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Synthesis and biological evaluation of new flavonoid fatty acid esters with anti-adipogenic and enhancing glucose consumption activities

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ABSTRACT

Oleoyl Formononetin (OF) has good weight loss activity and hypolipidemic activity, could improve insulin sensitivity and suppress adipogenesis. To acquire better biological activities, three series of flavonoid fatty acid esters were designed and synthesized by optimizing the structure of OF. Their bioactivities were assayed in vitro. Some of these novel compounds could effectively inhibit preadipocyte proliferation and adipogenesis. Moreover, they could enhance glucose consumption in adipocytes notably.

1. Introduction

Obesity, defined by the presence of excessive total body fat, has been recognized as a critical global health problem. It is a serious risk for many other diseases, such as dyslipidemia, non-insulin dependent diabetes, cardiovascular disease, non-alcoholic fatty liver disease, endocrine problems and various types of cancer.[1,2] Overall mortality due to any cause increased with the prevalence of obesity. Obesity is both an individual clinical condition and a serious public health and policy problem. The rapidly expanding obesity problem is leading to an enormous burden on global health care and welfare systems.[3]

Adipose tissue, especially adipocytes play a central role in the development of obesity. Obesity is characterized by increases in the number or size of adipocytes, or a combination of both. The proliferation and differentiation of adipocytes and fat accumulation are the direct cause of obesity.[4,5] There is growing evidence that adipose tissue is more than a passive storage of fat. It is also a key metabolic control and endocrine organ in the regulation of the body's energy homeostasis. Adipose tissue produce a wide range of hormones and cytokines involved in lipid metabolism (e.g., cholesterol ester transfer protein, CETP), glucose metabolism (e.g., adiponectin, resistin), inflammation (e.g., TNF-α, IL-6), coagulation (PAI-1), blood pressure (e.g., angiotensinogen, angiotensin II), and feeding behavior (leptin). These factors affect the metabolism and function of many organs and tissues including muscle, liver, vasculature, and brain.[6,7] Excessive adipogenesis is not only the culprit of obesity, but also a risk factor for metabolic diseases.

During adipogenesis, peroxisome proliferators-activated receptor gamma (PPARγ) highly expressed in adipose tissue, is an important transcription factor and plays a dominant role in fat tissue development.[8] PPARγ belongs to the nuclear receptor superfamily of ligand-activated transcription factors and is a target of the antidiabetic thiazolidinedione (TZD) drugs. PPARγ has been known for some time to regulate adipocyte differentiation, lipid storage, insulin sensitivity, glucose metabolism, macrophage maturation, and inflammation control. PPARγ is capable of inducing a variety of responses due to its numerous potential ligand-binding conformations and the large numbers of responsive genes.[9] PPARγ full agonists (e.g., pioglitazone) induce beneficial pharmacological effects, however, associated with inevitable adverse effects. Based on different ligands can induce different stimulatory or inhibitory responses, the concept of selective PPAR modulators (SPPARM) has been proposed. SPPARMs promote selective gene transcription in a gene-specific manner, with some genes induced to similar levels compared with those obtained with a full agonist, whereas others exhibit restricted activation.[10,11]

We have recently reported some isoflavone fatty acid esters showed good weight loss activity, hypolipidemic activity and low toxicity. Compound **1a** (Oleoyl Formononetin) significantly lowered total cholesterol (TC), triglyceride (TG), low density lipoproteins (LDL) cholesterol, plasma insulin and increased high density lipoproteins (HDL) cholesterol in plasma. Further, we found it could effectively inhibit adipogenesis and up-regulate PPARγ2-mRNA in vitro.[12,13] In our subsequent study, we found that

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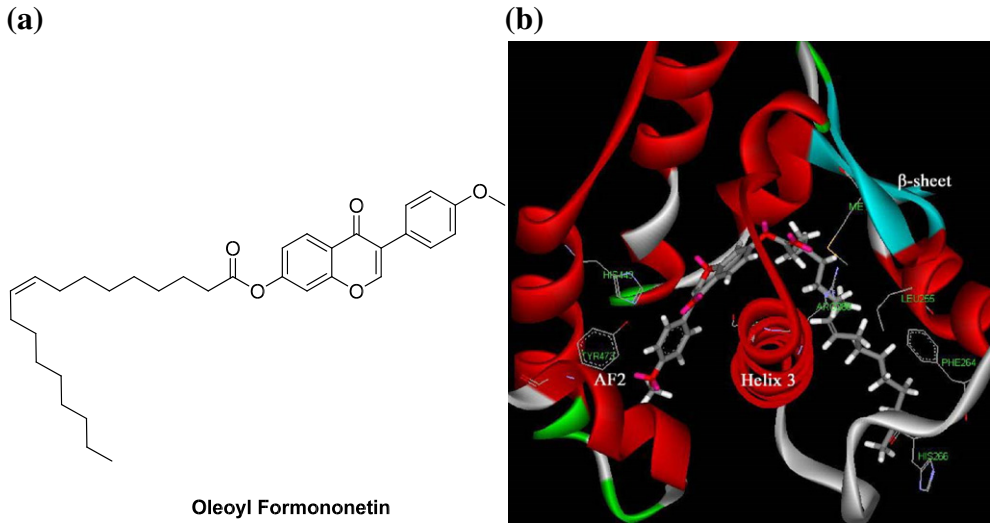


Figure 1. (a) Structure of Oleoyl Formononetin; (b) model of Oleoyl Formononetin bound to the PPARγ. Docking simulation was performed using the program GOLD and the crystal structure of PPARγ (PDB code: 2ZK4).

OF could reduce plasma glucose levels and improved insulin sensitivity. Also, OF was found to promote lipolysis of adipocytes, increased glycerol release notably during preadipocyte differentiation.[14] Therefore, we supposed that PPARγ was one of its potential targets, and it acted as a selective PPARγ modulator. To better understand the main binding interactions at the PPARγ binding sites, docking experiment was performed on Oleoyl Formononetin. As shown in Fig 1, its *p*-methoxyphenyl moiety oriented towards the ligand-regulated activation function (AF-2) domain, the benzopyrone moiety interacted with the helix 3, and the long oleoyl-chain formed hydrophobic interaction in the large pocket between helix 3 and β-sheet.

We hypothesized that increased flexibility between 3-phenyl group and the chromen moiety would enhance the activities of the compound. In order to validate our hypothesis, we designed three series compounds by replacing the Formononetin moiety with 2-benzylidene-benzo[*b*]furan-3-one and 3-(4-methoxybenzyl)-4-oxo-4*H*-chromen (Fig 2). Forty-one derivatives were synthesized, and their bioactivities were assayed.

2. Results and discussion

2.1. Chemical synthesis

The compounds of series 1 were synthesized from *p*-dihydroxybenzene (**1**), as shown in Scheme 1. *p*-Dihydroxybenzene (**1**) with acetic anhydride in the presence of sulfuric acid gave 1,4-diacetoxybenzene (**2**) in 98% yield. 2′,5′-Dihydroxyacetophenone (**3**) was got in the presence of aluminum chloride via Fries rearrangement.[15] Then 5′-position hydroxy was protected with the methoxymethyl (MOM) protecting group using MOM-Cl and K_2CO_3 in acetone. The chalcones (**5a–5g**) were synthesized by base catalyzed Claisen–Schmidt condensation of 2′-hydroxy-5′-(methoxymethoxy)acetophenone (**4**) with appropriate

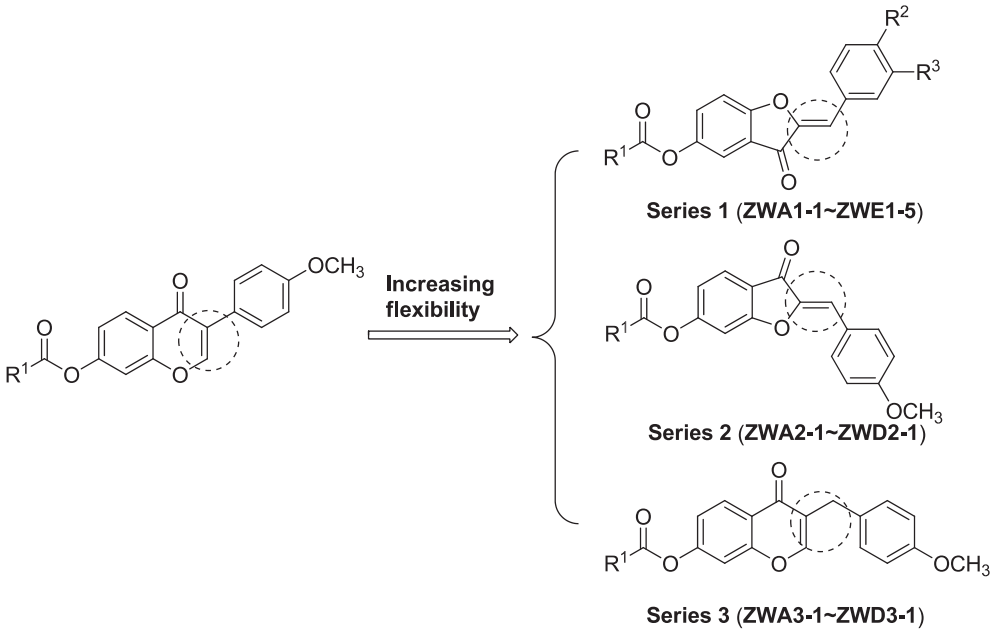
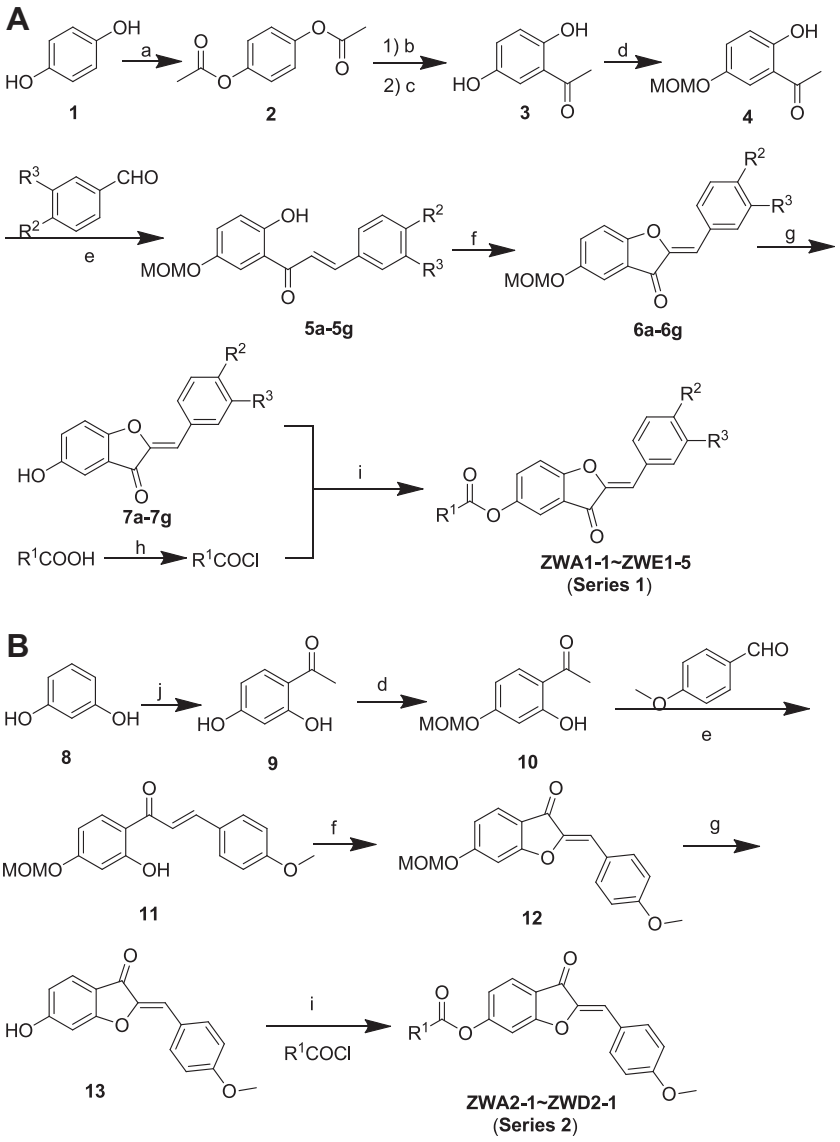
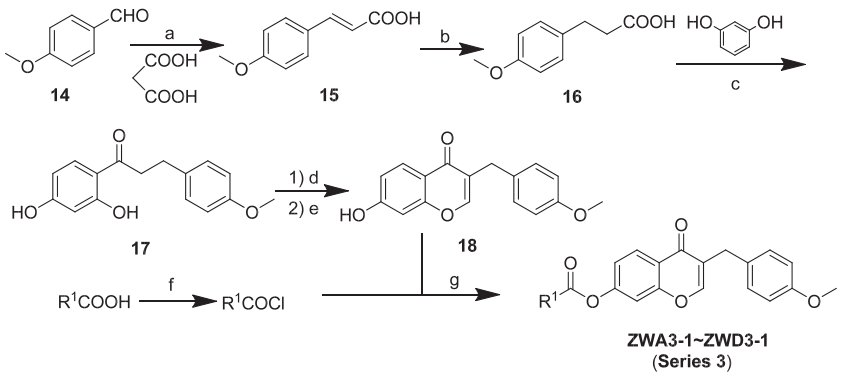


Figure 2. The design of new compounds by increasing flexibility.



Scheme 1. Synthesis of compounds (series 1 and 2). Reagents and conditions: (a) Ac_2O, H_2SO_4, rt, 15 min; (b) $AlCl_3$, NaCl, 180 °C, 3 h; (c) HCl, CH_3OH, reflux, 1 h; (d) CH_3OCH_2Cl, acetone, K_2CO_3, 30 °C, 2 h; (e) 20% aq KOH, EtOH, rt, 24–72 h; (f) $Hg(OAc)_2$, pyridine, 110 °C, 1 h; (g) 10% aqueous HCl, MeOH, reflux, 30 min; (h) PCl_3, 70 °C, 2 h; (i) CH_2Cl_2, pyridine, reflux, 1–2 h; (j) $ZnCl_2$, Ac_2O, reflux, 1 h.



Scheme 2. Synthesis of compounds (series 3). Reagents and conditions: (a) pyridine, piperidine, reflux, 2 h; (b) H_2, 10% Pd/C, rt, 5 h; (c) BF_3/Et_2O, 75 °C, 90 min; (d) $HC(OEt)_3$, $HClO_4$, rt, 5 h; (e) H_2O, reflux, 10 min; (f) PCl_3, 70 °C, 2 h; (g) CH_2Cl_2, pyridine, reflux, 2 h.

benzaldehydes. The aurones (**6a–6g**) were obtained via the oxidative cyclization methodology using mercury acetate in pyridine. In all cases the thermodynamically more stable *Z*-geometric isomer was obtained. Then, the aurone skeletons were obtained by removing the MOM protecting group in a solution of 10% aqueous HCl and methanol.[16,17]

The compounds of series 2 were synthesized from *m*-dihydroxybenzene (**8**) in a similar strategy as series 1.

The compounds of series 3 were synthesized from 4-methoxybenzaldehyde (**14**), as shown in Scheme 2. The 3-(4-methoxyphenyl)propanoic acid (**16**) was prepared by Knoevenagel condensation of the 4-methoxybenzaldehyde (**14**) with malonic acid, followed by Pd-catalyzed hydrogenation. The intermediate **17** was prepared from 3-(4-methoxyphenyl)propanoic acid (**16**) and resorcinol by Friedel–Crafts acylation using boron trifluoride etherate. Then the intermediate **17** was treated with triethylorthoformate, 70% perchloric acid and water to get the skeleton of series 3.[18]

The derivatives could be obtained by direct condensation of the skeletons and fatty acid chlorides in methylene chloride at boiling temperature, and pyridine as catalyst.

2.2. Biological evaluation

3T3-L1 cells were selected to assay the effects of our compounds on preadipocyte proliferation and differentiation. The testing compounds were compared with Oleoyl Formononetin.

The experiment of preadipocyte proliferation was assessed using the standard MTT assay, and the result was listed in Table 1. By comparing the long-chains of our compounds, the most active structural feature relevant to inhibit preadipocyte proliferation appeared to be the unsaturated fatty acid side chain. The compounds, with oleate side chain (**ZWA***), suppressed the proliferation of preadipocytes significantly (up to 22.57% inhibition ratio). Whereas, most of the compounds, with stearate side chain (**ZWC***), exhibited an opposite effect. For example, compound **ZWC3-1** promoted the proliferation of preadipocytes (inhibition ratio = −31.87%). The fact suggested that the fatty acid moiety might play an important role in biological effects, and the unsaturated bond was necessary.

The compounds were further characterized for its ability to inhibit adipogenesis during 3T3-L1 preadipocyte differentiation. Some compounds produced a significantly decrease in triglyceride accumulation, measured by Oil Red O Staining. The result was listed in Table 1. Most of the compounds with oleate side chain, except for **ZWA1-2** and **ZWA1-3**, exhibited better inhibiting adipogenesis activity (from 9.38% to 47.55%) than OF (2.52%). In

Table 1
Structures and the effects on the preadipocyte proliferation and adipogenesis

Compound No.	Structure				Inhibition ratio (%)	
	Series	R^1	R^2	R^3	MTT[a]	Adipogenesis[b]
ZWA1-1	1	CH_3–$(CH_2)_7$–CH=CH–$(CH_2)_7$	OCH_3	H	17.69 ± 1.35#	11.71 ± 1.52#
ZWA1-2	1	CH_3–$(CH_2)_7$–CH=CH–$(CH_2)_7$	H	OCH_3	−5.41 ± 0.34	0
ZWA1-3	1	CH_3–$(CH_2)_7$–CH=CH–$(CH_2)_7$	OCH_3	OCH_3	12.53 ± 1.15	0
ZWA1-4	1	CH_3–$(CH_2)_7$–CH=CH–$(CH_2)_7$	F	H	22.57 ± 3.55#	9.38 ± 0.87#
ZWA1-5	1	CH_3–$(CH_2)_7$–CH=CH–$(CH_2)_7$	Br	H	−6.62 ± 0.38	16.55 ± 3.23#
ZWA1-6	1	CH_3–$(CH_2)_7$–CH=CH–$(CH_2)_7$	H	Cl	20.76 ± 1.62#	0
ZWA1-7	1	CH_3–$(CH_2)_7$–CH=CH–$(CH_2)_7$	H	NO_2	17.55 ± 1.39#	15.58 ± 2.34#
ZWA2-1	2	CH_3–$(CH_2)_7$–CH=CH–$(CH_2)_7$	—	—	22.18 ± 1.87#	14.32 ± 1.12#
ZWA3-1	3	CH_3–$(CH_2)_7$–CH=CH–$(CH_2)_7$	—	—	1.88 ± 0.21	47.55 ± 4.55#
ZWB1-1	1	CH_3–$(CH_2)_4$–CH=CH–CH=CH–$(CH_2)_8$	OCH_3	H	−24.96 ± 1.75	22.56 ± 1.96#
ZWB1-2	1	CH_3–$(CH_2)_4$–CH=CH–CH=CH–$(CH_2)_8$	H	OCH_3	6.45 ± 0.73	—
ZWB1-3	1	CH_3–$(CH_2)_4$–CH=CH–CH=CH–$(CH_2)_8$	OCH_3	OCH_3	20.72 ± 1.32#	1.88 ± 0.09
ZWB1-4	1	CH_3–$(CH_2)_4$–CH=CH–CH=CH–$(CH_2)_8$	F	H	14.2 ± 1.03*	0
ZWB1-5	1	CH_3–$(CH_2)_4$–CH=CH–CH=CH–$(CH_2)_8$	Br	H	11.09 ± 1.52	—
ZWB1-6	1	CH_3–$(CH_2)_4$–CH=CH–CH=CH–$(CH_2)_8$	H	Cl	−11.46 ± 1.92	—
ZWB1-7	1	CH_3–$(CH_2)_4$–CH=CH–CH=CH–$(CH_2)_8$	H	NO_2	12.06 ± 1.62	15.08 ± 1.12#
ZWB2-1	2	CH_3–$(CH_2)_4$–CH=CH–CH=CH–$(CH_2)_8$	—	—	−2.4 ± 0.34	—
ZWB3-1	3	CH_3–$(CH_2)_4$–CH=CH–CH=CH–$(CH_2)_8$	—	—	−4.37 ± 0.31	—
ZWC1-1	1	CH_3–$(CH_2)_{16}$	OCH_3	H	−10.93 ± 0.76	—
ZWC1-2	1	CH_3–$(CH_2)_{16}$	H	OCH_3	−10.94 ± 0.67	—
ZWC1-3	1	CH_3–$(CH_2)_{16}$	OCH_3	OCH_3	−3.84 ± 0.43	—
ZWC1-4	1	CH_3–$(CH_2)_{16}$	F	H	−9.45 ± 0.32	—
ZWC1-5	1	CH_3–$(CH_2)_{16}$	Br	H	5.02 ± 0.19	—
ZWC1-6	1	CH_3–$(CH_2)_{16}$	H	Cl	−0.06 ± 0.03	—
ZWC1-7	1	CH_3–$(CH_2)_{16}$	H	NO_2	−5.03 ± 0.39	—
ZWC2-1	2	CH_3–$(CH_2)_{16}$	—	—	11.43 ± 1.56	0
ZWC3-1	3	CH_3–$(CH_2)_{16}$	—	—	−31.87 ± 2.38	1.95 ± 0.12
ZWD1-1	1	CH_2=CH–$(CH_2)_8$	OCH_3	H	−2.09 ± 0.30	—
ZWD1-2	1	CH_2=CH–$(CH_2)_8$	H	OCH_3	0.21 ± 0.07	—
ZWD1-3	1	CH_2=CH–$(CH_2)_8$	OCH_3	OCH_3	−22.17 ± 2.19	—
ZWD1-4	1	CH_2=CH–$(CH_2)_8$	F	H	16.67 ± 1.23#	25.35 ± 3.97#
ZWD1-5	1	CH_2=CH–$(CH_2)_8$	Br	H	1.25 ± 0.22	—
ZWD1-6	1	CH_2=CH–$(CH_2)_8$	H	Cl	57.4 ± 3.11#	28.68 ± 2.15#
ZWD1-7	1	CH_2=CH–$(CH_2)_8$	H	NO_2	−8.37 ± 0.82	—
ZWD2-1	2	CH_2=CH–$(CH_2)_8$	—	—	−1.39 ± 0.09	—
ZWD3-1	3	CH_2=CH–$(CH_2)_8$	—	—	−17.45 ± 2.10	—
ZWE1-1	1	CH_3–$(CH_2)_{14}$	OCH_3	H	1.19 ± 0.11	—
ZWE1-2	1	CH_3–$(CH_2)_{14}$	H	OCH_3	−20.26 ± 1.56	—
ZWE1-3	1	CH_3–$(CH_2)_{14}$	OCH_3	OCH_3	−3.72 ± 0.27	—
ZWE1-4	1	CH_3–$(CH_2)_{14}$	F	H	−0.47 ± 0.08	—
ZWE1-5	1	CH_3–$(CH_2)_{14}$	Br	H	1.05 ± 0.08	—
OF[c]	—	—	—	—	10.91 ± 0.67	2.52 ± 0.36

[a] Effect on the inhibition of 3T3-L1 preadipocyte proliferation by MTT assay.
[b] Effect on the inhibition of adipogenesis by Oil Red O Staining.
[c] Oleoyl Formononetin, as positive control drug.
* P <0.05 for comparisons against the OF group.
\# P <0.01 for comparisons against the OF group.

particular, compound **ZWA3-1** showed very good inhibitory activity on adipogenesis (47.55%), but had little effect on preadipocyte proliferation (1.88%). Thus, we could hypothesis that the mechanism by which these compounds suppress adipogenesis is different from preadipocyte proliferation. A look at the results of compounds with undecylenate side chain, we could found that two compounds (**ZWD1-4** and **ZWD1-6**) showed good effects on inhibiting adipogenesis and preadipocyte proliferation. The fact indicated that 18 carbons long-chain was not essential for the activities. When the substituent on the skeleton changed, the side chain could be shortened.

To examine whether our compounds affected glucose consumption, we treated 3T3-L1 cells with different doses of compounds in various concentrations of glucose culture medium and measured glucose consumption by the glucose-oxidase method. As shown in Table 2, the compounds enhanced the glucose consumption of differentiated adipocytes notably in a dose-dependent manner, and the effect in high-glucose culture medium was stronger than low-glucose culture medium. Compound **ZWA2-1** exhibited the best activity, a 2.39-fold increase, compared with untreated adipocytes. However, in promoting glucose consumption, there was no one beyond pioglitazone or OF.

Taken together, some compounds, such as **ZWA1-1**, **ZWA1-4**, **ZWA2-1**, **ZWA3-1**, **ZWB1-7**, **ZWD1-4**, and **ZWD1-6**, could inhibit adipogenesis and enhance glucose consumption in 3T3-L1 cells significantly. When the flexibility between substituted-phenyl group and the chromen moiety increased, the anti-adipogenic effect became stronger, but the promoting glucose consumption activity decreased slightly. Fortunately, we found compound **ZWA2-1**, whose suppressing preadipocyte proliferation activity and inhibiting adipogenesis activity were higher than OF, up to 2.03- and 5.68-fold, respectively. And it have a close activity in promoting glucose consumption with OF. Compared the activities of **ZWA1-1** and **ZWA2-1**, we could find that the change in the relative position between the side chain and the phenyl substituent appeared not to have a big impact on the activities (Fig. 3).

Table 2
Effects of compounds on glucose consumption in adipocytes

Compound	50 μg/mL	100 μg/mL
Low-glucose medium (10 mmol/L)		
Control	2.41 ± 0.22	2.35 ± 0.19
Pioglitazone	5.51 ± 0.38#	6.75 ± 0.46#
OF	5.01 ± 0.41#	6.95 ± 0.53#
ZWA1-1	4.48 ± 0.32#	5.05 ± 0.39#
ZWA1-4	3.81 ± 0.27#	4.35 ± 0.34#
ZWA1-7	2.93 ± 0.25	3.47 ± 0.35#
ZWA2-1	4.94 ± 0.29#	5.61 ± 0.42#
ZWA3-1	2.71 ± 0.24	3.38 ± 0.36*
ZWB1-3	2.99 ± 0.30	3.85 ± 0.39#
ZWB1-7	3.64 ± 0.37#	4.29 ± 0.44#
ZWD1-4	4.41 ± 0.43#	5.45 ± 0.48#
ZWD1-6	2.88 ± 0.29	3.56 ± 0.33#
High-glucose medium (25 mmol/L)		
Control	3.06 ± 0.29	3.12 ± 0.32
Pioglitazone	7.36 ± 0.53#	8.15 ± 0.61#
OF	6.52 ± 0.58#	7.49 ± 0.64#
ZWA1-1	4.91 ± 0.42#	5.67 ± 0.51#
ZWA1-4	4.07 ± 0.34*	4.82 ± 0.48#
ZWA1-7	3.35 ± 0.27	3.96 ± 0.35*
ZWA2-1	5.48 ± 0.47#	6.05 ± 0.59#
ZWA3-1	3.49 ± 0.36	4.23 ± 0.41*
ZWB1-3	3.71 ± 0.32	5.08 ± 0.45#
ZWB1-7	4.21 ± 0.33	4.95 ± 0.42#
ZWD1-4	5.16 ± 0.43#	5.85 ± 0.53#
ZWD1-6	3.86 ± 0.31*	4.49 ± 0.50*

Glucose consumptions (mmol/L) were measured after a 24-h incubation with different doses of compounds. Data are means ± SEM; $n = 3$ in each condition.
* $P < 0.05$ for comparisons against the control group.
# $P < 0.01$ for comparisons against the control group.

3. Conclusions

In order to improve the activities of Oleoyl Formononetin, we have designed and synthesized a series of compounds by increasing the flexibility of skeleton. Their activities have been evaluated. Some compounds presented significant inhibitory effects on preadipocyte proliferation and adipogenesis; moreover, they could enhance glucose consumption in adipocytes notably. Compound **ZWD1-6** exhibited the best activity in inhibiting preadipocyte proliferation (57.4%), good anti-adipogenic effect (28.68%) and moderate activity in promoting glucose consumption. Compound **ZWA3-1** presented satisfactory activity in inhibiting adipogenesis (47.55%), but only a little activity in inhibiting preadipocyte proliferation (1.88%). Compound **ZWA2-1** inhibited preadipocyte proliferation and adipogenesis by 22.18% and 14.32%, respectively, and enhanced glucose consumption 2.39 times of untreated group.

4. Experimental

4.1. Chemical synthesis

4.1.1. Materials

Most chemicals and solvents were of analytical grade and, when necessary, were purified and dried by standards methods. Reactions were monitored by thin-layer chromatography (TLC) using precoated silica gel plates (Silica Gel GF/UV 254), and spots were visualized under UV light (254 nm). Melting points (uncorrected) were determined on a Mel-TEMP II melting point apparatus and are uncorrected. Elemental analysis was determined on an Elementar Vario EL III elemental analyser. Infrared (IR) spectra (KBr) were

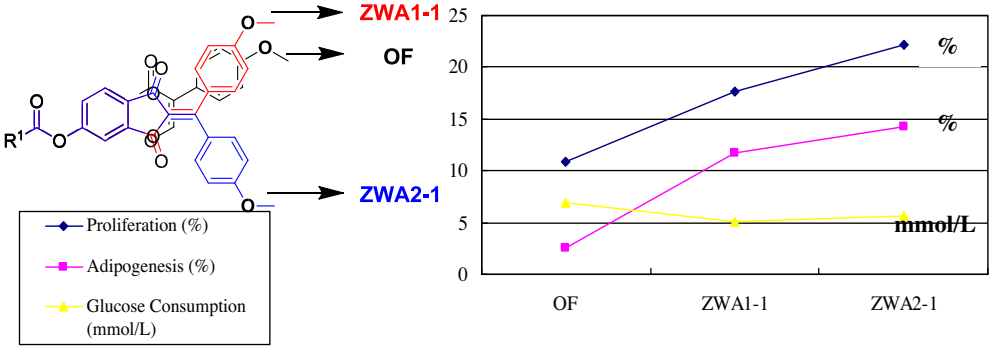


Figure 3. The change in the relative position between the side chain and the phenyl substituent appeared not to have a big impact on the activities.

recorded on a Nicolet Impact 410 instrument (KBr pellet). ^{1}H NMR spectra were recorded with a Bruker Avance 500 MHz spectrometer at 300 K. MS spectra were recorded on a Shimadzu GC–MS 2010 (EI) or a Mariner Mass Spectrum (ESI). Chemical shifts (δ) are expressed in parts per million relative to tetramethylsilane, which was used as an internal standard, coupling constants (J) are in hertz (Hz), and the signals are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br s, broad singlet.

4.1.2. 1,4-Diacetoxybenzene (2)

To a solution of *p*-dihydroxybenzene (**1**) (11.0 g, 0.10 mol) and acetic anhydride (28.3 mL, 0.30 mol) was added two drops of concentrated sulfuric acid. The reaction solution was stirred for 15 min at room temperature and then poured into water to produce a white precipitate. The precipitate was collected to give a white crystal of 19.0 g of 1,4-diacetoxybenzene (**2**) with a yield of 97.9%, mp 123–124 °C.

4.1.3. 2′,5′-Dihydroxyacetophenone (3)

A mixture of 1,4-diacetoxybenzene (**2**) (10.0 g, 51.1 mmol), NaCl (10 g) and anhydrous aluminum chloride (15.0 g, 0.112 mol) was heated for 3 h at 180 °C in an oil bath. The reaction mixture was cooled to room temperature and then was added 15 mL of concentrated hydrochloric acid and 200 mL of ice water to produce a precipitate. The precipitate was collected, washed with water, and dried. Then the dark product was stirred for 1 h with 4 mL of hydrogen chloride in 100 mL of methanol. The resulting solution was poured, with stirring, into ice water, the solid was filtered, and washed with water. The crude product was recrystallized from ethanol to give a dark-green crystal of 6.0 g of 2′,5′-dihydroxyacetophenone (**3**) with a yield of 76.6%, mp 198–200 °C. MS (EI): m/z = 152.

4.1.4. 2′-Hydroxy-5′-(methoxymethoxy)acetophenone (4)

To a mixture of 2′,5′-dihydroxyacetophenone (**3**, 7.0 g, 46.05 mmol) and anhydrous K_2CO_3 (20 g) in dry acetone (100 mL) was added dropwise chloromethyl methyl ether (4 mL, 53.6 mmol). The mixture was heated for 2 h at 30 °C in an oil bath, cooled to room temperature, filtered, washed with acetone and the solvent wad evaporated. The resulting yellowish oil was purified using flash column chromatography (petroleum ether/ethyl acetate 9:1) to give 2′-hydroxy-5′-(methoxymethoxy)acetophenone (**4**) as a low melting white solid. Yield: 6.0 g (66.5%).

4.1.5. General procedure for the synthesis of chalcones (5a–5g)

To a stirred solution of 2′-hydroxy-5′-(methoxymethoxy)acetophenone (**4**, 30.74 mmol) and a substituted benzaldehyde (33.82 mmol) in ethanol (50 mL) was added KOH (20% w/v aqueous solution, 20 mL) and the mixture was stirred at room temperature for 24–72 h. The reaction mixture was cooled to 0 °C (ice-water bath) and acidified with HCl (10% v/v aqueous solution). In most cases a yellow precipitate was formed, which was filtered and washed with 10% aqueous HCl solution. In the cases where an orange oil was formed, the mixture was extracted with CH_2Cl_2, the extracts were dried (Na_2SO_4) and the solvent was evaporated to give the chalcone as a soild. Each solid was recrystallized from ethanol.

4.1.5.1. 2′-Hydroxy-5′-(methoxymethoxy)-4-methoxy-chalcone (5a). Compound **5a** was obtained in 51.7% yield. Mp 67–69 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 12.58 (d, 1H, OH), 7.91 (d, 1H, J = 15.36 Hz, =CH–), 7.63 (d, 2H, J = 8.70 Hz, H-2, H-6), 7.57 (d, 1H, J = 2.82 Hz, H-6′), 7.50 (d, 1H, J = 15.39 Hz, –CH=), 7.24 (d, 1H, J = 5.34 Hz, H-3′), 6.95 (m, 3H, H-3, H-5, H-4′), 5.17 (s, 2H, –OCH_2O–), 3.88 (s, 3H, –OCH_3), 3.53 (s, 3H, –OCH_3); MS (EI): m/z = 314.

4.1.5.2. 2′-Hydroxy-5′-(methoxymethoxy)-3-methoxy-chalcone (5b). Compound **5b** was obtained in 88.1% yield. Mp 70–72 °C; MS (ESI): m/z = 313 $[M-H]^-$.

4.1.5.3. 2′-Hydroxy-5′-(methoxymethoxy)-3,4-dimethoxy-chalcone (5c). Compound **5c** was obtained in 57.2% yield, mp 90–92 °C.

4.1.5.4. 2′-Hydroxy-5′-(methoxymethoxy)-4-fluoro-chalcone (5d). Compound **5d** was obtained in 52.0% yield. Mp 99–101 °C; MS (EI): m/z = 302.

4.1.5.5. 2′-Hydroxy-5′-(methoxymethoxy)-4-bromo-chalcone (5e). Compound **5e** was obtained in 63.0% yield, mp 92–94 °C.

4.1.5.6. 2′-Hydroxy-5′-(methoxymethoxy)-3-chloro-chalcone (5f). Compound **5f** was obtained in 49.1% yield. Mp 77–78 °C; MS (ESI): m/z = 317 $[M-H]^-$.

4.1.5.7. 2′-Hydroxy-5′-(methoxymethoxy)-3-nitro-chalcone (5g). Compound **5g** was obtained in 53.3% yield. Mp 84–86 °C; MS (ESI): m/z = 328 $[M-H]^-$.

4.1.6. General procedure for the synthesis of *Z*-5-(methoxymethoxy)aurones 6a–6g

To a solution of mercuric acetate (19.0 mmol) in pyridine (50 mL) was added a substituted chalcone (16.0 mmol) at room temperature and the mixture was stirred at 110 °C for 1 h. The cooled reaction mixture was poured into ice cold water and acidified with HCl (10% v/v aqueous solution). The precipitated solid was extracted with CH_2Cl_2, the extracts were dried (Na_2SO_4) and the solvent was evaporated to give a solid which was recrystallized from ethanol.

4.1.6.1. *Z*-2-(4-Methoxybenzylidene)-5-(methoxymethoxy)benzo[*b*]furan-3-one (6a). Compound **6a** was obtained in 92.3% yield. Mp 120–122 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.90 (d, 2H, J = 8.7 Hz, H-2′, H-6′), 7.46 (d, 1H, J = 2.4 Hz, H-4), 7.34 (dd, 1H, $J_{6,7}$ = 8.85 Hz, $J_{4,6}$ = 2.4 Hz, H-6), 7.24 (m, 1H, H-7), 7.00 (d, 2H, J = 9.0 Hz, H-3′, H-5′), 6.84 (s, 1H, =CH–), 5.18 (s, 2H, –OCH_2O–), 3.88 (s, 3H, –OCH_3), 3.50 (s, 3H, –OCH_3); MS (EI): m/z = 312.

4.1.6.2. *Z*-2-(3-Methoxybenzylidene)-5-(methoxymethoxy)benzo[*b*]furan-3-one (6b). Compound **6b** was obtained in 95.7% yield, mp 132–134 °C.

4.1.6.3. *Z*-2-(3,4-Dimethoxybenzylidene)-5-(methoxymethoxy)-benzo[*b*]furan-3-one (6c). Compound **6c** was obtained in 96.0% yield, mp 143–145 °C.

4.1.6.4. *Z*-2-(4-Fluorobenzylidene)-5-(methoxymethoxy)benzo-[*b*]furan-3-one (6d). Compound **6d** was obtained in 94.1% yield, mp 152–154 °C.

4.1.6.5. *Z*-2-(4-Bromobenzylidene)-5-(methoxymethoxy)benzo-[*b*]furan-3-one (6e). Compound **6e** was obtained in 92.5% yield, mp 147–149 °C.

4.1.6.6. *Z*-2-(3-Chlorobenzylidene)-5-(methoxymethoxy)benzo-[*b*]furan-3-one (6f). Compound **6f** was obtained in 87.7% yield, mp 135–137 °C.

4.1.6.7. *Z*-2-(3-Nitrobenzylidene)-5-(methoxymethoxy)benzo-[*b*]furan-3-one (6g). Compound **6g** was obtained in 89.2% yield, mp 149–151 °C.

4.1.7. General procedure for the synthesis of *Z*-5-hydroxyaurones 7a–7g

The 5-(methoxymethoxy)aurone (**6a–6g**, 15 mmol) was added to methanol (250 mL) followed by dropwise addition of HCl (10% v/v aqueous solution, 30 mL). The mixture was refluxed for 30 min. The reaction mixture was cooled to room temperature. A yellow precipitate was formed, which was filtered and washed with methanol.

4.1.7.1. *Z* -2-(4-Methoxybenzylidene)-5-hydroxybenzo[*b*]furan-3-one (7a). Compound **7a** was obtained in 92.0% yield. Mp 240–242 °C; ^{1}H NMR (DMSO, 300 MHz): δ 9.79 (s, 1H, OH), 7.96 (d, 2H, J = 7.5 Hz, H-2′, H-6′), 7.40 (d, 1H, J = 8.7 Hz, H-7), 7.21 (d, 1H, J = 8.7 Hz, H-6), 7.08 (d, 2H, J = 7.2 Hz, H-3′, H-5′), 7.01 (s, 1H, H-4), 6.89 (s, 1H, H-10), 3.83 (s, 3H, –OCH_3); MS (EI): m/z = 268.

4.1.7.2. *Z* -2-(3-Methoxybenzylidene)-5-hydroxybenzo[*b*]furan-3-one (7b). Compound **7b** was obtained in 91.7% yield. Mp 178–180 °C; MS (ESI): m/z = 267 $[M-H]^-$.

4.1.7.3. *Z*-2-(3,4-Dimethoxybenzylidene)-5-hydroxybenzo[*b*]-furan-3-one (7c). Compound **7c** was obtained in 93.0% yield. Mp 227–230 °C; MS (ESI): m/z = 297 $[M-H]^-$.

4.1.7.4. *Z* -2-(4-Fluorobenzylidene)-5-hydroxybenzo[*b*]furan-3-one (7d). Compound **7d** was obtained in 90.5% yield. Mp 220–222 °C; MS (ESI): m/z = 255 $[M-H]^-$.

4.1.7.5. *Z* -2-(4-Bromobenzylidene)-5-hydroxybenzo[*b*]furan-3-one (7e). Compound **7e** was obtained in 94.5% yield. Mp 124–125 °C; ^{1}H NMR (DMSO, 300 MHz): δ 9.86 (s, 1H, OH), 8.17 (d, 2H, J = 7.2 Hz, H-2′, H-6′), 7.91 (d, 2H, J = 8.4 Hz, H-3′, H-5′), 7.39 (d, 1H, J = 9.0 Hz, H-7), 7.23 (dd, 1H, $J_{6,7}$ = 8.7 Hz, $J_{4,6}$ = 2.7 Hz, H-6), 7.03 (d, 1H, J = 2.7 Hz, H-4), 6.87 (s, 1H, H-10); MS (EI): m/z = 318.

4.1.7.6. *Z* -2-(3-Chlorobenzylidene)-5-hydroxybenzo[*b*]furan-3-one (7f). Compound **7f** was obtained in 90.7% yield. Mp 198–200 °C; MS (ESI): m/z = 271 $[M-H]^-$.

4.1.7.7. *Z* -2-(3-Nitrobenzylidene)-5-hydroxybenzo[*b*]furan-3-one (7g). Compound **7g** was obtained in 91.2% yield. Mp 252–254 °C; ^{1}H NMR (DMSO, 300 MHz): δ 9.86 (s, 1H, OH), 8.81 (s, 1H, H-2′), 8.4 (d, 1H, J = 7.8 Hz, H-4′), 8.27 (d, 1H, J = 7.8 Hz, H-6′), 7.78 (7, 1H, J = 8.1 Hz, H-5′), 7.44 (d, 1H, J = 9.0 Hz, H-7), 7.25 (dd, 1H, $J_{6,7}$ = 7.5 Hz, $J_{4,6}$ = 2.4 Hz, H-6), 7.06 (m, 2H, H-4, H-10); MS (ESI): m/z = 282 $[M-H]^-$.

4.1.8. General procedure for the synthesis of aurone fatty acid esters

A solution of fatty acid (3.75 mmol) and PCl_3 (1.5 mmol) was stirred at 70 °C for 3 h, and a yellow solution containing fatty acid chloride could be gotten.

A solution of aurone (1.86 mmol) and anhydrous pyridine (1 mL) in CH_2Cl_2 (10 mL) was stirred at boiling temperature, and the above reaction mixture was added slowly. Then the reaction continued for 1–2 h at boiling temperature. Washing with dilute hydrochloric acid (10% v/v aqueous solution), saturated $NaHCO_3$ solution, drying (Na_2SO_4) and removal of solvent under reduced pressure gives the crude product. The product was further purified either by flash column chromatography or recrystallization.

4.1.8.1. *Z*-2-(4-Methoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl (9*Z*)-oleate (ZWA1-1). Recrystallization from acetone afforded yellow crystals. Yield: 810 mg (81.9%). Mp 111–113 °C; IR (cm^{-1}): 3415, 2925, 2851, 1757, 1647, 1596, 1569, 1481, 1254, 835; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.90 (d, 2H, J = 8.4 Hz, H-2′, H-6′), 7.61 (s, 1H, H-4), 7.33 (m, 2H, H-6, H-7), 6.99 (d, 2H, J = 8.1 Hz, H-3′, H-5′), 6.91 (s, 1H, H-10), 5.37 (m, 2H, –CH=CH–), 3.88 (s, 3H, –OCH_3), 2.58 (t, 2H, J = 7.5 Hz, –CH_2CO–), 2.02 (m, 4H, –CH_2–CH=CH–CH_2–), 1.76 (m, 2H, –CH_2–CH_2CO–), 0.87 (m, 3H, –CH_3); MS (EI): m/z = 532. Anal. Calcd for $C_{34}H_{44}O_5$: C, 76.66; H, 8.33. Found: C, 76.54; H, 8.28.

4.1.8.2. *Z*-2-(3-Methoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl (9*Z*)-oleate (ZWA1-2). Recrystallization from acetone afforded yellow crystals. Yield: 720 mg (72.8%). Mp 92–94 °C; IR (cm^{-1}): 3416, 2917, 2850, 1761, 1706, 1649, 1601, 1575, 1480, 1267, 835; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.49 (m, 3H, H-7, H-4, H-5′), 7.35 (m, 3H, H-6, H-2′, H-6′), 6.98 (dd, 1H, $J_{4',5'}$ = 8.24 Hz, $J_{4',6'}$ = 2.52 Hz, H-4′), 6.88 (s, 1H, H-10), 5.37 (m, 2H, –CH=CH–), 3.89 (s, 3H, –OCH_3), 2.58 (t, 2H, J = 7.5 Hz, –CH_2CO–), 2.03 (m, 4H, –CH_2–CH=CH–CH_2–), 1.77 (m, 2H, –CH_2–CH_2CO–), 0.88 (m, 3H, –CH_3); MS (EI): m/z = 532. Anal. Calcd for $C_{34}H_{44}O_5{\cdot}H_2O$: C, 74.15; H, 8.42. Found: C, 74.65; H, 8.38.

4.1.8.3. *Z*-2-(3,4-Dimethoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl (9*Z*)-oleate (ZWA1-3). Recrystallization from acetone afforded yellow crystals. Yield: 750 mg (71.7%). Mp 90–92 °C; IR (cm^{-1}): 3428, 2923, 2852, 1752, 1697, 1650, 1592, 1525, 1483, 1201, 804; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.52 (m, 3H, H-7, H-4, H-6), 7.35 (m, 2H, H-2′, H-6′), 6.96 (d, 1H, J = 8.13 Hz, H-5′), 6.89 (s, 1H, H-10), 5.39 (m, 2H, –CH=CH–), 3.98 (s, 3H, –OCH_3), 3.96 (s, 3H, –OCH_3), 2.58 (t, 2H, J = 7.44 Hz, –CH_2CO–), 2.03 (m, 4H, –CH_2–CH=CH–CH_2–), 1.76 (m, 2H, –CH_2–CH_2CO–), 0.88 (m, 3H, –CH_3); MS (EI): m/z = 562. Anal. Calcd for $C_{35}H_{46}O_6$: C, 74.70; H, 8.24. Found: C, 74.21; H, 8.26.

4.1.8.4. *Z*-2-(4-Fluorobenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl (9*Z*)-oleate (ZWA1-4). Recrystallization from acetone afforded yellow crystals. Yield: 620 mg (64.1%). Mp 72–74 °C; IR (cm^{-1}): 3414, 2925, 2851, 1771, 1707, 1653, 1509, 1486, 1143, 838; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.92 (m, 2H, H-2′, H-6′), 7.51 (d, 1H, J = 1.8 Hz, H-4), 7.38 (m, 2H, H-6, H-7), 7.16 (t, 2H, J = 8.7 Hz, H-3′, H-5′), 6.88 (s, 1H, H-10), 5.37 (m, 2H, –CH=CH–), 2.58 (t, 2H, J = 7.5 Hz, –CH_2CO–), 2.02 (m, 4H, –CH_2–CH=CH–CH_2–), 1.76 (m, 2H, –CH_2–CH_2CO–), 0.88 (m, 3H, –CH_3); MS (EI): m/z = 520. Anal. Calcd for $C_{33}H_{41}FO_4{\cdot}0.5H_2O$: C, 74.83; H, 7.99. Found: C, 74.80; H, 8.11.

4.1.8.5. *Z*-2-(4-Bromobenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl (9*Z*)-oleate (ZWA1-5). Recrystallization from acetone afforded yellow crystals. Yield: 780 mg (72.2%). Mp 104–106 °C; IR (cm^{-1}): 3398, 2924, 2851, 1770, 1709, 1655, 1605, 1486, 1143, 841; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.78 (d, 2H, J = 8.4 Hz, H-2′, H-6′), 7.59 (d, 2H, J = 8.1 Hz, H-3′, H-5′), 7.52 (d, 1H, J = 2.4 Hz, H-4), 7.36 (m, 2H, H-6, H-7), 6.83 (s, 1H, H-10), 5.37 (m, 2H, –CH=CH–), 2.58 (t, 2H, J = 7.5 Hz, –CH_2CO–), 2.03 (m, 4H, –CH_2–CH=CH–CH_2–), 1.76 (m, 2H, –CH_2–CH_2CO–), 0.88 (m, 3H, –CH_3); MS (EI): m/z = 580. Anal. Calcd for $C_{33}H_{41}BrO_4$: C, 68.15; H, 7.11. Found: C, 68.12; H, 7.40.

4.1.8.6. *Z*-2-(3-Chlorobenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl (9*Z*)-oleate (ZWA1-6). Recrystallization from acetone afforded yellow crystals. Yield: 690 mg (69.1%). Mp 56–58 °C; IR (cm^{-1}): 3410, 2923, 2850, 1772, 1709, 1656, 1605, 1485, 1142, 839; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.95 (s, 1H, H-4), 7.74 (m, 1H, H-5′), 7.61 (s, 1H, H-2′), 7.40 (m, 4H, Ar-H), 6.82 (s, 1H, H-10), 5.36 (m, 2H, –CH=CH–), 2.58 (t, 2H, J = 7.5 Hz, –CH_2CO–), 2.02 (m, 4H, –CH_2–CH=CH–CH_2–), 1.76 (m, 2H, –CH_2–CH_2CO–), 0.88 (m, 3H, –CH_3); MS (EI): m/z = 536. Anal. Calcd for $C_{33}H_{41}ClO_4$: C, 73.79; H, 7.69. Found: C, 73.33; H, 7.99.

4.1.8.7. *Z*-2-(3-Nitrobenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl (9*Z*)-oleate (ZWA1-7). Recrystallization from acetone afforded yellow crystals. Yield: 730 mg (71.2%). Mp 82–84 °C; IR (cm^{-1}): 3410, 2921, 2851, 1747, 1709, 1656, 1614, 1529, 1479, 1351, 1111, 831; ^1H NMR ($CDCl_3$, 300 MHz): δ 8.82 (s, 1H, H-2′), 8.27 (d, 1H, J = 7.5 Hz, H-4′), 8.15 (d, 1H, J = 8.04 Hz, H-6′), 7.65 (t, 1H, J = 7.98 Hz, H-5′), 7.54 (s, 1H, H-4), 7.41 (m, 2H, H-6, H-7), 6.90 (s, 1H, H-10), 5.36 (m, 2H, –CH=CH–), 2.59 (t, 2H, J = 7.35 Hz, –CH_2CO–), 2.02 (m, 4H, –CH_2–CH=CH–CH_2–), 1.76 (m, 2H, –CH_2–CH_2CO–), 0.88 (m, 3H, –CH_3); MS (EI): m/z = 547. Anal. Calcd for $C_{33}H_{41}NO_6$: C, 72.37; H, 7.55; N, 2.56. Found: C, 72.44; H, 7.77; N, 2.45.

4.1.8.8. *Z*-2-(4-Methoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl (10*E*,12*Z*)-linoleate (ZWB1-1). Purified by flash column chromatography (petroleum ether/ethyl acetate 15:1) afforded yellow solid. Yield: 620 mg (62.9%). Mp 93–95 °C; IR (cm^{-1}): 3481, 2926, 2855, 1756, 1646, 1596, 1512, 1480, 1254, 1140, 839; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.89 (d, 2H, J = 8.85 Hz, H-2′, H-6′), 7.50 (d, 1H, J = 1.5 Hz, H-4), 7.33 (m, 2H, H-6, H-7), 6.99 (d, 2H, J = 8.88 Hz, H-3′, H-5′), 6.91 (s, 1H, H-10), 5.26–6.34 (m, 4H, –CH=CH–CH=CH–), 3.88 (s, 3H, –OCH_3), 2.58 (t, 2H, J = 7.53 Hz, –CH_2CO–), 2.10 (m, 4H, –CH_2–CH=CH–CH=CH–CH_2–), 1.75 (m, 2H, –CH_2–CH_2CO–), 0.88 (m, 3H, –CH_3); MS (EI): m/z = 530. Anal. Calcd for $C_{34}H_{42}O_5$: C, 76.95; H, 7.98. Found: C, 76.94; H, 8.10.

4.1.8.9. *Z*-2-(3-Methoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl (10*E*,12*Z*)-linoleate (ZWB1-2). Purified by flash column chromatography (petroleum ether/ethyl acetate 15:1) afforded yellow solid. Yield: 550 mg (55.8%). Mp 62–64 °C; IR (cm^{-1}): 3428, 2925, 2851, 1759, 1707, 1650, 1602, 1512, 1480, 1254, 1136, 835; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.50 (m, 3H, H-7, H-4, H-5′), 7.35 (m, 3H, H-6, H-2′, H-6′), 6.98 (d, 1H, J = 8.31 Hz, H-4′), 6.88 (s, 1H, H-10), 5.29–6.34 (m, 4H, –CH=CH–CH=CH–), 3.89 (s, 3H, –OCH_3), 2.58 (t, 2H, J = 7.53 Hz, –CH_2CO–), 2.10 (m, 4H, –CH_2–CH=CH–CH=CH–CH_2–), 1.75 (m, 2H, –CH_2–CH_2CO–), 0.87 (m, 3H, –CH_3); MS (ESI): m/z = 531 $[M+H]^+$. Anal. Calcd for $C_{34}H_{42}O_5$: C, 76.95; H, 7.98. Found: C, 76.94; H, 8.36.

4.1.8.10. *Z*-2-(3,4-Dimethoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl (10*E*,12*Z*)-linoleate (ZWB1-3). Purified by flash column chromatography (petroleum ether/ethyl acetate 15:1) afforded yellow solid. Yield: 450 mg (43.2%). Mp 94–96 °C; IR (cm^{-1}): 3422, 2926, 2854, 1754, 1698, 1650, 1592, 1524, 1481, 1267, 1143, 804; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.50 (m, 3H, H-7, H-4, H-6), 7.35 (m, 2H, H-2′, H-6′), 6.95 (d, 1H, J = 8.31 Hz, H-5′), 6.89 (s, 1H, H-10), 5.26–6.12 (m, 4H, –CH=CH–CH=CH–), 3.98 (s, 3H, –OCH_3), 3.96 (s, 3H, –OCH_3), 2.58 (t, 2H, J = 7.41 Hz, –CH_2CO–), 2.05 (m, 4H, –CH_2–CH=CH–CH=CH–CH_2–), 1.75 (m, 2H, –CH_2–CH_2CO–), 0.87 (m, 3H, –CH_3); MS (ESI): m/z = 561 $[M+H]^+$. Anal. Calcd for $C_{35}H_{44}O_6{\cdot}0.5H_2O$: C, 73.77; H, 7.96. Found: C, 73.63; H, 7.79.

4.1.8.11. *Z*-2-(4-Fluorobenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl (10*E*,12*Z*)-linoleate (ZWB1-4). Purified by flash column chromatography (petroleum ether/ethyl acetate 15:1) afforded yellow solid. Yield: 390 mg (40.5%). Mp 96–98 °C; IR (cm^{-1}): 3398, 2924, 2852, 1763, 1706, 1650, 1603, 1480, 1274, 1142, 819; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.90 (m, 2H, H-2′, H-6′), 7.51 (d, 1H, J = 2.1 Hz, H-4), 7.38 (m, 2H, H-6, H-7), 7.16 (t, 2H, J = 8.7 Hz, H-3′, H-5′), 6.87 (s, 1H, H-10), 5.29–6.34 (m, 4H, –CH=CH–CH=CH–), 2.58 (t, 2H, J = 7.53 Hz, –CH_2CO–), 2.10 (m, 4H, –CH_2–CH=CH–CH=CH–CH_2–), 1.76 (m, 2H, –CH_2–CH_2CO–), 0.88 (m, 3H, –CH_3); MS (ESI): m/z = 519 $[M+H]^+$. Anal. Calcd for $C_{33}H_{39}FO_4$: C, 76.42; H, 7.58. Found: C, 76.13; H, 7.69.

4.1.8.12. *Z*-2-(4-Bromobenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl (10*E*,12*Z*)-linoleate (ZWB1-5). Purified by flash column chromatography (petroleum ether/ethyl acetate 15:1) afforded yellow solid. Yield: 450 mg (41.8%). Mp 71–74 °C; IR (cm^{-1}): 3415, 2924, 2852, 1752, 1707, 1654, 1611, 1510, 1479, 1241, 1148, 830; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.78 (d, 2H, J = 8.4 Hz, H-2′, H-6′), 7.60 (d, 2H, J = 8.43 Hz, H-3′, H-5′), 7.51 (d, 1H, J = 2.16 Hz, H-4), 7.38 (m, 2H, H-6, H-7), 6.83 (s, 1H, H-10), 5.28–6.26 (m, 4H, –CH=CH–CH=CH–), 2.58 (t, 2H, J = 7.41 Hz, –CH_2CO–), 2.13 (m, 4H, –CH_2–CH=CH–CH=CH–CH_2–), 1.74 (m, 2H, –CH_2–CH_2CO–), 0.88 (m, 3H, –CH_3); MS (EI): m/z = 578. Anal. Calcd for $C_{33}H_{39}BrO_4{\cdot}H_2O$: C, 66.33; H, 6.92. Found: C, 66.34; H, 6.69.

4.1.8.13. *Z*-2-(3-Chlorobenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl (10*E*,12*Z*)-linoleate (ZWB1-6). Purified by flash column chromatography (petroleum ether/ethyl acetate 15:1) afforded yellow solid. Yield: 360 mg (36.2%). Mp 52–54 °C; IR (cm^{-1}): 3412, 2925, 2852, 1749, 1709, 1654, 1604, 1482, 1147, 824; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.95 (s, 1H, H-4), 7.74 (m, 1H, H-5′), 7.61 (s, 1H, H-2′), 7.39 (m, 4H, Ar-H), 6.82 (s, 1H, H-10), 5.22–6.43 (m, 4H, –CH=CH–CH=CH–), 2.58 (t, 2H, J = 7.5 Hz, –CH_2CO–), 2.12 (m, 4H, –CH_2–CH=CH–CH=CH–CH_2–), 1.76 (m, 2H, –CH_2–CH_2CO–), 0.87 (m, 3H, –CH_3); MS (EI): m/z = 534. Anal. Calcd for $C_{33}H_{39}ClO_4{\cdot}H_2O$: C, 71.66; H, 7.47. Found: C, 71.67; H, 7.42.

4.1.8.14. *Z*-2-(3-Nitrobenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl (10*E*,12*Z*)-linoleate (ZWB1-7). Purified by flash column chromatography (petroleum ether/ethyl acetate 15:1) afforded yellow solid. Yield: 670 mg (66.1%). Mp 98–100 °C; IR (cm^{-1}): 3410, 2924, 2853, 1750, 1712, 1656, 1532, 1481, 1351, 1111, 829; ^1H NMR ($CDCl_3$, 300 MHz): δ 8.81 (s, 1H, H-2′), 8.25 (d, 1H, J = 8.19 Hz, H-4′), 8.14 (d, 1H, J = 7.77 Hz, H-6′), 7.64 (t, 1H, J = 8.01 Hz, H-5′), 7.54 (s, 1H, H-4), 7.41 (m, 2H, H-6, H-7), 6.90 (s, 1H, H-10), 5.26–6.34 (m, 4H, –CH=CH–CH=CH–), 2.58 (t, 2H, J = 7.5 Hz, –CH_2CO–), 2.11 (m, 4H, –CH_2–CH=CH–CH=CH–CH_2–), 1.76 (m, 2H, –CH_2–CH_2CO–), 0.88 (m, 3H, –CH_3); MS (ESI): m/z = 546 $[M+H]^+$. Anal. Calcd for $C_{33}H_{39}NO_6{\cdot}0.5H_2O$: C, 71.46; H, 7.27; N, 2.53. Found: C, 71.08; H, 7.48; N, 2.41.

4.1.8.15. *Z*-2-(4-Methoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl stearate (ZWC1-1). Purified by flash column chromatography (petroleum ether/ethyl acetate 15:1) afforded yellow solid. Yield: 710 mg (71.5%). Mp 126–128 °C; IR (cm^{-1}): 3475, 2922, 2850, 1755, 1703, 1646, 1596, 1512, 1450, 1254, 1140, 836; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.89 (d, 2H, J = 8.79 Hz, H-2′, H-6′), 7.50 (d, 1H, J = 2.1 Hz, H-4), 7.34 (m, 2H, H-6, H-7), 6.99 (d, 2H, J = 8.82 Hz, H-3′, H-5′), 6.90 (s, 1H, H-10), 3.88 (s, 3H, –OCH_3), 2.57 (t, 2H, J = 7.44 Hz, –CH_2CO–), 1.76 (m, 2H, –CH_2–CH_2CO–), 0.88 (m, 3H, –CH_3); MS (EI): m/z = 534. Anal. Calcd for $C_{34}H_{46}O_5$: C, 76.37; H, 8.67. Found: C, 76.06; H, 8.72.

4.1.8.16. *Z*-2-(3-Methoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl stearate (ZWC1-2). Purified by flash column chromatography (petroleum ether/ethyl acetate 15:1) afforded yellow solid. Yield: 720 mg (72.5%). Mp 96–98 °C; IR (cm^{-1}): 3475, 2921, 2850, 1757, 1706, 1650, 1602, 1479, 1267, 1136, 1110, 786; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.50 (m, 3H, H-7, H-4, H-5′), 7.35 (m, 3H, H-6, H-2′, H-6′), 6.98 (d, 1H, J = 8.3 Hz, H-4′), 6.88 (s, 1H, H-10), 3.88 (s, 3H, –OCH_3), 2.58 (t, 2H, J = 7.41 Hz, –CH_2CO–), 1.76 (m, 2H, –CH_2–CH_2CO–), 0.88 (m, 3H, –CH_3); MS (EI): m/z = 534. Anal. Calcd for $C_{34}H_{46}O_5$: C, 76.37; H, 8.67. Found: C, 76.12; H, 8.64.

4.1.8.17. *Z*-2-(3,4-Dimethoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl stearate (ZWC1-3). Purified by flash column chromatography (petroleum ether/ethyl acetate 15:1) afforded yellow solid. Yield: 540 mg (55.3%). Mp 96–98 °C; IR (cm^{-1}):

3422, 2918, 2849, 1754, 1698, 1650, 1592, 1524, 1467, 1267, 1142, 804; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.51 (m, 3H, H-7, H-4, H-6), 7.34 (m, 2H, H-2′, H-6′), 6.95 (d, 1H, J = 8.13 Hz, H-5′), 6.89 (s, 1H, H-10), 3.98 (s, 3H, –OCH_3), 3.96 (s, 3H, –OCH_3), 2.58 (t, 2H, J = 7.41 Hz, –CH_2CO–), 1.75 (m, 2H, –CH_2–CH_2CO–), 0.88 (m, 3H, –CH_3); MS (EI): m/z = 564. Anal. Calcd for $C_{35}H_{48}O_6$: C, 74.44; H, 8.57. Found: C, 74.16; H, 8.87.

4.1.8.18. *Z*-2-(4-Fluorobenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl stearate (ZWC1-4). Purified by flash column chromatography (petroleum ether/ethyl acetate 15:1) afforded yellow solid. Yield: 680 mg (70.0%). Mp 91–93 °C; IR (cm^{-1}): 3415, 2922, 2851, 1762, 1705, 1648, 1509, 1471, 1134, 838; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.92 (m, 2H, H-2′, H-6′), 7.51 (d, 1H, J = 2.01 Hz, H-4), 7.35 (m, 2H, H-6, H-7), 7.16 (t, 2H, J = 8.7 Hz, H-3′, H-5′), 6.88 (s, 1H, H-10), 2.58 (t, 2H, J = 7.53 Hz, –CH_2CO–), 1.76 (m, 2H, –CH_2–CH_2CO–), 0.88 (m, 3H, –CH_3); MS (EI): m/z = 522. Anal. Calcd for $C_{33}H_{43}FO_4$: C, 75.83; H, 8.29. Found: C, 75.62; H, 8.14.

4.1.8.19. *Z*-2-(4-Bromobenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl stearate (ZWC1-5). Purified by flash column chromatography (petroleum ether/ethyl acetate 15:1) afforded yellow solid. Yield: 890 mg (82.1%). Mp 114–116 °C; IR (cm^{-1}): 3414, 2918, 2849, 1760, 1705, 1649, 1601, 1478, 1274, 1141, 1111, 819; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.77 (d, 2H, J = 8.52 Hz, H-2′, H-6′), 7.59 (d, 2H, J = 8.52 Hz, H-3′, H-5′), 7.51 (d, 1H, J = 2.07 Hz, H-4), 7.36 (m, 2H, H-6, H-7), 6.83 (s, 1H, H-10), 2.58 (t, 2H, J = 7.47 Hz, –CH_2CO–), 1.75 (m, 2H, –CH_2–CH_2CO–), 0.88 (m, 3H, –CH_3); MS (EI): m/z = 582. Anal. Calcd for $C_{33}H_{43}BrO_4$: C, 67.92; H, 7.43. Found: C, 67.79; H, 7.58.

4.1.8.20. *Z*-2-(3-Chlorobenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl stearate (ZWC1-6). Purified by flash column chromatography (petroleum ether/ethyl acetate 15:1) afforded yellow solid. Yield: 690 mg (68.9%). Mp 88–90 °C; IR (cm^{-1}): 3422, 2917, 2850, 1764, 1708, 1655, 1605, 1481, 1142, 833; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.95 (s, 1H, H-4), 7.74 (m, 1H, H-5′), 7.52 (m, 1H, H-2′), 7.39 (m, 4H, Ar-H), 6.82 (s, 1H, H-10), 2.58 (t, 2H, J = 7.5 Hz, –CH_2CO–), 1.76 (m, 2H, –CH_2–CH_2CO–), 0.88 (m, 3H, –CH_3); MS (EI): m/z = 538. Anal. Calcd for $C_{33}H_{43}ClO_4{\cdot}0.5CH_3COCH_3$: C, 72.93; H, 8.16. Found: C, 73.29; H, 8.62.

4.1.8.21. *Z*-2-(3-Nitrobenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl stearate (ZWC1-7). Purified by flash column chromatography (petroleum ether/ethyl acetate 15:1) afforded yellow solid. Yield: 705 mg (68.6%). Mp 104–106 °C; IR (cm^{-1}): 3449, 2915, 2851, 1760, 1707, 1654, 1614, 1531, 1478, 1350, 1136, 1108, 833; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.82 (s, 1H, H-2′), 8.25 (d, 1H, J = 7.53 Hz, H-4′), 8.15 (d, 1H, J = 7.95 Hz, H-6′), 7.64 (t, 1H, J = 7.95 Hz, H-5′), 7.54 (s, 1H, H-4), 7.41 (m, 2H, H-6, H-7), 6.90 (s, 1H, H-10), 2.58 (t, 2H, J = 7.44 Hz, –CH_2CO–), 1.76 (m, 2H, –CH_2–CH_2CO–), 0.88 (m, 3H, –CH_3); MS (EI): m/z = 549. Anal. Calcd for $C_{33}H_{43}NO_6$: C, 72.10; H, 7.88; N, 2.55. Found: C, 71.69; H, 7.79; N, 2.69.

4.1.8.22. *Z*-2-(4-Methoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl 10-undecylenate (ZWD1-1). Recrystallization from acetone afforded yellow crystals. Yield: 650 mg (80.5%). Mp 129–131 °C; IR (cm^{-1}): 3451, 2925, 2851, 1756, 1703, 1646, 1596, 1511, 1450, 1254, 1191, 1140, 838; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.89 (d, 2H, J = 8.7 Hz, H-2′, H-6′), 7.50 (d, 1H, J = 1.5 Hz, H-4), 7.35 (m, 2H, H-6, H-7), 6.99 (d, 2H, J = 8.7 Hz, H-3′, H-5′), 6.90 (s, 1H, H-10), 5.82 (m, 1H, =CH–), 5.04 (m, 2H, =CH_2), 3.88 (s, 3H, –OCH_3), 2.58 (t, 2H, J = 7.47 Hz, –CH_2CO–), 2.05 (m, 2H, =CH–CH_2–), 1.77 (m, 2H, –CH_2–CH_2CO–); MS (EI): m/z = 434. Anal. Calcd for $C_{27}H_{30}O_5{\cdot}0.5H_2O$: C, 73.12; H, 7.04. Found: C, 73.08; H, 7.09.

4.1.8.23. *Z*-2-(3-Methoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl 10-undecylenate (ZWD1-2). Recrystallization from acetone afforded yellow crystals. Yield: 540 mg (64.4%). Mp 95–97 °C; IR (cm^{-1}): 3340, 2926, 2850, 1760, 1707, 1651, 1603, 1479, 1267, 1135, 1111, 786; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.50 (m, 3H, H-7, H-4, H-5′), 7.36 (m, 3H, H-6, H-2′, H-6′), 6.98 (d, 1H, J = 8.1 Hz, H-4′), 6.88 (s, 1H, H-10), 5.81 (m, 1H, =CH–), 4.96 (m, 2H, =CH_2), 3.88 (s, 3H, –OCH_3), 2.58 (t, 2H, J = 7.2 Hz, –CH_2CO–), 2.05 (m, 2H, =CH–CH_2–), 1.76 (m, 2H, –CH_2–CH_2CO–); MS (EI): m/z = 434. Anal. Calcd for $C_{27}H_{30}O_5$: C, 74.63; H, 6.96. Found: C, 74.30; H, 6.92.

4.1.8.24. *Z*-2-(3,4-Dimethoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl 10-undecylenate (ZWD1-3). Recrystallization from acetone afforded yellow crystals. Yield: 680 mg (78.8%). Mp 110–112 °C; IR (cm^{-1}): 3493, 2923, 2852, 1754, 1698, 1650, 1592, 1524, 1482, 1267, 1143, 804; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.51 (m, 3H, H-7, H-4, H-6), 7.36 (m, 2H, H-2′, H-6′), 6.95 (d, 1H, J = 8.16 Hz, H-5′), 6.89 (s, 1H, H-10), 5.82 (m, 1H, =CH–), 4.96 (m, 2H, =CH_2), 3.98 (s, 3H, –OCH_3), 3.96 (s, 3H, –OCH_3), 2.58 (t, 2H, J = 7.47 Hz, –CH_2CO–), 2.05 (m, 2H, =CH–CH_2–), 1.76 (m, 2H, –CH_2–CH_2CO–); MS (EI): m/z = 464. Anal. Calcd for $C_{28}H_{32}O_6{\cdot}0.5H_2O$: C, 71.02; H, 7.02. Found: C, 70.73; H, 7.11.

4.1.8.25. *Z*-2-(4-Fluorobenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl 10-undecylenate (ZWD1-4). Recrystallization from acetone afforded yellow crystals. Yield: 560 mg (71.3%). Mp 82–84 °C; IR (cm^{-1}): 3410, 2926, 2852, 1752, 1707, 1655, 1509, 1478, 1241, 1147, 838; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.92 (m, 2H, H-2′, H-6′), 7.51 (d, 1H, J = 2.4 Hz, H-4), 7.36 (m, 2H, H-6, H-7), 7.16 (t, 2H, J = 8.4 Hz, H-3′, H-5′), 6.87 (s, 1H, H-10), 5.82 (m, 1H, =CH–), 4.96 (m, 2H, =CH_2), 2.58 (t, 2H, J = 7.5 Hz, –CH_2CO–), 2.05 (m, 2H, =CH–CH_2–), 1.76 (m, 2H, –CH_2–CH_2CO–); MS (EI): m/z = 422. Anal. Calcd for $C_{26}H_{27}FO_4{\cdot}0.75H_2O$: C, 71.62; H, 6.59. Found: C, 71.52; H, 6.65.

4.1.8.26. *Z*-2-(4-Bromobenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl 10-undecylenate (ZWD1-5). Recrystallization from acetone afforded yellow crystals. Yield: 550 mg (61.2%). Mp 122–124 °C; IR (cm^{-1}): 3416, 2924, 2851, 1761, 1705, 1649, 1602, 1478, 1274, 1140, 1111, 819; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.77 (d, 2H, J = 8.4 Hz, H-2′, H-6′), 7.59 (d, 2H, J = 8.4 Hz, H-3′, H-5′), 7.51 (d, 1H, J = 2.1 Hz, H-4), 7.36 (m, 2H, H-6, H-7), 6.83 (s, 1H, H-10), 5.82 (m, 1H, =CH–), 4.96 (m, 2H, =CH_2), 2.58 (t, 2H, J = 7.2 Hz, –CH_2CO–), 2.05 (m, 2H, =CH–CH_2–), 1.76 (m, 2H, –CH_2–CH_2CO–); MS (EI): m/z = 484. Anal. Calcd for $C_{26}H_{27}BrO_4{\cdot}0.5H_2O$: C, 63.42; H, 5.73. Found: C, 63.47; H, 5.83.

4.1.8.27. *Z*-2-(3-Chlorobenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl 10-undecylenate (ZWD1-6). Recrystallization from acetone afforded yellow crystals. Yield: 430 mg (65.0%). Mp 94–96 °C; IR (cm^{-1}): 3457, 2925, 2853, 1758, 1710, 1653, 1606, 1477, 1140, 1108, 821; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.95 (s, 1H, H-4), 7.74 (m, 1H, H-5′), 7.52 (m, 1H, H-2′), 7.38 (m, 4H, Ar-H), 6.82 (s, 1H, H-10), 5.82 (m, 1H, =CH–), 4.96 (m, 2H, =CH_2), 2.58 (t, 2H, J = 7.41 Hz, –CH_2CO–), 2.05 (m, 2H, =CH–CH_2–), 1.76 (m, 2H, –CH_2–CH_2CO–); MS (EI): m/z = 438. Anal. Calcd for $C_{26}H_{27}ClO_4{\cdot}1.5H_2O$: C, 67.02; H, 6.49. Found: C, 66.76; H, 6.22.

4.1.8.28. *Z*-2-(3-Nitrobenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl 10-undecylenate (ZWD1-7). Recrystallization from acetone afforded yellow crystals. Yield: 620 mg (74.2%). Mp 108–110 °C; IR (cm^{-1}): 3416, 2922, 2852, 1758, 1704, 1651, 1614, 1529, 1477, 1349, 1135, 1105, 834; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.82 (s, 1H, H-2′), 8.25 (d, 1H, J = 7.8 Hz, H-4′), 8.15 (d, 1H, J = 7.8 Hz, H-6′), 7.64 (t, 1H, J = 8.1 Hz, H-5′), 7.54 (s, 1H, H-4),

7.41 (m, 2H, H-6, H-7), 6.90 (s, 1H, H-10), 5.82 (m, 1H, =CH–), 4.96 (m, 2H, $=CH_2$), 2.58 (t, 2H, J = 7.5 Hz, $-CH_2CO-$), 2.05 (m, 2H, =CH–CH_2–), 1.76 (m, 2H, $-CH_2-CH_2CO-$); MS (EI): m/z = 449. Anal. Calcd for $C_{26}H_{27}NO_6\cdot H_2O$: C, 66.80; H, 6.25; N, 3.00. Found: C, 67.11; H, 6.13; N, 2.92.c

4.1.8.29. *Z*-2-(4-Methoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl palmitate (ZWE1-1). Recrystallization from acetone afforded yellow crystals. Yield: 810 mg (86.1%). Mp 138–140 °C; IR (cm^{-1}): 3469, 2923, 2851, 1755, 1703, 1647, 1596, 1512, 1480, 1255, 1141, 839; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.89 (d, 2H, J = 8.7 Hz, H-2′, H-6′), 7.50 (d, 1H, J = 1.35 Hz, H-4), 7.34 (m, 2H, H-6, H-7), 6.99 (d, 2H, J = 8.79 Hz, H-3′, H-5′), 6.90 (s, 1H, H-10), 3.88 (s, 3H, $-OCH_3$), 2.57 (t, 2H, J = 7.5 Hz, $-CH_2CO-$), 1.76 (m, 2H, $-CH_2-CH_2CO-$), 0.88 (m, 3H, $-CH_3$); MS (EI): m/z = 506. Anal. Calcd for $C_{32}H_{42}O_5\cdot H_2O$: C, 73.25; H, 8.45. Found: C, 73.30; H, 8.51.

4.1.8.30. *Z*-2-(3-Methoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl palmitate (ZWE1-2). Recrystallization from acetone afforded yellow crystals. Yield: 750 mg (79.7%). Mp 100–102 °C; IR (cm^{-1}): 3469, 2921, 2851, 1757, 1706, 1650, 1603, 1569, 1479, 1267, 1137, 1111, 786; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.50 (m, 3H, H-7, H-4, H-5′), 7.36 (m, 3H, H-6, H-2′, H-6′), 6.98 (dd, 1H, $J_{4',5'}$ = 8.07 Hz, $J_{4',6'}$ = 1.8 Hz, H-4′), 6.88 (s, 1H, H-10), 3.88 (s, 3H, $-OCH_3$), 2.58 (t, 2H, J = 7.5 Hz, $-CH_2CO-$), 1.76 (m, 2H, $-CH_2-CH_2CO-$), 0.88 (m, 3H, $-CH_3$); MS (EI): m/z = 506. Anal. Calcd for $C_{32}H_{42}O_5$: C, 75.86; H, 8.35. Found: C, 75.52; H, 8.45.

4.1.8.31. *Z*-2-(3,4-Dimethoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl palmitate (ZWE1-3). Recrystallization from acetone afforded yellow crystals. Yield: 770 mg (77.2%). Mp 114–116 °C; IR (cm^{-1}): 3428, 2919, 2850, 1755, 1697, 1650, 1592, 1524, 1481, 1267, 1145, 1030, 804; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.51 (m, 3H, H-7, H-4, H-6), 7.34 (m, 2H, H-2′, H-6′), 6.95 (d, 1H, J = 8.25 Hz, H-5′), 6.89 (s, 1H, H-10), 3.98 (s, 3H, $-OCH_3$), 3.96 (s, 3H, $-OCH_3$), 2.58 (t, 2H, J = 7.44 Hz, $-CH_2CO-$), 1.76 (m, 2H, $-CH_2-CH_2CO-$), 0.88 (m, 3H, $-CH_3$); MS (EI): m/z = 536. Anal. Calcd for $C_{33}H_{44}O_6\cdot 0.5H_2O$: C, 72.63; H, 8.31. Found: C, 72.73; H, 8.51.

4.1.8.32. *Z*-2-(4-Fluorobenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl palmitate (ZWE1-4). Recrystallization from acetone afforded yellow crystals. Yield: 670 mg (72.9%). Mp 100–102 °C; IR (cm^{-1}): 3415, 2923, 2851, 1760, 1705, 1648, 1508, 1473, 1134, 838; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.92 (m, 2H, H-2′, H-6′), 7.51 (d, 1H, J = 1.98 Hz, H-4), 7.35 (m, 2H, H-6, H-7), 7.16 (t, 2H, J = 8.67 Hz, H-3′, H-5′), 6.88 (s, 1H, H-10), 2.58 (t, 2H, J = 7.5 Hz, $-CH_2CO-$), 1.76 (m, 2H, $-CH_2-CH_2CO-$), 0.88 (m, 3H, $-CH_3$); MS (EI): m/z = 494. Anal. Calcd for $C_{31}H_{39}FO_4$: C, 75.27; H, 7.95. Found: C, 74.90; H, 8.37.

4.1.8.33. *Z*-2-(4-Bromobenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl palmitate (ZWE1-5). Recrystallization from acetone afforded yellow crystals. Yield: 860 mg (83.3%). Mp 124–126 °C; IR (cm^{-1}): 3414, 2918, 2848, 1761, 1705, 1649, 1603, 1479, 1274, 1200, 1112, 819; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.78 (d, 2H, J = 8.49 Hz, H-2′, H-6′), 7.59 (d, 2H, J = 8.46 Hz, H-3′, H-5′), 7.51 (d, 1H, J = 2.16 Hz, H-4), 7.36 (m, 2H, H-6, H-7), 6.83 (s, 1H, H-10), 2.58 (t, 2H, J = 7.65 Hz, $-CH_2CO-$), 1.76 (m, 2H, $-CH_2-CH_2CO-$), 0.88 (m, 3H, $-CH_3$); MS (EI): m/z = 554. Anal. Calcd for $C_{31}H_{39}BrO_4$: C, 67.02; H, 7.08. Found: C, 66.70; H, 7.12.

4.1.9. 2′,4′-Dihydroxyacetophenone (9)

A mixture of *m*-dihydroxybenzene (10.0 g, 51.1 mmol), $ZnCl_2$ (12 g) and glacial acetic acid (30 mL) was heated for 1 h at boiling temperature. The cooled reaction mixture was poured into ice cold water and acidified with HCl (10% v/v aqueous solution). The precipitate was collected, washed with water, and dried. Purified by flash column chromatography (petroleum ether/ethyl acetate 7:1) afforded 2′,4′-dihydroxyacetophenone (**9**). Yield: 450 mg (43.2%); mp 141–143 °C; MS (EI): m/z = 152.

4.1.10. 2′-Hydroxy-4′-(methoxymethoxy)acetophenone (10)

To a mixture of 2′,4′-dihydroxyacetophenone (**9**, 7.0 g, 46.05 mmol) and anhydrous K_2CO_3 (20 g) in dry acetone (100 mL) was added dropwise chloromethyl methyl ether (4 mL, 53.6 mmol). The mixture was heated for 2 h at 30 °C in an oil bath, cooled to room temperature, filtered, washed with acetone and the solvent wad evaporated. The resulting yellowish oil was purified using flash column chromatography (petroleum ether/ethyl acetate 9:1) to give 2′-hydroxy-4′-(methoxymethoxy)acetophenone (**10**) as a low melting white solid. Yield: 7.2 g (79.7%). MS (EI): m/z = 196.

4.1.11. 2′-Hydroxy-4′-(methoxymethoxy)-4-methoxy-chalcone (11)

To a stirred solution of 2′-hydroxy-4′-(methoxymethoxy)acetophenone (**10**, 30.74 mmol) and 4-methoxybenzaldehyde (33.82 mmol) in ethanol (50 mL) was added KOH (20% w/v aqueous solution, 20 mL) and the mixture was stirred at room temperature for 36 h. The reaction mixture was cooled to 0 °C (ice-water bath) and acidified with HCl (10% v/v aqueous solution). The yellow precipitate was formed, which was filtered and washed with 10% aqueous HCl solution. The solid was recrystallized from ethanol to give 2′-Hydroxy-4′-(methoxymethoxy)-4-methoxy-chalcone (**11**) with a yield of 51.0. Mp 85–87 °C; ^1H NMR ($CDCl_3$, 300 MHz): δ 13.39 (d, 1H, OH), 7.88 (d, 1H, J = 15.3 Hz, =CH–), 7.84 (d, 1H, J = 8.7 Hz, H-6′), 7.63 (d, 2H, J = 8.7 Hz, H-2, H-6), 7.46 (d, 1H, J = 15.3 Hz, –CH=), 6.95 (d, 2H, J = 8.4 Hz, H-3, H-5), 6.64 (d, 1H, J = 2.4 Hz, H-3′), 6.59 (dd, 1H, $J_{5,6}$ = 8.7 Hz, $J_{3,5}$ = 2.4 Hz, H-5′), 5.23 (s, 2H, $-OCH_2O-$), 3.87 (s, 3H, $-OCH_3$), 3.49 (s, 3H, $-OCH_3$); MS (EI): m/z = 314.

4.1.12. *Z*-2-(4-Methoxybenzylidene)-6-(methoxymethoxy)-benzo[*b*]furan-3-one (12)

To a solution of mercuric acetate (19.0 mmol) in pyridine (50 mL) was added 2′-hydroxy-4′-(methoxymethoxy)-4-methoxy-chalcone (**11**, 16.0 mmol) at room temperature and the mixture was stirred at 110 °C for 1 h. The cooled reaction mixture was poured into ice cold water and acidified with HCl (10% v/v aqueous solution). The precipitated solid was extracted with CH_2Cl_2, the extracts were dried (Na_2SO_4) and the solvent was evaporated to give a solid. Then the crude product was recrystallized from ethanol to give **12** with a yield of 95. Mp 132–134 °C; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.87 (d, 2H, J = 8.7 Hz, H-2′, H-6′), 7.72 (d, 1H, J = 8.4 Hz, H-4), 6.98 (m, 3H, =CH–, Ar-H), 6.84 (m, 2H, Ar-H), 5.29 (s, 2H, $-OCH_2O-$), 3.88 (s, 3H, $-OCH_3$), 3.52 (s, 3H, $-OCH_3$); MS (EI): m/z = 312.

4.1.13. *Z*-2-(4-Methoxybenzylidene)-6-hydroxybenzo[*b*]furan-3-one (13)

The *Z*-2-(4-Methoxybenzylidene)-6-(methoxymethoxy)benzo-[*b*]furan-3-one (**12**, 15 mmol) was added to methanol (150 mL) followed by dropwise addition of HCl (10% v/v aqueous solution, 30 mL). The mixture was refluxed for 30 min. The reaction mixture was cooled to room temperature. A yellow precipitate was formed, which was filtered and washed with methanol to give **13** with a yield of 90. Mp 256–258 °C; ^1H NMR (DMSO, 300 MHz): δ 11.17 (s, 1H, OH), 7.93 (d, 2H, J = 8.7 Hz, H-2′, H-6′), 7.63 (d, 1H, J = 8.4 Hz, H-4), 7.07 (d, 2H, J = 8.7 Hz, H-3′, H-5′), 6.86 (s, 1H,

=CH–), 6.72 (m, 2H, H-5, H-7), 7.01 (s, 1H, H-4), 3.96 (s, 3H, –OCH_3); MS (EI): m/z = 268.

4.1.14

4.1.14.1. *Z*-2-(4-Methoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-6-yl (9*Z*)-oleate (ZWA2-1). This compound was made using the same procedure as for the preparation of compound **ZWA1-1** using appropriate starting materials. Yield: 756 mg (76.4%). Mp 58–60 °C; IR (cm^{-1}): 3410, 2921, 2851, 1766, 1651, 1597, 1511, 1436, 1260, 1109, 839; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.88 (d, 2H, J = 8.7 Hz, H-2′, H-6′), 7.81 (d, 1H, J = 8.1 Hz, H-4), 7.15 (d, 1H, J = 1.8 Hz, H-7), 6.98 (d, 2H, J = 9.0 Hz, H-3′, H-5′), 6.94 (dd, 1H, $J_{4,5}$ = 8.1 Hz, $J_{5,7}$ = 1.8 Hz, H-5), 6.88 (s, 1H, H-10), 5.37 (m, 2H, –CH=CH–), 3.88 (s, 3H, –OCH_3), 2.60 (t, 2H, J = 7.5 Hz, –$COCH_2$–), 2.02 (m, 4H, –CH_2–CH=CH–CH_2–), 1.76 (m, 2H, –CH_2–CH_2CO–), 0.87 (m, 3H, –CH_3); MS (EI): m/z = 532. Anal. Calcd for $C_{34}H_{44}O_5$: C, 76.66; H, 8.33. Found: C, 76.58; H, 8.35.

4.1.14.2. *Z*-2-(4-Methoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-6-yl (10*E*,12*Z*)-linoleate (ZWB2-1). This compound was made using the same procedure as for the preparation of compound **ZWB1-1** using appropriate starting materials. Yield: 671 mg (68.1%). Mp 60–62 °C; IR (cm^{-1}): 3416, 2922, 2852, 1766, 1699, 1651, 1597, 1511, 1436, 1260, 1124, 839; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.87 (d, 2H, J = 8.7 Hz, H-2′, H-6′), 7.81 (d, 1H, J = 8.4 Hz, H-4), 7.15 (s, 1H, H-7), 6.98 (d, 2H, J = 8.7 Hz, H-3′, H-5′), 6.93 (d, 1H, J = 8.4 Hz, H-5), 6.88 (s, 1H, H-10), 5.26–6.34 (m, 4H, –CH=CH–CH=CH–), 3.88 (s, 3H, –OCH_3), 2.60 (t, 2H, J = 7.47 Hz, –$COCH_2$–), 2.05 (m, 4H, –CH_2–CH=CH–CH=CH–CH_2–), 1.76 (m, 2H, –CH_2–CH_2CO–), 0.87 (m, 3H, –CH_3); MS (EI): m/z = 530. Anal. Calcd for $C_{34}H_{42}O_5$: C, 76.95; H, 7.98. Found: C, 76.50; H, 7.92.

4.1.14.3. *Z*-2-(4-Methoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-6-yl stearate (ZWC2-1). This compound was made using the same procedure as for the preparation of compound **ZWC1-1** using appropriate starting materials. Yield: 700 mg (70.5%). Mp 88–90 °C; IR (cm^{-1}): 3420, 2918, 2851, 1765, 1699, 1652, 1597, 1512, 1436, 1260, 1125, 1109, 839; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.88 (d, 2H, J = 8.79 Hz, H-2′, H-6′), 7.81 (d, 1H, J = 8.31 Hz, H-4), 7.15 (d, 1H, J = 1.68 Hz, H-7), 6.98 (d, 2H, J = 8.79 Hz, H-3′, H-5′), 6.93 (dd, 1H, $J_{4,5}$ = 8.37 Hz, $J_{5,7}$ = 1.77 Hz, H-5), 6.88 (s, 1H, H-10), 3.88 (s, 3H, –OCH_3), 2.60 (t, 2H, J = 7.5 Hz, –$COCH_2$–), 1.78 (m, 2H, –CH_2–CH_2CO–), 0.88 (m, 3H, –CH_3); MS (EI): m/z = 534. Anal. Calcd for $C_{34}H_{46}O_5$: C, 76.37; H, 8.67. Found: C, 76.49; H, 8.36.

4.1.14.4. *Z*-2-(4-Methoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-6-yl 10-undecylenate (ZWD2-1). This compound was made using the same procedure as for the preparation of compound **ZWD1-1** using appropriate starting materials. Yield: 545 mg (67.5%). Mp 84–86 °C; IR (cm^{-1}): 3428, 2922, 2852, 1765, 1699, 1651, 1597, 1511, 1450, 1260, 1178, 1124, 1100, 839; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.88 (d, 2H, J = 9 Hz, H-2′, H-6′), 7.81 (d, 1H, J = 8.4 Hz, H-4), 7.15 (d, 1H, J = 1.2 Hz, H-7), 6.98 (d, 2H, J = 9 Hz, H-3′, H-5′), 6.93 (dd, 1H, $J_{4,5}$ = 8.4 Hz, $J_{5,7}$ = 1.8 Hz, H-5), 6.88 (s, 1H, H-10), 5.81 (m, 1H, =CH–), 4.92 (m, 2H, $=CH_2$), 3.88 (s, 3H, –OCH_3), 2.61 (t, 2H, J = 7.5 Hz, –CH_2CO–), 2.06 (m, 2H, =CH–CH_2–), 1.77 (m, 2H, –CH_2–CH_2CO–); MS (EI): m/z = 434. Anal. Calcd for $C_{27}H_{30}O_5 \cdot 0.25H_2O$: C, 73.87; H, 7.00. Found: C, 73.86; H, 7.24.

4.1.15. 3-(4-Methoxyphenyl)acrylic acid (15)

To a solution of 4-methoxybenzaldehyde (**14**, 35.6 mmol) in pyridine (20 mL) were added malonic acid (53.4 mmol) and piperidine (2 mL), and the reaction mixture was refluxed for 2 h. The cooled reaction mixture was poured, with stirring, into iced HCl (2 mmol/L aqueous solution). The precipitated solid was filtered, and washed with water. 3-(4-Methoxyphenyl)acrylic acid (**15**) was obtained in 80.5% yield, mp 170–172 °C.

4.1.16. 3-(4-Methoxyphenyl)propanoic acid (16)

To a solution of 3-(4-methoxyphenyl)acrylic acid (**15**, 14.7 mmol) in THF (50 mL) was added a catalytic amount of 10% palladium on activated carbon to carry out hydrogenation, followed by stirring for 5 h at room temperature. The mixture was filtered, and the filtrate was concentrated in vacuo to give a white solid with a yield of 95.2%, mp 102–103 °C.

4.1.17. 1-(2,4-Dihydroxyphenyl)-3-(4-methoxyphenyl)propan-1-one (17)

A mixture of 3-(4-methoxyphenyl)propanoic acid (**16**, 5.56 mmol), *m*-dihydroxybenzene (6.0 mmol) and BF_3/Et_2O (13.5 mL) was stirred at 75 °C for 90 min. The reaction mixture was then poured, with stirring, into saturated $NaHCO_3$ solution, and the solution was extracted with EtOAc. The combined EtOAc layer was washed with water, brine and dried over Na_2SO_4. The residue obtained after evaporation of the solvent was chromatographed over silica gel column (petroleum ether/ethyl acetate 7:1), and compound **17** was obtained in 66.1% yield, mp 58–60 °C.

4.1.18. 7-Hydroxy-3-(4-methoxybenzyl)-4*H*-chromen-4-one (18)

To a solution of 1-(2,4-dihydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (**17**, 5.56 mmol) in triethylorthoformate (7.3 mL) was added dropwise 70% perchloric acid (0.83 mL), and the reaction mixture was stirred for 5 h at room temperature. Then the reaction mixture was poured, with stirring, into ethyl ether (25 mL). The precipitated solid was filtered, dissolved in hot water (25 mL), and heated at reflux for 10 min. The mixture was then allowed to cool to room temperature then the solution was extracted with EtOAc (3 × 50 mL) and the combined EtOAc layer was washed with water (20 mL), brine (20 mL) and dried over Na_2SO_4. The residue obtained after evaporation of the solvent was chromatographed over silica gel column (petroleum ether/ethyl acetate 2:1), and compound **18** was obtained in 56.1% yield. Mp 162–164 °C; ^1H NMR($CDCl_3$) δ 10.76 (s, 1H, OH), 8.16 (s, 1H, H-2), 7.87 (d, 2H, J = 8.7 Hz, H-5), 7.20 (d, 2H, J = 8.4 Hz, H-2′, H-6′), 6.89 (dd, 1H, $J_{5,6}$ = 8.85 Hz, $J_{6,8}$ = 2.1 Hz, H-6), 6.83 (m, 3H, Ar-H), 3.69 (s, 3H, –OCH_3), 3.59 (s, 2H, H-11); MS (ESI): m/z = 283 $[M+H]^+$, 305 $[M+Na]^+$.

4.1.19

4.1.19.1. 3-(4-Methoxybenzyl)-4-oxo-4*H*-chromen-7-yl (9*Z*)-oleate (ZWA3-1). This compound was made using the same procedure as for the preparation of compound **ZWA1-1** using appropriate starting materials. Recrystallization from acetone afforded white crystals. Yield: 690 mg (67.9%). Mp 58–60 °C; IR (cm^{-1}): 3487, 2921, 2850, 1748, 1641, 1515, 1445, 1249, 1177, 1027, 809; ^1H NMR ($CDCl_3$, 300 MHz): δ 8.23 (d, 1H, J = 8.7 Hz, H-5), 7.56 (s, 1H, H-8), 7.22 (s, 1H, H-2), 7.20 (d, 2H, J = 6.3 Hz, H-2′, H-6′), 7.11 (dd, 1H, $J_{5,6}$ = 8.7 Hz, $J_{6,8}$ = 2.1 Hz, H-6), 6.86 (d, 2H, J = 8.7 Hz, H-3′, H-5′), 5.35 (m, 2H, –CH=CH–), 3.79 (s, 3H, –OCH_3), 3.75 (s, 2H, –CH_2-Ar), 2.58 (t, 2H, J = 7.5 Hz, –CH_2CO–), 2.01 (m, 4H, –CH_2–CH=CH–CH_2–), 1.76 (m, 2H, –CH_2–CH_2CO–), 0.88 (m, 3H, –CH_3); MS (EI): m/z = 546. Anal. Calcd for $C_{35}H_{46}O_5$: C, 76.89; H, 8.48. Found: C, 76.86; H, 8.49.

4.1.19.2. 3-(4-Methoxybenzyl)-4-oxo-4*H*-chromen-7-yl (10*E*,12*Z*)-linoleate (ZWB3-1). This compound was made using the same procedure as for the preparation of compound **ZWB1-1** using appropriate starting materials. Purified by flash column chromatography (petroleum ether/ethyl acetate 15:1) afforded white solid. Yield: 542 mg (53.6%). Mp 50–52 °C; IR (cm^{-1}): 3421, 2925, 2852, 1754, 1642, 1514, 1445, 1249, 1177, 1026, 808; ^1H NMR

($CDCl_3$, 300 MHz): δ 8.23 (d, 1H, J = 8.7 Hz, H-5), 7.56 (s, 1H, H-8), 7.22 (s, 1H, H-2), 7.20 (d, 2H, J = 6.6 Hz, H-2′, H-6′), 7.11 (dd, 1H, $J_{5,6}$ = 8.7 Hz, $J_{6,8}$ = 2.1 Hz, H-6), 6.85 (d, 2H, J = 8.4 Hz, H-3′, H-5′), 5.28–6.34 (m, 4H, –CH=CH–CH=CH–), 3.79 (s, 3H, $-OCH_3$), 3.75 (s, 2H, $-CH_2$-Ar), 2.58 (t, 2H, J = 7.2 Hz, $-CH_2CO-$), 2.09 (m, 4H, $-CH_2-CH=CH-CH=CH-CH_2-$), 1.75 (m, 2H, $-CH_2-CH_2CO-$), 0.88 (m, 3H, $-CH_3$); MS (EI): m/z = 544. Anal. Calcd for $C_{35}H_{44}O_5 \cdot H_2O$: C, 74.70; H, 8.24. Found: C, 74.59; H, 8.15.

4.1.19.3. 3-(4-Methoxybenzyl)-4-oxo-4*H*-chromen-7-yl stearate (ZWC3-1). This compound was made using the same procedure as for the preparation of compound **ZWC1-1** using appropriate starting materials. Purified by flash column chromatography (petroleum ether/ethyl acetate 15:1) afforded white solid. Yield: 560 mg (54.9%). Mp 86–88 °C; IR (cm^{-1}): 3415, 2917, 2849, 1748, 1641, 1515, 1445, 1250, 1178, 1027, 810; ^1H NMR ($CDCl_3$, 300 MHz): δ 8.23 (d, 1H, J = 8.7 Hz, H-5), 7.56 (s, 1H, H-8), 7.21 (m, 3H, H-2, H-2′, H-6′), 7.12 (d, 1H, J = 8.7 Hz, H-6), 6.85 (d, 2H, J = 8.4 Hz, H-3′, H-5′), 3.79 (s, 3H, $-OCH_3$), 3.75 (s, 2H, $-CH_2$-Ar), 2.58 (t, 2H, J = 7.5 Hz, $-CH_2CO-$), 1.75 (m, 2H, $-CH_2-CH_2CO-$), 0.88 (m, 3H, $-CH_3$); MS (EI): m/z = 548. Anal. Calcd for $C_{35}H_{48}O_5$: C, 76.61; H, 8.82. Found: C, 76.44; H, 8.85.

4.1.19.4. 3-(4-Methoxybenzyl)-4-oxo-4*H*-chromen-7-yl 10-undecylenate (ZWD3-1). This compound was made using the same procedure as for the preparation of compound **ZWD1-1** using appropriate starting materials. Purified by flash column chromatography (petroleum ether/ethyl acetate 15:1) afforded white solid. Yield: 510 mg (61.2%). Mp 64–66 °C; IR (cm^{-1}): 3415, 2923, 2850, 1750, 1642, 1515, 1445, 1250, 1177, 1026, 809; ^1H NMR ($CDCl_3$, 300 MHz): δ 8.23 (d, 1H, J = 8.7 Hz, H-5), 7.56 (s, 1H, H-8), 7.20 (m, 3H, H-2, H-2′, H-6′), 7.11 (dd, 1H, $J_{5,6}$ = 8.7 Hz, $J_{6,8}$ = 2.04 Hz, H-6), 6.85 (d, 2H, J = 8.67 Hz, H-3′, H-5′), 5.81 (m, 1H, =CH–), 4.95 (m, 2H, $=CH_2$), 3.79 (s, 3H, $-OCH_3$), 3.75 (s, 2H, $-CH_2$-Ar), 2.59 (t, 2H, J = 7.5 Hz, $-CH_2CO-$), 2.03 (m, 2H, =CH–CH_2–), 1.77 (m, 2H, $-CH_2-CH_2CO-$); MS (EI): m/z = 448. Anal. Calcd for $C_{28}H_{32}O_5$: C, 74.98; H, 7.19. Found: C, 75.09; H, 7.38.

4.2. Vitro experiment

4.2.1. Cell proliferation assay by MTT

3T3-L1 preadipocytes (5000 cells/well) were seeded in 96-well plates. After two days' conventional culture, the cells were incubated with Dulbecco's Modified Eagle's medium (DMEM, Gibco) containing the selected compounds (100 μmol/L) for 48 h. The culture solution containing 0.1% (V/V) ethanol (96%) was given to the control group. Twenty microliters of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL) was placed in each well for 4 h at 37 °C. Consecutively, 100 μL of dimethyl sulfoxide (DMSO) was added to extract the MTT formazan, and followed by agitation on a plate shaker for 15 min. The optical density (OD) was then measured on the multiwell enzyme-linked immunosorbent assay automatic spectrometer reader at 540 nm. The inhibition ratio was calculated through formula 1:

$$\text{Inhibition ratio} = (1 - \text{OD}_{\text{experimental group}}/\text{OD}_{\text{blank group}}) \times 100\% \quad (1)$$

4.2.2. Adipogenesis assay by Oil Red O staining

To induce cell differentiation, 3T3-L1 preadipocytes were cultivated in the growth medium until completely confluent. Two days after complete confluence was reached (defined as day 0), differentiation was induced using DMEM containing 15% fetal bovine serum (FBS), 0.5 mmol/L 3-isobutyl-1-methylxanthine (IBMX), 1 μmol/L dexamethasone (DEX), and 10 mg/L insulin for 2 days. On day 2, the medium was replaced with DMEM containing only 15% FBS and 10 mg/L insulin. The medium was replaced with new medium every 2 days. On day 4, insulin was removed, and the medium was replaced with DMEM containing the selected compounds (100 μmol/L).

On day 8, the cells were washed three times with phosphate-buffered saline (PBS) and then fixed for 30 min with 3.7% formaldehyde in PBS. Oil Red O (0.5%) in isopropanol was diluted with 2/3 volumes of water, filtered and added to the fixed cell monolayers for 1 h at room temperature. The cell monolayers were then washed with PBS, and the stained triglyceride droplets in the cells were visualized. The Oil Red O-stained triglyceride droplets were then extracted with isopropanol, and the OD at 492 nm was measured. The inhibition ratio of lipid production was calculated through formula 1.

4.2.3. Glucose consumption assay

3T3-L1 preadipocytes grown in 96-well plates were induced by the combination of IBMX, DEX, and insulin (MDI) to differentiate into adipocytes. Then the medium was replaced with DMEM containing 0.2% BSA, different doses of selected compounds (50 and 100 μg/mL) and glucose at various concentrations (10 and 25 mmol/L). The adipocytes were incubated at 37 °C for 24 h. Finally, the medium was removed and its glucose concentrations were determined by the glucose-oxidase method. The amount of glucose consumption (GC) was calculated by the glucose concentrations of blank wells subtracting the remaining glucose in cell plated wells.

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