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Indole-Based Fibrates as Potential Hypolipidemic and Antiobesity Agents

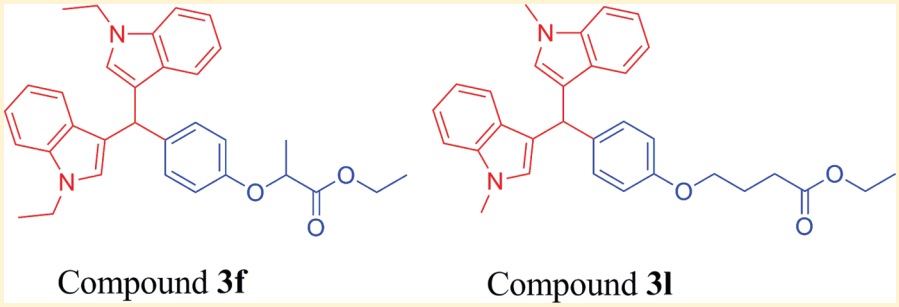
Koneni V. Sashidhara,*[,†] Manoj Kumar,[†] Ravi Sonkar,[‡] Bhanu Shankar Singh,[§] A. K. Khanna,[‡] and Gitika Bhatia[‡]

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

[‡]Biochemistry Division, CSIR-Central Drug Research Institute (CSIR-CDRI), Lucknow 226 001, India

[§]Medicinal Chemistry, National Institute of Pharmaceutical Education and Research (NIPER), Raebareli 229 010, India

Supporting Information



ABSTRACT: Hypolipidemic and antiobesity effects of the newly synthesized indole-based fibrates were evaluated in Triton WR-1339 and high fat diet (HFD)-induced hyperlipidemic rats. Preliminary screening of all the synthesized compounds was done by using an acute model (Triton model), in which compounds **3f** and **3l** showed significant antidyslipidemic activity. Furthermore, these compounds **3f** and **3l** were found to induce significant weight loss in the visceral fat mass of HFD-fed hyperlipidemic rats without affecting the normal feeding behavior. Histological examination of the liver of rats supplemented with **3f** and **3l** revealed a significant decrease in steatosis when compared to the effect of the standard drug fenofibrate. Additional effects such as an increase in lecithin cholesterol acyl-transferase (LCAT) enzyme level and increased receptor mediated catabolism of I^{131}-low density lipoproteins (LDL) confirm and reinforce the efficacy of both of these compounds as a new class of dual-acting hypolipidemic and antiobesity agents.

INTRODUCTION

Obesity has become a serious health problem in the industrialized world.[1] Analysis of worldwide epidemiological data reveals that the frequency of this disorder has gone up steeply, probably because of unhealthy living habits.[2] In addition, it has a huge impact on lifestyle-related disorders such as coronary heart disease, atherosclerosis, and diabetes.[3] Obesity involves an increased visceral fat mass and plasma lipid profile, as well as an increase in body weight. Most marketed antiobesity drugs are appetite suppressants (anorectics), which act directly on the central nervous system (CNS) and decrease food consumption by altering the central adrenergic or the serotonergic system.[4] Despite a rising worldwide epidemic of obesity, there are currently only a very small number of antiobesity drugs available to manage the problem. Orlistat[5] (lipase inhibitor; reduces dietary fat absorption) and sibutramine[6] (appetite suppressant; inhibits reuptake of serotonin and norepinephrine) are the only two Food and Drug Administration (FDA)-approved drugs that are currently available for the long-term treatment of obesity. Both of these drugs are of limited efficacy and are associated with severe side effects.[7] The primary dyslipidemia associated with obesity is characterized by elevated triglycerides, decreased high density lipoproteins (HDL) levels, and irregular LDL composition.[8] Furthermore, an increase in plasma lipids may lead to ischemic heart disease, myocardial infarction, and cerebrovascular incidents. These conditions are responsible for one-third of deaths in industrialized nations. Fibrates have been extensively used for the past several decades in the management of combined dyslipidaemia,[9] but they require high doses to show significant efficacy[10] and are also associated with primary muscle injury, especially when used in combination with a statin.[11,12] A more effective drug having both antiobesity properties and hypolipidemic activity is in great demand.

In the design of new drugs, the development of hybrid molecules through the combination of different pharmacophores may lead to compounds with interesting biological profiles. We have recently reported some coumarin-based hybrids, which have shown diverse biological properties such as anticancer, anti-inflammatory, and antithrombotic activities.[13] A series of coumarin-bis-indole hybrids has also been reported to

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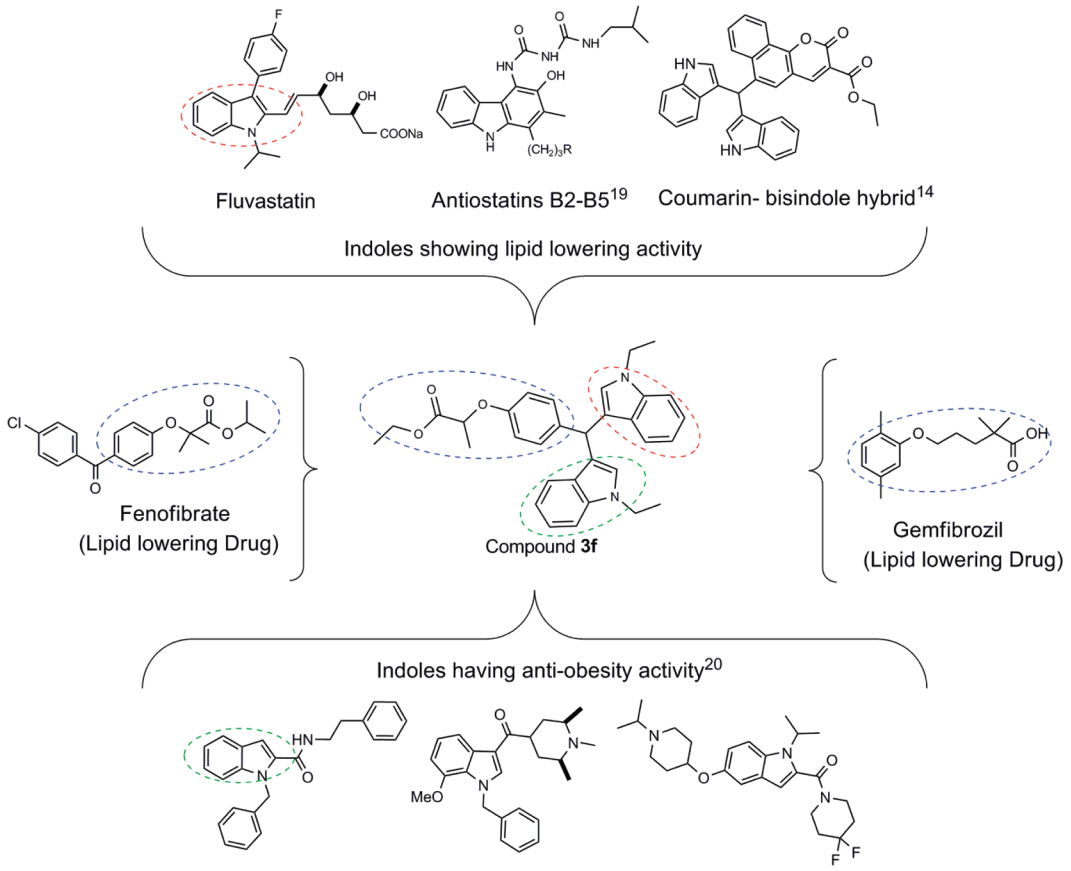

Figure 1. Designing of bis-indole−fibrate hybrids based on indole and fibrate scaffolds showing lipid lowering and antiobesity activity.

have significant antihyperlipidemic activity in HFD-fed hamsters.[14] The indole group is an essential part of a number of compounds with significant biological activity. Indole derivatives are known to exert antitubercular,[15] anticancer,[16,17] antiviral,[18] and antioxidant activities.[19] Fluvastatin [3-hydroxy-3-methyl-glutaryl (HMG)-CoA reductase inhibitor], which includes an indole moiety, is a synthetic member of the statin class of drug, used to lower cholesterol and prevent cardiovascular disease. The indole pharmacophore is also found in a variety of antiobesity agents.[20] Figure 1 shows chemical structures of some bioactive indoles with antiobesity and hypolipidemic activity. Herein, we wish to describe the synthesis of novel indole−fibrate hybrids and their utility as potential antiobesity and antidyslipidemic agents.

RESULTS AND DISCUSSION

The antidyslipidemic activities of bis-indole derivatives **3a−l** were evaluated in an in vivo Triton model.[21] Administration of Triton WR-1339 in rats induced marked hyperlipidemia as evidenced by an increase in the plasma level of total cholesterol (TC, 2.66-fold), phospholipids (PL, 2.36-fold), and triglyceride (TG, 3.02-fold) as compared to the control. Treatment of hyperlipidemic rats with bis-indole derivatives at a dose of 100 mg/kg po reversed the plasma levels of lipid with varying extents (Table 1). Compounds **3f** and **3l** significantly lowered the TC, PL, and TG by 22%, 23%, and 25%, and 20%, 14%, and 27% respectively, while compounds **3a−d**, **3g**, and **3i−k** showed mild activity. By comparison, the standard drug gemfibrozil at the same dose decreased the levels of TC, PL, and TG in plasma by 33%, 35%, and 35%, respectively.

Table 1. Percentage (%) Decrease of Plasma Lipids with the Treatment of Compound (3a−l) in Triton-Induced Hyperlipidemic Rats at the Dose of 100 mg/kg Body Weight[a]

compd	TC	PL	TG
3a	−12*	−25***	−10*
3b	−10*	−12*	−9 NS
3c	−12*	−6 NS	−10*
3d	−10*	−6NS	−8 NS
3e	−9 NS	−5 NS	−8 NS
3f	−22**	−23***	−25***
3g	−10*	−10*	−6 NS
3h	−9 NS	−9 NS	−6 NS
3i	−10*	−9 NS	−5 NS
3j	−11*	−9 NS	−9 NS
3k	−12*	−15*	−12*
3l	−20**	−14*	−27***
gemfibrozil	−33***	−35***	−35***

[a]Units are mean ± SD of six rats. The Triton-treated group was compared with the control group, and Triton plus compound-treated group was compared with the triton group only. ***$P < 0.001$. **$P < 0.01$. *$P < 0.05$ and NS = not significant.

With these promising activities in hand, dose-dependent (at 50, 100, 150 mg/kg body weight) studies on compounds **3f** and **3l** were performed on a different set of experimental animals, which exhibited concentration-dependent effects. Both compounds showed similar activity patterns, and at a dose of 150 mg/kg body weight, **3f** and **3l** showed activities comparable to those of the standard drug gemfibrozil (Table 2).

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Table 2. Dose−Response Effects of Compounds 3f and 3l in Triton-Induced Hyperlipidemic Rats on Different Lipid Parameters and Post-Heparin Lipolytic and Lecithin Cholesterol Acyl-Transferase Activities[a]

compd	dose	TC[b]	Pl[b]	TG[b]	PHLA[c]	LCAT[d]
Triton					−29***	−37***
3f	50 mg/kg	−16*	−15*	−12*	+11*	+8*
	100 mg/kg	−22**	−23***	−25***	+12*	+12*
	150 mg/kg	−33***	−29***	−26***	+15*	+18*
3l	50 mg/kg	−15*	−16*	−13*	+10*	+11*
	100 mg/kg	−20**	−14**	−27***	+15*	+15*
	150 mg/kg	−35***	−29***	−27***	+20*	+21*
gemfibrozil	100 mg/kg	−35***	−31***	−31***	+27***	+27***

[a]Units are the mean ± SD of six rats. Triton-treated group compared with the control group and triton plus compound-treated group compared with the Triton group only. ***P < 0.001. **P < 0.01. *P < 0.05. [b]Unit: mg/dL. [c]Unit: nmol cholesterol released/h/L plasma. [d]Unit: nmol free fatty acid formed/h/mL plasma.

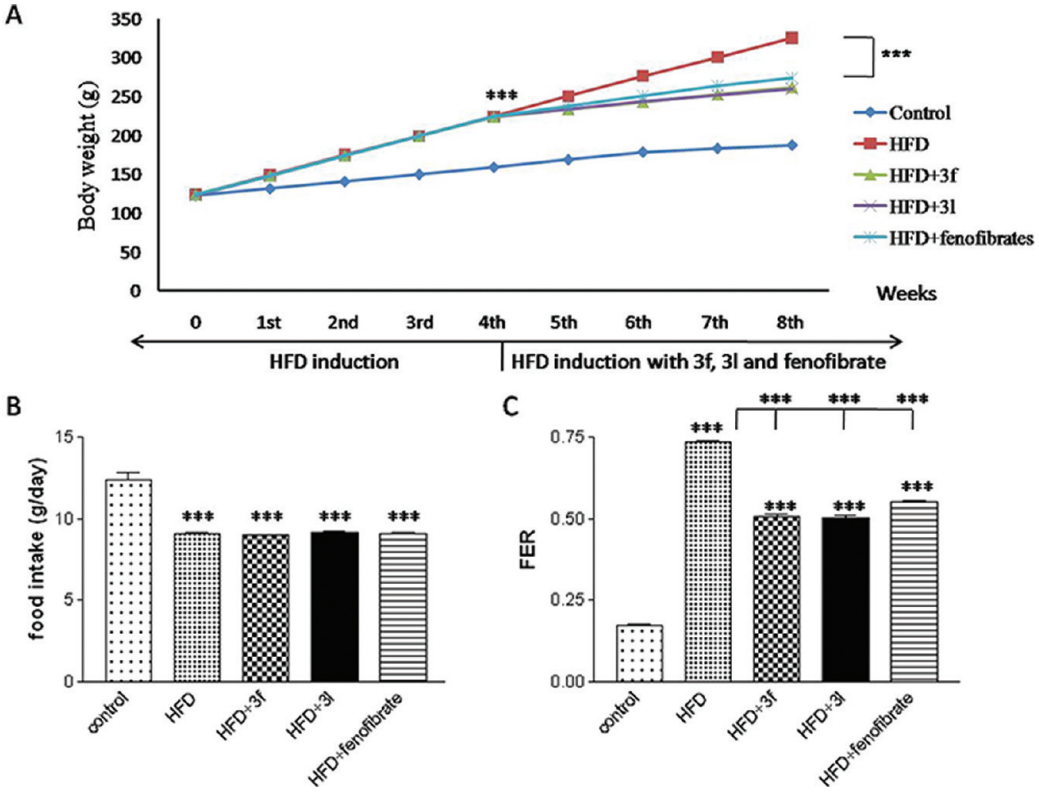

Figure 2. Effects of compounds **3f** and **3l** on body weight gain, food intake, and food efficiency ratio of HFD-fed hyperlipidemic rats Animals were sacrificed after 8 weeks of ND and HFD consumption with or without **3f**, **3l**, and fenofibrate (100 mg/kg/d). (A) Change in body weight gain (g), (B) food intake, and (C) FER. Values are expressed as the mean ± SD (n = 6). **P < 0.01; ***P < 0.001. FER = (body weight gain for the experimental period (g)/food intake for the experimental period (g)).

It is known that the LCAT enzyme converts free cholesterol into cholesteryl ester (a more hydrophobic form of cholesterol), which is subsequently converted into HDL. Compounds **3f** and **3l** showed this beneficiary effect as each significantly increased the level of LCAT enzyme in Triton-treated hyperlipidemic rats in a concentration-dependent manner. Furthermore, both compounds also increased post heparin lipolytic activities as shown in Table 2. Since compounds **3f** and **3l** showed significant activity in the Triton-induced hyperlipidemic model, these two compounds were further tested in the HFD-fed hyperlipidemic model, which is similar in feeding behavior to humans. Several biological experiments were carried out such as the study of their effects on lipolytic enzymes, hepatic biochemical parameters, specific binding of I^{125}-LDL, and fecal bile acid excretions in HFD-induced hyperlipidemic rats, and the results of various studies are discussed next.

Effects of 3f and 3l on Body Weight and Visceral Fat Mass of HFD-Induced Hyperlipidemic Rats. Adult Charles Foster rats were given HFD (to induce hyperlipidemia) or normal diet (ND) for four weeks. Subsequently, the ND rats were given ND + vehicle, and HFD-fed rats were divided into four groups and given HFD + vehicle, HFD + **3f**, and HFD + **3l**, and HFD + fenofibrate were given treatment for another four weeks. HFD increased the body weight in rats, but supplementing the HFD rats with either **3f** or **3l** (100 mg/kg/d) suppressed the gain in body weight without affecting the food intake, comparable in effect to the standard drug fenofibrate (Figure 2). The food efficiency ratio in the HFD + **3f** and HFD + **3l** groups was significantly lower than that in

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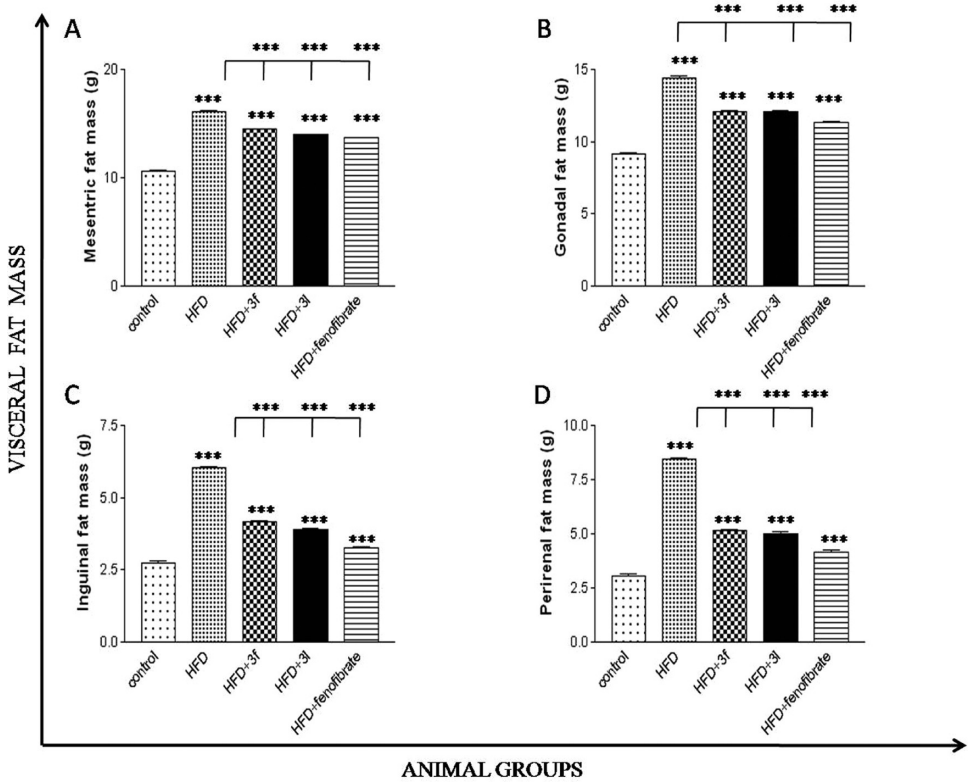

Figure 3. Effects of **3f** and **3l** on the visceral fat-pad weights of HFD-fed hyperlipidemic rats. Animals were sacrificed after 8 weeks of ND and HFD consumption with or without **3f**, **3l**, and fenofibrate (100 mg/kg/d). Visceral fat-pad weights in each group are shown. (A) Mesenteric, (B) gonadal, (C) inguinal, and (D) perirenal fat mass. Values are expressed as the mean ± SD (n = 6). $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 3. Effects of Fenofibrate Derivatives and Fenofibrates on Serum Lipids in HFD Induced Hyperlipidemic Rats[a]

parameters	control	HFD	HFD + **3f**	HFD + **3l**	HFD + fenofibrate
TC (mg/dL)	85.59 ± 7.66	240.39 ± 13.69*** (+2.80-fold over control)	180.49 ± 13.26*** (−25% over HFD)	173.46 ± 12.26*** (−28% over HFD)	153.05 ± 10.21*** (−36% over HFD)
PL (mg/dL)	81.85 ± 6.43	221.65 ± 6.38*** (+2.70-fold over control)	168.35 ± 10.25*** (−24% over HFD)	166.66 ± 9.17*** (−25% over HFD)	146.35 ± 6.38*** (−34% over HFD)
TG (mg/dL)	88.53 ± 9.67	350.37 ± 17.35*** (+3.95-fold)	262.30 ± 10.75*** (−25% over HFD)	256.65 ± 10.79*** (−27% over HFD)	233.89 ± 7.14*** (−33% over HFD)
LCAT (nmol cholesterol released/h/L plasma)	66.82 ± 3.04	37.06 ± 1.82*** (−41% over control)	54.57 ± 3.94*** (+32% over HFD)***	51.95 ± 4.40 (+29% over HFD)	56.44 ± 3.70*** (+34% over HFD)

[a]Each parameter represents pooled data from 6 rats/group, and values are expressed as the mean ± SD. $^{***}P < 0.001$, HFD group compared with the control group and HFD plus compound/fenofibrate groups compared with HFD.

the HFD group (Figure 2). The relative weights of the mesenteric, gonadal, perirenal, and inguinal fat-pads in HFD + **3f** and HFD + **3l** rats were significantly lower than those in the HFD-fed rats (Figure 3).

Effects of 3f and 3l on the Lipid Profile and LCAT Activity in HFD-Induced Hyperlipidemic Rats. The administration of HFD to rats increased their plasma levels of TC (2.8-fold), PL (2.7-fold), and TG (3.95-fold) followed by a decrease in LCAT activity (41%) as compared to control rats. As shown in Table 3, feeding of **3f** and **3l** significantly reversed the increased levels of TC, PL, and TG. Furthermore, compound **3f** increased the level of LCAT by 32%, whereas fenofibrate increased the level by 34% (Table 3).

Effects of 3f and 3l on the Lipid Composition in Serum Lipoproteins of HFD-Induced Hyperlipidemic Rats. As shown in Table 4, the analysis of hyperlipidemic serum of HFD administered rats showed marked increase in the level of lipids and apoproteins constituting β-lipoproteins, and these effects were pronounced for very low density lipoprotein (VLDL-TG, 2.18-fold) and low-density lipoprotein total cholesterol (LDL-TC, 4.08-fold). Treatment with **3f** and **3l** significantly reduced the increased levels of VLDL-TG (24% and 17%, respectively) as well as LDL-TC (24% and 22%), PL (25% and 20%), TG (25% and 21%), and apo-LDL (25% and 21%), respectively, in hyperlipidemic rats. Concurrently, the decreased levels of HDL lipid and apo-HDL in these animals were partially recovered (Table 4).

Effects of 3f and 3l on Hepatic Biochemical Parameters in HFD-Induced Hyperlipidemic Rats. As shown in Table 5, HFD administration to rats also caused marked accumulation of TC (2.16-fold), PL (1.92-fold), and TG (1.89-fold), followed by a decrease in LPL activity (39%) in liver tissue. However, treatment with **3f** and **3l** caused a decrease in the levels of TC (23% and 26%, respectively), PL (28% and 23%), and TG (27% and 24%), followed by reactivation of LPL activity (20% and 14%), respectively, in hyperlipidemic animals.

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Table 4. Effects of Bis-Indole Derivatives and Fenofibrate on Blood Lipids and Lipolytic Enzymes in HFD Induced Hyperlipidemic Rats[a]

parameters (mg/dL plasma)	control	HFD	HFD + 3f	HFD + 3l	HFD + fenofibrate
VLDL-TC	7.99 ± 1.20	25.77 ± 0.83*** (3.68-fold over control)	20.22 ± 1.34** (−21% over HFD)	21.99 ± 1.20* (−15% over HFD)	18.57 ± 0.68*** (−28% over HFD)
VLDL-PL	14.67 ± 1.24	35.01 ± 2.48*** (2.38-fold over control)	29.33 ± 1.98* (−16% over HFD)	31.10 ± 2.50* (−11% over HFD)	26.61 ± 3.53*** (−24% over HFD)
VLDL-TG	38.00 ± 1.83	83.2 ± 2.88*** (2.18-fold over control)	62.93 ± 2.57*** (−24% over HFD)	69.06 ± 4.40* (−17% over HFD)	58.13 ± 2.01*** (−30% over HFD)
apoprotein	6.12 ± 0.87	13.18 ± 0.88*** (2.15-fold over control)	9.68 ± 1.06*** (−26% over HFD)	8.85 ± 0.90*** (−33% over HFD)	8.80 ± 0.56*** (−35% over HFD)
LDL-TC	0.56 ± 1.00	52.59 ± 3.00*** (4.08-fold over control)	39.72 ± 0.86*** (−24% over HFD)	40.76 ± 1.17** (−22% over HFD)	37.73 ± 0.48*** (−28% over HFD)
LDL-PL	12.30 ± 0.72	43.92 ± 2.05*** (3.57-fold over control)	33.09 ± 2.35*** (−25% over HFD)	35.04 ± 2.40* (−20% over HFD)	31.52 ± 0.85*** (−28% over HFD)
LDL-TG	14.47 ± 1.49	36.03 ± 1.33*** (3.57-fold over control)	28.31 ± 0.98** (−21% over HFD)	26.89 ± 1.42*** (−25% over HFD)	26.22 ± 0.55*** (−27% over HFD)
apoprotein	16.92 ± 2.08	29.88 ± 2.08*** (1.76-fold over control)	22.36 ± 1.49*** (−25% over HFD	23.55 ± 1.49** (−21% over HFD)	21.37 ± 1.18*** (−28% over HFD)
HDL-TC	44.32 ± 3.22	34.08 ± 4.46** (−19% over control)	39.71 ± 3.28* (+14% over HFD)	38.57 ± 3.67* (+12% over HFD)	40.60 ± 3.26* (+16% over HFD)
HDL-PL	36.47 ± 2.35	29.55 ± 2.01*** (−23% over control)	33.98 ± 1.56* (+13% over HFD	32.28 ± 1.95^{NS} (+8% over HFD)	34.95 ± 1.56* (+15% over HFD)
HDL-TG	15.48 ± 1.72	11.60 ± 1.43*** (−25% over control)	13.10 ± 1.72* (+11% over HFD)	13.46 ± 1.60* (+14% over HFD)	13.82 ± 1.71* (+16% over HFD)
apoprotein	166.71 ± 5.56	28.35 ± 11.96*** (−23% over control)	152.48 ± 6.03* (+16% over HFD)	149.42 ± 5.75* (+14% over HFD)	155.64 ± 5.04* (+17% over HFD)

[a]Each parameter represents pooled data from 6 rats/group, and values are expressed as the mean ± SD. *P < 0.05. **P < 0.01. ***P < 0.001, HFD group compared with the control group and HFD plus compound/fenofibrate groups compared with HFD.

Effects of 3f and 3l on Liver Histopathology of HFD-Induced Rats. Figure 4 shows the effects of **3f** and **3l** on the hepatic morphology of rats fed with a high-fat diet. The control group showed normal hepatic histology. HFD administration caused macrovesicular steatosis in the centrilobular area, but liver fibrosis was not observed. The extent of steatosis was considerably reduced in rats fed with HFD plus either **3f** or **3l** (Figure 4).

Effects of Bis-Indole Derivatives and Fenofibrate on I^{125}-LDL Catabolism in Liver Plasma Membrane of HFD-Induced Hyperlipidemic Rats. Administration of HFD suppressed the specific binding of I^{125}-LDL in the liver plasma membrane by 54%. Compounds **3f** and **3l**, and fenofibrate reversed the receptor-mediated catabolism of LDL (24%, 18%, and 30%, respectively) in treated animals (Figure 5).

Effects of 3f and 3l on Fecal Excretion of Bile Acids. HFD administration to rats caused a significant decrease in the fecal excretion of cholic acid (41%) and deoxycholic acid (46%) over control, and these levels were shown to be recovered by the treatment with **3f** (26% and 21%) and **3l** (28% and 31%) in HFD-treated rats (Table 6).

Preliminary screening of bis-indole hybrids on Triton WR-1339-treated hyperlipidemic rats resulted in two promising compounds, **3f** and **3l**, in comparison to gemfibrozil by improving the lipid profile and enzymatic activity of plasma LPL and LCAT. Further studies on these two compounds were carried out using HFD-induced hyperlipidemic rats. The HFD-induced hyperlipidemic rat model is more suitable for study because the rats consume food and water ad libitum, similar to human eating patterns, rather than being force-fed. The assayed period of high-energy feeding induces a marked weight increase in rats. In this study, we have found that HFD feeding for four weeks resulted in hyperlipidemia, which was associated with increased body weight. Moreover, administration of **3f** and **3l** for the next four weeks significantly reduced body weight and visceral fat mass, which indicates the degradation of adipose tissue or inhibition of its formation. These beneficial effects of **3f** and **3l** were not because of decreased food intake, as the amount of food consumed per animal was unchanged. Additionally, the in vivo examined compounds did not show any toxic effects at the doses studied, and almost all the animals survived and looked normal both macroscopically and by autopsy, demonstrating low general toxicity of the synthesized compounds.

Dyslipidemia in obesity, characterized by increased triglycerides and LDL, and decreased HDL, is the most important factor that leads to coronary artery diseases.[22] In our study, **3f** and **3l** supplementation decreased the plasma TC, PL, and TG levels in HFD-fed rats, possibly by inhibition in of their exogenous absorption and formation. Compound **3f** and **3l** also increased the LCAT activity, which plays a key role in lipoprotein metabolism contributing to an increased level of HDL.

Increased endogenous production of TG-enriched hepatic VLDL, and decreased TG uptake in the peripheral tissues is the secondary factor contributing to hyperlipidemia in HFD rats.[23] TG enhances the ectopic accumulation of lipid stores in the liver and is associated with a number of diseases such as metabolic syndrome and type 2 diabetes.[24] High TC levels increase the risk of developing coronary heart disease (CHD), and high levels of LDL and VLDL are also a risk factor for CHD, while high HDL is helpful in transporting excess cholesterol to the liver for excretion in bile.[25]

The endogenous effects of **3f** and **3l** on lipid metabolism include increased receptor-mediated catabolism of LDL (Figure 6)[26] as well as in lipolytic activity of the liver and in the level of plasma HDL-TC, with a decrease of β-lipoprotein-lipid and improved hepatic lipid profile.

Compounds **3f** and **3l** enhanced the synthesis of LDL-apoprotein (Apo-B) as well as receptor protein to accelerate the

Table 5. Effects of Bis-Indole Derivatives and Fenofibrate on Hepatic Biochemical Parameters in HFD Induced Hyperlipidemic Rats[a]

parameters	control	HFD	HFD+3f	HFD+3l	HFD+fenofibrate
LPL activity (nmol free fatty acid formed/h/mg protein)	127.89 ± 14.35	77.49 ± 7.05*** (−39% over control)	97.10 ± 7.05** (+20% over HFD)	89.63 ± 8.40* (+14% over HFD)	104.57 ± 9.83*** (+26% over HFD)
TC (mg/g)	6.76 ± 0.84	14.76 ± 0.41*** (2.16-fold over control)	11.27 ± 0.96*** (−23% over HFD)	10.77 ± 0.79*** (−26% over HFD)	9.21 ± 1.00*** (−37% over HFD)
PL (mg/g)	22.77 ± 2.09	43.88 ± 2.09*** (1.92-fold over control)	31.52 ± 1.87*** (−28% over HFD)	33.88 ± 3.36*** (−23% over HFD)	29.44 ± 2.09*** (−33% over HFD)
TG (mg/g)	10.86 ± 1.20	20.54 ± 2.34*** (1.89-fold over control)	14.9 ± 1.70*** (−27% over HFD)	15.6 ± 1.58*** (−24% over HFD)	13.56 ± 1.45*** (−34% over HFD)

[a]Each parameter represents pooled data from 6 rats/group, and values are expressed as the mean ± SD. *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$, HFD group compared with the control group and HFD plus compound/fenofibrate groups compared with HFD.

turnover of cholesterol.[27] It also decreased the VLDL levels in plasma, which is mainly synthesized in the liver and is a carrier for various lipids. In addition, compounds **3f** and **3l** increased LPL levels in plasma, which catalyzes the hydrolysis of various lipids on lipoprotein particles such as chylomicrons and VLDL and turns them into smaller remnants that are rapidly cleared from the bloodstream. The decreased lipid levels in the liver were also complemented by increased bile acid secretion in feces.

In the liver, HFD increases fatty acid synthesis and facilitates the movement of free fatty acids from plasma.[28] It also decreases β-oxidation of free fatty acids, which may, in turn, cause TG accumulation in the liver.[29,30] The higher level of TG in hepatocytes causes cellular dysfunction and may damage the liver parenchyma.[31] On giving HFD, macrovasicular dysfunction (fatty degeneration) occurs in the liver. The risk factors from steatosis in the liver are diverse and are frequently associated with obesity since the liver is the primary organ for lipid metabolism. From our study, we have found that in HFD groups liver macrovasicular steatosis arises, but administration of compounds **3f** and **3l** to HFD-fed animals noticeably lowered the extent of steatosis (Figure 4). These results may indicate that compounds **3f** and **3l** could be beneficial for patients with hepatic steatosis.

CONCLUSIONS

In conclusion, a novel series of bis-indole based fibrates was synthesized and evaluated for their antihyperlipidemic and antiobesity potentials in two animal models. Compounds **3f** and **3l** emerged as promising agents for both dyslipidemia and obesity as these compounds significantly modulated blood plasma lipids and reduced visceral body masses without affecting the feeding pattern of the animals. Additional studies such as an increase in LCAT enzyme level and increased receptor mediated catabolism of I^{131}-LDL confirm and reinforce the efficacy of both these compounds as a new class of dual acting hypolipidemic and antiobesity agents. Furthermore, the compound supplement did not apparently adversely affect the animals. Compounds **3f** and **3l** seem to be good candidates for the development of a new type of dual-acting drug.

EXPERIMENTAL SECTION

General. 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), and silica gel plates with fluorescence F_{254} were used. Melting points were taken in open capillaries on a 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 Bruker Supercon Magnet DPX-200 and DRX-300 spectrometers (operating at 200 and 300 MHz for ^{1}H and 75 MHz for ^{13}C) using $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard. Chemical shifts are reported in parts per million. Electrospray ionization mass spectra (ESIMS) were recorded on Thermo Lcq Advantage Max-IT. High resolution mass spectra (HRMS) were recorded on 6520 Agilent Q Tof LC MS/MS (Accurate mass). Elemental analyses were performed on a Vario EL-III C, H, N, S analyzer (Germany), and values were within ±0.5% of the calculated values; therefore, these compounds meet the criteria of >95% purity. Additionally, purity of one of the key compounds was measured by a reverse phase HPLC with the following conditions, and purity of the compound was >95%. Analytical HPLC was performed using a C18 reverse phase column

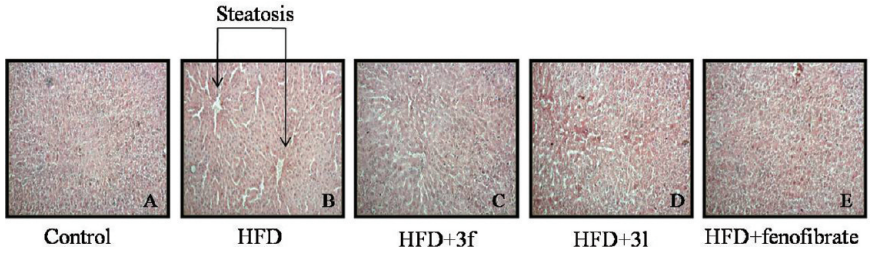

Figure 4. Histological findings of the livers of rats supplemented with or without **3f** and **3l**. In the HFD group, macrovesicular steatosis is present in the centrilobular area (H&E, 200×), whereas steatosis (H&E, 200×) decreases in the **3f**, **3l**, and fenofibrate supplemented HFD-fed rat group.

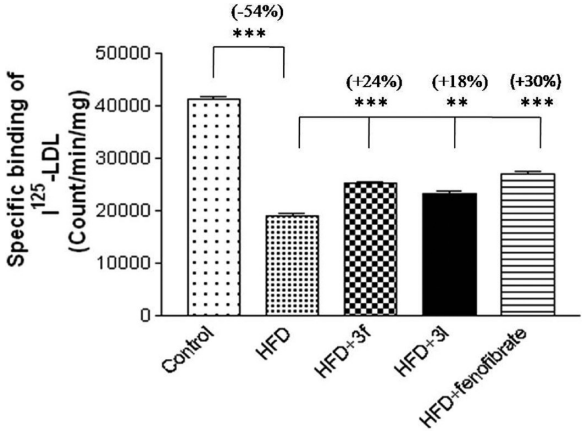

Figure 5. Effects of bis-indole derivatives and fenofibrate I^{125}-LDL catabolism in the liver plasma membrane of HFD-induced hyperlipidemic rats. Animals were sacrificed after 8 weeks of ND and HFD consumption with or without **3f**, **3l**, and fenofibrate (100 mg/kg/d). Compounds **3f** and **3l**, and fenofibrate increase the I^{125}-LDL catabolism in the plasma membrane of the liver in HFD-inducd hyperlipidemic rat. Values are expressed as the mean ± SD (n = 6). $^{**}P < 0.01$; $^{***}P < 0.001$.

(4.6 × 259 mm; Waters 5 μM) on a Merck Hitachi D-7000 instrument.

HPLC Conditions. Method: Isocratic solution of acetonitrile (80%) and 1% acetic acid–water; flow rate of 0.5 mL/min for 25 min; and was detected at UV 254 nm.

The route followed for the preparation of bis-indole derivatives from **1a–e** and **2a–d** is outlined in Scheme 1. Reaction of 4-hydroxybenzaldehyde with appropriate bromo esters in the presence of K_2CO_3 in acetonitrile under reflux conditions furnished substituted benzaldehydes (**2a–d**). An efficient electrophilic substitution of suitable indoles (**1a–e**) with these substituted benzaldehydes derivatives (**2a–d**) using catalytic iodine in acetonitrile at room temperature furnished final bis-indole-fibrate hybrids (**3a–l**) in good to excellent yields. The structures of the compounds were substantiated by ^{1}H NMR, ^{13}C NMR, mass spectrometry, and IR spectroscopy. The purity of these compounds was ascertained by TLC and spectral analysis, and furthermore, the purity of one of the most active compounds (**3l**) was determined by HPLC (see Supporting Information).

*Ethyl 2-(4-Formylphenoxy)acetate (**2a**).* To a mixture of 4-hydroxybenzaldehyde (12.3 mmol, 1.5 g), ethylbromoacetate (12.2 mmol, 2.05 g), and K_2CO_3 (24.6 mmol, 3.4 g) was added acetonitrile (50 mL). The reaction mixture was refluxed for 3 h. After completion of the reaction, K_2CO_3 was removed in a sintered funnel, and the filtrate was concentrated under vacuum and subjected to column chromatography (silica gel 100–200 mesh, EtOAc/hexane) to give pure **2a** as a colorless liquid.

Following the above procedure, other derivatives of 4-hydroxybenzaldehyde were synthesized. The structures of known intermediates were ascertained by spectroscopic methods and by comparison with the reported literature (**1a–e** and **2a–d**).[32,33]

*Ethyl 4-(4-(Bis(1-methyl-1H-indol-3-yl)methyl)phenoxy)-butanoate (**3l**).* A mixture of ethyl 4-(4-formylphenoxy)butanoate (**2d**) (1.1 mmol, 0.24 g), *N*-methylindole (**1c**) (2.2 mmol, 0.3 g), and I_2 (53.83 mg, 0.21 mmol) in acetonitrile (20 mL) was stirred at room temperature for 30 min. After completion of the reaction, the mixture treated with aq. $Na_2S_2O_3$ solution (5%, 10 mL) and the product was extracted with $CHCl_3$ (3 × 25 mL). The combined organic layers were dried with anhydrous sodium sulfate, concentrated in vacuo, and purified by column chromatography to afford **3l**.

The compounds (**3a–l**) were prepared in a manner similar to the procedure described above.

*Ethyl 2-(4-(Di(1H-indol-3-yl)methyl)phenoxy)acetate (**3a**).* Light brown gum; yield, 85%. IR (KBr): 3403, 3016, 1691, 1592, 1067 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.74 (brs, 2H), 7.32 (d, 2H, J = 7.9 Hz), 7.22–7.07 (m, 6H), 6.97–6.92 (m, 2H), 6.70 (d, 2H, J = 8.6 Hz), 6.39 (s, 1H), 6.38 (s, 1H), 5.75 (s, 1H), 4.50 (s, 2H), 4.21 (q, 2H, J = 7.1 Hz), 1.24 (t, 3H, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): 169.5, 156.2, 137.5, 136.7, 129.8, 127.1, 123.8, 121.8, 119.9, 119.6, 119.1, 114.4, 111.3, 65.6, 61.5, 39.3, 14.2. ESI-MS: (m/z) 423 $(M - H)^+$. Anal. Calcd for $C_{27}H_{24}N_2O_3$: C, 76.39; H, 5.70; N, 6.60. Found: C, 76.31; H, 5.81; N, 6.53.

*Ethyl 2-(4-(Bis(1-methyl-1H-indol-3-yl)methyl)phenoxy)acetate (**3b**).* Light brown gum; yield, 87%. IR (KBr): 3011, 1695, 1598, 1069 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.36 (d, 2H, J = 9.0 Hz), 7.28–7.15 (m, 6H), 7.00–6.95 (m, 2H), 6.80 (d, 2H, J = 8.6 Hz), 6.50 (s, 2H), 5.82 (s, 1H), 4.57 (s, 2H), 4.25 (q, 2H, J = 7.1 Hz), 3.65 (s, 6H), 1.27 (t, 3H, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): 169.2, 156.3, 137.9, 137.5, 129.8, 128.3, 127.5, 121.5, 120.1, 118.7, 118.5, 114.5, 109.2, 65.7, 61.4, 39.3, 32.7, 14.3. ESI-MS: (m/z) 451 (M –

Table 6. Effects of Fenofibrate Derivatives and Fenofibrates on Fecal Bile Acid Excretion in HFD Induced Hyperlipidemic Rats[a]

parameters (μg/g)	control	HFD	HFD+3f	HFD+3l	HFD+fenofibrate
cholic acid	81.90 ± 8.53	48.25 ± 6.69*** (−41%over control)	65.04 ± 3.93 ** (+26% over HFD)	60.82 ± 4.86 ** (+21%over HFD)	69.74 ± 3.71*** (+31% over HFD)
deoxycholic acid	51.82 ± 3.06	27.82 ± 3.11*** (−46%over control)	38.91 ± 4.07*** (+28%over HFD)	40.25 ± 3.38 *** (+31%over HFD)	1.76 ± 3.47*** (+33% over HFD)

[a]Each parameter represents pooled data from 6 rats/group, and values are expressed as the mean ± SD. $^{**}P < 0.01$. $^{***}P < 0.001$, HFD group compared with the control group and HFD plus compound/fenofibrate groups compared with HFD.

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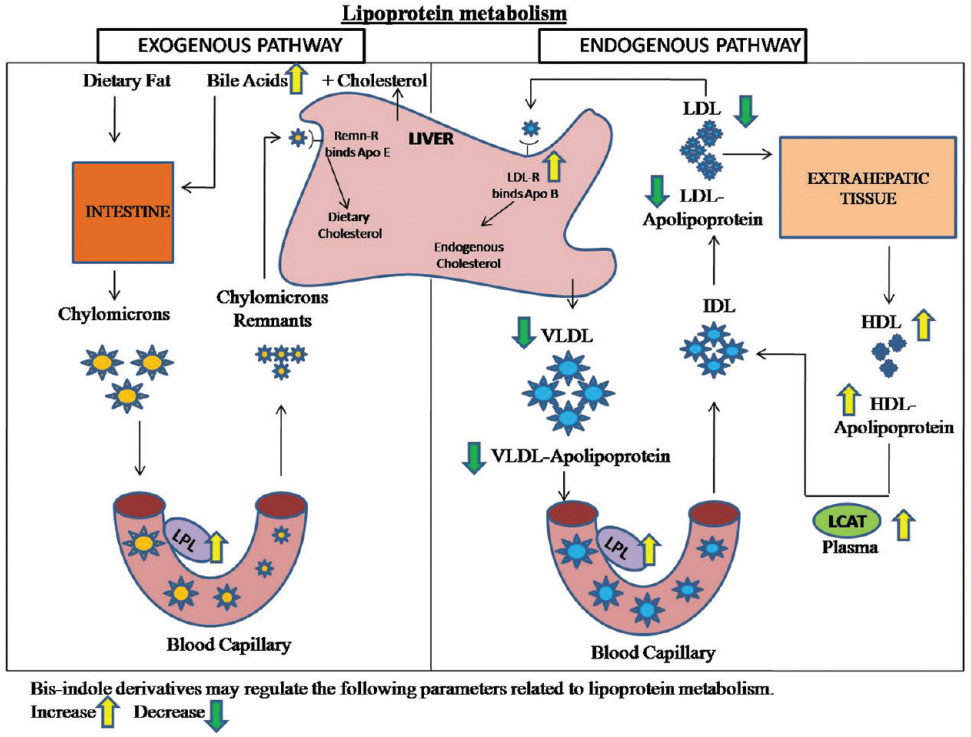

Figure 6. Effects of compounds **3f** and **3l** on lipoprotein metabolism, LCAT, LPL, and LDR receptor binding in HFD-induced rats.

H)$^+$. Anal. Calcd for $C_{29}H_{28}N_2O_3$: C, 76.97; H, 6.24; N, 6.19. Found: C, 76.82; H, 6.15; N, 6.24.

*Ethyl 2-(4-(Bis(1-ethyl-1H-indol-3-yl)methyl)phenoxy)acetate (**3c**).* Light brown solid; yield, 93%; mp 120−121 °C. IR (KBr): 3016, 1691, 1592, 1067 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.36−7.22 (m, 6H), 7.18−7.13 (m, 2H), 6.98−6.93 (m, 2H), 6.80 (d, 2H, J = 8.3 Hz), 6.56 (brs, 2H), 5.82 (s, 1H), 4.56 (brs, 2H), 4.24 (q, 2H, J = 7.1 Hz), 4.02 (q, 4H, J = 7.2 Hz), 1.34 (t, 6H, J = 7.2 Hz), 1.26 (t, 3H, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): 169.3, 156.3, 138.0, 136.5, 129.8, 127.7, 126.7, 121.3, 120.3, 118.6, 118.6, 114.5, 109.2, 65.8, 61.4, 40.9, 39.5, 15.6, 14.3. ESI-MS: (m/z) 479 (M − H)$^+$. Anal. Calcd for $C_{31}H_{32}N_2O_3$: C, 77.47; H, 6.71; N, 5.83. Found: C, 77.53; H, 6.75; N, 5.88.

*Ethyl 2-(4-(Bis(1-methyl-1H-indol-3-yl)methyl)phenoxy)-propanoate (**3d**).* Light brown solid; yield, 87%; mp 115−116 °C. IR (KBr): 3011, 1697, 1588, 1050 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.35 (d, 2H, J = 7.9 Hz), 7.28−7.15 (m, 6H), 6.99−6.95 (m, 2H), 6.78 (d, 2H, J = 8.6 Hz), 6.49 (brs, 2H), 5.81 (s, 1H), 4.69 (q, J = 8.6 Hz, 1H), 4.23−4.14 (m, 2H), 3.65 (brs, 6H), 1.58 (d, 3H, J = 6.8 Hz), 1.22 (t, 3H, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): 172.5, 156.1, 137.7, 137.5, 129.7, 128.3, 127.5, 121.5, 120.2, 118.7, 118.6, 115.0, 109.1, 61.3, 39.3, 32.7, 18.7, 14.2. ESI-MS: (m/z) 465 (M − H)$^+$. Anal. Calcd for $C_{30}H_{30}N_2O_3$: C, 77.23; H, 6.48; N, 6.00. Found: C, 77.32; H, 6.55; N, 5.91.

*Ethyl 2-(4-(Bis(2-methyl-1H-indol-3-yl)methyl)phenoxy)-propanoate (**3e**).* Light brown solid; yield, 92%; mp 185−186 °C. IR (KBr): 3390, 3016, 1691, 1595, 1045 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.65 (s, 2H), 7.23−7.12 (m, 5H), 7.03−6.95 (m, 4H), 6.85−6.80 (m, 2H), 6.75 (d, 2H, J = 8.7 Hz), 5.90 (s, 1H), 4.70 (q, 1H, J = 6.9 Hz), 4.24−4.15 (m, 2H), 1.99 (s, 6H), 1.59 (d, 3H, J = 6.8 Hz), 1.22 (t, 3H, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): 172.6, 156.0, 137.0, 135.2, 131.9, 130.1, 129.0, 120.7, 119.5, 119.1, 115.0, 113.7, 110.1, 61.3, 38.6, 18.7, 14.3, 12.5. ESI-MS: (m/z) 465 (M − H)$^+$. Anal. Calcd for $C_{30}H_{30}N_2O_3$: C, 77.23; H, 6.48; N, 6.00. Found: C, 77.29; H, 6.41; N, 6.05.

*Ethyl 2-(4-(Bis(1-ethyl-1H-indol-3-yl)methyl)phenoxy)-propanoate (**3f**).* Light brown solid; yield, 90%; mp 112−113 °C. IR (KBr): 3008, 1684, 1593, 1041 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.36−7.28 (m, 4H), 7.23−7.13 (m, 4H), 6.98−6.93 (m, 2H), 6.78 (d, 2H, J = 8.7 Hz), 6.55 (brs, 2H), 5.80 (s, 1H), 5.80 (s,1H), 4.70 (q, 1H, J = 6.7 Hz), 4.25−4.14 (m, 2H), 4.04 (q, 4H, J = 7.2 Hz), 1.59 (d, 4H, J = 6.8 Hz), 1.35 (t, 6H, J = 7.2 Hz), 1.22 (t, 3H, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): 172.6, 156.0, 137.8, 136.5, 129.8, 127.8, 126.7, 121.3, 120.4, 118.6, 115.0, 109.2, 61.3, 40.9, 39.5, 18.7, 15.6, 14.3. ESI-MS: (m/z) 493 (M − H)$^+$. HRMS m/z calcd for $C_{32}H_{34}N_2O_3$ (M − H)$^+$ 493.2490; found, 493.2478.

*Ethyl 2-(4-(Bis(1,2-dimethyl-1H-indol-3yl)methyl)phenoxy)-propanoate (**3g**).* Light brown gum; yield, 89%. IR (KBr): 2980, 1684, 1589, 1038 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.21−7.01 (m, 6H), 6.89−6.88 (m, 2H), 6.82−6.73 (m, 4H), 5.97 (s, 1H), 4.70 (q, 1H, J = 6.9 Hz), 4.22−4.15 (m, 2H), 3.60 (brs, 6H), 2.12 (brs, 6H), 1.58 (d, 3H, J = 6.8 Hz), 1.24−1.19 (m, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): 172.5, 155.9, 137.3, 136.7, 133.6, 130.1, 127.9, 120.1, 119.7, 118.7, 114.9, 113.3, 108.4, 61.3, 39.1, 29.8, 29.5, 18.7, 14.3, 10.8. ESI-MS: (m/z) 493 (M − H)$^+$. Anal. Calcd for $C_{32}H_{34}N_2O_3$: C, 77.70; H, 6.93; N, 5.66. Found: C, 77.78; H, 6.86; N, 5.75.

*Ethyl 2-(4-(di(1H-indol-3-yl)methyl)phenoxy)-2-methylpropa-noate (**3h**).* Light brown gum; yield, 85%. IR (KBr): 3404, 3002, 1696, 1590, 1034 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.84 (s, 2H), 7.14 (d, 2H, J = 7.9 Hz), 7.34−7.28 (m, 2H), 7.23−7.17 (m, 4H), 7.07−7.01 (m, 2H), 6.82 (d, 2H, J = 8.5 Hz), 6.52 (d, 2H, J = 1.6 Hz), 5.85 (s, 1H), 4.26 (q, 2H, J = 7.1 Hz), 1.64 (brs, 6H), 1.28 (t, 3H, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): 174.6, 153.7, 138.1, 136.7, 129.4, 127.1, 123.8, 121.9, 120.0, 119.3, 119.1, 111.2, 61.5, 39.5, 25.5, 14.2. ESI-MS: (m/z) 451 (M − H)$^+$. Anal. Calcd for $C_{29}H_{28}N_2O_3$: C, 76.97; H, 6.24; N, 6.19. Found: C, 76.86; H, 6.15; N, 6.29.

*Ethyl 2-(4-(Bis(1-methyl-1H-indol-3-yl)methyl)phenoxy)-2-meth-ylpropanoate (**3i**).* Light brown solid; yield, 86%; mp 110−111 °C. IR (KBr): 3012, 1696, 1599, 1040 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.34 (d, 2H, J = 7.9 Hz), 7.26 (d, 2H, J = 8.2 Hz), 7.19−7.14 (m, 4H), 6.98−6.93 (m, 2H), 6.75 (d, 2H, J = 8.6 Hz), 6.48 (s, 2H), 5.79 (s, 1H), 4.19 (q, 2H, J = 7.1 Hz), 3.63 (brs, 6H), 1.56 (brs, 6H), 1.20 (t, 3H, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): 174.5, 153.6, 138.2, 137.4, 129.3, 128.3, 127.4, 121.4, 120.1, 119.0, 118.6, 118.4, 109.1, 61.4, 39.3, 32.7, 25.5, 14.2. ESI-MS: (m/z) 479 (M − H)$^+$. Anal. Calcd for $C_{31}H_{32}N_2O_3$: C, 77.47; H, 6.71; N, 5.83. Found: C, 77.41; H, 6.79; N, 5.85.

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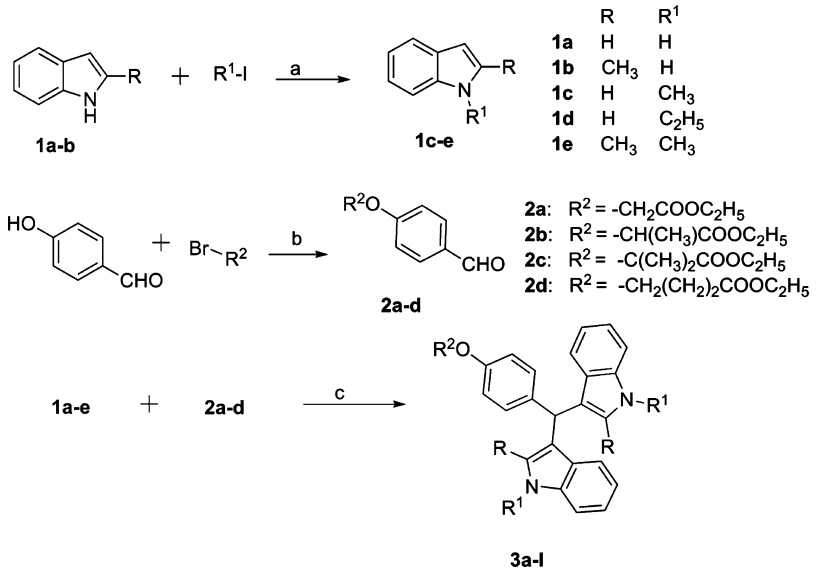
Scheme 1. Synthesis of Substituted Bis-Indoles[a]



Compound	R	R^1	R^2
3a	H	H	$-CH_2COOC_2H_5$
3b	H	CH_3	$-CH_2COOC_2H_5$
3c	H	C_2H_5	$-CH_2COOC_2H_5$
3d	H	CH_3	$-CH(CH_3)COOC_2H_5$
3e	CH_3	H	$-CH(CH_3)COOC_2H_5$
3f	H	C_2H_5	$-CH(CH_3)COOC_2H_5$
3g	CH_3	CH_3	$-CH(CH_3)COOC_2H_5$
3h	H	H	$-C(CH_3)_2COOC_2H_5$
3i	H	CH_3	$-C(CH_3)_2COOC_2H_5$
3j	H	C_2H_5	$-C(CH_3)_2COOC_2H_5$
3k	H	H	$-CH_2(CH_2)_2COOC_2H_5$
3l	H	CH_3	$-CH_2(CH_2)_2COOC_2H_5$

[a]Reagents and conditions: (a) NaH, DMF, 0 °C, 1 h. (b) K_2CO_3, CH_3CN, reflux, 2 h. (c) I_2, CH_3CN, rt, 30 min.

*Ethyl 2-(4-(Bis(1-ethyl-1H-indol-3-yl)methyl)phenoxy)-2-methylpropanoate (**3j**).* Light brown gum; yield, 91%. IR (KBr): 3008, 1685, 1597, 1038 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ: 7.35−7.28 (m, 4H), 7.20−7.13 (m, 4H), 6.95 (t, 2H, J = 7.5 Hz), 6.76 (d, 2H, J = 8.5 Hz), 6.55 (brs, 2H), 5.80 (s, 1H), 4.19 (q, 2H, J = 7.0 Hz), 4.02 (q, 4H, J = 7.2 Hz), 1.57 (brs, 6H), 1.34 (t, 6H, J = 7.2 Hz), 1.21 (t, 3H, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): 174.5, 153.7, 138.4, 136.5, 129.5, 127.7, 126.7, 121.8, 121.3, 120.3, 119.2, 118.6, 109.2, 79.2, 61.4, 40.9, 39.6, 32.0, 29.8, 29.5, 25.5, 22.8, 15.6, 14.2. ESI-MS: (m/z) 507 $(M - H)^+$. Anal. Calcd for $C_{33}H_{36}N_2O_3$: C, 77.92; H, 7.13; N, 5.51. Found: C, 77.84; H, 7.21; N, 5.62.

*Ethyl 4-(4-(Di(1H-indol-3-yl)methyl)phenoxy)butanoate (**3k**).* Light brown gum; yield, 89%. IR (KBr): 3403, 3013, 1694, 1593, 1049 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ: 7.82 (s, 2H), 7.35 (d, 2H, J = 7.9 Hz), 7.29 (d, 2H, J = 7.5 Hz), 7.21−7.10 (m, 5H), 6.97 (t, 2H, J = 7.3 Hz), 6.75 (d, 2H, J = 8.6 Hz), 6.54 (s, 1H), 6.54 (s, 2H), 5.79 (s, 1H), 4.12 (q, 2H, J = 7.1 Hz), 3.92 (t, 2H, J = 6.0 Hz), 2.48 (t, 2H, J = 7.3 Hz); 2.05 (t, 2H, J = 6.9 Hz), 1.23 (t, 3H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): 173.5, 157.3, 136.8, 136.5, 129.7, 127.2, 123.7, 122.0, 119.9, 120.1, 119.3, 114.3, 111.2, 66.8, 60.6, 39.5, 31.0, 24.9, 14.3. ESI-MS: (m/z); 451 $(M - H)^+$. Anal. Calcd for $C_{29}H_{28}N_2O_3$: C, 76.97; H, 6.24; N, 6.19. Found: C, 76.88; H, 6.15; N, 6.27.

*Ethyl 4-(4-(Bis(1-methyl-1H-indol-3-yl)methyl)phenoxy)-butanoate (**3l**).* Light brown solid; yield, 91%; mp 110−111 °C. IR (KBr): 3018, 1697, 1590, 1042 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ: 7.39−7.34 (m, 2H), 7.29−7.13 (m, 6H), 7.01−6.93 (m, 2H), 6.80−6.76 (m, 2H), 6.50 (s, 2H), 5.81 (s, 1H), 4.13 (q, 2H, J = 7.1 Hz), 3.95 (t, 2H, J = 6.1 Hz), 3.64 (s, 6H), 2.49 (t, 2H, J = 7.4 Hz), 2.11−2.01 (m, 2H), 1.24 (t, 3H, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): 173.4, 157.3, 137.6, 136.9, 129.7, 128.3, 127.6, 121.5, 120.2, 118.7, 114.2, 109.1, 66.8, 60.5, 39.4, 32.7, 31.0, 24.9, 14.4. ESI-MS: (m/z); 479 $(M - H)^+$. HRMS m/z calcd for $C_{31}H_{32}N_2O_3$ $(M - H)^+$ 479.2334; found, 479.2326. HPLC Purity >95%.

Biological Materials and Methods. *Animal and Diets.* In vivo experiments were conducted as per the guidelines provided by the animal ethics committee of the Central Drug Research Institute, Lucknow, India. Adult male Charles Foster rats (200−225 g) bred in the animal house of the institute were used. The animals were housed in polypropylene cages and kept in uniform hygienic conditions, at a temperature of 25−26 °C, relative humidity of 50−70%, and 12/12 h light/dark cycle. These were provided with standard rat pellet diet and water ad libitum for one week before their division into three weight-matched groups given ND and HFD. Hyperlipidemia was induced in three groups by feeding them HFD for 30 consecutive days. After induction of hyperlipidemia, bis-indole derivatives and fenofibrates were given by oral gavage to one HFD-fed groups (HFD + **3f**, HFD + **3l** ,and HFD + fenofibrate group), and the same volume of the vehicle (0.2% *Gum acacia*) was given in other HFD and ND groups. After 30 days of treatment, rats were fasted overnight. Blood was withdrawn from the retro-orbital plexus using a glass capillary in an EDTA coated tube (3 mg/mL blood). Thereafter, animals were sacrificed, and the liver was excised gently, washed with cold 0.15 M KCl, and kept at 4 °C until analysis. Blood was centrifuged to collect plasma/serum.

Biochemical Analysis of Plasma/Serum. Serum was fractionated into VLDL, LDL, and HDL by polyanionic precipitation methods.

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Serum and lipoproteins were analyzed for their TC, PL, TG, and total protein by the standard procedures reported earlier.[34]

Biochemical Analysis of the Liver. The liver was homogenized (10%, w/v) in cold 100 mM phosphate buffer at pH 7.2 and used for the assay of total lipolytic activity of LPL.[35] The lipid extract of each homogenate prepared in $CHCl_3/CH_3OH$ (2:1 v/v) was used for the estimation of TC, PL, TG, and total protein. Human serum LDL was prepared and radio-labeled with I^{125}, and the binding of this I^{125}LDL with liver plasma membrane preparation[36] was assayed as described previously.[37]

Histopathology of the Liver. Histopathological study of the rat liver of all groups was performed. A tissue section of 4 μm thickness was cut and stained with Erhlich's hematoxylin and eosin and examined by a light microscope.

Fecal Bile Acids. Rat feces was collected from all experimental groups from the day of dosing over 30 days and processed for the cholic and deoxycholic acid estimation test.[38]

■ ASSOCIATED CONTENT

Supporting Information

Detailed spectral analysis for all new compounds, structures, and 1D and 2D NMR. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +91 9919317940. Fax: +91-522-2628493. E-mail: sashidhar123@gmail.com; kv_sashidhara@cdri.res.in.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

HFD, high fat diet; LCAT, lecithin cholesterol acyl-transferase; LDL, low density lipoprotein; CNS, central nervous system; FDA, Food and Drug Administration; HDL, high density lipoprotein; HMG CoA reductase, 3-hydroxy-3-methyl-glutaryl-CoA reductase; TLC, thin layer chromatography; TMS, tetramethylsilane; ESIMS, electrospray ionization mass spectra; HRMS, high resolution mass spectra; HPLC, high-performance liquid chromatography; TC, total cholesterol; TG, triglycerides; PL, phospholipids; ND, normal diet; VLDL, very low density lipoprotein; CHD, coronary heart disease

■ REFERENCES

(1) (a) Wickelgren, I. Obesity: How Big a Problem? *Science* **1998**, *280*, 1364−1367. (b) Bray, G. A. Obesity: the disease. *J. Med. Chem.* **2006**, *49*, 4001−4007.

(2) Flegal, K. M.; Carroll, M. D.; Ogden, C. L.; Johnson, C. L. Prevalence and trends in obesity among US adults, 1999−2000. *JAMA* **2002**, *288*, 1723−1727.

(3) *The World Health Report 2002: Reducing Risks, Promoting a Healthy Life*; World Health Organization: Geneva, Switzerland, 2002.

(4) Jong, W. Y. Possible anti-Obesity drugs from nature- a review. *Phytochemistry* **2010**, *71*, 1625−1641.

(5) Drew, B. S.; Dixon, A. F.; Dixon, J. B. Obesity management: update on orlistat. *Vasc. Health Risk Manag.* **2007**, *3*, 817−821.

(6) Tziomalos, K.; Krassas, G. E.; Tzotzas, T. The use of sibutramine in the management of obesity and related disorders: an update. *Vasc. Health Risk Manag.* **2009**, *5*, 441−452.

(7) (a) De Simone, G.; D'Addeo, G. Sibutramine: balancing weight loss benefit and possible cardiovascular risk. *Nutr. Metab. Cardiovasc. Dis.* **2008**, *18*, 337−341. (b) Slovacek, L.; Pavlik, V.; Slovackova, B. The effect of sibutramine therapy on occurrence of depression symptoms among obese patients. *Nutr. Metab. Cardiovasc. Dis.* **2008**, *18*, e43−e44. (c) Karamadoukis, L.; Shivashankar, G. H.; Ludeman, L.; Williams, A. J. An unusual complication of treatment with orlistat. *Clin. Nephrol.* **2009**, *71*, 430−432.

(8) Agulera, C. M.; Gil-Compos, M.; Canete, R.; Gil, A. Alterations in plasma and tissue lipids associated with obesity and metabolic syndrome. *Clin. Sci.* **2008**, *114*, 183−193.

(9) Lalloyer, F.; Staels, B. Fibrates, glitazones, and peroxisome proliferator-activated receptors. *Arterioscler., Thromb., Vasc. Biol.* **2010**, *30*, 894−899.

(10) Davidson, M. H.; Armani, A.; McKenney, J. M.; Jacobson, T. A. Safety considerations with fibrate therapy. *Am. J. Cardiol.* **2007**, *99*, 3C−18C.

(11) Barker, B. J.; Goodenough, R. R.; Falko, J. M. Fenofibrate monotherapy induced rhabdomyolysis. *Diabetes Care* **2003**, *26*, 2482−2483.

(12) Shek, A.; Ferrill, M. J. Statin-fibrate combination therapy. *Ann. Pharmacother.* **2001**, *35*, 908−917.

(13) (a) Sashidhara, K. V.; Kumar, A.; Kumar, M.; Sarkar, J.; Sinha, S. Synthesis and in vitro evaluation of novel coumarin-chalcone hybrids as potential anticancer agents. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7205−7211. (b) Sashidhara, K. V.; Kumar, M.; Modukuri, R. K.; Sonkar, R.; Khanna, A. K.; Bhatia, G.; Rai, S.; Shukla, R. Synthesis and anti-inflammatory activity of novel biscoumarin-chalcone hybrids. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4480−4484. (c) Sashidhara, K. V.; Kumar, A.; Kumar, M.; Singh, S.; Jain, M.; Dikshit, M. Synthesis of novel 3-carboxamide-benzocoumarin derivatives as orally active antithrombotic agents. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 7034−7040.

(14) Sashidhara, K. V.; Kumar, A.; Kumar, M.; Srivastava, A.; Puri, A. Synthesis and antihyperlipidemic activity of novel coumarin bisindole derivatives. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6504−6507.

(15) Velezheva, V. S.; Brennan, P. J.; Marshakov, V. Y.; Gusev, D. V.; Lisichkina, I. N.; Peregudov, A. S.; Tchernousova, L. N.; Smirnova, T. G.; Andreevskaya, S. N.; Medvedev, A. E. Novel pyridazino[4,3-b]indoles with dual inhibitory activity against *Mycobacterium tuberculosis* and monoamine oxidase. *J. Med. Chem.* **2004**, *47*, 3455−3461.

(16) Andreani, A.; Burnelli, S.; Granaiola, M.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Varoli, L.; Landi, L.; Prata, C.; Berridge, M. V.; Grasso, C.; Fiebig, H. H.; Kelter, G.; Burger, A. M.; Kunkel, M. W. Antitumor activity of bis-indole derivatives. *J. Med. Chem.* **2008**, *51*, 4563−4570.

(17) Routier, S.; Peixoto, P.; Mérour, J. Y.; Coudert, G.; Dias, N.; Pierré, C. B. A.; Léonce, S.; Caignard, D. H. Synthesis and biological evaluation of novel naphthocarbazoles as potential anticancer agents. *J. Med. Chem.* **2005**, *48*, 1401−1413.

(18) Regina, G. L.; Coluccia, A.; Piscitelli, F.; Bergamini, A.; Sinistro, A.; Cavazza, A.; Maga, G.; Samuele, A.; Zanoli, S.; Novellino, E.; Artico, M.; Silvestri, R. Indolyl aryl sulfones as HIV-1 non-nucleoside reverse transcriptase inhibitors: role of two halogen atoms at the indole ring in developing new analogues with improved antiviral activity. *J. Med. Chem.* **2007**, *50*, 5034−5038.

(19) Knott, K. E.; Auschill, S.; Jäger, A.; Knölker, H. J. First total synthesis of the whole series of the antiostatins A and B. *Chem. Commun.* **2009**, *12*, 1467−1469.

(20) (a) Cowley, P. M.; Baker, J.; Barn, D. R.; Buchanan, K. I.; Carlyle, I.; Clark, J. K.; Clarkson, T. R.; Deehan, M.; Edwards, D.;

Goodwin, R. R.; Jaap, D.; Kiyoi, Y.; Mort, C.; Palin, R.; Prosser, A.; Walker, G.; Ward, N.; Wishart, G.; Young, T. The discovery of novel indole-2-carboxamides as cannabinoid CB1 receptor antagonists. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 497−501. (b) Morrison, A. J.; Adam, J. M.; Baker, J. A.; Campbell, R. A.; Clark, J. K.; Cottney, J. E.; Deehan, M.; Easson, A. M.; Fields, R.; Francis, S.; Jeremiah, F.; Keddie, N.; Kiyoi, T.; McArthur, D. R.; Meyer, K.; Ratcliffe, P. D.; Schulz, J.; Wishart, G.; Yoshiizumi, K. Design, synthesis, and structure-activity relationships of indole-3-heterocycles as agonists of CB1 receptor. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 506−509. (c) Pierson, P. D.; Fettes, A.; Freichel, C.; Gatti-McArthur, S.; Hertel, C.; Huwyler, J.; Mohr, P.; Nakagawa, T.; Nettekoven, M.; Plancher, J. M.; Raab, S.; Richter, H.; Roche, O.; Sarmiento, R. M. R.; Schmitt, M.; Schuler, F.; Takahashi, T.; Taylor, S.; Ullmer, C.; Wiegand, R. 5-Hydroxyindole-2-carboxylic acid amides: Novel histamine-3 receptor inverse agonists for the treatment of obesity. *J. Med. Chem.* **2009**, *52*, 3855−3868.

(21) Sashidhara, K. V.; Kumar, A.; Bhatia, G.; Khan, M. M.; Khanna, A. K.; Saxena, J. K. Antidyslipidemic and antioxidative activities of 8-hydroxyquinoline derived keto-enamine schiffs bases. *Eur. J. Med. Chem.* **2009**, *44*, 1813−1818.

(22) Howard, B. V.; Ruotolo, G.; Robbins, D. C. Obesity and dyslipidemia. *Endocrinol. Metab. Clin. North Am.* **2003**, *32*, 855−867.

(23) Colca, J. R.; Dailey, C. F.; Palazuk, B. J.; Hillman, R. M.; Dinh, D. M.; Melchior, G. W.; Spilman, C. H. Pioglitazone hydrochloride inhibits cholesterol absorption and lowers plasma cholesterol concentrations in cholesterol-fed rats. *Diabetes* **1991**, *40*, 1669−1674.

(24) Rijzewijk, L. J.; Van der Meer, R. W.; Lubberink, M.; Lamb, H. J.; Romijn, J. A.; Roos, A.; Twisk, J. W.; Heine, R. J.; Lammertsma, A. A.; Smit, J. W.; Diamant, M. Liver fat content in type 2 diabetes: relationship with hepatic perfusion and substrate metabolism. *Diabetes* **2010**, *59*, 2747−2754.

(25) Bhuiyan, M. J.; Do, H. V.; Mun, S.; Jun, H. J.; Lee, J. H.; Kim, Y. R.; Lee, S. J. Hypocholesterolemic and hypoglycemic effects of enzymatically modified carbohydrates from rice in high-fat-fed C57BL/6J mice. *Mol. Nutr. Food Res.* **2011**, *55*, S214−S226.

(26) Lees, R. Hyperlipidemia and Atherosclerosis HST.151: *Principles of Pharmacology*; http://ocw.mit.edu/courses (Accessed on October 16, 2011).

(27) Windler, E. E.; Kovanen, P. T.; Chao, Y. S.; Brown, M. S.; Havel, R. J. The estradiol-stimulated lipoprotein receptor of rat liver. A binding site that membrane mediates the uptake of rat lipoproteins containing apoproteins B and E. *J. Biol. Chem.* **1980**, *255*, 10464−10471.

(28) Anstee, Q. M.; Goldin, R. D. Mouse models in non-alcoholic fatty liver disease and steatohepatitis research. *Int. J. Exp. Pathol.* **2006**, *87*, 1−16.

(29) Rahman, S. M.; Wang, Y.; Yotsumoto, H.; Cha, J.; Han, S.; Inoue, S.; Yanagita, T. Effects of conjugated linoleic acid on serum leptin concentration, body-fat accumulation, and beta-oxidation of fatty acid in OLETF rats. *Nutrition* **2001**, *17*, 385−390.

(30) Reddy, J. K.; Rao, M. S. Lipid metabolism and liver inflammation. II. Fatty liver disease and fatty acid oxidation. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2006**, *290*, G852−G858.

(31) Jeon, J. R.; Kim, J. Y. Effects of pine needle extract on differentiation of 3T3-L1 preadipocytes and obesity in high-fat diet fed rats. *Biol. Pharm. Bull.* **2006**, *29*, 2111−2115.

(32) (a) Xu, X. H.; Liu, G. K.; Azuma, A.; Tokunaga, E.; Shibata, N. Synthesis of indole and biindolyl triflones: Trifluoromethanesulfonylation of indoles with Tf_2O/TTBP (2,4,6-tri-tert-butylpyridine) system. *Org. Lett.* **2011**, *13*, 4854−4857. (b) Seefeld, M. A.; Miller, W. H.; Newlander, K. A.; Burgess, W. J.; Dewolf, W. E. Jr.; Elkins, P. A.; Head, M. S.; Jakas, D. R.; Janson, C. A.; Keller, P. M.; Manley, P. J.; Rittenhouse, S. F.; Uzinskas, I. N.; Wallis, N. G.; Huffman, W. F. Indole naphthyridinones as inhibitors of bacterial enoyl-ACP reductases FabI and FabK. *J. Med. Chem.* **2003**, *46*, 1627−1635.

(33) (a) Bhaumik, J.; Yao, Z.; Borbas, K. E.; Taniguchi, M.; Lindsey, J. S. Masked imidazoyl-dipyrromethanes in the synthesis of imidazole-substituted porphyrins. *J. Org. Chem.* **2006**, *71*, 8807−8817. (b) Sierra, M. L.; Beneton, V.; Boullay, A. N.; Boyer, T.; Brewster, A. G.; Donche, F.; Forest, M. C.; Fouchet, M. H.; Gillibert, F. J.; Grillot, D. A.; Lambert, M. H.; Laroze, A.; Grumelec, C. L.; Linget, J. M.; Montana, V. G.; Nguyen, V. L.; Nicodeme, E.; Patel, V.; Penfornis, A.; Pineau, O.; Pohin, D.; Potvain, F.; Paulain, G.; Ruault, C. B.; Saunders, M.; Taum, J.; Xu, H. E.; Xu, R. X.; Pianetti, P. M. Substituted 2-[(4-Aminomethyl)phenoxy]-2-methylpropionic acid PPARα agonists. 1. Discovery of a novel series of potent HDLc raising agents. *J. Med. Chem.* **2007**, *50*, 685−695. (c) Fraga-Dubreuil, J.; Bazureau, J. P. Efficient combination of task-specific ionic liquid and microwave dielectric heating applied to one-pot three component synthesis of a small library of 4-thiazolidinones. *Tetrahedron* **2003**, *59*, 6121−6130.

(34) Khanna, A. K.; Chander, R.; Singh, C.; Srivastava, A. K.; Kapoor, N. K. Hypolipidemic activity of Terminalia chebula in rats. *Fitoterpia* **1993**, *64*, 351−356.

(35) Wing, D. R.; Robinson, D. S. Clearing-factor lipase in adipose tissue. A possible role of adenosine 3′,5′-(cyclic)-monophosphate in the regulation of its activity. *Biochem. J.* **1968**, *109*, 841−849.

(36) Kovanen, P. T.; Brown, M. S.; Goldstein, J. L. Increased binding of low density lipoprotein to liver membranes from rats treated with 17 alpha-ethinyl estradiol. *J. Biol. Chem.* **1979**, *254*, 11367−11373.

(37) Singh, V.; Kaul, S.; Chander, R.; Kapoor, N. K. Stimulation of low density lipoprotein receptor activity in liver membrane of guggulsterone treated rats. *Pharmacol. Res.* **1990**, *22*, 37−44.

(38) Mosbach, E. H.; Klenisky, H. J.; Hal, P.; Kendall, F. E. Determination of deoxycholic acid cholic acid in bile. *Arch. Biochem. Biophys.* **1954**, *51*, 402−409.