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SHORT COMMUNICATION

Novel phenoxyalkylcarboxylic acid derivatives as hypolipidaemic agents

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Abstract

Novel phenoxyalkylcarboxylic acid derivatives based on the natural scaffolds, flavonoids, or resveratrol were designed, synthesized, and evaluated for hypolipidaemic activity. Among the compounds, **30b** lowered the triglycerides by 48.5% ($P < 0.05$) and total cholesterol by 44.2% ($P < 0.05$), respectively, and was more effective than the reference drug fenofibric acid in a Triton WR-1339-induced hyperlipidaemic mice model orally (300 mg/kg body weight). **30b** also showed 59.4% triglycerides lowering in an alloxan-induced diabetic mice model orally (150 mg/kg body weight). Receptor docking studies revealed that compound **30b** could interact with the amino acid residues in the ligand-binding domain essential for the activation of the PPARα. The results indicate that resveratrol should be a better scaffold to derive a new class of hypolipidaemic agents in comparison with a flavonoid scaffold.

Keywords: Scaffolds, flavonoids, resveratrol, hypolipidaemic, PPARα

Introduction

Hyperlipidaemia is the major risk factor for coronary heart disease (CHD), ischaemic cerebrovascular diseases, and peripheral vascular diseases.[1–3] Phenoxyalkylcarboxylic acid derivatives (fibrates), such as fenofibrate (2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoic acid isopropyl ester, **1a**), gemfibrozil (5-(2,5-dimethylphenoxy)-2,2-dimethyl-pentanoic acid, **2**) (Figure 1), have been widely used in the clinic as an important class of lipid-modifying agents by lowering serum triglycerides and raising HDL cholesterol (HDLc) and remain the first choice for the treatment of severe hypertriglyceridaemia.[4–6] The effects of fibrates are attributed to the activation of the peroxisome proliferator-activated receptor alpha (PPARα), which is a known target for the treatment of dyslipidaemia.[7–9] However, most fibrates can cause myopathy (muscle pain with creatine phosphokinase elevations). Moreover, in combination with statin drugs, fibrates can cause an increased risk of rhabdomyolysis, idiosyncratic destruction of muscle tissue, leading to renal failure.[10–12] Elevated serum transaminase concentrations may occur with fenofibrate.[13] Fenofibrate retains some hepatotoxicity, especially in the rodents.[14] As a consequence, novel molecular scaffolds are expected to provide a superior clinical profile compared with that of existing fibrates for therapeutic intervention in dyslipidaemia and other metabolic disorders.[15]

Flavonoids are naturally occurring phenolic compounds that are widely distributed in plants fulfilling many functions, such as antioxidant, antiallergic, anti-inflammatory, antimicrobial, and anticancer activities.[16–18] Consumers and food manufacturers have become interested in flavonoids for their medicinal properties, especially their potential role in the prevention of cancers and cardiovascular diseases.[19] They are relatively of low toxicity compared with other active plant compounds (for instance, alkaloids). The chemical structure of flavonoids are based on a C_{15} skeleton with a chromone

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ring bearing a second aromatic ring B in position 2 or 3 (Figure 2). Various subgroups of flavonoids including flavones, isoflavonoids, chalcones, and flavanones are classified according to the substitution patterns of ring C (Figure 1).

The stilbene-based components, or stilbenoids, have been suggested to have many health benefits including antioxidative, anti-inflammatory, antileukemic, antibacterial, antifungal, antiplatelet aggregation, vasodilator, and antitumour activities.[20–27] Resveratrol (*trans*-3,5,4′-trihydroxystilbene), the most widely studied stilbene, is a phytoalexin produced naturally by several plants when attacked by pathogens such as bacteria or fungi. Resveratrol is found in the skin of red grapes and is a constituent of red wine, and some scientists believe it is one of the factors behind the French Paradox.[28,29] In mouse and rat experiments, anticancer, anti-inflammatory, blood sugar-lowering and other beneficial cardiovascular effects of resveratrol have been reported.[30] Resveratrol protects our heart and blood vessels by directly scavenging oxidants, which could cause oxidation of lipids, and by preventing oxidative stress-induced apoptosis of endothelial cells.[31,32] It may also help to prevent heart damage after a cardiac arrest. Reduced platelet aggregation by resveratrol can reduce the risk of atherosclerosis.[33] Resveratrol has also been demonstrated to reduce blood lipid levels in animals.[34] Some naturally occurring or synthetic resveratrol analogues have shown to lower plasma lipid levels when fed to hamsters or to significantly activate PPARα.[35]

These beneficial effects of flavonoids and resveratrol and their diverse chemical structures have attracted much attention, and they may provide novel scaffolds as therapeutic drugs. In this article, novel hypolipidaemic agents based on the flavonoid and resveratrol scaffolds were studied. The structure–activity relationship (SAR) on the fibrates shows that they consist of an acidic head group and a large lipophilic backbone.[36] The classical acidic head group could be 2,2-dimethyloxyvaleric acid or oxyisobutyric acid.[37] Here, we described the design and synthesis of novel hypolipidaemic agents by the combination of the fibrate head groups and the flavonoid or resveratrol scaffolds, which probably would provide synergistic pharmacological effects. The flavone, isoflavonoid, chalcone, flavanone, and resveratrol backbones were selected as the scaffolds in this study.

Materials and methods

Chemistry

All common laboratory chemicals were purchased from commercial sources and used without further purification. Melting points (uncorrected) were obtained by using an XT4 MP apparatus (Taike Corp., Beijing, China). ^{1}H NMR and ^{13}C NMR were measured on a JNM-ECA-400 NMR spectrometer (at 400 MHz for 1H and 100 MHz for ^{13}C) using TMS as an internal standard. Coupling constants (*J*) are given in hertz. Electrospray mass spectra (ESI-MS) were recorded on an API3000 spectrometer. Thin-layer chromatography (TLC) was carried out on

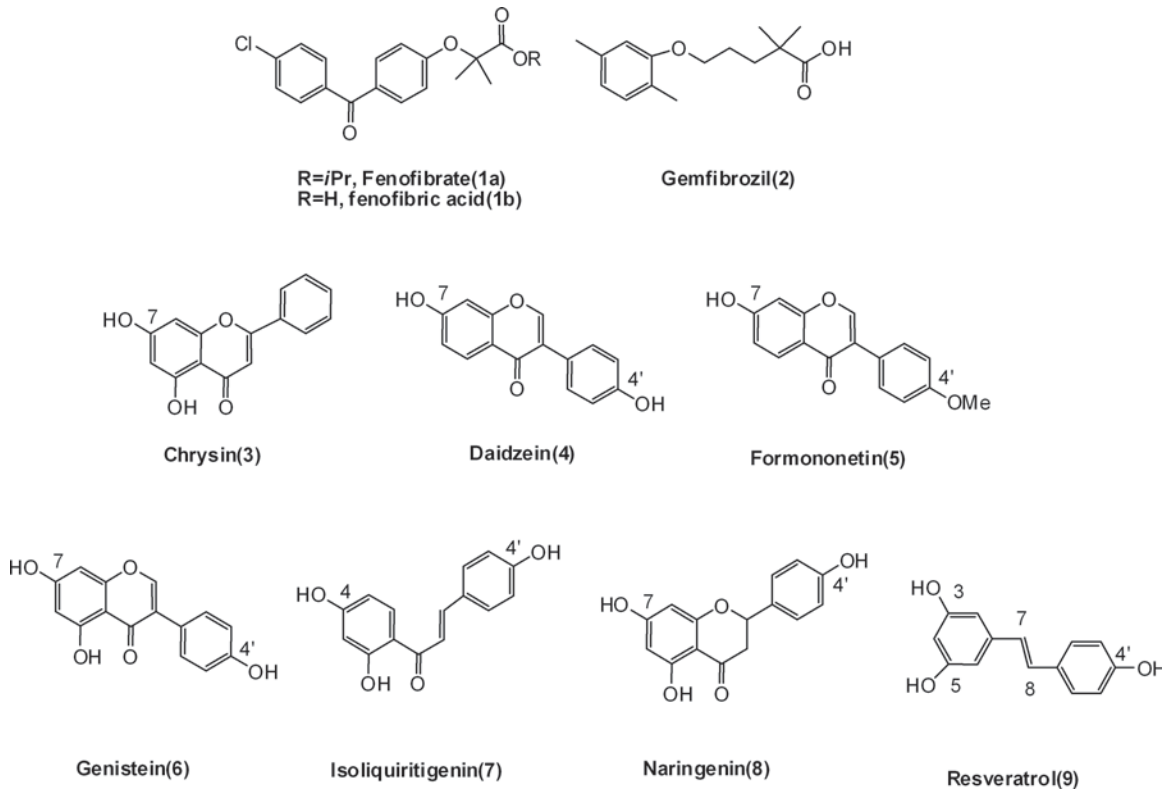


Figure 1. Synthetic triglyceride-lowering drug (fenofibrate, fenofibric acid, and gemfibrozil); naturally occurring flavone (chrysin), isoflavonoids (daidzein, formononetin, genistein), chalcone (isoliquirtigenin), flavanone (naringenin), and resveratrol.

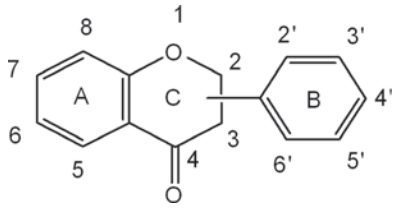


Figure 2. Chemical structures of flavonoids.

silica gel plates with a fluorescence indicator of F254 (0.2 mm, Qingdao Haiyang), and the spots were visualized in UV light. TOF-HRMS spectra were determined on an Agilent 1100 instrument.

The following illustrated the synthesis and characteristics of three representative target compounds (**11**, **17a**, **17b**), and the details of the synthesis of the other compounds, compound characterization data are available in the Supporting Materials.

5-Hydroxy-7-(1-methyl-1-ethoxycarboxylethoxy)flavone (10)

Ethyl 2-bromo-2-methylpropanate (2.21 mL, 15 mmol) was added to a solution of 5,7-dihydroxyflavone (1.90 g, 7.5 mmol) with anhydrous K_2CO_3 (3.14 g, 22.5 mmol) and KI (0.25 g, 1 mmol) in anhydrous dimethylformamide (DMF) (40 mL), and stirring was continued for 12 h at 120°C. The reaction mixture was cooled and filtered. The filtered solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (PE/EtOAc 85:15) to afford **10** (2.07 g, 75% yield) as a yellow solid. Mp. 231–233°C; ^{1}H NMR (DMSO-d_6) δ 1.18 (3H, m, CH_3), 1.66 (6H, m, $2CH_3$), 4.22 (2H, m, CH_2), 6.14 (1H, d, J=2 Hz, Ar-H), 6.55 (1H, d, J=2 Hz, Ar-H), 7.04 (1H, s, Ar-H), 7.60 (3H, m, Ar-H), 8.10 (2H, m, Ar-H), 12.73 (1H, br.s, 5-OH); $C_{21}H_{20}O_6$, MW calcd. 368.38; ESI-MS (MeOH): 369 $[M+H]^+$.

5-Hydroxy-7-(1-methyl-1-carboxylethoxy) flavone (11)

K_2CO_3 (0.41 g, 3.00 mmol) was added to a solution of **10** (0.37 g, 1.00 mmol) in MeOH/H_2O (1:1, 20 mL) and the solution was stirred and refluxed for 12 h. The mixture was concentrated under reduced pressure. The residue was poured into ice water (10 mL), acidified with a 3N HCl aqueous solution to pH 3–4 and extracted with EtOAc (3 × 20 mL). The organic extracts were washed with brine, dried over Na_2SO_4, and filtered. The solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (PE/EtOAc 7:3) to afford **11** (0.22 g, 65% yield) as a white solid. Mp. 264–266°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.65(6H, m, $2CH_3$), 6.17(1H, d, J=2 Hz, Ar-H), 6.51 (1H, d, J=2 Hz, Ar-H), 7.02 (1H, s, Ar-H), 7.57–7.63 (3H, m, Ar-H), 8.09 (2H, m, Ar-H), 12.71 (1H, br.s, 5-OH), 13.21 (1H, br.s, COOH); $C_{19}H_{16}O_6$, MW calcd. 340.33; ESI-MS (MeOH): 341$[M+H]^+$; TOF-HRMS m/z=341.1023 $[M+H]^+$ ($C_{19}H_{17}O_6$ requires 341.1025).

4′-Hydroxy-7-methoxymethoxyisoflavone (14a)

Chloromethyl methyl ether (3.17 mL, 39.4 mmol) was added dropwise to a solution of 4′,7-dihydroxyisoflavone (10 g, 39.4 mmol) with anhydrous K_2CO_3 (4.35 g, 31.5 mmol) in anhydrous acetone (250 mL), and the solution was stirred and refluxed for 12 h. The reaction mixture was filtered and the filtrate was concentrated. The residue was purified by silica gel chromatography (PE/EtOAc 85:15) to afford **14a** (3.2 g, 27.4% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.81 (3H, s, OCH_3), 5.27 (2H, s, OCH_2O), 6.80–6.84 (3H, m, Ar-H), 6.95 (1H, dd, J=9.0, 2.2 Hz, Ar-H), 7.36 (2H, d, J=8.4 Hz, Ar-H), 7.98 (1H, d, J=8.6 Hz, Ar-H), 8.32 (1H, s, Ar-H), 9.50 (1H, s, 4′-OH); $C_{17}H_{14}O_5$, MW calcd. 298.29; ESI-MS (MeOH): 299 $[M+H]^+$.

4′,5-Dihydroxy-7-methoxymethoxyisoflavone (14b)

The titled compound was prepared from 4′,5,7-trihydroxyisoflavone and ethyl 2-bromo-2-methylpropanate according to the method of compound **14a** with the following yield: 44.8 %; yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.78 (3H, s, OCH_3), 5.23 (2H, s, OCH_2O), 6.21 (1H, d, J=2 Hz, Ar-H), 6.39 (1H, d, J=2 Hz, Ar-H), 6.85 (2H, m, Ar-H), 7.49 (2H, m, Ar-H), 8.41 (1H, s, Ar-H), 9.54 (1H, s, 4′-OH), 12.89 (1H, s, 5-OH); $C_{17}H_{14}O_6$, MW calcd. 314.29; ESI-MS (MeOH): 315 $[M+H]^+$.

4′-(1-Methyl-1-ethoxycarboxylethoxy)-7-methoxymethoxyisoflavone (15a)

The titled compound was prepared from **14a** and ethyl 2-bromo-2-methylpropanate according to the method of compound **10** with the following yield: 60%; white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.16 (3H, m, CH_3), 1.64 (6H, s, $2CH_3$), 3.83 (3H, s, OCH_3), 4.25 (2H, m, CH_2), 5.33 (2H, s, OCH_2O), 6.81–6.84 (3H, m, Ar-H), 6.97 (1H, dd, J=9.0, 2.2 Hz, Ar-H), 7.37 (2H, d, J=8.4 Hz, Ar-H), 8.02 (1H, d, J=8.6 Hz, Ar-H), 8.34 (1H, s, Ar-H); $C_{23}H_{24}O_7$, MW calcd. 412.43; ESI-MS (MeOH): 413 $[M+H]^+$.

4′-(1-Methyl-1-ethoxycarboxylethoxy)-5-hydroxy-7-methoxymethoxyisoflavone (15b)

The titled compound was prepared from **14b** and ethyl 2-bromo-2-methylpropanate according to the method of compound **10** with the following yield: 50.3%; yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.17 (3H, m, CH_3), 1.63 (6H, s, $2CH_3$), 4.24 (2H, m, CH_2), 3.78 (3H, s, OCH_3), 5.24 (2H, s, OCH_2O), 6.22 (1H, d, J=2 Hz, Ar-H), 6.40 (1H, d, J=2 Hz, Ar-H), 6.84 (2H, m, Ar-H), 7.45 (2H, m, Ar-H), 8.42 (1H, s, Ar-H), 12.90 (1H, s, 5-OH); $C_{23}H_{24}O_8$, MW calcd. 428.43; ESI-MS (MeOH): 429 $[M+H]^+$.

4′-(1-Methyl-1-ethoxycarboxylethoxy)-7-hydroxyisoflavone (16a)

Six millilitres of 10% aqueous HCl was added to a solution of **15a** (1 g, 2.4 mmol) in MeOH (30 mL) and the mixture was stirred and refluxed for 30 min. The solution was poured onto water (40 mL), filtered, washed with water (100 mL), and dried in vacuum to give **16a** as a white solid (0.75 g, 85% yield) without further purification.

4′-(1-Methyl-1-ethoxycarboxylethoxy)-5,7-dihydroxyisoflavone (16b)

The titled compound was prepared from **15b** according to the method of compound **16a** with 90% yield as a yellow powder without further purification.

4′-(1-Methyl-1-carboxylethoxy)-7-hydroxyisoflavone (17a)

The titled compound was prepared from **16a** according to the method of compound **11** with the following yield: 72 %; white solid; Mp. 246–248°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.54 (6H, m, 2CH$_3$), 6.85–6.88 (3H, m, Ar-H), 6.94 (1H, dd, J=8.8, 2.4 Hz, Ar-H), 7.48 (2H, m, Ar-H), 7.97 (1H, d, J=8.8 Hz, Ar-H), 8.35 (1H, s, Ar-H), 10.80 (1H, s, 7-OH), 13.02 (1H, br.s, COOH); $C_{19}H_{16}O_6$, MW calcd. 340.33; ESI-MS (MeOH): 341 $[M+H]^+$, 363 $[M+Na]^+$; TOF-HRMS m/z=363.0843 $[M+Na]^+$ ($C_{19}H_{16}NaO_6$ requires 363.0845).

4′-(1-Methyl-1-carboxylethoxy)-5,7-dihydroxy isoflavone (17b)

The titled compound was prepared from **16b** according to the method of compound **11** with the following yield: 72%; yellow powder; Mp. 222–224°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.54 (6H, m, 2CH$_3$), 6.23 (1H, d, J=2 Hz, Ar-H), 6.40 (1H, d, J=2 Hz, Ar-H), 6.87 (2H, m, Ar-H), 7.46 (2H, m, Ar-H), 8.39 (1H, s, Ar-H), 10.94 (1H, s, 7-OH), 12.91 (1H, s, 5-OH), 13.14 (1H, s, COOH); $C_{19}H_{16}O_7$, MW calcd. 356.33; ESI-MS (MeOH):357 $[M+H]^+$, 379 $[M+Na]^+$; TOF-HRMS m/z=379.0792 $[M+Na]^+$ ($C_{19}H_{16}NaO_7$ requires 379.0794).

Biology

In vivo *hypolipidaemic activity evaluation in Triton WR-1339-induced hyperlipidaemic mice*

Male Kunming mice (18–22 g) bred in the animal house of the institute were divided into Triton, Triton plus drug-treated groups containing 10 mice in each. Overnight fasted mice of experimental groups were administered suspension of the desired test samples (made in 0.5% CMC-Na) at 300 mg/kg (i.g.) or 100 mg/kg (i.p.) dose. After 30 min, Triton WR-1339 (Sigma, St. Louis, MO) was administered (400 mg/kg body weight) by injecting via tail vein. Blood was withdrawn 22 h later from retro-orbital plexus and serum was separated by centrifugation at low speed and assayed for total cholesterol and triglycerides. The analysis of serum biochemical parameters was performed on **a** Semi-automatic Vital Microlab 300 Biochemistry Analyzer by standard enzymatic methods. The assay kits were purchased from BioSino Biotechnology and Science Inc., Changping, China. Differences between two groups were analysed using unpaired Student's t-test and one-way ANOVA for multiple comparisons. P<0.05 was considered significant.

In vivo *hypolipidaemic activity evaluation in alloxan-induced diabetic mice*

Male Kunming mice (18–22 g) bred in the animal house of the institute were divided into diabetic control, diabetic plus drug-treated groups containing 10 mice in each. Mice of experimental groups were administered suspension of the desired test samples orally (made in 0.5% CMC-Na) at 150 mg/kg dose daily from Day 1 to 3. Animals of control group were given an equal amount of 0.5% CMC-Na. On the second day, after 30 min of administration of overnight fasted mice, alloxan was administered (110 mg/kg body weight) by injecting via tail vein. The blood was collected on Day 4, 48 h after alloxan administration and serum was separated by centrifugation at low speed and assayed for triglycerides and glucose. The analysis of serum biochemical parameters was performed on a Semi-automatic Vital Microlab 300 Biochemistry Analyzer by standard enzymatic methods. The assay kits were purchased from BioSino Biotechnology and Science Inc.. Differences between two groups were analysed using unpaired Student's t-test and one-way ANOVA for multiple comparisons. P<0.05 was considered significant.

Molecular modelling

The crystal structure of PPARα in complex with GW409544 (1K7L) recovered from the Brookhaven Protein Data Bank was used as the target for molecular docking. The docking calculations of compounds with PPARα were performed with the AutoDock Vina1.0 program (Oleg Trott et al., The Scripps Research Institute, La Jolla, CA). These images were generated using the Discovery Studio 2.5 Visualizer program (Accelrys Software Inc.).

Results and discussion

Chemistry

The flavone and isoflavonoid derivatives were synthesized starting with the natural scaffolds, chrysin (5,7-dihydroxyflavone, **3**), daidzein (4′,7-dihydroxyisoflavone, **4**), formononetin (7-hydroxy-4′-methoxyisoflavone, **5**), genistein (4′,5,7-trihydroxyisoflavone, **6**), one common natural flavone and three common natural isoflavonoids. The target compounds were prepared by the alkylation of the phenols with ethyl 2-bromo-2-methylpropanate, and then hydrolysis of the esters as shown in Schemes 1 and 2 and S1 (in Supplementary Materials). As the 7-hydroxyl group is more reactive than 4′-hydroxyl group, the latter could be selectively alkylated following MOM protection of the 7-hydroxyl group. MOM deprotection and then hydrolysis of the esters provided the isoflavonoid derivatives **17a** and **17b**.

The method for the synthesis of chalcone derivatives is shown in S2 (in Supplementary Materials). After aldol condensation and MOM deprotection, the chalcone scaffolds were obtained and the phenols were alkylated with ethyl 2-bromo-2-methylpropanate. Alkaline hydrolysis of the esters gave the target compounds **23a** and **23b**.

Cyclization of **21b** by treatment with sodium acetate and then MOM deprotection gave the flavanone scaffold. The subsequent alkylation and hydrolysis afforded the

target compound **26** as shown in S3 (in Supplementary Materials).

In previous studies, simple groups were introduced at 4′ position and the methoxy groups were kept at the 3 and 5 positions of resveratrol.[38] In our target compounds, the classical fibrate head groups were introduced at the 3, 5, and/or 4′ positions of resveratrol by the alkylation of the phenols with either ethyl 2-bromo-2-methylpropanate or isobutyl 5-chloro-2,2-dimethylvalerate, and the remaining phenolic hydroxyl groups could be methylated by iodomethane or not. The compounds with two or three fibrate head groups were also obtained by the one-pot synthesis method in order to better understand the SAR. Furthermore, as the reactivity of the 4′-hydroxyl group of resveratrol was comparable with the 3-hydroxyl group, the resveratrol-4′-O- and 3-O-monosubstitutes were synthesized by the one-pot synthetic approach. The synthesis routes are shown in S4 and S5 (in Supplementary Materials). All the target compounds above were confirmed by spectroscopy. Like resveratrol, the *trans* configuration of the target compounds were confirmed as the relatively large coupling constant (~16 Hz) between H-7 and H-8.

Biological activity

The ability of the compounds to affect lipid/cholesterol homeostasis was first examined on Triton WR-1339-induced hyperlipidaemia in mice. Table 1 shows the activity of flavonoid derivatives at the dose of 300 mg/kg body weight orally. Out of these eight derivatives, isoflavonoid derivative **17a** and chalcone derivative **23a** were the two most potent compounds. **17a** lowered the triglycerides by 20.2% ($P<0.05$), total cholesterol by 16.6% ($P<0.05$), and **23a** lowered 19.8% ($P<0.05$) in triglycerides, 16.3% ($P<0.05$) in total cholesterol quantity. Moreover, the isoflavonoid derivatives **17a** and **17b** showed more potent than their parent compounds daidzein and genistein, respectively. These results suggested that the enhanced hypolipidaemic activities of **17a** and **17b** can be attributed to the presence of the fibrate head moieties and the modification can lead to increased activity. But none of the synthesized compounds was more potent than the reference drug fenofibric acid (2-[4-(4-chlorobenzoyl) phenoxy]-2-methylpropanoic acid, **1b**), the active metabolite of fenofibrate.

The *in vivo* hypolipidaemic activity of resveratrol derivatives in a Triton WR-1339-induced hyperlipidaemic mice model is shown in Table 2. The synthesized compounds, employing the 2,2-dimethyloxyvaleric acid group, or the oxyisobutyric acid group, showed more potent than the parent compound, resveratrol (**9**) by intraperitoneal injection (100 mg/kg body weight) or orally (300 mg/kg body weight). The reference drug fenofibric acid was the most potent followed by **30b** at the dose of 100 mg/kg when administration by intraperitoneal injection. But at the dose of 300 mg/kg orally, **30b** was identified as the most potent and lowered the triglycerides by 48.5% ($P<0.05$), total cholesterol by 44.2% ($P<0.05$), whereas fenofibric acid lowered the triglycerides by 38.2% ($P<0.05$), total cholesterol by 30.8% ($P<0.05$). Moreover, interestingly, methylation of the remaining phenolic hydroxyl groups led to increased activities: **30a** was more effective than **28a**, and **34** was more effective than **28c**, and **33b** was more effective than **28g**. The compounds, employing the 2,2-dimethyloxyvaleric acid group, showed more potent than the compounds employing the oxyisobutyric acid group at the corresponding positions of the compounds (for instance, **28e** > **28b**, **28g** > **28c**). Furthermore, **34** exhibited more effective than **30a** suggesting the substitution position of the fibrate head group could influence the activity. **28b** bearing two oxyisobutyric acid moieties showed more potent than the compounds **28a** (i.g., 300 mg/kg) and **28c** (i.v., 100 mg/kg) which have only one oxyisobutyric acid moiety in their molecules.

Compounds **28e**, **28f**, **30a**, and **30b** were further tested for their hypolipidaemic activity in an alloxan-induced diabetic mice model at a dose of 150 mg/kg body weight orally. In this experiment, the model mice exhibited hypertriglyceridaemia and hyperglycaemic. These compounds showed good lowering in triglycerides, and **30b** was the most potent among them. **30b** lowered 59.4% ($P<0.01$) in triglycerides, which was slightly more active than fenofibric acid (Table 3). In addition, **28e** (53.5%),

Table 1. *In vivo* hypolipidaemic activity of flavonoid derivatives in Triton WR-1339-induced hyperlipidaemic mice.

Compound	Dose (mg/kg)	TG (mM)	% Reduction, TG	TC (mM)	% Reduction, TC
11	300	15.0 ± 2.0	8.8	16.4 ± 0.9	12.3
12	300	14.4 ± 2.6	12.3	17.0 ± 2.3	9.4
13	300	14.2 ± 3.5	13.7	16.2 ± 3.2	13.4
17a	300	13.2 ± 2.8*	20.2*	15.8 ± 1.1*	15.6*
17b	300	13.6 ± 2.1*	17.6*	15.6 ± 1.3*	16.6*
23a	300	13.2 ± 2.8*	19.8*	15.7 ± 1.2*	16.3*
23b	300	13.3 ± 2.6*	19.0*	16.6 ± 1.4	11.7
26	300	15.4 ± 1.9	6.2	17.8 ± 1.5	4.9
4	300	16.0 ± 2.6	3.0	18.1 ± 0.4	3.7
6	300	16.1 ± 1.7	2.4	18.6 ± 0.3	1.1
1b	300	10.9 ± 2.2*	33.7*	14.7 ± 2.0*	21.6*
Triton model	—	16.5 ± 1.2	—	18.8 ± 2.0	—

*$P<0.05$, compared with model group.

30a (52.6%), and fenofibric acid (54.1%) showed similar triglyceride-lowering activity. Like fenofibric acid, none of the compounds exhibited any blood glucose-lowering activity in this model.

Docking studies

Molecular modelling (docking) studies were carried out to gain an insight into the likely binding interactions between the most potent compound **30b** and PPARα. Our proposed binding pose of **30b** superimposed on the co-crystal structure of GW409544 inside the PPARα ligand-binding domain (PDB:1K7L) is illustrated in Figure 3A. GW409544 is a full PPARα and PPARγ agonist.[39] Docking studies revealed that one of the carboxylate oxygens of **30b** could form a bifurcated H-bond with the Tyr464 OH and the Tyr314 OH groups of the PPARα. The other carboxylate oxygen of **30b** was at H-bonding distance from Ser280 OH. Ser280, Tyr314, Tyr464 are known to be the key interaction residues for the activation of the PPARα.[39] The gem-dimethyl substituents of **30b** were directed into a lipophilic pocket bounded by Phe273, Gln277, Val444, and Leu456, a region at the top end of the so-called "benzophenone" pocket. The remaining two phenyl rings were docked into the hydrophobic pocket formed by the helices 2′, 3, and β sheet, and one methoxy group at the 3 or 5 position of **30b** could produce a hydrogen bond interaction with Tyr334 OH. In *in vivo* studies, **30b** showed good hypolipidaemic activity, so it can be predicted that the activity may be due to the activation of the PPARα. However, unlike compound **30b**, docking of fenofibric acid, the marketed activator of PPARα, showed the two phenyl rings were docked into the hydrophobic pocket formed by the helices 3, 6, and 10 adjacent to the AF-2 helix, also known as "benzophenone" pocket (Figure 3B). These docking models revealed that **30b** may bind to the receptor in a different mode from fenofibric acid.

Table 2. *In vivo* hypolipidaemic activity of resveratrol derivatives in Triton WR-1339-induced hyperlipidaemic mice.

	i.g., 300 mg/kg		i.p., 100 mg/kg	
Compound	% Reduction, TG	% Reduction, TC	% Reduction, TG	% Reduction, TC
28a	21.2*	2.7	ND	ND
28b	31.3*	17.8	43.4*	35.8*
28c	ND	ND	13.9	15.5
28e	35.2*	21.3*	49.7*	38.7*
28f	23.6*	19.2*	26.5*	14.3*
28g	23.0*	22.9*	23.8*	17.9*
30a	26.4*	22.9*	29.1*	29.8*
30b	48.5*	44.2*	50.3*	50.0*
33a	21.8*	24.5*	21.8*	21.4*
33b	35.8*	25.0*	38.4*	25.6*
34	33.9*	33.0*	35.1*	32.1*
1b	38.2*	30.8*	59.6*	46.4*
9	13.9	8.3	16.1	10.4

ND: not detected. *$P < 0.05$, compared with model group.

Table 3. *In vivo* hypolipidaemic activity of compounds in alloxan-induced diabetic mice.

Compound	Dose (mg/kg)	% Reduction, TG	% Reduction, GLU
28e	150	53.5**	14.3
28f	150	35.9*	8.1
30a	150	52.6**	8.9
30b	150	59.4**	10.2
1b	150	54.1**	17.2

*$P < 0.05$, or **$P < 0.01$, compared with model group.

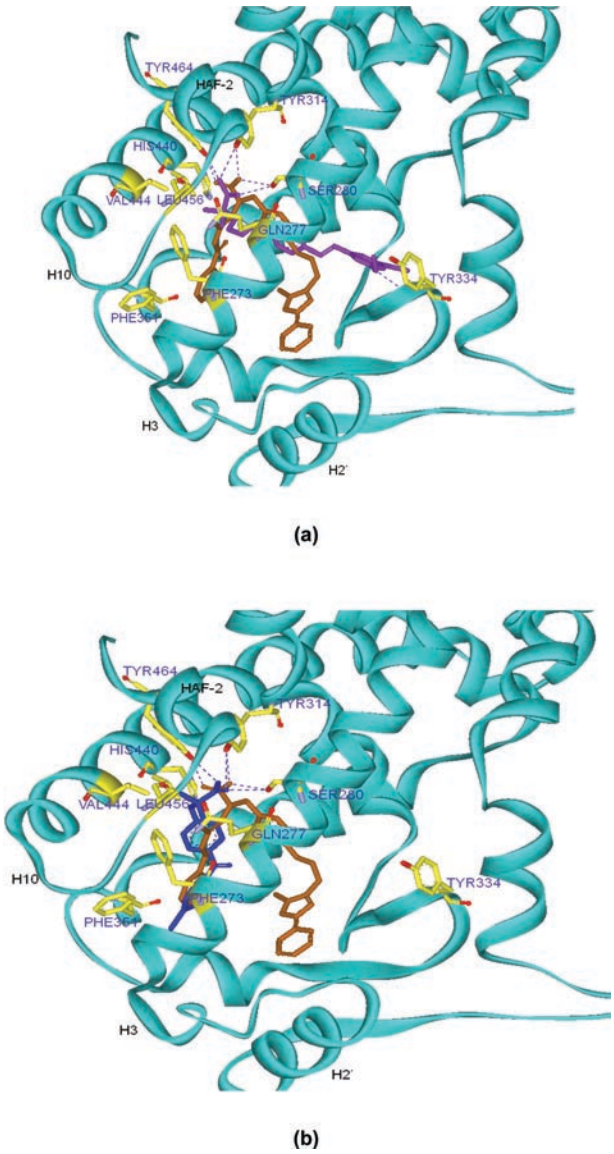
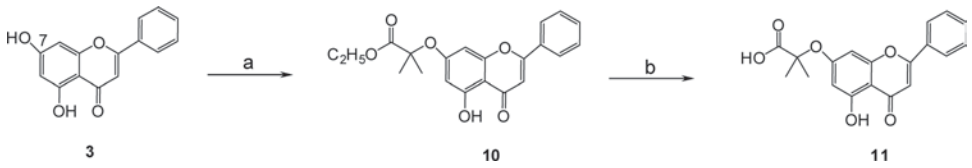
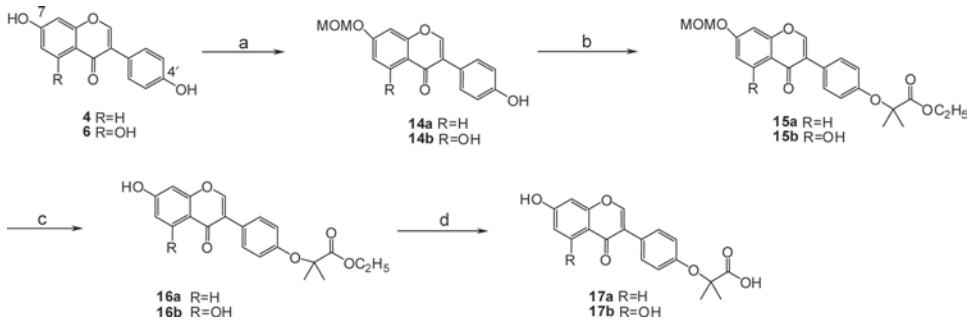


Figure 3. (A) Proposed binding pose of **30b** (purple) superimposed on the co-crystal structure of GW409544 (golden) inside PPARα ligand-binding domain. Blue dotted line represents hydrogen bonding. Hydrogen atoms are not shown for clarity. The protein backbone is shown as cyan ribbon. Important residues are shown in yellow. (B) Proposed binding pose of fenofibric acid (blue) superimposed on the co-crystal structure of GW409544 (golden) inside PPARα ligand-binding domain. Blue dotted line represents hydrogen bonding. Hydrogen atoms are not shown for clarity. The protein backbone is shown as cyan ribbon. Important residues are shown in yellow.



Scheme 1. (a) $BrC(CH_3)_2COOC_2H_5$, K_2CO_3, DMF and (b) (i) K_2CO_3, $MeOH/H_2O$, reflux, (ii) aqueous HCl.



Scheme 2. (a) MOMCl, acetone, K_2CO_3, reflux; (b) $BrC(CH_3)_2COOC_2H_5$, K_2CO_3, DMF; (c) 10% HCl/MeOH, reflux, 0.5 h; and (d) (i) K_2CO_3, $MeOH/H_2O$, reflux, (ii) aqueous HCl.

Conclusions

In conclusion, novel phenoxyalkylcarboxylic acid derivatives based on the natural scaffolds, flavonoids or resveratrol, were prepared and screened for their hypolipidaemic activity *in vivo*. Compound **30b** was the most potent among the synthesized compounds and was orally more effective than the reference drug fenofibric acid in two animal models. Docking studies revealed that compound **30b** could interact with the amino acid residues in the ligand-binding domain essential for the activation of the PPARα and may bind to the receptor in a different mode from fenofibric acid. The results of this study indicate that resveratrol can be used as a better scaffold to derive a new class of hypolipidaemic agents compared with a flavonoid scaffold. Further work is in progress to find out the mechanism of action of **30b** in hypolipidaemic activity, and whether **30b** can indeed activate the PPARα through interactions with the ligand-binding domain of the receptor as predicted by these modelling studies. Moreover, as we mentioned above, there should be further research to find whether the compounds could produce synergistic pharmacological effects by the combination of the pharmacophore moieties and the natural scaffolds. We hope this information might be helpful for the discovery of new hypolipidaemic agents.

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Declaration of interest

The authors report no conflict of interest, and the authors alone are responsible for the content of this article.

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