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Original article

Synthesis and biological evaluation of ester prodrugs of benzafibrate as orally active hypolipidemic agents

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ABSTRACT

A series of bezafibrate ester prodrugs **1**–**7** were synthesized and evaluated for hypolipidemic activity in Swiss Albino mice (SAM). Bezafibrate (**1a**), a hypolipidemic drug was used as a reference compound for data comparison. Among the synthesized compounds, prodrug **7** showed superior activities in decreasing triglyceride up to 30% in mice plasma after oral administration of 50 mg/kg/day for 8 days. Prodrugs **2**, **3**, **5**, **6**, and **7** were found to be more lipophilic than bezafibrate (**1a**), indicated by partition coefficients measured in octanol-buffer system at pH 7.4. On the basis of *in vivo* studies, prodrug **7** emerged as new potent hypolipidemic agent.

1. Introduction

High-serum low-density lipoprotein (LDL) and elevated total cholesterol levels are the most prevalent indicators for susceptibility to atherosclerotic heart disease [1,2]. Hypercholesterolemia is now considered a major risk factor in the development of premature atherosclerosis. Past efforts to slow or even reverse this disease process have focused almost exclusively on the development of potent hypocholesterolemic agents. As a result of these efforts, several classes of hypolipidemic agents are now available for the control of hypercholesterolemia. These include the most recent class of agents, the HMG CoA reductase inhibitors [3], the second-generation fibric acid derivatives [4], and the bile acid sequestrants [5]. These various agents can be separated on the basis of their different mechanisms of action, and the combination of cholesterol-lowering drugs from different classes represents a currently accepted therapy for aggressive cholesterol-lowering therapy.

Fibrate class of drugs, discovered a decade ago, are effective in reducing the serum triglyceride and increasing the high-density lipoprotein (HDL) cholesterol in humans. Bezafibrate (**1a**, Fig. 1) is fibric acid derivative, corresponds to the nomenclature of 2-(4-{2-[(4-chlorobenzoyl)amino]ethyl}phenoxy)-2-methylpropanoic acid and well-known activator of peroxisome proliferator-activated receptors (PPARs), that can activate both PPAR-α and PPAR-β. It is used to reduce triglyceride and cholesterol in the management of hyperlipidemias, including type IIa, type IIb, type III, type IV, and type V hyperlipoproteinemias [6]. The usual dose is 200 mg three times daily by mouth taken with or after food. Bezafibrate reduce triglycerides by lowering the concentration of very-low-density lipoprotein (VLDL). They reduce low-density lipoprotein cholesterol (LDLC) to a lesser extent, although the effect is variable, and may also increase high-density lipoprotein cholesterol (HDLC) [7].

New strong fibrates with piperidine moiety showed very superior activities in decreasing triglyceride and cholesterol compared to bezafibrate in mice and rats [8]. α-Asarone bioisosteric analogs of fibrates such as clofibrate, bezafibrate, and fenofibrate have significant hypocholesterolemic activity [9–11]. A prodrug is a pharmacological substance (drug) administered in an inactive (or significantly less active) form. The rationale behind the use of

Abbreviations: HPLC, high performance liquid chromatography; GC, gas chromatograph; API, active pharmaceutical ingredient; DMAc, dimethylacetamide; Ar, aryl; PPAR-α, peroxisome proliferator-activated receptor alpha; PPAR-β, peroxisome proliferator-activated receptor beta; SE, standard error; TC, total cholesterol; TG, triglyceride; HDLC, high-density lipoprotein cholesterol; LDLC, low-density cholesterol; VLDL, very-low-density lipoprotein; SAM, Swiss albino mice; RT, retention time; BDL, below detection limit; CAD, coronary artery diseases; PSD, particle size distribution.

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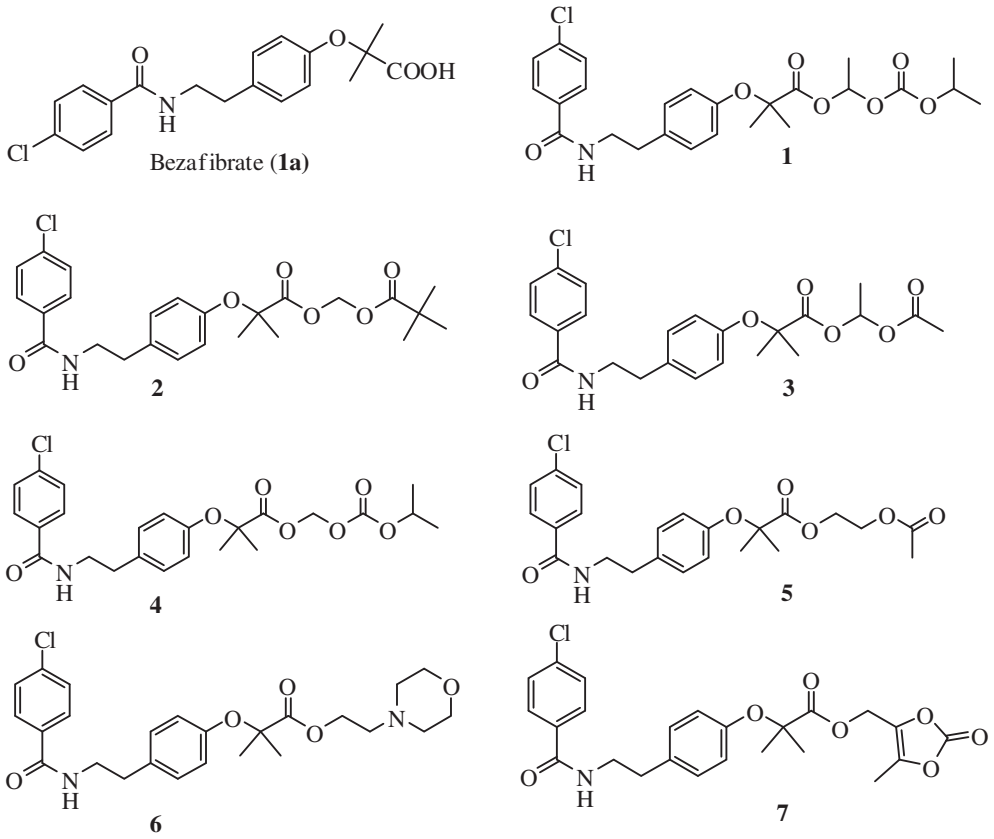


Fig. 1. Chemical structures of bezafibrate (**1a)** and prodrugs **1**–**7**.

a prodrug is generally for absorption, distribution, metabolism, and excretion (ADME) optimization. Prodrugs are usually designed to improve oral bioavailability, eventually to improve the efficacy as well.

Once administered, the prodrug is metabolized *in vivo* into an active metabolite. Thus, prodrugs are molecules that must undergo biotransformation prior to exhibiting their therapeutic effects. Our strategy to synthesize new bezafibrate prodrugs **1**–**7** (Fig. 1) consists of modifying the carboxyl function by selected bulkier derivatives and studying the impact of such modifications on the hypolipidemic activity in male Swiss albino mice (SAM). The metabolic product (i.e. parent drug) subsequently elicits the desired pharmacological response [12,13].

The physicochemical properties of a drug plays major role in the development of formulation and bioavailability. Thus, physicochemical parameters like partition coefficient (log *P*), aqueous stability and particle size distribution were studied.

2. Results and discussion

2.1. Chemistry

A series of novel bezafibrate ester prodrugs **1**–**7** were readily synthesized in good yields (88%, 85%, 74%, 83%, 87%, 76%, 73%) by straight forward condensation of bezafibrate (**1a**) with appropriate promoiety **a**–**g** (Fig. 2), in the presence of 1,1,3,3,-tetramethyl guanidine (TMG) or sodium carbonate or potassium carbonate in dimethylacetamide (DMAc), according to the Scheme 1. Promoieties **a**–**g** were synthesized as per the procedures given in our earlier published articles [14,15]. The structures of all prodrugs were established by IR, ^{1}H NMR, ^{13}C NMR, mass spectrometry, elemental analysis, and their purity in excess of 99% was confirmed by HPLC analysis (Supporting Information).

2.2. Pharmacological evaluation

Owing to interest in the synthesis of new hypolipidemic agents, series of bezafibrate ester prodrugs **1**–**7** were synthesized and evaluated for triglyceride and cholesterol-lowering potential in Swiss albino mice (SAM), a moderate hypertriglyceride model [16–20]. Normal mice (chow fed) were used for primary screening of hypolipidemic agents. The mice were treated with prodrugs **1**–**7** and bezafibrate (**1a)** by oral gavage at a dose of 50 mg/kg/day for 8 days. The *in vivo* profile of compounds **1**–**7** was compared with a reference drug bezafibrate (**1a)** for hypolipidemic activity. The prodrugs **2**, **3**, **6**, and **7** were found more potent in lowering triglyceride than bezafibrate (**1a**) and the prodrug **7** alone showed a significant reduction in mice plasma triglyceride level (TG). Total cholesterols (TCs) did not show significant changes in the bezafibrate as well as prodrug-treated animals (Table 1, Fig. 3). The TG lowering activity of bezafibrate (**1a**) was improved by esterification with 4-chloromethyl-5-methyl-1,3-dioxol-2-one and the lowering effect of TG in mice plasma was the highest among all prodrugs. This may be because of improvement in the oral bioavailability of the compound eventually better efficacy.

2.3. Dose response of prodrug **7** in Swiss albino mice

Prodrug **7** alone lowered the mice triglyceride level significantly ($P < 0.01$) as compared to normal control and hence a dose effect relationship for triglyceride (TG), and for total cholesterol (TC) at the doses of 10, 30, 100 mg/kg/day was studied and the results were

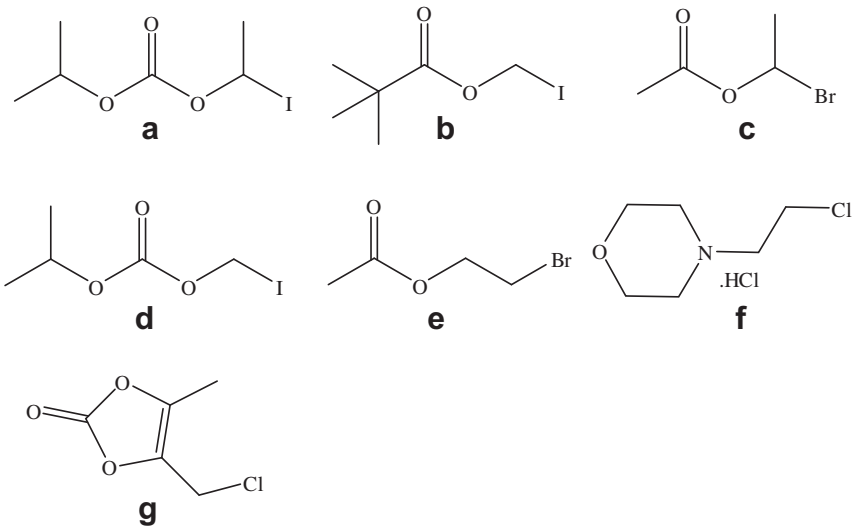


Fig. 2. Chemical structures of (a) iodoethylisopropyl carbonate, (b) iodomethyl pivalate, (c) 1-acetoxyethyl bromide, (d) iodomethylisopropyl carbonate, (e) 2-acetoxyethyl bromide, (f) 4-(2-chloroethyl) morpholine hydrochloride, and (g) 4-chloromethyl-5-methyl-1,3-dioxol-2-one.

summarized in Table 2. A dose 30 mg/kg per day, showed the significant TG lowering activity (about 27%), which was identical to dose 50 mg/kg/day and dose 100 mg/kg/day (Table 2). There were no significant changes observed in total cholesterol (TC) at all doses in mice plasma (Table 2).

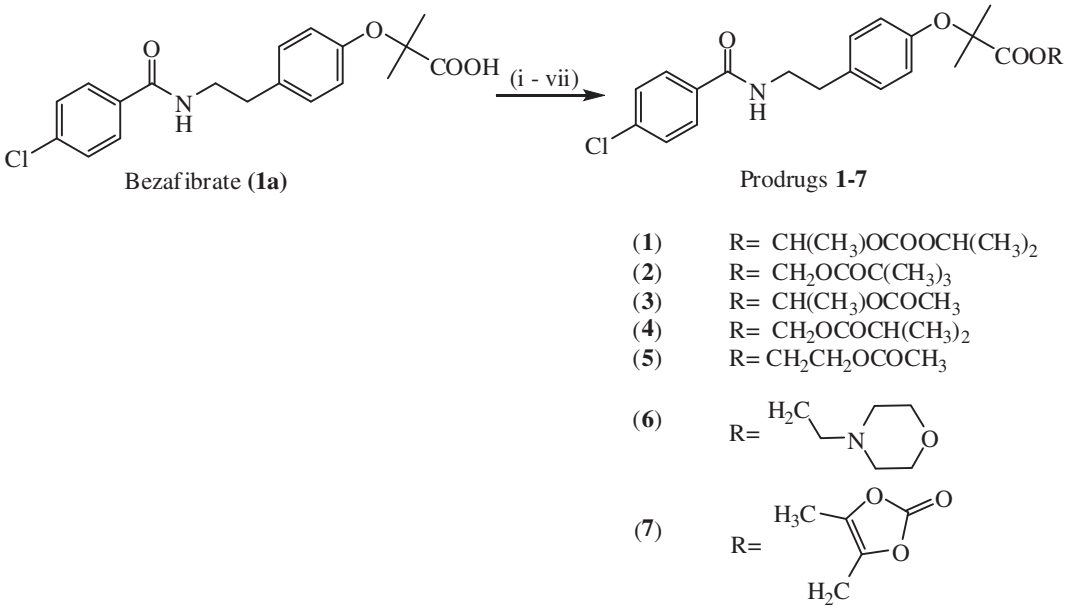
2.4. Physicochemical properties

2.4.1. Particle size distribution (PSD)

To improve the bioavailability, active pharmaceutical ingredients (API) are converted to fine particle size either by chemical synthesis or by physical operations like micronization/ spray drying etc. Lower PSD of compounds improves its oral bioavailability eventually better efficacy. Pharmacological profiles of prodrugs in lowering triglyceride (Table 1) indicate that prodrugs **2**, **3**, **6**, and **7** are more potent than bezafibrate (**1a**) even though these prodrugs compounds have either higher or identical particle size distribution (Table 3). Efficacy of these prodrugs may improve further upon micronization.

2.4.2. Partition coefficient (log P)

To examine the fundamental process of the transfer of drug to the biological membrane, the partition coefficient of prodrugs **1**–**7** and the reference drug bezafibrate (**1a)** was determined by HPLC method in octanol-buffer system at 25 °C. Partition coefficient experimental value of bezafibrate (**1a**) was in line with the value reported in the literature [21]. Higher log *P* values of all prodrugs indicated that prodrugs are more lipophilic than bezafibrate (**1a**) and prodrug **7** is less lipophilic than the prodrugs **1**–**6** (Table 4).



Scheme 1. Synthesis of bezafibrate ester prodrugs **1**–**7**: reagents and conditions: (i) $ICH(CH_3)OCOOCH(CH_3)_2$, DMAc, TMG, −5 °C, 30 min; (ii) $ICH_2OCO(CH_3)_3$, DMAc, TMG, −10 °C, 30 min; (iii) $BrCH(CH_3)OCOCH_3$, Na_2CO_3, DMAc, 30 °C, 3.5 h; (iv) $ICH_2OCOO(CH_3)_2$, DMAc, TMG, −10 °C, 30 min; (v) $BrCH_2CH_2OCOCH_3$, Na_2CO_3, DMAc, 55 °C, 8 h; (vi) promoiety **f**, K_2CO_3, DMAc, 65 °C, 6 h; (vii) promoiety **g**, Na_2CO_3, DMAc, 32 °C, 70 h.

Table 1
Hypolipidemic effect of prodrugs **1**–**7** and bezafibrate (**1a**) on triglyceride (TG) and total cholesterol (TC) level in Swiss albino mice (SAM)[a].

Compound	Dose (mg/kg)	TG		TC	
		(mg/dL)	% fall	(mg/dL)	% fall
Control	–	172.3 ± 8.0	–	123.8 ± 3.0	–
1a	50	152.7 ± 10.4	11.4	110.9 ± 4.2	10.4
1	50	165.6 ± 11.5	3.9	130.7 ± 4.4	−5.6[b]
2	50	39.9 ± 6.6	18.8	119.6 ± 2.3	3.4
3	50	142.6 ± 7.6	17.2	118.5 ± 4.3	4.3
4	50	167.1 ± 8.0	3.0	120.1 ± 3.5	3.0
5	50	155.7 ± 9.3	9.6	120.8 ± 3.8	2.4
6	50	152.0 ± 8.2	11.8	120.1 ± 2.8	3.0
7	50	120.3 ± 11.2	30.2	107.7 ± 3.1	13.0

[a] Each value represents the mean ± SE ($n = 10$). The percentage of TG and TC lowering action are shown relative to normal control.
[b] Negative value increase in the level of measured parameter, % fall and % rise are calculated for groups in relation to the control group.

2.4.3. Aqueous stability

Prodrug candidates **2**, **3**, **5**, **6**, and **7** were evaluated for physiological stability in buffers ranging from pH 1 to 9, so that they can be formulated in a stable dosage form. Buffers employed in chemical hydrolysis study covered pH of microclimate of stomach (pH 1–2), duodenum (pH 4.0–5.5), jejunum (pH 5.5–7.0), and systemic circulation (pH ~7.4). Results of hydrolysis of prodrugs in aqueous buffer at pH 1–9 are summarized in Table 5. All prodrugs were found highly stable at pH 1 and 3. Marginal hydrolysis of prodrugs was observed at pH 5.2, 7.4 and 9.0. Chemical stability of prodrugs **1**–**7** indicates that all these compounds have sufficient stability, ensuring them to be absorbed as intact moieties from the gastrointestinal tract (Table 5).

3. Conclusions

Novel bezafibrate ester prodrugs **1**–**7** were synthesized, and evaluated for hypolipidemic activity by known experimental techniques [16–18] in male Swiss albino mice (SAM) in comparison with reference drug bezafibrate (**1a**). On the basis of *in vivo* evaluation prodrug 7 demonstrated better efficacy in lowering triglyceride (30.2%) than reference drug bezafibrate (11.4%). This may be because of improvement in the oral bioavailability of the compound eventually better efficacy. Thus, on the basis of *in vivo* studies, prodrug candidate **7** has an improved therapeutic profile *in vivo* over their parent drug bezafibrate (**1a**) in SAM. Hence, a promising novel approach, prodrug concept, has been successfully worked out, yielding therapeutically better compounds for long-term oral hypolipidemic therapy.

Table 2
Hypolipidemic effect of prodrug **7** in Swiss albino mice (SAM).

Compound	Dose (mg/kg)	TG		TC	
		(mg/dL)	% fall	(mg/dL)	% fall
Control	–	117 ± 9.5	–	100.50 ± 8.47	–
7	10	113.5 ± 11	3.51	95.63 ± 3.26	4.9
7	30	85.8 ± 6.5	27.1	98.50 ± 2.19	2.0
7	100	91.9 ± 10.6	22.3	96.50 ± 5.34	4.0

Each value represents the mean ± SE ($n = 8$). The percentage of TG and TC lowering action are shown relative to normal control.

4. Experimental section

4.1. Materials and methods

Melting points were determined on an MR VIS (Lab India) melting point apparatus and are uncorrected. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Advance spectrophotometer using $CDCl_3$ and DMSO solvent. The chemical shifts are reported in ppm downfield from zero, and coupling constants are reported in hertz (Hz). IR spectra were acquired by using an FTIR Perkin-Elmer model RXI and FTIR spectrophotometer Nicolet 380 (Thermo Nicolet); software was Omnic. Mass spectra were recorded on a PE-SCIEX API-3000 LCMS/MS (Applied Biosystem) spectrophotometer. HPLC analysis was performed by using a Waters Alliance system, pump model 2695 with UV detector model 2487 and Shimadzu LC 2010 CHT with UV detector. Particle size distribution analysis was performed on Malvern particle size analyzer model Master sizer S. All prodrug compounds were analyzed by HPLC, and their purity was confirmed to be excess of 98.0%. *In vivo* study was performed on Clinical Chemistry analyzer (Erba XL 300) by using ERBA diagnostics kit and was carried out by using protocol approved by the Institutional Animal Ethics Committee (IAEC) which compiled with National Institute of Health (NIH) guidelines on handling of experimental animals. An inbreed colony (at Orchid Research Laboratory, India) of male Swiss albino mice (SAM) of *Mus Musculus* strain were used for the study. Commercially available promoieties **e** and **f** were gifted by Orex Pharma Private Limited, India and M/s Matrix Laboratories, India, respectively. Bezafibrate (EP grade) sample was gifted by D. K. Pharmachem Pvt. Ltd., India.

4.2. 2-[4-[2-[(4-Chlorobenzoyl)amino]ethyl]phenoxy}-2-methylpropanoic acid 1-isopropoxycarbonyloxyethyl ester (*1*)

A solution of **1a** (2 g, 5.52 mmol) in DMAc (10 mL) was treated with TMG (0.68 g, 5.87 mmol) at −5 °C for 20 min under N_2 atmosphere.

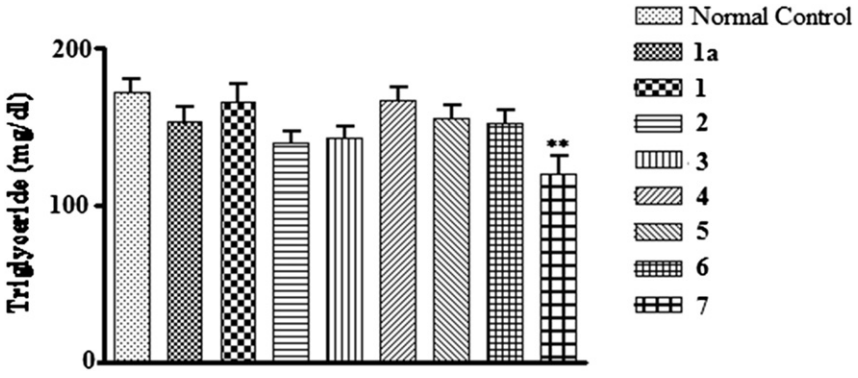


Fig. 3. Plasma triglyceride levels in Swiss albino mice treated via oral gavages for 8 days with bezafibrate (**1a**) and prodrugs **1**–**7**.

Table 3
Particle size distribution of bezafibrate (**1a**) and prodrugs **1**–**7**.

Compound	Particle size distribution (in μm)				
	<10%	<50%	<75%	<90%	<100%
1a	5	36	92	170	386
1	15	79	126	178	314
2	6	92	145	209	795
3	26	116	176	231	386
4	1	48	98	152	314
5	2	42	113	184	350
6	1	12	89	168	348
7	1	41	104	168	348

Iodoethylisopropyl carbonate (1.43 g, 5.54 mmol) was added at −5 °C, and stirred for 30 min at −5 °C under N_2 atmosphere. Reaction progress was monitored by HPLC. The reaction mixture was transferred to a mixture of ethyl acetate (25 mL), water (80 mL) and sodium thiosulfate (0.5 g) under vigorous stirring. The organic phase was separated, washed with brine solution (4 × 55 mL), treated with activated carbon (0.4 g), filtered and solvent was evaporated *in vacuo* at 35–40 °C to get solid residue. Water (50 mL) was added, the slurry was stirred at 5 °C for 30 min, filtered, washed with water (50 mL), and dried *in vacuo* at 50 °C for 4 h to afford the title compound **1** as white crystalline solid (3.5 g, 88%). Chromatographic purity (HPLC) 99.86%; mp 125.4 °C; MS (ESI^+) $m/z = 492$ $[M+H]^+$; IR (KBr) cm^{-1} 3272, 1759, 1633, 1263, 1073. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.30 (d, 6H, $J = 6.24$ Hz, $-OCH(CH_3)_2$), 1.50 (d, 3H, $J = 5.36$ Hz, $-OCH(O)CH_3$), 1.58 (s, 6H, $-C(CH_3)_2$), 2.87 (t, 2H, $J = 6.8$ Hz, $-NHCH_2CH_2Ar$), 3.7 (t, 2H, $J = 6.8$ Hz, $-HNCH_2CH_2Ar$), 4.89 (sep, 1H, $J = 6.24$ Hz, $-OCH(CH_3)_2$), 6.11 (bs, 1H, −N*H*), 6.8 (q, 1H, $J = 5.36$ Hz, $-OCH(O)CH_3$), 6.84–7.62 (m, 8H, Ar−*H*); ^{13}C NMR (DMSO, 100 MHz) δ 18.96 ($-OCH(O)CH_3$), 21.29 (2C, $-OCH(CH_3)_2$), 24.92 (2C, $ArOCH(CH_3)_2$), 34.18 ($-HNCH_2CH_2Ar$), 40.99 ($-HNCH_2CH_2Ar$), 72.51($-OCH(CH_3)_2$), 78.38 ($ArOCH(CH_3)_2$), 91.78 ($-OCH(O)CH_3$), 119.02–151.98 (12C, Ar), 153.8 (−OCOO), 165.07 (ArCONH−), 171.54 (−CHCOO). Anal. Calcd. for $C_{25}H_{30}ClNO_7$: C, 61.03; H, 6.15; N, 2.85. Found: C, 60.96; H, 6.12; N, 2.83.

4.3. 2-[4-[2-[(4-Chlorobenzoyl)amino]ethyl]phenoxy}-2-methylpropanoic acid 2,2-dimethylpropionyloxymethyl ester (**2**)

A solution of **1a** (5 g, 13.81 mmol) in DMAc (25 mL) was treated with TMG (1.68 g, 14.5 mmol) at −10 °C for 20 min under N_2 atmosphere. Then, iodomethyl pivalate (3.41 g, 14.1 mmol) was added at −10 °C and stirred for 30 min under N_2 atmosphere. The reaction progress was monitored by HPLC. The reaction mixture was transferred to a mixture of ethyl acetate (65 mL), water (200 mL) and sodium thiosulfate (0.5 g) under vigorous stirring. The pH of reaction mixture was adjusted to 10.0 with 2% aqueous ammonia solution and stirred for 10 min at 25 °C. The organic phase was separated, washed with brine solution (3 × 100 mL), treated with activated carbon (1.5 g), filtered and solvent was evaporated *in vacuo* 40 °C to get solid residue. To the residue, water (250 mL) was added, stirred for 30 min at 2 °C, filtered, washed with water (50 mL), and dried *in vacuo* at 45 °C for 5 h to afford the title compound **2** as white crystalline solid (5.6 g, 85%). Chromatographic purity (HPLC) 100%; mp 133 °C; MS (ESI^+) $m/z = 476.2$ $[M+H]^+$; IR (KBr) cm^{-1} 3251, 2977, 2939, 1755, 1634, 1094; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.17 (s, 9H, $-C(CH_3)_3$), 1.61 (s, 6H, $-OC(CH_3)_2$), 2.87 (t, 2H, $J = 6.8$ Hz, $-NHCH_2CH_2Ar$), 3.68 (t, 2H, $J = 6.8$ Hz, $-NHCH_2CH_2Ar$), 5.84 (s, 2H, $-OCH_2O$), 6.11 (bs, 1H, N−*H*), 6.81–7.63 (m, 8H, Ar−*H*); ^{13}C NMR (DMSO, 100 MHz) δ 24.81 (2C, $ArOCH(CH_3)_2$), 26.46 (3C, $-COCH(CH_3)_3$), 34.15 ($-HNCH_2CH_2Ar$), 38.22 ($-COCH(CH_3)_3$), 41.00 ($-HNCH_2CH_2Ar$), 78.38 ($ArOCH(CH_3)_2$), 79.80 ($-OCH_2O$), 118.96–153.11 (12C, Ar), 165.06 (ArCONH), 172.35 ($-OCOCH(CH_3)_3$), 176.08 (−OCHCOO). Anal. Calcd. for $C_{25}H_{30}ClNO_6$: C, 63.09; H, 6.35; N, 2.94. Found: C, 62.97; H, 6.32; N, 2.92.

Table 4
Partition coefficient of bezafibrate (**1a**) and prodrugs (**2**, **3**, **5**, **6**, **7**) in aqueous solution.

Compound	Partition coefficient (log *P*)[a]
	Phosphate buffer pH 7.4 (0.2 M)
1a	1.5
1a[b]	2.0 ± 0.2
2	5.1
3	4.5
5	3.8
6	5.1
7	3.1

[a] Experimental results are expressed as the mean of three tests ($n = 3$).
[b] Partition coefficient reported in the literature [20].

Table 5
Aqueous stability of prodrugs (**2**, **3**, **5**, **6**, **7**) in buffer solutions at 40 °C for 4 h.

Compound	Formation of bezafibrate by hydrolysis (μg/mL)				
	pH 1.0	pH 3.0	pH 5.2	pH 7.4	pH 9.0
2	BDL	BDL	1.7	2.2	0.8
3	BDL	BDL	0.2	0.9	6.6
5	BDL	BDL	0.7	1.8	2.5
6	BDL	BDL	6.3	6.2	8.8
7	BDL	BDL	0.2	0.5	2.6

BDL: below detection limit.

4.4. 2-[4-[2-[(4-Chlorobenzoyl)amino]ethyl]phenoxy}-2-methylpropanoic acid 1-acetoxyethyl ester (**3**)

A solution of **1a** (5 g, 13.82 mmol) in DMAc (25 mL) was treated with micronized sodium carbonate (1.46 g, 13.82 mmol) for 60 min at 30 °C under N_2 atmosphere. 1-Acetoxyethyl bromide (5.92 g, 35.46 mmol) was added and stirred at 30 °C for 3.5 h. Reaction progress was monitored by HPLC. The reaction mixture was added under stirring into the mixture of ethyl acetate (60 mL), water (200 mL) and sodium thiosulfate (1.0 g). pH of reaction mixture was adjusted to 10.5 with sodium carbonate, and stirred for 10 min at 20–25 °C. The organic phase was separated, washed brine solution (3 × 100 mL), treated with activated carbon (2.0 g) for 10 min, filtered and solvent was evaporated completely under vacuum at 35–40 °C to give solid product. The solid product was stirred with water (150 mL) for 15 min at 15 °C, filtered, washed with water (75 mL), and dried to get the title compound **3** as pale yellow crystalline solid (8.0 g, 74%). Chromatographic purity (HPLC) 99.47%; mp 115 °C; MS (ESI+) $m/z = 448.1$ $[M+H]^+$ IR (KBr) cm^{-1} 3245, 1759, 1741, 1633, 1488, 1091, 942; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.47 (d, 3H, $J = 5.4$ Hz, $-OCHCH_3$), 1.58 (s, 6H, $-OCCH_3)_2$), 2.02 (s, 3H, $-OCOCH_3$), 2.87 (t, 2H, $J = 6.8$ Hz, $-NHCH_2CH_2Ar$), 3.72 (t, 2H, $J = 6.8$ Hz, $-NHCH_2CH_2Ar$), 6.16 (bs, 1H, −N−*H*), 6.92 (q, 1H, $J = 5.4$ Hz, $-OCHCH_3$), 6.81–7.63 (m, 8H, Ar−*H*); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.4 ($-OCOCH_3$), 20.89 ($-OCH(O)CH_3$), 25.4 (2C, $-OCH(CH_3)_2$), 34.9 ($-HNCH_2CH_2Ar$), 41.41 ($-HNCH_2CH_2Ar$), 79.08 ($ArOCH(CH_3)_2$), 89.37 ($-OCH(CH_3)O$), 119.66–154.11 (12C, Ar), 166.55 (ArCONH), 169.03 ($-OCOCH_3$), 172.47 (−OCHCOO). Anal. Calcd. for $C_{23}H_{26}ClNO_6$: C, 61.67; H, 5.85; N, 3.13. Found: C, 61.55; H, 5.82; N, 3.11.

4.5. 2-[4-[2-[(4-Chlorobenzoyl)amino]ethyl]phenoxy}-2-methylpropanoic acid isopropoxycarbonyloxymethyl ester (**4**)

The compound **1a** (2.0 g, 5.5 mmol) was dissolved in DMAc (10 mL) at 30 °C. The reaction mixture was cooled to −15 °C under

N_2 atmosphere. TMG (0.672 g, 5.8 mmol) was added once and stirred for 15 min at −15 °C. Then, 1-iodomethylisopropyl carbonate (1.35 g, 5.52 mmol) was added at once. The reaction mass was stirred for 30 min at −15 to −10 °C. Reaction progress was monitored by HPLC. The reaction mixture was transferred to a mixture of ethyl acetate (30 mL), water (80 mL) and sodium thiosulfate (0.5 g) under vigorous stirring. Then, pH of reaction mixture was adjusted to 10.5 with sodium carbonate. The organic phase was separated, washed with brine (4 × 30 mL), decolorized with activated carbon (0.5 g), and filtered. After evaporation of solvent *in vacuo* solid product was obtained in the flask. Then, water (75 mL) was added, and stirred to get homogeneous slurry, filtered, washed with water (50 mL) and dried *in vacuo* at 45 °C to afford the desired product **4** as off white solid (2.2 g, 83%). Chromatographic purity (HPLC) 100%; mp 104.2 °C; MS (ESI^+) $m/z = 478.2$ $[M+H].^+$; IR (KBr) cm^{-1} 3269, 2990, 2940, 1770, 1632; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.28 (d, 6H, $J = 6.2$ Hz, −OCH(CH_3)$_2$), 1.60 (s, 6H, −OC(CH_3)$_2$), 2.87 (t, 2H, $J = 6.4$ Hz, −NHCH$_2$CH_2Ar), 3.69 (t, 2H, $J = 6.4$ Hz, −NHCH_2CH$_2$Ar), 4.9 (sept, 1H, $J = 6.2$ Hz, −OCH(CH$_3$)$_2$), 5.82 (s, 2H, −OCH_2O), 6.1(bs, 1H, −NH), 6.8–7.63 (m, 8H, Ar−H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.26 (2C, −OCH(CH_3)$_2$), 24.71 (2C, −OCH(CH_3)$_2$), 34.17 (−HNCH$_2$CH$_2$Ar), 40.96 (−HNCH$_2$CH$_2$Ar), 72.76 (−OCH(CH$_3$)$_2$), 78.44 (ArOCH(CH$_3$)$_2$), 82.40 (−OCH$_2$O), 119.14–153.01 (12C, Ar), 152.61 (−OCOO), 165.06 (ArCONH), 172.23 (−OCHCOO). Anal. Calcd. for $C_{24}H_{28}ClNO_7$: C, 60.31; H, 5.91; N, 2.93. Found: C, 60.25; H, 5.88; N, 2.91.

4.6. 2-[4-[2-[(4-Chlorobenzoyl)amino]ethyl]phenoxy}-2-methylpropanoic acid 2-acetoxyethyl ester (**5**)

A solution of **1a** (3 g, 8.29 mmol) in DMAc (15 mL) was treated with sodium carbonate (0.59 g, 5.55 mmol) and 2-acetoxyethyl bromide (1.73 g, 10.36 mmol) at room temperature. The reaction mixture was heated to 55 °C, and stirred for 8 h under N_2 atmosphere. Reaction progress was monitored by HPLC. The reaction mixture was added under stirring into the mixture of ethyl acetate (41 mL), water (120 mL) and sodium thiosulfate (0.5 g) under vigorous stirring. The organic phase was separated, washed with brine solution (3 × 38 mL), filtered, and solvent was evaporated *in vacuo* at 35–40 °C to get solid residue. Then, water (50 mL) was added, stirred at 5 °C for 30 min, filtered, washed with water (50 mL), and dried *in vacuo* to afford the title compound **5** as white fluffy solid (3.2 g, 87%). Chromatographic purity (HPLC) 98.13%; mp 108.9 °C; MS (ESI^+) $m/z = 448.2$ $[M+H].^+$; IR (KBr) cm^{-1} 3256, 1737, 1634. ^1H NMR (DMSO, 400 MHz) δ 1.49 (s, 6H, −OC(CH_3)$_2$), 1.94 (s, 3H, −OCOCH_3), 2.79 (t, 2H, $J = 7.44$ Hz, −NHCH$_2$CH_2Ar), 3.46 (t, 2H, $J = 7.44$ Hz, −NHCH_2CH$_2$Ar), 4.34 (m, 4H, −OCH_2CH_2O), 6.73–7.84 (m, 8H, Ar−H), 8.6 (bs, 1H, −N−H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.8 (−OCOCH_3), 25.49 (2C, −OCH(CH_3)$_2$), 34.85 (−HNCH$_2$CH$_2$Ar), 41.43 (−HNCH$_2$CH$_2$Ar), 62.08 (−OCH$_2$CH$_2$O), 62.87 (−OCH$_2$CH$_2$O), 79.2 (ArOCH(CH$_3$)$_2$), 119.38–154.16 (12C, Ar), 166.59 (ArCONH), 170.87 (−OCOCH$_3$), 174.19 (−OCHCOO). Anal. Calcd. for $C_{23}H_{26}ClNO_6$: C, 61.67; H, 5.85; N, 3.13. Found: C, 61.58; H, 5.82; N, 3.11.

4.7. 2-[4-[2-[(4-Chlorobenzoyl)amino]ethyl]phenoxy}-2-methylpropanoic acid 2-morpholin-4-yl-ethyl ester (**6**)

A solution of 4-(2-chloroethyl) morpholine hydrochloride (0.27 g, 1.451 mmol) in DMAc (2.5 mL) was treated with potassium carbonate (0.3245 g, 2.279 mmol) and **1a** (0.5 g, 0.1381 mmol) for 6 h at 65 °C under N_2 atmosphere. Reaction progress was monitored by HPLC. The reaction mixture was transferred to a mixture of ethyl acetate (12 mL) and water (40 mL) under vigorous stirring. The pH of reaction mixture was adjusted to 10.5 with sodium carbonate and stirred for 10 min at 25 °C. The organic phase was separated, washed with brine solution (2 × 30 mL), filtered, and solvent was evaporated *in vacuo* at 40 °C to get residual solid in the flask. Then, water (25 mL) was added, stirred for 10 min at 4 °C, filtered, washed with water (40 mL), and dried *in vacuo* at 45 °C to afford the title compound **7** as white fluffy solid (0.5 g, 76%). Chromatographic purity (HPLC) 99.95%; mp 141.9 °C; MS (ESI^+) $m/z = 475.1$ $[M+H].^+$; IR (KBr) cm^{-1} 3257, 1727, 1633. ^1H NMR (DMSO, 400 MHz) δ 1.49 (s, 6H, −OC(CH_3)$_2$), 2.35 (t, 4H, $J = 4.4$ Hz, −N(CH$_2$)$_2$), 2.52 (t, 2H, $J = 5.5$ Hz, −OCH$_2$CH_2N), 2.78 (t, 2H, $J = 7.6$ Hz, −NCH$_2$CH_2Ar), 3.33 (t, 2H, $J = 7.6$ Hz, −NCH_2CH$_2$Ar), 3.50 (t, 4H, $J = 4.4$ Hz, −O(CH_2)$_2$), 4.23 (t, 2H, $J = 5.5$ Hz, −OCH_2CH$_2$N), 6.75–7.84 (m, 8H, Ar−H), 8.64 (bs, 1H, −N−H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 25.09 (2C, ArOCH(CH_3)$_2$), 34.15 (−HNCH$_2$CH$_2$Ar), 40.99 (−HNCH$_2$CH$_2$Ar), 53.22 (2C, −N(CH$_2$)$_2$), 56.31 (−OCH$_2$CH$_2$N), 61.96 (−OCH$_2$CH$_2$N), 66.17 (2C, −O(CH$_2$)$_2$), 78.55 (−OCHCOO), 118.86–153.4 (12C, Ar), 165.03 (ArCONH), 173.25 (−OCHCOO). Anal. Calcd. for $C_{25}H_{31}ClN_2O_5$: C, 63.22; H, 6.58; N, 5.90. Found: C, 63.15; H, 6.55; N, 5.88.

4.8. 2-[4-[2-[(4-Chlorobenzoyl)amino]ethyl]phenoxy}-2-methylpropanoic acid 5-methyl-2-oxo-[1,3]dioxol-4-yl methyl ester (**7**)

A solution of **1a** (2.0 g, 5.52 mmol) in DMAc (10 mL) was treated with micronized sodium carbonate (0.44 g, 4.1 mmol) and 5-methyl-2-oxo-1, 3-dioxolene-4-yl-4-methylene chloride (0.9 g, 6.06 mmol) for 70 h at 32 °C under N_2 atmosphere. Reaction progress was monitored by HPLC. The reaction mixture was transferred to a mixture of ethyl acetate (50 mL) and water (100 mL) under vigorous stirring. The pH of reaction mixture was adjusted to 11.0 with sodium carbonate, and stirred for 10 min at 25 °C. The organic phase was separated, washed with brine solution (2 × 100 mL), filtered, and solvent was evaporated completely under vacuum at 35–40 °C to give solid residue. Then, water (50 mL) was added, stirred for 10 min, filtered, washed with water (50 mL), and dried *in vacuo* at 45 °C to afford the title compound **7** as white solid (1.9 g, 73%). Chromatographic purity (HPLC) 98.87%; mp 129.8 °C; MS (ESI^+) $m/z = 474.1$ $[M+H].^+$; IR (KBr) cm^{-1}: 3282, 1823, 1743, 1634; ^1H NMR (DMSO, 400 MHz) δ 1.55 (s, 6H, −OC(CH_3)$_2$), 2.13 (s, 3H, −C=CCH_3), 2.77 (t, 2H, $J = 7.7$ Hz, −NHCH$_2$CH_2Ar), 3.45 (t, 2H, $J = 7.7$ Hz, −NHCH_2CH$_2$Ar), 5.07 (s, 2H, −OCH_2), 6.69–7.84 (m, 8H, Ar−H), 8.65 (bs, 1H, −NH); ^{13}C NMR (DMSO, 100 MHz) δ 8.80 (−CCH$_3$), 24.96 (2C, −OCH(CH_3)$_2$), 34.21 (−HNCH$_2$CH$_2$Ar), 40.96 (−HNCH$_2$CH$_2$Ar), 54.47 (−OCH$_2$C), 78.67 (−OCHCOO), 118.73–153.19 (12C, Ar + 2C, ethylene), 151.71 (−OCOO), 165.04 (ArCONH), 172.89 (−OCHCOO). Anal. Calcd. for $C_{24}H_{24}ClNO_7$: C, 60.83; H, 5.10; N, 2.96. Found: C, 60.77; H, 5.07; N, 2.94.

4.9. HPLC analysis

Testing procedure for in-process analysis, aqueous solubility, chromatographic purity, partition coefficient analysis, and limit of detection (LOD). A reverse phase HPLC method was used. Instruments used: HPLC, Waters Alliance, pump model 2695, UV detector model 2487. Mobile phase A was prepared by mixing glacial acetic acid (2.0 mL in Milli Q water 1000 mL), filtered through 0.45 μm filter, and degassed by sonication (pH of this solution is around 3.0). Mobile phase B, involved filtered and degassed methanol (HPLC grade). Chromatographic parameters were as follows: column, YMC-Pack C-8, 100 mm length, 4.6 mm internal diameter, 3 μm particle size; flow rate, 0.8 mL/min; wavelength, 254 nm; injection volume, 20 μL; run time, 30 min. The gradient elution program is given below

Time (min)	Mobile phase A	Mobile phase B
0–2	90	10
2–3	30	70
3–12	30	70
12–15	90	10
15–20	90	10

4.10. Hypolipidemic activity

Hypolipidemic activity of the test compounds **1**–**7** and reference drug bezafibrate (**1a)** were evaluated in male Swiss albino mice (SAM) model reported previously [18–20].

Materials. Carboxy methyl cellulose (CMC) was purchased from Aldrich Chemicals (St. Louis, USA), Tween-80 was purchased from Sigma Aldrich chemicals and the Triglyceride kit from ERBA Diagnostics.

Animals. Male Swiss albino mice (SAM) of 21–30 g body weight were obtained from in-house animal facility of Orchid Research Laboratories, India. Animals were kept for 1 week at a controlled temperature 22–24 °C under 12 h light and 12 h dark cycle for accommodation with free access to standard laboratory chow and water *ad libitum*. All experiments were carried out by using protocol approved by the Institutional Animals Ethics Committee (IAEC) and compiled with National Institutes of Health (NIH) guidelines on handling of experimental animals.

Formulation. All the test compounds were suspended in vehicle (Tween-80, 5 μL/mL in 0.5% CMC, 10 mL/kg).

Biochemical analysis. Plasma triglyceride and cholesterol were measured spectrophotometrically using commercially available ERBA kit.

Statistical analysis. Mean values of treated groups were compared to those of the vehicle treated group. Statistical significance was determined by one-way analysis of variance (ANOVA), followed by Dunnett's test. Results were expressed as mean ± SE from 10 animals in each group. A P value <0.05 was considered significant.

Method. Male Swiss albino mice (SAM) were grouped into nine groups ($n = 10$).

Group 1: Normal control untreated SAM received 0.5% CMC (control vehicle).
Group 2: Administered bezafibrate (**1a**) orally and considered as reference drug.
Group 3: Administered test compound **1** orally.
Group 4: Administered test compound **2** orally.
Group 5: Administered test compound **3** orally.
Group 6: Administered test compound **4** orally.
Group 7: Administered test compound **5** orally.
Group 8: Administered test compound **6** orally.
Group 9: Administered test compound **7** orally.

Animals were treated orally with reference compound **1a** and test compounds **1**–**7** at a dose of 50 mg/kg body weight for 8 days. After 8 days of treatment, animals were fasted for 12 h. On the ninth day after giving respective treatment, blood samples were collected 1 h post-administration of test compounds **1**–**7** as well as **1a**. Blood was collected under mild ether anesthesia. Plasma was separated by centrifugation at 4000 rpm for 10 min for measurement of triglyceride (TG) and total cholesterol (TC) level by spectrophotometrically using a commercially available ERBA kit. The results obtained were expressed as mean ± SE. Statistical analysis was done by one-way analysis of variance (ANOVA), followed by Dunnett's test, and $P < 0.05$ was considered as statistically significant.

4.11. Determination of partition coefficient (log P)

The lipophilic character of bezafibrate (**1a**) and prodrugs **2**, **3**, **5**, **6**, **7** were assessed by determining its partition coefficient between 1-octanol and water (0.2 M phosphate buffer, pH 7.4) by HPLC method at 25 °C. Before use, the 1-octanol and buffer solution were mutually saturated for 24 h by shaking at 400 rpm on mechanical shaker at 25 °C. A known concentration of compounds in 1-octanol (5 mL) and buffer solution (5 mL) was shaken on mechanical shaker for 60 min at 400 rpm at 25 °C and centrifuged at 3500 rpm for 5 min. The concentration of the test compound (solute) in both phases was analyzed quantitatively by HPLC at 254 nm. Each experiment was repeated in triplicate and mean value was considered for log P estimation. The partition coefficient (log P) was calculated by following equation.

$$\log P_{\mathrm{oct/wat}} = \log\left(\frac{[\mathrm{solute}]_{\mathrm{octanol}}}{[\mathrm{solute}]^{\mathrm{un-ionized}}_{\mathrm{water}}}\right)$$

4.12. Particle size distribution

Instrument used. Malvern particle size analyzer, Model Master Sizer S.

Analytical condition. RPM in dispersion unit, 2000; Sample time, 20 s (10,000 sweeps); % obscuration, 25 ± 2.5. Sample measurement stability duration, 7 min; presentation, 3 OHD; dispersant, purified water pH 7.0–7.5 (pH adjusted with dilute ammonia solution).

Sample preparation. Test sample (~100 mg) was taken in 100 mL size beaker, and dispersant (10 drops) was added followed by addition of Tween-80 (2 drops). Thick paste was obtained. About 50 mL of dispersant was added to get homogeneous suspension and then sonicated for 3–4 min.

Procedure. Dispersion unit and flow cell was cleaned with n-hexane, and then about 100 mL of dispersion medium was poured into the dispersion unit. RPM was set (2000). After stabilization, instrument was aligned. Further sample was added gradually till ~25% obscuration attained. After 7 min of stabilization, particle size distribution was measured.

4.13. Aqueous stability

The solution stability of the prodrug compounds **2**, **3**, **5**, **6**, and **7** was studied at 40 °C in 0.1 M boric acid buffer at pH 9.0, 0.2 M phosphate buffer at pH 7.4, 0.1 M phosphate buffer at pH 5.2, 0.1 M citric acid buffer at pH 3.0, 0.2 M hydrochloric acid buffer at pH 1.0. Test compound (10 mg each) in buffer solution (10 mL) was incubated at 40 °C for 4 h at 400 rpm on mechanical shaker. The solution was filtered through 0.20 μm membrane filter, and the filtrate solution was analyzed quantitatively by HPLC at wavelength 254 nm for % hydrolyzed compound.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.08.036

References

[1] N.S. Habib, K.A. Ismail, A.A. El-Tombary, T. Abd El-Aziem, Pharmazie 55 (2000) 495–499.
[2] V. Fuster, P.W. Alexander, R.A. O'Rourke, S.B. King, H.J. Wellens, Anthrogenesis and its determinants, in: Hurst's the heart, vol. 3, 10th ed., Mc Graw-Hill, New York, 2001. 1065.
[3] D.R. Illingworth, J.A. Tobert, Clin. Ther 16 (1994) 366–385.
[4] C.R. Sirtori, G. Franceschini, Pharmacol. Ther 37 (1988) 167–191.
[5] K. Einarsson, S. Ericsson, S. Ewerth, E. Reihner, M. Rudling, D. Stahlberg, B. Angelin, Eur. J. Clin. Pharmacol 40 (1991) S53–S58.
[6] S.C. Sweetman, The complete drug reference. Electronic version, in: Martindale, 35th ed., Pharmaceutical Press, London, 2005.
[7] A.G. Gillman, J.G. Hardman, L.E. Limbird (Eds.), The pharmacological basis of therapeutics, 11th ed., McGraw-Hill, 2006, pp. 892–894.
[8] T. Komoto, H. Hirota, M. Otsuka, J. Kotake, S. Hasegawa, H. Koya, S. Sato, T. Sakamoto, Chem. Pharm. Bull. 48 (2000) 1978–1985.
[9] F. Labarrios, L. Garduno, M.R. Vidal, R. Garcia, M. Salazar, E. Martinez, F. Diaz, G. Chamorro, J. Tamariz, J. Pharm. Pharmacol. 51 (1999) 1–7.
[10] D. Hernandez, P. Bernal, A. Cruz, Y. Garciafigueroa, L. Garduno, M. Salazar, F. Díaz, G. Chamorro, J. Tamariz, Drug Dev. Res. 61 (2004) 19–36.
[11] C. Zuniga, L. Garduno, M. del Carmen Cruz, M. Salazar, R. Perez-Pasten, G. Chamorro, F. Labarrios, J. Tamariz, Drug. Dev. Res. 64 (2005) 28–40.
[12] A. Albert, Nature 182 (1958) 421–423.
[13] H. Bundgaard, Drug Future 16 (1991) 443–458.
[14] B.P. Bandgar, R.J. Sarangdhar, S. Vishwakarma, A.A. Fakrudeen, J. Med. Chem. 54 (2011) 1191–1201.
[15] B.P. Bandgar, R.J. Sarangdhar, A.A. Fakrudeen, S. Viswakarma, J. Med. Chem. 54 (2011) 1202–1210.
[16] S.K. Das, K.A. Reddy, C. Abbineni, J. Iqbal, J. Suresh, M. Premkumar, R. Chakrabarti, Bioorg. Med. Chem. Lett. 13 (2003) 399–403.
[17] K.A. Reddy, B.B. Lohray, V. Bhushan, A.S. Reddy, N.V. Rao Mamidi, P.P. Reddy, V. Saibaba, N.J. Reddy, A. Suryaprakash, P. Misra, R.K. Vikramadithyan, R. Rajagopalan, J. Med. Chem. 42 (1999) 3265–3278.
[18] B.P. Bandgar, R.J. Sarangdhar, F. Khan, M. Jeyamurugan, P. Shetty, G. Singh, J. Med. Chem. 54 (2011) 5915–5926.
[19] R.L. Buchanan, V. Sprancmanis, R.A. Partyka, J. Med. Chem. 12 (1969) 1001–1006.
[20] J.N. Moss, E.Z. Dajani, Antihyperlipidemic agents, in: R.A. Turner, P. Hebbon (Eds.), Screening methods in pharmacology, Academic Press, New York, 1971, pp. 121–143.
[21] R.M. Facino, M. Carini, R. Bertuletti, Pharmacol. Res. Comm. 13 (1981) 121–131.