European Journal of Medicinal Chemistry 44 (2009) 2679–2684

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Design, synthesis and hypolipidemic activity of novel 2-(*m*-tolyloxy) isobutyric acid derivatives

Gamal A. Idrees, Gamal El-Din A. Abuo-Rahma*, Omar M. Aly, Mohamed F. Radwan

Department of Medicinal Chemistry, Faculty of Pharmacy, Minia University, 47 Adnan El-Malky, 61519 Minia, Egypt

ARTICLE INFO

Article history:
Received 19 July 2008
Received in revised form
5 November 2008
Accepted 8 December 2008
Available online 24 December 2008

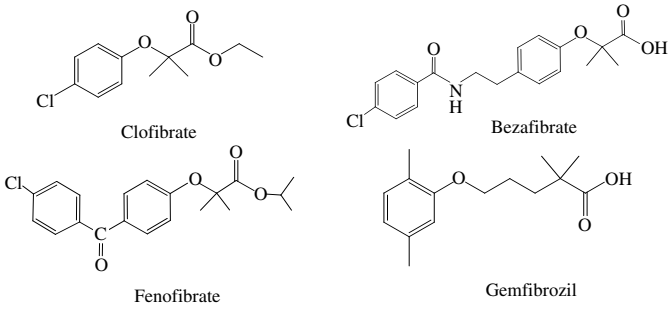
Keywords:
Fibrates
Cholesterol
Triglycerides
Hypolipidemic

ABSTRACT

Novel 2-substituted isobutyric acid derivatives were synthesized and their hypolipidemic activity was evaluated in high cholesterol diet fed rat model. The amide **5a** was found to decrease the levels of serum total cholesterol, LDL cholesterol and triglycerides in hyperlipidemic rats to a greater degree than the reference gemfibrozil.

1. Introduction

Derivatives of 2-methyl-2-phenoxypropanoic acid (fibric acid) as clofibrate, bezafibrate and fenofibrate are called fibrates [1]. The frequently prescribed hypolipidemic agent, gemfibrozil having a 5-phenoxypentanoic acid moiety instead of the fibric acid moiety is also considered a fibrate because of having almost similar pharmacological properties as the other classical fibrates [2].



In general, the major lipoprotein effects of fibrates are to reduce the levels of plasma triglycerides by 30–50% and to increase levels of HDL cholesterol by 5–6%. The magnitude of their effect is directly related to the severity of lipoprotein abnormalities at baseline [3–5].

The fibrates' primary mode of action is to selectively activate the alpha-isotype of the receptors peroxisome proliferator-activated receptors (PPARs) [6]. Activation of PPAR-α modulates the expression of several genes involved in lipoprotein metabolism. The activity of lipoprotein lipase is increased and results in an increase in the clearance of circulating triglyceride-rich lipoproteins [7]. It is established that the apolipoprotein C-III (apoC-III) inhibits lipoprotein lipase [8]. The biosynthesis of apoC-III is decreased by fibrates [7]. Hence, low apoC-III levels will further enhance the clearance of triglyceride-rich lipoproteins. In addition to the anti-hyperlipidemic effect, the fibrates have anti-inflammatory action as evidenced by a reduction in acute phase reactants such as C-reactive protein as well as a number of cytokines, IL-6, TNF-alpha and interferon-gamma. This pleiotropic effect of fibrates contributes in their coronary risk reducing ability [9–11]. Moreover, several studies indicate that fibrates decrease the levels of factors promoting coagulation and increase fibrinolysis. The dual hypolipidemic/antiplatelet effect of fibrates renders them interesting candidates for reducing the risk of atherosclerosis and its thrombotic complications [12], which are the major cause of coronary artery diseases [13,14]. Recent studies revealed the ability of fibrates to inhibit the activity of aldose reductase enzyme that makes them reliable agents in preventing the progression of secondary diabetic complications [15]. These interesting findings motivated us to prepare new fibrate analogs that might hold promising antidyslipidemic and antiatherosclerotic benefits. Our strategy to develop new fibrates consists of modifying the fibric acid moiety by replacing the phenyl group by an *m*-tolyl one and

* Corresponding author. Tel.: +20 103069431; fax: +20 862369075.
E-mail address: gamalaburahma@yahoo.com (G.E.-DinA. Abuo-Rahma).

0223-5234/$ – see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2008.12.009

the carboxyl function by selected bulkier derivatives and studying the impact of such modifications on the hypolipidemic activity.

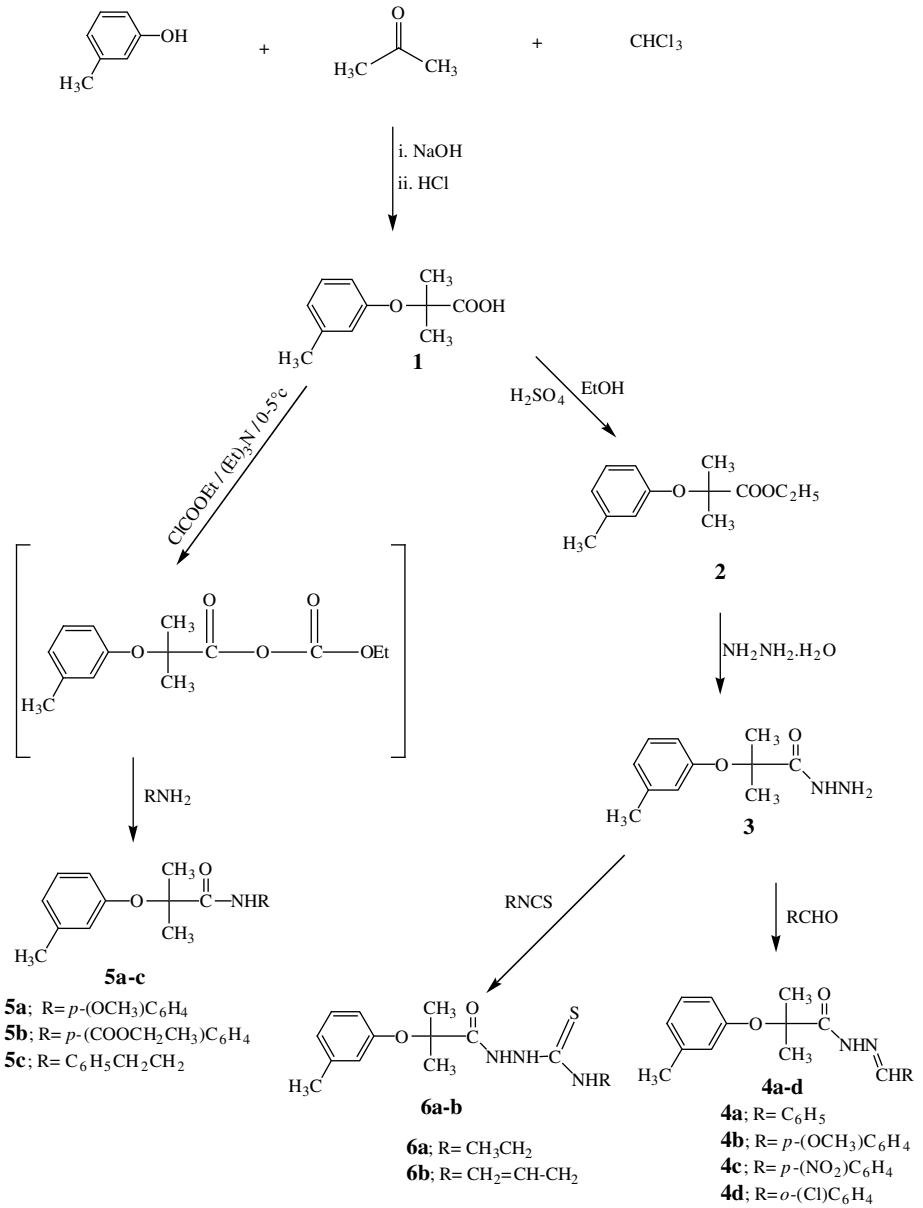
2. Results and discussion

2.1. Chemistry

The syntheses of the designed compounds are outlined in Scheme 1. Following reported procedures, treating *m*-cresol with acetone and chloroform in the presence of sodium hydroxide afforded 2-(*m*-tolyloxy)isobutyric acid **1** that was converted into the hydrazide **3** by treating its ethyl ester **2** with hydrazine hydrate [16]. Condensation of the hydrazide **3** with the appropriate carbonyl compounds afforded the arylidenes **4a–d**. The acid **1** was converted to the corresponding carboxamides **5a–c** by the mixed anhydride method using ethyl chloroformate [17]. The desired 1,4-disubstituted thiosemicarbazides **6a**,**b** were obtained by treating the hydrazide **3** with the appropriate alkyl isothiocyanate. Our attempts to cyclize the 1,4-disubstituted thiosemicarbazides **6a**,**b** into the corresponding mercaptotriazoles by piperidine in ethanol or aqueous 2 N sodium hydroxide or sodium metal in methanol, were all unsuccessful. This might be explained by the steric hindrance created by the two methyl groups on the carbon alpha to the carbonyl group which in turn prevents the intramolecular cyclization.

Regarding the prepared arylidenes **4a–d**, it was noticed that aromatic carbonyl compounds having electron-withdrawing substituents (e.g. NO_2 and Cl) give higher yields than those having electron-releasing ones (e.g. OCH_3). This could be explained by the ability of electron attractors to intensify the positive charge on the carbonyl carbon thereby increasing its susceptibility for nucleophilic attack while electron donors are likely to neutralize that positive charge with the result that the carbonyl carbon becomes less attractive to nucleophiles.

The structures of the prepared compounds were substantiated by ^{1}H NMR, ^{13}C NMR spectra as well as elemental microanalyses.



Scheme 1.

The ^{1}H NMR spectra of the arylidenes **4a–d** are characterized by the presence of azomethine and amidic protons in the range of 8.09–8.35 ppm and 8.59–10.04 ppm, respectively, whereas their ^{13}C NMR spectra show a characteristic azomethine carbon (CH=N) ranging from 146.9 to 148.8 ppm. The appearance of amidic proton at 8.49–9.11 ppm is a hallmark of the ^{1}H NMR spectra of the amides **5a–c**. An interesting finding in the ^{1}H NMR spectrum of compound **5c** is that it contains a quartet of 2 protons at 3.60 ppm for the methylene group attached to NH that could be explained by the slow exchange rate of the NH proton allowing splitting of the methylene proton signals by the adjacent NH and CH_2. The ^{1}H NMR spectra of compounds **6a**,**b** showed 3 amidic NH protons beyond 7 ppm. Furthermore, the ^{13}C NMR spectra of compounds **6a**,**b** showed a characteristic thione (C=S) around 181 ppm. The ^{13}C NMR spectra of all synthesized compounds show a characteristic C=O group above 170 ppm.

2.2. Hypolipidemic activity

The hypolipidemic activity of the synthesized compounds was studied in the high cholesterol diet (HCD) fed hyperlipidemic rat model [18] against hyperlipidemic control [i.e., rats fed with HCD and given drug vehicle (0.5% CMC) only], by oral administration of 20 mg/kg of the compounds. The results were compared with that obtained by the reference hypolipidemic agent gemfibrozil (Table 1). The obtained results revealed that feeding rats with high cholesterol diet for 14 days significantly elevated the serum level of total cholesterol, triglycerides and LDL-C by 59.58%, 71.76% and 102.36%, respectively, when compared with normal control rats. Moreover, induction of hyperlipidemia significantly decreased serum HDL-C level by 34.77% from that of normal control rats. The obtained data revealed that the tested compounds produced variable effects on the serum levels of TC (Fig. 1), TG (Fig. 2), and LDL-C (Fig. 3), as compared with the hyperlipidemic control group. Compound **5a** exhibited the maximum hypolipidemic activity expressed as 23.24% and 25.97% reduction in the levels of TC and TG, respectively, that was more active than the reference gemfibrozil which afforded 4.68% and 22.23% reduction in the levels of TC and TG, respectively. The acid **1** was effective in reducing the levels of TC and TG by 15.89% and 17.98%, respectively, while compound **5c** reduced only TC by 12.95%. No significant lipid lowering activity was produced by either the arylidenes **4a–d** or the 1,4-disubstituted thiosemicarbazides **6a**,**b**. Also, there was no significant change in HDL-C values in any of the treated animals (Fig. 4). The reduction of the level of LDL-C (28.67%) by compound **5a** is an interesting finding because the LDL fraction is generally thought to carry cholesterol to the tissues and is responsible for the atherogenesis process [19]. Decreasing the ratio of LDL-C (bad cholesterol) to that of HDL-C (good cholesterol) plays a pivotal role in reducing the risk of atherosclerosis [20]. Results showed that the lowest LDL-C/HDL-C ratio was obtained by compound **5a**.

3. Experimental

3.1. Chemistry

Melting points were determined on Stuart electrothermal melting point apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a JEOL Lambda 600 spectrometer (600 MHz), on a Mercury 300BB NMR spectrometer (300 MHz), and on a GEMINI-200 NMR spectrometer (200 MHz) with TMS as internal standard and $CDCl_3$ as a solvent. ^{13}C NMR spectra were recorded on a JEOL Lambda 600 spectrometer (600 MHz) with TMS as internal standard and $CDCl_3$ as a solvent. Elemental microanalysis was performed on Hereaus Vario EL apparatus.

3.1.1. General procedure for the preparation of 2-methyl-2-m-tolyloxypropionic acid (arylidene)hydrazides (**4a–d**)

A mixture of 2-methyl-2-*m*-tolyloxypropionic acid hydrazide (2.08 g, 10 mmol) and the appropriate carbonyl compound (10 mmol) in absolute ethanol (20 mL) was heated at reflux for 3 h. The reaction mixture was cooled and the precipitated solid was filtered off, dried and recrystallized from ethanol.

*3.1.1.1. 2-Methyl-2-*m*-tolyloxypropionic acid benzylidene hydrazide (**4a**).* Colorless solid; m.p. 139–140 °C; yield 70% (2.07 g); ^{1}H NMR ($CDCl_3$, 200 MHz, δ, ppm): 1.62 (s, 6H, O–C(CH_3)$_2$), 2.36 (s, 3H, Ar-CH_3), 6.80–7.80 (m, 9H, Ar-H), 8.17 (s, 1H, azomethine proton), 9.80 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm): 21.5 (Ar-CH_3), 25.2 (O–C(CH_3)$_2$), 81.7 (O–C(CH_3)$_2$), 118.8, 122.7, 124.8, 127.9, 128.8, 129.2, 130.7, 133.6, 139.7 and 153.8 (aromatic carbons), 148.8 (CH=N), 171.2 (C=O); Anal. Calcd. for $C_{18}H_{20}N_2O_2$ (%): C, 72.95; H, 6.80; N, 9.45. Found: C, 73.06; H, 6.51; N, 9.12.

*3.1.1.2. 2-Methyl-2-*m*-tolyloxypropionic acid (4-methoxybenzylidene) hydrazide (**4b**).* Colorless solid; m.p. 134–136 °C; yield 69% (2.25 g); ^{1}H NMR ($CDCl_3$, 200 MHz, δ, ppm): 1.61 (s, 6H, O–C(CH_3)$_2$), 2.34 (s, 3H, Ar-CH_3), 3.85 (s, 3H, OCH_3), 6.79–7.74 (m, 8H, Ar-H), 8.09 (s, 1H, azomethine proton), 9.73 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm): 21.5 (Ar-CH_3), 25.2 (O–C(CH_3)$_2$), 55.4 (OCH_3), 81.7 (O–C(CH_3)$_2$), 114.2, 118, 122.7, 124.7, 126.2, 129.2, 129.5, 139.6, 153.8 and 161.7 (aromatic carbons), 148.6 (CH=N), 170.9 (C=O); Anal. Calcd. for

Table 1
Effect of tested compounds on lipid profile of hyperlipidemic rats.

Groups	TC (mg/dL)	% Fall	TG (mg/dL)	% Fall	HDL-C (mg/dL)	% Rise	LDL-C (mg/dL)	% Fall	LDL/HDL
Control	71.0 ± 2.65		55.0 ± 5.57		19.67 ± 5.13		40.33 ± 2.64		2.05
HCD fed	113.3 ± 3.22		94.47 ± 3.25		12.83 ± 2.02		81.61 ± 1.67		6.36
HCD fed + gemfibrozil	108.0 ± 8.54	4.68	73.47 ± 3.14**	22.23	16.07 ± 1.01	25.18	77.24 ± 9.77	5.35	4.81
HCD fed + 1	95.33 ± 4.73**	15.89	77.48 ± 9.28*	17.98	14.21 ± 0.79	10.68	65.63 ± 3.15	19.56	4.62
HCD fed + 4a	108.7 ± 2.52	4.12	92.07 ± 3.72	2.54	15.20 ± 1.31	18.47	75.05 ± 1.53	8.02	4.94
HCD fed + 4b	113.3 ± 6.51	0.00	95.72 ± 6.53	−1.32[a]	14.70 ± 1.54	14.58	79.49 ± 6.26	2.60	5.41
HCD fed + 4c	113.0 ± 9.54	0.29	95.26 ± 2.63	−0.84[a]	14.00 ± 0.89	9.12	79.95 ± 9.03	2.30	5.71
HCD fed + 4d	120.7 ± 4.73	−6.47[a]	85.94 ± 8.91	9.03	14.87 ± 0.81	15.82	89.28 ± 5.00	−9.39[a]	6.00
HCD fed + 5a	87.00 ± 4.58**	23.24	70.11 ± 4.49**	25.79	14.80 ± 0.57	15.28	58.18 ± 6.35**	28.67	3.93
HCD fed + 5b	110.3 ± 10.50	2.65	94.06 ± 5.54	0.43	14.77 ± 0.91	15.04	76.75 ± 12.50	5.94	5.20
HCD fed + 5c	98.67 ± 2.52	12.95	93.34 ± 3.52	1.20	15.72 ± 0.67	22.45	64.28 ± 2.63*	21.21	4.09
HCD fed + 6a	114.0 ± 7.55	−0.59	89.33 ± 2.00	5.44	13.58 ± 0.73	5.85	82.55 ± 7.65	−1.16[a]	6.08
HCD fed + 6b	115.3 ± 5.69	−1.77[a]	95.99 ± 4.58	−1.61[a]	13.70 ± 1.32	6.78	82.43 ± 4.99	−1.00[a]	6.02

TC, total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.
*Significantly different from hyperlipidemic control group at $P < 0.05$.
**Significantly different from hyperlipidemic control group at $P < 0.01$.
[a] Negative values indicate increase in the level of the measured parameter % fall and % rise are calculated for groups in relation to the hyperlipidemic control group.

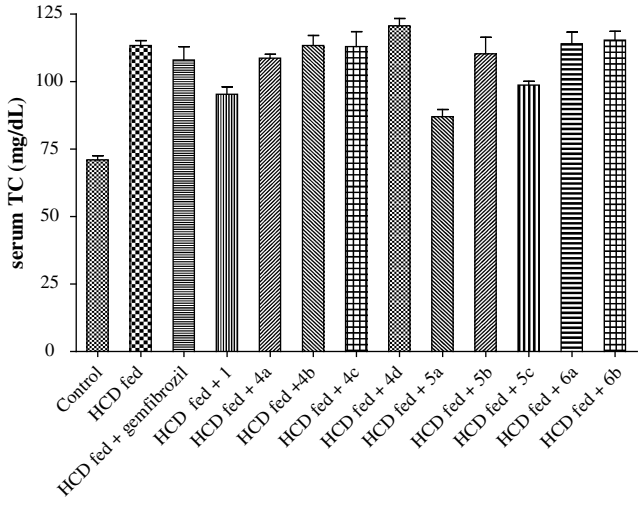


Fig. 1. Effect of the tested compounds and gemfibrozil on serum TC of hyperlipidemic rats.

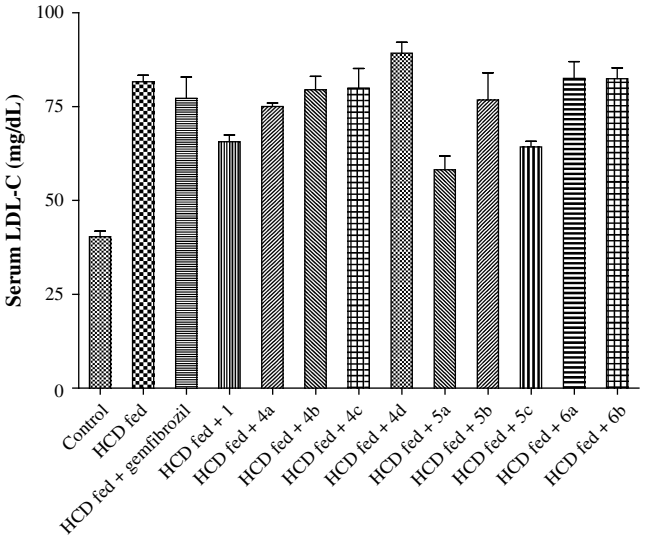


Fig. 3. Effect of the tested compounds and gemfibrozil on serum LDL-C of hyperlipidemic rats.

$C_{19}H_{22}N_2O_3$ (%): C, 69.92; H, 6.79; N, 8.58. Found: C, 69.87; H, 6.91; N, 8.37.

*3.1.1.3. 2-Methyl-2-*m*-tolyloxypropionic acid (4-nitrobenzylidene) hydrazide (**4c**).* Yellow solid; m.p. 156–157 °C; yield 76% (2.60 g); ^{1}H NMR ($CDCl_3$, 200 MHz, δ, ppm): 1.62 (s, 6H, O–C(CH_3)$_2$), 2.35 (s, 3H, Ar-CH_3), 6.78–8.29 (m, 8H, Ar-H), 8.35 (s, 1H, azomethine proton), 10.04 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm): 21.5 (Ar-$\underline{C}H_3$), 25.2 (O–C($\underline{C}H_3$)$_2$), 81.8 (O–$\underline{C}$(CH_3)$_2$), 118.9, 122.8, 124.1, 125.0, 128.3, 129.2, 139.8, 145.9 and 153.5 (aromatic carbons), 148.8 (CH=N), 171.6 (C=O); Anal. Calcd. for $C_{18}H_{19}N_3O_4$ (%): C, 63.33; H, 5.61; N, 12.31. Found: C, 63.11; H, 5.54; N, 12.34.

*3.1.1.4. 2-Methyl-2-*m*-tolyloxypropionic acid (2-chlorobenzylidene) hydrazide (**4d**).* Colorless solid; m.p. 140–142 °C; yield 75% (2.48 g); ^{1}H NMR ($CDCl_3$, 200 MHz, δ, ppm): 1.60 (s, 6H, O–C(CH_3)$_2$), 2.35 (s, 3H, Ar-CH_3), 6.81–7.34 (m, 8H, Ar-H), 8.23 (s, 1H, azomethine proton), 8.59 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm): 21.5 (Ar-$\underline{C}H_3$), 25.2 (O–C($\underline{C}H_3$)$_2$), 55.4 (OCH_3), 81.7 (O–$\underline{C}$(CH_3)$_2$), 119.2, 124.2, 126.5, 128.7, 129.6, 130.8, 131.5, 132.3, 133.1, 136.5, 141.5 and 155.4 (aromatic carbons), 146.9 (CH=N), 173.8 (C=O); Anal. Calcd. for $C_{18}H_{19}ClN_2O_2$ (%): C, 65.35; H, 5.79; N, 8.47. Found: C, 65.29; H, 5.63; N, 8.29.

*3.1.2. General procedure for the preparation of 2-methyl-2-*m*-tolyloxy-*N*-substituted propionamides (**5a–c**)*

To a stirred solution of 2-methyl-2-*m*-tolyloxypropionic acid (1.94 g, 10 mmol), in 30 ml dry chloroform at 0–5 °C, triethylamine (1.01 g, 10 mmol) was added, followed by ethyl chloroformate (1.08 g, 10 mmol) in a drop wise manner, the mixture was stirred in the ice bath for 30 min, and then the appropriate amine (10 mmol) in dry chloroform was added drop wise. The mixture was stirred for additional 12 h at room temperature, the solvent was evaporated under reduced pressure and the crude product was dissolved in 30 ml chloroform, washed with water (2 × 30 ml), 5% $NaHCO_3$ solution (2 × 20 ml), 1 N HCl (2 × 20 ml), water (2 × 30 ml) and

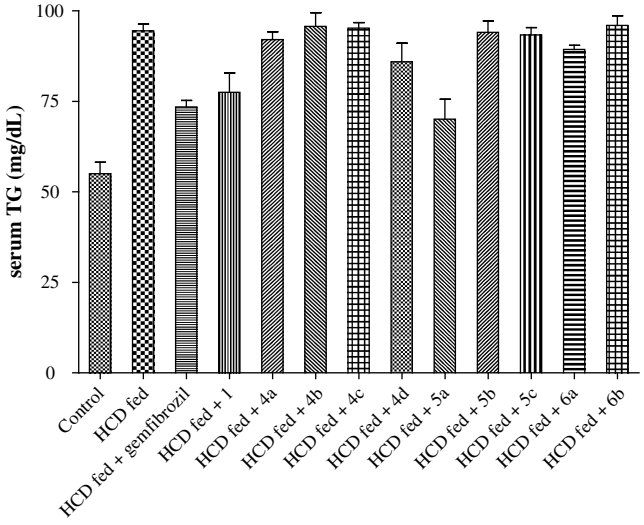


Fig. 2. Effect of the tested compounds and gemfibrozil on serum TG of hyperlipidemic rats.

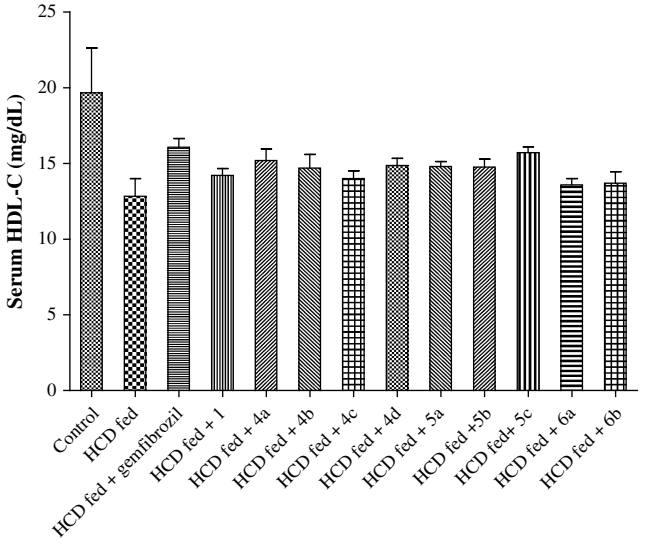


Fig. 4. Effect of the tested compounds and gemfibrozil on serum HDL-C of hyperlipidemic rats.

finally with brine (2 × 30 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The product was collected and recrystallized from aqueous ethanol.

3.1.2.1. N-*(4-Methoxyphenyl)-2-methyl-2*-m-*tolyloxypropionamide (**5a**).* Colorless solid; m.p. 125–126 °C; yield 74% (2.22 g); ^{1}H NMR ($CDCl_3$, 200 MHz, δ, ppm): 1.59 (s, 6H, O–C(CH_3)$_2$), 2.34 (s, 3H, Ar-CH_3), 3.81 (s, 3H, OCH_3), 6.79–7.51 (m, 8H, Ar-H), 8.49 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm): 21.5 (Ar-CH_3), 25.2 (O–C(CH_3)$_2$), 55.6 (OCH_3), 81.9 (O–C(CH_3)$_2$), 114.3, 118.8, 121.6, 122.7, 124.7, 129.1, 130.8, 139.6, 154.1 and 156.6 (aromatic carbons), 172.9 (C=O); Anal. Calcd. for $C_{18}H_{21}NO_3$ (%): C, 72.22; H, 7.07; N, 4.68. Found: C, 72.27; H, 7.14; N, 4.49.

3.1.2.2. 4-(2-Methyl-2-m-*tolyloxypropionylamino)benzoic acid ethyl ester (**5b**).* Colorless solid; m.p. 118–120 °C; yield 62% (2.11 g); ^{1}H NMR ($CDCl_3$, 200 MHz, δ, ppm): 1.70 (t, 3H, J = 7 Hz, $COOCH_2CH_3$), 1.88 (s, 6H, O–C(CH_3)$_2$), 2.64 (s, 3H, Ar-CH_3), 4.65 (q, 2H, J = 7 Hz, $COOCH_2CH_3$), 7.07–8.36 (m, 8H, Ar-H), 9.11 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm): 14.4 (CH_3CH_2), 21.5 (Ar-CH_3), 25.1 (O–C(CH_3)$_2$), 61.0 (CH_3CH_2), 82.0 (O–C(CH_3)$_2$), 118.96, 118.99, 122.91, 124.99, 126.2, 129.2, 130.9, 139.7, 141.7 and 153.7 (aromatic carbons), 166.21 (CONH), 173.5 (COO); Anal. Calcd. for $C_{20}H_{23}NO_4$ (%): C, 70.36; H, 6.79; N, 4.10. Found: C, 70.31; H, 6.96; N, 3.89.

3.1.2.3. 2-Methyl-N-*phenethyl-2*-m-*tolyloxypropionamide (**5c**).* Colorless solid; m.p. 124–126 °C; yield 66% (1.96 g); ^{1}H NMR ($CDCl_3$, 200 MHz, δ, ppm): 1.51 (s, 6H, O–C(CH_3)$_2$), 2.34 (s, 3H, Ar-CH_3), 2.86 (t, 2H, J = 7 Hz, CH_2CH_2NH), 3.60 (q, 2H, J = 7 Hz, CH_2CH_2NH), 6.66–7.31 (m, 9H, 8 Ar-H + NH); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm): 21.5 (Ar-CH_3), 25.2 (O–C(CH_3)$_2$), 35.8 (ArCH_2), 40.5 (CH_2NH), 81.4 (O–C(CH_3)$_2$), 118.1, 122.0, 124.0, 126.6, 128.7, 128.8, 129.0, 138.9, 139.4 and 154.3 (aromatic carbons), 174.9 (C=O); Anal. Calcd. for $C_{19}H_{23}NO_2$ (%): C, 76.73; H, 7.80; N, 4.71. Found: C, 76.82; H, 7.67; N, 4.54.

3.1.3. General procedure for the preparation of 1-(2-methyl-2-m-*tolyloxy)propionyl-4-substituted thiosemicarbazides (**6a**,**b**)*

A mixture of 2-methyl-2-*m*-tolyloxypropionic acid hydrazide (2.08 g, 10 mmol) and the appropriate alkyl isothiocyanate (10 mmol) in absolute ethanol was refluxed for 4 h. The mixture was cooled and the precipitated solid was filtered, dried and recrystallized from methanol to give the title compounds.

3.1.3.1. 1-(2-Methyl-2-m-*tolyloxypropionyl)-4-ethylthiosemicarbazide (**6a**).* Colorless solid; m.p. 140–142 °C; yield 75% (2.21 g); ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.15 (t, 3H, J = 6.9 Hz, CH_2CH_3), 1.56 (s, 6H, O–C(CH_3)$_2$), 2.34 (s, 3H, Ar-CH_3), 3.56 (q, 2H, J = 6.9 Hz, CH_2CH_3), 6.92–7.27 (m, 5H, 4 Ar-H + NH), 9.09 (s, 1H, NH), 9.59 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm): 14.6 (CH_2-CH_3), 21.5 (Ar-CH_3), 25.3 (O–C(CH_3)$_2$), 41.6 (CH_2-CH_3), 81.0 (O–C(CH_3)$_2$), 115.7, 120.6, 123.0, 127.5, 136.4 and 150.5 (aromatic carbons), 170.5 (C=O), 181.5 (C=S); Anal. Calcd. for $C_{14}H_{21}N_3O_2S$ (%): C, 56.92; H, 7.17; N, 14.22. Found: C, 56.82; H, 7.26; N, 14.04.

3.1.3.2. 1-(2-Methyl-2-m-*tolyloxypropionyl)-4-allylthiosemicarbazide (**6b**).* Colorless solid; m.p. 148–149 °C; yield 76% (2.33 g); ^{1}H NMR ($CDCl_3$, 600 MHz, δ, ppm): 1.63 (s, 6H, O–C(CH_3)$_2$), 2.31 (s, 3H, Ar-CH_3), 4.21 (t, 2H, J = 5.4 Hz, CSNHCH_2), 5.83–5.87 (m, 1H, CH_2–CH=CH_2), 5.14 (dd, 1H, J = 1.02 Hz and 9.30 Hz, CH_2–CH=CH_2), 5.20 (dd, 1H, J = 1.02 Hz and 18.00 Hz, CH_2–CH=CH_2), 6.84–7.25 (m, 7H, 4 Ar-H + 3 NH); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm): 21.5 (Ar-CH_3), 25.3 (O–C(CH_3)$_2$), 47.1 (CH_2–CH=CH_2), 81.0 (O–C(CH_3)$_2$), 118.8 (CH_2–CH=CH_2), 133.3 (CH_2–CH=CH_2), 117.5, 122.9, 125.0, 129.2, 139.8 and 153.6 (aromatic carbons), 171.5 (C=O), 180.5 (C=S); Anal. Calcd. for $C_{15}H_{21}N_3O_2S$ (%): C, 58.61; H, 6.89; N, 13.67. Found: C, 58.26; H, 7.30; N, 13.61.

3.2. Hypolipidemic activity

Male albino rats of Wistar strain weighing 160–180 g were used in this study. Rats were obtained from the animal house of National Research Centre, Dokki, Cairo. The animals were kept for one week in the laboratory under 12 h day and night cycle for accommodation with free access to food and water *ad libitum*. Rats were divided into control (fed normal rodent chow), hyperlipidemic control and hyperlipidemic plus compound treated groups. Each group consisted of six animals. Hyperlipidemia was induced by feeding rats with high cholesterol diet (HCD) prepared by mixing normal rodent chow with 2% cholesterol (Sigma, USA) and 1% cholic acid (Sigma, USA). Hyperlipidemic rats had a free access to the HCD and water *ad libitum* during the entire period of the experiment. The tested compounds as well as the standard drug gemfibrozil were given orally at a dose of 20 mg/kg using 0.5% carboxymethylcellulose as a drug vehicle from day 8 to day 14 (7 days) in the HCD fed rats. Control and hyperlipidemic control rats (fed with HCD) received only the drug vehicle from day 8 to day 14 of the experiment. At the end of the experiment, that is, on the 14th day, the animals were fasted for 12 h and the tail blood was collected under mild ether anesthesia. Serum was separated by centrifugation at 3000 rpm for 10 min for measurement of total cholesterol (TC), triglycerides (TGs) and HDL cholesterol (HDL-C) using Randox kits (UK) on a Jenway UV–vis spectrophotometer. Serum LDL–cholesterol concentration was determined according to Friedewald's formula [21].

The results obtained were expressed as mean ± SD. The difference between the groups was evaluated for its significance by one-way ANOVA test. Mean, standard deviation calculations and ANOVA test were performed using "GraphPad Prism version 4.0" software. A P value < 0.05 was considered significant.

4. Conclusion

Novel 2-substituted isobutyric acid derivatives were prepared and their hypolipidemic activity was screened in hyperlipidemic rats. Among the synthesized compounds, the amide **5a** was found to be the most active hypolipidemic agent affording significant hypocholesterolemic and hypotriglyceridemic activities and seems to be a good candidate for developing a new strong fibrate derivative with good hypolipidemic and antiatherosclerotic benefits.

Acknowledgement

The authors are grateful to Prof. Dr. Jochen Lehmann, Institute of Pharmacy, Department of Pharmaceutical/Medicinal Chemistry, Friedrich-Schiller University, Jena, Germany, for carrying out the elemental microanalysis.

References

[1] A. Gaw, J. Shepherd, Curr. Opin. Lipidol. 2 (1991) 39.
[2] T. Komoto, H. Hirota, M. Otsuka, et al., Chem. Pharm. Bull. 48 (2000) 1978.
[3] M.H. Frick, O. Elo, K. Haapa, et al., N. Engl. J. Med. 317 (1987) 1237.
[4] H.B. Rubins, S.J. Robins, D. Collins, et al., N. Engl. J. Med. 341 (1999) 410.
[5] The Diabetes Atherosclerosis Intervention Study Investigators, a randomized study, Lancet 357 (2001) 905 [erratum appears in Lancet 357 (2001) 1890].
[6] J.C. Fruchart, Am. J. Cardiol. 88 (2001) 24N.
[7] B. Staels, J. Dallongeville, J. Auwerx, et al., Circulation 98 (1998) 2088.
[8] K. Willems van Dijk, P.C.N. Rensen, P.J. Voshol, et al., Curr. Opin. Lipidol. 15 (2004) 239.
[9] T.D. Wang, W.J. Chen, J.W. Lin, et al., Atherosclerosis 170 (2003) 315.
[10] J.P. Despres, I. Lemieux, A. Pascot, et al., Arterioscler. Thromb. Vasc. Biol. 23 (2003) 702.
[11] A. Madej, B. Okopien, J. Kowalski, et al., Int. J. Clin. Pharmacol. Ther. 36 (1998) 345.

[12] A. Ammazzalorso, R. Amoroso, M. Baraldi, et al., Eur. J. Med. Chem. 40 (2005) 918.
[13] V. Fuster, L. Badimon, M. Cohen, et al., Circulation 77 (1988) 1213.
[14] S. Batra, P.B. Amiya, S.J. Bhawani, et al., Bioorg. Med. Chem. 9 (2001) 3093.
[15] G.K. Balendiran, B. Rajkumar, Biochem. Pharmacol. 70 (2005) 1653.
[16] T. Ramalingam, P.B. Sattur, Ind. J. Chem. 28B (1989) 611.
[17] G. Kokotos, Synthesis (Stuttg) 4 (1990) 299.
[18] V. Sudhahar, S.A. Kumar, P.T. Sudharsan, et al., Vascul. Pharmacol. 46 (2007) 412.
[19] R. Russel, N. Engl. J. Med. 314 (1986) 488.
[20] J. Popławski, B. Łozowicka, A.T. Dubis, et al., J. Med. Chem. 43 (2000) 3671.
[21] W.T. Friedewald, R.I. Levy, D.S. Fredrickson, Clin. Chem. 18 (1972) 499.