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ARTICLE TYPE

Hybrids of coumarin-indole: Design, synthesis and biological evaluation in triton WR-1339 and high-fat diet induced hyperlipidemic rat models†

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In this study, a series of coumarin-indole hybrids have been synthesized and evaluated for their lipid lowering activity. Preliminary biological screening of synthesized compounds was undertaken in an *in vitro* model of HMG-CoA reductase enzyme and the activity was confirmed in Triton WR-1339 induced hyperlipidemic rats. Among the hybrids, compound **26** was found to be the most persuasive as it significantly reduced the serum and hepatic lipid profile in an HFD-fed hyperlipidemic rat model. The mechanism of action seems to be associated with the regulation of HMG-CoA reductase activity in liver, which is in good agreement with its binding mode studies. Compound **26** exhibited favorable pharmacokinetic behavior for its oral administration, which underscores the potential of this template as a new class of hypolipidemic agents.

Introduction

Hyperlipidemia is a group of disorders that involve elevated levels of lipids in the blood, including serum cholesterol and triglycerides. Epidemiological evidence indicates that hyperlipidemia is the most important risk factors for the progression of cardiovascular diseases, including coronary heart disease.[1] Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is a key enzyme, which plays central role in the cholesterol biosynthesis. Inhibitors of HMG-CoA reductase induce the expression of low density lipoprotein (LDL) receptors in the liver and the subsequent clearance of LDL particles from the bloodstream.[2] Current therapies for the treatment of hyperlipidemia and mixed dyslipidemia include statins,fibrates, niacin, bile acid resins, and ezetimibe.[3,5]

.Statins, which are HMG-CoA reductase inhibitors, are widely prescribed for lowering low density lipoprotein cholesterol (LDL-C) levels and reducing cardiovascular event rates.[6]However, they are associated with serious drawbacks like myalgia, muscle cramp, and rhabdomyolysis.[7] The fibrate drugs are used clinically to reduce the level of triglycerides (TG), free fatty acid (FFA) levels and they enhance high-density lipoprotein cholesterol (HDL-C).[8] However, fibrates are known to cause myopathy and increase creatine kinase activity.[9] Statins and fibrate class of drugs have played a major role in the treatment of dyslipidemia for the past two decades. However, a combination of fibrate and statins has met with the potential increased risk for myopathy, which may occasionally be accompanied by rhabdomyolysis and renal failure.[10] Therefore, there is a urgent need of the development of new class of lipid lowering agents which could lower the elevated serum cholesterol levels without causing side effects.

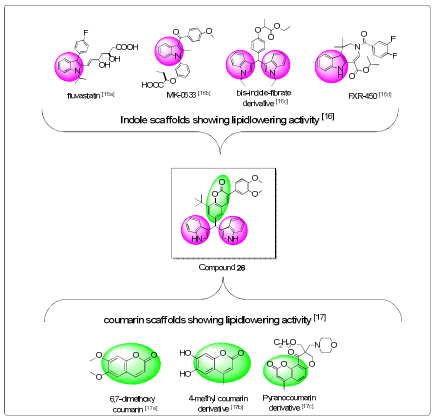

Fig. 1 Designing of coumarin-indole based hybrids by pharmacophore hybridization.

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The concept of hybridization is an attractive approach in medicinal chemistry as it generates new scaffolds with improved bioactivity.[11] Here two distinct pharmacophores are covalently linked into a single chemical entity so as to produce new hybrid compound with improved efficacy and safety. In the present context, we have combined coumarin and indole subunits to produce novel hybrid derivatives. The indole structure is a key component of various pharmacological agents and its derivatives play a prominent role in medicinal chemistry due to its high receptor binding affinity.[12-14] We had previously developed some coumarin-based hybrids, which showed diverse biological properties.[15a-i] Our previous encouraging laboratory experience on these scaffolds and the literature survey revealed that coumarin and indole pharmacophores occur in a variety of potential antidyslipidemic agents (Fig. 1).[16,17]

Thus keeping in view the lipid lowering potential of coumarin and indoles, it was envisaged that the synthesis of their hybrids is worth the attempt. Herein, we describe the design, synthesis and biological evaluation of novel coumarin-indole hybrids as potential antidyslipidemic agents.

Results and discussion

Chemistry

The synthesis of targeted hybrids was achieved as shown in Scheme 1. The synthesis of dicarbaldehyde intermediate (**5-8**) was achieved by the Duff reaction on commercially available ortho-substituted phenols in the presence of hexamethylenetetraamine (HMTA) and trifluoroacetic acid (TFA) at 120°C.[18] Subsequent condensation of these dicarbaldehyde intermediates (**5-8**) with the appropriately substituted phenylacetic acids in the presence of cyanuric chloride and N-methyl morpholine (NMM) in DMF for 1 hr gave the 3-arylcoumarin intermediates (**9-17**) in good yields.[19] Further, these 3-arylcoumarin intermediates (**9-17**) were subjected to electrophilic substitution with substituted indoles using iodine in acetonitrile for 30 min at room temperature, cleanly furnished the desired coumarin–indole hybrids (**18-29**).[20]The structures of all the compounds were confirmed by ^{1}H NMR, ^{13}C NMR, and Mass spectrometry (Supporting information).

Biological evaluation

Inhibition of HMG-CoA reductase enzyme

Initially, to determine the effects of the synthesized coumarin-indole hybrids in cholesterol biosynthesis, we evaluated them for their HMG-CoA reductase inhibition using standard protocol.[21] The percentages of inhibitory activities of hybrids at a dose of 10μM are depicted in Fig. 2A, and results compared with the standard drug atorvastatin. Interestingly, compounds **26** and **19** showed similar potency to that of standard drug atorvastatin as they are exhibiting 56% and 55% of inhibition compared to that of standard drug atorvastatin, which showed 60% of inhibition. In case of remaining compounds, inhibition profile was modest. To have more insight into the inhibition activity of most active compounds, we carried out a dose-response study compared to the atorvastatin.

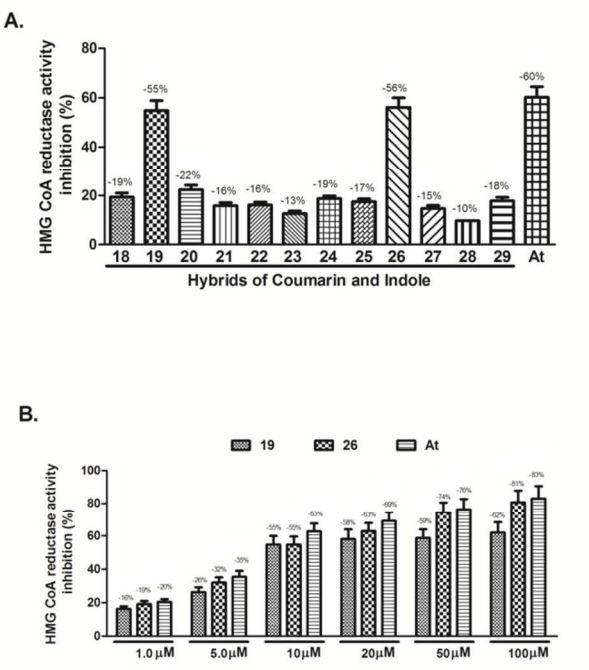

Fig.2 *In vitro* HMG-CoA activity assay: (A) Percentage inhibition of HMG-CoA reductase activity in the presence of coumarin-indole hybrids and atorvastatin at a dose of 10 μM. (B) Percentage inhibition of HMG-CoA reductase activity at different doses (1.0-100 μM) of active compounds (**19** and **26**) and atorvastatin. **P < 0.01; ***P < 0.001 *vs* control.

The complete dose-response study at different concentrations ranging between 1 μM to 100 μM is presented in Fig. 2B.From the inhibition results, it is observed that up to 10 μM concentration, activity remains similar to that of standard drug atorvastatin but at higher concentrations wide variation in inhibition was observed. Both active compounds **26** and **19** exhibited significant 63% and 58% inhibition at 20 μM concentrations, 74% and 59% of inhibition at 50 μM concentrations respectively, whereas atorvastatin displayed 69% and 76% inhibition at 20 μM and 50 μM concentrations respectively. Interestingly, at 100 μM concentration the active compounds **26** and **19** inhibited 81% and 62% of enzyme respectively, while atorvastatin showed 83% of inhibition. From the primary findings, it is clear that compounds **26** and **19** inhibited HMG-CoA reductase enzyme in a dose- dependent manner. However, since the concentrations shown to inhibit the in vitro HMG-CoA are on the higher side, other underlying mechanism of these hybrids cannot be ruled out.

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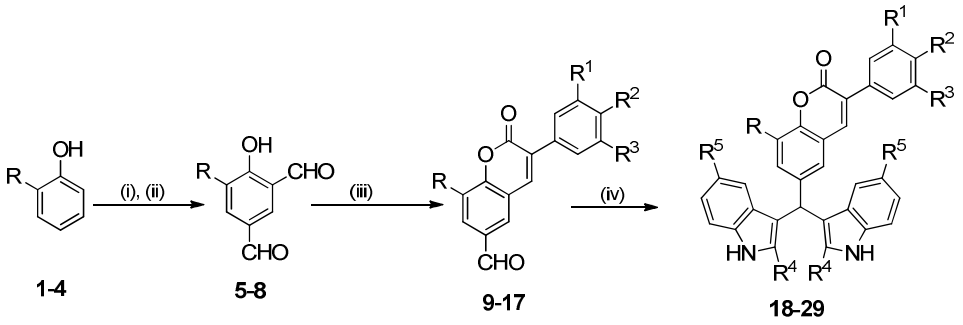

Scheme 1 Synthesis of coumarin-indole derivatives. Reagents and conditions: (i) HMTA, TFA, 120°C, 4h; (ii) aq H_2SO_4, 100°C, 2 h; (iii) Phenylacetic acid derivatives, cyanuric chloride, *N*-Methylmorpholine, DMF, reflux, 1 h; (iv) Substituted indoles, I_2, CH_3CN, rt, 30 min.

Effect of coumarin-indole hybrids in triton induced hyperlipidemic rats

Due to the reliability and simplicity of Triton WR-1339 model, it has been widely used for screening antihyperlipidemic drugs.[22] Thus, to investigate the potential of the coumarin-indole derivatives on lipid parameters, we employed Triton WR-1339 which induced acute hyperlipidemia in test animals. The data in Table 1 showed that intraperitoneal (I.P) injection of triton WR-1339 (400 mg/kg b.w) led to a remarkable raise in the level of total cholesterol (TC), phospholipids (PL) and triglycerides (TG) approximately by 3.5, 2.9 and 3.1 folds respectively. After administration of coumarin-indole hybrids at dose a of 100 mg/kg in triton induced hyperlipidemic rats, compounds **19** and **26** registered potent activity profile compared with the standard drugs atorvastatin and fenofibrate. Compounds **19** and **26** diminished the levels of TC by 30% and 32%, PL by 31% and 33%, TG by 29% and 33%, respectively. Whereas under the same experimental conditions, atorvastatin (at a dose of 10 mg/kg/d) and fenofibrate (100 mg/kg/d) diminished the levels of TC by 36% and 35%, PL by 35% and 37%, TG by 35% and 35%, respectively. Compound **26** had a noteworthy effect on all lipidemic indices, comparable to that of both standard drugs. Although compound **19** was somewhat less active than compound **26**, both compounds were considered as lead molecules among the series. These remarkable results guided and motivated us to carry out further dose-response studies on these two lead compounds.

Table 2 represents hypolipidemic activities of active lead compounds in triton induce hyperlipidemic rats treated with doses of 50 mg/kg, 100 mg/kg and 150 mg/kg body weight. The results revealed the dose dependent activity of both the lead compounds in all the studied lipid parameters. However of the two leads, **26** was preferred choice for further detailed studies.

Thus, compound **26** was next evaluated in a chronic hyperlipidemia model in HFD-fed rats, as the feeding behavior of these animals is similar to humans. A series of experiments were carried out including the study of their effects on body weight gain, plasma and hepatic lipid profile, receptor mediate catabolism of LDL, and fecal bile acid excretions in HFD-fed hyperlipidemic rats. Findings of these experiments are discussed next.

Table 1.Hypolipidemic activity of synthetic compounds in triton induce hyperlipidemic rats at 100 mg/kg dose

Compound no.	Serum lipid profile		
	TC (mg/dL)	**PL (mg/dL)**	**TG (mg/dL)**
Control	84.37±6.92	80.47±6.12	86.28±8.00
Triton (T) + Vehicle	300.62±25.17[c] (+3.56 F)	240.61±18.64[c] (+2.99 F)	270.17±17.30[c] (+3.13 F)
T+**18**	260.38±22.77 (-13)*	210.75±13.70 (-12)*	225.55±23.00 (-17)*
T+**19**	210.63±16.77 (-30)***	166.10±12.60 (-31)***	190.92±17.17 (-29)***
T+**20**	253.37±21.11 (-16)*	225.18±20.17 (-6) [NS]	250.30±21.14 (-7) [NS]
T+**21**	270.92±21.93 (-10)*	200.83±18.88 (-16)*	232.20±18.37 (-14)*
T+**22**	266.66±20.00 (-11)*	222.22±19.30 (-8) [NS]	240.30±18.99 (-11)*
T+**23**	277.50±24.84 (-8)[NS]	217.55±16.08 (-10)*	248.62±20.69 (-8) [NS]
T+**24**	260.52±23.07 (-13)*	216.66±17.17 (-10)*	240.14±23.10 (-11)*
T+**25**	266.21±24.00 (-11)*	211.11±17.00 (-12)*	250.44±20.70 (-7) [NS]
T+**26**	205.10±1.63 (-32)***	160.43±13.00 (-33)***	180.22±18.93 (-33)***

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T+**27**	270.66±20.51 (-10)*	206.12±18.10 (-14)*	233.31±18.99 (-14)*
T+**28**	280.93±25.17 (-7) ^{NS}	212.63±16.08 (-12)*	244.39±20.10 (-9) ^{NS}
T+**29**	262.20±23.44 (-13)*	210.60±15.00 (-12)*	234.40±21.18 (-13)*
T+**Atorvastatin** (At) (10 mg/Kg)	190.17±16.77 (-36)***	155.10±12.14 (-35)***	175.18±13.00 (-35)***
T+**Fenofibrate** (100 mg/kg)	195.40± 15.83 (-35)***	151.58± 11.31(-37)***	175.14±14.26 (-35)***

Each parameter represents pooled data from 6 rats/group and values are expressed as mean±S.D.$^{c}P< 0.001$, Triton treated + Vehicle rats group compared with control group and $^{*}P< 0.05$; $^{**}P< 0.01$; $^{***}P< 0.001$ Triton plus compounds groups compared with Triton treated group only. NOTE: NS (non significant) and F (Fold change over control group)

Table 2. Hypolipidemic activity of active compounds **26** and **19** in triton induced hyperlipidemic rats.

Compound no. and dose	Lipid parameters[a]			
	TC (mg/dL)	HDL-C (mg/dL)	PL (mg/dL)	TG (mg/dL)
Control	80.31±7.04	98.88±4.41	83.73±8.04	81.24±7.32
Triton	280.71±25.51^{c} (3.50 Folds)	73.35±2.82^{c} (-26%)	253.12±19.62^{c}(3.02 Folds)	250.49±21.18^{c} (3.08 Folds)
26 50 mg/kg	224.56±19.42** (-20)	84.35±3.83* (+15)	197.43±18.88** (22)	200.39±18.82** (-20)
100 mg/kg	190.88±21.06*** (-32)	90.22±3.44*** (+23)	169.59±11.26*** (-33)	167.82±12.41*** (-33)
150 mg/kg	188.07±12.54*** (-33)	91.68±4.37*** (+25)	164.52±15.92*** (-35)	168.12±15.92*** (-33)
19 50 mg/kg	221.76±21.04** (-21)	82.15±3.32* (+12)	205.02±19.62* (-19)	200.21±18.66** (-20)
100 mg/kg	196.49±17.28*** (-30)	89.48±3.49** (+22)	174.65±13.81*** (-31)	177.84±10.52*** (-29)
150 mg/kg	191.13±20.08*** (-32)	89.76±4.04** (+22)	174.37±17.35*** (-31)	175.34±18.28*** (-30)
Atorvastatin (10 mg/kg)	179.65±15.36*** (-36)	89.59±4.61** (+22)	164.14±14.82*** (-35)	162.81±14.84*** (-35)
Fenofibrate(100 mg/kg)	182.46±12.72*** (-35)	93.15±3.80*** (+27)	159.46±15.56*** (-37)	162.61±15.05*** (-35)

Each parameter represents pooled data from 6 rats/group and values are expressed as mean±S.D.$^{c}P< 0.001$, Triton treated +Vehicle rats group compared with control group and $^{*}P< 0.05$; $^{**}P< 0.01$; $^{***}P< 0.001$ Triton plus compounds groups compared with Triton treated group only. NOTE: NS (non significant) and F (Fold change over control group

Effect of compound 26andatorvastatinon body weight in hyperlipidemic rats

Charles Foster (CF) rats fed with HFD attained significant increase in their body weight to induce hyperlipidemia in 4 weeks. Oral supplementation of compound **26** (100 mg/kg b.w/d) for next 4 weeks along with HFD significantly reduced the body weight gain compared to the value for the HFD rats without change in food intake during treatment period (Table 3).

Effect of compound 26 and atorvastatin on serum biochemistry and lipoprotein metabolism in HFD-fed rat

Compound **26** showed significantly lower serum concentrations of TC (-24%), TG (-26%), AST (-28%) and ALT (-26%) in

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comparison with the values for HFD-fed rats (Table 4). Compound **26** reversed the detrimental effects of HFD feeding on the hepatic accumulation of TC (-27%) and TG (-29%) respectively. Additionally, continuous feeding of HFD in rats caused a remarkable reduction in the fecal bile acids excretion of cholic and deoxycholic acids and these levels were improved by the treatment of compound **26** by +31% and +39% respectively in HFD-fed hyperlipidemic rats, the similar effect was also observed in atorvastatin treated rats. HFD administered rat serum, showed significantly increase in the contents of cholesterol and TG in VLDL and LDL respectively.

Table 3. Effect of Compound **26** and atorvastatin on body weight and food intake in hyperlipidemic rats.

	ND+Vehicle	**HFD+Vehicle**	**HFD+Compound26** (100 mg/kg)	**HFD+Atorvastatin** (10 mg/kg)
Body weight (g)	186.38±13.86	263.52±21.56*** over ND+Vehicle	228.19±19.28** over HFD+Vehicle	255.73±22.61^{NS}over HFD+Vehicle
Food intake (g/d)	12.68±1.03	9.81±0.61** over ND+Vehicle	9.63±0.78^{NS} over HFD+Vehicle	9.71±0.59^{NS} over HFD+Vehicle

Each parameter represents pooled data from 6 rats per group and values are expressed as mean ± S.D, $^{***}P< 0.001$; $^{**}P< 0.01$ and ^{NS} non significant.

Table 4.Effect of compound **26** and atorvastatin on serum and hepatic biochemical parameters and fecal bile acids excretion in hyperlipidemicrats.

	ND+Vehicle	**HFD+Vehicle**	**HFD+** Comp **26** (100 mg/kg)	**HFD+ At** (10 mg/kg)
Serum				
TC (mg/dL)	83.26±6.31	188.33±15.62*** (+2.2 fold over ND)	143.13±11.62*** (-24% over HFD)	141.24±9.81*** (-25% over HFD)
TG (mg/dL)	88.10±4.92	193.46±14.73*** (+2.1 fold over ND)	143.16±10.39*** (-26% over HFD)	143.10±12.71*** (-26% over HFD)
AST (IU/L)	75.38±5.83	129.38±10.61*** (+1.7 fold over ND)	93.15±6.26*** (-28% over HFD)	91.85±6.39*** (-29% over HFD)
ALT ((IU/L)	41.08±2.91	77.32±4.91*** (+1.8 fold over ND)	57.21±3.82*** (-26%) over HFD	57.99±4.13*** (-25%) over HFD
Liver				
TC (mg/g)	9.06±0.86	17.73±1.43*** (+1.9 fold over ND)	12.94±1.08*** (-27% over HFD)	12.76±0.96*** (-28% over HFD)
TG (mg/g)	14.75±1.04	25.91±1.86*** (-1.7 over ND)	18.39±1.41*** (-29%) over HFD	18.13±1.48*** (-30%) over HFD
Fecal bile acids				
Cholic acid (μ/g)	71.63±4.26	33.28±2.51*** (-54% over ND)	43.46±2.93*** (+31% over HFD)	44.26±3.86*** (+33% over HFD)
Deoxycholic acid (μ/g)	43.82±3.68	29.39±2.08*** (-33% over ND)	40.85±3.61*** (+39% over HFD)	41.14±2.61*** (+40% over HFD)

Each parameter represents pooled data from 6 rats per group and values are expressed as mean ± S.D, $^{***}P< 0.001$.

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Treatment of compound **26** and atorvastatin considerably reversed the levels of TC and TG in VLDL and LDL, in HFD-fed hyperlipidemic rats (Fig 3**A** and **B**). Interestingly, the decreased levels of HDL-TC and HDL-TG in these animals were partially improved (Fig 3**C**). HFD feeding caused significant decrease in serum LCAT activity and lipolytic activity of LPL in liver (Fig3 **D** and **E**). Treatment of compound **26** and atorvastatin moderately reactivated this lipolytic action in serum and liver of HFD-fed animals. Additionally, increase in hepatic and circulatory lipids in HFD-fed rats suppressed receptor mediate catabolism of LDL in the liver membrane. Compound **26** and atorvastatin significantly reversed the receptor mediated catabolism of LDL in HFD-fed rats (Fig 3 **F**).

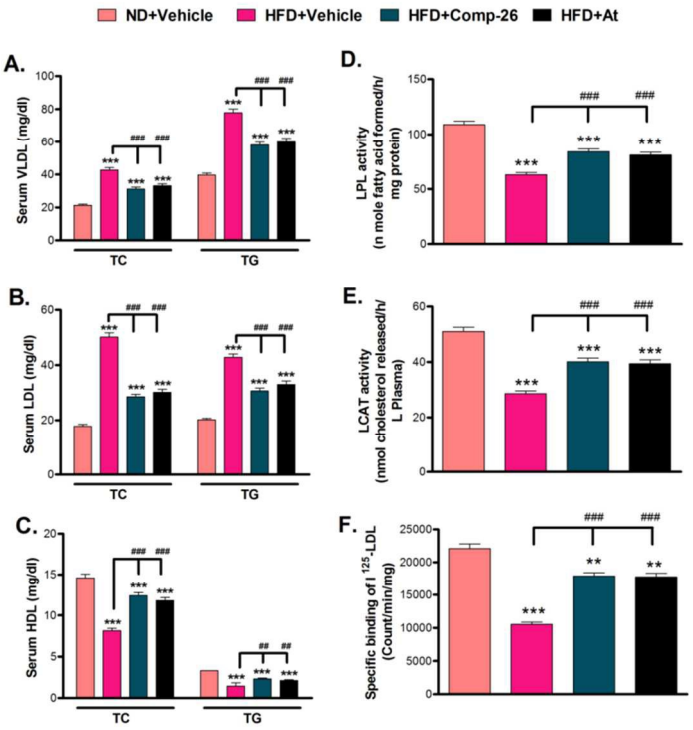

Fig 3. Compound **26** (100 mg/kg/d) and At (atorvastatin) (10 mg/kg/d) improve serum lipoprotein metabolism in HFD-fed rat. (A) Serum VLDL-TC and VLDL-TG, (B) Serum LDL-TC and LDL- TG, (C) Serum HDL- TC and HDL- TG, (D) LPL activity, (E) LCAT activity, and (F) I^{125}-LDL catabolism in the liver plasma membrane of HFD-induced hyperlipidemic rats, Values are expressed as mean±SD of six animals per group. ***P*< 0.01; ****P*< 0.001 *vs*ND+Vehicle and $^{\#\#}P$ < 0.01; $^{\#\#\#}P$< 0.001 *vs*HFD+Vehicle groups.

Effects of compound 26 and atorvastatin on hepatic HMG-CoA reductase activity in HFD-induced hyperlipidemic rats

The HMG-CoA reductase activity was measured as HMG CoA-Mevalonate ratio in order to determine whether the decrease in cholesterol levels was due to the suppression of HMG-CoA reductase activity. As expected, feeding of HFD significantly increased the activity of HMG-CoA reductase (Figure 4) in HFD-fed hyperlipidemic rats. However the treatment with compound **26** and atorvastatin significantly reduced the enzymatic activity in HFD-fed hyperlipidemic rats (Figure 4).

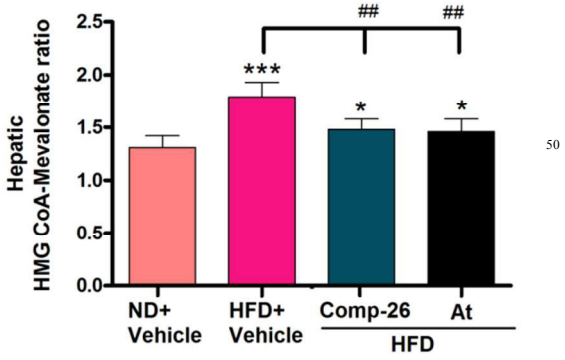

Fig 4. Effect of Compound **26** (100 mg/kg/d) and At (10 mg/kg/d) on hepatic HMG-CoA reductase activity of HFD-fed hyperlipidemic rats. Values are expressed as the mean ± SD of six animals per group. *P < 0.05; ***P < 0.001 *vs*ND+Vehicle and $^{\#\#}$P < 0.01 *vs*HFD+Vehicle groups.

***In-silico* binding mode prediction of compound 26**

Next, the docking studies were carried out to corroborate the above findings and investigate the possible bound conformation of compound **26** in the active site of HMG-CoA. Conformation of atorvastatin generated after re-docking was found to be almost identical to co-crystallized conformation (Figure 5**A**), indicating that the docking protocol was good enough to generate the correct binding pose of atorvastatin and can be used further to predict the binding mode of compound **26** under study. Proposed binding mode of compound **26** is shown in Figure 5**B**. Surfelx docking score of compound **26** was lower (7.21) than the atorvastatin (15.92), which shows that compound **26** binds to the target with lesser affinity than the standard drug atorvastatin.

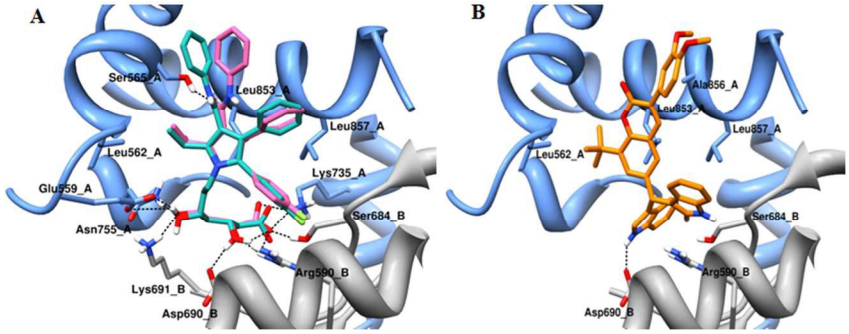

Fig 5. Overlay of docked (magenta) and co-crystallized (cyan) conformations of atrovastatin (A) and proposed binding mode of compound **26** (orange) (B) in the active site of HMG-CoA reductase. For clear representation chain A (grey) and chain B (sky blue) are shown in different colours.

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Detailed interaction study revealed that the bound conformation is mostly stabilized by hydrophobic interaction contributed by residues Leu562_A, Leu853_A, Ala856_A and Leu857_A. One of the indole rings of compound **26** was involved in H-bonding with Asp690_A. Compound **26** showed many similar hydrophobic interactions as seen in the case of atorvastatin. Coumarin moiety occupies the same position as the pyrrole ring of atorvastatin, also one of the hydrophobic indole rings project towards Leu857_A similar to the 4-flurophenyl ring of atorvastatin and other indole ring occupies the deep pocket where HMG like moiety of atorvastatin is present. We also observed possible cation-π interactions between the Arg590_B residue and two indole rings of the compound **26**, which may stabilize its binding within the HMG-CoA active site.

Stability in simulated gastrointestinal fluids

SGF and SIF stability studies were next carried out in order to evaluate the compound stability in gastro-intestinal (GI) fluids before membrane permeation. Compound **26** was found to be stable in SGF and SIF fluids (Figure 6) and this data supports its oral administration.

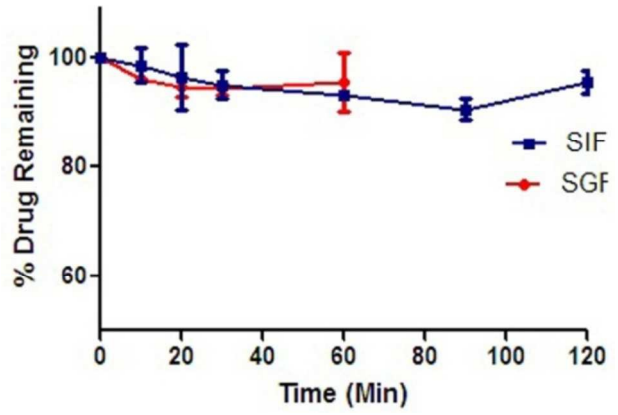

Fig 6. Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) stability of compound **26**. Data are shown as mean ± S.D (n=3)

***In Vitro* Metabolic Stability**

Drug loss in the body is mostly due to its metabolism in the liver. Liver metabolism plays significant role in clearance of the drug from the body. The first pass metabolism in the liver is one of the factors for low oral bioavailability. Compound **26** was found stable in the control reaction in absence of cofactor confirmed the compound stability as well as cofactor dependent degradation (Figure 7).The calculated microsomal intrinsic clearance (CL_{int}, vitro) of compound **26** was 0.0058 mL/min/mg microsomal protein. The predicted in vitro hepatic intrinsic clearance CL_{int} was 12.81 ± 0.53 mL/min* kg of body weight. Compound **26** was stable in simulated gastric and intestinal fluid and it was mainly metabolized by the enzymes involved in major metabolizing cellular organelles such as hepatocytes or enterocyte.

***In vivo* pharmacokinetic studies in male *Sprague-Dawley* rats**

Plasma concentration−time profile obtained after administration of compound **26** as a single oral 100 mg/kg dose was given in Figure 8. The main pharmacokinetic parameters are summarized in Table 5. The mean peak concentration (C_{max}) 78.97 ± 6.47 ng/mL was achieved at 4.0 hr after oral administration. The apparent clearance (Cl/F) was found to be 241.37 ± 18.10. The liver and peripheral tissue are usually active site for antihyperlipidmic drugs, thus compound **26** may have good distribution at target site. The compound **26** was resistant to enzymes in digestive fluid and plasma but was subject to metabolism in the liver microsomes. Apparent mean residence time (MRT) of compound **26** was found to be 4.46 hr in plasma, which indicated prolong efficacy following single dose administration. Thus, the pharmacokinetic behavior of compound**26** is favorable for its oral administration and supports its efficacy for anti-hyperlipidemic activity.

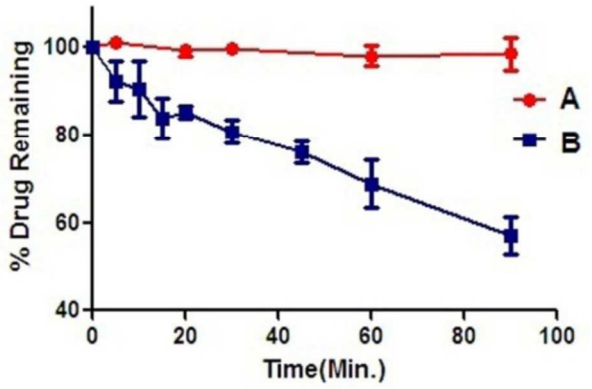

Fig 7. Metabolic Stability of compound **26** of in rat liver microsomes (RLM). Metabolic elimination profiles (% turnover or amount remaining vs. incubation time) for: (A) without NADPH and (B) with NADPH. Data are shown as mean ± S.D (n=3).

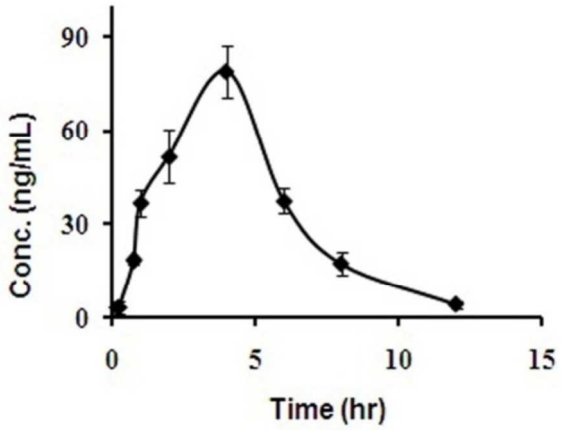

Fig 8. Mean plasma concentration−time profile of compound **26**, after a single oral administration of (100 mg/kg). Data are shown as mean ± S.D (n=3).

Discussion

The compound **26** exhibited potent antihyperlipidemic activity in both models of triton WR-1339 and HFD induced hyperlipidemic rats. Atorvastatin (well known inhibitor of the HMG-CoA reductase) was used as positive control in this study [23]. The weight gain in the rats treated with atorvastatin was similar to that of HFD-induced hyperlipidemic rats, while in contrast compound **26** significantly reduced the gain in body weight when compared to the hyperlipidemic rats. Both compound **26** and atorvastatin significantly decreased biochemical parameters of TC, PL, TG, VLDL, LDL, AST and ALT and significantly increased HDL concentration in the HFD-induced hyperlipidemic rats(Figure 3, Table 4). As expected due to the complications that follow the obesity induced hyperlipidemia, the serum lipids and lipoproteins

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level of rats was found to be elevated in this study. However, compound **26** reduced the development of diet induced obesity as indicated by the decreased body weight without change in food intake pattern. The rats fed with HFD showed increased levels of serum TC, TG, LDL-TC, VLDL-TC and decreased level of HDL-TC. Administration of compound **26** to HFD-induced hyperlipidemic rats significantly decreased the level of serum lipids TC, TG, LDL-TC, VLDL-TC and increased the HDL-C.

Table 5. Pharmacokinetic estimation of compound **26** in rat after single oral 100 mg/kg dose administration, each value represents Mean ± SD

Parameter	Units	Estimates
C_{max}	*n*g/mL	78.97 ± 6.47
T_{max}	h	4.0 ± 0.0
$AUC_{0-\infty}$	h*ng/mL	416.57 ± 30.56
Cl/F	L/h/kg	241.37 ± 18.10
V_d/F	L/kg	683.91 ± 93.83
MRT	h	4.46 ± 0.14

Moreover the LPL activity in liver of rats fed with HFD were found to be decreased. Compound **26** caused significant increase in LPL activity, which separates free fatty acids from TGs present in VLDL and chylomicrones. Serum LCAT activity was also increased by compound **26**, which is the main modulator of HDL-TC and plays an important role in the reverse cholesterol transport system. The bovine serum albumin (BSA) interaction studies indicate that the lead compound has strong affinity for albumin protein and forms a ground state complex (supporting information). These drug like effects clearly underscore the antihyperlipidemic potential of compound **26**.

The antihyperlipidemic activity of compound **26** may be due to reduced anabolism of cholesterol in the liver. Specifically it may be due to the decrease in the activity of hepatic HMG-CoA reductase. The present results showed that treatment of Compound **26** in hyperlipidemic rat caused significant reduction in hepatic HMG-CoA reductase activity, which strongly suggests that the activity is mediated by inhibition of HMG-CoA reductase in the hyperlipidemic rat liver and the results corroborated well with *in vitro* data and molecular docking analysis. The schematic representation of mechanism of action of compound **26** is shown in Figure 9.

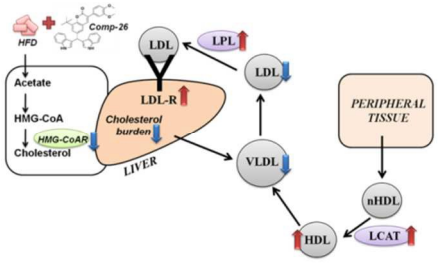

Fig 9. Coumarin-indole derivative may regulate lipoprotein metabolism via inhibition on HMG-CoA reductase activity in liver.

Conclusion

In conclusion, a series of coumarin-indole derivatives have been designed, synthesized and evaluated using the HMG-CoA reductase inhibitor assay. The hypolipidemic activity of the most potent compound **26** was confirmed in two different animal models. Compound **26** remarkably reduced the body weight, liver mass, plasma and hepatic lipid level followed by the increase in excretion of fecal bile acids without change in food intake of experimental rats. It also balanced the lipoprotein metabolism by increasing the LPL, LCAT, receptor mediated catabolism of I^{125}-LDL. We are now in the process of elucidating the precise molecular mechanism of action of this compound and also synthesize analogs of **26** for better understanding of the structure activity relationships, so as to advance this new prototype as a new class of hypolipidemic agents. The exquisite potency, favorable pharmacokinetic and structural novelty of **26** suggests it to be a new class of lipid lowering agent.

Experimental

Analysis and instruments

All reagents were commercial and were used without further purification. Chromatography was carried on silica gel (60-120 and 100-200 mesh). All reactions were monitored by thin-layer chromatography (TLC), silica gel plates with fluorescence F254 were used. Melting points were taken in open capillaries on Complab melting point apparatus and are presented uncorrected. Infrared spectra were recorded on a Perkin-Elmer FT-IR RXI spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded using BrukerSupercon Magnet DRX-300 spectrometer (operating at 300, 400MHz for ^{1}H and 100, 75MHz for ^{13}C) using DMSO-d_6 or $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard. Chemical shifts are reported in parts per million. Electrospray ionization mass spectra (ESI-MS) were recorded on Thermo Lcq Advantage Max-IT. High resolution mass spectra (HRMS) were recorded on 6520 Agilent Q T of LC MS/MS (Accurate mass). Additionally, purity of compounds was measured by a RP- HPLC with the following conditions, and purity of the compounds was >95%. The Waters HPLC system, Milford USA consisted of a binary pump (model Waters 515 HPLC pump), auto sampler (model 717 plus Auto sampler) and PDA detector (Waters 2996). Data collection and analysis were performed using Empower pro 2 software. The resolution and better peak shape were achieved on a Symmetry-Shield C18 (5µm, 4.6 X 150 mm) column, protected with a C_{18} guard column. The system was analyzed in isocratic mode with a mobile phase consisting of Methanol: 0.5% aqueous formic acid (52: 48, v/v) at a flow rate of 1.0 mL/min.

General procedure for the synthesis of compounds (5–8**)**
Synthesis of dicarbaldehyde substrates (5-8) were achieved by our previously reported protocol.[18]

4-hydroxy-5-methylisophthalaldehyde (5**)**
White solid, Yield: 60%; mp 125-127ºC; IR (KBr) : 3262, 1703, 1626, 745 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ :11.83 (s, 1H), 9.98 (s, 1H), 9.90 (s, 1H), 7.97(d, J = 1.96 Hz, 1H), 7.93 (brs, 1H), 2.34 (s, 3H) ; ^{13}C NMR ($CDCl_3$, 100MHz): 196.66, 189.97, 165.20, 137.46, 134.93, 128.91, 119.96, 15.40 ; ESI-MS (*m/z)*: 165 $[M+H]^+$.

4-hydroxy-5-isopropylisophthalaldehyde (6**)**
White solid, Yield: 65%; mp 163-164ºC; IR (KBr) : 3023, 1693, 1655, 758 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ:11.94 (s, 1H), 9.97

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(s, 1H), 9.91 (s, 1H), 7.99 (brs, 1H), 7.96 (s, 1H), 3.41-3.34 (m, 1H), 1.27 (d, J = 6.92 Hz, 6H); ^{13}C NMR ($CDCl_3$, 75MHz): 196.62, 189.90, 164.34, 138.90, 134.86, 133.28, 129.06, 119.94, 26.54, 22.11; ESI-MS (*m/z)*: 193 **[**M+H]$^+$.

5-tert-butyl-4-hydroxyisophthalaldehyde (7)

Oily, Yield: 60%; IR (KBr) : 3381, 1692, 1653, 778 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz) δ:12.39 (s, 1H), 9.97 (s, 1H), 9.91 (s, 1H), 8.05 (d, J =1.4 Hz, 1H), 7.97(d, J = 1.5 Hz, 1H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$, 75MHz): 196.62, 189.90, 164.34, 138.90, 134.86, 133.28, 129.06, 119.94, 26.54, 22.11 ; ESI-MS (*m/z)*: 207 **[**M+H]$^+$.

5-(sec-butyl)-4-hydroxyisophthalaldehyde (8)

Oily, Yield 65%, IR (neat): 3267, 3062, 1709, 1622, 1018 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200MHz) δ: 11.99 (s, 1H), 10.51 (s, 1H), 9.96 (s, 1H), 8.08 (brs, 1H), 7.99 (s, 1H), 3.27-3.10 (m, 1H), 1.74–1.57 (m, 2H), 1.25 (d, J = 7.0 Hz, 3H), 0.87 (t, J = 7.3 Hz, 3H); ^{13}C NMR: ($CDCl_3$, 75MHz) δ: 196.47, 189.43, 163.98, 137.19, 134.50, 133.49, 128.70, 119.64, 32.77, 28.89, 19.39, 11.58; ESI-MS: m/z: 207 **[**M+H]$^+$.

General procedure for the synthesis of 3-phenylcoumarins (9-17).[19]

To a solution of cyanuric chloride (4.85 mmol) in N-methylmorholine (7.28 mmol) was added phenylacetic acid (4.85 mmol), and the solution was stirred at room temperature for 10 min. To the above solution was added DMF solution of dialdehyde (4.85 mmol), and the reaction mixture was stirred 100 °C for 1.5 h. The mixture was extracted with EtOAc (50 mL × 3). The combined EtOAc extracts were dried (Na_2SO_4) and concentrated to yield the the crude products, which were purified by column chromatography on silica gel yielded the desired compounds.

8-(tert-butyl)-3-(4-methoxyphenyl)-2-oxo-2H-chromene-6-carbaldehyde (9)

White solid, yield: 63%; mp 154-155ºC; IR (KBr): 3014, 1705, 1285, 776 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 10.03 (s, 1H), 8.02 (d, J = 1.8 Hz, 1H), 7.91 (d, J = 1.8 Hz, 1H), 7.82 (s, 1H), 7.71 (d, J = 8.8 Hz, 2H), 6.99 (d, J = 8.8 Hz, 2H), 3.86 (s, 3H), 1.58 (s, 9H); ^{13}C NMR: ($CDCl_3$, 75MHz) δ: 190.87, 160.58, 159.41, 155.90, 139.14, 138.47, 132.27, 129.93, 128.88, 128.85, 128.17, 126.49, 120.68, 114.18, 55.50, 35.35, 29.82; ESI-MS: m/z: 337 **[**M+H]$^+$.

8-tert-butyl-3-(3,4-dimethoxyphenyl)-2-oxo-2H-chromene-6-carbaldehyde (10)

White solid, yield: 75%; mp 163-164ºC; IR (KBr): 3020, 1697, 1272, 757 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ:10.01 (s, 1H), 8.01 (d, J = 1.7 Hz, 1H), 7.92 (d, J = 1.6 Hz, 1H), 7.85 (s, 1H), 7.35-7.29 (m, 2H), 6.94 (d, J = 8.4 Hz, 1H), 3.93 (s, 3H), 3.92 (s, 3H), 1.56 (s, 9H); ^{13}C NMR ($CDCl_3$, 100MHz): 190.82, 159.40, 155.84, 150.16, 148.90, 139.13, 138.70, 132.28, 128.96, 128.84, 128.13, 126.78, 121.31, 120.60, 111.84, 111.22, 56.12, 56.08, 35.33, 29.79; ESI-MS (*m/z)*: 367 **[**M+H]$^+$.

8-tert-butyl-2-oxo-3-(3,4,5-trimethoxyphenyl)-2H-chromene-6-carbaldehyde (11)

Pale yellow solid, yield: 69%; mp 181-182ºC; IR (KBr): 3042, 1647, 1242, 754 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 10.02 (s, 1H), 8.03 (d, J = 1.8 Hz, 1H), 7.94 (d, J =1.8 Hz, 1H), 7.87 (s, 1H), 6.97 (s, 2H), 3.91 (s, 6H), 3.89 (s, 3H), 1.56 (s, 9H); ^{13}C NMR ($CDCl_3$, 100MHz): 190.75, 159.26, 155.92, 153.33, 139.58, 139.28, 139.22, 132.35, 129.51, 129.25, 128.96, 128.34, 120.42, 106.08, 61.04, 56.41, 35.35, 29.78; ESI-MS (*m/z)*: 397 **[**M+H]$^+$.

3-(4-methoxyphenyl)-8-methyl-2-oxo-2H-chromene-6-carbaldehyde (12)

Light yellow solid, yield: 65%; mp 148-150ºC; IR (KBr): 3033, 1725, 1705, 1639, 1021 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 9.98 (s, 1H), 7.86 (d, J = 4.6 Hz, 2H), 7.78 (s, 1H), 7.68 (d, J = 8.8 Hz, 2H), 6.96 (d, J = 8.8 Hz, 2H), 3.84 (s, 3H), 2.53 (s, 3H); ^{13}C NMR ($CDCl_3$, 75MHz) δ: 190.55, 160.53, 159.94, 155.38, 137.94, 132.43, 132.30, 129.90, 128.63, 128.07, 127.26, 126.48, 119.84, 114.10, 55.44, 15.58; ESI-MS: m/z: 295 **[**M+H]$^+$.

3-(3,4-dimethoxyphenyl)-8-methyl-2-oxo-2H-chromene-6-carbaldehyde (13)

Light yellow solid yield: 60%; mp 178-180ºC; IR (KBr): 3053, 1730, 1695, 1634, 1026 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 10.01 (s, 1H), 7.89 (d, J = 7.2 Hz, 2H), 7.83 (s, 1H), 7.31–7.28 (m, 2H), 6.95 (d, J = 8.9 Hz, 1H), 3.95 (s, 3H), 3.93 (s, 3H), 2.56 (s, 3H); ^{13}C NMR ($CDCl_3$, 75MHz) δ: 190.49, 159.88, 155.35, 150.16, 148.89, 138.17, 132.45, 132.39, 128.66, 128.03, 127.26, 126.80, 121.40, 119.78, 111.84, 111.18, 56.10, 56.04, 15.54; ESI-MS: m/z: 325 **[**M+H]$^+$.

8-methyl-2-oxo-3-(3,4,5-trimethoxyphenyl)-2H-chromene-6-carbaldehyde (14)

White solid, yield: 60%; mp 196-198ºC; IR (KBr): 3074, 1728, 1695, 1632, 1010 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 10.02 (s, 1H), 7.93-7.91 (m, 2H), 7.86 (s, 1H), 6.95 (s, 2H), 3.93 (s, 6H), 3.90 (s, 3H), 2.57 (s, 3H); ^{13}C NMR ($CDCl_3$, 75MHz) δ: 190.48, 159.82, 155.53, 153.36, 139.38, 139.38, 132.77, 132.58, 129.62, 129.04, 128.19, 127.44, 119.67, 106.20, 61.04, 56.44, 15.63; ESI-MS: m/z: 355 **[**M+H]$^+$.

3-(3,4-dimethoxyphenyl)-8-isopropyl-2-oxo-2H-chromene-6-carbaldehyde (15)

Pale yellow solid, yield: 79%; mp 144-145ºC; IR (KBr): 3078, 1647, 1229, 746 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ:10.01 (s, 1H), 7.95 (s, 1H), 7.89 (s, 1H), 7.84 (s, 1H), 7.30-7.25 (m, 2H), 6.93 (d, J = 8.0 Hz, 1H), 3.93 (s, 3H), 3.91 (s, 3H), 3.71-3.64 (m, 1H), 1.35 (d, J = 6.8 Hz, 6H);^{13}C NMR ($CDCl_3$, 100MHz): 190.71, 159.90, 154.44, 150.16, 148.90, 138.47, 137.72, 132.73, 128.65, 128.39, 128.11, 126.86, 121.39, 119.96, 111.84, 111.20, 56.12, 56.07, 26.76, 22.59; ESI-MS (*m/z)*: 353 **[**M+H]$^+$.

8-isopropyl-2-oxo-3-(3,4,5-trimethoxyphenyl)-2H-chromene-6-carbaldehyde (16)

Pale yellow solid, yield: 75%; mp 131-132ºC; IR (KBr): 3028, 1677, 1229, 768 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ:10.02 (s, 1H), 7.97 (s, 1H), 7.92 (s, 1H), 7.87 (s, 1H), 6.94 (s, 2H), 3.91 (s, 6H), 3.88 (s, 3H), 3.71-3.64 (m, 1H), 1.36 (d, J = 6.9 Hz, 6H);^{13}C NMR ($CDCl_3$, 100MHz): 190.65, 159.76, 154.54, 153.33, 139.36, 139.29, 137.81, 132.80, 129.62, 128.90, 128.70, 128.24, 119.79, 106.13, 61.03, 56.41, 26.81, 22.59; ESI-MS (*m/z)*: 383 **[**M+H]$^+$.

8-sec-butyl-3-(3,4-dimethoxyphenyl)-2-oxo-2H-chromene-6-carbaldehyde (17)

Light yellow solid, yield: 59%;mp 140-142ºC; IR (KBr): 3065, 1728, 1697, 1607, 1032 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 10.03 (s, 1H), 7.92 (d, J = 1.6 Hz, 2H), 7.86 (s, 1H), 7.32–7.29 (m, 2H), 6.97 (d, J = 8.5 Hz, 1H), 3.96 (s, 3H), 3.94 (s, 3H), 3.53–3.44 (m, 1H), 1.81–1.71 (m, 2H), 1.34 (d, J = 6.9 Hz, 3H), 0.90 (t, J = 7.3 Hz, 3H) ; ^{13}C NMR ($CDCl_3$, 75MHz) δ: 190.75, 159.96, 154.79, 150.24, 148.98, 138.55, 136.81, 132.77, 129.19, 128.77, 128.02, 126.94, 121.44, 120.06, 111.94, 111.28, 56.18, 56.13, 33.54, 29.77, 20.58, 12.14; ESI-MS: m/z: 367 [M]$^+$.

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General procedure for the synthesis of final compounds (18–29)

To a magnetically stirred solution of 3-phenylcoumarins (9-17) (1.06 mmol) in acetonitrile (20 mL) were added different substituted indoles (2.12 mmol) and iodine (0.21 mmol) and stirred for 30min at rt. The formed solids were collected by filtration and obtained solids (18-29) were dried invacuo.

6-(di(1H-indol-3-yl)methyl)-3-(4-methoxyphenyl)-8-methyl-2H-chromen-2-one (18)

Yellow solid, yield: 68%; mp 168-169ºC; IR (KBr) : 3008, 2915, 1648, 1570, 1559, 736 cm^{-1}; ^1H NMR (DMSO-d_6, 300MHz) δ: 10.85 (s, 2H), 8.08 (s, 1H), 7.68-7.65 (d, J = 8.7 Hz, 2H), 7.51 (d, J = 9.6 Hz, 2H) , 7.37-7.30 (m, 4H), 7.07-7.02 (m, 2H), 6.99 (s, 2H), 6.90-6.85 (m, 4H), 5.89 (s, 1H), 3.78 (s, 3H), 2.34 (s, 3H);^{13}C NMR (DMSO-d_6, 75MHz): 160.02, 159.43, 149.45, 140.91, 139.59, 136.61, 132.73, 129.72, 126.92, 126.50, 125.76, 125.09, 124.17, 123.75, 120.95, 118.98, 118.27, 117.70, 113.57, 111.49, 55.16, 15.02 ; HRMS (*m/z*): calcd for $C_{34}H_{26}N_2O_3[M+H]^+$ 511.2022, found: 511.2027

6-(bis(5-nitro-1H-indol-3-yl)methyl)-3-(4-methoxyphenyl)-8-methyl-2H-chromen-2-one (19)

Pale yellow solid, yield: 75%; mp 180-181ºC;IR (KBr) : 3047, 2928, 1680, 1555, 1512, 758 cm^{-1}; ^1H NMR (DMSO-d_6, 400MHz)δ: 11.71 (s, 2H), 8.38 (d, J = 2.2 Hz, 2H), 8.13 (s, 1H), 7.99 (dd, $J_{1,3}$ = 8.9 Hz, $J_{1,2}$ = 2.2 Hz, 2H), 7.68 (d, J = 8.9 Hz, 2H), 7.57 (s, 1H), 7.55 (m, 3H), 7.15 (d, J = 1.8 Hz, 2H), 6.99 (d, J = 8.9 Hz, 2H), 6.30 (s, 1H), 3.79 (s, 3H), 2.38 (s, 3H);^{13}C NMR (DMSO-d_6, 100MHz): 159.95, 159.49, 149.78, 140.31, 139.85, 139.67, 139.45, 132.51, 129.74, 127.89, 126.86, 126.01, 125.72, 125.06, 124.70, 120.28, 119.28, 116.69, 116.12, 113.57, 112.14, 55.14, 37.73, 15.03; HRMS (*m/z*): calcd for $C_{34}H_{24}N_4O_7[M+H]^+$ 601.1723, found: 601.1719.

6-(di(1H-indol-3-yl)methyl)-3-(3,4-dimethoxyphenyl)-8-methyl-2H-chromen-2-one (20)

Orange solid, yield: 73%;mp 155-156ºC;IR (KBr) : 3012, 2938, 1645, 1588, 1527, 785 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ:10.85 (s, 2H), 8.15 (s, 1H), 7.52 (s, 2H), 7.37-7.31 (m, 6H), 7.07-6.98 (m, 2H), 6.91 (s, 1H), 6.88 (s, 2H), 6.86 (s, 2H), 5.90 (s, 1H), 3.78 (s, 6H), 2.35 (s, 3H);^{13}C NMR (DMSO-d_6, 75MHz): 159.96, 149.42, 149.18, 148.31, 140.94, 139.77, 136.63, 132.82, 127.08, 126.51, 125.79, 125.06, 124.18, 123.78, 121.23, 120.98, 118.98, 118.30, 117.71, 112.09, 111.51, 111.31, 55.58, 55.53, 15.04; HRMS (*m/z*): calcd for $C_{35}H_{28}N_2O_4[M+H]^+$ 541.2127, found: 541.2171.

6-(bis(2-methyl-1H-indol-3-yl)methyl)-8-methyl-3-(3,4,5-trimethoxyphenyl)-2H-chromen-2-one (21)

Red solid, yield: 65%;mp 170-171ºC; IR (KBr): 3036, 2948, 1548, 1600, 1209, 720 cm^{-1}; ^1H NMR (DMSO-d_6, 300MHz)δ: 10.79 (s, 2H), 8.17 (s, 1H), 7.34 (d, J = 10.5 Hz, 2H), 7.23 (d, J=7.9 Hz, 2H), 7.05 (s, 2H), 6.93-6.84 (m, 4H), 6.72-6.67 (m, 2H), 6.00 (s, 1H), 3.79 (s, 6H), 3.68 (s, 3H), 2.32 (s, 3H), 2.07 (s, 6H); ^{13}C NMR (DMSO-d_6, 75MHz): 159.85, 152.47, 149.54, 140.85, 140.42, 137.85, 135.10, 133.56, 132.39, 130.08, 128.20, 125.98, 125.39, 124.19, 119.66, 118.70, 118.31, 118.12, 111.59, 110.44, 106.18, 60.06, 55.98, 38.03, 15.07, 12.05; HRMS (*m/z*): calcd for $C_{38}H_{34}N_2O_5[M+H]^+$ 599.2546, found: 599.2542

6-(di(1H-indol-3-yl)methyl)-3-(3,4-dimethoxyphenyl)-8-isopropyl-2H-chromen-2-one (22)

Pale yellow solid, yield: 75%; mp 180-181ºC; IR (KBr) : 3047, 2928, 1680, 1555, 1512, 758 cm^{-1}; ^1H NMR (DMSO-d_6, 300MHz)δ:10.83 (s, 2H), 8.15 (s, 1H), 7.63 (s, 1H), 7.51 (s, 1H), 7.37-7.29 (m, 6H), 7.07-6.98 (m, 3H), 6.90-6.85 (m, 4H), 5.93 (s, 1H), 3.78 (s, 6H), 3.46-3.42 (m, 1H), 1.24 (d, J= 6.8 Hz, 6H); ^{13}C NMR (DMSO-d_6, 75MHz): 159.86, 149.17, 148.31, 141.08, 140.01, 136.63, 134.32, 128.79, 127.08, 126.52, 125.68, 125.16, 123.67, 121.22, 120.95, 119.15, 119.02, 118.24, 117.83, 112.12, 111.49, 111.33, 55.59, 55.53, 26.38, 22.40; HRMS (*m/z*): calcd for $C_{37}H_{32}N_2O_4[M+H]^+$ 569.2440, found: 569.2437.

6-(bis(2-methyl-1H-indol-3-yl)methyl)-8-isopropyl-3-(3,4,5-trimethoxyphenyl)-2H-chromen-2-one (23)

Yellow solid, yield: 66%;mp 140-142ºC; IR (KBr) : 3028, 2936, 1663, 1566, 1218, 701 cm^{-1}; ^1H NMR (DMSO-d_6, 300MHz) δ:10.77 (s, 2H), 8.14 (s, 1H), 7.48 (s, 1H), 7.30 (s, 1H), 7.22 (d, J=7.9 Hz, 2H), 7.04 (s, 2H), 6.92-6.83 (m, 4H), 6.71-6.67 (m, 2H), 6.03 (s, 1H), 3.78 (s, 6H), 3.68 (s, 3H), 3.39 (brs, 1H), 2.07 (s, 6H), 1.16 (d, J= 6.8 Hz, 6H);^{13}C NMR (DMSO-d_6, 75MHz): 159.74, 152.45, 148.46, 141.03, 140.39, 137.88, 135.09, 134.36, 132.27, 130.04, 129.69, 128.14, 125.89, 125.32, 119.64, 118.85, 118.32, 117.99, 111.74, 110.39, 106.23, 60.02, 55.97, 38.14, 26.35, 22.32, 12.03; HRMS (*m/z*): calcd for $C_{40}H_{38}N_2O_5[M+H]^+$ 627.2859, found: 627.2854.

6-(bis(2-methyl-1H-indol-3-yl)methyl)-8-tert-butyl-3-(4-methoxyphenyl)-2H-chromen-2-one (24)

White solid, yield: 69%;mp 253-254ºC;IR (KBr): 3021, 1705, 1645, 1595, 1285, 768 cm^{-1}; ^1H NMR (DMSO-d_6, 400MHz)δ:10.80 (s, 2H), 8.06 (s, 1H), 7.70(d, J= 8.8 Hz, 2H), 7.54 (d, J=1.8 Hz, 1H), 7.36 (s, 1H), 7.24 (d, J= 8.0 Hz, 2H), 6.96 (d, J=7.0 Hz, 2H), 6.94-6.87 (m, 4H), 6.72-6.68 (m, 3H), 3.77 (s, 3H), 2.09 (s, 6H), 1.35 (s, 9H); ^{13}C NMR (DMSO-d_6, 75MHz): 159.57, 159.42, 149.69, 140.21, 139.74, 135.65, 135.08, 132.18, 129.71, 128.10, 126.70, 125.96, 125.13, 119.61, 118.32, 117.94, 113.55, 111.84, 110.36, 55.12, 38.21, 34.32, 29.49, 12.04; HRMS (*m/z*): calcd for $C_{39}H_{36}N_2O_3[M+H]^+$ 581.2804, found: 581.2810.

6-(bis(5-nitro-1H-indol-3-yl)methyl)-8-tert-butyl-3-(4-methoxyphenyl)-2H-chromen-2-one (25)

Yellow solid, yield: 69%;mp 209-210ºC;IR (KBr): 3021, 2927, 1645, 1550, 1285, 768 cm^{-1}; ^1H NMR (DMSO-d_6, 300MHz)δ:11.69 (s, 2H), 8.37 (s, 2H), 8.13 (s, 1H), 8.00-7.96 (m, 2H), 7.75 (s, 1H), 7.69 (d, J = 8.7 Hz, 2H), 7.59 (s, 1H), 7.57-7.54 (m, 2H), 7.22 (s, 2H), 6.99 (d, J= 8.7 Hz, 2H), 6.34 (s, 1H), 3.79 (s, 3H), 1.44 (s, 9H); ^{13}C NMR (DMSO-d_6, 75MHz): 159.49, 159.46, 149.91, 140.25, 140.13, 139.85, 139.48, 136.14, 129.71, 128.98, 127.65, 126.73, 126.07, 125.74, 125.37, 120.32, 120.15, 116.66, 116.26, 113.61, 112.14, 55.14, 38.12, 34.52, 29.57; HRMS (*m/z*): calcd for $C_{37}H_{30}N_4O_7[M+H]^+$ 643.2193, found: 643.2198.

8-tert-butyl-6-(di(1H-indol-3-yl)methyl)-3-(3,4-dimethoxyphenyl)-2H-chromen-2-one (26)

White solid, yield: 65%;mp 272-273ºC;IR (KBr); 3015, 2935, 1662, 1570, 1274, 756 cm^{-1}; ^1H NMR (DMSO-d_6, 400MHz) δ: 10.84 (s, 2H), 8.17 (s, 1H), 7.67 (s, 1H), 7.56 (s, 1H), 7.38-7.32 (m, 6H), 7.07-6.98 (m, 3H), 6.90-6.87 (m, 4H), 5.95 (s, 1H), 3.79 (s, 6H), 1.43 (s, 9H); ^{13}C NMR (DMSO-d_6, 75MHz): 159.62, 149.70, 149.26, 148.40, 140.76, 140.47, 136.72, 135.88, 129.26, 127.03, 126.62, 125.95, 125.29, 123.76, 121.28, 121.09, 119.85, 119.15, 118.38, 117.95, 112.14, 111.62, 111.43, 55.67, 55.60, 34.50, 29.69; HRMS (*m/z*): calcd for $C_{38}H_{34}N_2O_4$ $[M+H]^+$ 583.2597, found: 583.2593.

6-(bis(2-methyl-1H-indol-3-yl)methyl)-8-tert-butyl-3-(3,4-dimethoxyphenyl)-2H-chromen-2-one (27)

Pale yellow solid, yield: 69%;mp 248-249ºC;IR (KBr): 3021, 2927, 1645, 1585, 1285, 768 cm^{-1}; ^1H NMR (DMSO-d_6, 400MHz) δ : 10.80 (s, 2H), 8.13(s, 1H), 7.54 (d, J= 1.8 Hz, 1H), 7.38-7.34 (m, 3H), 7.23 (d, J = 8.0 Hz, 2H), 6.98 (d, J= 8.5 Hz, 1H), 6.92-6.85 (m, 4H), 6.72-6.68 (m, 2H), 6.04 (s, 1H), 3.77 (s, 6H), 2.09 (s, 6H), 1.36 (s, 9H);^{13}C NMR (DMSO-d_6, 75MHz):

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159.50, 149.66, 149.16, 148.31, 140.37, 139.76, 135.66, 135.09, 132.21, 129.81, 128.11, 126.89, 125.92, 125.21, 121.19, 119.62, 118.33, 117.95, 112.13, 111.82, 111.32, 110.37, 55.57, 55.51, 38.21, 34.32, 29.49, 12.04; HRMS (*m/z*): calcd for $C_{40}H_{38}N_2O_4$[M+H]$^+$ 611.2910, found: 611.2917.

6-(bis(5-nitro-1H-indol-3-yl)methyl)-8-tert-butyl-3-(3,4-dimethoxyphenyl)-2H-chromen-2-one (28)

Pale yellow solid, yield: 75%;mp 160-161ºC; IR (KBr); 3043, 2912, 1670, 1532, 1246, 751 cm^{-1}; ^{1}H NMR (DMSO-d$_6$, 300MHz) δ : 11.69 (s, 2H), 8.36 (s, 2H), 8.17 (s, 1H), 8.00-7.97 (m, 2H), 7.75 (s, 1H), 7.57 (s, 2H), 7.54 (s, 1H), 7.36-7.33(m, 2H), 7.21 (s, 2H), 7.01-6.99 (m, 1H), 6.34 (s, 1H), 3.78 (s, 6H), 1.44 (s, 9H) ;^{13}C NMR (DMSO-d$_6$, 75MHz): 159.38, 149.86, 149.22, 148.31, 140.25, 139.85, 139.50, 136.13, 129.03, 127.66, 126.89, 126.04, 125.73, 125.40, 121.19, 120.32, 120.12, 116.66, 116.25, 112.15, 112.08, 111.34, 55.58, 55.51, 38.10, 34.52, 29.57 ; HRMS (*m/z*): calcd for $C_{38}H_{32}N_4O_8$[M+H]$^+$ 673.2298, found: 673.2284.

6-(bis(5-nitro-1H-indol-3-yl)methyl)-8-sec-butyl-3-(3,4-dimethoxyphenyl)-2H-chromen-2-one (29)

Pale yellow solid, yield: 76%; mp 130-131ºC; IR (KBr): 3012, 2921, 1710, 1627, 1210, 719 cm^{-1}; ^{1}H NMR (DMSO-d$_6$, 300MHz) δ :11.70 (s, 2H), 8.34 (s, 2H), 7.99 (d, J = 8.6 Hz, 2H), 7.67-7.54 (m, 4H), 7.35-7.31 (m, 2H), 7.19 (s, 2H), 6.99 (d, J = 8.4 Hz, 1H), 6.33 (s, 1H), 5.75 (s, 1H), 3.78 (s, 6H), 1.66-1.61 (m, 2H), 1.22 (d, J = 6.8 Hz, 3H), 0.75 (t, J = 7.1 Hz, 3H,) ;^{13}C NMR (DMSO-d$_6$, 75MHz): 159.81, 149.20, 148.91, 148.30, 140.24, 139.87, 133.41, 129.29, 127.71, 127.64, 127.04, 125.96, 125.74, 125.38, 121.25, 120.37, 120.22, 119.57, 116.70, 116.24, 112.20, 112.10, 111.31, 55.58, 55.54, 38.04, 33.13, 28.99, 20.47, 11.74; HRMS (*m/z*): calcd for $C_{38}H_{32}N_4O_8$ [M+H]$^+$ 673.2298, found: 673.2293.

Ethical statement

In vivo experiments were conducted as per the guidelines provided by the animal ethics committee (IAEC) of the Central Drug Research Institute, Lucknow, India.

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Hybrids of coumarin-indole: Design, synthesis and biological evaluation in triton WR-1339 and high-fat diet induced hyperlipidemic rat models

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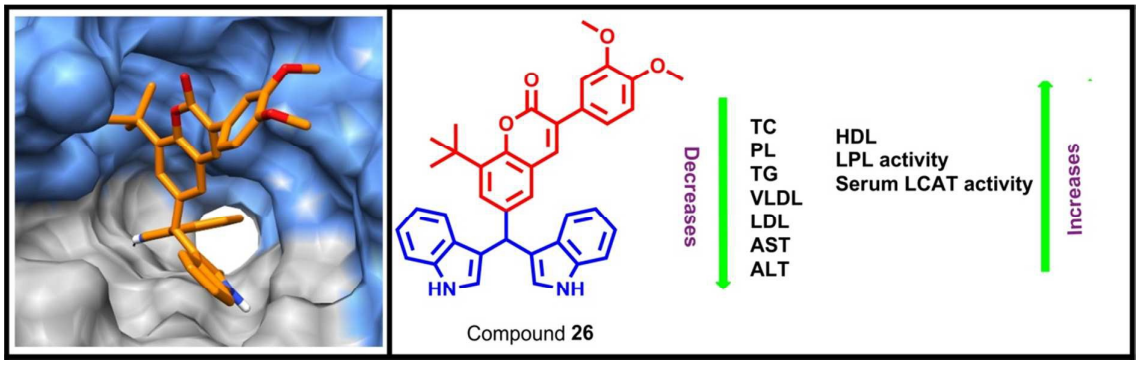

Herein, we have described the *in vitro* and *in vivo* investigations of novel coumarin-indole hybrids and showed their promising lipid lowering activity.