Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry
Bioorganic & Medicinal Chemistry 14 (2006) 584–591

Dual PPAR-α and -γ activators derived from novel benzoxazinone containing thiazolidinediones having antidiabetic and hypolipidemic potential☆

Gurram R. Madhavan,[a,*] Ranjan Chakrabarti,[b,*] K. Anantha Reddy,[a] B. M. Rajesh,[a] V. Balraju,[a] P. Bheema Rao,[a] R. Rajagopalan[b] and Javed Iqbal[a]

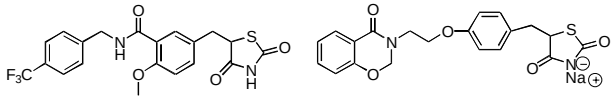
[a]*Discovery Chemistry, Dr. Reddy's Laboratories Ltd., Bollarum Road, Miyapur, Hyderabad 500049, India*
[b]*Discovery Biology, Dr. Reddy's Laboratories Ltd., Bollarum Road, Miyapur, Hyderabad 500049, India*

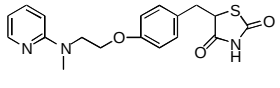
Received 6 July 2005; revised 18 August 2005; accepted 19 August 2005
Available online 28 September 2005

Abstract—2,4-Thiazolidinedione derivatives of 1,3-benzoxazinone were synthesized and evaluated for their PPAR-α and -γ dual activation. DRF-2519, a compound obtained through SAR of TZD derivatives of benzoxazinone, has shown potent dual PPAR activation. In ob/ob mice, it showed better efficacy than the comparator molecules. In fat fed rat model, it showed significant improvement in lipid parameters, which was better than fibrates.

1. Introduction

Success of thiazolidinediones (TZDs), the ligand of peroxisome proliferator-activated receptor γ (PPARγ), has generated a lot of interest for PPARs as target for new drug discovery. PPARs exist in three subtypes[1] viz., α, γ, and δ. PPAR-α is reported to be involved primarily in hepatic lipid metabolism. Lipid lowering drug fibrates are the ligands for PPAR-α.[2] PPAR-γ plays a central role in adipogenesis and glucose homeostasis, and as mentioned, antidiabetic drug TZDs are the synthetic ligands for PPAR-γ.[3] PPAR-δ is believed to be involved in lipid metabolism, energy homeostasis, and atherosclerosis.[4] To date, no PPAR-δ ligand is available in the market. Thiazolidinediones, the first insulin sensitizers to be marketed, decrease glucose levels while simultaneously reducing circulating insulin and free fatty acids.[5] A large number of type II diabetes patients also suffer from dyslipidemia. Unfortunately, TZDs have only marginal effect on lipids. It was visualized that a dual PPAR-α and -γ activator could be an effective drug for insulin resistance, hyperglycemia, and dyslipidemia. Several drugs of such dual activity were in clinical development, for example, Ragaglitazar (Dr. Reddy's group), KRP-297 (Kyorin), Tesaglitazar (Astra), and Muraglitazar (BMS).[6] Unfortunately, the first two were discontinued following instances of carcinogenicity in rodent models. NDA has recently been filed for Muraglitazar. Interestingly, among all these molecules, only KRP-297 is a TZD, others belong to different chemical classes. In our thiazolidinedione program, we synthesized a series of benzoxazinone heterocycle derivatives of thiazolidinediones and discovered a compound (DRF-2519) which exhibited potent dual PPAR-α and -γ activity. It showed significant antidiabetic and lipid lowering activities, which was better than the standard drugs.


KRP-297 DRF-2519


Rosiglitazone

Keywords: PPAR; TZD; Knoevenagel condensation; Plasma glucose; Lipids.
☆ DRL publication No. 453-A.
* Corresponding authors. Tel: +91 40 23045439; fax: +91 40 2304543; e-mail addresses: madhavangr@rediffmail.com (G.R.M); ranjanchakrabarti@drreddys.com (R.C)

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.08.043

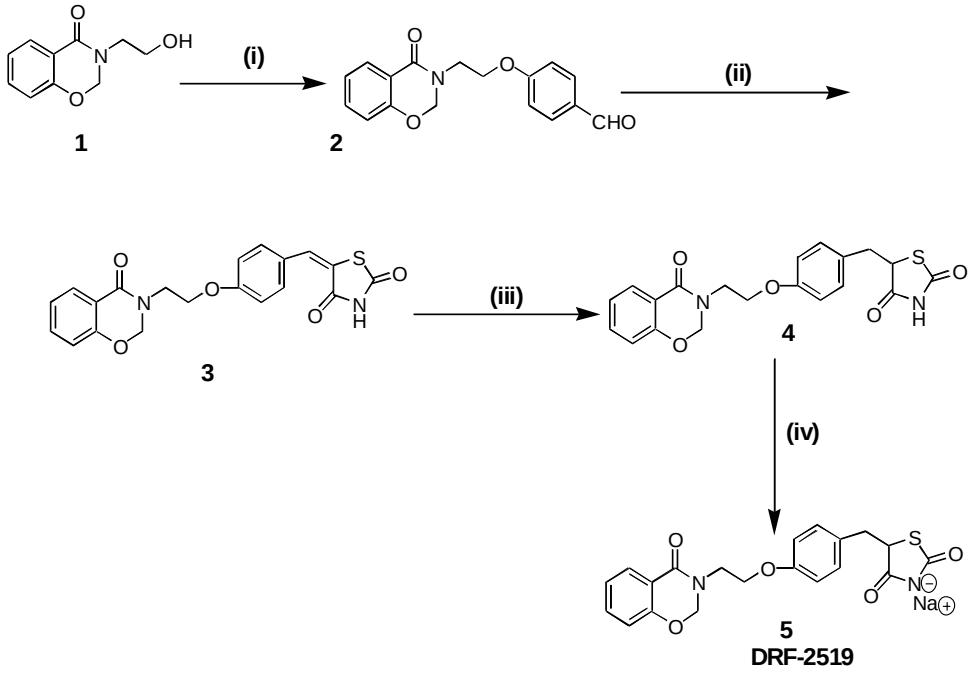
2. Chemistry

Compounds **1**–**5** were synthesized as described in Scheme 1. The starting material, 3-(2-hydroxyethyl)-2*H*-1,3-benzoxazin-4(3*H*)-one **1**[7], was reacted with 4-fluorobenzaldehyde under SN2 conditions to give rise to the aldehyde **2**. The aldehyde was condensed with 2,4-thiazolidinedione following Knoevenagel reaction conditions.[8] The unsaturated compound **3** was converted to its saturated compound **4** under hydrogenation conditions by using excess Pd/C to avoid the effect of catalyst poisoning. To have better physical properties like solubility in water and high melting point, compound **4** was converted to its sodium salt **5**.

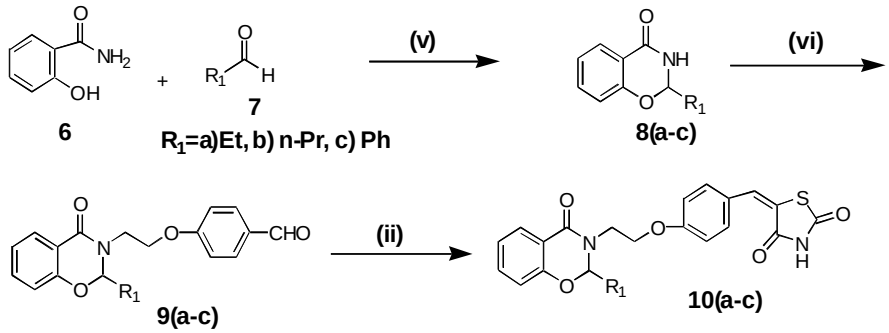
2-Substituted 1,3-benzoxazinones were synthesized from salicylamide and aldehydes, by dehydration reaction using PPE in refluxing chloroform. Subsequently, the 1,3-benzoxazinones were *N*-alkylated with 4-(2-bromoethoxy)benzaldehyde to produce aldehydes **9**(**a**–**c**). These aldehydes were condensed with thiazolidine-2,4-dione in a similar manner as given in Scheme 1 to give compounds **10**(**a**–**c**) [Scheme 2]. Preparations of compounds linking from 2-position of benzoxazinones (**13a** and **b**) were carried out by condensation of *N*-alkyl salicylamide (**11a** and **b**) with the 5-[4-[2,2-diethoxy]phenylmethyl]thiazolidinedione (**12**) in the presence of PPE (Scheme 3), in a manner similar to that in the case of preparation for the compounds in Scheme 1.

3. Results and discussion

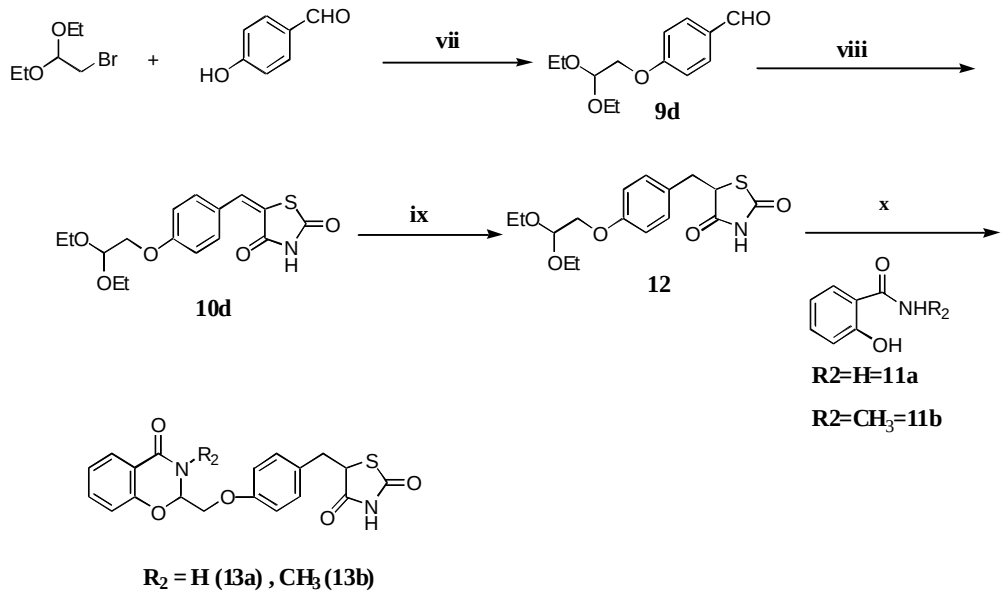
Initially the unsaturated TZD compounds **3**, **10a**–**c** were synthesized and assessed for their PPAR-α and -γ transactivation, compound **3** showed moderate activity for PPAR-α and mild activity for PPAR-γ. Compound **10a** which is a 2-ethyl derivative of benzoxazinone showed no PPAR-α activity and only mild PPAR-γ activity. The propyl derivative (compound **10b**) lost even the PPAR-γ activity considerably. Then we changed the 2-position substitution to phenyl group which too did



Scheme 1. Reagents and conditions: (i) 4-fluorobenzaldehyde, NaH, DMF, rt, 3 h, 75%; (ii) 2,4-thiazolidinedione, $PhCO_2H$, piperidine, tolune, reflux, 2 h, 86%; (iii) H_2, Pd/C, dioxane, rt, 60 h, 74%; (iv) NaOMe, MeOH, rt, 0.5 h, 77%.



Scheme 2. Reagents and conditions: (v) PPE, $CHCl_3$, reflux, 6 h, 60–90%; (vi) 4(2-bromoethoxy)benzaldehyde, K_2CO_3, DMF, rt, 5 h, 40%.



Scheme 3. Reagents and conditions: (vii) NaH, DMF, 25–60 °C, 48 h, 58%; (viii) 2,4-thiazolidinedione, $PhCO_2H$, piperidine, tolune, reflux, 2 h, 70%; (ix) H_2, 10% Pd/C, dioxane, 60 psi, 60 h, 67%; (x) PPE, $CHCl_3$, reflux, 3 h, 81%.

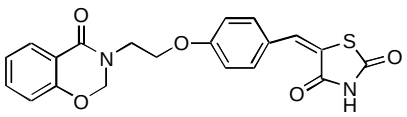
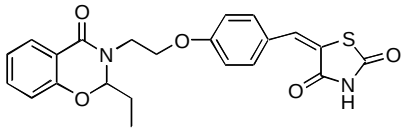
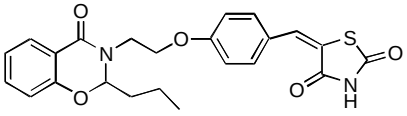
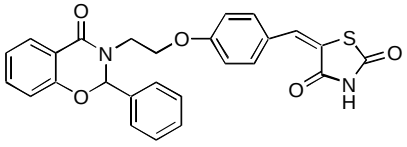
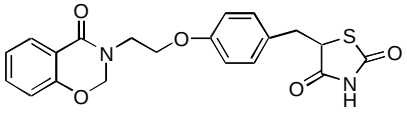
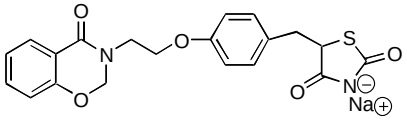
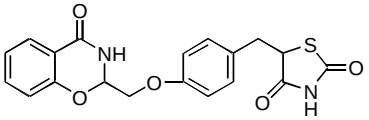
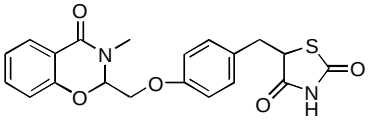
not improve PPAR-α; but this compound (**10c**) did show PPAR-γ activity. Among the four compounds, as **3** showed moderate PPAR-α along with mild PPAR-γ activity we converted it to its saturated version (compound **4**). We knew from our previous work on thiazolidinediones[9] that saturated version of TZDs shows a better profile as compared to their unsaturated versions. The compound **4** showed potent PPAR-α and -γ activity. The compound **4** was converted to its sodium salt (**5**, DRF-2519) to have better solubility. We did not attempt to make the saturated TZDs of **10a–c** as their in vitro potency was less than that of compound **3**. Then, the linker attachment was changed to 2-position of the 1,3-4(3*H*)benzoxazinone ring by synthesizing compounds **13a** and **b**, and we found that they have less PPAR activity as compared to DRF-2519. Compound **13a** showed significantly reduced PPAR-γ activity, while retaining the PPAR-α activity. Compound **13b** lost even the PPAR-α activity considerably (Table 1). There was no significant PPAR-δ activity observed for any of these compounds. Finally, among all the compounds DRF-2519 was selected for profiling in animal models.

From the in vitro dose–response curves of DRF-2519 (Fig. 1) for PPAR-α and -γ, it was clear that **DRF-2519** was almost equally potent for PPAR-α compared to the standard compound WY 14,643; but the potency for PPAR-γ was less compared to rosiglitazone (Rosi). Ob/ob mice are a well-established model for obese insulin resistance, hyperglycemia, and dyslipidemia.[10] DRF-2519 administered orally in ob/ob mice showed a dose-dependent reduction in plasma glucose (PG), triglyceride (TG), free fatty acids (FFA), and insulin levels after 14 days of treatment. ED_{50} values were calculated and compared with those of Rosi and KRP-297 (Table 2). DRF-2519 showed better efficacy than the standard compounds. It is relevant to note that KRP has a low affinity for murine PPAR-α,[11] though there is no such concern for PPAR-γ. No significant difference in body weight change was observed in DRF-2519 treated groups compared to control, although PPAR-γ agonist Rosi showed increase in body weight. These data suggested that DRF-2519 due to its dual PPAR-α and -γ modulating effects in vivo showed significant glucose, lipid, and insulin lowering activity. For further confirmation of effects on lipid parameters, it was tested in high fat fed rat model at 3 and 10 mg/kg/day dose and showed significant reduction in TG, total cholesterol, and LDL-cholesterol, and increase in HDL-cholesterol (Table 3), indicating that DRF-2519 had considerable effect on the lipid parameters. Effect of DRF-2519 (10 mg/kg) was comparable to that of Fenofibrate (30 mg/kg). Fenofibrate did not show any significant effect below 30 mg/kg dose. Standard PPAR-γ activators-rosiglitazone or pioglitazone did not show any significant activity in this model (data not shown). It is interesting to note that although PPAR-γ activity of DRF-2519 was less than that of Rosi, in vivo effect of DRF-2519 was better than that of Rosi. We believe, PPAR-α activity of DRF-2519 is also contributing to its overall activity. The pharmacokinetic studies did not show any significant difference in pharmacokinetic profiles of DRF-2519 and rosiglitazone, which indicates that the different pharmacodynamic effects of these two molecules were not caused by any pharmacokinetic issue. Based on the in vitro and in vivo results, DRF-2519 was selected for further development.

4. Conclusions

SAR studies of thiazolidinedione derivative of benzoxazinones were carried out by synthesizing TZDs from Knoevenagel conditions followed by hydrogenation of the double bond. We have identified DRF-2519 as a

Table 1. SAR of the thiazolidinedione derivatives of benzoxazinones

S. No.	Compound number	Structure	PPAR	
			α (50 μM)	γ (1 μM)
1	**3**		1.7	7.0
2	**10a**		0.9	6.7
3	**10b**		1.0	3.6
4	**10c**		1.0	8.0
5	**4**		4.2	36
6	**5**		5.0	34
7	**13a**		3.7	19.6
8	**13b**		1.5	20
9		WY-14643	4.5	—
10		Rosiglitazone	—	46.0

HEK 293T cells were transfected with PPAR-γ and PPAR-α constructs as mentioned in Section 5. These values are an average of three experiments conducted in triplicate.

potent dual PPAR-α and -γ activator. It showed significant plasma glucose, insulin, and lipid lowering activity in ob/ob mice, which was better than those of the standard compounds. Additionally, it also showed significant improvement in lipid parameters in fat fed rats, which was better than that of fibrate.

5. Experimental

5.1. Animals and treatment

All animals were maintained under 12 h light and 12 h dark cycle at 25 ± 1 °C. All animals were given standard chow (National Institute of Nutrition, India) and water ad libitum. All animal experiments were carried out in accordance with internationally valid guidelines. All experimental protocols were approved by DRF Animal Ethics Committee.

C57 BL/6J-ob/ob mice were obtained from Jackson Laboratory, USA. They were used at the age of 10 weeks, DRF-2519, rosiglitazone, and KRP-297 were administered orally for 14 days at 0.3, 1, 3, and 10 mg/kg/day. All compounds were triturated to fine particles, before making suspension. Animals in the control group received vehicles only (0.25% CMC, 10 ml/kg). Blood samples were collected from animals (in fed state) under mild ether anesthesia from retro-orbital sinus 1 h after drug administration. Plasma samples were separated for glucose, triglycerides, and insulin measurement. Sprague–Dawley rats were bred at Dr. Reddy's Laboratories, Discovery Research Animal House Facility. All animals were maintained under 12 h light and 12 h dark

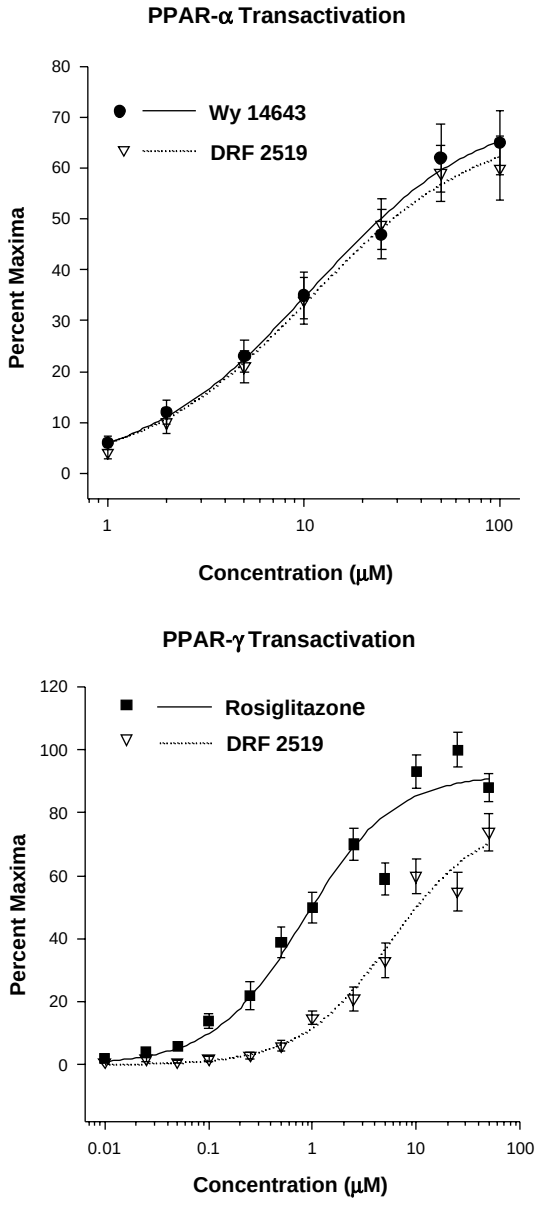


Figure 1. PPAR-α and -γ transactivation of DRF-2519. PPAR activation was carried out as mentioned in Section 5. Fourfold activation by WY 14643 at 25 μM and 87-fold by rosiglitazone at 25 μM has been considered as maximum effect of PPAR-α and -γ, respectively. Values are expressed as means ± SE from three experiments conducted in triplicate.

Table 2. Comparative effects of DRF-2519 with rosiglitazone in ob/ob mice

Plasma parameters	ED_{50} (mg/kg) for DRF-2519	ED_{50} (mg/kg) for rosiglitazone	ED_{50} (mg/kg) for KRP-297
PG	1.2	3.6	3
TG	1.5	10	10
FFA	2.0	>10	10
Insulin	1.1	5.0	8

ED_{50} was calculated according to the regression analysis of the dose–response curve.

cycle. All animals were given standard Laboratory chow and water ad libitum. Male SD rats weighing 180–200 g were made hyperlipidemic by feeding a high fat diet containing 2% cholesterol and 1% sodium cholate mixed with standard Laboratory chow. Hyperlipedemic Sprague–Dawley rats were treated orally with DRF-2519 for 6 days. Plasma samples were separated for lipid measurement. All plasma parameters were measured in Merck vitalab autoanalyzer. Plasma insulin was measured with a rodent RIA kit from LINCO research (USA). Blood samples were collected in fed state from the animals under mild ether anesthesia from retro-orbital sinus, 1 h after drug administration.

5.2. PPAR transactivation assay

The response element, upstream activated sequence of galactose DNA binding domain yeast transcription factor (UASGAL4 × 5) is present upstream of pFR-Luc reporter (Promega, Madison, USA) that contains simian virus early promoter for luciferase assay. GAL4 fusions were made by fusing human PPAR-γ or PPAR-α ligand binding domain (amino acids: 174–475) to the C-terminal end of the yeast GAL4 DNA binding domain (amino acids: 1–147) of pM1 vector. pAdvantage (Promega) vector was used to enhance luciferase expression. HEK 293T cells were transfected with relevant plasmids by superfect as per the supplier's instruction manual. Forty-two hours after transfection, cells were treated for 18 h with the test compounds. DMSO (1:1000) was used as blank. Luciferase activity was determined as fold activation relative to untreated cells by using Luclite kit (Packard Instrument Co, Meriden, CT, USA) in a Packard Top Count (Packard Instrument Co.).

5.3. Synthesis

Melting points were determined with a Veego melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin–Elmer FT-IR 1600 spectrometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian Gemini 200 MHz spectrometer with TMS as the internal standard. Mass spectra were recorded on a HP-5989A mass spectrometer. Column chromatography was performed by using silicagel (200–400 mesh, SRL) with the indicated solvent. 4-(2-Bromo ethoxy) benzaldehyde[12] was prepared according to the general method.

5.3.1. Preparation of 2-ethyl-3,4-dihydro-2*H*-benzo[1,3]-oxazaine-4-one (8a). To a stirred solution of PPE (6.62 g, 15.32 mmol) was added a suspension of salicylamide (2 g, 14.6 mmol) in 20 ml of dry chloroform, followed by a solution of propionaldehyde (1.20 ml, 16.05 mmol) in 5 ml chloroform. The mixture was refluxed for a period of 4 h (reaction monitored by TLC). Chloroform was evaporated completely and the residue was cooled, made alkaline by using 10% aqueous NaOH solution to pH 10, when a cream-colored solid precipitated out. The solid was filtered off and washed with water and pet. ether to afford the title compound as a solid. Yield: 2.08 g (81%); mp: 108–110 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.95–7.91 (m, 1H), 7.50–7.41 (m, 1H), 7.13–7.00 (m, 1H), 6.97 (d, J = 7.50 Hz, 1H), 5.28–5.22 (m, 1H), 2.05–1.90 (m, 2H), 1.13 (t, J = 7.50 Hz, 3H); IR (KBr): 1683, 3405 cm^{-1}; CIMS (*m*/*z*): 178 (M+H)$^+$.

Table 3. Effects of DRF-2519 on high fat fed Sprague–Dawley rats

Group	TG (mg/dl)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Control	116 ± 18	342 ± 59	22 ± 2	297 ± 58
DRF-2519 (3 mg/kg/day)	59 ± 2.8*	226 ± 11*	24 ± 4	184 ± 7
DRF-2519 (10 mg/kg/day)	38 ± 4*	168 ± 25*	32 ± 2*	132 ± 10*
Fenofibrate (30 mg/kg/day)	49 ± 4*	177 ± 15*	80 ± 3*	130 ± 11*

Male Sprague–Dawley rats were made hyperlipidemic as mentioned in Section 5. Compounds were given for 6 days at respective doses. Values are expressed as means ± SE ($n = 5$ per group). TG, triglyceride; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein.
* $p < 0.05$ as compared to control group (ANOVA).

In the same manner, the following compounds were obtained.

5.3.2. Preparation of 2-propyl-3,4-dihydro-2*H*-benzo[1,3]-oxazaine-4-one (8b). Yield: 75%; mp: 98 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.95–7.91 (m, 1H), 7.50–7.41 (m, 1H), 7.14–7.05 (m, 1H), 6.97 (d, $J = 8.30$ Hz, 1H), 5.33–5.26 (m, 1H), 2.03–1.90 (m, 2H), 1.86–1.62 (m, 2H), 1.02 (t, $J = 7.35$ Hz, 3H); IR (KBr): 3470, 1683, 1469 cm^{-1}; CIMS (*m*/*z*): 192 $(M+H)^+$.

5.3.3. Preparation of 2-phenyl-3,4-dihydro-2*H*-benzo[1,3]-oxazaine-4-one (8c). Yield: 68%; mp: 166–167 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.00–7.95 (m, 1H), 7.6–7.51 (m, 2H), 7.49–7.42 (m, 4H), 7.18–7.10 (m, 1H), 6.98 (d, $J = 8.06$ Hz, 1H), 6.58 (br s, 1H), 6.25 (s, 1H); IR (KBr): 3186, 1685, 1610, 1467 cm^{-1}; CIMS (*m*/*z*): 225 $(M+H)^+$.

5.3.4. Preparation of 4-[2-(4-oxo-3,4-dihydro-2*H*-benz[1,3]oxazin-3-yl)ethoxy]benzaldehyde (2). To an ice-cold stirred solution of 3-(2-hydroxyethyl)-2*H*-1,3-benzoxazin-4(3*H*)one[4] (0.5 g, 2.59 mmol) in dry DMF (5 ml) was added portionwise NaH (60%). After stirring for 10 min at room temperature, 4-fluorobenzaldehyde (0.35 g, 2.85 mmol) was added dropwise at 0 °C. The reaction was stirred at room temperature for 3 h and quenched by adding ice pieces when a cream colored solid separated out, which was filtered, washed with water, and dried to afford the title product. Yield: 0.58 g (75.4%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.89 (s, 1H), 7.95 (d, $J = 7.60$ Hz, 1H), 7.84 (d, $J = 8.62$ Hz, 2H), 7.45 (t, $J = 7.05$ Hz, 1H), 7.12 (t, $J = 7.40$ Hz, 1H), 7.0 (d, $J = 8.62$ Hz, 2H), 6.98 (d, $J = 8.07$ Hz, 1H), 5.37 (s, 2H), 4.30 (t, $J = 4.82$ Hz, 2H), 3.99 (t, $J = 4.82$ Hz, 2H); IR (KBr): 1697, 1650 cm^{-1}; CIMS (*m*/*z*): 297 (M+).

5.3.5. Preparation of 4-[2-(2-ethyl-4-oxo-3,4-dihydro-2*H*-benzo[1,3]oxazin-3-yl)ethoxy]benzaldehyde (9a). A mixture of 2-ethyl-3,4-dihydro-2*H*-benzo[1,3]oxazaine-4-one (1 g, 5.64 mmol), fused K_2CO_3 (2.35 g, 16.94 mmol) in 10 ml of dry DMF was stirred for a period of 30 min followed by the addition of a solution of 4-(2-bromoethoxy)benzaldehyde (1.29 g, 5.64 mmol) in 5 ml of dry DMF at room temperature. The reaction mixture was then stirred at 70 °C for a period of 24 h. The reaction mixture was diluted with ethyl acetate (100 ml) and washed with water (3×50 ml), brine (50 ml). The organic layer was dried over anhydrous Na_2SO_4, evaporated, and chromatographed using (1:3) EtoAc/pet. ether system to afford 30% yield of title product as a colorless gum.

^{1}H NMR (200 MHz, $CDCl_3$): δ 9.88 (s, 1H), 7.94–7.85 (m, 1H), 7.82 (d, $J = 6.71$ Hz, 2H), 7.48–7.39 (m, 1H), 7.11–6.92 (m, 4H), 5.42–5.36 (m, 1H), 4.38–4.22 (m, 3H), 3.67–3.60 (m, 1H), 2.07–1.82 (m, 2H), 1.03 (t, $J = 7.35$ Hz, 3H); IR (neat): 1662, 1601 cm^{-1}; CIMS (*m*/*z*): 326 $(M+H)^+$.

Following aldehydes were prepared in a similar manner mentioned above.

5.3.6. Preparation of 4-[2-(2-propyl-4-oxo-3,4-dihydro-2*H*-benzo[1,3]oxazin-3-yl)ethoxy]benzaldehyde (9b). Yield: 30%; ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.87 (s, 1H), 7.94–7.89 (m, 1H), 7.85–7.78 (m, 2H), 7.52–7.35 (m, 1H), 7.14–6.86 (m, 4H), 5.51–5.44 (m, 1H), 4.40–4.19 (m, 3H), 3.68–3.55 (m, 1H), 1.90–1.70 (m, 2H), 1.57–1.3 (m, 2H), 1.02 (t, $J = 7.35$ Hz, 3H); IR (neat): 1663, 1601, 1471 cm^{-1}; CIMS (*m*/*z*): 340 $(M+H)^+$.

5.3.7. Preparation of 4-[2-(2-phenyl-4-oxo-3,4-dihydro-2*H*-benzo[1,3]oxazin-3-yl)ethoxy]benzaldehyde (9c). Yield: 20%; ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.89 (s, 1H), 9.33 (d, $J = 8.81$ Hz, 1H), 8.23 (d, $J = 8.81$ Hz, 1H), 7.83 (d, $J = 7.79$ Hz, 2H), 7.75 (d, $J = 9.72$ Hz, 1H), 7.59 (t, $J = 5.58$ Hz, 2H), 7.43–7.29 (m, 3H), 7.00–6.50 (m, 3H), 6.51 (m, 1H), 4.44–4.34 (m, 4H); IR (KBr): 3390, 1676, 1600 cm^{-1}; CIMS (*m*/*z*): 373 (M^+).

5.3.8. Preparation of 4-[(2,2-diethoxy)ethoxy]benzaldehyde (9d). To a stirred suspension of 98% sodium hydride (2.5 g, 100 mmol) in DMF (100 ml) was added a solution of 4-hydroxybenzaldehyde (10 g, 82 mmol) in DMF (100 ml) slowly dropwise at room temperature and stirred for 30 min and bromoacetaldehyde diethylacetal (19.7 g, 100 mmol) was added to the reaction mixture, and the mixture was heated at 60 °C for 48 h. The reaction mixture was cooled to room temperature, quenched with water (200 ml), and extracted with ethyl acetate (3×300 ml). The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated. The crude compound was purified by chromatography using EtOAc/pet. ether (1:2) as eluent to yield the title compound (12.65 g, 58%) as a brown colored liquid.

^{1}H NMR (200 MHz, $CDCl_3$): δ 9.88 (s, 1H), 7.82 (d, $J = 8.63$ Hz, 2H), 7.02 (d, $J = 8.63$ Hz, 2H), 4.85 (t, $J = 5.17$ Hz, 1H), 4.08 (d, $J = 7.17$ Hz, 2H), 3.88–3.50 (m, 4H), 1.24 (t, $J = 7.03$ Hz, 6H); IR (neat): 1670, 1560; CIMS (*m*/*z*): 239 $(M+H)^+$.

5.3.9. Preparation of 5-[4-[2-[2*H*-1,3-benzoxazin-4(3*H*) one-3-yl]ethoxy]phenylmethylene]thiazolidine-2,4-dione (3). A mixture of 4-[2-(4-oxo-3,4-dihydro-2*H*-benz[1,3]oxazin-3-yl)ethoxy]benzaldehyde (2.82 g, 9.49 mmol), 2,4-thiazolidinedione (1.10 g, 9.49 mmol), benzoic acid (0.15 g, 1.23 mmol), and piperidine (0.12 g, 1.42 mmol) in 50 ml toluene was refluxed with continuous removal of water formed during the reaction for 2 h. The reaction mixture was cooled and the yellow solid was filtered off and dried to afford the title compound. Yield: 3.23 g, 86%; mp: 188–190 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.96 (d, J = 7.88 Hz, 1H), 7.79 (s, 1H), 7.45 (d, J = 8.39 Hz, 2H), 7.12 (d, J = 7.47 Hz, 1H), 7.00 (d, J = 7.50 Hz, 2H), 6.99 (d, J = 8.39 Hz, 2H), 5.37 (s, 2H), 4.28 (t, J = 4.78 Hz, 2H), 3.98 (t, J = 4.78 Hz, 2H); IR (KBr): 3437, 1694, 1661 cm^{-1}; CIMS (*m*/*z*): 396 (M^+). Analysis calculated for $C_{20}H_{16}N_2O_5S$: C, 60.65; H, 4.06; N, 7.07. Found: C, 60.45; H, 3.95; N, 7.11%.

Following compounds (**10a**–**c**) and **16** were prepared in the similar procedure mentioned above.

5.3.10. Preparation of 5-[4-[2-[2-ethyl-1,3-benzoxazin-4(3*H*)one-3- yl]ethoxy]phenyl methylene]thiazolidine-2,4-dione (10a). Yield: 79%; mp: 156–158 °C; ^{1}H NMR (200 MHz $CDCl_3$): δ 8.19 (br s, 1H), 7.91 (d, J = 7.89 Hz, 1H), 7.77 (s, 1H), 7.43 (d, J = 8.63 Hz, 2H), 7.11–6.91 (m, 5H), 5.41–5.35 (m, 1H), 4.24 (t, J = 7.90 Hz, 2H), 3.64 (t, J = 7.90 Hz, 2H), 2.02–1.88 (m, 2H), 1.03 (t, J = 7.40 Hz, 3H); IR (KBr): 1740, 1703, 1640 cm^{-1}; CIMS (*m*/*z*): 425 $(M+H)^+$. Analysis calculated for $C_{22}H_{20}N_2O_5S$: C, 62.31; H, 4.75; N, 6.60. Found: C, 62.28; H, 4.76; N, 6.55%.

5.3.11. Preparation of 5-[4-[2-[2-propyl-1,3-benzoxazin-4(3*H*)one-3-yl]ethoxy]phenylmethylene]thiazolidine-2,4-dione (10b). Yield: 81%; mp: 160–162 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.80 (br s, 1H), 7.90 (d, J = 7.80 Hz, 1H), 7.77 (s, 1H), 7.50–7.39 (m, 3H), 7.15–6.90 (m, 4H), 5.51–5.40 (m, 1H), 4.40–4.10 (m, 4H), 3.81–3.5 (m, 2H), 2.10–1.70 (m, 2H), 0.98 (t, J = 7.40 Hz, 3H); IR (KBr): 3035, 1745, 1703, 1641 cm^{-1}; CIMS (*m*/*z*): 439 $(M+H)^+$. Analysis calculated for $C_{23}H_{22}N_2O_5S$: C, 63.06; H, 4.05; N, 6.39. Found: C, 62.83; H, 4.93; N, 6.60%.

5.3.12. Preparation of 5-[4-[2-[2-phenyl-1,3-benzoxazin-4(3*H*)one-3-yl]ethoxy]phenylmethylene]thiazolidine-2,4-dione (10c). Yield: 85%; mp:170–172 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.41 (d, J = 9.60 Hz, 1H), 8.90 (br s, 1H, D_2O exchangeable), 8.32 (d, J = 8.0 Hz, 1H), 7.89 (d, J = 8.0 Hz, 1H), 7.86 (s, 1H), 7.80–7.60 (m, 1H), 7.58–7.25 (m, 5H), 7.27–6.80 (m, 4H), 6.60 (s, 1H), 4.60–4.30 (m, 4H); IR (KBr): 3368, 1740, 1701, 1597 cm^{-1}; CIMS (*m*/*z*): 473$(M+H)^+$. Analysis calculated for $C_{26}H_{20}N_2O_5S$: C, 66.15; H, 4.26; N, 5.93. Found: C, 65.97; H, 4.17; N, 5.99%.

5.3.13. 5-[4-[(2,2-Diethoxy)]phenyl]methylene]thiazolidine-2,4-dione (10d). A mixture of 4-[2,2-diethoxy]ethoxy]benzaldehyde (10.6 g, 44.53 mmol), thiazolidine-2,4-dione (5.21 g, 44.53 mmol), benzoic acid (0.7 g, 5.78 mmol), and piperidine (0.64 ml, 6.7 mmol) in toluene (150 ml) was refluxed for 2 h under continuous removal of water. The reaction mixture was cooled to room temperature and diluted with EtOAc (150 ml). The mixture was washed with water, brine, dried over sodium sulfate, and concentrated. The crude compound was purified by column chromatography using EtOAc/pet. ether (1:2) as eluent to afford the title compound (12.54 g, 70%) as a brown colored oil.

^{1}H NMR (200 MHz, $CDCl_3$): δ 8.70 (br s, 1H, D_2O exchangeable), 7.80 (s, 1H), 7.45 (d, J = 8.72 Hz, 2H), 4.87 (t, J = 5.21 Hz, 1H), 4.08 (d, J = 5.21 Hz, 2H), 3.90–3.52 (m, 4H), 1.26 (t, J = 7.02 Hz, 6H); IR (neat): 1740, 1700, 1620 cm^{-1}; CIMS (*m*/*z*): 338 $(M+H)^+$.

5.3.14. Preparation of 5-[4-[2-[2*H*-1,3-benzoxazin-4(3*H*)one-3-yl]ethoxy]phenylmethyl]thiazolidine-2,4-dione (4). A solution of 5-[4-[2-[2*H*-1,3-benzoxazin-4(3*H*)one-3-yl]ethoxy]phenylmethylene]thiazolidine-2,4-dione (3.2 g, 8.08 mmol) in 75 ml of 1,4-dioxan and 10% Pd/C (8 g) was taken in a 500 ml parr hydrogenation flask and hydrogenated at 60 psi for 48 h at room temperature. The catalyst was filtered off through a Celite bed and washed with dioxane. Combined organic layers were evaporated to afford the product, which was crystallized from dichloromethane/hexane. Yield: 2.4 g, 74.6%; mp: 110–112 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.96 (d, J = 7.56 Hz, 1H), 7.45 (t, J = 7.20 Hz, 1H), 7.15 (d, J = 8.63 Hz, 2*H*), 7.08 (t, J = 7.40 Hz, 1H), 6.95 (d, J = 7.40 Hz, 1H), 6.84 (d, J = 8.63 Hz, 2H), 5.38 (s, 2H), 4.50 (dd, J = 9.20 and 3.87 Hz, 1H), 4.20 (t, J = 4.60 Hz, 2H), 3.97 (t, J = 4.60 Hz, 2H), 3.44 (dd, J = 14.11 and 4.15 Hz, 1H), 3.12 (dd, J = 14.10 and 9.40 Hz, 1H); IR (KBr): 1756, 1701, 1650 cm^{-1}; CIMS (*m*/*z*): 399 $(M+H)^+$. Analysis calculated for $C_{20}H_{18}N_2O_5S$: C, 60.35; H, 4.55; N, 7.04. Found: C, 60.29; H, 4.40; N, 7.02%.

5.3.15. 5-[4-[[4-oxo-3,4-dihydro-(2*H*)-1,3-benzoxazine-2-yl]methoxy]phenylmethyl]thiazolidine-2,4-dione (13a). To a stirred solution of polyphosphonate ethyl ester (PPE) (3.15 g, 7.29 mmol) in chloroform (4 ml) was added salicylamide (0.5 g, 3.65 mmol) followed by addition of a solution of 5-[4-[(2,2-diethoxy]phenylmethylene]thiazolidine-2,4-dione (**10d**) (1.36 g, 4.0 mmol) in chloroform (10 ml) dropwise at room temperature. The reaction mixture was refluxed for 3 h, cooled to room temperature, and $CHCl_3$ was removed under reduced pressure. To the resultant residue aq saturated $NaHCO_3$ solution was added (25 ml) and stirred for 30 min. The precipitated brown colored solid was filtered and purified by column chromatography by EtOAc/pet. ether (1:1) to yield the title compound, (1.15 g, 81%). mp: 134–138 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.40 (br s, 1H, D_2O exchangeable), 7.90 (d, J = 7.50 Hz, 1H), 7.15 (d, J = 8.30 Hz, 2H), 7.05 (t, J = 7.50 Hz, 1H), 6.90 (d, J = 7.50 Hz, 1H), 6.80 (d, J = 8.30 Hz, 2H), 5.80 (t, J = 5.30 Hz, 1H), 4.42 (dd, J = 9.50, 3.80 Hz, 1H), 4.30–4.10 (m, 2H), 3.34 (dd, J = 14.10, 3.80 Hz, 1H), 3.02 (dd, J = 14.10, 9.50 Hz, 1H); IR (KBr): 1751, 1699, 1650 cm^{-1}; CIMS (*m*/*z*): 385 (*m*/*z*): 385 $(M)^+$. Analysis calculated for

$C_{19}H_{16}N_2O_5S$: C, 59.42; H, 4.19; N, 7.29. Found: C, 59.39; H, 4.11; N, 7.14%.

5.3.16. 5-[4-[[3-Methyl-4-oxo-3,4-dihydro-(2*H*)-1,3-benzoxazine-2-yl]methoxy]phenylmethyl]thiazolidine-2,4-dione (13b). This compound was prepared in a similar manner as described for **13a** starting from *N*-methyl salicylamide. Yield: 60%; mp: 187 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.23 (br s, 1H, D_2O exchangeable), 7.95 (d, J = 7.50 Hz, 1H), 7.43 (t, J = 7.50 Hz, 1H), 7.12 (d, J = 8.54 Hz, 2H), 7.08 (t, J = 5.39 Hz, 1H), 6.93 (d, J = 7.50 Hz, 1H), 6.77 (d, J = 8.54 Hz, 2H), 5.62 (t, J = 5.39 Hz, 1H), 3.21 (d, J = 3.83 Hz, 3H), 3.10 (dd, J = 14.05, 9.04 Hz, 1H); IR (KBr): 1757, 1702, 1646 cm^{-1}; CIMS (*m*/*z*): 399 $(M+H)^+$. Analysis calculated for $C_{20}H_{18}N_2O_5S$: C, 60.35; H, 4.55; N, 7.04. Found: C, 60.22; H, 4.58; N, 7.14%.

5.3.17. Preparation of 5-[4-[2-[2*H*-1,3-benzoxazin-4(3*H*)one-3-yl]ethoxy]phenylmethyl]thiazolidine-2,4-dione sodium salt (5; DRF-2519). To a stirred solution of 5-[4-[2-[2*H*-1,3-benzoxazin-4(3*H*)one-3-yl]ethoxy]phenylmethyl]thiazolidine-2,4-dione (0.65 g, 1.63 mmol) in 9 ml of dry MeOH was added NaOMe (freshly prepared by dissolving 150 mg sodium in 6 ml MeOH) and stirred for 30 min. About 25 ml of dry ether was added to the reaction mixture when a white solid separated out, which was filtered and dried. Yield: 0.53 g, 77.3%; mp: 277 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.80 (d, J = 7.50 Hz, 1H), 7.49 (t, J = 7.50 Hz, 1H), 7.18 (t, J = 7.50 Hz, 1H), 7.09 (d, J = 8.53 Hz, 2H), 7.05 (d, J = 7.50 Hz, 1H), 6.84 (d, J = 8.53 Hz, 2H), 5.40 (s, 2H), 4.11 (t, J = 5.39 Hz, 2H), 4.03 (dd, J = 10.47 and 3.33 Hz, 1H), 3.84 (t, J = 5.39 Hz, 2H), 3.33 (dd, J = 14.02 and 3.33 Hz, 1H), 2.60 (dd, J = 14.02 and J = 10.47 Hz, 1H); IR (KBr): 1666, 1612, 1586 cm^{-1} ; CIMS (*m*/*z*): 398 (M^+; for metal free ligand).

Acknowledgment

We are thankful for Dr. Reddy's management and the encouragement and support provided.

References and notes

1. Schoonjans, K.; Peinado-Onsurbe, J.; Lefebvre, A. M.; Heyman, R. A.; Briggs, M.; Deeb, S.; Staels, B.; Auwerx, J. *EMBO J.* **1996**, *15*, 5336.
2. Staels, B.; Dallongeville, J.; Auwerx, J.; Schoonjans, K.; Leitersdorf, E.; Fruchart, J. C. *Circulation* **1998**, *98*, 2088.
3. Staels, B.; Auwerx, J. *Curr. Pharm. Des* **1997**, *3*, 1.
4. Li, A. C.; Binder, C. J.; Gutierrez, A.; Brown, K. K.; Plotkin, C. R.; Pattison, J. W.; Valledor, A. F.; Davis, R. A.; Willson, T. M.; Witztum, J. L.; Palinski, W.; Glass, C. K. *J. Clin. Invest.* **2004**, *114*, 1564.
5. Perry, C.; Petrie, J. R. *Emerging Drugs* **1998**, *3*, 247.
6. Brad, R. H. *J. Med. Chem* **2004**, *47*, 4118.
7. Francesca, B.; Georgio, B.; Roberta, C.; Giancarlo, D.; Gianni, G.; Silvio, L.; Jacques, M.; Alberto, S. *J. Med. Chem* **1995**, *38*, 130.
8. Johnson, J. R. *Org. React.* **1942**, *1*, 210.
9. Madhavan, G. R.; Chakrabarti, R.; Vikramadithyan, R. K. V.; Rao, N. V. S. M.; Balaraju, V.; Rajesh, B. M.; Misra, P.; Kumar, K. B. S.; Lohray, B. B.; Lohray, V. B.; Rajagopalan, R. *Biorg. Med. Chem* **2002**, *10*, 2671.
10. Genuth, S. M.; Przybylski, R. J.; Rosenberg, D. M. *Endocronology* **1971**, *88*, 1230.
11. Miyachi, H. *Yakugaku Zasshi* **2004**, *124*, 803.
12. Nate, H.; Matsuki, K.; Tsuna Shina, A.; Ohtsuka, H.; Sekine, Y. *Chem. Pharm. Bull.* **1987**, *35*, 2394.