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Novel coumarin derivatives as potential antidyslipidemic agents

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ABSTRACT

A series of novel benzocoumarin derivatives were synthesized and evaluated for their in vivo antidyslipidemic and in vitro antioxidant activities. Among 11 compounds tested, 2 compounds showed potent antidyslipidemic activity and 3 compounds showed potent antioxidant activity.

Atherosclerotic cardiovascular disease is the leading cause of death in both developed and developing countries. Epidemiological studies have indicated that dyslipidemia and coagulation disturbances are among the most significant risk factors of the development of atherosclerotic condition.[1]

Current therapies mostly focus on lowering LDL-cholesterol and statins (HMG-CoA reductase inhibitors) used for this purpose are pretty effective. However, most patients still experience adverse coronary events despite statin therapy. In addition, recent reports of undesirable side effects (myopathy) of some 'super statins' indicate that the scope of improving the potency of this class of drugs may be modest.[2] Fibrate class (PPARα agonists) of drugs, which are mostly used to treat hyper triglyceridemia and low HDL-cholesterol, requires high doses to show significant efficacy.[3] In addition, a combination of fibrate and statins has met with serious safety concerns as exemplified by the withdrawal of Cerivastatin in 2001. Therefore, there is a constant need for a different class of potent compounds to treat dyslipidemia without severe side effects. Figure 1 shows the chemical structures of some clinically used fibrates.

Hyperlipidemia may also induce other abnormalities like oxidation of free fatty acids, leading to the formation of ketone bodies as well as masking liver and muscles resistance to insulin which initiates the progress of diabetes in patients.[4] Furthermore, in hyperglycemic patients, several non-enzymatic glycosylations occurs accompanied by glucose oxidation catalyzed by Cu^{2+} and Fe^{2+} resulting in the formation of $O_2^{\cdot}$ and $^{\cdot}OH$ radicals which further accelerates the risk of cardiac diseases in dyslipidemic subjects.[5] Therefore, it is envisaged that, beside cholesterol lowering property, a hypolipidemic agent that incorporates antioxidant activity will be able to protect endothelial and myocardial function could serve as a better anti-atherosclerotic agent.

Coumarins are an elite class of oxygen heterocycles, which occupy a special role in nature. Coumarins and their derivatives have attracted intense interest in recent years because of their diverse pharmacological properties.[6–11] In addition many coumarin derivatives have the special ability to scavenge reactive oxygen species (ROS) and to influence processes involving free radical injury.[12] Furthermore, coumarins (umbelliferone) and its derivatives are shown to have lipid lowering potential.[13,14] The coumarins are extremely variable in structure, due to the various types of substitutions in their basic structure, which in turn can influence their biological activity.

Recently, we have reported the synthesis and biological evaluation of benzocoumarin derivatives as potential antioxidant and lipid lowering activity.[15] Our initial studies towards the identification of a new lead for antidyslipidemia indicated compound **3** (1-hydroxy-4-methyl-naphthalene-2-carbaldehyde) as biologically important scaffold (compound **3** significantly lowered the TC, PL and TG by 24%, 25%, 23%, respectively) thus a series of novel derivatives of benzocoumarins from compound **3** were synthesized and all the derivatives were evaluated for their potential lipid lowering activity in vivo and antioxidant activity in vitro.

The Duff reaction on naphthalen-1-ol resulted in expected compound **2** and unexpected compound **3** that was characterized ambiguously by us.[16] As previously revealed compound **3**

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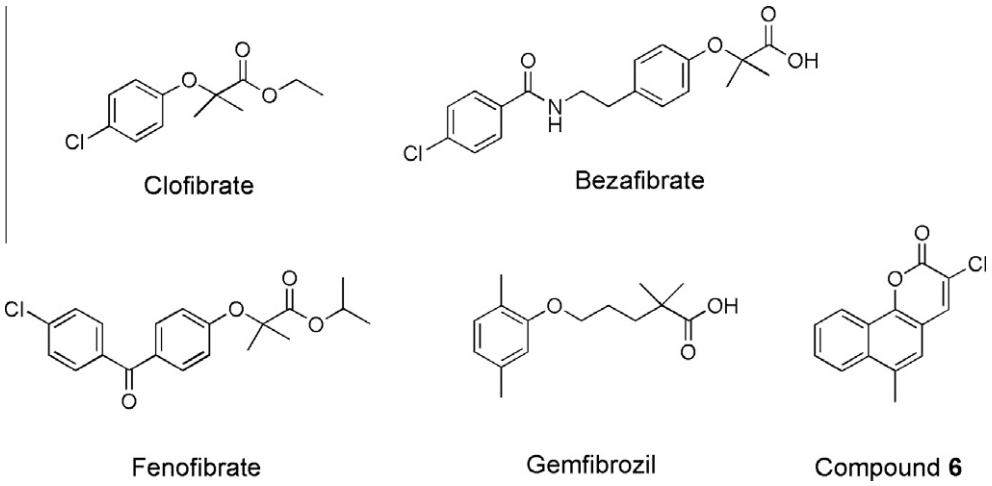


Figure 1. Structures of fibrates and benzocoumarin (compound **6**) as lipid lowering agents.

(1-hydroxy-4-methyl-naphthalene-2-carbaldehyde) exhibited promising antidyslipidemic activity, it prompted us to take the compound **3** as a molecular template and an active pharmacophore for further diversification. The Knoevenagel condensation on **3** that was catalyzed by piperidine resulted in the formation of its benzocoumarin derivatives (**4**–**8**).[17] Furthermore, compound **8** on alkaline hydrolysis furnished compound **9**, which on its esterification resulted in compounds **10**–**13** (Scheme 1). All the compounds were characterized using ^{1}H NMR, ^{13}C NMR, Mass and IR spectroscopy. The purity of these compounds was ascertained by TLC and spectral analysis (see Supplementary data).

The antidyslipidemic and post heparin lipolytic activity of benzocoumarin derivatives **4**–**13** were evaluated in an in vivo Triton model.[18–20] Administration of Triton WR-1339 in rats induced marked hyperlipidemia as evidenced by increase in the plasma level of total cholesterol TC (4.05-fold), phospholipids PL (3.31-fold) and triglyceride TG (2.67-fold) as compared to control. Triton induced rats caused inhibition of post heparin lipolytic activity plasma PHLA (−28%) as compared to control.[21] Treatment of hyperlipidemic rats with benzocoumarin derivatives at the dose of 100 mg/kg po reversed the plasma levels of lipid with varying extents.[22]

The synthesized derivatives inhibited cholesterol biosynthesis and potentiated the activity of lipolytic enzymes to early clearance of lipids from circulation in triton—induced hyperlipidemia. Compound **3** significantly lowered the TC, PL and TG by 24%, 25%, 23% and however, the compound **6** (the benzocoumarin derivative of **3** having a chlorine at position 3) was the most potent in the series as it showed 27%, 26% and 26% lowering in TC, PL and TG, respectively, while compounds **4**, **5, 7**–**13** showed mild activity (Fig. 2). These data are comparable with standard drug Gemfibrozil at the dose of 100 mg/kg which decreased level of TC, PL and TG in plasma by 34%, 38% and 35%, respectively. In PHLA enzyme activity, again compound **3** and **6** showed significant reversal of PHLA in plasma of hyperlipidemic rats by 26% and 25%, respectively, comparable to Gemfibrozil, which caused 28% reversal of activity of this enzyme as compared to control group.

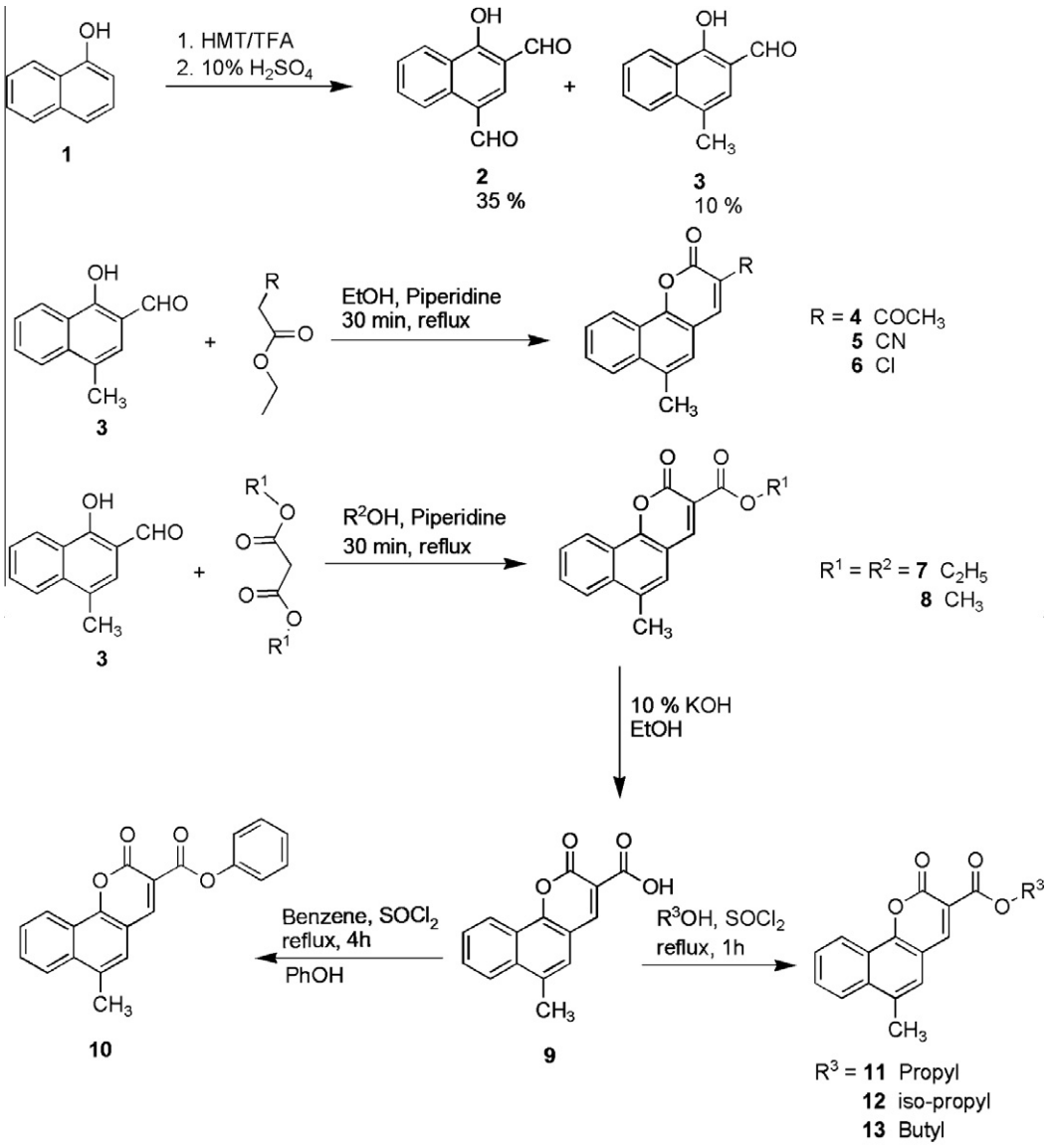
In another experiment, antioxidant activities of compounds **3**–**13** were evaluated by generating free radicals in vitro in the absence and presence of these compounds.[23] The scavenging potential of benzocoumarins **3**–**13** at 200 μg/mL against formation of $O_2^{\cdot}$ and ·OH in non-enzymic systems were studied. Further, their effect on lipid peroxidation in microsomes were also studied (Fig. 3). Compounds **3** and **6** showed significant decrease in superoxide anions inhibition by 27% and 32%, hydroxyl radicals inhibition by 41% and 49% and microsomal lipid peroxidation inhibition by 41% and 38%, respectively. The standard drug Alloperinol at 20 μg/mL showed 80% inhibition in superoxide anions. Manitol and α-Tocopherol at the dose of 100 μg/ml showed 52% and 47% inhibition of hydroxyl ions and microsomal lipid peroxidation, respectively. The scavenging potential of other derivatives were modest. The wide variation in the free radical scavenging potential may be due to the variation in the proton-electron transfer by the derivatives due to difference in their structures and stability.

The frequently prescribed hypolipidemic agent, Gemfibrozil having a 5-phenoxypentanoic acid moiety instead of the fibric acid moiety is also considered a fibrate because of having almost similar pharmacological properties as the other classical fibrates.[24] The fibrates primary mode of action is to selectively activate the α-isotype of the receptors peroxisome proliferator-activated receptors (PPARs).[25] Though efforts are ongoing to delineate the precise mechanism of action of the synthesized compounds, we believe it to be acting on the same target as Gemfibrozil.

In terms of SAR, the minimum structural requirement for binding of the derivatives to the target includes an aromatic or heteroaromatic ring (ring B, C), which is believed to participate in Π–Π stacking with aromatic amino acids residue of the receptor. Substituents in ring B have varied effect on binding. It is generally true, however that an electron releasing group (–CH_3) substituted at the 7 position as in compound **3** markedly increases the antidyslipidemic activity, as in the compound **2** having electron withdrawing group (–CHO) was found to be inactive. In the ring A neither the acid or ester group at position 3 is required for in vivo dyslipidemic activity. Though the electron withdrawing group at position 3 is desired, the lack of activity for compound **5** having –CN group might be due to its conversion back to –C=N. Substitution of Cl for CN increases the activity which we believe is due to the better selectivity.

Compound **6** exhibited interesting most promising activity in both lipid lowering and antioxidant experiments. Initial studies indicate compound **6** to be devoid of cytotoxicity in normal cells. Further studies on **6** including dose optimization are in progress and will be published in the future.

In conclusion, a series of novel substituted benzocoumarin derivatives (**4**–**13**) have been synthesized from compound **3** (1-hydroxy-4-methyl-naphthalene-2-carbaldehyde) by Knoevenagel condensation. Among the synthesized compounds, compound **6** was found to be the most active hypolipidemic agent in addition to having potent antioxidant and thus represent a new class of antidyslipidemic agents.



Scheme 1. Synthesis of novel benzocoumarin derivatives **4**–**13**.

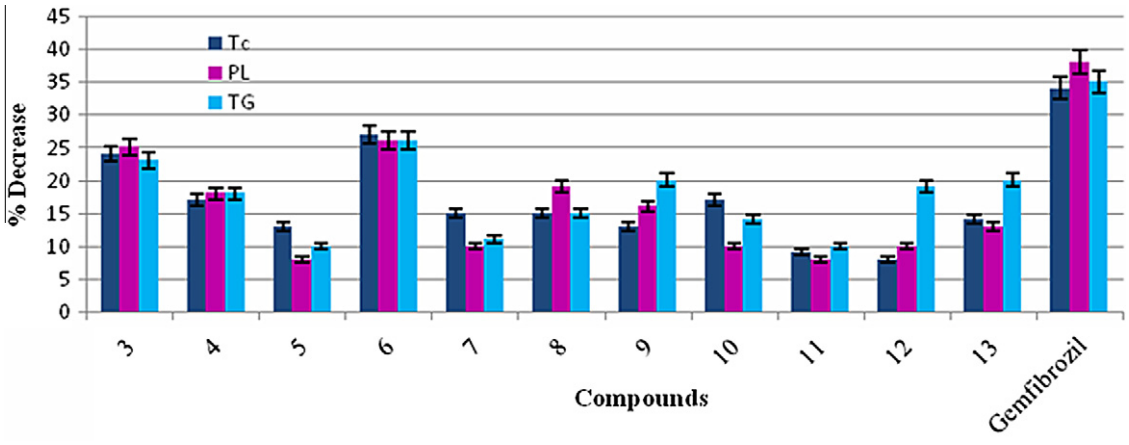


Figure 2. The lipid lowering activity of novel benzocoumarin derivatives (100 mg/kg) in Triton treated hyperlipidemic rats. Triton treated group is compared with control and drug treated group is compared with triton group (units—mg/dL). Values are mean ± SD of six animals.

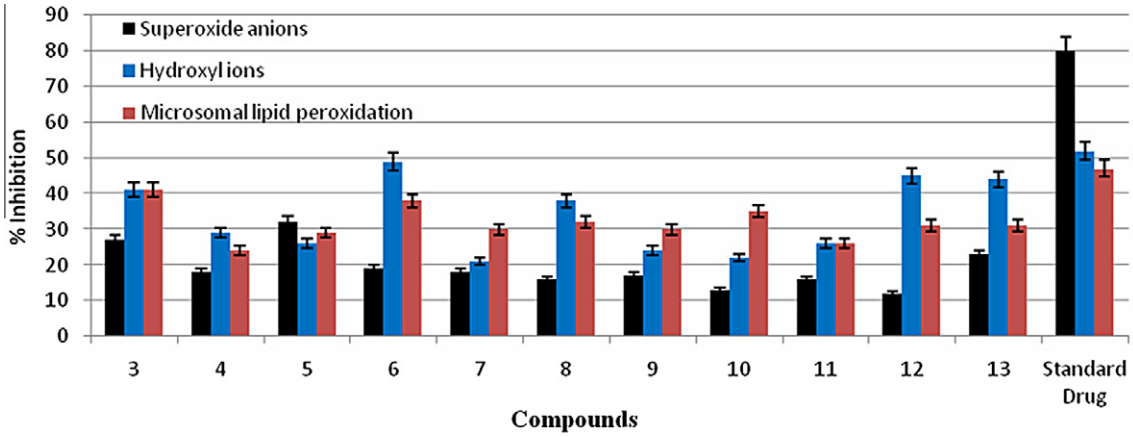


Figure 3. The effect of novel benzocoumarin derivatives (200 μg/mL) on superoxide ion (nmol formazone formed/min), hydroxyl ion (nmol MDA formed/h) and lipid peroxidation in microsomes (nmol MDA formed/mg protein) is shown (standard drugs for superoxide anions-Alloperinol (20 μg/mL), hydroxyl ions-Manitol and for microsomal lipid peroxidation-α-tocopherol (100 μg/mL) were used). Values are mean ± SD of six animals.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.023.

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17. Representative procedure for the synthesis of compound **4** (3-acetyl-6-methyl-2*H*-benzo[*h*]chromen-2-one):
To an equivalent mixture of 1-hydroxy-4-methyl-naphthalene-2-carbaldehyde **3** (100 mg, 0.54 mmol) and ethylacetoacetate (70 mg, 0.54 mmol) were dissolved in ethanol (20 mL) and to this piperidine (0.1 mL) was added and the reaction mixture was refluxed for 30 min. After completion of the reaction, ethanol was removed through high vacuo. The residue was neutralized with acetic acid, diluted with water and extracted with $CHCl_3$ (75 mL × 3). The organic layer was washed with water, brine, and dried over anhydrous Na_2SO_4 and concentrated through high vacuum. The crude product thus obtained was purified by column chromatography (60–120 mesh) to furnish (147 mg, 92% yield) of pure **4** (3-acetyl-6-methyl-2*H*-benzo[*h*]chromen-2-one) as light yellow solid.
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22. Lipid lowering activity: Adult male Charles Foster rats (200–225 g) bred in the animal house of the institute were used for the lipid lowering activity. Rats were divided in control, triton induced, triton plus compounds and Gemfibrozil (100 mg/kg) treated groups containing six rats in each. Hyperlipidemia was developed by administration of Triton WR-1339 (Sigma chemical Co., St. Louis, USA) at a dose of 400 mg/kg body wt. intraperitoneally to animals of all groups except the control. Compounds **3–13** were macerated with gum acacia (0.2% w/v), suspended in water and fed simultaneously with triton at a dose of 100 mg/kg po to the animals of treated groups. Animals of the control and triton group without treatment with test compounds were given same amount of gum acacia suspension (vehicle). After 18 h of treatment (50 mg/kg b. wt.) 1.0 mL blood was withdrawn from retro-orbital sinus using glass capillary in EDTA coated eppendorf tube (3.0 mg/mL blood). The blood was centrifuged (at 2500*g*) at 4 °C for 10 min and the plasma was separated. Plasma was diluted with normal saline (ratio 1:3) and used for analysis of total cholesterol (TC), phospholipids (PL), triglycerides (Tg) and post heparin lipolytic activity (PHLA) using spectrophotometer, Beckmann auto-analyzer and standard kits purchased from Beckmann Coulter International, USA.
23. Antioxidant activity (generation of free radicals): Super oxide anions were generated enzymatically by xanthine (160 mM), xanthine oxidase (0.04 U), and nitroblue tetrazolium (320 μM) in absence or presence of compounds **3–13** (100 μg/mL) in 100 mM phosphate buffer (pH 8.2). Fractions were sonicated well in phosphate buffer before use. The reaction mixtures were incubated at 37 °C and after 30 min the reaction was stopped by adding 0.5 mL glacial acetic acid. The amount of formazone formed was calculated spectrophotometrically. In another set of experiment effect of compounds on the generation of hydroxyl radical was also studied by non-enzymatic reactants. Briefly, OH^- were generated in a non-enzymatic system comprising deoxy ribose (2.8 mM), $FeSO_4{\cdot}7H_2O$ (2 mM), sodium ascorbate (2.0 mM) and H_2O_2 (2.8 mM) in 50 mM KH_2PO_4 buffer (pH 7.4) to a final volume of 2.5 mL. The above reaction mixtures in the absence or presence of test compounds (200 μg/mL) were incubated at 37 °C for 90 min. The test compounds were also studied for their inhibitory action against microsomal lipid peroxidation in vitro by non-enzymatic inducer. Reference tubes and reagents blanks were also run simultaneously. Malondialdehyde (MDA) contents in both experimental and reference tubes were estimated spectrophotometrically by thiobarbituric acid (Okhawa, H.; Ohishi, N.; Yagi, K. *Anal. Biochem.* **1978**, *95*, 351). Alloprinol, Mannitol and α-tocopherol were used as standard drugs for superoxide, hydroxylations and microsomal lipid peroxidation. All experimental data were analyzed using Student's *t*-test. Oxidized LDL was compared with the test compounds treated oxidized LDL. The generation of oxygen free radicals was compared in the presence and absence of test compounds. The hyperlipidemic group was compared with control and hyperlipidemic plus drug treated groups $P < 0.05$ was considered to be significant.
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