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Spiralisones A–D: acylphloroglucinol hemiketals from an Australian marine brown alga, *Zonaria spiralis*†

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An intertidal sample of the Australian marine brown alga, *Zonaria spiralis*, exhibited promising kinase inhibitory and antibacterial activity. Chemical analysis returned six phloroglucinol-derived lipids, the new hemiketal spiralisones A–D (**1–4**) and the known chromones **5–6**, and the known norsesquiterpenoid apo-9′-fucoxanthinone (**7**). Structures **1–7** were assigned on the basis of detailed spectroscopic analysis, biosynthetic considerations and total synthesis. Spiralisones undergo facile acid-mediated dehydration to yield the corresponding chromones, revealing for the first time that brown algal chromones may be handling artifacts rather than natural products. Hemiketals **1** and **2**, and chromone **6**, displayed inhibitory activity against the neurodegenerative disease kinase targets CDK5/p25, CK1δ and GSK3β, while hemiketals **1**, **3** and **4**, and chromone **6**, displayed growth inhibitory activity against the Gram-positive bacteria *Bacillus subtilis* (ATCC 6051 and 6633). The promising kinase inhibitory and antibacterial properties of the *Z. spiralis* extract were attributed to the cumulative effect of many moderately potent phloroglucinol-derived lipid co-metabolites.

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Introduction

Neurodegenerative diseases represent a very significant challenge to modern healthcare, none more so than Alzheimer's disease where patients are confronted with progressive and irreversible deterioration of cognitive ability, and a pathology characterized by the aggregation and deposition of misfolded proteins.[1] More specifically, on autopsy the brains of Alzheimer's patients exhibit amyloid pathology, mediated by β-secretase (BACE) processing of the essential neuronal transmembrane amyloid precursor protein (APP) to generate amyloid plaques,[2] and tau pathology mediated by kinases such as casein kinase 1δ (CK1δ), cyclin-dependent kinase 5 (CDK5) and glycogen synthase kinase 3β (GSK3β), that hyperphosphorylate highly soluble microtubule-associated tau protein to generate neurofibrillary tangles.[3] Not surprisingly, efforts to develop clinically useful drugs to treat Alzheimer's diseases have included the search for inhibitors of BACE, CK1δ, CDK5 and GSK3β. Another major challenge to modern healthcare is that of infectious diseases, where clinically important antibiotics are increasingly compromised and rendered less effective by drug resistance.

In an effort to address the dual healthcare challenges associated with neurodegenerative and infectious diseases, we implemented a marine biodiscovery program in which ~2600 southern Australian and Antarctic marine invertebrates and algae were screened for inhibitory activity against BACE (27, 1%), CK1δ (44, 1.7%), CDK5 (26, 1%) and GSK3β (69, 2.6%), as well as for antibiotic activity. This approach has proved successful in the past, as evidenced by our recent reports on biologically active alkaloids spanning the ianthellidone,[4] dictyodendrin,[5] lamellarin,[6] ningalin,[7] and massadine[8] structure classes. In this report we describe our investigations into an intertidal collection of the southern Australian brown alga, *Zonaria spiralis*, which exhibited promising inhibitory activities against BACE, CDK5 and GSK3β, as well as Gram-positive antibacterial activity. Comprehensive chemical analysis yielded four new phloroglucinol-derived hemiketal lipids, spiralisones A–D (**1–4**), as well as the known chromone co-metabolites 5,7-dihydroxy-2-tridecanyl chromone (**5**)[9] and 5,7-dihydroxy-2-(4*Z*,7*Z*,10*Z*,13*Z*,16*Z*-nonadecapentaenyl)-chromone (**6**),[10] and the degraded norterpene apo-9′-

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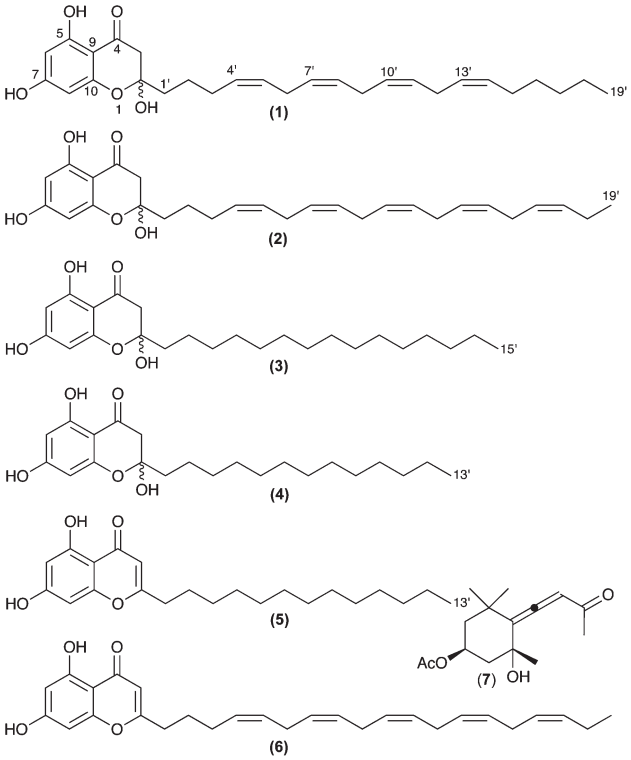
fucoxanthinone (**7**).[11]



Results and discussion

A portion of the aqueous EtOH extract derived from *Zonaria spiralis* was decanted, concentrated *in vacuo* and subjected to sequential solvent trituration and reversed phase chromatography (SPE and HPLC) to yield the new spiralisones A–D (**1–4**), along with the known co-metabolites **5–7**. Structures were assigned to **1–7** on the basis of detailed spectroscopic analysis supported by chemical synthesis. An account of the structure elucidation of spiralisones A–D (**1–4**), 5,7-dihydroxy-2-tridecanyl chromone (**5**)[9] and 5,7-dihydroxy-2-(4*Z*,7*Z*,10*Z*,-13*Z*,16*Z*-nonadecapentaenyl) chromone (**6**),[10] inclusive of synthetic studies, is outlined below.

High resolution ESI(+)MS analysis of spiralisone A (**1**) revealed an adduct ion ($[M + Na]^+$) consistent with a molecular formula ($C_{28}H_{38}O_5$, Δmmu −0.6) requiring ten double bond equivalents (DBE). Analysis of the NMR ($CDCl_3$) data for **1** (Table 1) revealed resonances attributable to a ketone (δ_C 195.0), a tetrasubstituted benzene bearing three oxy substituents (δ_C 164.7, 164.2, 160.2, 102.9, 97.0 and 96.2) and four 1,2-disubstituted double bonds (δ_C 130.8, 129.2 (x2), 128.9, 128.5, 128.3, 128.1 and 127.7), accounting for nine double bond equivalents (DBE) and requiring an additional ring system. A quaternary hemiketal carbon (δ_C 102.5) accounted for the final oxygen atom and permitted assembly of the required ring system, while exchangeable ^1H NMR resonances were attributed to non-hydrogen bonded (δ_H 5.70) and hydrogen bonded (δ_H 11.92) phenol protons, respectively. Key HMBC correlations (Fig. 1) allowed assembly of the structure elements listed above, revealing spiralisone A (**1**) as an acylated phloroglucinol further cyclized to a hemiketal bearing a pendant nonadeca-4,7,10,13-tetraenyl sidechain. The methylene interrupted regiochemistry and *Z* geometry of the sidechain double bonds were inferred from diagnostic overlapping resonances for H_2-6′, H_2-9′ and H_2-12′ (δ_H 2.77–2.83); shielded signals for C-6′, C-9′ and C-12′ (δ_C 25.92, 25.89, and 25.89);[12] HMBC correlations (Fig. 1); and spectroscopic comparisons with authentic unsaturated fatty acids such as arachidonic acid. With a zero optical rotation spiralisone A (**1**) was assigned the racemic structure as shown.

High resolution ESI(+)MS analysis of spiralisone B (**2**) revealed an adduct ion ($[M + Na]^+$) consistent with a molecular formula ($C_{28}H_{36}O_5$, Δmmu −0.6) indicative of a dehydro analogue of **1**. Comparison of the NMR data for **2** and **1** (Table 1) confirmed this hypothesis with the only significant differences being attributed to an additional methylene interrupted $\Delta^{16'}$ double bond (δ_H 5.30 and 5.38; δ_C 127.2 and 132.3) in **2**. These observations, together with a zero optical rotation, permitted structure assignment of spiralisone B (**2**) as indicated (Fig. 2).

High resolution ESI(+)MS analysis of spiralisones C (**3**) and D (**4**) revealed adduct ions ($[M + Na]^+$) consistent with molecular formulae ($C_{24}H_{38}O_5$ and $C_{22}H_{34}O_5$; Δmmu 0.5 and −0.7, respectively) indicative of lower saturated homologues of **1**. Comparison of the NMR data for **3** and **4** with **1** (Table 1) confirmed this hypothesis, with the differences being attributable to pentadecanyl and tridecanyl sidechains as indicated. These considerations, together with zero optical rotations, permitted assignment of the racemic structures to spiralisones C (**3**) and D (**4**) as indicated.

High resolution ESI(+)MS analysis of **5** and **6** revealed adduct ions ($[M + Na]^+$) consistent with molecular formulae ($C_{22}H_{32}O_4$ and $C_{28}H_{34}O_4$; Δmmu 0.8 and 0.6, respectively) indicative of dehydration analogues of **4** and **2**, respectively. Comparison of the NMR data for **5** with **4**, and **6** with **2** (Table 1), revealed resonances diagnostic for chromone moieties, permitting identification of **5** as 5,7-dihydroxy-2-tridecanyl chromone, and **6** as 5,7-dihydroxy-2-(4*Z*,7*Z*,10*Z*,13*Z*,16*Z*-nonadecapentaenyl) chromone. First reported in 1987 from a mass spectrometric analysis of a rhododendron lace bug,[9] our re-isolation of **5** provided the first comprehensive spectroscopic analysis and synthesis (see below). First reported in 1982 from the Pacific brown alga *Zonaria tournefortii*,[10a] **6** was the subject of a 2009 total synthesis from 2,4,6-trihydroxybenzaldehyde and the ethyl ester of eicosapentaenoic acid.[10b]

Discovery of chromones **5** and **6**, as co-metabolites with hemiketals **4** and **2**, raised the possibility that the former may be handling artifacts (dehydration products) of the latter. Indeed, during our studies we noted that hemiketals **4** and **2** underwent ready dehydration to return chromones **5** and **6** when exposed to mildly acidic conditions, such as acidic chromatographic media (*e.g.* silica) and spectroscopic solvents (*e.g.* $CDCl_3$). This observation prompted us to propose a plausible biosynthesis (Fig. 3), and develop a short biomimetic synthesis (Scheme 1).

Table 1 NMR data ($CDCl_3$, 600 MHz) for spiralisones A–D (**1–4**)

	1		**2**		**3**		**4**	
No.	δ_C	δ_H (m., *J* in Hz)	δ_C	δ_H (m., *J* in Hz)	δ_C	δ_H (m., *J* in Hz)	δ_C	δ_H (m., *J* in Hz)
2	102.5		102.5		102.6		102.6	
3	45.1	a 2.88 (d, 16.9) b 2.74 (d, 16.9)	45.1	a 2.88 (d, 17.2) b 2.74 (d, 17.2)	45.1	a 2.88 (d, 16.9) b 2.75 (d, 16.9)	45.1	a 2.88 (d, 16.9) b 2.74 (d, 16.9)
4	195.0		195.1		195.1		195.1	
5	164.2		164.1		164.2		164.2	
6	97.0	5.96 (br s)	97.0	5.95 (d, 1.7)	96.9	5.96 (d, 1.6)	96.9	5.96 (br s)
7	164.7		164.9		164.7		164.6	
8	96.2	5.89 (br s)	96.3	5.89 (d, 1.7)	96.1	5.89 (d, 1.6)	96.2	5.89 (br s)
9	102.9		102.8		103.0		102.9	
10	160.2		160.2		160.2		160.3	
1′	40.8	1.89 (br t, 8.1)	40.8	1.89 (br t, 8.2)	41.4	1.87 (br t, 7.6)	41.4	1.87 (br t, 7.6)
2′	23.4	1.58 (m)	23.4	1.58 (m)	23.4	1.48 (m)	23.4	1.48 (m)
3′	27.0	2.15 (m)	27.0	2.14 (m)	29.73[a]	1.34 (m)	29.73[a]	1.34 (m)
4′	129.2	5.30–5.43 (m)	129.17	5.32–5.44 (m)	29.92[a]	1.20–1.30 (m)	29.90[a]	1.21–1.30 (m)
5′	129.2[a]	5.30–5.43 (m)	129.23[a]	5.32–5.44 (m)	29.91[a]	1.20–1.30 (m)	29.87[a]	1.21–1.30 (m)
6′	25.92[a]	2.77–2.85 (m)	25.91[a]	2.77–2.85 (m)	29.90[a]	1.20–1.30 (m)	29.87[a]	1.21–1.30 (m)
7′	128.9[a]	5.30–5.43 (m)	128.8[a]	5.32–5.44 (m)	29.88[a]	1.20–1.30 (m)	29.8[a]	1.21–1.30 (m)
8′	128.5[a]	5.30–5.43 (m)	128.5[a]	5.32–5.44 (m)	29.87[a]	1.20–1.30 (m)	29.69[a]	1.21–1.30 (m)
9′	25.89[a]	2.77–2.85 (m)	25.88[a]	2.77–2.85 (m)	29.8[a]	1.20–1.30 (m)	29.68[a]	1.21–1.30 (m)
10′	128.3[a]	5.30–5.43 (m)	128.4[a]	5.32–5.44 (m)	29.69[a]	1.20–1.30 (m)	29.6[a]	1.21–1.30 (m)
11′	128.1[a]	5.30–5.43 (m)	128.34[a]	5.32–5.44 (m)	29.68[a]	1.20–1.30 (m)	32.1	1.24 (m)
12′	25.89[a]	2.77–2.85 (m)	25.86[a]	2.77–2.85 (m)	29.6[a]	1.20–1.30 (m)	22.9	1.28 (m)
13′	127.7	5.31 (m)	128.29[a]	5.32–5.44 (m)	32.2	1.24 (m)	14.3	0.86 (t, 7.0)
14′	130.8	5.38 (m)	128.1[a]	5.32–5.44 (m)	22.9	1.27 (m)		
15′	27.5	2.03 (m)	25.8[a]	2.77–2.85 (m)	14.3	0.86 (t, 7.0)		
16′	29.5	1.34 (m)	127.2	5.30 (m)				
17′	31.7	1.26 (m)	132.3	5.38 (m)				
18′	22.8	1.29 (m)	20.8	2.05 (m)				
19′	14.3	0.87 (t, 7.0)	14.5	0.95 (t, 7.5)				
5-OH		11.92 (s)		11.91 (s)		11.93 (s)		11.93 (s)
7-OH		5.70 (br s)		5.99 (br s)		5.52 (br s)		5.63 (br s)

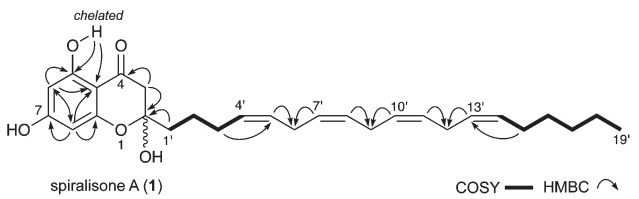


Fig. 1 Key 2D NMR correlations for spiralisone A (**1**).

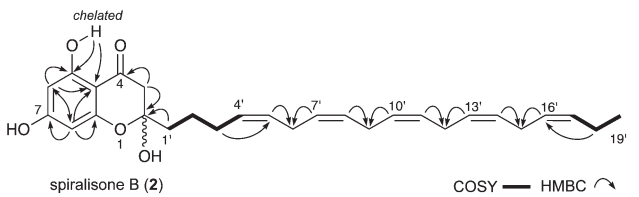


Fig. 2 Key 2D NMR correlations for spiralisone B (**2**).

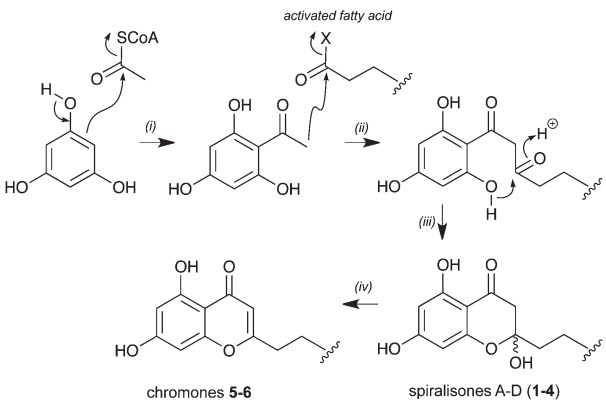
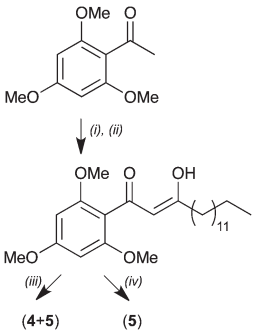


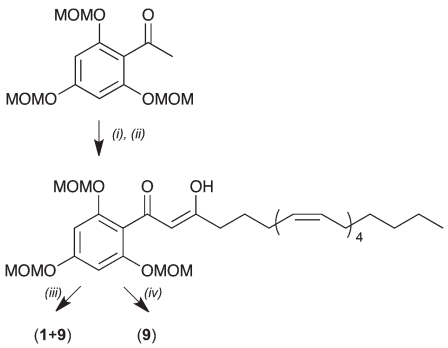
Fig. 3 Plausible mixed polyketide/fatty acid biosynthesis of the hemiketal spiralisones A–D (**1–4**) and the chromones **5–6**.

The biosynthetic pathway outlined in Fig. 3 proceeds from the common algal polyketide precursor phloroglucinol, engaging with fatty acid biosynthesis. We hypothesize that (*i*) condensation of acetyl-CoA and phloroglucinol can generate an acylphloroglucinol intermediate, which in turn undergoes (*ii*) sidechain elongation by a Claisen-type condensation with an array of activated unsaturated/saturated fatty acids, for example where X = coenzyme A [SCoA] or acyl carrier protein [ACP], to yield acylphloroglucinol β-diketone intermediates. The latter undergo rapid non-enzyme mediated (*iii*) intramolecular cyclization to yield hemiketals (spiralisones), and (*iv*) dehydration to deliver chromones. The racemic nature of spiralisone hemiketals supports a non-enzyme mediated cyclization pathway.

Our biomimetic synthesis of spiralisone D (**4**) and the chromone **5** (Scheme 1) draws on our proposed biosynthesis, and



Scheme 1 Biomimetic synthesis of spiralisone D (**4**) and the chromone **5**. (*i*) tetradecanal, LDA, THF, −78 °C; (*ii*) DMP, CH_2Cl_2, 0 °C; (*iii*) BBr_3, CH_2Cl_2, −78 °C ~ rt; (*iv*) *n*PrSLi, HMPA, 80 °C.



Scheme 2 Biomimetic synthesis of spiralisone A (**1**) and its corresponding chromone (**9**). (*i*) arachidonal, LDA, THF, −78 °C; (*ii*) DMP, CH_2Cl_2, 0 °C; (*iii*) HCl/MeOH, rt; (*iv*) TFA, CH_2Cl_2.

involves (*i*) aldol condensation of tridecanal and permethylated acetylphloroglucinol, followed by (*ii*) oxidation of the resulting alcohol to deliver the β-diketone product as an equilibrium mixture of keto–enol tautomers. Deprotection with (*iii*) BBr_3 in CH_2Cl_2 initiated spontaneous cyclization to deliver the target hemiketal **4** (30%), along with a methyl hemiketal side-product (40%) and the chromone **5** (10%), whereas deprotection with (*iv*) lithium propane-1-thiolate in HMPA returned a high yield of the chromone **5** (75%). Synthetic samples of **4** and **5** were identical in all respects with those isolated from *Zonaria spiralis*.

While (*i*) and (*ii*) aldol coupling (Scheme 2) of arachidonal with permethylated acetylphloroglucinol was successful, the arachidonyl side chain proved unstable to the harsh methyl ether deprotection protocols. Fortunately, the corresponding methoxymethyl (MOM) protected acetylphloroglucinol did undergo successful deprotection with (*iii*) HCl/MeOH, to return low yields of **1** (16%) and its corresponding chromone **9** (33%), and (*iv*) TFA in CH_2Cl_2, to return a high yield of **9** (85%).

Whereas the two new hemiketals **1**, **2** and the chromone **6** showed inhibitory activity against CDK5/p25 (IC_{50} 10, 3.0 and 10.0 μM, respectively), CK1δ (all IC_{50} < 10 μM) and GSK3β (all IC_{50} < 10 μM), **3–5** and **7** failed to exhibit inhibitory activity up to 30 μM (ESI Table S8†). None of the *Zonaria spiralis* metabolites **1–7** inhibited BACE up to 10 μM. We attribute the promising kinase inhibitory potency detected in the crude *Zonaria spiralis* extract as arising from the cumulative effect of multiple moderately potent spiralisone inhibitors.

The co-metabolites **1–7** were assessed for antibacterial and antifungal activities (ESI Table S8†). The hemiketals **1**, **3**, and **4**, and the chromone **6**, all exhibited moderate growth inhibitory activity against *Bacillus subtilis* (ATCC 6051 and 6633) (IC_{50} 2.5–10 μM), while none of the *Zonaria spiralis* metabolites **1–7** inhibited the Gram-positive bacteria *Staphylococcus aureus* (ATCC 9144 and 25923), the Gram-negative bacteria *Escherichia coli* (ATCC 11775) and *Pseudomonas aeruginosa* (ATCC 10145), or the fungus *Candida albicans* (ATCC 90028). As with the kinase inhibitory activity, the antibacterial properties of the crude *Zonaria spiralis* extract was attributed to the "cumulative effect" of multiple weakly antibacterial co-metabolites.

Conclusions

In summary, our bioassay guided investigation into the chemistry of an Australian marine brown alga *Zonaria spiralis* led to the discovery of an unprecedented class of acylphloroglucinol hemiketals, the spiralisones A–D (**1–4**) which undergo facile dehydration to chromones under mildly acidic conditions. Isolation and characterization of these metabolites necessitated great care, and highlighted the possibility that previously reported algal chromones might be handling (dehydration) artifacts, rather than natural products as previously supposed. We proposed a plausible mixed polyketide/fatty acid biosynthesis, and used this to develop successful biomimetic syntheses of the hemiketals **1** and **4**, and their respective chromones **5** and **9**. The spiralisones were established as the dominant kinase inhibitory and antibacterial agents in the crude *Zonaria spiralis* extract, with the potency displayed by the crude extract being attributed to the cumulative concentration effect of multiple mildly bioactive members of this class of mixed biosynthesis co-metabolites.

Experimental section

General experimental procedures

Optical rotations were measured on a JASCO P-1010 polarimeter with a 10 cm length cell. UV spectra were obtained on a Cary 50 UV-visible spectrophotometer with 1 cm pathway cell. NMR experiments were performed on a Bruker Avance DRX600 spectrometer and referenced to residual ^1H signals in the deuterated solvents. ESIMS experiments were carried out on an Agilent 1100 series LC/MSD instrument. HR-ESIMS data were acquired on a Bruker micrOTOF mass spectrometer by direct infusion in MeCN at 3 μL min^{-1} using sodium formate clusters as an internal calibrant. All HPLC analyses and purifications were performed on Agilent 1100 series LC instruments with corresponding detectors, collectors and software inclusively.

All chemicals were purchased from Merck, Sigma-Aldrich or Fluka. Solvents used for general purposes were of at least analytical grade, and solvents used for HPLC were of HPLC grade. Agilent Zorbax SB-C_8 (5 μm, 4.6 × 150 mm (analytical)

and 9.4 × 250 mm (semi-preparative)) columns were used for HPLC analyses and separations. A normal phase Biotage SNAP 50 g cartridge and an Alltech SPE 5 g C_{18} cartridge were also used for crude fractionation. Flash column chromatography was performed on silica gel LC60A (40–63 mm, Davisil).

Collection and taxonomy

The alga material identified as *Zonaria Spiralis* (Order: Dictyotales; Family: Dictyotaceae) was collected in January 1993 by the author (Capon) from intertidal rock platforms, near North Walkerville, Victoria, Australia.

Extraction and isolation

A portion (*ca.* 200 mL) of the EtOH extract of *Zonaria spiralis* was decanted and concentrated *in vacuo* to return a dark brown solid (1.7 g), which was subsequently partitioned between *n*-BuOH (2 × 40 mL) and H_2O (50 mL). Bioassay analysis localized the active agents in the *n*-BuOH solubles (1.3 g), which was sequentially triturated with *n*-hexane (6 × 5 mL), CH_2Cl_2 (4 × 5 mL) and MeOH (2 × 5 mL) and concentrated *in vacuo* to yield 93 mg, 211 mg and 873 mg solvent triturations respectively.

The CH_2Cl_2 solubles were eluted through a normal phase Biotage SNAP cartridge using gradient elution from 92% *n*-hexane/EtOAc to 34% *n*-hexane/EtOAc to return fractions I (6.4 mg), II (143 mg) and III (62 mg). A portion (80 mg) of fraction II was subjected to HPLC fractionation (Zorbax SB-C_8 semi-preparative column, 4.0 mL min^{-1} gradient elution from 25% H_2O/MeCN to 15% H_2O/MeCN over 15 min) to yield, in order of elution, spiralisone B (**2**, 38.8 mg), spiralisone D (**4**, 2.0 mg), spiralisone A (**1**, 2.3 mg), chromone **6** (11.7 mg), spiralisone C (**3**, 1.1 mg), and chromone **5** (1.2 mg). Fraction III (62 mg) was subjected to SPE fractionation (Alltech C_{18} SPE cartridge using a 10 mL stepwise gradient elution from 50% H_2O/MeOH to 100% MeOH) to return fractions IIIA–G. Fraction IIIB (5.1 mg) was further fractionated by HPLC (Zorbax SB-C_8 semi-preparative column, 4.2 mL min^{-1} gradient elution from 70% H_2O/MeCN to 60% H_2O/MeCN over 15 min) to yield apo-9′-fucoxanthinone (**7**, 0.5 mg).

(*Note* – metabolite % yields were calculated as a weight to weight estimate against the total *n*-BuOH solubles (1.3 g); **1** (0.32%), **2** (5.3%), **3** (0.15%), **4** (0.28%), **5** (0.17%), **6** (1.6%) and **7** (0.04%).)

Spiralisone A (**1**). Off-white solid; $[\alpha]_D^{22}$ +0.9 (*c* 0.23, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 227 (4.28) and 288 (4.20) nm; ^1H and ^{13}C NMR ($CDCl_3$, 600 MHz) Table 1 and ESI Table S1;† ESI(+)MS *m*/*z* 455 $[M + H]^+$, ESI(−)MS *m*/*z* 453 $[M - H]^-$; HR-ESI(+)MS *m*/*z* 477.2617 $[M + Na]^+$ (calcd for $C_{28}H_{38}O_5Na$, 477.2611).

Spiralisone B (**2**). Off-white solid; $[\alpha]_D^{22}$ +0.3 (*c* 0.5, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 227 (4.13) and 288 (4.20) nm; ^1H and ^{13}C NMR ($CDCl_3$, 600 MHz) Table 1 and ESI Table S2;† ESI(+)MS *m*/*z* 453 $[M + H]^+$ and 475 $[M + Na]^+$, ESI(−)MS *m*/*z* 451 $[M - H]^-$; HR-ESI(+)MS *m*/*z* 475.2461 $[M + Na]^+$ (calcd for $C_{28}H_{36}O_5Na$, 475.2455).

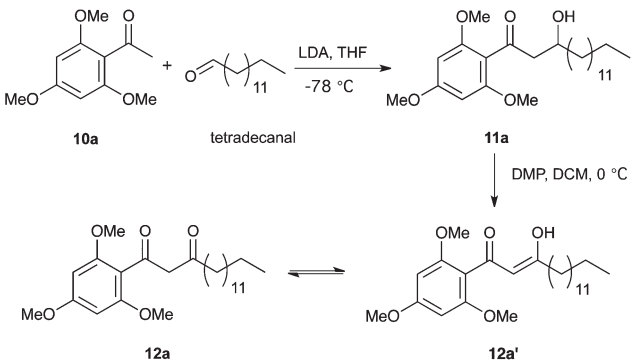
Spiralisone C (**3**). Off-white solid; $[\alpha]_D^{22}$ −4.4 (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 226 (4.02) and 288 (4.04) nm; ^1H and ^{13}C NMR ($CDCl_3$, 600 MHz) Table 1 and ESI Table S3;† ESI(+)MS *m*/*z* 407 $[M + H]^+$, ESI(−)MS *m*/*z* 405 $[M - H]^-$; HR-ESI(+)MS *m*/*z* 429.2606 $[M + Na]^+$ (calcd for $C_{24}H_{38}O_5Na$, 429.2611).

Spiralisone D (**4**). Off-white solid; $[\alpha]_D^{22}$ +0.8 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 225 (4.22) and 288 (4.23) nm; ^1H and ^{13}C NMR ($CDCl_3$, 600 MHz) Table 1 and ESI Table S4;† ESI(+)MS *m*/*z* 379 $[M + H]^+$, ESI(−)MS *m*/*z* 377 $[M - H]^-$; HR-ESI(+)MS *m*/*z* 401.2305 $[M + Na]^+$ (calcd for $C_{22}H_{34}O_5Na$, 401.2298).

5,7-Dihydroxy-2-tridecanyl chromone (**5**).[9] Off-white solid; UV (MeOH) λ_{max} (log ε) 227 (4.14), 248 (4.14), 255 (4.12) and 292 (3.79) nm; ^1H and ^{13}C NMR ($CDCl_3$, 600 MHz) ESI Table S5;† ESI(+)MS *m*/*z* 361 $[M + H]^+$, ESI(−)MS *m*/*z* 359 $[M - H]^-$; HR-ESI(+)MS *m*/*z* 383.2185 $[M + Na]^+$ (calcd for $C_{22}H_{32}O_4Na$, 383.2193).

5,7-Dihydroxy-2-(4*Z*,7*Z*,10*Z*,13*Z*,16*Z*-nonadecapentaenyl) chromone (**6**).[10] Off-white solid; UV (MeOH) λ_{max} (log ε) 221 (4.12), 238 (4.12), 245 (4.10) and 279 (3.76) nm; ^1H and ^{13}C NMR ($CDCl_3$, 600 MHz) ESI Table S6;† HR-ESI(+)MS *m*/*z* 435.2527 $[M + H]^+$ (calcd for $C_{28}H_{35}O_4$, 435.2530).

Apo-9′-fucoxanthinone (**7**).[11] Off-white solid; $[\alpha]_D^{22}$ −23.0 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.40), 218 (3.47) nm; ^1H and ^{13}C NMR (CD_3OD, 600 MHz) ESI Table S7;† HR-ESI(+)MS *m*/*z* 289.1402 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_4Na$, 289.1410).

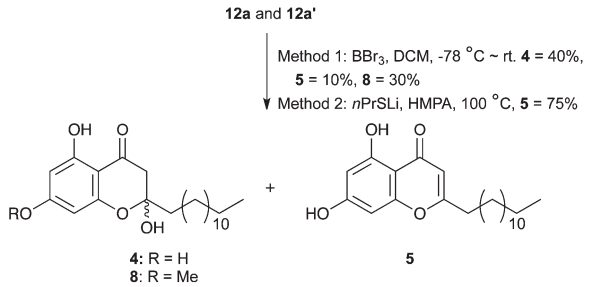


Synthetic studies

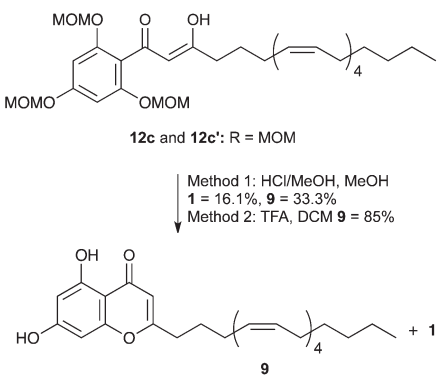
General procedures of the synthesis of keto–enol tautomers

*Keto–enol (**12a**, **12a'**).* LDA (0.5 mL, 1.0 mmol, 2.0 M in THF) was added to a solution of 2-acetyl-1,3,5-trimethoxybenzene **10a** (100 mg, 0.5 mmol) in THF (15 mL) at −78 °C, and the reaction mixture was stirred at this temperature for 30 min. Tetradecanal (303 mg, 1.4 mmol in THF) was then added dropwise and the resulting reaction mixture was stirred for a further 3 h at −78 °C before quenching by addition of a saturated aqueous solution of NH_4Cl (5 mL). **11a** was subsequently obtained by extraction with EtOAc (3 × 5 mL), drying over anhydrous $MgSO_4$ and concentration *in vacuo*. The crude sample of **11a** was dissolved in CH_2Cl_2 (10 mL) at 0 °C without further purification, and following the addition of DMP (212 mg, 0.5 mmol) the reaction mixture was stirred at 0 °C for 3 h before quenching by addition of saturated aqueous solutions of Na_2SO_3 (5 mL) and $NaHCO_3$ (5 mL). The quenched reaction mixture was extracted with CH_2Cl_2 (3 × 5 mL), and the

combined organic phases was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to yield, after silica gel chromatography (EtOAc/hexane = 1 : 6), the keto–enol equilibrium mixture (**12a**, **12a′**) (150 mg, 75% for 2 steps) as a white solid.



Spiralisone D (**4**) and chromone (**5**). Two different deprotection/cyclization methods were employed. (1) BBr_3 (1.4 mL, 1.4 mmol, 1 M in CH_2Cl_2) was added dropwise to a solution of keto–enol (**12a**, **12a′**, 12 mg, 0.03 mmol) in dry CH_2Cl_2 (5 mL) at −78 °C, and the reaction mixture was allowed to reach ambient temperature overnight before quenching with water at 0 °C. The quenched reaction mixture was extracted with CH_2Cl_2 (3 × 5 mL), and the combined organic phases was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The resulting residue was fractionated by HPLC (Zorbax SB-C_8 semi-preparative column, gradient elution at 3.5 mL min^{-1} over 15 min from 30% H_2O/MeCN to 100% MeCN) to yield **4** (4.3 mg, 40%) and **5** (1.0 mg, 10%) whose spectral data were in accord with those of the natural products, and **8** (3.4 mg, 30%). (2) Lithium propane-1-thiolate (0.48 mL, 0.24 mmol, 0.5 M in HMPA) was added to a solution of keto–enol (**12a**, **12a′**, 10 mg, 0.024 mmol) in dry HMPA (0.1 mL), and the reaction mixture was maintained at 100 °C overnight before quenching with HCl (4 ml, 0.4 mmol, 0.1 M). The resulting mixture was extracted with CH_2Cl_2 (3 × 5 mL), and the combined organic phases was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The resulting residue was fractionated by HPLC (Zorbax SB-C_8 semi-preparative column, gradient elution 3.5 mL min^{-1} over 15 min, from 30% H_2O/MeCN to 100% MeCN) to yield **5** (6.4 mg, 75%).



Spiralisone A (**1**) and chromone (**9**). Following the general procedure of synthesis of keto–enol tautomers, keto–enol (**12c**, **12c′**, 67%) were prepared. Two different deprotection/cyclization methods were employed. (1) HCl (545 μL, 0.68 mmol, 1.25 M in MeOH) was added to a solution of keto–enol (**12c**, **12c′**, 8.0 mg, 0.013 mmol) in MeOH (1 mL), and the reaction mixture was stirred overnight. After removal of MeOH *in vacuo*, the residue was fractionated by HPLC (Zorbax SB-C_8 semi-preparative column, gradient elution 3.5 mL min^{-1} over 15 min from 30% H_2O/MeCN to 100% MeCN) to yield **1** (1.0 mg, 16.1%) whose spectral data were in accord with those of the natural product, and the corresponding chromone **9** (2.0 mg, 33.3%). (2) TFA (5.2 μL, 0.68 mmol) was added to a solution of keto–enol (**12c**, **12c′**, 8.0 mg, 0.013 mmol) in CH_2Cl_2 (1 mL), and then the reaction mixture was stirred for 2 h. After removal of CH_2Cl_2 *in vacuo*, the residue was fractionated by HPLC (Zorbax SB-C_8 semi-preparative column, gradient elution 3.5 mL min^{-1} over 15 min from 30% H_2O/MeCN to 100% MeCN) to yield **9** (5.0 mg, 85.0%) as colorless oil.

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