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Discovery and synthesis of novel substituted benzocoumarins as orally active lipid modulating agents ☆

Koneni V. Sashidhara [a,*], Manoj Kumar [a], Ram K. Modukuri [a], Anuj Srivastava [b], Anju Puri [b]

[a] Medicinal and Process Chemistry Division, Central Drug Research Institute (CSIR-CDRI), Lucknow 226 001, India
[b] Biochemistry Division, Central Drug Research Institute (CSIR-CDRI), Lucknow 226 001, India

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ABSTRACT

The synthesis of a series of benzocoumarin keto-enamine schiff bases is reported. The novel compounds were evaluated for their antihyperlipidemic activity in the hyperlipidemic hamster model. The compound **11** at a dose of 10 mg/kg body weight significantly lowered the plasma triglyceride levels (TG) by 70%, total cholesterol (TC) by 47%, accompanied by an increase in HDL-C/TC ratio by 80% in hyperlipidemic hamsters to a greater degree than the reference drugs atorvastatin and lovastatin.

Dyslipidemia and coagulation disturbances are among the most significant risk factors of the development of atherosclerotic condition.[1] Elevated plasma concentration of cholesterol, especially low-density lipoprotein (LDL) and triglyceride is recognized as a leading cause in the development of atherosclerosis and coronary heart disease.[2] Furthermore, hypertriglyceridemia is the most frequent form of hyperlipidemia observed in type 2 diabetes, because it co-exists with hyperinsulinemia in the general population.

Statins and fibrate class of drugs are the most widely used candidates for treatment of dyslipidemia. Statins (HMG-CoA reductase inhibitors) used for lowering LDL-cholesterol are pretty effective. However, most patients still experience adverse coronary events despite statin therapy. Furthermore, current reports of unwanted side effects (myopathy) of some 'super statins' point out that the scope of improving the potency of this class of drugs may be modest.[3] The fibrate class (PPARα agonists) of drugs, which are mostly used to treat hypertriglyceridemia and low HDL-cholesterol, requires high doses to show significant efficacy.[4] Additionally, 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.

In continuation of our efforts to explore the chemical diversity space around coumarin scaffold as antidyslipidemic agents, previously we have synthesized 3 and 4 substituted coumarins and some of which have shown good lipid lowering profile in comparison to standard drug.[5] That study revealed that, such attempts on benzocoumarins based pharmacophores which is a biologically important scaffold might result in identification of new lead for antidyslipidemia.

Furthermore, in the synthesis of a series of keto-enamine schiff bases of benzocoumarin and its evaluation as potential lipid lowering agents, indicated that the keto-enamine side chain is crucial for activity.[6] However, it remained unclear as to what substituents would be desirable to enhance the activity. To answer this question, we synthesized derivatives with different side chain modifications, that took into account the homologation, chain branching, ring chain transformed analogs, etc. and studying the impact of such modification on the hypolipidemic activity. This study underscores the importance of the side chain modification on coumarin scaffold that seem to modulate the activity dramatically. In this communication, we describe the details of this study. A graphical representation of the evolution of our work on coumarins resulting in the current series of molecules is depicted in Figure 1.

The synthetic strategy started with Pechmann reaction between naphthalene 1,5-diol **1**, and ethyl acetoacetate to give 7-hydroxy-4-methyl-benzo(h)chromene-2-one **2**. The coumarin **2** was subjected to the Duff formylation to furnish 7-hydroxyl-4-methyl-2-oxo-2H-benzo(h)chromene-8,10 dicarbaldehyde **3**. This dicarbaldehyde was then condensed with various primary amines to give final compounds (**4–13**).[7] The detailed synthetic methodology of which has been previously reported by us and the reaction conditions are outline in Scheme 1. The structures of all the novel compounds were

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* Corresponding author. Tel.: +91 9919317940; fax: +91 522 2623405.
E-mail addresses: kv_sashidhara@cdri.res.in, sashidhar123@gmail.com (K.V. Sashidhara).

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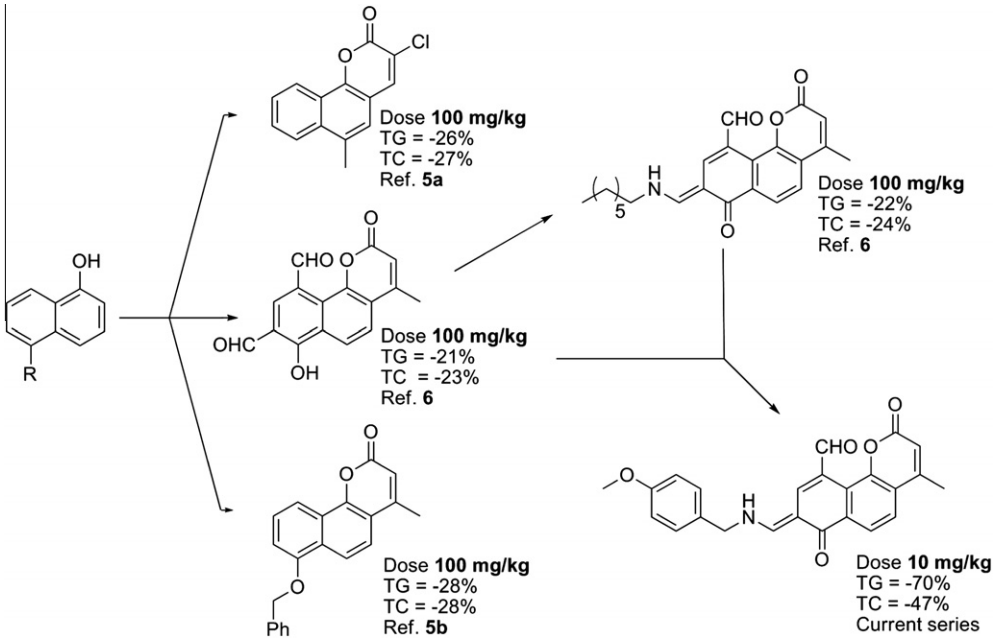
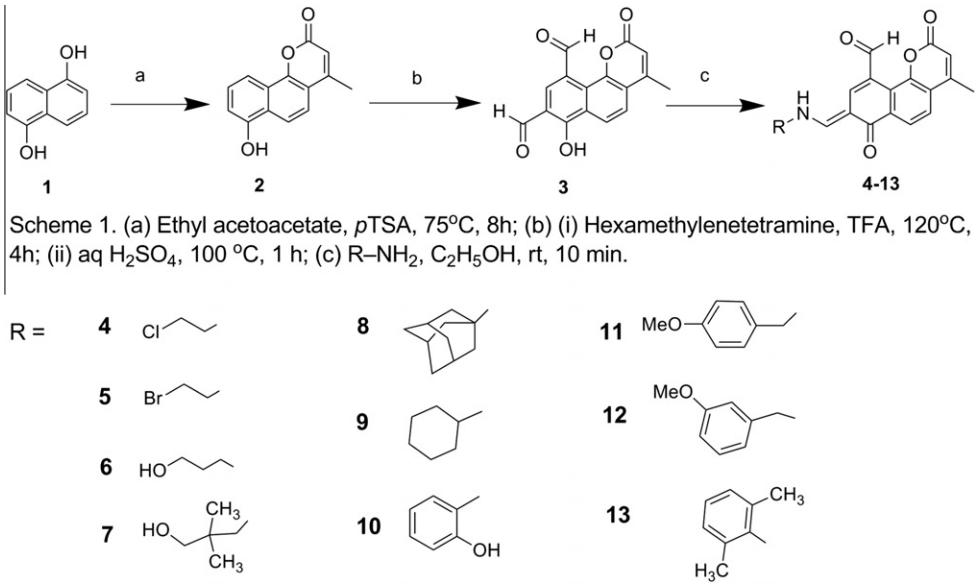


Figure 1. Graphical depiction of the evolution of our work on coumarins leading to the current series of substituted coumarins.



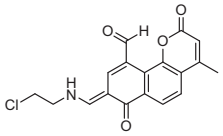
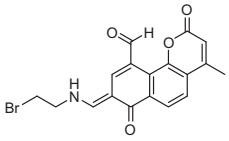
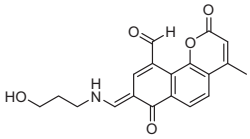
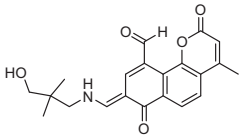
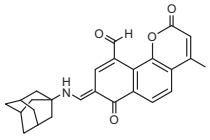
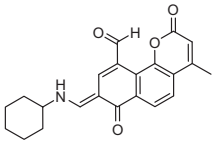
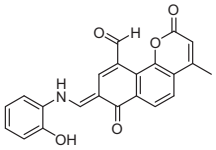
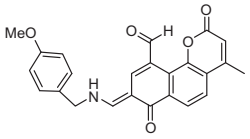
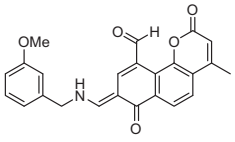
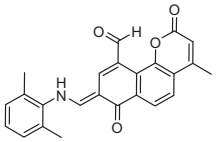
Scheme 1. Synthesis of benzocoumarins (**4**–**13**).

confirmed by NMR and Mass Spectrometry (see Supplementary data).

In the present study, we have carried out experiments to investigate the antidyslipidemic activity of benzocoumarins (**4–13**) in the high fat diet (HFD) fed dyslipidemic hamster model,[8] which has been reported as an ideal in vivo model for evaluating antidyslipidemic drugs. The development of dyslipidemic hamster model is based on diet induced dyslipidemia in normal animals with an intact metabolism. The high fat diet (HFD) in combination with fructose is found to give rapidly induced dyslipidemia with hyper-cholesteremia and hyper triglyceridemia.[9,10] In the high fat diet (HFD) fed dyslipidemic hamsters, the administration of benzocoumarins caused a distinct decrease in the serum triglyceride, total cholesterol, LDL and increase in HDL and HDL-C/TC ratio (Table 1). No significant differences were observed in the food intake and weight gain between the groups (data not shown). As such, the compounds (**4–13**) supplement did not apparently adversely affect the hamsters. All the compounds were administered orally at the dose of 10 mg/kg body weight for seven consecutive days. Normal hamsters fed with HFD and given drug vehicle (gum acacia) only served as control animals.

The role of amino alkane is important in metabolic disorders like diabetes and dyslipidemia.[11] In our current series diversification with alkane amine side chain shown interesting biological profile with desired parameters. The analysis of the lipid profile data (TC, TG, HDL, LDL, and H/C) of different groups clearly suggests that most of the synthesized compounds exerted excellent hypolipidemic activity. The most promising compound in the series was

Table 1
Percentage (%) decrease/increase of plasma lipids with the treatment of compound (**4**–**13**) in dyslipidemic hamsters at the dose of 10 mg/kg body weight

Compounds	Structures	TG	TC	HDL	LDL	H/C
4		−47***	−17	+19	−14	+9
5		−40*	−13	−1	−10	+15
6		−50***	−26	+3	−21	+39
7		−49***	−36*	+1	−36	+61
8		−48***	−48***	+1	−59***	+93
9		−62***	−14	+1	−10	+17
10		−42***	−3	+15	+12	+19
11		−70***	−47***	+1	−54*	+80
12		−52***	−47***	+3	−59***	+93
13		−16	−30*	+1	−40***	+43
	Atorvastatin	−67***	−25	+2	−23	+83
	Lovastatin[a]	−29	−9	+3	−11	+12

Values represented are % change with respect to HFD-fed hamster group (group of eight animals).
H/C = HDL/TC.
[a] Lovastatin at the dose of 25 mg/kg body weight.
* $p < 0.05$.
*** $p < 0.001$.

the compound **11** which at a oral dose of 10 mg/kg body weight significantly lowered the plasma triglyceride levels (TG) by 70%, total cholesterol (TC) by 47%, low density lipoprotein-cholesterol (LDL-C) by 54% and increased HDL-C/TC ratio by 80%, in comparison to atorvastatin at the same dose lowered TG by 67%, TC by 25%, LDL-C by 23% and increased HDL-C/TC ratio by 83%, while lovastatin at the

Table 2
Antioxidant potential of compounds (**4–13**) using the TEAC assays

Compounds	TEAC value (mM)
4	0.38
5	0.10
6	1.46
7	1.90
8	0.99
9	0.30
10	0.36
11	1.36
12	1.26
13	0.99
Ascorbic acid	3.13

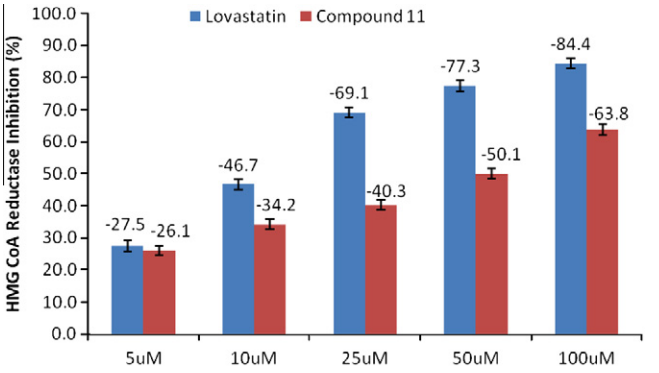


Figure 3. In vitro dose response study of compound **11** and lovastatin for HMG-CoA inhibitory activity.

higher dose of 25 mg/kg body weight in the same model, decreased the level of TG by 29%, total cholesterol by 9% and increase in HDL-C/TC ratio by 12%. Additionally compounds **7**, **8**, and **12** lowered TG by 49%, 48%, and 52%, TC by 36%, 48%, and 47%, LDL by 36%, 59%, and 59% increased the HDL-C/TC ratio by 61%, 93%, and 93% respectively, which is considered a beneficiary effect in the treatment of dyslipidemia condition. The reduction of the level of LDL-C by the compounds **8**, **11**, and **12** by 59%, 54%, and 59% respectively is an interesting finding because the LDL fraction is generally thought to carry cholesterol to the tissues and is responsible for the artherogenesis process.[12] The higher TG levels and lower the HDL-C increase the risk of coronary heart disease. In latter case, the high density lipoproteins (HDL) mediate the reverse transport of cholesterol from peripheral tissues to the liver, which will disallow the slow accumulation of lipids in artery walls. The compound **11** exhibited both the above beneficiary effects as it has decreased the TG by 70% and increased the HDL-C/TC ratio by 80% at a dose of 10 mg/kg body weight. Furthermore, these synthesized compounds were also evaluated for their antioxidant potential using TEAC assay.[13] Antioxidant activities of benzocoumarins in comparison to ascorbic acid are given in Table 2. Oxidative stress is one of the causative factors that link hypercholesterolemia with atherogenesis. However, the antioxidant potential of the synthesized compound was found to be modest.

Structure–activity relationship reveals that incorporation of halogen atom (like compounds **4** and **5**) to benzocoumarin showed mild protection. Whereas, increased lipophilicity as in compound **8** with adamantyl group proved admirable activity. But most interesting results were obtained in benzyl amine substituted derivatives as compound **11** with 4-methoxy benzyl amine and compound **12** with 3-methoxy benzyl amine group showed better activity than reference drug atorvastatin and lovastatin.

With these promising activities in hand, dose dependent (different doses 2.5, 5, 10, and 25 mg/kg body weight) study on the compound **11** was performed on a different set of experimental animals which exhibited concentration dependent effects (Fig. 2). To investigate the mechanistic pathway of the synthesized benzocoumarins, we have carried out HMG-CoA inhibitory activity[8] of compound **11** in comparison to standard drug lovastatin. The inhibitory activity was evaluated at five different concentrations ranging from 5 to 100 μM (Fig. 3). Compound **11** showed 26.10% inhibition of HMG-CoA reductase at 5 μM concentration which was similar to lovastatin, thus suggesting the plasma lipid lowering action of the compound **11** seemed partly due to the inhibition of HMG-CoA reductase. Somewhat parallel results were obtained at further higher concentrations of 10, 25, 50, and 100 μM. Maximum inhibition of 63.80% was shown by **11** at 100 μM concentration. However, more extensive investigation of compound **11** is still required to further elucidate the mechanism and usefulness of this synthetic derivative in different animal models and humans.

In conclusion, novel substituted benzocoumarins derivatives were prepared and their hypolipidemic activity was screened in hyperlipidemic hamsters. Among the synthesized compounds, the compound **11** was found to be most active hypolipidemic agent affording significant hypocholesterolemic and hypotriglyceridemic

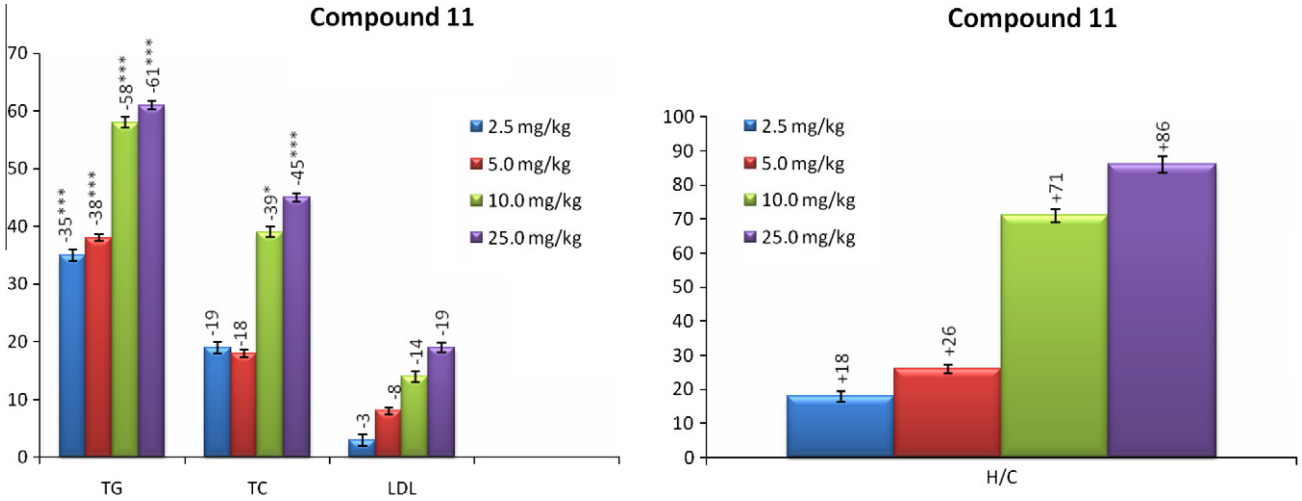


Figure 2. In vivo dose response study of compound **11** on TG, TC, LDL, and HDL-C/TC of hamster model.

activities and seems to be a good candidate for developing a new lead with good hypolipidemic and antiatherosclerotic benefits. Further studies on compound **11** are under progress to advance it as a novel antidyslipidemic lead compound.

Acknowledgments

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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.2011.09.053.

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7. *Synthetic procedure and spectral data of 8-((1-adamantaneamino)methylene)-4-methyl-2,7-dioxo-7,8-dihydro-2H-benzo[h]chromene-10-carbaldehyde* (**8**): To an equivalent mixture of 3 and 1-adamantanamine, absolute ethanol (10 ml) was added and stirred at room temperature. After completion of the reaction solvent was evaporated and the residue was washed with hexane to afford compound **8** in 87% yield.
Yellow solid, yield: 87%; mp 265–266 °C; IR (KBr): 3411, 1713, 1620, 1501, 1315 cm^{-1}, ^1H NMR (CDCl3 300 MHz) δ 13.76 (br s, 1H, —NH), 11.13 (s, 1H, —CHO), 8.42 (d, *J* = 8.6 Hz, 1H), 8.14–8.08 (m, 2H), 7.62 (d, J = 8.6 Hz, 1H), 6.44 (br s, 1H), 2.56 (br s, 3H), 2.30 (s, 3H), 2.03 (br s, 6H), 1.86–1.73 (m, 6H), ^{13}C NMR (CDCl3, 75 MHz) δ 191.5, 179.1, 159.8, 159.2, 153, 137.3, 132.9, 124.6, 121.9, 120.9, 120.1, 119.9, 115.7, 108.7, 56.1, 42.6, 35.6, 29.1, 19.5, ESI-MS: (*m*/*z*): 416 $(M+H)^+$.
8. *Animals:* Golden Syrian hamsters (*Mesocricetus auratus*), male, 12 week old, wt 110 ± 10 g were used. Animals were kept in a room-controlled temperature at 25–26 °C, relative humidity 60–80% and 12:12 h light/dark cycle light (on from 8.00 AM to 8.00 PM) under hygienic conditions. The animal had free access to the diet and water ad libitum. Experimental protocols were approved by our institutional ethical committee, which follows guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), which complies with international norms of INSA.
Antidyslipidemic activity in high fat diet fed hamster model: Feeding with high fat diet- (HFD) developed hyperlipidemia in hamster. Hamsters were divided into – HFD fed, HFD fed, and benzocoumarins treated group containing eight animals in each group. High fat diet was given daily from day 1 to 10 to animals of all the groups. Hamsters were treated with benzocoumarins orally at a dose of 10 mg/kg body weight once a day for seven consecutive days, from day 4 to 10. HFD fed animals treated with vehicle (0.1% gum acacia) served as controls. Body weight of animals was recorded daily.
Collection of blood samples and biochemical analysis from plasma: After the last day of treatment blood was collected in EDTA coated tubes from the retro orbital plexus of the hamsters. The samples were centrifuged at 4000 rpm for 5 min and plasma was separated. The plasma samples were used for the assay of total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL)-cholesterol (HDL-C) and low-density lipoprotein (LDL)-cholesterol by standard enzymatic methods using Synchron CX-5 Clinical System Beckmann Coulter auto analyzer. All assay kits were purchased from Beckman Coulter International, USA.
Statistical analysis: Biological data were analyzed using student's t-test on Graph Pad Prism ver 3.02 data templates. Significance (*p* values) is calculated using one-way analysis of variance of ANOVA program. Value $p < 0.05$ was considered as significant.
HMG-CoA reductase assay: The HMG-CoA reductase assay was performed using the HMG-CoA reductase assay kit from Sigmae–Aldrich. HMG-CoA (substrate), NADPH, assay buffer and enzyme HMGR were supplied with the assay kit.
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