, d, ^2J = 16.6, 2-H_A), 3.88 (1H, d, ^2J = 16.6, 2-H_B), 3.81 (3H, s, 5′-OCH_3), 3.70 (3H, s, 1-OCH_3), 2.62 (1H, dd, ^2J = 16.5, ^3J = 11.6, 3′-H_A), 2.54 (1H, dd, ^2J = 16.5, ^3J = 4.1, 3′-H_B), 1.81 (1H, m, 1″-H_A), 1.65 (1H, m, 1″-H_B), 1.5–1.1 (6H, m, 2″-4″-CH_2), 0.90 (3H, t, ^3J = 6.6, 5″-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 192.5 (4′-C), 171.8 (1-C), 165.1 (7′-C), 164.5 (8a′-C), 138.4 (5′-C), 113.9 (6′-C), 113.4 (4a′-C), 100.4 (8′-C), 77.6 (2′-C), 55.6 (7′-OCH_3), 51.9 (1′-OCH_3), 43.6 (2-CH_2), 41.2 (3′-CH_2), 34.9, 31.6, 24.6, 22.6, 14.1 (5″-CH_3). ESI-MS: 343 (100, $[M + Na]^+$), 289 (60). HRESI-MS: Found *m*/*z* of 343.1522 (calculated for $C_{18}H_{24}O_5{}^{23}Na$ $[M + Na]^+$ 343.1521). IR (ν_{max}): 2933, 1725, 1601.

Methyl 2-(2-pentyl-7-methoxy-4-oxo-4*H*-chromen-5-yl)acetate (14)

Prepared in an analogous manner to **13**. The title compound was obtained as an amorphous solid (89%). ^{1}H NMR (300 MHz): δ 6.77 (1H, d, ^4J = 2.5, 8′-H), 6.68 (1H, d, ^4J = 2.5, 6′-H), 6.00 (1H, s, 3′-H), 4.14 (2H, s, 2-CH_2), 3.87 (3H, s, 7′-OCH_3), 3.71 (3H, s, 1-OCH_3), 2.52 (2H, t, ^3J = 7.5, 1″-CH_2), 1.69 (2H, m, 2″-CH_2), 1.4–1.1 (6H, m, 2″-4″-CH_2), 0.90 (3H, t, ^3J = 6.6, 5″-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 179.3 (4′-C), 171.9 (1-C), 167.9 (2′-C), 162.6 (7′-C), 159.7 (8a′-C), 137.2 (5′-C), 122.0 (6′-C), 117.6 (4a′-C), 110.7 (3′-C), 100.0 (8′-C), 55.7 (7′-OCH_3), 51.9 (1-OCH_3), 41.0 (2-C), 33.8, 31.2, 26.5, 22.4, 14.0 (5″-C). ESI-MS: 341 (100, $[M + Na]^+$), 287 (60). HRESI-MS: Found *m*/*z* of 341.1364 (calculated for $C_{18}H_{22}O_5{}^{23}Na$ $[M + Na]^+$ 341.1365). IR (ν_{max}): 1649, 1607.

Pestalotiopsone F: methyl 2-(2-pentyl-7-hydroxy-4-oxo-4*H*-chromen-5-yl)acetate (4)

Prepared in an analogous manner to **1**. The title compound was obtained as an amorphous white solid (85%). Purity was confirmed *via* HPLC on a phenomenex LUNA 5μ C18 analytical column using 70% methanol in water as solvent with a flow rate of 1.5 mL min^{-1} resulting in a retention time of 8.34 min. ^{1}H NMR (300 MHz, acetone-d_6): δ 6.82 (1H, d, ^4J = 2.4, 8′-H), 6.76 (1H, d, ^4J = 2.4, 6′-H), 5.94 (1H, s, 3′-H), 4.11 (2H, s, 2-CH_2), 3.60 (3H, s, 1-OCH_3), 2.59 (2H, t, 1″-CH_2), 1.72 (2H, m, 2″-CH_2), 1.4–1.2 (4H, m, 3″-4″-CH_2), 0.90 (3H, t, ^3J = 7.0, 5″-CH_3). ^{13}C NMR (75 MHz, acetone-d_6): δ 179.1 (4′-C), 171.9 (1-C), 168.4 (2′-C), 161.7 (7′-C), 160.4 (8a′-C), 138.8 (5′-C), 118.6 (6′-C), 116.1 (4a′-C), 110.8 (3′-C), 102.7 (8′-C), 51.6 (1-OCH_3), 40.9 (2-CH_2), 34.1, 31.9, 27.1, 23.0, 14.2 (5″-CH_3). ESI-MS: 305 (10, $[M + H]^+$), 273 (100), 245 (60). HRESI-MS: Found *m*/*z* of 305.1385 (calculated for $C_{17}H_{21}O_5$ $[M + H]^+$ 305.1389). IR (ν_{max}): 3385, 1698, 1572. UV (CH_3OH) λ_{max} (log ε): 215 (4.17), 242 (4.03), 250 (4.05), 290 (3.87).

2-(2-Heptyl-7-methoxy-4-oxochroman-5-yl)acetic acid (15)

To a solution of **11** (100 mg, 0.290 mmol) in methanol (20 mL) was added 1 M NaOH until the solution became slightly cloudy. Methanol was then added until the solution returned to translucency. The reaction was then heated to reflux for 16 hours, at which time it was cooled to room temperature and diluted with water. The solution was washed with CH_2Cl_2 and the aqueous layer was then acidified to pH 1 with 5 M HCl. After extraction with CH_2Cl_2 the organic layer was dried with $MgSO_4$, filtered and concentrated to yield a colourless amorphous solid (96%, 93.0 mg). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.46 (1H, d, ^4J = 2.3, 6′-H), 6.40 (1H, d, ^4J = 2.3, 8′-H), 4.42 (1H, m, 2′-H), 3.96 (1H, d, ^2J = 15.8, 2-H_A), 3.90 (1H, d, ^2J = 15.8, 2-H_B), 3.83 (3H, s, 5′-OCH_3), 2.76–2.58 (2H, m, 3′-H_A & 3′-H_B), 1.83 (1H, m, 1″-H_A), 1.69 (1H, m, 1″-H_B), 1.48 (2H, m, 2″-CH_2), 1.4–1.2 (8H, m, 3″-6″-CH_2), 0.89 (3H, t, ^3J = 7.1, 7″-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.5 (4′-C), 175.6 (1-C), 165.3 (7′-C), 164.9 (8a′-C), 137.8 (5′-C), 114.1 (6′-C), 113.2 (4a′-C), 100.6 (8′-C), 77.7 (2′-C), 55.7 (7′-OCH_3), 43.5 (2-CH_2), 41.4 (3′-CH_2), 34.9, 31.8, 29.4, 29.2, 24.9, 22.7, 14.1 (7″-CH_3). ESI-MS: 357 (100, $[M + Na]^+$), 335 (20, $[M + H]^+$). HRESI-MS: Found *m*/*z* of 335.1859 (calculated for $C_{19}H_{27}O_5$ $[M + H]^+$ 335.1858). IR (ν_{max}): 2917, 1698, 1605.

2-(2-Heptyl-7-methoxy-4-oxo-4*H*-chromen-5-yl)acetic acid (16)

Prepared in an analogous manner to **13**. Purified *via* reverse phase ODS column chromatography using 40% acetonitrile in water (R_f = 0.1). The title compound was obtained as an amorphous solid (73%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.91 (1H, d, ^4J = 1.9, 8′-H), 6.83 (1H, d, ^4J = 1.9, 6′-H), 6.19 (1H, s, 3′-H), 4.12 (2H, s, 2-CH_2), 3.89 (3H, s, 7′-OCH_3), 2.61 (2H, t, ^3J = 7.5, 1″-CH_2), 1.72 (2H, m, 2″-CH_2), 1.45–1.20 (8H, m, 3″-6″-CH_2), 0.88 (3H, t, ^3J = 6.8, 7″-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.2 (4′-C), 172.1 (1-C), 170.0 (2′-C), 163.5 (7′-C), 160.1 (8a′-C), 136.3 (5′-C), 118.2 (6′-C), 115.5 (4a′-C), 110.3 (3′-C), 100.7 (8′-C), 56.0 (7′-OCH_3), 42.3 (2-CH_2), 34.0 (1″-CH_2), 31.7, 29.0, 29.0, 26.8, 22.7, 14.1 (7″-CH_3). ESI-MS: 333 (100, $[M + H]^+$), 289 (70). HRESI-MS: Found *m*/*z* of 333.1700 (calculated for $C_{19}H_{25}O_5$ $[M + H]^+$ 333.1702). IR (ν_{max}): 2912, 1653, 1606.

Pestalotiopsone C: 2-(2-heptyl-7-hydroxy-4-oxo-4*H*-chromen-5-yl)acetic acid (3)

To a solution of **1** (20.0 mg, 60.0 μmol) in THF (1 mL) was added water (5 mL) and H_2SO_4 (3 drops). The solution was heated to reflux and stirred for 16 hours. The reaction was cooled to room temperature and extracted with EtOAc. The organic layer was dried with $MgSO_4$, filtered and concentrated *in vacuo* to yield the title compound as a colourless amorphous solid (99%, 18.9 mg). Purity was confirmed *via* HPLC on a

phenomenex LUNA 5μ C18 analytical column using 70% methanol in water as solvent with a flow rate of 1.5 mL min^{-1} resulting in a retention time of 7.60 min. ^{1}H NMR (400 MHz, CD_3OD): δ 6.78 (1H, d, ^4J = 2.3, 8′-H), 6.70 (1H, d, ^4J = 2.3, 6′-H), 6.04 (1H, s, 3′-H), 4.10 (2H, s, 2-CH_2), 2.62 (2H, t, ^3J = 7.4, 1″-CH_2), 1.73 (1H, m, 2″-H_A), 1.59 (1H, m, 2″-H_B), 1.45–1.35 (4H, m, 5″-6″-CH_2), 1.35–1.25 (4H, m, 3″-4″-CH_2), 0.88 (3H, t, ^3J = 6.7, 7″-CH_3). ^{13}C NMR (75 MHz, CD_3OD): δ 181.4 (4′-C), 173.9 (1-C), 170.7 (2′-C), 163.4 (7′-C), 161.3 (8a′-C), 138.7 (5′-C), 119.6 (6′-C), 115.6 (4a′-C), 110.4 (3′-C), 103.0 (8′C), 41.8 (2-CH_2), 34.6 (1″-CH_2), 32.9, 30.0, 27.9, 23.7, 14.4 (7″-CH_3). ESI-MS: 319 (70, $[M + H]^+$), 301 (100), 247 (60). HRESI-MS: Found *m*/*z* of 317.1382 (calculated for $C_{18}H_{21}O_5$ $[M - H]^-$ 317.1389). IR (ν_{max}): 3085, 2927, 1627, 1559. UV (CH_3OH) λ_{max} (log ε): 222 (4.38), 243 (4.22), 251 (4.27), 274 (4.36).

Alternatively, pestalotiopsone C: 2-(2-heptyl-7-hydroxy-4-oxo-4*H*-chromen-5-yl)acetic acid (**3**) can be prepared from **16** in an analogous manner to **1**. The title compound was obtained as an amorphous solid (62%).

Determination of the sensitivity of selected bacterial strains

The sensitivity of four resistant clinical bacterial strains (*S. epidermidis*, *E. coli*, *P. aeruginosa* and *M. smegmatis*), were tested by a turbidity assay OD_{600} (optical density at λ = 600 nm). To prepare the inoculum, the bacterial suspension adjusted to equal the density of 0.5 McFarland standard (OD_{600} = 0.08) was diluted 1 : 100 with Mueller Hinton broth (MHB) and 150 μL was used as an inoculum. Forty microlitres of MHB was added to wells 1B to 1H of sterile 96-well plates. 80 μL of the unfiltered test sample (2 mg mL^{-1}) or appropriate antibiotic control (5 mg mL^{-1}) was dispensed into well 1A and 40 μL removed and serial two-fold dilutions of the test sample were prepared directly on the plate. The plate was read at A_{600} to control for pre-existing turbidity of the samples before incubation. The plate was incubated at 37 °C for 18 hours on a shaker incubator. After incubation, the plate was read at A_{600} 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*: higher concentration of test compound of the two points on the graph that brackets 50% inhibition, *B*: lower concentration of test compound of the two points on the graph that brackets 50% inhibition, *C*: inhibitory activity (%) at the concentration *B*, *D*: inhibitory activity (%) at the concentration *A*. Inhibitory activity (%) was calculated by:

$$[1 - (OD_{sample} - OD_{blank})/\text{average OD growth control}] \times 100.$$

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