Bioorganic & Medicinal Chemistry 18 (2010) 8066–8075

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Redox-silent tocotrienol esters as breast cancer proliferation and migration inhibitors

Fathy A. Behery, Ahmed Y. Elnagar, Mohamed R. Akl, Vikram B. Wali, Bilal Abuasal, Amal Kaddoumi, Paul W. Sylvester, Khalid A. El Sayed *

Department of Basic Pharmaceutical Sciences, College of Pharmacy, University of Louisiana at Monroe, Monroe, LA 71201, United States

ARTICLE INFO

Article history:
Received 19 May 2010
Revised 25 August 2010
Accepted 2 September 2010
Available online 19 September 2010

Keywords:
Anti-migratory
Antiproliferative
Breast cancer
Chemical stability
Semisynthetic esterification
Tocotrienol
Vitamin E
Water solubility

ABSTRACT

Tocotrienols are vitamin E members with potent antiproliferative activity against preneoplastic and neoplastic mammary epithelial cells with little or no effect on normal cell growth or functions. However, physicochemical and pharmacokinetic properties greatly limit their use as therapeutic agents. Tocotrienols' chemical instability, poor water solubility, NPC1L1-mediated transport, and rapid metabolism are examples of such obstacles which hinder the therapeutic use of these valuable natural products.

Vitamin E esters like α-tocopheryl succinate were prepared to significantly improve chemical and metabolic stability, water solubility, and potency. Thus, 12 semisynthetic tocotrienol ester analogues **4**–**15** were prepared by direct esterification of natural tocotrienol isomers with various acid anhydrides or chlorides. Esters **4**–**15** were evaluated for their ability to inhibit the proliferation and migration of the mammary tumor cells +SA and MDA-MB-231, respectively. Esters **5**, **9**, and **11** effectively inhibited the proliferation of the highly metastatic +SA rodent mammary epithelial cells with IC_{50} values of 0.62, 0.51, and 0.86 μM, respectively, at doses that had no effect on immortalized normal mouse CL-S1 mammary epithelial cells. Esters **4**, **6**, **8**–**10**, and **13** inhibited 50% of the migration of the human metastatic MDA-MB-231 breast cancer cells at a single 5 μM dose in wound-healing assay. The most active ester **9** was 1000-fold more water-soluble and chemically stable versus its parent α-tocotrienol (**1**). These findings strongly suggest that redox-silent tocotrienol esters may provide superior therapeutic forms of tocotrienols for the control of metastatic breast cancer.

1. Introduction

Vitamin E is composed of two groups of lipid-soluble compounds, tocopherols and tocotrienols.[1] Both groups share the same chemical structure, but differ in the degree of unsaturation of their side chains. Tocopherols have saturated phytyl side chain while tocotrienols possess unsaturated isoprenoid side chain, with all E Δ3′,7′,11′ systems. Each group possesses four isomeric members designated as α, β, γ, and δ, depending on the number and position of the methyl groups on the chromanol ring.[2]

The lipid-soluble vitamin E members are essential nutrients for normal growth and development and antioxidants in animals.[3] Tocotrienols are unique vitamin E members, which occur only in few natural sources including palm oil, barley, oat, and rice bran.[4] Vitamin E deficiency can cause severe degenerative diseases.[5] Tocotrienols showed better antioxidant activity versus tocopherols in membranes.[6–9] They also exhibited a wide range of biological effects in humans, independently of their radical-scavenging abilities.[10,11] These include antiproliferative, anti-angiogenic, cholesterol-lowering, neuroprotective, and other activities.[6,11] These redox-silent activities may be attributed to their unique effects on various signal transduction, mevalonate–cholesterol biosynthetic pathways, and gene expressions.[4,6]

Vitamin E esters like α-tocopheryl succinate or oxyacetate showed potent growth-inhibitory and apoptotic-inducing activities in several in vitro and in vivo cancer models including breast, prostate, neuroblastoma, and mesothelioma compared to their parent α-tocopherol.[12–14] These tocopheryl esters act as 'mitocans' by selectively targeting cancer cells' mitochondria whereas normal cells are less affected.[12–15] This broad spectrum of action in conjunction with low toxicity underlies the translational potential of vitamin E esters for cancer control and prevention.[15]

Natural vitamin E members are relatively unstable toward air, heat, light, alkali, and metal ions.[16,17] Therefore, several synthetic stabilized vitamin E analogues, mainly esters, for example, α-tocopheryl acetate and tocopheryl succinate, were synthesized and became commercially available for use in supplements and cosmetics.[17–19] Esters are less susceptible to oxidation and therefore more appropriate for food and pharmaceutical applications compared to the free form.[17–19]

The poor bioavailability of γ-tocotrienol was attributed to its NPC1L1-mediated intestinal uptake and their lipophilicity and

* Corresponding author. Tel.: +1 318 342 1725; fax: +1 318 342 1737.
E-mail address: elsayed@ulm.edu (K.A. El Sayed).

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.09.009

poor water solubility.[20–22] A commercially available water- soluble vitamin E ester was produced by Eastman Chemical Company by esterification of polyethylene glycol-1000 onto RRR-α-tocopheryl succinate.[23] This ester has the ability to form miscible micelles in water due to its amphiphilic properties. This approach enhanced tocopherols' bioavailability in animals and humans via improving their water solubility and absorption.[23]

After exerting their antioxidant activity, vitamin E isomers are usually rapidly metabolized and excreted.[16,17,24–26] Esterification of tocotrienols affords redox-silent analogues, which will prevent their rapid metabolic inactivation via masking the free phenolic group required for chromanoxy radical formation. Generally, esters will slowly hydrolyze to release their parent natural phenol, which decrease the rate of metabolism and enhance the metabolic stability of tocotrienols.[2,16] Intestinal or epidermal esterases will catalyze ester hydrolysis and thus can be considered as pro-vitamins or prodrugs of the natural tocotrienols. Esterification of tocotrienols is expected not only to maintain the bioactivity but also to improve its solubility and chemical stability.[12–14,27] Therefore, this study aims at the preparation of new highly polar esters of tocotrienols with expected enhanced water solubility, stability, and bioactivity.

2. Results and discussion

2.1. Semisynthesis of tocotrienol ester analogues

α-, γ-, and δ-Tocotrienols (**1**–**3**, respectively) were isolated from a tocotrienol-rich fraction of palm oil using normal phase vacuum liquid chromatography. New esters **4**–**15** were semisynthesized by direct esterification with carboxylic acid anhydrides or chlorides. Acids with terminal ionizable groups were used to esterify tocotrienol isomers. 1,2,4-Benzenetricarboxylic, maleic, and 3-methylglutaric anhydrides successfully reacted with tocotrienols. The naturally occurring caffeic and gallic acids did not react with tocotrienol isomers before activation with oxalyl chloride after protecting their phenolic groups by acetylation. The identity of each new tocotrienol ester was established by spectral techniques.

The HREIMS of **4** and **6** suggested the molecular formula $C_{38}H_{48}O_7$ and possible esterification of **1** with 1,2,4-benzenetricarboxylic anhydride. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) further supported this conclusion. Esterification induced downfield shifting of C-5 ($\Delta\delta$ +4.7) and C-7 ($\Delta\delta$ +4.2) in **4**, compared to its parent **1**. Similar downfield shift was also observed in **6** for C-5 and C-7 ($\Delta\delta$ +4.7 and 4.1, respectively). A +4.0 ppm downfield shift for C-6 was also observed in both compounds. The hypsochromic shift (−2 and −6 nm in **4** and **6**, respectively) in the UV spectrum further confirmed the C-6 esterification.[16] The HMBC spectra of **4** and **6** showed correlation contours between the *o*- and *m*-coupled aromatic proton H-7″ (δ_H 8.03 and 8.75 in **4** and **6**, respectively) and the downfield carbonyl carbon C-1″ (δ_C 165.6 and 164.8 in **4** and **6**, respectively). Thus, compounds **4** and **6** were determined to be 4-(((*R*)-2,5,7,8-tetramethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-yloxy)-carbonyl)-isophthalic acid and 2-(((*R*)-2,5,7,8-tetramethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-yloxy)carbonyl)terephthalic acid, respectively.

The HREIMS of **5** and **7** suggested the molecular formula $C_{37}H_{46}O_7$ and possible esterification with 1,2,4-benzenetricarboxylic anhydride. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) further supported this conclusion. Esterification induced the downfield shifting of C-5 and H-5 ($\Delta\delta$ +6.4, +0.44, respectively) and C-7 ($\Delta\delta$ +1.3) in **5** compared to its parent **2**. Similar downfield shift was also observed in **7** for C-5, H-5 and C-7 ($\Delta\delta$ +6.5, +0.39, and +1.5, respectively). Carbon C-6 was also downfield shifted in **5** and **7** ($\Delta\delta$ +3.7 and +3.5, respectively). The UV spectra of **5** and **7** also showed hypsochromic shift as previously described, further confirming the esterification. The HMBC spectra of **5** and **7** connected the *o*- and *m*-coupled aromatic proton H-7″, respectively, with the ester carbonyl C-1″. Thus, compounds **5** and **7** were determined to be 4-(((*R*)-2,7,8-trimethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-yloxy)carbonyl)isophthalic acid and 2-(((*R*)-2,7,8-trimethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-yloxy)carbonyl)terephthalic acid, respectively.

Table 1
^{1}H and ^{13}C NMR data of **4** and **5**[a]

Position	**4**		**5**	
	δ_C, mult.	δ_H (*J*)	δ_C, mult.	δ_H (*J*)
2	75.0, qC		76.8, qC	
2-CH_3	17.7, CH_3	1.22, s	24.1, CH_3	1.26, s
3	31.2, CH_2	1.76, m	31.1, CH_2	1.76, m
4	20.7, CH_2	2.59, m	22.3, CH_2	2.74, m
5	123.3, qC		118.6, CH	6.81, s
5-CH_3	11.9, CH_3	2.05, s		
6	149.7, qC		150.0, qC	
7	125.4, qC		127.2, qC	
7-CH_3	13.1, CH_3	2.09, s	12.1, CH_3	2.13, s
8	127.1, qC		126.1, qC	
8-CH_3	12.2, CH_3	2.08, s	12.8, CH_3	2.12, s
9	117.6, qC		118.8, qC	
10	140.6, qC		141.6, qC	
1′	39.8, CH_2	1.53, 1.60, m	39.8, CH_2	1.56, 1.64, m
2′	22.2, CH_2	2.07, 2.14, m	22.3, CH_2	2.11, 2.15, m
3′	124.4, CH	5.06, m	124.5, CH	5.09, m
4′	135.0, qC		135.3, qC	
4′-CH_3	15.9, CH_3	1.55, s	16.0, CH_3	1.58, s
5′	39.8, CH_2	1.94, m	39.8, CH_2	1.96, m
6′	26.8, CH_2	2.02, m	26.8, CH_2	2.06, m
7′	124.2, CH	5.06, m	124.3, CH	5.09, m
8′	134.9, qC		135.1, qC	
8′-CH_3	16.0, CH_3	1.55, s	16.1, CH_3	1.58, s
9′	39.8, CH_2	1.94, m	39.8, CH_2	1.96, m
10′	26.6, CH_2	2.02, m	26.7, CH_2	2.06, m
11′	124.3, CH	5.06, m	124.3, CH	5.09, m
12′	131.4, qC		130.5, qC	
12′a-CH_3	16.6, CH_3	1.57, s	17.8, CH_3	1.60, s
12′b-CH_3	25.7, CH_3	1.63, s	25.8, CH_3	1.66, s
1″	165.6, qC		166.6, qC	
2″	135.3, qC		138.0, qC	
3″	133.8, qC		131.8, qC	
3″-*C*OO	169.1, qC		170.4, qC	
4″	130.7, CH	8.44, d, (1.4)	131.0, CH	8.71, d (1.4)
5″	133.5, qC		131.3, qC	
5″-*C*OO	167.2, qC		169.6, qC	
6″	132.2, CH	8.23, dd (1.8, 8.1)	133.9, CH	8.37, dd (1.5, 8.0)
7″	129.4, CH	8.03, d (8.0)	129.2, CH	7.92, d (8.0)

[a] In $CDCl_3$, *J* in Hertz. 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR. Carbon multiplicities were determined by APT experiments, C = quaternary, CH = methine, CH_2 = methylene, CH_3 = methyl carbons.

The HREIMS of **8** suggested the molecular formula $C_{36}H_{44}O_7$ and possible esterification of **3** with 1,2,4-benzenetricarboxylic anhydride. The ^{1}H and ^{13}C NMR data (Table 3) further supported this conclusion. The esterification with terephthalate was further confirmed by UV, ^{1}H, ^{13}C NMR chemical shifts, and HMBC data. Thus, compound **8** was determined to be 2-(((*R*)-2,8-dimethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)-chroman-6-yloxy)-carbonyl)terephthalic acid.

The HREIMS of **9** suggested the molecular formula $C_{33}H_{46}O_5$ and possible maleate esterification. The ^{1}H and ^{13}C NMR data (Table 3) further supported this conclusion. The proton doublet H-3″ (δ_H 7.06) showed a ^{3}*J*-HMBC coupling with the carbonyl carbon C-1″ and COSY coupling with proton H-2″ (δ_H 7.13). The later showed a ^{3}*J*-HMBC coupling with the carbonyl C-4″, confirming the identity of the maleate ester. Thus, compound **9** was determined to be (*Z*)-4-oxo-4-((*R*)-2,5,7,8-tetramethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-yloxy)but-2-enoic acid.

Table 2
^{1}H and ^{13}C NMR data of **6** and **7**[a]

Position	6		7	
	δ_C, mult.	δ_H (*J*)	δ_C, mult.	δ_H (*J*)
2	75.0, qC		75.9, qC	
2-CH_3	17.8, CH_3	1.26, s	24.1, CH_3	1.24, s
3	31.2, CH_2	1.80, m	31.1, CH_2	1.74, m
4	22.3, CH_2	2.61, m	22.2, CH_2	2.71, m
5	123.3, qC		118.7, CH	6.76, s
5-CH_3	12.0, CH_3	2.05, s	—	
6	149.7, qC		149.8, qC	
7	125.3, qC		127.4, qC	
7-CH_3	13.1, CH_3	2.12, s	12.0, CH_3	2.10, s
8	127.0, qC		126.0, qC	
8-CH_3	12.3, CH_3	2.10, s	12.7, CH_3	2.07, s
9	117.6, qC		118.6, qC	
10	140.6, qC		141.7, qC	
1′	39.8, CH_2	1.57, 1.62, m	39.8, CH_2	1.50, 1.57, m
2′	20.7, CH_2	2.07, 2.14, m	22.3, CH_2	2.04, 2.08, m
3′	124.5, CH	5.09, m	124.4, CH	5.07, m
4′	135.3, qC		135.3, qC	
4′-CH_3	16.0, CH_3	1.58, s	15.9, CH_3	1.55, s
5′	39.8, CH_2	1.96, m	39.8, CH_2	1.93, m
6′	26.8, CH_2	2.04, m	26.8, CH_2	2.01, m
7′	124.3, CH	5.09, m	124.2, CH	5.07, m
8′	135.1, qC		135.1, qC	
8′-CH_3	16.1, CH_3	1.58, s	16.0, CH_3	1.55, s
9′	39.8, CH_2	1.96, m	39.8, CH_2	1.93, m
10′	26.7, CH_2	2.04, m	26.6, CH_2	2.01, m
11′	124.3, CH	5.09, m	124.2, CH	5.07, m
12′	130.4, qC		131.4, qC	
12′a-CH_3	16.5, CH_3	1.60, s	17.7, CH_3	1.57, s
12′b-CH_3	25.8, CH_3	1.66, s	25.7, CH_3	1.63, s
1″	164.8, qC		166.4, qC	
2″	132.4, qC		133.1, qC	
3″	138.3, qC		136.5, qC	
3″-*C*OO	169.7, qC		168.8, qC	
4″	129.1, CH	7.81, d (8.1)	129.5, CH	7.86, d (8.0)
5″	133.4, CH	8.29, dd (1.5, 8.0)	132.7, CH	8.21, dd (1.5, 7.7)
6″	131.4, qC		134.3, qC	
6″-*C*OO	167.3, qC		167.1, qC	
7″	131.2, CH	8.75, d (1.5)	130.4, CH	8.51, d (1.5)

[a] In $CDCl_3$, *J* in Hertz. 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR. Carbon multiplicities were determined by APT experiments, C = quaternary, CH = methine, CH_2 = methylene, CH_3 = methyl carbons.

Table 3
^{1}H and ^{13}C NMR data of **8** and **9**[a]

Position	8		9	
	δ_C, mult.	δ_H (*J*)	δ_C, mult.	δ_H (*J*)
2	76.0, qC		75.0, qC	—
2-CH_3	24.1, CH_3	1.24, s	24.2, CH_3	1.25, s
3	31.1, CH_2	1.75, m	31.1, CH_2	1.76, m
4	22.5, CH_2	2.73, m	20.7, CH_2	2.60, m
5	119.0, CH	6.77, d (2.9)	123.3, qC	
5-CH_3			11.9, CH_3	1.96, s
6	150.0, qC		149.8, qC	
7	121.0, CH	6.82, d (2.6)	124.8, qC	
7-CH_3			13.1, CH_3	2.10, s
8	127.5, qC		126.6, qC	
8-CH_3	16.1, CH_3	2.13, s	12.2, CH_3	2.00, s
9	121.1, qC		117.6, qC	
10	142.6, qC		140.3, qC	
1′	39.8, CH_2	1.50, 1.59, m	39.8, CH_2	1.54, 1.62, m
2′	22.2, CH_2	2.06, 2.10, m	22.3, CH_2	2.08, m
3′	124.4, CH	5.06, m	124.5, CH	5.09, m
4′	135.4, qC		135.3, qC	
4′-CH_3	15.9, CH_3	1.55, s	16.1, CH_3	1.59, s
5′	39.8, CH_2	1.92, m	39.8, CH_2	1.96, m
6′	26.8, CH_2	2.02, m	26.8, CH_2	2.05, m
7′	124.2, CH	5.06, m	124.3, CH	5.09, m
8′	135.1, qC		135.1, qC	
8′-CH_3	16.0, CH_3	1.55, s	16.0, CH_3	1.59, s
9′	39.8, CH_2	1.92, m	39.8, CH_2	1.96, m
10′	26.6, CH_2	2.02, m	26.7, CH_2	2.05, m
11′	124.2, CH	5.06, m	124.3, CH	5.09, m
12′	131.4, qC		131.3, qC	
12′a-CH_3	17.7, CH_3	1.56, s	17.8, CH_3	1.58, s
12′b-CH_3	25.7, CH_3	1.63, s	25.8, CH_3	1.67, s
1″	166.8, qC		163.8, qC	
2″	133.2, qC		133.4, CH	7.13, d (15.8)
3″	136.0, qC		135.6, CH	7.06, d (15.8)
3″-*C*OO	168.6, qC			
4″	129.5, CH	7.89, d (8.0)	168.7, qC	
5″	133.2, CH	8.21, dd (1.5, 7.0)		
5″-*C*OO	167.1, qC			
6″	134.4, qC			
7″	130.4, CH	8.45, d (1.4)		

[a] In $CDCl_3$, *J* in Hertz. 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR. Carbon multiplicities were determined by APT experiments, C = quaternary, CH = methine, CH_2 = methylene, CH_3 = methyl carbons.

The HREIMS of **10** and **11** suggested the molecular formula $C_{32}H_{44}O_5$ and $C_{31}H_{42}O_5$, respectively, suggesting possible maleate esterification of **2** and **3**, respectively. Their ^{1}H and ^{13}C NMR data (Table 4) further supported this conclusion. Thus, compounds **10** and **11** were determined to be (*Z*)-4-oxo-4-((*R*)-2,7,8-trimethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)-chroman-6-yloxy)but-2-enoic acid and (*Z*)-4-((*R*)-2,8-dimethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-yloxy)-4-oxo-but-2-enoic acid, respectively.

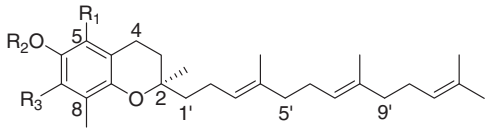
The HREIMS of **12** suggested the molecular formula $C_{34}H_{50}O_5$ and possible esterification with 3-methylglutaric anhydride. The ^{1}H and ^{13}C NMR data (Table 5) further supported this conclusion. The methyl doublet H_3-6″ (δ_H 1.16) showed ^3J-HMBC couplings with the methylene carbons C-2″ and C-4″ (δ_C 40.6 and 40.5, respectively). It also showed a ^2J-HMBC coupling with the methine carbon C-3″ (δ_C 27.4). Proton H-3″ showed ^3J-HMBC couplings with the carbonyl carbons C-1″ and C-5″ (δ_C 171.5 and 178.2, respectively) and COSY couplings with the methylene protons H_2-2″ and H_2-H-4″, confirming the 3-methyl-glutaryl ester. Thus, compound **12** was determined to be 3-Methyl-5-oxo-5-((*R*)-2,7,8-trimethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-yloxy)pentanoic acid.

The HREIMS of **13** suggested the molecular formula $C_{30}H_{46}O_2$ and possible benzoylation. The ^{1}H and ^{13}C NMR data (Table 5) further supported this conclusion. The proton doublet H-3″/H-7″ (δ_H 8.21) showed ^3J-HMBC couplings with the carbonyl ester C-1″ and the aromatic methine carbon C-5″ (δ_C 165.7 and 133.4, respectively). Protons H-3″/H-7″ also showed a COSY coupling with proton doublet of doublets H-4″/H-6″ (δ_H 7.50), confirming the identity of the benzoate moiety. Thus, compound **13** was determined to be (*R*)-2,7,8-trimethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-yl benzoate.

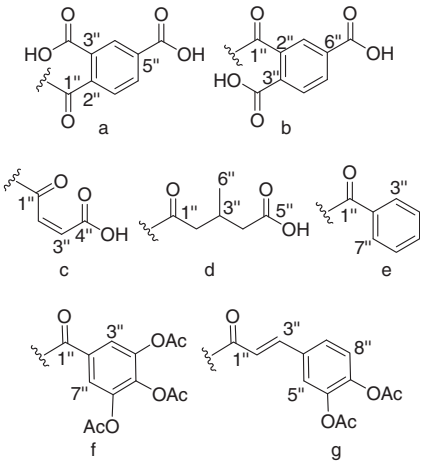
The HREIMS of **14** suggested the molecular formula $C_{41}H_{52}O_9$ and possible esterification with 3,4,5-triacetoxybenzoyl chloride. The proton singlet H-3″/H-7″ (δ_H 7.95) showed ^3J-HMBC couplings with the carbonyl ester C-1″ and the aromatic oxygenated quaternary carbon C-5″ (δ_C 163.5 and 139.1, respectively). The C-4″, C-5″, and C-6″ acetoxy methyl singlets (δ_H 2.31, 2.32, and 2.31, respectively) showed ^2J-HMBC couplings with their corresponding carbonyl carbons (δ_C 167.8, 166.5, and 167.8, respectively). There was no enough available spectral evidence to unambiguously assign these acetoxy groups. Thus, compound **14** was determined to be 5-(((*R*)-2,7,8-trimethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-yloxy)carbonyl)benzene-1,2,3-triyl triacetate.

The HREIMS of **15** suggested the molecular formula $C_{41}H_{52}O_7$ and possible esterification with 3,4-diacetylcaffeoyl chloride. The ^{1}H and ^{13}C NMR data (Table 6) further supported this conclusion. The proton doublet H-3″ (δ_H 7.79) showed ^3J-HMBC couplings with the carbonyl ester C-1″ and the aromatic methine carbons C-5″ and

C-9″ (δ_C 165.7, 123.0, and 126.7, respectively). Proton H-3″ showed a COSY coupling with the proton doublet H-2″ (δ_H 6.59). Proton H-9″ (δ_H 7.46) showed ^3J-HMBC couplings with C-5″ and the oxygenated quaternary aromatic carbon C-7″ (δ_C 143.8). It also showed COSY coupling with proton doublet H-8″ (δ_H 7.25). The later proton showed ^3J-HMBC couplings with the quaternary aromatic carbons C-4″ and C-6″ (δ_C 133.3 and 143.4, respectively), confirming the identity of the caffeoyl moiety. The C-6″and C-7″ acetoxymethyl singlets (δ_H 2.31 and 2.32, respectively) showed ^2J-HMBC couplings with their corresponding carbonyl carbons (δ_C 168.2 and 168.1, respectively). There was no enough available spectral evidence to unambiguously assign these acetoxy groups. Thus, compound **15** was determined to be 4-((*E*)-3-oxo-3-((*R*)-2,7,8-trimethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-yloxy)-prop-1-enyl)-1,2-phenylene diacetate.



Compound	R_1	R_2	R_3
1	CH_3	H	CH_3
2	H	H	CH_3
3	H	H	H
4	CH_3	a	CH_3
5	H	a	CH_3
6	CH_3	b	CH_3
7	H	b	CH_3
8	H	b	H
9	CH_3	c	CH_3
10	H	c	CH_3
11	H	c	H
12	H	d	CH_3
13	H	e	CH_3
14	H	f	CH_3
15	H	g	CH_3



2.2. Biological activity

Esters **4**–**15** have been tested for their antiproliferative activity on malignant +SA and normal CL-S1 mouse mammary epithelial cells using MTT assay (Figs. 1 and 2). All compounds displayed antiproliferative activity except **12**, **13**, and **15** on +SA mammary tumor cells. However, treatment with similar doses of these compounds had no effect on the growth or the viability of immortalized normal CL-S1 mammary epithelial cells. The presence of hydrogen bond donating (HBD) and/or accepting (HBA) group may play a role in the activity. Despite the presence of a free carboxylic group in compound **12**, it was inactive. Compound **12** differ than the other active compounds in that it lacks the α,β-unsaturation and the acid is five-carbons long. This may indicate that an acid that will afford a maximum of four-carbon distance between the C-6 oxygen and the HBD and/or HBA group is preferred for activity. In addition, the coexistence of both α,β-unsaturation and HBD and/or HBA group is required for high activity. The data are consistent with literature where unsaturated dicarboxylic acids (e.g., maleate or fumarate) esters or amides of tocopherols showed greater apoptotic activity than the saturated tocopheryl esters like succinate.[14,28] The most active esters **5**, **9**, and **11** potently inhibited the proliferation of +SA mammary epithelial cells with IC_{50} values of 0.62, 0.51, and 0.86 μM, respectively (Table 7) while the IC_{50} values of **1**–**3** are 5 μM, 4 μM, and 3 μM, respectively, against +SA cells.[29] The remarkable activity of α-tocotrienol maleate (**9**) was of special interest because its parent natural product (α-tocotrienol, **1**) was the least active versus other tocotrienols. This remarkable activity improvement opens new horizons for future development of tocotrienol redox-silent ester analogues as potential anticancer drugs.

Esters **4**–**15** have been tested for their anti-migratory activity against the human highly metastatic MDA-MB-231 breast cancer cells in wound-healing assay (Figs. 3 and 4). Compounds **4**, **6**, **8**–**10**, and **13** showed inhibition of nearly 50% of the migrated MDA-MB231 cells at a single 5 μM dose (Figs. 3 and 4). The presence of terminal free ionizable groups together with aromatic or α,β-unsaturated ester moiety was also correlated with the anti-migratory activity. It is also worth noting the potent anti-migratory activity of α-tocotrienol isophthalate, terephthalate, and maleate (**4**, **6**, and **9**, respectively) compared to its inactive parent natural α-tocotrienol. The activity of γ-tocotrienol benzoate was consistent with the reported activity of γ-tocotrienol benzyl carbamate,[27] which suggests a possible π-stacking interaction role for the benzyl moiety, which improve the binding affinity at specific molecular target(s) when located at optimized distance from C-6 oxygen.

2.3. Solubility and stability

The water solubility of the most active ester **9** and its parent **1** was investigated in phosphate buffer (pH 7.4, 25 °C). The solubility of α-tocotrienol maleate (**9**) was nearly 1000-fold that of its parent **1**. The stability of **9** in phosphate buffer (pH 7.4, 37 °C) was also examined. α-Tocotrienol maleate (**9**) showed 100% chemical stability relative to the zero time concentration over 24 h. On contrary, the parent **1** was highly unstable under the same conditions and started decomposition 1 h after dissolution. α-Tocotrienol (**1**) was completely undetectable after 24 h, in the buffer solution and other peaks were detected by HPLC with shorter retention times. In rat plasma, ester **9** concentration was 100% stable over the first 3 h. Its concentration then started to decrease at the 4th hour and

Table 4
^{1}H and ^{13}C NMR data of **10** and **11**[a]

Position	10		11	
	δ_C, mult.	δ_H (J)	δ_C, mult.	δ_H (J)
2	76.8, qC	—	76.8, qC	
2-CH_3	24.2, CH_3	1.27, s	24.2, CH_3	1.27, s
3	31.1, CH_2	1.76, m	31.0, CH_2	1.80, m
4	22.3, CH_2	2.71, m	22.2, CH_2	2.74, m
5	118.7, CH	6.62, s	118.8, CH	6.67, d (2.6)
5-CH_3	—		—	
6	149.9, qC		150.1, qC	
7	126.9, qC		120.9, CH	6.72, d (2.2)
7-CH_3	12.1, CH_3	2.12, s	—	
8	126.1, qC		127.6, qC	
8-CH_3	12.8, CH_3	2.02, s	16.2, CH_3	2.15, s
9	118.5, qC		121.1, qC	
10	141.4, qC		142.1, qC	
1′	39.8, CH_2	1.54, 1.62, m	39.8, CH_2	1.53, 1.64, m
2′	22.3, CH_2	2.09, m	22.5, CH_2	2.11, m
3′	124.5, CH	5.10, m	124.5, CH	5.11, m
4′	135.3, qC		135.4, qC	
4′-CH_3	16.1, CH_3	1.59, s	16.1, CH_3	1.59, s
5′	39.8, CH_2	1.97, m	39.8, CH_2	1.96, m
6′	26.8, CH_2	2.04, m	26.8, CH_2	2.04, m
7′	124.3, CH	5.10, m	124.2, CH	5.11, m
8′	135.0, qC		135.1, qC	
8′-CH_3	16.0, CH_3	1.59, s	16.0, CH_3	1.59, s
9′	39.8, CH_2	1.97, m	39.8, CH_2	1.96, m
10′	26.7, CH_2	2.04, m	26.7, CH_2	2.04, m
11′	124.3, CH	5.10, m	124.2, CH	5.11, m
12′	131.3, qC		131.4, qC	
12′a-CH_3	17.7, CH_3	1.58, s	17.8, CH_3	1.59, s
12′b-CH_3	25.8, CH_3	1.67, s	25.8, CH_3	1.67, s
1″	164.1, qC		164.0, qC	
2″	134.2, CH	7.15, d (15.8)	134.8, CH	7.08, d (15.8)
3″	134.8, CH	7.04, d (15.8)	134.2, CH	6.99, d (15.8)
4″	168.2, qC		168.5, qC	

[a] In $CDCl_3$, J in Hertz. 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR. Carbon multiplicities were determined by APT experiments, C = quaternary, CH = methine, CH_2 = methylene, CH_3 = methyl carbons.

5th hour to become 81% and 77% of the initial concentration, respectively. After 24 h, only 43% of the initial concentration of **9** was detectable. The slow rate of hydrolysis of **9** to its parent **1** bodes well for expected enhanced metabolic stability.

3. Conclusions

In conclusion, 12 new tocotrienol esters **4**–**15** were semisynthesized. At doses that had no effect on the normal mouse CL-S1 cells, esters **5**, **9**, and **11** effectively inhibited the proliferation of the highly metastatic +SA mammary epithelial cells at nM doses. Esters **4**, **6**, **8**–**10**, and **13** potently inhibited the wound-healing migration of MDA-MB231, the metastatic human breast cancer cell line. Terminal HBD and/or HBA group together with aromatic or α,β-unsaturation in the acylating acid can greatly improve the antiproliferative and anti-migratory activities. Tocotrienol esters showed remarkable enhancement of water solubility, chemical stability, and slow decomposition rate in rat plasma.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured on a Rudolph Research Analytical Autopol III polarimeter. IR spectra were recorded on a Varian 800 FT-IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, using TMS as an internal standard, on a JEOL Eclipse-400 NMR spectrometer, operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. The HREIMS experiments were conducted at Louisiana State University on a 6200-TOF LC–MS (Agilent) equipped with multimode source (mixed source that can ionized the compounds alternatively by ESI and APCI). TLC analysis was carried on precoated Si gel 60 F_{254} 500 μm TLC plates (EMD Chemicals), using variable proportions of *n*-hexane–ethyl acetate and ethyl acetate–methanol–water as a mobile phase. 1% Vanillin in concentrated H_2SO_4 was used as visualizing reagent. For column chromatography, Si gel 60 (Natland, 63–200 μm) was used.

4.2. Biological material

Tocotrienol-rich fraction 50 g (Palm TRF 70%, low in tocopherol from First Tech International Ltd, Hong Kong) was fractionated on Si gel 60 using *n*-hexane–ethyl acetate (gradient elution).

4.3. Chemical reactions

4.3.1. Preparation of triacetylgallic and diacetylcaffeic acid chlorides[30]

To a solution of gallic or caffeic acids (20 mmol, each) was added acetic anhydride (6 equiv) and 2 mL pyridine (Scheme 1). Each mixture was then stirred for 4 h, at rt and then poured onto 10 mL 1 M H_3PO_4 cold solution. Each mixture was extracted with ethyl acetate (3 × 10 mL). The organic layers were washed with brine, saturated aqueous $NaHCO_3$, and H_2O. The combined organic solution was dried using anhydrous $MgSO_4$, filtered, and the solvent was removed under a vacuum to afford 3,4,5-triacetoxybenzoic acid, and 3,4-diacetoxycinnamic acid. These products were identified by ^{1}H and ^{13}C NMR analysis and comparison with the literature.[31,32] To a solution of each acetylated acid (5 mmol) in dry CH_2Cl_2 (10 mL), 0.7 mL oxalyl chloride was added and each mixture was stirred for 8 h at rt. Each mixture was concentrated under vacuum to give the 3,4,5-triacetoxybenzoic and 3,4-diacetoxycinnamic acid chlorides.

4.3.2. Esterification of tocotrienols using acid chlorides[30]

A mixture of dry CH_2Cl_2 (10 mL), triethylamine (5 mL), and tocotrienol (1.2 equiv) was added to acid chloride concentrate at 0 °C (Scheme 2). Each reaction mixture was stirred over night and then poured onto ice water. Each mixture was extracted three times with ethyl acetate (3 × 10 mL). The combined organic phase was dried over anhydrous $MgSO_4$, filtered, and the solvent was removed under vacuum. Each residue was purified by column chromatography on Si gel 60.[33]

4.3.3. Esterification of tocotrienols using acid anhydrides

To a dry pyridine solution of tocotrienol (4.8 mmol), 5.7 mmol of acid anhydrides, and 5.7 mmol of dicyclohexylcarbodiimide (DCC) were added (Scheme 2). The reaction mixture was stirred at rt for 20 h and the dicyclohexylurea formed was removed by filtration. After solvent evaporation, the residue was treated with 100 mL H_2O and alkalinized by adding $NaHCO_3$. The solution was then extracted with EtOAc (100 mL × 3). The organic layer was dried over anhydrous $MgSO_4$ and evaporated. The residue was purified by column chromatography either over Si gel 60 using isocratic ethyl acetate–methanol (92.5:7.5) as a mobile system, or over C18-RP Si gel, using MeOH–H_2O, gradient elution.

4.3.3.1. 4-(((*R*)-2,5,7,8-Tetramethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)-chroman-6-yloxy)carbonyl)isophthalic acid (4). Yellow viscous oil, UV (MEOH) λ_{max} (log ε) 288 (6.09); $[\alpha]_D^{25}$ +2.5 (*c* 0.16, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3626, 2926.4, 1740, 1706.9, 1230.3 cm^{-1}; ^{1}H and ^{13}C NMR: see Table 1; HREIMS *m*/*z* 615.3327, $[M-H]^+$ (calcd for $C_{38}H_{47}O_7$, 615.3323).

Table 5
^{1}H and ^{13}C NMR data of **12** and **13**[a]

Position	12		13	
	δ_C, mult.	δ_H (J)	δ_C, mult.	δ_H (J)
2	76.8, qC		75.9, qC	
2-CH_3	24.2, CH_3	1.59, s	24.2, CH_3	1.29, s
3	31.1, CH_2	1.73, m	29.7, CH_2	1.77, m
4	22.3, CH_2	2.71, m	22.3, CH_2	2.74, m
5	118.9, CH	6.55, s	119.1, CH	6.70, s
6	149.6, qC		149.7, qC	
7	127.1, qC		127.4, qC	
7-CH_3	12.1, CH_3	2.10, s	12.1, CH_3	2.14, s
8	126.0, qC		126.0, qC	
8-CH_3	12.9, CH_3	2.01, s	12.9, CH_3	2.04, s
9	118.5, qC		118.6, qC	
10	141.5, qC		141.9, qC	
1′	39.8, CH_2	1.54, 1.63, m	39.8, CH_2	1.50, 1.62, m
2′	22.3, CH_2	2.07, m	22.3, CH_2	2.12, m
3′	124.5, CH	5.10, m	124.5, CH	5.11, m
4′	135.3, qC		135.3, qC	
4′-CH_3	16.1, CH_3	1.60, s	16.1, CH_3	1.59, s
5′	39.8, CH_2	1.97, m	39.8, CH_2	1.97, m
6′	26.8, CH_2	2.05, m	26.8, CH_2	2.04, m
7′	124.3, CH	5.10, m	124.3, CH	5.11, m
8′	135.1, qC		135.1, qC	
8′-CH_3	16.0, CH_3	1.60, s	16.0, CH_3	1.59, s
9′	39.8, CH_2	1.97, m	39.8, CH_2	1.97, m
10′	26.7, CH_2	2.05, m	26.7, CH_2	2.04, m
11′	124.3, CH	5.10, m	124.3, CH	5.11, m
12′	131.3, qC		131.3, qC	
12′a-CH_3	17.8, CH_3	1.59, s	17.8, CH_3	1.61, s
12′b-CH_3	25.8, CH_3	1.67, s	25.8, CH_3	1.67, s
1″	171.5, qC		165.7, qC	
2″	40.6, CH_2	2.60, dd (6.2, 12.8), 2.66, d (5.5)	129.9, qC	
3″	27.4, CH_2	2.54, m	130.2, CH	8.21, dd (1.1, 7.3)
4″	40.5, CH_2	2.37, dd (7.0, 15.4), 2.49, d (7.0)	128.6, CH	7.50, dd (7.7, 7.7)
5″	178.2, qC		133.4, CH	7.62, br t (7.3)
6″	20.0, CH_3	1.16, d (6.2)	128.6, CH	7.50, dd (7.7, 7.7)
7″			130.2, CH	8.21, dd (1.1, 7.3)

[a] In $CDCl_3$, J in Hertz. 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR. Carbon multiplicities were determined by APT experiments, C = quaternary, CH = methine, CH_2 = methylene, CH_3 = methyl carbons.

4.3.3.2. 4-(((*R*)-2,7,8-Trimethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-yloxy)carbonyl)isophthalic acid (5). Yellowish white semisolid, UV (MEOH) λ_{max} (log ε) 286 (6.48); $[\alpha]_D^{25}$ 0.0 (c 0.08, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3623, 2832.9, 2305.4, 1720.5, 1263.3 cm^{-1}; ^{1}H and ^{13}C NMR: see Table 1; HREIMS m/z 601.3165, $[M-H]^+$ (calcd for $C_{37}H_{45}O_7$, 601.3171).

4.3.3.3. 2-(((*R*)-2,5,7,8-Tetramethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-yloxy)carbonyl)terephthalic acid (6). Yellowish white semisolid, UV (MEOH) λ_{max} (log ε) 284 (6.38); $[\alpha]_D^{25}$ −2.3 (c 0.04, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3625, 3054, 2986, 2305, 1738, 1605, 1422, 1018 cm^{-1}; ^{1}H and ^{13}C NMR: see Table 2; HREIMS m/z 615.3328, $[M-H]^+$ (calcd for $C_{38}H_{47}O_7$, 615.3327).

4.3.3.4. 2-(((*R*)-2,7,8-Trimethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-yloxy)carbonyl)terephthalic acid (7). Yellowish white semisolid, UV (MEOH) λ_{max} (log ε) 290 (6.25); $[\alpha]_D^{25}$ 0.0 (c 0.19, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2927, 1742, 1705, 1228, 1102 cm^{-1}; ^{1}H and ^{13}C NMR: see Table 2; HREIMS m/z 601.3170, $[M-H]^+$ (calcd for $C_{37}H_{45}O_7$, 601.3171).

4.3.3.5. 2-(((*R*)-2,8-Dimethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-yloxy)-carbonyl)terephthalic acid (8). Yellowish white semisolid, UV (MEOH) λ_{max} (log ε) 290 (6.52); $[\alpha]_D^{25}$ −2.5 (c 0.04, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3054, 2986, 2305, 1741, 1705, 1422, 1154 cm^{-1}; ^{1}H and ^{13}C NMR: see Table 3; HREIMS m/z 587.3014, $[M-H]^+$ (calcd for $C_{36}H_{43}O_7$, 587.3014).

4.3.3.6. (*Z*)-4-Oxo-4-((*R*)-2,5,7,8-tetramethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-yloxy)but-2-enoic acid (9). Yellow viscous oil, UV (MEOH) λ_{max} (log ε) 284 (6.22); $[\alpha]_D^{25}$ −4.8 (c 0.08, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3054, 2933, 2857, 2305, 1736, 1710, 1230, 1152 cm^{-1}; ^{1}H and ^{13}C NMR: see Table 3; HREIMS m/z 521.3269, $[M-H]^+$ (calcd for $C_{33}H_{45}O_5$, 521.3272).

4.3.3.7. (*Z*)-4-Oxo-4-((*R*)-2,7,8-trimethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)-chroman-6-yloxy)but-2-enoic acid (10). Yellow viscous oil, UV (MEOH) λ_{max} (log ε) 288 (6.23); $[\alpha]_D^{25}$ −36.6 (c 0.03, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3054, 2987, 2685, 2360, 2306, 1734, 1605, 1421, 1157 cm^{-1}; ^{1}H and ^{13}C NMR: see Table 4; HREIMS m/z 507.3118, $[M-H]^+$ (calcd for $C_{32}H_{43}O_5$, 507.3116).

4.3.3.8. (*Z*)-4-((*R*)-2,8-Dimethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-yloxy)-4-oxobut-2-enoic acid (11). Yellow viscous oil, UV (MEOH) λ_{max} (log ε) 288 (6.18); $[\alpha]_D^{25}$ 12.8 (c 0.023, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3054, 2987, 2685, 2360, 2306, 1734, 1605, 1421, 11266 cm^{-1}; ^{1}H and ^{13}C NMR: see Table 4; HREIMS m/z 493.2960, $[M-H]^+$ (calcd for $C_{31}H_{41}O_5$, 493.2959).

4.3.3.9. 3-Methyl-5-oxo-5-((*R*)-2,7,8-trimethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-yloxy)pentanoic acid (12). White yellowish viscous oil, UV (MEOH) λ_{max} (log ε) 288 (5.57); $[\alpha]_D^{25}$ -2.3 (c 0.39, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3626, 2927, 2856, 1711, 1476, 1377, 1198 cm^{-1}; ^{1}H and ^{13}C NMR: see Table 5; HREIMS m/z 537.3585, $[M-H]^+$ (calcd for $C_{34}H_{49}O_5$, 537.3585).

Table 6
^{1}H and ^{13}C NMR data of **14** and **15**[a]

Position	14		15	
	δ_C, mult.	δ_H (J)	δ_C, mult.	δ_H (J)
2	76.8, qC		76.8, qC	
2-CH_3	24.2, CH_3	1.28, s	24.2, CH_3	1.27, s
3	31.3, CH_2	1.78, m	31.1, CH_2	1.76, m
4	22.3, CH_2	2.72, m	22.3, CH_2	2.73, m
5	118.9, CH	6.64, s	118.9, CH	6.64, s
6	149.8, qC		149.7, qC	
7	127.2, qC		126.9, qC	
7-CH_3	12.1, CH_3	2.12, s	12.1, CH_3	2.12, s
8	126.1, qC		126.6, qC	
8-CH_3	12.9, CH_3	2.04, s	12.8, CH_3	2.04, s
9	118.6, qC		118.5, qC	
10	141.6, qC		141.6, qC	
1′	39.8, CH_2	1.54, m 1.62, m	39.8, CH_2	1.57, m 1.62, m
2′	22.2, CH_2	2.10, m	22.3, CH_2	2.10, m
3′	124.5, CH	5.11, m	124.5, CH	5.11, m
4′	135.3, qC		135.3, qC	
4′-CH_3	16.0, CH_3	1.59, s	16.1, CH_3	1.59, s
5′	39.8, CH_2	1.97, m	39.8, CH_2	1.96, m
6′	26.8, CH_2	2.07, m	26.8, CH_2	2.06, m
7′	124.3, CH	5.11, m	124.3, CH	5.11, m
8′	135.1, qC		135.0, qC	
8′-CH_3	16.1, CH_3	1.59, s	16.0, CH_3	1.59, s
9′	39.8, CH_2	1.97, m	39.8, CH_2	1.96, m
10′	26.7, CH_2	2.07, m	26.7, CH_2	2.06, m
11′	124.3, CH	5.11, m	124.3, CH	5.11, m
12′	131.4, qC		131.3, qC	
12′a-CH_3	17.8, CH_3	1.60, s	17.8, CH_3	1.60, s
12′b-CH_3	25.8, CH_3	1.67, s	25.8, CH_3	1.67, s,
1″	163.5, qC		165.7, qC	
2″	128.1, qC		118.8, CH	6.59, d (16.1)
3″	122.8, CH	7.95, s	144.2, CH	7.79, d (16.1)
4″	143.6, qC		133.3, qC	
4″-OCO(CH_3)	167.8,[b] qC			
4″-OCO(CH_3)	20.7,[c] CH_3	2.31,[b] s		
5″	139.1, qC		123.0, CH	7.42, d (1.8)
5″-OCO(CH_3)	166.5,[b] qC			
5″-OCO(CH_3)	20.3,[c] CH_3	2.32,[b] s		
6″	143.6, qC		143.4, qC	
6″-OCO(CH_3)	167.8,[b] qC		168.2,[b] qC	
6″-OCO(CH_3)	20.7,[c] CH_3	2.31,[b] s	20.8,[c] CH_3	2.31,[b] s
7″	122.8, CH	7.95, s	143.8, qC	
7″-OCO(CH_3)			168.1,[b] qC	
7″-OCO(CH_3)			20.7,[c] CH_3	2.32,[b] s
8″			124.1, CH	7.25, d (8.1)
9″			126.7, CH	7.46, dd (1.8, 8.4)

[a] In $CDCl_3$, J in Hertz. 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR. Carbon multiplicities were determined by APT experiments, C = quaternary, CH = methine, CH_2 = methylene, CH_3 = methyl carbons.
[b,c] Interchangeable in the same column.

4.3.3.10. (*R*)-2,7,8-Trimethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-yl benzoate (13). White yellowish viscous oil, UV (MEOH) λ_{max} (log ε) 282 (6.40); $[\alpha]_D^{25}$ −4.6 (c 0.065, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2927, 2855, 1731, 1602, 1229, 1094 cm^{-1}; ^{1}H and ^{13}C NMR: see Table 5; HREIMS m/z 437.3421, $[M-H]^+$ (calcd for $C_{30}H_{45}O_2$, 437.3425).

4.3.3.11. 5-(((*R*)-2,7,8-Trimethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-yloxy)carbonyl)benzene-1,2,3-triyl triacetate (14). Yellowish white semisolid, UV (MEOH) λ_{max} (log ε) 286 (6.52); $[\alpha]_D^{25}$ -6.0 (c 0.13, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2928, 2856, 2303, 1782, 1736, 1612, 1493, 1371, 1326, 1190 cm^{-1}; ^{1}H and ^{13}C NMR: see Table 6; HREIMS m/z 689.3690, $[M+H]^+$ (calcd for $C_{41}H_{53}O_9$, 689.3684).

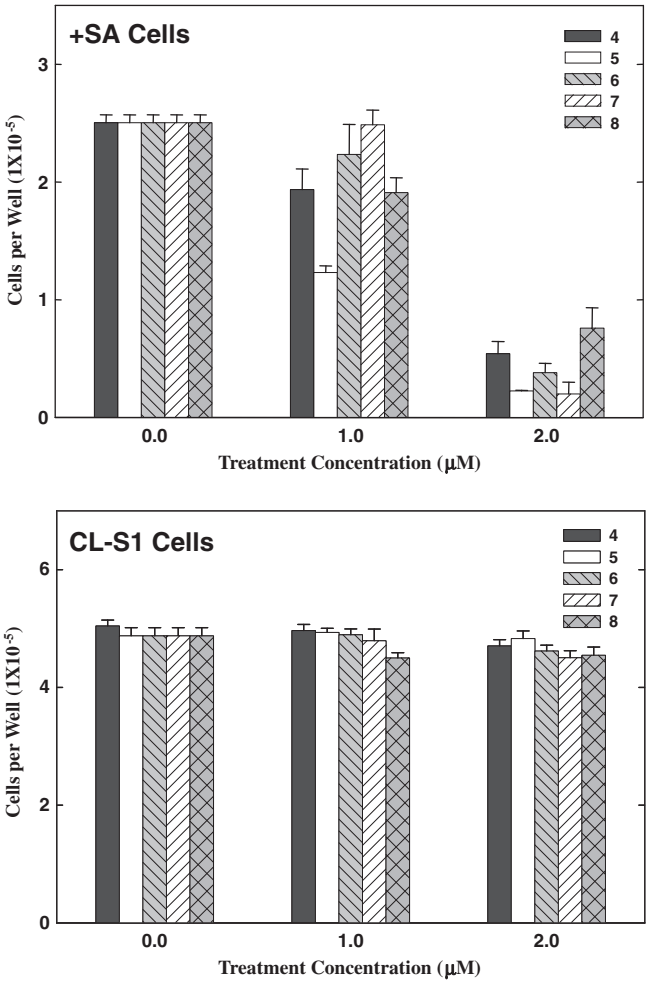


Figure 1. Effects of tocotrienol analogues **4**–**8** on the viability of +SA mammary tumor (top) and CL-S1 cells (bottom).

4.3.3.12. 4-((*E*)-3-Oxo-3-((*R*)-2,7,8-trimethyl-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-yloxy)prop-1-enyl)-1,2-phenylene diacetate (15). Yellow oil, UV (MEOH) λ_{max} (log ε) 284 (6.96); $[\alpha]_D^{25}$ 10 (c 0.03, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3054, 2927, 2855, 1774, 1725, 1641, 1205 cm^{-1}; ^{1}H and ^{13}C NMR: Table 6; HREIMS m/z 657.3772, $[M+H]^+$ (calcd for $C_{41}H_{53}O_7$: 657.3786).

4.4. Biological assays

4.4.1. MTT assay

All materials were purchased from Sigma Chemical Company (St. Louis, MO) unless otherwise stated. The highly malignant +SA mouse mammary epithelial cell line were serially passaged at subconfluent cell density. The +SA mammary tumor cell line was derived from an adenocarcinoma that developed spontaneously in a BALB/c female mouse.[34] The normal CL-S1 mammary epithelial cell line is immortal in culture tube, but does not grow in soft agarose or form solid tumors upon transportation back into the mammary pad of syngeneic BALB/c mice.[29,34] Cell culture conditions have been previously described in detail.[35] Briefly, +SA cells were maintained in serum-free defined medium consisting of Dulbecco's modified Eagle's medium (DMEM)/F12 containing 5 mg/mL bovine serum albumin (BSA), 10 mg/mL transferrin, 100 U/mL soybean

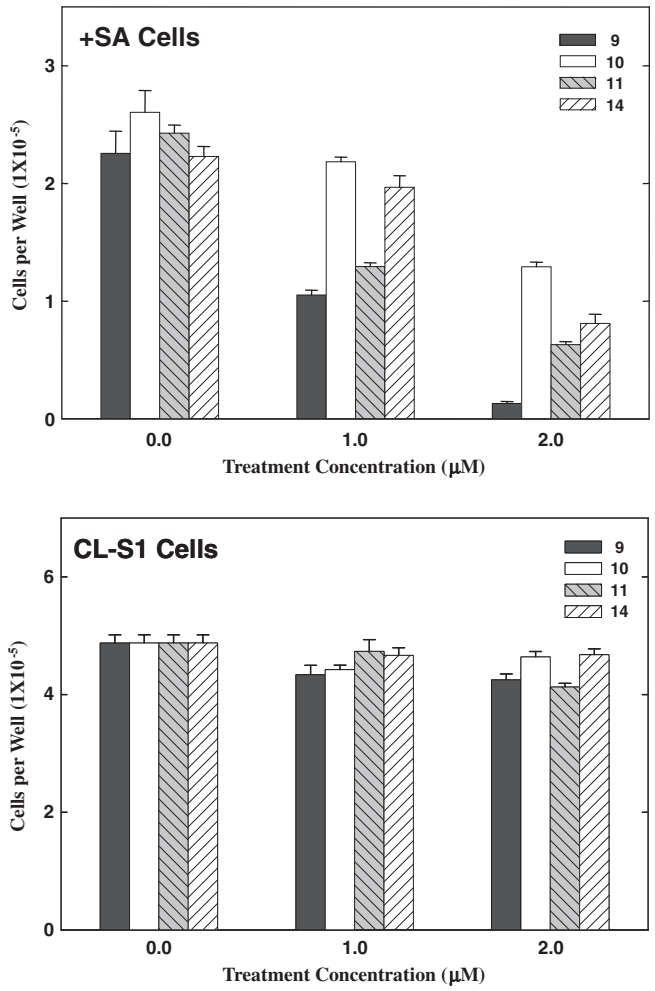


Figure 2. Effects of tocotrienol analogues **9**–**11** and **14** on the viability of +SA mammary tumor (top) and CL-S1 cells (bottom).

Table 7
Antiproliferative activity of tocotrienol esters **4**–**15** against the malignant mice +SA mammary cells

Compound	IC_{50} (μM)
4	1.50
5	0.62
6	1.46
7	1.44
8	1.50
9	0.52
10	2.30
11	0.87
12	19.19
13	6.55
14	1.72
15	>40

trypsin inhibitor, 100 U/mL penicillin G, 0.1 mg/mL streptomycin, 10 mg/mL insulin, and 10 ng/mL epidermal growth factor. While CL-S1 were maintained in DMEM/F12 media containing 10% bovine calf serum, 100 U/mL penicillin G, 0.1 mg/mL streptomycin and 10 mg/mL insulin. For subculturing, cells were rinsed twice with sterile Ca^{2+} and Mg^{2+}-free phosphate buffered saline (PBS) and incubated in 0.05% trypsin containing 0.025% EDTA in PBS for 5 min at 37 °C. The released cells were centrifuged, resuspended in fresh media and counted using hemocytometer. A stock solution of the compounds was prepared in DMSO. Once dissolved, this solution was added to a small volume of sterile 10% BSA in water and incubated overnight at 37 °C. This solution conjugated to BSA was used to prepare various concentrations (0–40 μM) of tocotrienol analogues. DMSO was added to all treatment media such that the final DMSO concentration was the same in all treatment groups within a given experiment and was always less than 0.1%. For cytotoxic studies, cells were seeded at a density of 5×10^4 cells/well (+SA cells) or 1×10^5 cells/well (CL-S1 cells) (six wells/group) in 24-well culture plates and allowed to grow in their respective control media. After a 3-day incubation period (approximately 70% confluency), cells were divided equally into various treatment groups and exposed to their respective treatments for a 24-h incubation period. Following the 24-h treatment period, cell viability was measured using the MTT assay.

+SA Mammary epithelial cell viable number was determined by the 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay as described previously.[35,36] Briefly, on the assay day, treatment medium was replaced with fresh control medium containing 0.42 mg/mL MTT, and the cells in 24-well plates were incubated at 37 °C for 4 h. Afterward, the medium was removed, and the MTT crystals were dissolved in isopropyl alcohol (1 mL/well). The optical density of each sample was read at 570 nm on a microplate reader (SpectraCount, Packard BioScience Company), against a blank prepared from cell-free cultures. The number of cells/well was calculated against a standard curve prepared by plating various concentrations of cells, as determined by hemocytometer, at the start of each experiment.[36]

Differences among the various treatment groups in +SA cell cytotoxic studies were determined by analysis of variance (ANOVA) followed by Dunnett's *t*-test. The difference of $P < 0.05$ was considered to be statistically significant as compared with vehicle-treated controls or as defined in figure legends. Linear regression analysis of treatment effects on viable cell number in growth and cytotoxicity studies was used to determine the 50% growth inhibition concentration (IC_{50}) for individual treatments.

4.4.2. Wound-healing assay[27]

The highly metastatic human breast cancer MDA-MB-231 cells were cultured in RPMI 1640 medium containing 10 mM HEPES, 4 mM L-glutamine, 10% fetal bovine serum, penicillin (100 IU/mL), and streptomycin (50 μg/mL), and grown in a 5% CO_2 atmosphere at 37 °C. Cells were plated onto sterile 24-well and allowed to recover for a confluent cell monolayer formed in each well (>95% confluence). Wounds were then inflicted to each cell monolayer using a sterile 200 μL pipette tip. Media were removed, cells monolayers were washed twice with PBS, and then fresh media containing test compounds were added to each well. Test compounds were prepared in DMSO at 5 μM concentration and added to the plates, each in triplicate using DMSO as negative control. The incubation was carried out for 24 h under serum-starved conditions, after which media was removed and cells were fixed and stained using Diff Quick staining (Dade Behring Diagnostics, Aguada, Puerto Rico). The number of cells migrated on the scratched wound were counted under the microscope in three or more randomly selected fields (magnification: 400×). Final results are expressed as mean ± SEM per 400× field.

4.5. Solubility and stability studies

4.5.1. Water solubility

About 5.6 mg of each of **1** and **9** were added to 3 mL of phosphate buffer (pH 7.4) in amber vials. Tested solutions were maintained shaking under at 25 °C for 24 h in a constant-temperature water bath. Each solution was then centrifuged at 5000 rpm for 10 min before collecting samples from the clear supernatant at 0,

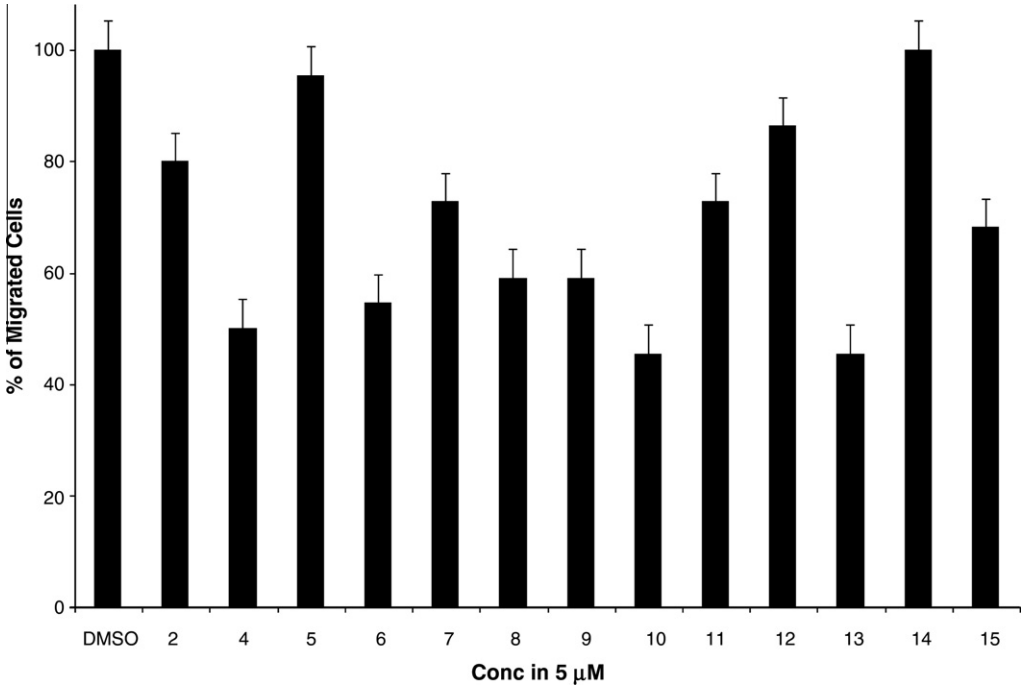


Figure 3. Anti-migratory activity of tocotrienol esters **4**–**15** against the human breast cancer cells MDA-MB-231 in wound-healing assay.

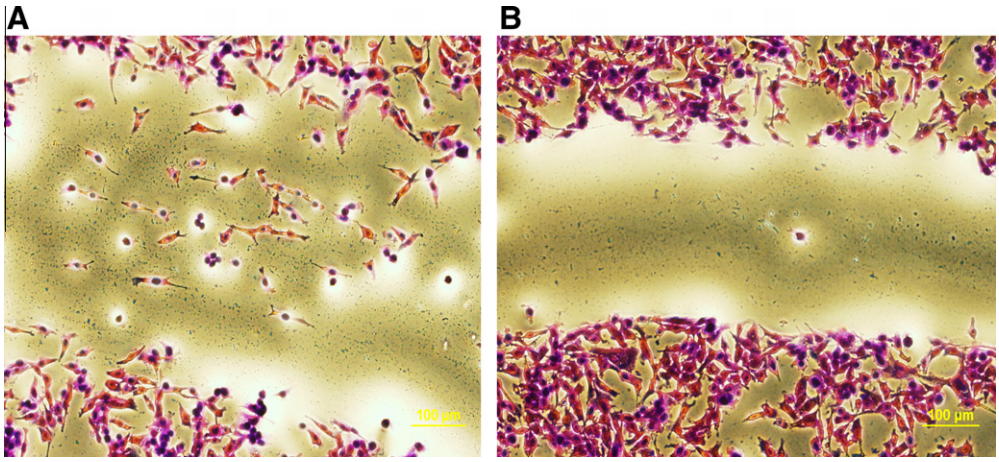
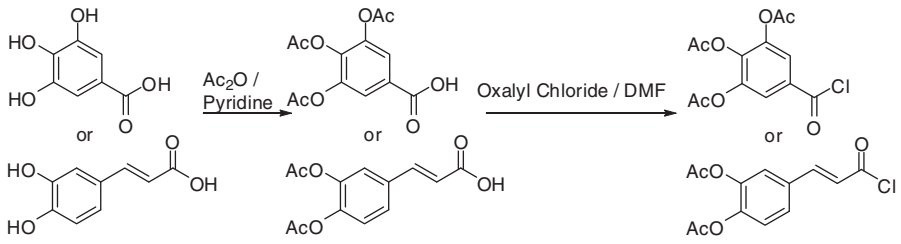
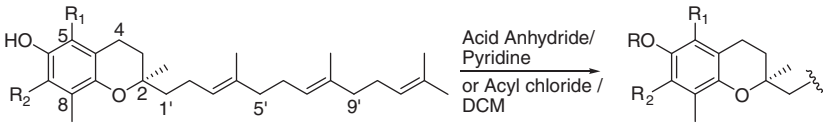


Figure 4. Wound-healing assay model using the human metastatic breast cancer cell line MDA-MB231. (A) Vehicle control (DMSO). (B) Active tocotrienol treatment.



Scheme 1. Preparation of acetylated caffeic and gallic acid chlorides.



Scheme 2. Esterification of tocotrienols **1**–**3**.

1, 2, 4, 6, and 24 h. Each compound concentration in the aspirates was determined using the HPLC method described below.

4.5.2. Stability studies

4.5.2.1. Stability in phosphate buffer. The stability of each of **1** and **9** was studied at 37 °C in phosphate buffer (pH 7.4). In amber vials, 500 μg/mL of each compound were dissolved in the buffer and the solution was incubated at 37 °C. At interval times (0, 0.5, 1, 2, 4, 6, and 24 h), 50 μL samples were collected and equal volumes of methanol were added to each sample followed by HPLC analysis.

4.5.2.2. Stability in rat plasma. The stability of each of **1** and **9** was studied at 37 °C in rat plasma. In amber vials, 50 μg/mL of each compound were dissolved in the plasma. The solution was incubated at 37 °C, and at interval times (0, 0.5, 1, 2, 4, 6, and 24 h), 50 μL samples were taken and mixed with 100 μL of methanol followed by centrifugation at 14,000 rpm for 10 min. Each supernatant was analyzed by HPLC.

4.5.3. HPLC analysis of 1 and 9

A Shimadzu HPLC system (Columbia, MD) was used for quantification of **1** and **9**. This system is composed of SIL 20-AHT autosampler, SPD-20A UV/vis detector, and LC-20AB pump connected to a Dgu-20A3 degasser. Data acquisition was achieved by LC Solution software version 1.22 SP1 Shimadzu. The following chromatographic conditions were used: Luna 5μ C18 column (250 × 4.6 mm id; Phenomenex, Torrance, CA), flow rate was adjusted to 1.0 mL/min, and λ was set at 295 nm. For compound **1** solubility and stability studies, an isocratic elution with MeOH–EtOH–CH_3CN (40:30:30, v/v/v) as mobile phase for 10 min. Compound **1** was eluted at 6.7 min. For the simultaneous separation of compounds **1** and **9** the following gradient elution was also used starting with H_2O–MeOH–$N(Et)_3$ (10:90:0.05, v/v/v) for 5 min, followed by isocratic MeOH–$N(Et)_3$ (100:0.05, v/v) for another 10 min. The retention times were 4 and 14.1 for **9** and **1**, respectively. Standard curves for **1** and **9** in MeOH were prepared in the range of 0.4–50 μg/mL and 1–1000 μg/mL, respectively. Each compound was quantified using its calibration curve for compounds peak area versus its concentration.

4.6. Chemicals and reagents

All reagents have been purchased from Sigma–Aldrich. (TRF) 50 g (Palm TRF 70%, low in tocopherol from First Tech International Ltd, Hong Kong) was fractionated using Si gel 60 VLC using *n*-hexane/EtOAc (gradient elution) as a mobile phase.

Acknowledgment

This publication was supported in part by First Tech International Ltd (Hong Kong).

References and notes

1. Packer, L. *Am. J. Clin. Nutr.* **1993**, *53*, 1050S.
2. Kamal-Eldin, A.; Appelqvist, L. *Lipids* **1996**, *31*, 671.
3. Burton, G. W. *Proc. Nutr. Soc.* **1994**, *53*, 251.
4. Sen, C. K.; Khanna, S.; Roy, S. *Mol. Aspects Med.* **2007**, *28*, 692.
5. Sies, H. Oxidative Stress: Introduction. In *Oxidative Stress: Oxidants and Antioxidants*; Academic Press: London, 1991; p xv.
6. Packer, L.; Weber, S. U.; Rimbach, G. *J. Nutr.* **2001**, *131*, 369S.
7. Serbinova, E.; Kagen, V.; Han, D.; Packer, L. *Free Radical Biol. Med.* **1991**, *10*, 263.
8. Packer, L. Nutrition and Biochemistry of the Lipophilic Antioxidants, Vitamin E and Carotenoids. In *Nutrition, Lipids, Health and Disease*; Ong, A. S. H., Niki, E., Packer, L., Eds.; American Oil Chemists Society: Champaign, IL, 1995; p 8. Chapter 2.
9. Sen, C. K.; Khanna, S.; Roy, S. *Life Sci.* **2006**, *78*, 2088.
10. Kamat, J. P.; Devasagayam, T. P. A. *Neurosci. Lett.* **1995**, *195*, 179.
11. Xu, Z.; Hua, N.; Godber, J. S. *J. Agric. Food Chem.* **2001**, *49*, 2077.
12. Basu, A.; Imrhan, V. *Nutr. Rev.* **2005**, *63*, 247.
13. Kline, K.; Yu, W.; Sanders, B. G. *J. Nutr.* **2004**, *134*, 3458S.
14. Neuzil, J.; Tomasetti, M.; Zhao, Y.; Dong, L. F.; Birringer, M.; Wang, X. F.; Low, P.; Wu, K.; Salvatore, B. A.; Ralph, S. J. *Mol. Pharmacol.* **2007**, *71*, 1185.
15. Wang, X. F.; Dong, L.; Zhao, Y.; Tomasetti, M.; Wu, K.; Neuzil, J. *Mol. Nutr. Food Res.* **2006**, *50*, 675.
16. Eitenmiller, R.; Lee, L. *Vitamin E: Food Chemistry, Composition, and Analysis*; Marcel Dekker Inc.: New York, Basil, 2004. pp 89–135.
17. Chow, C. K. Vitamin E. In *Hand Book of Vitamins*; Bruker, R. B., Suttie, J. W., McCormick, D. B., Machlin, L. J., Eds.; Marcel Dekker Inc.: New York, Basel, 2001; p 165.
18. Repka, M. A.; McGinity, J. W. *Int. J. Pharm.* **2000**, *202*, 63.
19. Zingg, J. M. *Mini-Rev. Med. Chem.* **2007**, *7*, 543.
20. Hayes, K. C.; Pronczuk, A.; Liang, J. S. *Proc. Soc. Exp. Biol. Med.* **1993**, *202*, 353.
21. Yap, S. P.; Yuen, K. H.; Wong, J. W. *J. Pharm. Pharmacol.* **2001**, *53*, 67.
22. Abuasal, B.; Sylvester, P. W.; Kaddoumi, A. *Drug Metab. Dispos.* **2010**, *38*, 939.
23. Sokol, R. J.; Butler-Simon, N.; Conner, C.; Heubi, J. E.; Sinatra, F. R.; Suchy, F. J.; Heyman, M. B.; Perrault, J.; Rothbaum, R. J.; Levy, J.; Iannaccone, S. T.; Shneider, B. L.; Koch, T. K.; Narkewicz, M. R. *Gastroenterology* **1993**, *104*, 1727.
24. Csallany, A. S.; Draper, H. H. *Arch. Biochem. Biophys.* **1963**, *100*, 335.
25. Draper, H. H.; Csallany, A. H.; Chiu, M. *Lipids* **1967**, *2*, 47.
26. Schltz, N.; Leist, M.; Petrzika, M.; Gassmann, B.; Breigelius-Flohe, R. *Am. J. Clin. Nutr.* **1995**, *62*, 1527S.
27. Elnagar, A. Y.; Wali, V. B.; Sylvester, P. W.; El Sayed, K. A. *Bioorg. Med. Chem.* **2010**, *18*, 755.
28. Birringer, M.; Eytina, J. H.; Salvatore, B. A.; Neuzil, J. *Br. J. Cancer* **2003**, *88*, 1948.
29. McIntyre, B. S.; Briski, K. P.; Gabor, A.; Sylvester, P. W. *Proc. Soc. Exp. Biol. Med.* **2000**, *224*, 292.
30. Lin, C. F.; Chang, T. C.; Chiang, C.-C.; Tsai, H. I.; Hsu, L. Y. *Chem. Pharm. Bull.* **2005**, *53*, 1402.
31. Inayama, S.; Harimaya, K.; Hori, H.; Ohkura, T.; Kawamata, T.; Hikichi, M.; Yokokura, T. *Chem. Pharm. Bull.* **1984**, *32*, 1135.
32. Andrus, M. B.; Liu, J.; Meredith, E. L.; Nartey, E. *Tetrahedron Lett.* **2003**, *44*, 4819.
33. Takata, J.; Hidaka, R.; Yamasaki, A.; Hattori, A.; Fukushima, T.; Tanabe, M.; Matsunaga, K.; Karube, Y.; Imai, K. *Lipid Res.* **2002**, *43*, 2196.
34. Anderson, L. W.; Danielson, K. G.; Hosick, H. L. *In Vitro* **1979**, *15*, 841.
35. Wali, V. B.; Sylvester, P. W. *Lipids* **2007**, *42*, 1113.
36. Sylvester, P. W.; Birkenfeld, H. P.; Hosick, H. L.; Briski, K. P. *Exp. Cell Res.* **1994**, *214*, 145.