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ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · JULY 2013

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2013.07.009 · Source: PubMed

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Original article

Hybrid benzofuran–bisindole derivatives: New prototypes with promising anti-hyperlipidemic activities☆

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ARTICLE INFO

Article history:
Received 26 March 2013
Received in revised form
15 July 2013
Accepted 21 July 2013
Available online 30 July 2013

Keywords:
Benzofuran–bisindole
Lipid lowering activity
Lipoprotein metabolism
LCAT activity
Triton model

ABSTRACT

A series of different benzofuran–bisindole hybrids were synthesized and evaluated *in vitro* for their antioxidant and *in vivo* for antidyslipidemic activity in triton WR-1339 induced hyperlipidemic rats. Among the series, compounds **4a**, **4c**, **4h** and **4j** showed significant decrease in plasma levels of total cholesterol (TC), phospholipids (PL) and triglycerides (TG) followed by increase in post heparin lipolytic activity (PHLA). In addition, the active hybrids possessed moderate antioxidant properties and increased the plasma lecithin cholesterol acyltransferase (LCAT) activity, which plays a key role in lipoprotein metabolism contributing to an increased level of HDL-C in serum. These results indicate that these hybrids constitute novel prototypes for the management of dyslipidemia.

1. Introduction

Hyperlipidemia, a key feature of the metabolic syndrome and is the major cause of heart disease, stroke and death in most industrialized world [1]. It is estimated that nearly 31.9 million US adults have total serum cholesterol levels ≥240 mg/dL, and 13.8% are with a prevalence of cardiovascular risk and this is set to rise in future [2]. The typical characteristics of hyperlipidemia are high plasma triglyceride (TG) concentration, low high density lipoprotein cholesterol (HDL-C) concentration and increased concentration of small density lipoprotein cholesterol (LDL-C) particles [3]. Current available therapies for hyperlipidemia include statins, fibrates, niacin/nicotinic acid and bile acid sequestrants [4]. Statins such as atorvastatin, lovastatin, fluvastatin, simvastatin, and pravastatin work via inhibition of HMG-CoA reductase, a key enzyme in cholesterol biosynthetic pathway, leading to a reduced cholesterol concentration and a consequent increase in expression of the low-density lipoprotein receptor (LDLR), the main receptor involved in the hepatic clearance of LDL cholesterol [5]. Atorvastatin (Lipitor generic name) still remains the best selling branded lipid lowering drug worldwide [6]. However, disorders of muscles, (rhabdomyolysis) leading to withdrawal of cerivastatin in 2001 and in light of the recent warning by the US FDA, (February 28th, 2012. http://www.fda.gov/safety Alerts) that statins may increase the risk of diabetes mellitus, clearly underscores the constant need for new class of drug to combat this dreaded metabolic disorder without severe side effects [7].

Increase in oxidative stress has been frequently implicated in the pathogenesis of several other metabolic risk factors such as diabetes and coronary heart disease (CAD) [8]. Reactive oxygen species, such as hydrogen peroxide (H_2O_2), superoxide anions ($O_2^{\cdot-}$) or hydroxyl radicals (·OH), which are a by-product of oxidative metabolism, can damage the cells resulting in lipid peroxidation, modification of protein and nucleic acids. Excess of hydroxyl free radicals are the major factor for the peroxidative damage to lipoproteins present in the blood, which are responsible for the initiation and progression of atherosclerosis in the hyperlipidemic conditions [9]. Due to multifactorial nature of the metabolic syndrome, it is envisaged that compounds endowed with both hypolipidemic and antioxidant properties will be able to offer a better therapeutic benefit.

Indoles are an important structural motif found in numerous natural products and they exhibit diverse biological activities including antioxidant properties [10]. Also, it is interesting to note

☆ Part XXV in the series, "Advances in drug design and discovery".

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http://dx.doi.org/10.1016/j.ejmech.2013.07.009

the presence of this important scaffold in fluvastatin, which is a statin class of drug. Our previous laboratory experiences with this framework clearly demonstrated its promising lipid lowering properties [11]. On the other hand, benzofuran derivatives are versatile biodynamic agents that can be used to design and develop new potentially useful therapeutic agents [12]. In addition, the benzofuran containing natural product tourefolic acids A and B have shown potent anti-lipid-peroxidative properties [13]. Furthermore, a literature survey and based on the framework reveals that benzofuran ring containing moiety, exhibit anti-hyperlipidemic activity (Fig. 1) [14]. These studies, suggested that compounds containing this ring might have a potential lipid-lowering effect. In continuation of our drug discovery program on new antidyslipidemic agents, and our laboratory experiences on molecular hybridization approach [15,16], a series of new benzofuran based bisindoles prototypes were synthesized (our prototype in Fig. 1) and evaluated for their anti-hyperlipidemic activity.

2. Chemistry

The synthesis of target and intermediate compounds is outlined in the Scheme 1. The modified Duff reaction [17] on *ortho*-substituted phenols (**1a**–**f**) in the presence of hexamethylenetetramine (HMTA) and TFA at 120 °C followed by hydrolysis using 10% aqueous H_2SO_4 solution gave the dicarbaldehyde intermediates (**2a**–**f**). The Rap–Stoermer reaction [18] on compounds (**2a**–**f**) with different phenacylbromides in the presence of K_2CO_3 furnished benzofuran carbaldehyde derivatives (**3a**–**m**) in quantitative yields. Further, the introduction of indole template was achieved by the reaction of different indoles in the presence of iodine and acetonitrile as a solvent at room temperature to give the final benzofuran–bisindole hybrids (**4a**–**s**) in excellent yields. All the synthesized compounds were characterized by using ^{1}H NMR, ^{13}C NMR, IR spectroscopy and ESI-MS.

3. Pharmacology

3.1. Animals used

Rats (Charles Foster strain, male, adult, body weight 200–225 g) were kept in a room with controlled temperature (25–26 °C), humidity (60–80%) and 12/12 h light/dark cycle (light on from 8.00 A.M. to 8.00 P.M.) under hygienic conditions. Animals, which were acclimatized for one week before starting the experiment, had free access to the normal diet and water.

3.2. Lipid lowering and plasma post heparin lipolytic activity

Rats were divided into twelve groups control, triton induced, triton plus **4a**–**s** and gemfibrozil (100 mg/kg) treated groups, containing six rats in each group. In this experiment of 18 h, hyperlipidemia was developed by administration of triton WR-1339 (Sigma chemical company, St. Louis, MO, USA) at a dose of 400 mg/kg body weight intraperitoneally to animals of all the groups except the control. These derivatives were macerated with gum acacia (0.2% w/v), suspended in water and fed simultaneously with triton with a dose of 100 mg/kg p.o. to the animals of treated group and the diet being withdrawn. Animals of control and triton group without treatment with amide based fibrate compounds were given same amount of gum acacia suspension (vehicle). After 18 h of treatment the animals were anaesthetized with thiopentone solution (50 mg/kg b.w.) prepared in normal saline and then 1.0 mL blood was withdrawn from retro-orbital sinus using glass capillary in EDTA coated eppendorf tube (3.0 mg/ml blood). The blood was centrifuged (at 2500 g) at 4 °C for 10 min and plasma was separated. Plasma was diluted with normal saline (ratio of 1:3) and used for analysis of total cholesterol (TC), triglycerides (TG) and phospholipids (PL) by standard enzymatic methods [19] and post heparin lipolytic activity (PHLA) were assayed [20] using spectrophotometer, Beckmann auto-analyzer and standard kits purchased from Beckmann Coulter International, USA.

3.3. Lipoprotein measurement in blood plasma of triton induced hyperlipidemic rats

Plasma was fractionated into very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) by polyanionic precipitation methods. Plasma and lipoproteins were analyzed for their TC, PL and TG [11].

3.4. Measurement of lipoprotein cholesterol level and LCAT activity

Serum was fractionated into very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) by polyanionic precipitation methods. Plasma and lipoproteins were analyzed for their TC, PL and TG by standard

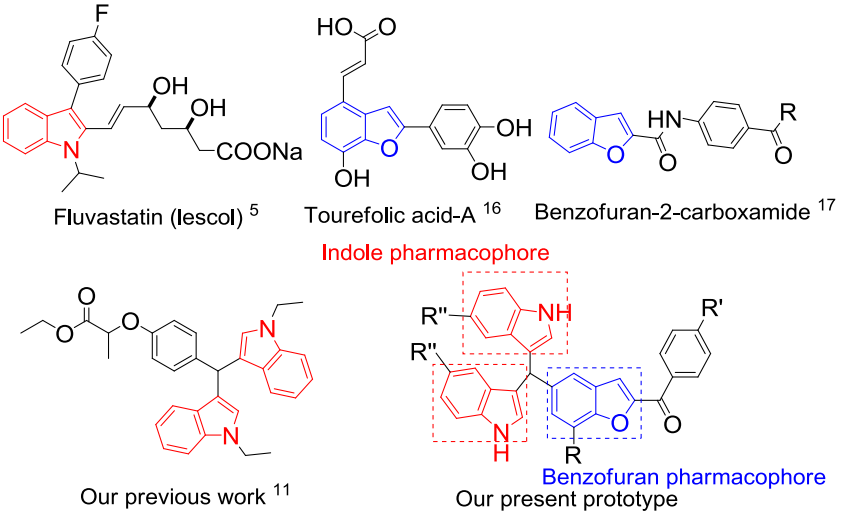
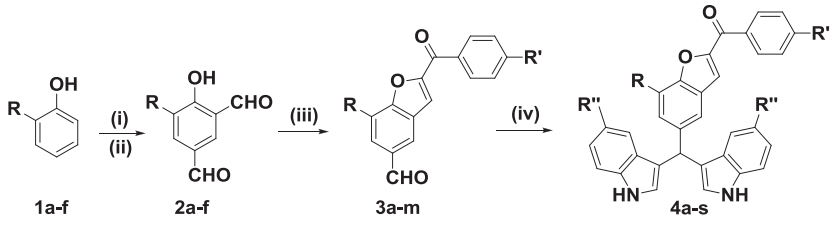


Fig. 1. Chemical structure of some potent lipid modulating indoles, benzofurans and general structure of our prototype.



compd	R	R'	compd	R	R'	R''	compd	R	R'	R''
1a,2a	CH_3-	-	3h	$CH_3(CH_2)_2$-	CH_3O-	-	4h	$CH_3(CH_2)_2$-	CH_3O-	CH_3O-
1b,2b	$(CH_3)_2CH$-	-	3i	CH_3-	CN-	-	4i	CH_3-	CN-	H-
1c,2c	$(CH_3)_3C$-	-	3j	CH_3CH_2-	CH_3O-	-	4j	CH_3CH_2-	CH_3O-	CH_3O-
1d,2d	CH_3CH_2-	-	3k	CH_3-	Cl-	-	4k	CH_3-	Cl-	H-
1e,2e	$CH_3(CH_2)_2$-	-	3l	$(CH_3)_3C$-	Cl-	-	4l	$(CH_3)_3C$-	Cl-	H-
1f,2f	Cl-	-	3m	CH_3-	CH_3-	-	4m	CH_3-	CH_3-	H-
3a	CH_3-	CH_3O-	4a	CH_3-	CH_3O-	CH_3O-	4n	$(CH_3)_2CH$-	CH_3O-	NO_2-
3b	$(CH_3)_2CH$-	CH_3O-	4b	$(CH_3)_2CH$-	CH_3O-	H-	4o	CH_3CH_2-	CH_3O-	NO_2-
3c	$(CH_3)_3C$-	CH_3O-	4c	$(CH_3)_3C$-	CH_3O-	CH_3O-	4p	CH_3CH_2-	CH_3O	H-
3d	CH_3CH_2	Cl-	4d	CH_3CH_2-	Cl-	H-	4q	$CH_3(CH_2)_2$-	CH_3O-	OH-
3e	Cl-	CH_3O-	4e	Cl-	CH_3O-	CH_3O-	4r	$(CH_3)_3C$-	CH_3O-	H-
3f	$(CH_3)_2CH$-	Cl-	4f	$(CH_3)_2CH$-	Cl-	H-	4s	CH_3-	CH_3O-	H-
3g	$(CH_3)_2CH$-	CN-	4g	$(CH_3)_2CH$-	CN-	H-				

Reagents and conditions: (i) HMTA / TFA, 120 ºC, 3h; (ii) 10% H_2SO_4, 90-100 ºC, 2h (iii) Different substituted phenacyl bromides, K_2CO_3/CH_3CN, reflux, 3h (iv) Different substituted indoles, I_2/CH_3CN, r.t, 0.5h.

Scheme 1. Synthesis of novel benzofuran–bisindole hybrids.

procedures reported earlier [21]. Serum lecithin: cholesterol acetyltransferase (LCAT) activity was measured using method reported earlier [22].

3.5. Antioxidant activity (generation of free radicals)

Superoxide anions (O_2^-) were generated enzymatically [23] by xanthine (160 mM), xanthine oxidase (0.04 U) and nitroblue tetrazolium (320 μM) in absence or presence of compounds (200 μg/ml) in 100 mM phosphate buffer (pH 8.2). Fractions were sonicated well in phosphate buffer before use. The reaction mixtures were incubated at 37° C and after 30 min the reaction was stopped by adding 0.5 mL glacial acetic acid. The amount of formazone formed was measured at 560 nm on a spectrophotometer. Percentage inhibition was calculated taking absorption coefficient of formazone as $7.2 \times 10^3\ M^{-1}\ 1cm^{-1}$. In another set of experiment, an effect of compounds on generation of hydroxyl radicals (•OH) was also studied by non-enzymic reactants [24]. Briefly (•OH) were generated in a non-enzymic system comprised of deoxy ribose (2.8 mM), $FeSO_4 \cdot 7H_2O$ (2 mM), sodium ascorbate (2.0 mM) and H_2O_2 (2.8 mM) in 50 μM KH_2PO_4 buffer, pH 7.4 to a final volume of 2.5 mL. The above reaction mixtures in the absence or presence of compounds (200 μg/ml) were incubated at 37 °C for 90 min. Reference samples and reagent blanks were also run simultaneously. Malondialdehyde (MDA) content in both experimental and reference samples were estimated spectrophotometrically by thiobarbituric acid method as mentioned above [25].

3.6. Statistical evaluation

Data were analyzed using student's *t*-test. The hyperlipidemic groups were compared with control drug treated groups. $P < 0.05$ was considered to be significant.

4. Results and discussion

4.1. The lipid lowering activity of benzofuran–bisindole hybrids

Administration of triton WR–1339 in rats induced markedly increased the plasma level of TC (4.0 fold), PL (3.3 fold) and TG (2.6 fold) and PHLA (30%) as compared to control. Treatment of hyperlipidemic rats with benzofuran–bisindole hybrids **4**(**a**–**s**) at the dose of 100 mg/kg p.o. reversed the plasma levels of lipids with varying extents. However among these, the activity of **4a**, **4c**, **4h** and **4j** was more pronounced. Compound **4j** was found to be the most potent in the series as it exhibited 28%, 29%, and 28% lowering in TC, PL and TG respectively. The lipid lowering levels of these four actives were comparable with that of the standard hypolipidemic drug gemfibrozil, which at the same dose of 100 mg/kg decreased levels of TC, PL and TG in plasma by 34%, 35% and 34%, respectively. Compounds **4a**, **4c**, **4h** and **4j** showed significant reversal of PHLA in plasma of hyperlipidemic rats by 16%, 17%, 16% and 18% respectively, comparable to gemfibrozil, which caused 20% reversal of activity of this enzyme as compared to control group and the results are summarized in Table 1.

Table 1
Percentage (%) change of plasma lipids with the treatment of benzofuran–bisindole hybride molecules in triton-induced hyperlipidemic rats at the dose of 100 mg/kg body weight.

Compound no.	Lipid profile			PHLA[b]
	Total cholesterol (TC)[a]	Phospholipids (PL)[a]	Triglyceride (TG)[a]	
Control	93.74 ± 8.51	89.31 ± 8.06	90.64 ± 6.98	17.42 ± 1.64
Triton	184.21 ± 15.81[c] (+1.9Fold)	206.88 ± 18.62[c] (+2.3Fold)	216.11 ± 20.02[c] (+2.3 Fold)	13.21 ± 1.26[c] (−23)
4a	141.84 ± 10.74*** (−23)	159.29 ± 15.11*** (−23)	164.24 ± 12.48*** (−24)	15.32 ± 1.11* (+16)
4b	152.89 ± 11.69* (−17)	175.84 ± 11.59* (−15)	181.53 ± 17.39* (−16)	14.79 ± 1.28* (+12)
4c	139.99 ± 9.63*** (−24)	159.29 ± 10.08*** (−23)	166.40 ± 10.28*** (−23)	15.45 ± 1.09* (+17)
4d	147.36 ± 13.77** (−20)	163.43 ± 10.94** (−21)	170.72 ± 17.08** (−21)	15.05 ± 1.44* (+14)
4e	145.52 ± 11.36** (−21)	165.50 ± 12.86** (−20)	168.56 ± 12.65** (−22)	15.19 ± 1.32* (+15)
4f	162.10 ± 13.35* (−12)	186.19 ± 14.86* (−10)	194.40 ± 18.63* (−10)	13.77 ± 1.20 NS (+4)
4g	167.63 ± 11.94 NS (−9)	186.19 ± 14.57* (−10)	193.81 ± 16.86* (−10)	13.75 ± 1.26 NS (+4)
4h	139.99 ± 12.33*** (−24)	159.29 ± 10.66*** (−23)	166.40 ± 9.64*** (−23)	15.32 ± 1.41* (+16)
4i	171.25 ± 18.01NS (−7)	190.11 ± 18.16 NS (−8)	200.98 ± 18.88 NS (−7)	13.87 ± 1.18 NS (+5)
4j	132.63 ± 10.76*** (−28)	146.88 ± 11.23*** (−29)	155.59 ± 11.75*** (−28)	15.58 ± 1.18* (+18)
4k	163.94 ± 14.16* (−11)	186.19 ± 17.12* (−10)	192.33 ± 17.72* (−11)	14.39 ± 1.11 NS (+9)
4l	165.78 ± 12.86* (−10)	187.91 ± 12.93 NS (−9)	194.49 ± 19.04* (−10)	14.37 ± 1.09 NS (+9)
4m	169.47 ± 12.83 (−8)	190.32 ± 13.86 (−8)	196.66 ± 15.23 (−9)	14.26 ± 1.29 (+8)
4n	167.63 ± 11.94 NS (−9)	186.19 ± 14.57* (−10)	193.81 ± 16.86* (−10)	13.75 ± 1.26 NS (+4)
4o	176.84 ± 16.38 NS (−4)	194.46 ± 19.02 NS (−6)	207.46 ± 18.69 NS (−4)	13.47 ± 1.31 NS (+2)
4p	169.47 ± 12.83 (−8)	190.32 ± 13.86 (−8)	196.66 ± 15.23 (−9)	14.26 ± 1.29 (+8)
4q	143.68 ± 8.82** (−22)	161.36 ± 9.58** (−22)	172.88 ± 10.43** (−20)	15.19 ± 1.28* (+15)
4r	171.31 ± 17.08 NS (−7)	190.32 ± 18.18 NS (−8)	198.82 ± 16.62 NS (−8)	13.73 ± 1.22 NS (+4)
4s	162.10 ± 14.58* (−12)	184.12 ± 12.72* (−11)	194.49 ± 15.37* (−10)	13.71 ± 1.22 NS (+4)
Gemfibrozil	121.57 ± 10.82*** (−34)	134.47 ± 12.81*** (−35)	142.63 ± 12.26*** (−34)	15.85 ± 1.27** (+20)

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; NS = Non significant. The lipid lowering activity of Benzofuran–bisindole hybrids (100 mg/kg) in Triton treated hyperlipidemic rats. Triton treated group is compared with control and drug treated group is compared with triton group.

[a] mg/dl.

[b] *n* mol of free fatty acids formed/h/ml of plasma; values are mean ± SD of six rats.

4.2. Effect of compounds **4a**, **4c**, **4h** and **4j** on lipid lowering at different doses in triton induced hyperlipidemic rat

After the confirmation of the most active hybrids in primary screening we further, evaluated the activity of compounds **4a**, **4c**, **4h** and **4j** at different doses at 50–150 mg/kg body weight. Among the four potent compounds, the compound **4j** lowered the TC by 22%–29%, PL by 20%–29%, and TG by 20%–30% respectively in dose dependent manner (Fig. 2.) The synthesized derivatives inhibited cholesterol biosynthesis and enhanced the activity of lipolytic enzymes to early clearance of lipids from circulation in triton induced hyperlipidemia.

4.3. Effect of compounds **4a**, **4c**, **4h** and **4j** on lipoprotein cholesterol level and LCAT activity in triton induced hyperlipidemic rat

The analysis of hyperlipidemic plasma of triton administered rats showed a significant increase in the level of lipoprotein lipids and these effects were pronounced for VLDL and LDL followed by a decrease in HDL as compared to control rats. Pleasingly, the treatment with active compounds **4a**, **4c**, **4h** and **4j** significantly reversed the VLDL, LDL and HDL levels as shown in Fig. 3. Furthermore, triton administration markedly decreased the level of LCAT in hyperlipidemic rats, and which on treatment with compounds **4a**, **4c**, **4h** and **4j** were found to be significantly increased at par with the standard drug gemfibrozil (Fig. 4). The role of LCAT activity in lipoprotein metabolism which contributes to an increased level of HDL-C in plasma is well known [22].

4.4. Effect of compounds **4a–4s** on superoxide anions, hydroxyl radicals and lipid-peroxidation

Antioxidant activities of compounds **4a–4s** at 200 μg/mL were evaluated by generating free radicals [superoxide ions ($O_2^{\cdot-}$), hydroxyl radicals (•OH), microsomal lipid peroxidation] *in vitro* in the absence and presence of these compounds. The results of this study are shown in Fig. 5. Compounds **4a** and **4j** significantly suppressed the superoxide ions by 33% and 31%, hydroxyl radical's by 30% and 27% and microsomal lipid peroxidation by 31% and 32%, respectively. The standard drug Alloperinol, at 200 μg/mL, showed 51% inhibition in superoxide ions, whereas Mannitol and α-tocopherol, at the same dose, showed 49% and 52% inhibition of hydroxyl ions and microsomal lipid peroxidation, respectively in *in vitro* system. The scavenging potential of the other derivatives was modest at best.

Interestingly, in terms of structure activity relationship, among the series of benzofuran–bisindole hybrids synthesized (**4a–4s**), the presence of electron donating groups (methoxy substituted) on both indole and benzofuran rings (**4a**, **4c**, **4h** and **4j**) exhibited potent activity, while the absence of these or its replacement with electron withdrawing groups resulted in diminished activity, as evidenced in compounds (**4b**, **4f**, **4g**, **4i**, **4k**, **4l** and **4m**).

5. Conclusion

In conclusion, the pathological investigation has revealed that the novel hybrids **4a**, **4c**, **4h** and **4j** have significant anti-hyperlipidemic activity and they decreased the total cholesterol (TC), phospholipids (PL) and triglycerides (TG) in plasma of hyperlipidemic rats. In parallel, the treatment with active hybrids effectively reversed the levels of VLDL, LDL and HDL and also increased the LCAT activity, which plays a key role in lipoprotein metabolism contributing to an increased level of HDL-C in plasma. Furthermore, the synthesized hybrids compounds **4a** and **4j** exhibited potent antioxidant properties. Thus, taken together our findings strongly suggest that these novel hybrid prototypes (with unique chemical structures) improve lipid abnormalities and would be a potential new leads against dyslipidemia.

6. Experimental

6.1. General information

All reagents were commercial and were used without further purification. Chromatography was carried on silica gel (60–120 and

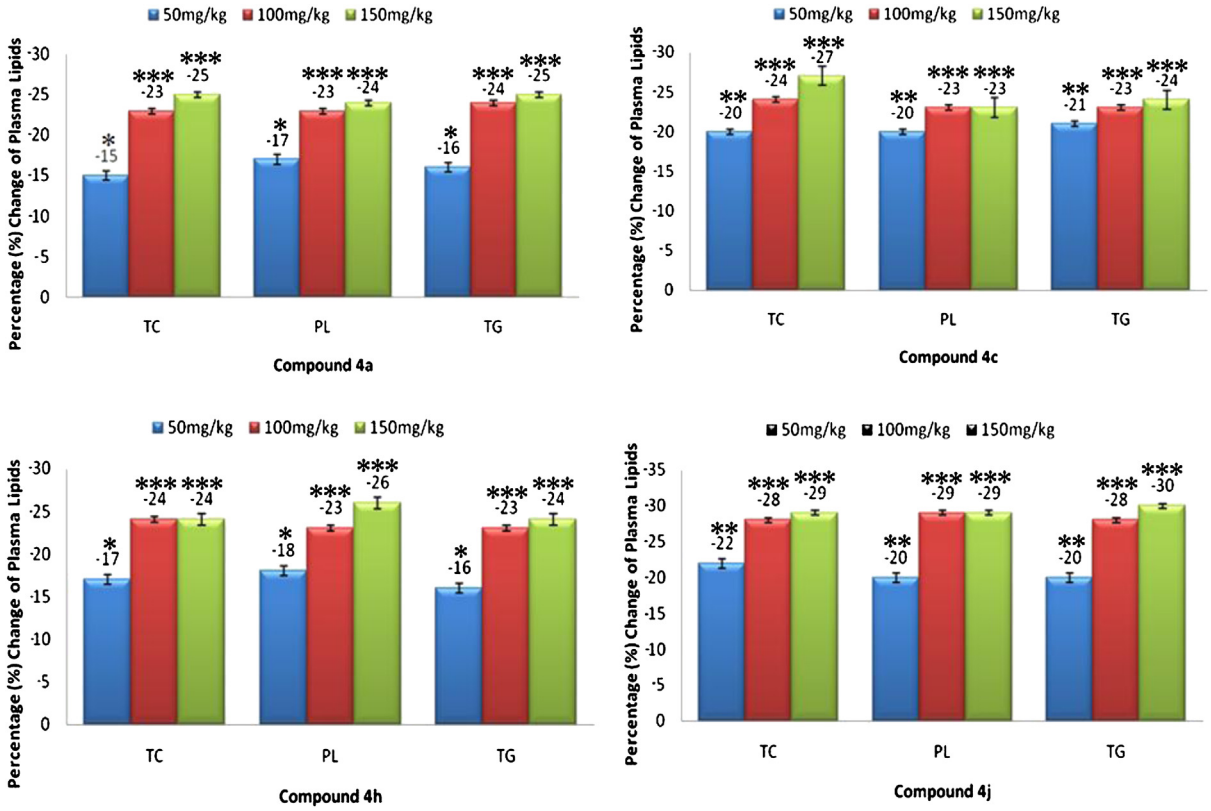


Fig. 2. Percentage (%) change of plasma lipids with the treatment of compounds **4a**, **4c**, **4h** and **4j** in triton-induced hyperlipidemic rats at the different doses. Each parameter represents pooled data from 6 rats/group and values are expressed as mean ± S.D. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ compared b/w triton and triton plus compound treated different doses. (50, 100 and 150 mg/kg b.wt).

100–200 mesh). All reactions were monitored by TLC; silica gel plates with fluorescence F254 were used. Melting points were uncorrected. The ^{1}H NMR, 2D-NMR (COSY, HMBC, HSQC) and ^{13}C NMR spectra were determined on 200, 300, MHz and 50, 75, MHz, respectively, using $CDCl_3$ and DMSO- d_6 as solvents and TMS as internal standard. All chemical shifts were given in ppm. IR spectra were recorded on in the range of 500–4000 cm^{-1}, and multiplicity (s = singlet, brs = broad singlet, d = doublet, brd = broad doublet, dd = double doublet, t = triplet, q = quartet, m = multiplet).

6.2. Starting materials (**1a**–**f**)

Starting materials (**1a**–**f**) were commercially available and were used without further purification.

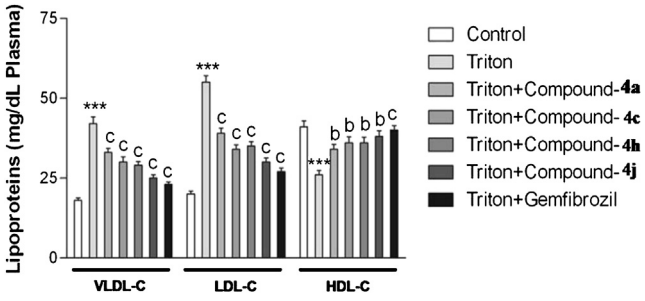


Fig. 3. Effect of compounds **4a**, **4c**, **4h** and **4j** (100 mg/kg) improves the lipoprotein cholesterol level in triton induced hyperlipidemic rats. Each parameter represents pooled data from 6 rats/group and values are expressed as mean ± S.D. $^{***}P < 0.001$ compared between control and triton treated rats group only, $^{b}P < 0.01$; $^{c}P < 0.001$ between triton and triton plus compounds treated groups, gemfibrozil is taken as standard drug.

6.3. General synthetic procedure for preparation of 4-hydroxy-5-alkyl isophthalaldehydes. (**2a**–**f**)

2-Alkyl phenol (1.0 equiv.) and hexamethylenetetramine (1.2 equiv.) were dissolved in TFA (25 mL) and the solution was heated at 120 °C for 3 h. After cooling to room temperature 10% aq. H_2SO_4 (25 mL) was added and again the temperature maintained (at 90–100 °C) for two more hours. The solution was basified with $NaHCO_3$ to pH 8 and extracted 3-fold with 50 mL of $CHCl_3$. The combined organic layers were dried on Na_2SO_4, filtered, and

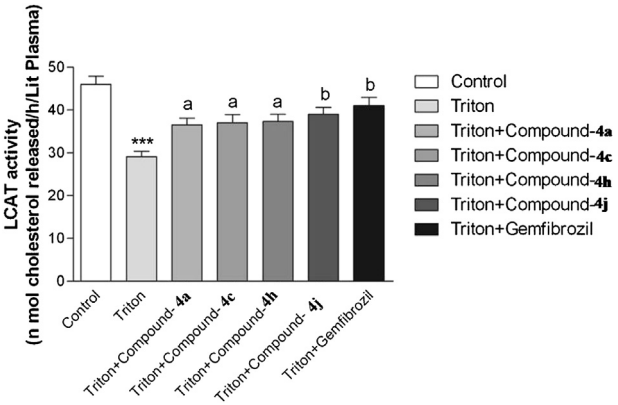


Fig. 4. Compounds **4a**, **4c**, **4h** and **4j** (100 mg/kg) re-activate LCAT activity in triton induced hyperlipidemic rats. Each parameter represents pooled data from 6 rats/group and values are expressed as mean ± S.D. $^{***}P < 0.001$ between control and triton treated rats group only, $^{a}P < 0.05$; $^{b}P < 0.01$ between triton and triton plus compounds treated groups, gemfibrozil is taken as standard drug.

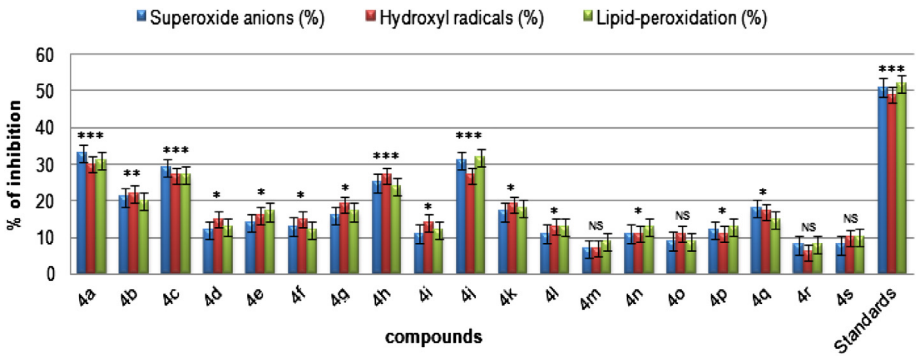


Fig. 5. The effect of benzofuran–bisindole hybrids (200 μg/ml) on superoxide ion (nmol. formazone formed/min), hydroxyl ion (nmol. MDA formed/h) and lipid peroxidation in microsomes (nmol. MDA formed/mg protein) was shown (standard drugs for superoxide anions-alloperinol (200 μg/ml), hydroxyl ions-manitol and for microsomal lipid peroxidation-α-tocopherol (200 μg/ml) were used. Each value is mean ± SD of six values, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ experimental values compared with control values. NOTE: ^{NS} (non significant).

concentrated to dryness under reduced pressure. The crude product was purified on a silica gel column (100–200 mesh) using ethylacetate-hexane (12:88, v/v) as eluent to afford compounds **2a**–**f** in good yields.

*6.3.1. 4-Hydroxy-5-methylisophthalaldehyde (**2a**)*

Compound **2a** was synthesized from **1a,** after purification on a silica gel eluted with ethylacetate-hexane (12:88, v/v), compound **2a** was obtained. White solid; Yield: 60%; mp: 125–127 °C; IR (neat): 3262, 2865, 1703, 1626, 1013 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 11.82 (s, 1H), 9.97 (s, 1H), 9.90 (s, 1H), 7.97 (d, $J = 1.8$ Hz, 1H), 7.93 (brs, 1H), 2.33 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): 196.4, 189.9, 165.0, 137.2, 134.8, 128.7, 125.2, 119.7, 15.1; ESI-MS: *m/z*: 165 $(M + H)^+$. HRMS *m/z* calcd for $C_9H_8O_3$ $(M + H)^+$ 165.0552, found 165.0559.

*6.3.2. 4-Hydroxy-5-isopropylisophthalaldehyde (**2b**)*

Compound **2b** was synthesized from **1b,** after purification on a silica gel eluted with ethylacetate-hexane (12:88, v/v), compound **2b** was obtained. White solid, yield: 65%; mp: 163–164 °C; IR (KBr): 3028, 1677, 1665, 1615, 1229, 768 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 11.94 (s, 1H), 9.97 (s, 1H), 9.91 (s, 1H), 7.99 (brs, 1H), 7.96 (d, $J = 1.47$ Hz, 1H), 3.43–3.33 (m, 1H), 1.27 (d, $J = 5.19$ HZ, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) : 196.6, 189.9, 164.3, 138.9, 134.8, 133.2, 129.0, 119.9, 26.5, 22.1; ESI-MS: *m/z*: 193 $(M + H)^+$. HRMS *m/z* calcd for $C_{11}H_{12}O_3$ $(M + H)^+$ 193.0865, found 193.0861.

*6.3.3. 5-Tert-butyl-4-hydroxyisophthalaldehyde (**2c**)*

Compound **2c** was synthesized from **1c,** after purification on a silica gel eluted with ethylacetate-hexane (10:90, v/v), compound **2c** was obtained.. Oily; Yield: 65%; IR (neat): 3252, 2865, 1703, 1626, 1013 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 12.39 (s, 1H), 9.99 (s, 1H), 9.93 (s, 1H), 8.07 (brs, 1H), 7.99 (brs, 1H), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz); 196.4, 190.0, 166.1, 140, 135.4, 133.9, 128.6, 120.4, 35.2, 29.1; ESI-MS: *m/z*: 207 $(M + H)^+$; HRMS *m/z* calcd for $C_{12}H_{14}O_3$ $(M + H)^+$ 207.1021, found 207.1018.

*6.3.4. 5-Ethyl-4-hydroxyisophthalaldehyde (**2d**)*

Compound **2d** was synthesized from **1d,** after purification on a silica gel eluted with ethylacetate-hexane (12:89, v/v), compound **2d** was obtained. White solid, yield: 69%; mp: 173–174 °C; IR (neat): 3259, 2870, 1700, 1628, 1013 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 11.85 (s, 1H), 9.98 (s, 1H), 9.91 (s, 1H), 7.97–7.95 (m, 2H), 2.78–2.72 (m, 2H), 1.26 (*t*, $J = 7.5$ Hz, 3H); ESI-MS: *m/z*: 179 $(M + H)^+$; HRMS *m/z* calcd for $C_{10}H_{10}O_3$ $(M + H)^+$ 179.0708, found 179.0711.

*6.3.5. 4-Hydroxy-5-propylisophthalaldehyde (**2e**)*

Compound **2e** was synthesized from **1e,** after purification on a silica gel eluted with ethylacetate-hexane (08:92, v/v), compound **2e** was obtained. Oily; Yield: 62%; IR (neat): 3259, 2872, 1710, 1620, 1010 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 11.85 (s, 1H), 9.96 (s, 1H), 9.89 (s, 1H), 7.96 (d, $J = 2.0$ Hz, 1H), 7.91 (d, $J = 2.0$ Hz, 1H), 2.68 (*t*, $J = 6.6$ Hz, 2H), 1.71–1.61 (m, 2H), 0.96 (*t*, $J = 7.3$ Hz, 3H); ESI-MS: *m/z*: 193 $(M + H)^+$; HRMS *m/z* calcd for $C_{11}H_{12}O_3$ $(M + H)^+$ 193.0865, found 193.0869.

*6.3.6. 5-Chloro-4-hydroxyisophthalaldehyde (**2f**)*

Compound **2f** was synthesized from **1f,** after purification on a silica gel eluted with ethylacetate-hexane (15:85, v/v), compound **2f** was obtained. White solid; Yield: 70%; IR (neat): 3263, 2880, 1721, 1632, 1013 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 12.05 (s, 1H), 10.00 (s, 1H), 9.91 (s, 1H), 8.15 (d, $J = 1.9$ Hz, 1H), 8.07 (d, $J = 1.9$ Hz, 1H); ESI-MS: *m/z*: 185 $(M + H)^+$; HRMS *m/z* calcd for $C_8H_5ClO_3$ $(M + H)^+$ 185.0005, found 185.0011.

*6.4. General synthetic procedure for preparation of 2-(4-chlorobenzoyl)-7-methylbenzofuran-5-carbaldehyde (**3a**)*

To a mixture of 4-hydroxy-5-methylisophthalaldehyde (**2a**) (1.0 g, 6.09 mmol), 4-chlorophenacylbromide (1.6 g, 7.31 mmol) and K_2CO_3 (0.8 g, 6.08 mmol), acetonitrile (20 mL) was added. 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 with EtOAc:Hexane (10:90, v/v) to give pure **3a** as a pale yellow colored solid.

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

*6.4.1. 2-(4-Chlorobenzoyl)-7-methylbenzofuran-5-carbaldehyde (**3a**)*

Compound **3a** was synthesized from **2a,** after purification on a silica gel eluted with ethylacetate-hexane (10:90, v/v), compound **3a** was obtained. Pale yellow solid, yield; 85%; mp 168–169 °C; IR (KBr); 3020, 1691, 1646, 1600, 1217, 777 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ; 10.04 (s, 1H), 8.09 (s, 1H), 8.03 (d, $J = 8.6$ Hz, 2H), 7.86 (s, 1H), 7.63 (s, 1H), 7.53 (d, $J = 8.6$ Hz, 2H), 2.65 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz); 191.4, 182.5, 158.2, 153.3, 139.8, 135.0, 133.4, 131.0, 129.1, 128.9, 126.8, 124.9, 124.1,116.7, 15.2; ESI-MS (*m/z*) 299 $(M + H)^+$; HRMS *m/z* calcd for $C_{17}H_{11}ClO_3$ $(M + H)^+$ 299.0475, found 299.0461.

*6.4.2. 7-Methyl-2-(4-methylbenzoyl) benzofuran-5-carbaldehyde (**3j**)*

Compound **3j** was synthesized from **2j,** after purification on a silica gel eluted with ethylacetate-hexane (15:85, v/v), compound

3j was obtained. Pale yellow solid, yield; 80%; mp 165–166 °C; IR (KBr): 3043, 1684, 1659, 1629, 1204, 755 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ; 10.05 (s, 1H), 8.08 (s, 1H), 7.99 (d, $J = 8.1$ H, 2Hz), 7.84 (s, 1H), 7.60 (s, 1H), 7.35 (d, $J = 8.1$ Hz, 2H), 2.66 (s, 3H), 2.47(s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): 191.4, 183.5, 158.2, 153.8, 144.3, 134.2, 133.3, 128.7, 126.9, 124.8, 124.1, 116.3, 21.8, 15.2; ESI-MS: (*m/z*) 279 $(M + H)^+$; HRMS *m/z* calcd for $C_{18}H_{14}O_3$ $(M + H)^+$ 279.1021, found 279.1017.

6.5. General synthetic procedure for preparation of (5-(bis(5-methoxy-1H-indol-3-yl)methyl)-7-methylbenzofuran-2-yl)(4-methoxyphenyl) methanone (**4a**)

A mixture of ethyl 2-(4-chlorobenzoyl)-7-methylbenzofuran-5-carbaldehyde (**3a**) (1.0 g, 3.4 mmol), 5-methoxyindole (0.9 g, 6.8 mmol), and I_2 (17.21 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 **4a**.

The compounds (**4a**–**s**) were prepared in a manner similar to the procedure described above.

6.5.1. (5-(Bis(5-methoxy-1H-indol-3-yl)methyl)-7-methylbenzofuran-2-yl)(4-methoxyphenyl) methanone (**4a**)

Compound **4a** was synthesized from **3a,** after purification on a silica gel eluted with ethylacetate-hexane (20:80, v/v), compound **4a** was obtained. Yellow solid; yield: 84%; mp: 179–180 °C; IR(KBr, cm^{-1}): 3413, 3019, 1631, 1259, 1216, 770; ^1H NMR (DMSO-d_6, 300 MHz) δ: 10.71 (s, 2H), 8.08 (d, $J = 8.1$ Hz, 2H), 7.72 (s, 1H), 7.58 (s, 1H), 7.46 (s, 1H), 7.30–7.13 (m, 4H), 6.87–6.73 (m, 6H), 5.90 (s, 1H), 3.91 (s, 3H), 3.63 (s, 6H), 2.54 (s, 3H); ^{13}C NMR (DMSO-d_6, 50 MHz); 181.7, 163.1, 153.0, 152.6, 151.7, 141.1, 131.8, 131.6, 130.0, 129.3, 126.1, 124.4, 121.1, 126.17, 124.4, 121.1, 119.6, 117.7, 116.5, 113.9, 112.0, 110.5, 101.4, 55.2, 14.8. ESI-MS: *m/z*: 571 $(M + H)^+$; Anal Calcd for $C_{36}H_{30}N_2O_5$: C, 75.77; H, 5.30; N, 4.91; Found: C, 75.79; H, 5.37; N, 4.89.

6.5.2. (5-(Di(1H-indol-3-yl)methyl)-7-isopropylbenzofuran-2-yl)(4-methoxyphenyl)methanone (**4b**)

Compound **4b** was synthesized from **3b,** after purification on a silica gel eluted with ethylacetate-hexane (20:80, v/v), compound **4b** was obtained. Yellow solid; yield: 72%; mp: 182–183 °C; IR(KBr,cm^{-1}): 3413, 3016, 2922, 1629, 768; ^1H NMR (DMSO-d_6, 300 MHz) δ: 10.83 (s, 2H), 8.07 (d, $J = 8.1$ Hz, 2H), 7.69 (s, 1H), 7.56 (s, 2H), 7.40–7.32 (m, 4H), 7.15–7.04 (m, 4H), 6.92–6.87 (m, 4H), 6.01 (s, 1H), 3.90 (s, 3H), 3.33 (s, 1H), 1.36 (d, $J = 6.3$ Hz, 6H); ^{13}C NMR (DMSO-d_6, 75 MHz); 182.3, 163.7, 152.5, 152.3, 141.7, 137.1, 132.4, 132.2, 129.9, 127.1, 126.9, 124.2, 121.4, 120.3, 119.6, 118.8, 118.7, 116.8, 114.5, 111.9, 56.0, 29.2, 22.9. ESI-MS: *m/z*: 539 $(M + H)^+$; Anal Calcd for $C_{36}H_{30}N_2O_3$: C, 80.27; H, 5.61; N, 5.20; Found: C, 80.24; H, 5.59; N, 5.24.

6.5.3. (5-(Bis(5-methoxy-1H-indol-3-yl)methyl)-7-tert-butylbenzo furan-2-yl)(4-methoxy phenyl) methanone (**4c**)

Compound **4c** was synthesized from **3c,** after purification on a silica gel eluted with ethylacetate-hexane (20:80, v/v), compound **4c** was obtained. Yellow solid; yield: 84%; mp: 175–176 °C; IR(KBr, cm^{-1}): 3429, 3018, 1634, 1218, 765; ^1H NMR (DMSO-d_6, 300 MHz) δ: 10.71 (s, 2H), 8.05 (d, $J = 8.0$ Hz, 2H), 7.72 (s, 1H), 7.62 (d, $J = 4.7$ Hz, 2H), 7.29 (d, $J = 8.7$ Hz, 2H), 7.14 (d, $J = 8.7$ Hz, 2H), 6.90 (d, $J = 2.0$ Hz, 2H), 6.81 (d, $J = 2.0$ Hz, 2H), 6.76–6.72 (m, 2H), 5.94 (s, 1H), 3.90 (s, 3H), 3.62 (s, 6H), 1.50 (s, 9H). ESI-MS: *m/z*: 613 $(M + H)^+$; Anal Calcd for $C_{39}H_{36}N_2O_5$: C, 76.45; H, 5.92; N, 4.57; Found: C, 76.51; H, 5.98; N, 4.62.

6.5.4. (4-Chlorophenyl)(5-(di(1H-indol-3-yl)methyl)-7-ethylbenzo furan-2-yl)methanone (**4d**)

Compound **4d** was synthesized from **3d,** after purification on a silica gel eluted with hexane ethylacetate-hexane (20:80, v/v), compound **4d** was obtained. yellow solid; yield: 84%; mp: 190–191 °C; IR(KBr, cm^{-1}): 3414, 3013, 2143, 1639, 1219, 770; ^1H NMR (DMSO-d_6, 300 MHz) δ: 10.87 (s, 2H), 7.86 (d, $J = 7.8$ Hz, 2H), 7.76 (s, 1H), 7.66 (d, $J = 7.8$ Hz, 2H), 7.58 (s, 2H), 7.54 (s, 2H), 7.40–7.32 (m, 4H), 7.07 (*t*, $J = 6.3$ Hz, 2H), 6.92–6.87 (m, 4H), 6.00 (s, 1H), 2.92 (d, $J = 7.0$ Hz, 2H), 1.31 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz); 182.7, 153.3, 151.6, 141.9, 138.3, 137.1, 136.0, 131.5, 129.4, 129.2, 127.9, 127.1, 126.9, 124.2, 121.4, 120.5, 119.6, 118.7, 118.6, 118.3, 111.9, 22.9, 14.4. ESI-MS: *m/z*: 530 $(M + H)^+$; Anal Calcd for $C_{34}H_{25}ClN_2O_2$: C, 77.19; H, 4.76; N, 5.30; Found: C, 77.17; H, 4.73; N, 5.28.

6.5.5. (5-(Bis (5-methoxy-1H-indol-3-yl) methyl)-7-chlorobenzo furan-2-yl) (4-methoxy phenyl)methanone (**4e**)

Compound **4e** was synthesized from **3e,** after purification on a silica gel eluted with ethylacetate-hexane (20:80, v/v), compound **4e** was obtained. Yellow solid; yield: 84%; mp: 130–131 °C; IR(KBr, cm^{-1}): 3424, 3014, 1635, 1216, 769; ^1H NMR (DMSO-d_6, 300 MHz) δ: 10.78 (s, 2H), 8.10 (d, $J = 8.8$ Hz, 2H), 7.84 (s, 1H), 7.77 (s, 1H), 7.69 (s, 1H), 7.30 (d, $J = 8.7$ Hz, 2H), 7.15 (d, $J = 8.8$ Hz, 2H), 6.93 (d, $J = 2$ Hz, 2H), 6.83 (d, $J = 2.0$ Hz, 2H), 6.78–6.74 (m, 2H), 6.00 (s, 1H), 3.91 (s, 3H), 3.65 (s, 6H) ESI-MS: *m/z*: 591 $(M + H)^+$; Anal Calcd for $C_{35}H_{27}ClN_2O_5$: C, 71.12; H, 4.60; N, 4.74; Found: C, 71.17; H, 4.67; N, 4.71.

6.5.6. (4-Chlorophenyl)(5-(di(1H-indol-3-yl)methyl)-7-isopropyl benzofuran-2-yl)methanone (**4f**)

Compound **4f** was synthesized from **3f,** after purification on a silica gel eluted with ethylacetate-hexane (20:80, v/v), compound **4f** was obtained. Yellow solid; yield: 84%; mp: 170–171 °C; IR(KBr, cm^{-1}): 3412, 3011, 1630, 770; ^1H NMR (DMSO-d_6, 300 MHz) δ: 10.84 (s, 2H), 8.04 (d, $J = 7.8$ Hz, 2H), 7.76–7.58 (m, 5H), 7.40–7.32 (m, 4H), 7.08 (*t*, $J = 6.0$ Hz, 2H), 6.91–6.87 (m, 4H), 6.01 (s, 1H), 3.34 (m, 1H), 1.36 (d, $J = 6.0$ Hz, 6H); ^{13}C NMR (DMSO-d_6, 75 MHz); 182.7, 152.7, 151.6, 141.9, 138.3, 137.1, 136.0, 132.4, 129.2, 127.4, 127.1, 127.0, 124.4, 121.4, 119.6, 118.7, 118.6, 118.3, 111.9, 22.9, 22.8. ESI-MS: *m/z*: 543 $(M + H)^+$; Anal Calcd for $C_{35}H_{27}ClN_2O_2$: C, 77.41; H, 5.01; N, 5.16; Found: C, 77.48; H, 5.07; N, 5.14.

6.5.7. 4-(5-(Di (1H-indol-3-yl) methyl)-7-isopropylbenzofuran-2-carbonyl)benzonitrile (**4g**)

Compound **4g** was synthesized from **3g,** after purification on a silica gel eluted with ethylacetate-hexane (20:80, v/v), compound **4g** was obtained. Yellow solid; yield: 84%; mp: 168–169 °C; IR(KBr, cm^{-1}): 3410, 3018, 2965, 1645, 1548, 1457, 1096, 767; ^1H NMR (DMSO-d_6, 300 MHz) δ: 10.83 (s, 2H), 8.07 (d, $J = 9.5$ Hz, 4H),7.75 (s, 1H), 7.55 (d, $J = 7.4$ Hz, 2H), 7.36–7.28 (m, 4H), 7.03 (m, $J = 6.5$ Hz, 2H), 6.88–6.83 (m, 4H), 5.98 (s, 1H), 3.35 (s, 1H), 1.32 (d, $J = 6.2$ Hz, 6H); ^{13}C NMR (DMSO-d_6, 75 MHz); 182.8, 153.0, 151.3, 142.0, 141.1, 137.1, 133.1, 132.5, 130.2, 127.8, 127.1, 124.1, 121.4, 120.7, 119.1, 118.7, 115.3, 112.0, 29.1, 22.8; ESI-MS: *m/z*: 534 $(M + H)^+$; Anal Calcd for $C_{36}H_{27}N_3O_2$: C, 81.03; H, 5.10; N, 7.87; Found: C, 81.08; H, 5.14; N, 7.90.

6.5.8. (5-(Bis (5-methoxy-1H-indol-3-yl) methyl)-7-propylbenzo furan-2-yl) (4-methoxyphenyl) methanone (**4h**)

Compound **4h** was synthesized from **3h,** after purification on a silica gel eluted with ethylacetate-hexane (20:80, v/v), compound **4h** was obtained. Yellow solid; yield: 84%; mp: 175–176 °C; IR(KBr,

cm^{-1}): 3410, 3018, 2965, 1645, 1548, 1457, 1096, 769; ^1H NMR (DMSO-d_6, 300 MHz) δ: 10.71 (s, 2H), 8.06 (d, $J = 8.8$ Hz, 2H), 7.71 (s, 1H), 7.60 (d, $J = 1.4$ Hz, 1H), 7.49 (d, $J = 1.4$ Hz, 1H), 7.30 (s,1H), 7.28 (s,1H), 7.14 (d, $J = 8.8$ Hz, 2H), 6.87 (d, $J = 2.3$ Hz, 2H), 6.80 (d, $J = 2.3$ Hz, 2H), 6.76–6.72 (m, 2H), 5.92 (s, 1H), 3.90 (s, 3H), 3.62 (s, 6H), 2.89 (*t*, $J = 6.0$ Hz, 2H), 1.80–1.72 (m, 2H), 0.94(*t*, $J = 7.4$ Hz, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz); 182.3, 141.6, 132.4, 129.9, 127.5, 126.9, 126.1, 124.9, 120.5118.4, 116.9, 114.5, 112.6, 111.1, 102.0, 56.0, 55.8, 31.7, 22.9, 14.1. ESI-MS: *m/z*: 599 $(M + H)^+$; Anal Calcd for $C_{38}H_{34}N_2O_5$: C, 76.23; H, 5.72; N, 4.68; Found: C, 76.18; H, 5.70; N, 4.65.

6.5.9. 4-(5-(Di (1H-indol-3-yl) methyl)-7-methylbenzofuran-2-carbonyl) benzonitrile (**4i**)

Compound **4i** was synthesized from **3i,** after purification on a silica gel eluted with ethylacetate-hexane (20:80, v/v), compound **4i** was obtained. Yellow solid; yield: 84%; mp: 180–181 °C; IR(KBr, cm^{-1}): 3412, 3015, 1643, 1219, 770; ^1H NMR (DMSO-d_6, 300 MHz) δ: 10.88 (s, 2H), 8.12–8.09 (m, 4H), 7.78 (s, 1H), 7.58 (s, 1H), 7.51 (s, 1H), 7.40–7.32 (m, 4H), 7.09–7.05 (m, 2H), 6.91–6.87 (m, 4H), 5.98 (s, 1H), 2.53 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz); 182.8154.0, 151.3, 141.9, 141.0, 137.1, 133.1, 131.3, 130.2, 127.0, 126.7, 124.2, 121.4, 120.5, 119.5, 119.3, 118.7, 118.5, 115.3, 111.9, 15.3; ESI-MS: *m/z*: 506 $(M + H)^+$; Anal Calcd for $C_{34}H_{23}N_3O_2$: C, 80.77; H, 4.59; N, 8.31; Found: C, 80.81; H, 4.63; N, 8.36.

6.5.10. (5-(Bis (5-methoxy-1H-indol-3-yl) methyl)-7-ethylbenzofuran-2-yl) (4-methoxyphenyl) methanone (**4j**)

Compound **4j** was synthesized from **3j,** after purification on a silica gel eluted with ethylacetate-hexane (20:80, v/v), compound **4j** was obtained. Yellow solid; yield: 84%; mp: 177–178 °C; IR(KBr, cm^{-1}): 3418, 3015, 1633, 1219, 771; ^1H NMR (DMSO-d_6, 300 MHz) δ: 10.71 (s, 2H), 8.07 (d, $J = 8.8$ Hz, 2H), 7.72 (s, 1H), 7.58 (s, 1H), 7.52 (s, 1H), 7.28 (d, $J = 8.7$ Hz, 2H), 7.15 (d, $J = 8.8$ Hz, 2H), 6.87 (d, $J = 3.0$ Hz, 2H), 6.75 (d, $J = 3.0$ Hz, 3H), 6.76–6.72 (m, 2H), 5.91 (s, 1H), 3.90 (s, 3H), 3.62 (s, 6H), 2.97–2.90 (m, 2H), 1.29 (d, $J = 1.2$Hz, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz); 182.3, 132.4, 132.2, 129.9, 128.9, 127.8, 127.5, 126.9, 124.9, 120.4, 118.4, 116.9, 114.6, 112.6, 111.1, 102.0, 56.1, 55.8, 22.9, 14.6; ESI-MS: *m/z*: 585 $(M + H)^+$; Anal Calcd for $C_{37}H_{32}N_2O_5$: C, 76.01; H, 5.52; N, 4.79; Found: C, 76.04; H, 5.57; N, 4.82.

6.5.11. (4-Chlorophenyl) (5-(di (1H-indol-3-yl) methyl)-7-methyl benzofuran-2-yl) methanone (**4k**)

Compound **4k** was synthesized from **3k,** after purification on a silica gel eluted with ethylacetate-hexane (20:80, v/v), compound **4k** was obtained. Yellow solid; yield: 84%; mp: 168–169 °C; IR(KBr, cm^{-1}): 3435, 3017, 1636, 1219, 774; ^1H NMR (DMSO-d_6, 300 MHz) δ: 10.86 (s, 2H), 8.04 (d, $J = 7.8$ Hz, 2H), 7.76–7.66 (m, 3H), 7.58 (s, 1H), 7.50 (s, 1H), 7.36 (m, 4H), 7.04 (*t*, $J = 6.6$ Hz, 2H), 6.93 (m, 4H), 5.99 (s, 1H), 2.54 (s, 3H); ESI-MS: *m/z*: 516 $(M + H)^+$; Anal Calcd for $C_{33}H_{23}ClN_2O_2$: C, 76.96; H, 4.50; N, 5.44; Found: C, 76.99; H, 4.54; N, 5.42.

6.5.12. (7-Tert-butyl-5-(di(1H-indol-3-yl)methyl)benzofuran-2-yl)(4-chlorophenyl)methanone (**4l**)

Compound **4l** was synthesized from **3l,** after purification on a silica gel eluted with ethylacetate-hexane (20:80, v/v), compound **4l** was obtained. yellow solid; yield: 84%; mp: 158–159 °C; IR(KBr, cm^{-1}): 3429, 3018, 2950, 1638, 1219, 770; ^1H NMR (DMSO-d_6, 300 MHz) δ: 10.83 (s, 2H), 8.04 (d, $J = 7.8$ Hz, 2H), 7.77–7.60 (m, 5H), 7.40–7.32 (m, 4H), 7.07–6.87 (m, 6H), 6.02 (s, 1H), 1.47 (s, 9H); ESI-MS: *m/z*: 558 $(M + H)^+$; Anal Calcd for $C_{36}H_{29}ClN_2O_2$: C, 77.62; H, 5.25; N, 5.03; Found: C, 77.67; H, 5.29; N, 5.09.

6.5.13. (5-(Di (1H-indol-3-yl) methyl)-7-methylbenzofuran-2-yl) (p-tolyl) methanone (**4m**)

Compound **4m** was synthesized from **3m,** after purification on a silica gel eluted with ethylacetate-hexane (20:80, v/v), compound **4m** was obtained. Yellow solid; yield: 84%; mp: 130–131 °C; IR(KBr, cm^{-1}): 3413,3012, 2142, 1636, 766; ^1H NMR (DMSO-d_6, 300 MHz) δ: 10.88 (s, 2H), 7.94 (d, $J = 7.3$ Hz, 2H), 7.71 (s, 1H), 7.58 (s, 1H), 7.48–7.33 (m, 6H), 7.07 (s, 2H), 6.92–6.87 (m, 4H), 5.98 (s, 1H), 2.54 (s, 3H), 2.45 (s, 3H); ^{13}C NMR (DMSO-d_6, 50 MHz); 182.9, 153.1, 151.5, 143.4, 141.1, 136.6, 134.2, 130.2, 129.2, 126.5, 126.2, 123.7, 121.2, 120.9, 119.0, 118.1, 117.2, 111.4, 21.12, 14.8; ESI-MS: *m/z*: 495 $(M + H)^+$; Anal Calcd for $C_{34}H_{26}N_2O_2$: C, 82.57; H, 5.30; N, 5.66; Found: C, 82.52; H, 5.28; N, 5.61.

6.5.14. (5-(Bis(5-nitro-1H-indol-3-yl)methyl)-7-isopropylbenzofuran-2-yl)(4-methoxyphenyl) methanone (**4n**)

Compound **4n** was synthesized from **3b,** after purification on a silica gel eluted with ethylacetate-hexane (20:80, v/v), compound **4n** was obtained. Yellow solid; yield: 84%; mp: 137–138 °C; IR(KBr, cm^{-1}): 3409, 3021, 2966, 1218, 1030, 765; ^1H NMR (DMSO-d_6, 300 MHz) δ: 11.70 (s, 2H), 8.38 (s, 2H), 8.09–8.00 (m, 4H), 7.72 (s, 1H), 7.64–7.57 (m, 4H), 7.21–7.13 (m, 4H), 6.39 (s, 1H), 3.90 (s, 3H), 3.44–3.41 (m, 1H), 1.38 (d, $J = 6.2$ Hz, 6H); ^{13}C NMR (DMSO-d_6, 50 MHz); 182.7, 163.2, 152.1, 151.9, 140.2, 139.9, 139.8, 132.3, 131.6, 129.2, 127.6, 126.9, 126.0, 125.7, 120.7, 119.9, 116.6, 116.2, 113.9, 112.1, 55.5, 28.5, 22.3; ESI-MS: *m/z*: 630 $(M + H)^+$; Anal Calcd for $C_{36}H_{28}N_4O_7$: C, 68.78; H, 4.49; N, 8.91; Found: C, 68.79; H, 4.51; N, 8.94.

6.5.15. (5-(Bis(5-nitro-1H-indol-3-yl)methyl)-7-ethylbenzofuran-2-yl)(4-methoxyphenyl)methanone (**4o**)

Compound **4o** was synthesized from **3j,** after purification on a silica gel eluted with ethylacetate-hexane (20:80, v/v), compound **4o** was obtained. Yellow solid; yield: 84%; mp: 178–179 °C; IR(KBr, cm^{-1}): 3429, 3019, 2954, 1633, 1512, 772; ^1H NMR (DMSO-d_6, 300 MHz) δ: 11.71 (s, 2H), 8.39 (s, 2H), 8.08–8.00 (m, 4H), 7.73 (s, 1H), 7.60–7.57 (m, 4H), 7.19–7.13 (m, 4H), 6.39 (s, 1H), 3.90 (s, 3H), 2.95 (d, $J = 7.0$ Hz, 2H), 1.33 (*t*, $J = 6.0$ Hz, 3H); ^{13}C NMR (DMSO-d_6, 50 MHz); 181.7, 163.1, 152.7, 151.9, 140.2, 139.9, 139.8, 131.6, 129.2, 128.0, 127.7, 126.7, 125.7, 120.7, 119.9, 116.6, 116.2, 113.9, 112.1, 55.5, 22.4, 13.9; ESI-MS: *m/z*: 615 $(M + H)^+$; Anal Calcd for $C_{35}H_{26}N_4O_7$: C, 68.40; H, 4.26; N, 9.12; Found: C, 68.44; H, 4.28; N, 9.16.

6.5.16. (5-(Di(1H-indol-3-yl)methyl)-7-ethylbenzofuran-2-yl)(4-methoxyphenyl)methanone (**4p**)

Compound **4p** was synthesized from **3j,** after purification on a silica gel eluted with ethylacetate-hexane (20:80, v/v), compound **4p** was obtained. Yellow solid; yield: 84%; mp: 165–166 °C; IR(KBr, cm^{-1}): 3419, 3015, 2916, 1253, 768; ^1H NMR (DMSO-d_6, 300 MHz) δ: 10.8 (s, 2H), 8.00 (d, $J = 8.7$ Hz, 2H), 7.69 (s, 1H), 7.57 (s, 1H), 7.51 (s, 1H), 7.40–7.33 (m, 4H), 7.15–7.05 (m, 4H), 6.92–6.87 (m, 4H), 6.00 (s, 1H), 3.90 (s, 3H), 2.96–2.89 (m, 2H), 1.26 (m,3H); ESI-MS: *m/z*: 525 $(M + H)^+$; Anal Calcd for $C_{35}H_{28}N_2O_3$: C, 80.13; H, 5.38; N, 5.34; Found: C, 80.16; H, 5.39; N, 5.36.

6.5.17. (5-(Bis (5-hydroxy-1H-indol-3-yl) methyl)-7-propylbenzofuran-2-yl) (4-methoxyphenyl) methanone (**4q**)

Compound **4q** was synthesized from **3h,** after purification on a silica gel eluted with ethylacetate-hexane (20:80, v/v), compound **4q** was obtained. Yellow solid; yield: 84%; mp: 134–135 °C; IR(KBr, cm^{-1}): 3435, 3019, 1634, 1219, 777; ^1H NMR (DMSO-d_6, 300 MHz) δ: 10.53 (s, 2H), 8.52 (s, 2H), 8.08 (d, $J = 8.8$ Hz, 2H), 7.72 (s, 1H), 7.53 (d, $J = 1.3$ Hz, 1H), 7.44 (d, $J = 1.1$ Hz, 1H), 7.19–7.14 (m, 4H), 6.69–6.65 (m, 4H), 6.61–6.58 (m, 2H), 5.74 (s, 1H), 3.91 (s, 3H), 2.94–2.77 (m, 2H), 1.81–1.73 (m, 2H), 0.97 (*t*, $J = 7.2$ Hz, 3H); ESI-MS: *m/z*: 571

$(M + H)^+$; Anal Calcd for $C_{36}H_{30}N_2O_5$: C, 75.77; H, 5.30; N, 4.91; Found: C, 75.79; H, 5.34; N, 4.95.

*6.5.18. (7-Tert-butyl-5-(di(1H-indol-3-yl)methyl)benzofuran-2-yl)(4-methoxyphenyl) methanone (**4r**)*

Compound **4r** was synthesized from **3c,** after purification on a silica gel eluted with ethylacetate-hexane (20:80, v/v), compound **4r** was obtained. Yellow solid; yield: 84%; mp: 160–161 °C; IR(KBr, cm^{-1}): 3420, 3022, 2933, 1260, 775; ^1H NMR (DMSO-d_6, 300 MHz) δ: 10.87 (s, 2H), 8.07 (d, J = 8.8 Hz, 2H), 7.70 (s, 1H), 7.60 (s, 2H), 7.41–7.35 (m, 4H), 7.15–7.05 (m, 4H), 6.92–6.88 (m, 4H), 6.04 (s, 1H), 3.89 (s, 3H), 1.49 (s, 9H); ESI-MS: m/z: 553 $(M + H)^+$; Anal Calcd for $C_{37}H_{32}N_2O_3$: C, 80.41; H, 5.84; N, 5.07; Found: C, 80.46; H, 5.89; N, 5.09.

*6.5.19. (5-(Di (1H-indol-3-yl) methyl)-7-methylbenzofuran-2-yl) (4-methoxyphenyl) methanone (**4s**)*

Compound **4s** was synthesized from **3a,** after purification on a silica gel eluted with ethylacetate-hexane (20:80, v/v), compound **4s** was obtained. Yellow solid; yield: 84%; mp: 140–141 °C; IR(KBr, cm^{-1}): 3425, 3011, 2921, 1263, 774; ^1H NMR (DMSO-d_6, 300 MHz) δ: 10.88 (s, 2H), 8.07 (d, J = 8.1 Hz, 2H), 7.71 (s, 1H), 7.70 (s, 1H), 7.58 (s, 1H), 7.47 (s, 1H), 7.47–7.33 (m, 4H), 7.16–7.08 (m, 4H), 6.92–6.87 (m, 4H), 5.98 (s, 1H), 3.91 (s, 3H), 2.54 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz); 182.3, 141.6, 137.1, 132.1, 130.5, 129.8, 127.1, 126.7, 124.2121.7121.4, 120.2, 119.5, 118.7, 116.9, 114.5, 111.9, 56.0, 15.4; ESI-MS: m/z: 511 $(M + H)^+$; Anal Calcd for $C_{34}H_{26}N_2O_3$: C, 79.98; H, 5.13; N, 5.49; Found: C, 79.97; H, 5.14; N, 5.46.

Acknowledgments

The authors are grateful to the Director, CDRI, Lucknow, India for constant encouragement in drug development program, S.P. Singh for technical support, SAIF for NMR, IR, and Mass spectral data. R.K.M, K.B.R. and R.S. are thankful to CSIR, New Delhi, India for financial support. This is CSIR-CDRI communication number 8496.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.07.009.

References

[1] O. Guardamagna, F. Abello, P. Saracco, V. Baracco, E. Rolfo, M. Pirro, Atherosclerosis 207 (2009) 471–475.
[2] A.S. Go, D. Mozaffarian, V.L. Roger, E.J. Benjamin, J.D. Berry, W.B. Borden, et al., Circulation 127 (2013) e6–e245.
[3] (a) G.I. Uwaifo, R.E. Ratner, Am. J. Cardiol. 99 (2007) e51–e67;
(b) R.K. Semple, V.K. Chatterjee, S.O. Rahilly, J. Clin. Invest. 116 (2006) e581–e589.
[4] R.L. Pollex, T.R. Joy, R.A. Hegele, Expert Opin. Emerging Drugs 2 (2008) 363–381.
[5] J.B. Green, J.S. Ross, C.A. Jackevicius, N.D. Shah, H.M. Krumholz, JAMA. Intern. Med. 3 (2013) 229–232.
[6] B. Staels, M. Maes, A. Zambon, Nat. Clin. Pract. Cardiovasc. Med. 5 (2008) 542–553.
[7] H. Ledford, Nature 480 (2011) 16–17.
[8] D.J. Graham, J.A. Staffa, D. Shatin, S.E. Andrade, S.D. Schech, L. Grenade, J.H. Gurwitz, K.A. Chan, M.J. Goodman, R. Platt, JAMA 292 (2004) e2585–e2590.
[9] K. Sugamura, J.F. Keaneyjunior, Free Radical Biol. Med. 51 (2011) 978–992.
[10] V. Sharma, P. Kumar, D. Pathak, J. Heterocycl. Chem. 47 (2010) 491–502.
[11] K.V. Sashidhara, M. Kumar, R. Sonkar, B.S. Singh, A.K. Khanna, G. Bhatia, J. Med. Chem. 55 (2012) 2769–2779.
[12] M. Kamal, A.K. Shakya, T. Jawaid, Int. J. Med. Pharm. Sci. 1 (2011) 1–15.
[13] P. Nguyen, T. Nguyen, T. Dao, H. Kang, N. Derek-Tantoh, J. Mbafor, W. Oh, J. Nat. Prod. 73 (2010) 598–602.
[14] (a) T. Al-Qirim, G. Shattat, K. Sweidan, W. El-Huneidi, G.A. Sheikha, R. Abu Khalaf, S. Hikmat, Arch. Pharm. Chem. Life Sci. 345 (2012) 401–406;
(b) G. Shattat, T. Al-Qirim, K. Sweidan, M. Shahwan, W. El-Huneidi, Y. Al-Hiari, J. Enzyme. Inhib. Med. Chem. 25 (2010) 751–755.
[15] (a) K.V. Sashidhara, A. Kumar, M. Kumar, J. Sarkar, S. Sinha, Bioorg. Med. Chem. Lett. 20 (2010) 7205–7211;
(b) K.V. Sashidhara, G.R. Palnati, R.P. Dodda, R. Sonkar, A.K. Khanna, G. Bhatia, Eur. J. Med. Chem. 57 (2012) 302–310.
[16] (a) K.V. Sashidhara, M. Kumar, R.K. Modukuri, A. Srivastava, A. Puri, Bioorg. Med. Chem. Lett. 21 (2011) 6709–6713;
(b) K.V. Sashidhara, A. Kumar, M. Kumar, A. Srivastava, A. Puri, Bioorg. Med. Chem. Lett. 20 (2010) 6504–6507;
(c) K.V. Sashidhara, A. Kumar, M. Kumar, R. Sonkar, G. Bhatia, A.K. Khanna, Bioorg. Med. Chem. Lett. 20 (2010) 4248–4251.
[17] K.V. Sashidhara, A. Kumar, R.P. Dodda, N.N. Krishna, P. Agarwal, K. Srivastava, S.K. Puri, Bioorg. Med. Chem. Lett. 22 (2012) 3926–3930.
[18] J.R. Stille, J.A. Ward, C. Leffelman, K.A. Sullivan, Tetrahedron Lett. 37 (1996) 9267–9270.
[19] (a) R. Deeg, J. Ziegehorn, Clin. Chem. 29 (1983) 1798–1802;
(b) G. Buccolo, H. David, Clin. Chem. 19 (1973) 476–482;
(c) D.B. Zilversmit, A.K. Davis, B.S. Memphis, Tenn, J. Lab. Clin. Med. 35 (1950) 155–160.
[20] E.H. Mosbach, H.J. Klenisky, P. Hal, F.E. Kendall, Arch. Biochem. Biophys 51 (1954) 402–409.
[21] A.K. Khanna, R. Chander, C. Singh, Fitoterapia 64 (1993) 103–109.
[22] T. Nagasaki, Y. Akanuma, Clin. Chim. Acta 75 (1977) 371–375.
[23] A. Bindoli, M. Valente, L. Cavallini, Pharmacol. Res. Commun. 17 (1985) e831–e840.
[24] B. Halliwell, J.M.C. Gutteridge, O. Arouma, Anal. Biochem. 165 (1987) e215–e219.
[25] H. Okhawa, N. Ohishi, K. Yagi, Anal. Biochem. 95 (1978) e351–e358.