



Kent Academic Repository

Ojike, Fredrick O., Lavignac, Nathalie and Casely-Hayford, Maxwell A. (2018) *Synthesis and in vitro Bioactivity of Polyunsaturated Fatty Acid Conjugates of Combretastatin A-4*. *Journal of Natural Products*, 81 . pp. 2101-2105. ISSN 0163-3864.

Downloaded from

<https://kar.kent.ac.uk/69154/> The University of Kent's Academic Repository KAR

The version of record is available from

<https://doi.org/10.1021/acs.jnatprod.7b01062>

This document version

Author's Accepted Manuscript

DOI for this version

Licence for this version

UNSPECIFIED

Additional information

Versions of research works

Versions of Record

If this version is the version of record, it is the same as the published version available on the publisher's web site. Cite as the published version.

Author Accepted Manuscripts

If this document is identified as the Author Accepted Manuscript it is the version after peer review but before type setting, copy editing or publisher branding. Cite as Surname, Initial. (Year) 'Title of article'. To be published in *Title of Journal*, Volume and issue numbers [peer-reviewed accepted version]. Available at: DOI or URL (Accessed: date).

Enquiries

If you have questions about this document contact ResearchSupport@kent.ac.uk. Please include the URL of the record in KAR. If you believe that your, or a third party's rights have been compromised through this document please see our [Take Down policy](https://www.kent.ac.uk/guides/kar-the-kent-academic-repository#policies) (available from <https://www.kent.ac.uk/guides/kar-the-kent-academic-repository#policies>).

Synthesis and in vitro Bioactivity of Polyunsaturated Fatty Acid Conjugates of Combretastatin A-4

Fredrick O. Ojike, Nathalie Lavignac* and Maxwell A. Casely-Hayford*

Medway School of Pharmacy, University of Kent, Central Avenue, Chatham Maritime, ME4 4TB, United Kingdom

ABSTRACT: Combretastatin A-4 (CA-4) (**1**) is a plant-derived anticancer agent binding to the tubulin colchicine site. Polyunsaturated fatty acids (PUFA) are readily taken up by cancer cells and have been used to improve cell targeting. In the present study, four CA-4-PUFA conjugates were synthesized by coupling combretastatin A-4 (**1**) with several polyunsaturated fatty acids. The conjugates (**2a-d**) were characterized using spectroscopic methods. Their cytotoxicity was evaluated against human breast cancer cells (MCF-7) and the inhibition of tubulin polymerization was determined in vitro. All conjugates influenced tubulin polymerization with the arachidonic acid conjugate (**2c**) displaying cytotoxicity similar in potency to the natural product CA-4 (**1**).

Microtubules are polymeric intracellular structures composed of tubulin and are important for a variety of cellular functions, including intracellular transport, maintenance of cell shapes and cell division.¹ During cell division, microtubules form the mitotic spindle in which the dynamics are highly sensitive to microtubule-binding agents. Modification of microtubule dynamics has been among the most promising approaches in the search for new and more efficient cytotoxic drugs and has led to the development of tubulin-binding agents such as the taxanes and vinca alkaloids, which stabilize or destabilize microtubules.² However, the search for new agents demonstrating improved cancer cell specificity, less neurotoxicity and increased efficacy in chemoresistant cancer cells is still ongoing.³ Combretastatins hold considerable promise in this regard, offering a potential wider therapeutic window or activity against multi-drug-resistant cancer cells,⁴ and much effort has been made to develop them as novel tubulin-binding agents for cancer chemotherapy.

The combretastatins are a class of natural stilbene derivatives isolated from the bark of the South African bush willow tree *Combretum caffrum* Kuntze (Combretaceae).⁵ The *cis*-stilbenoid derivative combretastatin A-4 (CA-4, **1**) (Figure 1) was found to be the most potent among this class of natural products⁶ and the search for more effective analogues has led to the development of numerous derivatives,^{4c, 7} including amino derivatives⁸ such as combretastatin A-4 amine and its serine prodrug AVE8062, which has shown improved pharmacological activities when compared to the natural product. Water-soluble analogues such as combretastatin A-4 phosphate,⁹ and combretastatin A-1 phosphate¹⁰ have also been developed and have been used in different clinical trials either on their own or as part of combination therapy. Mechanistically, CA-4 and its derivatives are potent inhibitors of microtubule polymerization, leading to arrest in the G₂/M-phase and subsequent apoptotic cell death.¹¹ In addition to their cytotoxic effects on cancer cells,^{4c, 7b, 11a} combretastatins exert a selective effect on proliferating endothelial cells leading to a substantial vascular-disruptive activity on tumor blood vessels.^{4b, 6, 12}

Although CA-4 and its analogues have demonstrated their potential as cancer chemotherapeutic agents, they are not completely devoid of some of the problems associated with treatments using cytotoxic vascular-disrupting agents. Indeed, cardiac toxicity has been identified as a dose-limiting toxicity for CA-4 analogues in several phase I clinical trials.^{9c, 11a, 13} Selective tumor targeting of cytotoxic agents is one of the most effective protocols developed to alleviate many of the unwanted side effects encountered with untargeted chemotherapy.¹⁴ Developing a method to selectively deliver combretastatins into cancer cells could minimize some of the associated side effects.

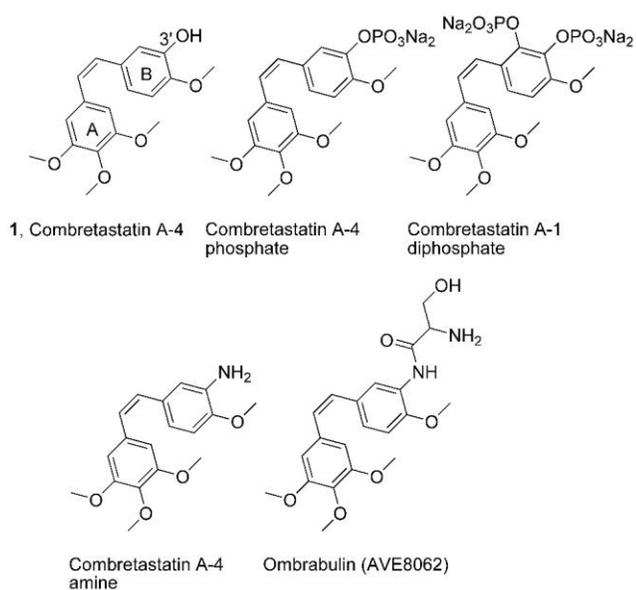


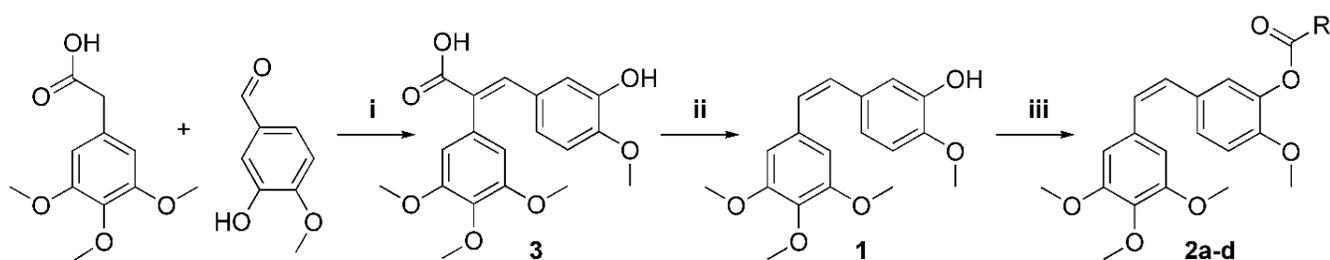
Figure 1. Structures of Combretastatin-type Anti-Tubulin Agents

Polyunsaturated fatty acids (PUFAs) are important nutritional constituents of dietary vegetable oils and cold water fish and therefore are considered to be safe. Studies have shown that there is a selective uptake of PUFAs into cancerous cells compared to healthy cells and that the incorporation of PUFAs into the lipid bilayer of the cancer cells disrupts their membrane structure and fluidity, which as a result seems to modify their chemosensitivity.¹⁵ In the context of targeted therapies, conjugation of PUFAs to

conventional cytotoxic drugs has been a promising strategy ¹⁶ with Taxoprexin (paclitaxel/docosahexaenoic acid) reaching phase III clinical trials.¹⁷ Applying this same general concept to combretastatins could minimize some of the unwanted side effects and improve their therapeutic indices.

Herein are reported the synthesis of four combretastatin A-4/polyunsaturated fatty acid (CA-4-PUFA, **2a-d**) conjugates (Scheme 1) and their biological effects on human breast cancer cells (MCF-7) and microtubule polymerization.

Scheme 1. Synthesis of Combretastatin A-4-PUFA Conjugates



compound	R	yield
2a		82%
2b		82%
2c		90%
2d		57%

(i) Ac₂O, TEA, reflux, 3 h (ii) Cu, C₉H₇N, 210 °C, 2 h (iii) linoleic acid (**2a**), linolenic acid (**2b**), arachidonic acid (**2c**), docosahexaenoic acid (**2d**), DIC, DMAP, CH₂Cl₂, r.t., overnight.

Studies have shown that the *cis* configuration of the stilbene unit is required for highest compound cytotoxic potency.^{7b, 18} Z-Combretastatin A-4 (**1**) was therefore synthesized, in a 41% overall yield, using the stereoselective Perkin condensation method of Gaukroger et al. with subsequent decarboxylation ¹⁸

(Figures S1 and S2, Supporting Information). The alkene bridge proton signals occurred as two doublets at 6.47 ppm and 6.38 ppm ($J = 12.0$ Hz), which is distinctive of vicinal olefinic protons and indicative of a *cis*-stilbene (Figure S2a, Supporting Information). Indeed, *cis* coupling is expected between 6 and 12 Hz and *trans* between 12 and 18 Hz. Further data on the geometrical isomerism was gained from the UV spectra of CA-4 (**1**) and compound **3**. Beale et al. and others have shown that *trans* and *cis* stilbenes can be identified by UV spectroscopy.¹⁹ Extinction coefficients ($\log \epsilon$) obtained from UV spectra are much greater for stilbenes with structures where the aromatic moieties are *trans* to each other ($\log \epsilon \sim 4.48$) compared with those with *cis* aromatic rings ($\log \epsilon \sim 4.00$).¹⁹ Extinction coefficients were calculated for intermediate **3** and CA-4 (**1**) from their UV spectrum (Figures S1d and S2e, Supporting Information) and were found to be $\log \epsilon = 4.13$ and $\log \epsilon = 4.03$, respectively. This is in agreement with the literature and confirmed that the synthesized CA-4 (**1**) has a *cis* configuration. With CA-4 in hand, conjugation to PUFAs was accomplished using the Steglich esterification method to give CA-4-PUFA conjugates (**2a-d**) with yields between 57-90%. The synthesized compounds were characterized by HRMS, ¹H NMR, ¹³C NMR and IR spectroscopy (Figures S3-S6, Supporting Information).

Combretastatin A-4 has demonstrated some cytotoxic activity towards various cancer cells.^{4a, 7b, 11a} Interference with the cellular microtubule dynamics generally leads to cell-cycle arrest and cell death via mitotic catastrophe, although other studies have demonstrated an apoptotic effect.⁶ The cytotoxicity of compounds **2a-2d** was evaluated against the MCF7 human breast cancer cell line using an MTS assay (Figure 2). The cells were treated with different concentrations of each substance (0 – 10 μ M) and their metabolic activity was evaluated after 72 h. The antiproliferative activity of CA-4 (**1**) was confirmed with an IC_{50} value of 0.2 μ M, in good agreement with the literature.²⁰ One of the first PUFA conjugates was developed by Bradley et al.^{16a} who conjugated docosahexaenoic acid (DHA) to paclitaxel. In vitro, the PUFA analogue was less active by three orders of magnitude. However, in vivo, the conjugate

demonstrated increased antitumor activity when compared with paclitaxel due to an improved pharmacokinetic profile. Since then, other potential PUFA anticancer agents have been synthesized (e.g. curcumin, phenstatin, doxorubicin) and have displayed various activities against cancer cells.^{16b-e, 21}

In the present study, four CA-4 conjugates were synthesized using four different PUFA derivatives (i.e., linoleic acid, linolenic acid, arachidonic acid and docosahexaenoic acid) (Scheme 1). The arachidonic acid conjugate (**2c**) displayed the highest in vitro activity and showed some concentration dependent potency similar to the natural product (**1**) with an IC₅₀ of 0.7 μM. The other conjugates (**2a**, **2b** and **2d**) also displayed some cytotoxicity (~ 65-75% cell viability) but at higher concentrations (5-10 μM) (Figure 2). These results are consistent with the reduction of in vitro activity also observed for other PUFA conjugates when compared to the free lead compounds.^{16a, 16c, 16e, 21}

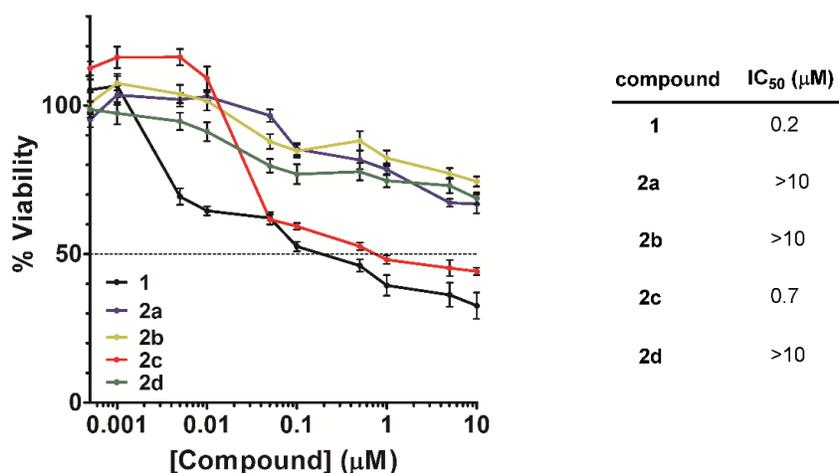


Figure 2. Antiproliferative effects of CA-4 and PUFA analogues for MCF-7 human breast cancer cells. The cells (5,000 cells/well) were treated with different concentrations of the test compounds [combretastatin A-4 (**1**), linoleic acid conjugate (**2a**); linolenic acid conjugate (**2b**); arachidonic acid conjugate (**2c**) and docosahexaenoic acid conjugate (**2d**)] and their metabolic activity was assessed after 72 h using an MTS assay. Viability of the MCF-7 cells was determined relative to untreated cells. Mean values \pm SEM, $n = 18$.

The cytotoxicity of CA-4 (**1**) has been associated generally with the inhibition of microtubule formation due to the binding of the compound to the colchicine binding site on β -tubulin². Structure-activity studies have identified chemical features linked to the activity of the molecule.^{4a, 5} Whereas the *cis* configuration and the trimethoxybenzene moiety on the A ring are essential for high cytotoxicity and efficient tubulin binding, the B ring is amenable to structural modifications. Indeed, studies have indicated that the 4-methoxy substituent is important but that the substituent at the C-3' position (Figure 1) can be modified without loss of activity.^{4a, 7b, 8, 22} In this context, some CA-4 derivatives have displayed cytotoxicity without any *in vitro* tubulin activity.^{7b, 7d, 23} This has also been observed with DHA paclitaxel conjugates.^{16a}

To determine if the antiproliferative activities of the conjugates produced herein correlated with their interaction with β -tubulin, the effect of the synthesized CA-4-PUFAs on tubulin polymerization/depolymerization was determined in vitro, using a turbidity assay^{20b} (Figure 3). The amount of tubulin polymerized is directly proportional to the area under the curve (AUC). To quantify the conjugates' effect on tubulin polymerization when compared to CA-4 (**1**), the AUCs of the samples were normalized to that of combretastatin A-4. Values above 1.0 indicated a lower inhibition of microtubule polymerization. At low concentration (1 μ M), all conjugates displayed a profile similar to **1** (Figures 3a and 3c). The lag time for the onset of the tubulin polymerization was between 15-20 min, followed by a similar rate of microtubule propagation. These results also indicated that, contrary to DHA paclitaxel, the in vitro toxicity of **2c**, which displayed similar activity to **1** (Figure 2), seems to correlate with its interference with tubulin polymerization. At the highest concentration (10 μ M), it was apparent that all compounds displayed a lower inhibitory activity compared to CA-4 (**1**) (Figures 3b and 3c).

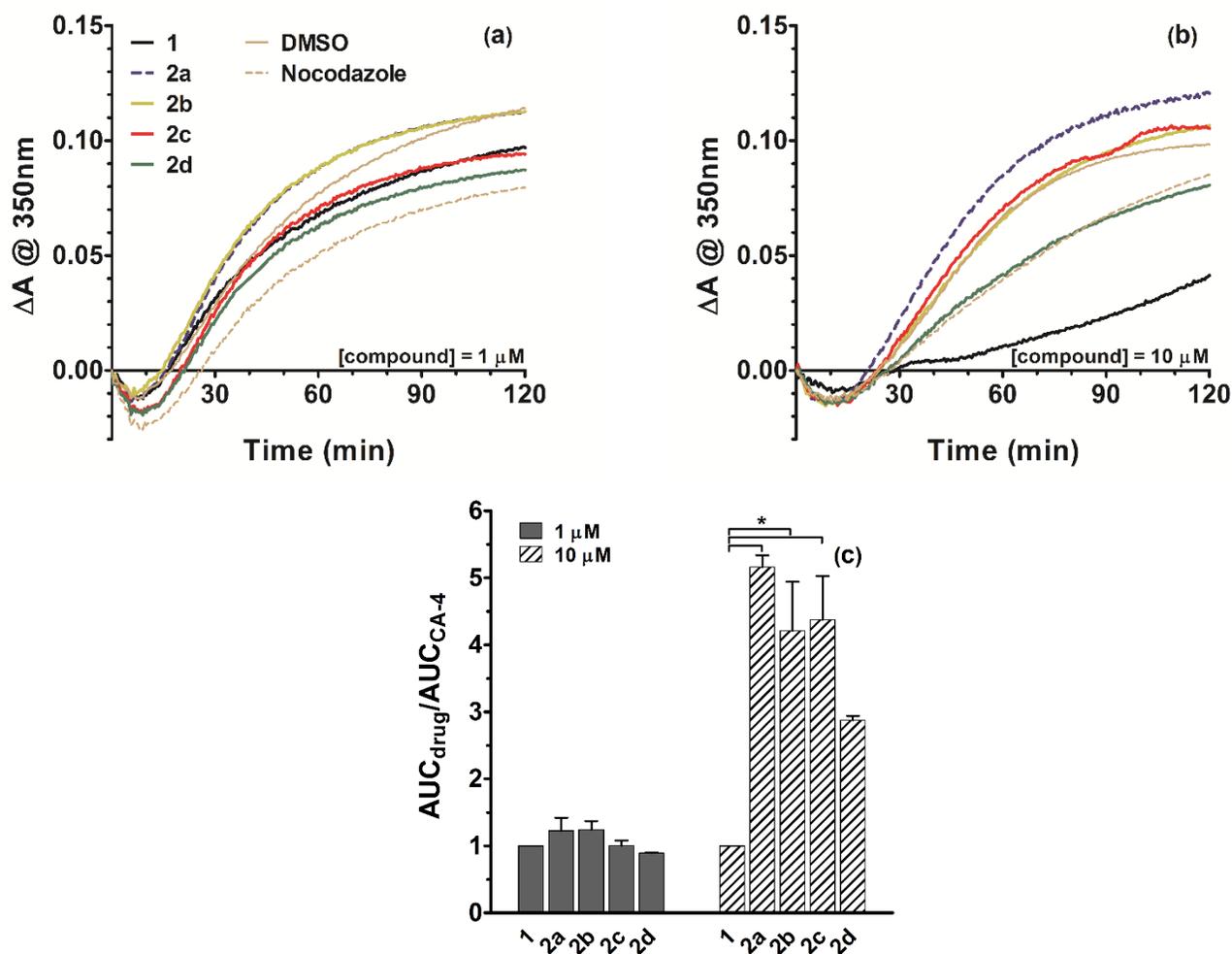


Figure 3. In vitro tubulin polymerization curves in the presence of combretastatin A-4 (**1**) and combretastatin A-4 PUFA analogues at concentrations of 1 μM (a) and 10 μM (b). Area under the curves of the conjugates (* $p < 0.05$) (c). [Combretastatin A4 (CA-4, **1**), CA-4 linoleic acid conjugate (**2a**); CA-4 linolenic acid conjugate (**2b**); CA-4 arachidonic acid conjugate (**2c**) and CA-4 docosahexaenoic acid conjugate (**2d**)]. Tubulin polymerization ([Tubulin] = 26.7 μM (2.94 mg/mL)) was followed at 37 $^{\circ}\text{C}$ by measuring the turbidity variation at 350 nm every 30 sec for 2 h. Nocodazole and DMSO were used as controls. Assays were performed in duplicate. Statistical analysis: one way analysis of variance with Bonferroni post-test.

EXPERIMENTAL SECTION

General Experimental Procedures. Reactions were carried out in oven-dried glassware and moisture-sensitive procedures were performed under N₂. Melting points were measured using capillary tubes on a Stuart SMP3 instrument and are uncorrected. UV spectra were acquired on a Shimadzu UV-1700 spectrophotometer. The extinction coefficients of **3** (4.16 x10⁻⁵ M) and **1** (3.55 x10⁻⁵ M) were determined at the maximum wavelength using methanol as the solvent. IR spectra were obtained on a Perkin Elmer Spectrum One FTIR spectrometer (ATR). ¹H NMR spectra and ¹³C NMR spectra were obtained on a Varian NMR spectrometer at 400 MHz and 101 MHz or 500 MHz and 126 MHz, respectively. Chemical shifts are relative to TMS. Exact mass measurements and CHN analysis were performed at University College London, School of Pharmacy, using a Micromass Q-TOF Premier Tandem mass spectrometer and a Carlo-Erba EA 1108 apparatus respectively. Thin-layer chromatography (silica gel 60 UV₂₅₄ on aluminum plates) was used to monitor reactions and was observed under UV light (254 nm and 365 nm), or visualized by phosphomolybdic acid (0.1 mg.mL⁻¹ in absolute ethanol) with heating. Column chromatography was performed using Merck silica gel 60 (230-400 mesh).

(E)-3-(3'-Hydroxy-4'-methoxyphenyl)-2-(3'',4'',5''-trimethoxyphenyl)prop-2-enoic acid (3). Compound **3** was synthesized using the method developed by Gaukroger et al.¹⁸ A mixture of 3,4,5-trimethoxyphenylacetic acid (2.00 g, 8.84 mmol), 3-hydroxy-4-methoxybenzaldehyde (0.6 g, 4.4 mmol), acetic anhydride (4 mL), and triethylamine (2 mL) was heated under reflux for 3 h. After acidification with concentrated hydrochloric acid (6 mL), the resulting solid was filtered off and recrystallized from ethanol to give the propenoic acid derivative (**3**) as fine yellow needles (950 mg, 60%): yellow needles (EtOH); mp 237–239 °C; UV (MeOH) λ_{max} (log ε) 324 (4.13) nm; IR (ATR) ν_{max} 3320, 2941, 1667, 1608, 1586, 1504 cm⁻¹; ¹H NMR (DMSO-*d*₆, 500 MHz) δ 12.43 (1H, s, COOH), 8.95 (1H, s, ArOH), 7.57 (1H, s), 6.81 (1H, d, *J* = 8.5 Hz), 6.61 (1H, dd, *J* = 8.6, 2.3 Hz), 6.54 (1H, d, *J* = 2.3 Hz), 6.44 (2H, s), 3.73

(3H, s), 3.72 (3H, s), 3.69 (6H, s); ^{13}C NMR (DMSO- d_6 , 126 MHz) δ 168.6, 153.1, 148.9, 145.9, 139.1, 137.0, 132.2, 130.4, 127.1, 122.98, 117.2, 111.5, 106.7, 60.2, 56.0, 55.5; ESIMS m/z 361.2 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{19}\text{H}_{21}\text{O}_7^+$, 360.1).

***cis*-Combretastatin A-4 (1)**. Compound **3** (2.00 g, 5.56 mmol) was added to powdered copper (1.84 g, 28.95 mmol) in quinoline (20 mL, 170 mmol) and the resulting mixture was heated at 210 °C for 2 h. Upon cooling, ether (50 mL) was added and the copper was filtered off through Celite. The filtrate was washed with 1 M hydrochloric acid and the aqueous layer was recovered and further extracted with diethylether. The combined organic layers were washed with saturated sodium carbonate solution, water, and brine, dried over MgSO_4 and concentrated under a vacuum. Column chromatography (25% ethyl acetate in hexane) and recrystallization from ethyl acetate and hexane afforded *cis*-combretastatin A-4 (**1**) as a pale yellow crystalline solid (1.19 g, 68%): yellow solid ($\text{C}_4\text{H}_8\text{O}_2/\text{C}_6\text{H}_{14}$); mp 118–119 °C; UV (MeOH) λ_{max} (log ϵ) 299 (4.03) nm; IR (ATR) ν_{max} 3505, 3288, 2995, 2940, 2839, 1579, 1504 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) δ 6.92 (1H, d, $J = 2.0$ Hz), 6.79 (1H, dd, $J = 2.0, 8.0$ Hz), 6.73 (1H, d, $J = 8.0$ Hz), 6.53 (2H, s), 6.48-6.40 (2H, dd, $J = 12.0, 12.0$ Hz), 5.53 (1H, s) 3.86 (3H, s), 3.84 (3H, s), 3.70 (6H, s); ^{13}C NMR (CDCl_3 , 101 MHz) δ 152.8, 145.8, 137.2, 132.7, 130.6, 129.1, 121.1, 115.1, 110.3, 106.1, 60.9, 56.0; ESIMS m/z 317.3 $[\text{M}+\text{H}]^+$; HRESIMS m/z 317.1372 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{18}\text{H}_{21}\text{O}_5^+$, 317.1389).

Linoleic Acid Conjugate (2a). General method A: A solution of combretastatin A-4 (**1**) (0.15 g, 0.47 mmol), linoleic acid (0.324 g, 1.16 mmol) and *N, N*-diisopropylcarbodiimide (0.183 mL, 1.18 mmol) in dry CH_2Cl_2 (6 mL) was stirred for 15 min under N_2 . 4-Dimethylaminopyridine (0.017 g, 0.141 mmol) in minimal CH_2Cl_2 was added and the reaction was stirred overnight at room temperature under N_2 . The reaction mixture was transferred into a separating funnel and 20 mL of CH_2Cl_2 were added, followed by washing with water (2 x 20 mL). The organic layer was collected and the aqueous layer further extracted with dichloromethane (3 x 20 mL). The combined organic layers were dried over anhydrous MgSO_4 ,

filtered and concentrated under vacuum. Column chromatography (40% ethyl acetate - 60% hexane) afforded the conjugate (**2a**) as an oil (263 mg, 82% yield): IR (ATR) ν_{\max} 3006, 2927, 2865, 1763, 1577, 1508 cm^{-1} ; ^1H NMR (CDCl_3 , 500 MHz) δ 7.11 (1H, d, $J = 5$ Hz), 7.00 (1H, d, $J = 0.15$ Hz), 6.84 (1H, d, $J = 8.5$ Hz), 6.50 (2H, s), 6.45 (2H, dd, $J = 12.2$ Hz), 5.39–5.32 (4H, m), 3.83 (3H, s), 3.79 (3H, s), 3.70 (6H, s), 2.77 (2H, t, $J = 6.5$ Hz), 2.52 (2H, t, $J = 7.5$ Hz), 2.07–2.03 (4H, m), 1.72 (2H, quint., $J = 7.4$ Hz), 1.38–1.28 (14H, m), 0.89 (3H, t, $J = 6.8$ Hz); ^{13}C NMR (CDCl_3 , 126 MHz) δ 171.8, 153.1, 150.4, 139.7, 137.3, 132.6, 130.3, 130.2, 129.6, 128.7, 128.2, 128.0, 127.7, 123.3, 112.1, 106.0, 61.0, 56.0, 34.1, 31.6, 29.7, 29.5, 29.28, 29.25, 29.1, 27.3, 25.7, 25.1, 22.7, 14.2; ESIMS m/z 580 $[\text{M}+\text{H}]^+$; HRESIMS m/z 579.3658 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{36}\text{H}_{51}\text{O}_6^+$, 579.3686).

Linolenic Acid Conjugate (2b). Compound **2b** was prepared according to general method A using **1** (0.15 g, 0.47 mmol), linolenic acid (0.329 g, 1.18 mmol), *N,N*-diisopropylcarbodiimide (0.183 mL, 1.18 mmol), and 4-dimethylaminopyridine (0.017 g, 0.141 mmol). Compound **2b** was isolated as an oil (303 mg, 82% yield): IR (ATR) ν_{\max} 2930, 2864, 1826, 1763, 1613, 1579, 1508 cm^{-1} ; ^1H NMR (CDCl_3 , 500 MHz) δ 7.11 (1H, d, $J = 2.1$ Hz), 7.00 (1H, d, $J = 2.1$ Hz), 6.84 (1H, d, $J = 8.5$ Hz), 6.50 (2H, s), 6.45 (2H, dd, $J = 12.2$ Hz), 5.42–5.32 (6H, m), 3.83 (3H, s), 3.79 (3H, s), 3.70 (6H, s), 2.81 (4H, t, $J = 6.2$ Hz), 2.52 (2H, t, $J = 7.5$ Hz), 2.09–2.04 (4H, m), 1.71 (2H, quint., $J = 7.4$ Hz), 1.37–1.33 (8H, m), 0.97 (3H, t, $J = 7.5$ Hz); ^{13}C NMR (CDCl_3 , 126 MHz) δ 171.7, 153.1, 150.4, 137.3, 129.6, 128.7, 128.4, 127.7, 127.2, 123.3, 112.1, 104.0, 61.0, 56.0, 34.0, 29.7, 29.3, 29.1, 25.7, 25.1, 20.6, 14.4; ESIMS m/z 578 $[\text{M}+\text{H}]^+$; HRESIMS m/z 611.3134 $[\text{M}+\text{Cl}]^-$; (calcd for $\text{C}_{36}\text{H}_{48}\text{O}_6\text{Cl}^-$, 611.3140).

Arachidonic Acid Conjugate (2c). Compound **2c** was prepared according to general method A using **1** (0.045 g, 0.142 mmol), arachidonic acid (0.108 g, 0.355 mmol), *N,N*-diisopropylcarbodiimide (0.120 mL, 0.355 mmol), and 4-dimethylaminopyridine (0.011 g, 0.042 mmol). Compound **2c** was isolated as an oil (77 mg, 90% yield): IR (ATR) ν_{\max} 3010, 2929, 2856, 1763, 1613, 1579, 1508 cm^{-1} ; ^1H NMR (CDCl_3 ,

500 MHz) δ 7.11 (1H, d, $J = 2.1$ Hz), 7.00 (1H, d, $J = 2.2$ Hz), 6.84 (1H, d, $J = 8.5$ Hz), 6.50 (2H, s), 6.45 (2H, dd, $J = 12.2$ Hz), 5.42–5.34 (8H, m), 3.83 (3H, s), 3.79 (3H, s), 3.70 (6H, s), 2.84–2.79 (6H, m), 2.54 (2H, t, $J = 7.5$ Hz), 2.21–2.17 (2H, m), 2.07–2.03 (2H, m), 1.83 (2H, quint., $J = 7.5$ Hz), 1.36–1.28 (6H, m), 0.88 (3H, t, $J = 6.8$ Hz); ^{13}C NMR (CDCl_3 , 126 MHz,) δ 171.6, 153.1, 150.4, 139.6, 137.3, 132.6, 130.6, 130.2, 129.6, 129.0, 128.7, 128.4, 128.0, 127.7, 123.3, 112.1, 106.0, 61.0, 56.0, 33.5, 31.6, 29.4, 27.3, 26.6, 25.7, 25.0, 22.7, 14.2; ESIMS m/z 603 $[\text{M}+\text{H}]^+$; HRESIMS m/z 603.3676 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{38}\text{H}_{51}\text{O}_6^+$, 603.3686).

Docosaehaenoic Acid Conjugate (2d). Compound **2d** was prepared according to general method A using **1** (100 mg, 0.316 mmol), docosaehaenoic acid (100 mg, 0.304 mmol), *N,N*-diisopropylcarbodiimide (0.097 mL, 0.632 mmol), and 4-dimethylaminopyridine (11 mg, 0.042 mmol). Compound **2d** was isolated as an oil (113 mg, 57% yield): IR (ATR) ν_{max} 3011, 2963, 2934, 2838, 2118, 1764, 1579, 1508 cm^{-1} ; ^1H NMR (CDCl_3 , 500 MHz) δ 7.11 (1H, d, $J = 2.2$ Hz), 7.01 (1H, d, $J = 2.1$ Hz), 6.84 (1H, d, $J = 8.5$ Hz), 6.50 (2H, s), 6.45 (2H, dd, $J = 12.2$ Hz), 5.45–5.36 (12H, m), 3.83 (3H, s), 3.79 (3H, s), 3.70 (6H, s), 2.87–2.79 (10H, m), 2.60 (2H, t, $J = 7$ Hz), 2.51–2.47 (2H, m), 2.10–2.04 (2H, m), 0.88 (3H, t, $J = 7.5$ Hz); ^{13}C NMR (CDCl_3 , 126 MHz) δ 171.1, 153.1, 150.4, 139.6, 137.3, 132.5, 132.1, 130.2, 129.6, 129.5, 128.7, 128.4, 128.21, 128.17, 128.0, 127.8, 127.7, 127.1, 123.3, 112.1, 106.0, 61.0, 56.0, 33.9, 25.7, 22.9, 20.6, 14.4; ESIMS m/z 627 $[\text{M}^+]$; HRESIMS m/z 661.3284 $[\text{M}+\text{Cl}]^-$ (calcd for $\text{C}_{38}\text{H}_{50}\text{O}_6\text{Cl}^-$, 661.3296).

Cell Viability Assays. MCF-7 cells were cultured in DMEM supplemented with 10% heat inactivated FBS, penicillin, streptomycin, L-glutamine and 1 x MEM NEAA at 37 °C in a humidified incubator with a 5% CO_2 -containing atmosphere. Stock solutions of the compounds (**1**, **2a-d**) were prepared in DMSO and diluted in DPBS to appropriate concentrations (0 - 210 μM). MCF-7 cells (5000 cells/well) were seeded into 96-well plates. After 24 h, the medium was replaced by 100 μL of fresh medium and 5 μL of

appropriate compound diluted solutions (final: 0 - 10 μM). After 72 h incubation at 37 $^{\circ}\text{C}$, the supernatants were removed and fresh medium without FBS was added (200 μL). MTS/PMS reagent (10 μL ; 2/0.92 mg/mL in DPBS) was added and the cells were left at 37 $^{\circ}\text{C}$ for 1 h. Absorbance was read at 490 nm on an Infinite M200 Pro spectrophotometer from Tecan. Viability was expressed as percentage of untreated control cells. IC_{50} values were determined graphically. Assays were performed using six replicates in three independent experiments.

Tubulin Assays. The assay was based on a Millipore tubulin polymerization kit. Stock solutions of the compounds (**1**, **2a-d**) were prepared in DMSO (0.7 mM) and diluted to 70 μM (or 7 μM) in deionized water. Fresh G-PB buffer was prepared by mixing 150 μL of polymerization buffer (5x PB) with 5 μL of 200 mM GTP in deionized water (598 μL) and glycerol (117 μL). All solutions were kept on ice. Then 10 μL of each compound diluted solutions were added to an ice-chilled 96-well plate (half-area). Next, 130 μL of tubulin solution (240 μM) was added to the G-PB buffer (870 μL) and 60 μL of this diluted tubulin solution were added to each well. Tubulin polymerization was followed at 37 $^{\circ}\text{C}$ by measuring the turbidity variation at 350 nm every 30 s for 2 h. Nocodazole and DMSO (1.43% or 0.14% v/v) were used as controls. Assays were performed in duplicate.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: XXX.

Copies of UV, IR, ^1H NMR, ^{13}C NMR and HRMS spectra (pdf)

AUTHOR INFORMATION

Corresponding Authors

* Tel (M. Casely-Hayford) +44 (0)1634 20 2947. Fax: +44 (0)1634 88 3927. E-mail: M.A.Casely-Hayford@kent.ac.uk - Tel (N. Lavignac): +44 (0)1634 88 8828. Fax: +44 (0)1634 88 3927. E-mail: N.Lavignac@kent.ac.uk

ORCID

Maxwell A. Casely-Hayford: 0000-0003-4054-4371

Nathalie Lavignac: 0000-0002-9259-7603

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We would like to thank the University of Greenwich for providing access to the NMR and MS facilities.

REFERENCES

- (1) Muroyama, A.; Lechler, T. *Development* **2017**, *144*, 3012-3021.
- (2) Kingston, D. G. *J. Nat. Prod.* **2009**, *72*, 507-515.
- (3) Fanale, D.; Bronte, G.; Passiglia, F.; Calo, V.; Castiglia, M.; Di Piazza, F.; Barraco, N.; Cangemi, A.; Catarella, M. T.; Insalaco, L.; Listi, A.; Maragliano, R.; Massihnia, D.; Perez, A.; Toia, F.; Cicero, G.; Bazan, V. *Anal. Cell. Pathol.* **2015**, 690916.
- (4) (a) Woods, J. A.; Hadfield, J. A.; Pettit, G. R.; Fox, B. W.; McGown, A. T. *Br. J. Cancer* **1995**, *71*, 705-711. (b) Tozer, G. M.; Kanthou, C.; Baguley, B. C. *Nat. Rev. Cancer* **2005**, *5*, 423-435. (c) Lee, H.

Y.; Chang, J. Y.; Nien, C. Y.; Kuo, C. C.; Shih, K. H.; Wu, C. H.; Chang, C. Y.; Lai, W. Y.; Liou, J. P. *J. Med. Chem.* **2011**, *54*, 8517-8525.

(5) (a) Jaroch, K.; Karolak, M.; Gorski, P.; Jaroch, A.; Krajewski, A.; Ilnicka, A.; Sloderbach, A.; Stefanski, T.; Sobiak, S. *Pharmacol. Rep.* **2016**, *68*, 1266-1275. (b) Bukhari, S. N. A.; Kumar, G. B.; Revankar, H. M.; Qin, H. L. *Bioorg. Chem.* **2017**, *72*, 130-147.

(6) Tozer, G. M.; Kanthou, C.; Parkins, C. S.; Hill, S. A. *Int. J. Exp. Pathol.* **2002**, *83*, 21-38.

(7) (a) Pettit, G. R.; Singh, S. B.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Schmidt, J. M.; Hogan, F. *J. Med. Chem.* **1995**, *38*, 1666-1672. (b) Pettit, G. R.; Rhodes, M. R.; Herald, D. L.; Hamel, E.; Schmidt, J. M.; Pettit, R. K. *J. Med. Chem.* **2005**, *48*, 4087-4099. (c) Tron, G. C.; Pirali, T.; Sorba, G.; Pagliai, F.; Busacca, S.; Genazzani, A. A. *J. Med. Chem.* **2006**, *49*, 3033-3044. (d) Devkota, L.; Lin, C. M.; Strecker, T. E.; Wang, Y.; Tidmore, J. K.; Chen, Z.; Guddneppanavar, R.; Jelinek, C. J.; Lopez, R.; Liu, L.; Hamel, E.; Mason, R. P.; Chaplin, D. J.; Trawick, M. L.; Pinney, K. G. *Bioorg. Med. Chem.* **2016**, *24*, 938-956.

(8) Pettit, G. R.; Anderson, C. R.; Herald, D. L.; Jung, M. K.; Lee, D. J.; Hamel, E.; Pettit, R. K. *J. Med. Chem.* **2003**, *46*, 525-531.

(9) (a) Siemann, D. W.; Chaplin, D. J.; Walicke, P. A. *Expert Opin. Invest. Drugs* **2009**, *18*, 189-197. (b) Liu, P.; Qin, Y.; Wu, L. Y.; Yang, S.; Li, N.; Wang, H. J.; Xu, H. Y.; Sun, K. L.; Zhang, S. X.; Han, X. H.; Sun, Y.; Shi, Y. K. *Anti-Cancer Drugs* **2014**, *25*, 462-471. (c) Grisham, R.; Ky, B.; Tewari, K. S.; Chaplin, D. J.; Walker, J. *Gynecol. Oncol. Res. Pract.* **2018**, *5*, 1.

(10) Tanpure, R. P.; Nguyen, B. L.; Strecker, T. E.; Aguirre, S.; Sharma, S.; Chaplin, D. J.; Siim, B. G.; Hamel, E.; Lippert, J. W.; Pettit, G. R.; Trawick, M. L.; Pinney, K. G. *J. Nat. Prod.* **2011**, *74*, 1568-1574.

(11) (a) Simoni, D.; Romagnoli, R.; Baruchello, R.; Rondanin, R.; Rizzi, M.; Pavani, M. G.; Alloatti, D.; Giannini, G.; Marcellini, M.; Riccioni, T.; Castorina, M.; Guglielmi, M. B.; Bucci, F.; Carminati, P.;

Pisano, C. *J. Med. Chem.* **2006**, *49*, 3143-3152. (b) Shen, C. H.; Shee, J. J.; Wu, J. Y.; Lin, Y. W.; Wu, J. D.; Liu, Y. W. *Br. J. Pharmacol.* **2010**, *160*, 2008-2027.

(12) (a) Su, M.; Huang, J.; Liu, S.; Xiao, Y.; Qin, X.; Liu, J.; Pi, C.; Luo, T.; Li, J.; Chen, X.; Luo, Z. *Sci. Rep.* **2016**, *6*, 28139. (b) Siemann, D. W.; Chaplin, D. J.; Horsman, M. R. *Cancer Invest.* **2017**, *35*, 519-534.

(13) Subbiah, I. M.; Lenihan, D. J.; Tsimberidou, A. M. *Oncologist* **2011**, *16*, 1120-1130.

(14) Ojima, I. *Acc. Chem. Res.* **2008**, *41*, 108-119.

(15) (a) Sauer, L. A.; Dauchy, R. T. *Br. J. Cancer* **1992**, *66*, 297-303. (b) Grammatikos, S. I.; Subbaiah, P. V.; Victor, T. A.; Miller, W. M. *Br. J. Cancer* **1994**, *70*, 219-227.

(16) (a) Bradley, M. O.; Webb, N. L.; Anthony, F. H.; Devanesan, P.; Witman, P. A.; Hemamalini, S.; Chander, M. C.; Baker, S. D.; He, L. F.; Horwitz, S. B.; Swindell, C. S. *Clin. Cancer Res.* **2001**, *7*, 3229-3238. (b) Wang, J. H.; Luo, T. W.; Li, S. B.; Zhao, J. *Expert Opin. Drug Delivery* **2012**, *9*, 1-7. (c) Chen, J. H.; Brown, D. P.; Wang, Y. J.; Chen, Z. S. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 5119-5122. (d) Liang, C. H.; Ye, W. L.; Zhu, C. L.; Na, R.; Cheng, Y.; Cui, H.; Liu, D. Z.; Yang, Z. F.; Zhou, S. Y. *Mol. Pharmaceutics* **2014**, *11*, 1378-1390. (e) Potter, E.; Jha, M.; Bhullar, K. S.; Rupasinghe, H. P. V.; Balzarini, J.; Jha, A. *Bioorg. Med. Chem.* **2015**, *23*, 411-421.

(17) Bedikian, A. Y.; DeConti, R. C.; Conry, R.; Agarwala, S.; Papadopoulos, N.; Kim, K. B.; Ernstoff, M. *Ann. Oncol.* **2011**, *22*, 787-793.

(18) Gaukroger, K.; Hadfield, J. A.; Hepworth, L. A.; Lawrence, N. J.; McGown, A. T. *J. Org. Chem.* **2001**, *66*, 8135-8138.

(19) Beale, R. N.; Roe, E. M. F. *J. Chem. Soc.* **1953**, *0*, 2755-2763.

(20) (a) Romagnoli, R.; Baraldi, P. G.; Salvador, M. K.; Preti, D.; Tabrizi, M. A.; Brancale, A.; Fu, X. H.; Li, J.; Zhang, S. Z.; Hamel, E.; Bortolozzi, R.; Basso, G.; Viola, G. *J. Med. Chem.* **2012**, *55*, 475-488.

(b) Salehi, M.; Ostad, S. N.; Riazi, G. H.; Assadieskandar, A.; Cheraghi-Shavi, T.; Shafiee, A.; Amini, M. *Med. Chem. Res.* **2014**, *23*, 1465-1473.

(21) Wang, Y. Q.; Li, L. F.; Jiang, W.; Yang, Z. Q.; Zhang, Z. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2974-2977.

(22) (a) Ma, M.; Sun, L.; Lou, H.; Ji, M. *Chem. Cent. J.* **2013**, *7*, 179. (b) Kamal, A.; Kumar, G. B.; Polepalli, S.; Shaik, A. B.; Reddy, V. S.; Reddy, M. K.; Reddy Ch, R.; Mahesh, R.; Kapure, J. S.; Jain, N. *ChemMedChem* **2014**, *9*, 2565-2579.

(23) Borrel, C.; Thoret, S.; Cachet, X.; Guenard, D.; Tillequin, F.; Koch, M.; Michel, S. *Bioorg. Med. Chem.* **2005**, *13*, 3853-3864.

Graphical abstract

