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Acylphloroglucinol and xanthones from *Hypericum ellipticum*

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ABSTRACT

An acylphloroglucinol, elliptophenone A, and two xanthones, elliptoxanthone A and elliptoxanthone B, were isolated from the aerial portions of *Hypericum ellipticum* together with three known xanthones, 1,3,7-trihydroxy-8-(3-methyl-2-butenyl)-9*H*-xanthen-9-one, 1,6-dihydroxy-4-methoxy-9*H*-xanthen-9-one, and 1,4,5-trihydroxy-9*H*-xanthen-9-one. Their structures were determined by spectroscopic analyses. The acylphloroglucinol and xanthones were evaluated for cytotoxicity using three human colon cancer cell lines cell lines (HT-29, HCT-116 and Caco-2) and a normal human colon cell line (CCD-18Co).

1. Introduction

The *Hypericum* genus (family Clusiaceae) contains over 400 species distributed worldwide. Phytochemical studies of several members of the *Hypericum* genus, including *H. perforatum* (St. John's wort), have yielded a number of structurally diverse secondary metabolites from the naphthodianthrone, acylphloroglucinol, flavonoid, and xanthone classes (Avato, 2005). These compounds exhibit a wide range of biological properties including anticancer, antimicrobial, anti-inflammatory, anti-HIV and antioxidant activities (Avato, 2005; Ciochina et al., 2006; El-Seedi et al., 2010).

As part of a study to characterize biologically active secondary metabolites from *Hypericum* species growing in the state of Pennsylvania, USA, we recently reported the isolation of a benzophenone glucoside, 3′-*O*-β-D-3″,4″,6″-triacetyl-glucopyranosyl-2,4,5′,6-tetrahydroxybenzophenone from an acetone extract of *H. ellipticum*, pale St. John's wort (Petrunak et al., 2009). During the isolation of the benzophenone glucoside, minor components belonging to the xanthone and acylphloroglucinol classes were detected by ^{1}H NMR spectroscopy. However, owing to the paucity of plant extracts, these compounds could not be isolated in sufficient quantities for characterization. We now report the isolation of a new acylphloroglucinol derivative, elliptophenone A (**1**), two new xanthones, elliptoxanthone A (**2**) and elliptoxanthone B (**3**), and three known xanthones, 1,3,7-trihydroxy-8-(3-methyl-2-butenyl)-9*H*-xanthen-9-one (**4**), 1,6-dihydroxy-4-methoxy-9*H*-xanthen-9-one (**5**) and 1,4,5-trihydroxy-9*H*-xanthen-9-one (**6**) from an acetone extract of *H. ellipticum*. Herein, we describe the structure elucidation of compounds **1–3** and cytotoxicity evaluation of compounds **1–2** and **4–6** against three human colon cancer cell lines and one normal colon cell line.

2. Results and discussion

The acetone extract of the aerial portions of *H. ellipticum* was subjected to column chromatography with silica gel followed by reversed-phase HPLC purification to afford one previously uncharacterized acylphloroglucinol (**1**) and two xanthone derivatives (**2** and **3**) (Fig. 1). In addition, three known xanthones, 1,3,7-trihydroxy-8-(3-methyl-2-butenyl)-9*H*-xanthen-9-one (**4**) (Morel et al., 2002), 1,6-dihydroxy-4-methoxy-9*H*-xanthen-9-one (**5**) (Seo et al., 2002) and 1,4,5-trihydroxy-9*H*-xanthen-9-one (**6**) (Minami et al., 1995) were isolated and identified by NMR spectroscopic analyses. The structures of compounds **4–6** were confirmed by comparison of the spectroscopic data with literature values.

The molecular formula of compound **1** was deduced to be $C_{24}H_{28}O_4$ based on HRESIMS data. The IR spectrum displayed absorption bands at 3424 and 1653 cm^{-1} indicating the presence of hydroxyl groups and conjugated carbonyl carbons. The ^{1}H NMR spectrum (Table 1) of compound **1** displayed three aromatic signals at 7.45, 7.40 and 7.35 ppm, integrating for five protons. HMBC correlations (Table 1) indicated that these protons

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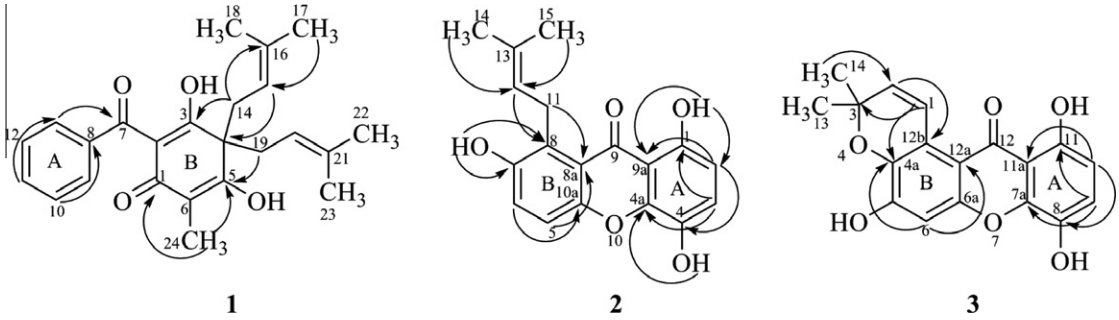


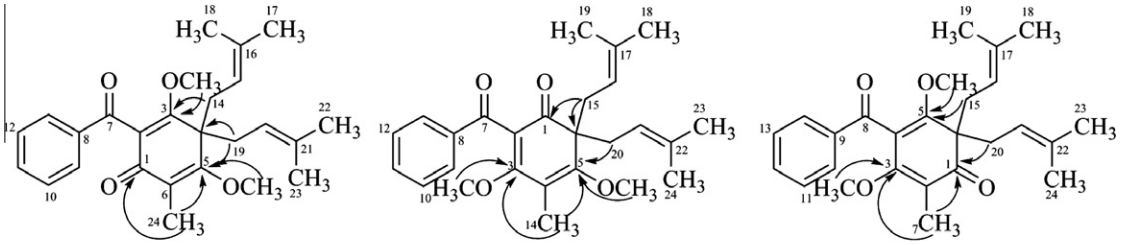
Fig. 1. Structures and important HMBC correlations for compounds **1**, **2** and **3**.

Table 1
NMR spectroscopic data of elliptophenone A (**1**) and dimethyl elliptophenone A (**1a**).

Position	**1** [$(CD_3)_2CO$]			**1a** [$CDCl_3$]	
	δ_C	δ_H [a]	HMBC[b]	δ_C	δ_H[a]
1	189.5		24	188.6	
2	106.5			118.3	
3	193.7		14,19	169.9	
4	57.4		14, 15, 19, 20	53.8	
5	173.2		14, 19, 24	170.2	
6	107.6		24	119.5	
7	195.7		9, 13	196.6	
8	139.5		10,12	138.6	
9	128.0	7.40 (1H, d, 7.3)	11	129.3	7.88 (1H, d, 7.0)
10	127.4	7.35 (1H, dd, 7.3, 7.3)	12	128.7	7.41 (1H, dd, 7.0, 7.0)
11	130.5	7.45 (1H, dd, 7.3, 7.3)	9,13	133.1	7.50 (1H, dd, 7.0, 7.0)
12	127.4	7.35 (1H, dd, 7.3, 7.3)	10	128.7	7.41 (1H, dd, 7.0, 7.0)
13	128.0	7.40 (1H, d, 7.3)	11	129.3	7.88 (1H, d, 7.0)
14	37.3	2.65 (2H, br d, 7.4)	15	35.8	2.61 (2H, m)
15	118.7	4.93 (1H, t, 7.4)	14, 17, 18	118.7	4.93 (1H, t, 6.4)
16	134.2		14, 17, 18	134.7	
17	25.2	1.62 (3H, s)	15, 18	26.0	1.71 (3H, s)
18	17.3	1.59 (3H, s)	15, 17	18.0	1.60 (3H, s)
19	37.3	2.65 (2H, br d, 7.4)	20	35.8	2.61 (2H, m)
20	118.7	4.93 (1H, t, 7.4)	19, 22, 23	118.7	4.93 (1H, t, 6.4)
21	134.2		19, 22, 23	134.7	
22	25.2	1.62 (3H, s)	20, 23	26.0	1.71 (3H, s)
23	17.3	1.59 (3H, s)	20, 22	18.0	1.60 (3H, s)
24	6.6	1.90 (3H, s)		9.8	1.89 (3H, s)
3-OH		18.49 (1H, br s)			
5-OH		9.87 (1H, br s)			
3-OCH_3				59.6	3.56 (3H, s)
5-OCH_3				61.5	3.90 (3H, s)

[a] Coupling contants (*J*, Hz) given in parentheses.
[b] Data for compound **1**. Proton correlating to carbon shift.

comprised a monosubstituted aromatic ring, A. The protons at 7.40 ppm showed HMBC correlations to the carbonyl carbon at 195.7 ppm in the ^{13}C NMR spectrum (Table 1), confirming that the carbonyl group was directly attached to ring A. The presence of the modified phloroglucinol ring B was suggested by the ^{13}C NMR spectroscopic data (193.7, 189.5, 173.2, 107.6, 106.5 and 57.4 ppm) (Zhao et al., 2005). The following ^1H NMR signals suggested the presence of two 3-methyl-2-butenyl groups: 4.93 ppm (2H, t), 2.65 ppm (4H, br d), 1.62 ppm (6H, s) and 1.59 ppm (6H, s). These data were corroborated by characteristic ^{13}C NMR resonances at 134.2, 118.7, 37.3, 25.2 and 17.3 ppm, coupled with HMBC correlations (Fig. 1). A singlet at 1.90 ppm in the ^1H NMR spectrum, integrating for three protons, confirmed the presence of an additional methyl group. The points of attachment of the methyl group and the two 3-methyl-2-butenyl groups to ring B were determined based on HMBC correlations. The protons of the prenyl moieties, H_2-14/H_2-19 (2.65 ppm) and H-15/H-20 (4.93 ppm), showed long-range correlations with the aliphatic quaternary carbon at 57.4 ppm. Additionally, H_2-14/H_2-19 showed HMBC correlations to C-3 (193.7 ppm) and C-5 (173.2 ppm), establishing the placement of the two 3-methyl-2-butenyl groups at position 4. The methyl protons at 1.90 ppm showed long-range correlations to the quaternary carbons at C-1 (189.5 ppm), C-5 (173.2 ppm), and C-6 (107.6 ppm), allowing the placement of the methyl group at C-6. A proton singlet at 18.49 ppm, was attributed to the hydroxyl group at position 3, with the proton in a hydrogen bonding relationship to the C-7 carbonyl carbon. Based on the foregoing data, compound **1** was determined to be 2-benzoyl-3,5-dihydroxy-6-methyl-4,4-bis-(3-methyl-2-butenyl)cyclohexa-2,5-dienone, named elliptophenone A (Fig. 1). Although elliptophenone A can exist in more than one tautomeric form, only a single tautomer was observed in acetone and DMSO. The compound is prone to oxidative decomposition, so the hydroxyl groups were methylated in order to obtain the compound in a more stable form. Methylation of compound **1** using diazomethane in ether afforded three di-*O*-methylated derivatives, **1a**, **1b** and **1c** (Fig. 2), with **1a** being the major form.



1a **1b** **1c**

Fig. 2. Structures and important HMBC correlations for compounds **1a**, **1b** and **1c**.

The structures of **1a–1c** were confirmed by extensive NMR spectroscopic analyses. The ^{1}H and ^{13}C NMR data of **1a** (Table 1) are similar to those of compound **1**, except that the hydroxyl proton signals in compound **1** were replaced by methoxyl proton signals at 3.90 and 3.56 ppm. The placement of these protons at C-5 and C-3, respectively, was based on HMBC correlations between the protons at 3.90 ppm and C-5 and between the protons at 3.56 ppm and C-3 (Fig. 2). The NMR data of compound **1a** are consistent with those for dimethyl grandone (de Oliveira et al., 1996), which contains a 3-methyl-2-butenyl in place of the C-24 methyl group. The structures of **1b** and **1c** were distinguished by examining the long-range correlations between the protons in the groups attached directly to ring B and the carbon atoms in the ring (Fig. 2). For compound **1b**, the methylene protons at C-15/C-20 showed correlations to the C-1 carbonyl carbon and C-5, whereas the methyl protons at C-14 showed correlations to C-3 and C-5. For compound **1c**, the methyl protons at C-7 were correlated to both the C-1 carbonyl carbon and C-3, whereas the methylene protons C-15/C-20 were correlated to the C-1 carbonyl carbon and C-5. The UV spectra of compounds **1b** and **1c** displayed absorption bands at 341 and 345 nm, respectively. These absorptions bands are indicative of an extended conjugated system containing a dienone moiety (Porto et al., 2000), distinguishing **1b** and **1c** from **1a** which contains a cross-conjugated dienone moiety.

Compound **1** is structurally related to the hops bitter acid, lupulone, which like grandone contains a modified phloroglucinol ring with three C-prenyl groups attached. Incubation studies have established that lupulone is biosynthesized from an acylphloroglucinol and three units of dimethylallyl pyrophosphate (DMAPP), catalyzed by prenyltransferases (Zuurbier et al., 1995, 1998). Compound **1** may be biosynthesized by a similar process with the C-24 methyl group being introduced by *S*-adenosylmethionine (SAM), catalyzed by a methyltransferase enzyme.

Compound **2** was assigned the molecular formula $C_{18}H_{16}O_5$ on the basis of HRESIMS data. The UV spectrum with absorption maxima at 409, 335, 269 and 239 nm indicated a xanthone skeleton (Nguyen and Harrison, 1998). The IR spectrum exhibited absorption bands at 3447 and 1653 cm^{-1} indicative of one or more hydroxyl groups and a conjugated carbonyl, respectively. The conjugated carbonyl was confirmed by a ^{13}C NMR signal at 184.8 ppm. A sharp singlet at 12.40 ppm in the ^{1}H NMR spectrum (Table 2) indicated that the carbonyl group was hydrogen bonded to a phenolic proton (OH-1). This relationship was confirmed by HMBC correlations between OH-1 and C-1, C-2 and C-9a (Fig. 1). The ^{1}H NMR spectrum also displayed signals corresponding to two additional phenolic protons (9.80 and 9.50 ppm), two pairs of *ortho* coupled aromatic protons (7.23/6.57 ppm and 7.43/7.35 ppm; $J = 8.8$ Hz), and a 3-methyl-2-butenyl group (5.29 ppm, 1H, t; 4.16 ppm, 2H, d; 1.82 ppm, 3H, s; 1.63 ppm, 3H, s). Both the protons at 7.23 and 6.57 ppm showed HMBC correlations to C-1 and C-4, establishing the placement of these two protons at C-2 and C-3, with a phenolic group at position 4. The placement of the phenolic group at position 4 was further supported by HMBC correlation between the C-4 phenolic hydrogen and C-4a. The points of attachment of the 3-methyl-2-butenyl group and the remaining hydroxyl substituent to ring B were established based on HMBC correlations. The placement of the 3-methyl-2-butenyl group at C-8 was based on correlations between the methylene protons (4.16 ppm) and C-8 and C-8a. The methylene protons appear downfield of typical values owing to the deshielding effect of the carbonyl group (Duan et al., 2010). The location of the hydroxyl group at position 7 was determined by correlations between the hydroxyl proton and C-8. The foregoing data required that the remaining set of *ortho* coupled protons (7.43 and 7.35 ppm) be placed at positions 5 and 6 on ring B, confirmed by HMBC correlations between both protons and C-10a and between H-5 and C-8a. Based on the spectroscopic data, compound **2** was identified as 1,4,7-trihydroxy-8-(3-methyl-2-butenyl)-9*H*-xanthen-9-one, named elliptoxanthone A.

Table 2
NMR spectroscopic data for elliptoxanthone A (**2**) and elliptoxanthone B (**3**) in $(CD_3)_2CO$.

2			**3**		
Position	δ_C	δ_H (J in Hz)[a]	Position	δ_C	δ_H (J in Hz)[a]
1	154.0		1 2 3	120.6	8.03 (1H, d, 10.2)
2	108.3	6.57 (1H, d, 8.8)	2	133.0	5.93 (1H, d, 10.2)
3	122.7	7.23 (1H, d, 8.8)	3	76.1	
4	136.7		4a	138.3	
4a	143.7		5	153.5	
5	116.4	7.35 (1H, d, 8.8)	6	102.7	6.87 (1H, s)
6	123.9	7.43 (1H, d, 8.8)	6a	153.5	
7	151.6		7a	143.5	
8	128.2		8	136.7	
8a	118.9		9	122.3	7.20 (1H, d, 8.8)
9	184.8		10	108.7	6.55 (1H, d, 8.8)
9a	109.4		11	153.9	
10a	151.5		11a	109.2	
11	25.5	4.16 (2H, d, 7.0)	12	183.5	
12	123.3	5.29 (1H, t, 7.0)	12a	107.9	
13	130.8		12b	120.1	
14	25.2	1.63 (3H, s)	13	26.3	1.46 (3H, s)
15	17.5	1.82 (3H, s)	14	26.3	1.46 (3H, s)
1-OH		12.40 (1H, s)	5-OH		9.50 (1H, s)
4-OH		9.50 (1H, s)[b]	8-OH		9.50 (1H, s)
6-OH			11-OH		12.38 (1H, s)
7-OH		9.80 (1H, s)[b]			

[a] Coupling contants (J, Hz) given in parentheses.
[b] Observed in DMSO-d_6.

The molecular formula of compound **3** was determined to be $C_{18}H_{14}O_6$ on the basis of HRESIMS data. The UV spectrum with absorption maxima at 410, 321 and 291 nm indicated a xanthone nucleus (Nguyen and Harrison, 1998). The IR spectrum was similar to that of compound **2** with absorption bands indicative of hydroxyl (3406 cm^{-1}) and conjugated carbonyl (1653 cm^{-1}) groups. The ^{1}H NMR spectroscopic data (Table 2) showed signals at 12.38 ppm (s), 9.50 (br, s), 7.20 ppm (d) and 6.55 ppm (d), indicating a

dihydroxylated pattern in ring A, identical to that of compound **2**. Two proton doublets (J = 10.2 Hz) at 8.03 ppm (H-1) and 5.93 ppm (H-2) coupled with a singlet at 1.46 ppm (H_3-13, H_3-14), integrating for six protons, indicated the presence of a *gem*-dimethylpyran ring. The downfield position of H-1 indicated that it was located near to the carbonyl carbon (Ishiguro et al., 1993). A third phenolic group (9.50 ppm) was positioned at C-5. A sharp singlet at 6.87 ppm was attributed to a hydrogen at position 6. These placements were based on HMBC correlations between the proton signal at 6.87 ppm and C-4a, C-5, C-6a and C-12a. The spectroscopic data led to the identification of compound **3** as 5,8,11-trihydroxy-3,3-dimethyl-3*H*,12*H*-pyrano[3,2-a]xanthen-12-one, named elliptoxanthone B. The ^{1}H and ^{13}C resonances for ring B and the pyran ring were in close agreement with those reported for toxyloxanthone (Ishiguro et al., 1993). Toxyloxanthone is an isomer of elliptoxanthone containing a hydroxyl group at position 9 instead of position 8.

Compounds **1–2**, **4–6**, and the positive control, etoposide, were examined for cytotoxicity against three human colon cancer cell lines, namely, HT-29, HCT-116 and Caco-2 as well as a normal colon cell line (CCD-18Co). Compound **3** was not tested owing to the limited supply of the sample. All five compounds showed moderate activity against the four cell lines. Overall, the acylphloroglucinol, **1**, and prenylxanthones, **2** and **4**, showed weaker activity (IC_{50}: 84–104 μg/mL) than the two non-prenylated xanthone derivatives, **5** and **6**, (IC_{50}: 71–95 μg/mL). The HCT-116 and Caco-2 tumor cells lines were more sensitive to all of the compounds compared to CCD-18Co normal colon cells. However, there was no significant difference between the IC_{50} values for the HT-29 colon tumor cells and the CCD-18Co normal colon cells. Etoposide showed greater selectivity for the three tumor cells lines (IC_{50}: 9.5–16.2 μg/mL) relative to the normal cells (IC_{50}: 43.9 μg/mL). However, unlike compounds **1**, **2**, **4**, **5** and **6**, it showed greatest activity with the HT-29 cells (IC_{50}: 9.5 μg/mL).

Several prenylated and non-prenylated xanthones have exhibited moderate to strong activity against HT-29 and HCT-116 tumor cell lines (El-Seedi et al., 2010), but anticancer studies of these compounds against Caco-2 cells is limited. However, studies have been conducted with Caco-2 cells and xanthones when the Caco-2 monolayer is utilized as an epithelial cell culture model to assess absorption (Bumrungpert et al., 2009).

2.1. Concluding remarks

An acylphloroglucinol and five xanthones were isolated from the aerial portions of *H. ellipticum*. The acylphloroglucinol and four of the xanthone derivatives were evaluated for cytotoxicity against three human colon tumor cell lines (HCT-116, HT-29 and Caco-2) and a normal colon cell line (CCD-18Co). All tested compounds demonstrated selective but moderate cytotoxicity for the HCT-116 and Caco-2 tumor cells when compared to normal colon cells (CCD-18Co) indicating a possible cytotoxic selectivity towards colon cancer cells.

3. Experimental

3.1. General experimental procedures

UV data were obtained using a Varian Cary 4000 UV–Vis spectrophotometer. FTIR data were measured using a Nicolet Magna IR 560 spectrometer. NMR spectra were recorded on a JEOL ECP 400 MHz spectrometer (^{1}H, 400 MHz; ^{13}C, 100 MHz) using $CDCl_3$, $(CD_3)_2CO$ and DMSO-d_6 as solvents and TMS as internal standard. Gradient HMQC and HMBC data were obtained using standard pulse programs. HRMS data were acquired using electrospray ionization on an Agilent G6520A Q-TOF high resolution mass spectrometer (NIH: 1S10RR022649-01 and CUNY Instrumentation Fund).

Preparative HPLC was performed using a Waters Delta 600 system equipped with a Waters 2487 dual wavelength absorbance detector. Column chromatography (CC) was performed using Fisher Scientific silica gel (230–400 mesh), and analytical TLC was performed using Sigma–Aldrich polyester backed plates precoated with silica gel UV_{254}. All solvents were HPLC grade and were obtained from Fisher Scientific and Aldrich Chemical Co.

3.2. Plant material

The aerial parts of *H. ellipticum* were collected in Clearfield County, Pennsylvania, USA, in August 2009. The plant material was authenticated by Joseph and Bonnie Isaac and a voucher specimen (20714) has been deposited at the Carnegie Museum Herbarium in Pittsburgh, Pennsylvania, USA.

3.3. Extraction and isolation

Aerial parts of *H. ellipticum* were oven dried at 38 °C and ground to a fine powder using a coffee grinder. The plant material (459 g) was extracted sequentially at room temperature using hexanes (3 × 1 L), acetone (3 × 1 L) and MeOH (3 × 1 L). Each 1 L aliquot of solvent added was allowed to percolate for 24 h. The combined extracts for each solvent (e.g. hexanes) were concentrated *in vacuo*. The acetone extract (14.6 g) was fractionated using silica gel CC to afford fractions A1–A11, eluted with a solvent gradient of $(CH_3)_2CO$-hexanes (5:95 to 100:0, v/v). Fraction A3 (509 mg), eluted with $(CH_3)_2CO$-hexanes (1:3, v/v) was further fractionated with EtOAc-hexanes (1:4 to 1:3, v/v) to afford a brown solid (145 mg) which was purified by HPLC (Atlantis dC18 column, 5 μm, 19 × 150 mm, MeOH–H_2O (4:1, v/v) isocratic elution, flow rate 10 mL/min) to afford compound **1** (28.0 mg). Fraction A4 (1.05 g), eluted with $(CH_3)_2CO$-hexanes (25:75 to 40:60, v/v) was further fractionated by silica gel CC using EtOAc-hexanes (30:70 to 35:65, v/v) to afford a yellow-orange solid (310 mg). The solid was re-applied to a silica gel column using EtOAc-hexanes (1:4, v/v) to afford a yellow solid (87.0 mg), containing predominantly compound **1**. The sample was treated with excess diazomethane in ether, prepared from diazald according to standard protocol (Chen, 1988), and purified by HPLC (Phenomenex Luna C18 column, 5 μm, 10 × 250 mm, MeOH–H_2O (85:15, v/v), flow rate 2.0 mL/min) to afford compounds **1a** (11.9 mg), **1b** (5.9 mg) and **1c** (6.4 mg). Subfractions B1–B7 resulted from further fractionation of A6 (655 mg) using *tert*-butyl methyl ether-hexanes (40:60 to 65:35, v/v). Fractions B2 (34.7 mg), B3 (67.3 mg) and B5 (108.8 mg) were purified by HPLC (Atlantis dC18 column, flow rate 10 mL/min). Fraction B2 was eluted with MeOH–H_2O (70:30, v/v) to afford compound **5** (3.4 mg), fraction B3 was eluted with MeOH–H_2O (63:37 to 65:35, v/v) to give compound **4** (5.1 mg), and fraction B5 was eluted with MeOH–H_2O (60:40, v/v) to yield compounds **2** (21.1 mg), **3** (2.4 mg) and **6** (4.2 mg).

3.4. Cytotoxicity assays

Cell lines included three human colon cancer cells: HT-29 (human colon adenocarcinoma), HCT-116 (human colon carcinoma) and Caco-2 (human epithelial colorectal adenocarcinoma). In addition, normal human colon cells were included: CCD-18Co (human colon fibroblasts). All cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and maintained at the University of Rhode Island. Caco-2 and CCD-18Co cells were grown in EMEM medium supplemented with 10% v/v fetal bovine serum, 1% v/v nonessential amino acids, 1% v/v L-glutamine and 1%

v/v antibiotic solution (Sigma). HT-29 and HCT-116 cells were grown in McCoy's 5a medium supplemented with 10% v/v fetal bovine serum, 1% v/v nonessential amino acids, 2% v/v Hepes and 1% v/v antibiotic solution. For each cell line, passage numbers between 22 and 35 were used. Cells were maintained at 37 °C in an incubator under a 5% CO_2/95% air atmosphere at constant humidity and maintained in the linear phase of growth.

Cytotoxicity studies were carried out using a MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfenyl)-2*H*-tetrazolium, inner salt] assay as described previously (Cory et al., 1991). Briefly, test samples and a positive control, etoposide 4 μg/mL (Sigma), were solubilized in DMSO by sonication. All samples were diluted with media to the desired treatment concentration and the final DMSO concentration per well did not exceed 0.5%. Control wells were also included on all plates. Following a 72 h drug-incubation period at 37 °C with serially diluted test compounds, MTS, in combination with the electron coupling agent, phenazine methosulfate, was added to the wells and cells were incubated at 37 °C in a humidified incubator for 3 h. Absorbance at 490 nm (OD_{490}) was monitored with a spectrophotometer (SpectraMax M2, Molecular Devices Corp., operated by SoftmaxPro v.4.6 software, Sunnyvale, CA, USA) to obtain the number of surviving cells relative to control populations. The results are expressed as the median cytotoxic concentrations (IC_{50} values) and were calculated from six-point dose response curves using 4-fold serial dilutions. Each point on the curve was tested in. Data are expressed as means ± SE for three replications on each cell line.

3.5. 2-Benzoyl-3,5-dihydroxy-6-methyl-4,4-bis(3-methyl-2-butenyl)-cyclohexa-2,5-dienone

(**1**): white amorphous solid; UV (MeOH) λ_{max} (log ε) 249 (3.89) nm; IR (KBr) ν_{max} 3424, 1653, 1588, 1560, 1508, 1493, 1448 cm^{-1}; For ^1H NMR, ^{13}C NMR and HMBC spectroscopic data, see Table 1; HRESIMS: *m/z* 379.1919, $[M-H]^-$ (calcd for $C_{24}H_{27}O_4$: 379.1909).

3.6. 2-Benzoyl-3,5-dimethoxy-6-methyl-4,4-bis(3-methyl-2-butenyl)cyclohexa-2,5-dienone

(**1a**): pale yellow oil; UV (MeOH) λ_{max} (log ε) 250 (4.38) nm; IR (NaCl) ν_{max} 1674, 1656, 1607, 1458 cm^{-1}; For ^1H NMR and ^{13}C NMR spectroscopic data, see Table 1; HRESIMS: *m/z* 409.2375, $[M+H]^+$ (calcd for $C_{26}H_{33}O_4$: 409.2379).

3.7. 2-Benzoyl-3,5-dimethoxy-4-methyl-6,6-bis(3-methyl-2-butenyl)cyclohexa-2,4-dienone

(**1b**): pale yellow oil; UV (MeOH) λ_{max} (log ε) 247 (4.53), 341 (4.08) nm; IR (NaCl) ν_{max} 1653, 1558, 1457 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.85 (2H, d, *J* = 7.0 Hz, H-9/13), 7.50 (1H, dd, *J* = 7.0, 7.0 Hz, H-11), 7.39 (2H, dd, *J* = 7.0, 7.0 Hz, H-10/12), 4.91 (2H, t, *J* = 7.2 Hz, H-16/21), 3.84 (3H, s, 5-OCH_3), 3.68 (3H, s, 3-OCH_3), 2.59 (2H, dd, *J* = 14.0, 7.2 Hz, H-15/20), 2.43 (2H, dd, *J* = 14.0, 7.2 Hz, H-15/20), 1.95 (3H, s, H_3-14), 1.68 (3H, s, H_3-18/23), 1.53 (3H, s, H_3-19/24); ^{13}C NMR ($CDCl_3$) δ: 199.9 (C-1), 196.7 (C-7), 171.8 (C-3), 167.9 (C-5), 138.8 (C-8), 134.5 (C-17, C-22), 133.0 (C-11), 129.4 (C-9, C-13), 128.5 (C-10, C-12), 118.8 (C-16, C-21), 115.6 (C-4), 114.5 (C-2), 61.9 (5-OCH_3), 60.7 (3-OCH_3), 58.7 (C-6), 37.7 (C-15, C-20), 26.0 (C-18, C-23), 18.2 (C-19, C-24), 10.7 (C-14); HRESIMS: *m/z* 409.2368 $[M+H]^+$ (calcd for $C_{26}H_{33}O_4$: 409.2379).

3.8. 4-Benzoyl-3,5-dimethoxy-2-methyl-6,6-bis(3-methyl-2-butenyl)cyclohexa-2,4-dienone

(**1c**): pale yellow oil; UV (MeOH) λ_{max} (log ε) 249 (4.30), 345 (3.59) nm; IR (NaCl) ν_{max} 1653, 1559, 1457 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.95 (2H, d, *J* = 7.0 Hz, H-10/14), 7.56 (1H, dd, *J* = 7.0, 7.0 Hz, H-12), 7.48 (2H, t, *J* = 7.0, 7.0 Hz, H-11/13), 4.93 (2H, t, *J* = 7.0 Hz, H-16/21), 3.55 (3H, s, 5-OCH_3), 3.48 (3H, s, 3-OCH_3), 2.61 (4H, m, H_2-15/20), 1.79 (3H, s, H_3-7), 1.68 (3H, s, H_3-18/23), 1.58 (3H, s, H_3-19/24); ^{13}C NMR ($CDCl_3$) δ: 202.0 (C-1), 195.4 (C-8), 169.5 (C-3), 165.7 (C-5), 138.8 (C-9), 134.5 (C-17, C-22), 133.5 (C-12), 129.2 (C-10, C-14), 128.9 (C-11, C-13), 118.8 (C-16, C-21), 115.6 (C-2), 112.2 (C-4), 61.1 (5-OCH_3), 59.6 (3-OCH_3), 58.8 (C-6), 39.2 (C-15, C-20), 26.0 (C-18, C-23), 17.9 (C-19, C-24), 8.7 (C-7); HRESIMS: *m/z* 409.2358 $[M+H]^+$ (calcd for $C_{26}H_{33}O_4$: 409.2379).

3.9. 1,4,7-Trihydroxy-8-(3-methyl-2-butenyl)-9H-xanthen-9-one

(**2**): yellow solid; UV (MeOH) λ_{max} (log ε) 239 (3.35), 269 (3.34), 335 (1.72), 409 (1.81) nm; IR (KBr) ν_{max} 3447, 1653, 1613, 1593, 1494 cm^{-1}; For ^1H NMR and ^{13}C NMR spectroscopic data, see Table 2; HRESIMS *m/z* 313.1069 $[M+H]^+$ (calcd for $C_{18}H_{17}O_5$: 313.1076).

3.10. 5,8,11-Trihydroxy-3,3-dimethyl-3H,12H-pyrano[3,2-a]xanthen-12-one

(**3**): yellow solid; UV (MeOH) λ_{max} (log ε) 291 (3.61), 321 (3.59), 410 (3.22) nm; IR (KBr) ν_{max} 3406, 1653, 1647, 1619, 1576, 1506, 1472, 1456 cm^{-1}; For ^1H NMR and ^{13}C NMR spectroscopic data, see Table 2; HRESIMS *m/z* 327.0867 $[M+H]^+$ (calcd for $C_{18}H_{15}O_6$: 327.0869).

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