Introduction

There is a great deal of experimental, epidemiological, and clinical evidence suggesting that hyperlipidemia plays an important role in the pathogenesis of atherosclerosis and cardiovascular diseases (Goldstein et al., 1973; Frishman, 1998). This lipid metabolism disorder is characterized by elevated concentrations of circulating lipids including triglycerides, total cholesterol, very low-density lipoprotein, and low-density lipoprotein (Raasch, 1988). Hyperlipidemia is usually asymptomatic and is regularly detected during routine screening. The increased incidence of cardiovascular disease (CVD) among patients with elevated concentrations of circulating lipids has led to increased recognition of hyperlipidemia as important targets of therapy.

In the course of the present study, Triton WR-1339-induced hyperlipidemic rats were used as an experimental model to investigate the potential activity of some synthetic hypolipidemic agents. Previous studies showed that a single parenteral administration of Triton WR-1339 to adult rats produces hyperlipidemia in which cholesterol, triglycerides, and phospholipids levels increase to a maximum within about 20 hours and decrease thereafter (Schurr et al., 1972). This activity of Triton WR-1339 was found to be due to its inhibitory effect on lipoprotein lipase (Schotz et al., 1957).

Currently hyperlipidemia is treated with different groups of drugs including fibrates and their derivatives (such as bezafibrate) (Frick et al., 1987). Fibric acids enhance the fatty acid catabolism and accordingly reduce plasma lipid levels, predominantly triglyceride levels (Rubins et al., 1999). The main mechanism was found to be through decreasing the synthesis of apoC-III and increasing the activity of lipoprotein lipase, which together enhance the clearance of circulating triglyceride-rich lipoproteins (Schoonjans et al., 1996).

Benzothiophene and its derivatives have been reported to show activity in treating various medical conditions, such as hyperlipidemia, breast cancer, and uterus cancer (Charles et al., 1984). On the other hand, other groups of compounds such as benzophenone, aminopyridine, and amino-
noimidazole have been investigated for their potential hypolipidemic activity (Al-Qirim et al., 2009; Shahwan et al., 2010; Bosies et al., 1980; Sher and Ellsworth, 2004; Kopin et al., 2006; Dasseux and Oniciu, 2002). But to the best of our knowledge, a combination of benzothiophene with other groups of compounds such as benzophenone, aminopyridine, and aminoimidazole has not been investigated before as potential lipid-lowering agents.

Taking into consideration the correlation between hyperlipidemia and heart diseases, development of new agents with the potential capability to reduce elevated blood lipid levels is considered by medical authorities to be extremely important for the treatment and prevention of heart diseases. Therefore, this study aimed to evaluate the possible lipid-lowering activity of novel benzothiophene carboxamide derivatives of aminobenzophenone, aminopyridine, aminobenzimidazole, and aniline derivatives.

**Material and Methods**

**Chemical studies**

The benzothiophene carboxamide derivatives of aminobenzophenones, aminopyridines, and anilines, compounds 1–8, were synthesized in one step by melting benzothiophene-2-carbonyl chloride (10) with the corresponding aminobenzophene-
G. Abu Sheikha et al. · Novel Potent Antihypertriglyceridemic Agents

nones 11–15 at 130 °C, as shown in Schemes 1 and 2, and with 2- and 3-aminopyridines 16 and 17, and with p-chloroaniline 18 at 150 °C for 20 min, according to Scheme 3. The mixtures of reactions were furthermore purified by using column chromatography to afford the desired compounds in high yields (75–90%). Each compound was characterized by $^1$H NMR, $^{13}$C NMR, IR, elemental, and mass spectroscopic analyses, and was found to have the correct structure. Compound 9 was prepared by reaction of benzothiophene-2-carbonyl chloride (10) with 2-aminobenzimidazole (19) in the presence of triethylamine in DMF at 160 °C (Scheme 3).

Melting points were measured using a Gallenkamp melting point apparatus (Watford, UK) and are uncorrected. $^1$H NMR and $^{13}$C NMR spectra were collected on a Varian Oxford NMR 300 spectrometer (Santa Clara, USA). The samples were dissolved in CDCl$_3$ at a content of 0.3–0.7 wt-% and placed in 5-mm NMR tubes. High-resolution mass spectra (HRMS) were measured in the positive ion mode using the electrospray ion trap (ESI) technique by collision-induced dissociation on a Bruker Apex-4 (Tesla) instrument (Bremen, Germany). The samples were dissolved in acetonitrile, diluted in spray solution (methanol/water, 5:4.9, v/v + 0.1 formic acid) and infused
using the syringe pump with a flow rate of 2 μl/min. External calibration was conducted using an arginine cluster in the mass range m/z = 175–871.

IR spectra were recorded using a Shimadzu IR affinity-1 spectrophotometer (Kyoto, Japan). The samples were dissolved in CHCl₃ and analysed as thin solid films using NaCl plates. Analytical thin layer chromatography (TLC) was carried out using pre-coated aluminum plates and the bands were visualized by UV light (at 254 and/or 360 nm). Elemental analysis was performed using an EuroVector elemental analyzer (Milan, Italy).

Chemicals and solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were used without further purification.

**Preparation of benzothiophene-2-carboxyl chloride (10)**

Benzo thioph ene -2 -carboxylic acid (1.0 g, 5.6 mmol) was treated with thionyl chloride (5 ml, 68.0 mmol). The reaction mixture was refluxed for 3 h at 130 °C, and then evaporated under reduced pressure. The product was washed twice with

Preparation of N-(2-benzoylphenyl)-benzothiophene-2-carboxamide (1)

Benzothiophene-2-carbonyl chloride (0.5 g, 2.8 mmol) was treated with 2-aminobenzenophenone (1.11 g, 5.6 mmol). The mixture was heated at 150 °C on a hot plate until complete fusion and left for 20 min. The resulting residue was then dissolved in CHCl₃ and extracted from water, and the organic layer was purified by column chromatography using CHCl₃ as eluent to afford the title compound as yellow solid (1.44 g, 79%). – M.p. 174 – 176 °C. – Rf = 0.66 (100% CHCl₃). – ¹H NMR (CDCl₃): δ = 12.10 (1 H, br s, NHCO), 8.0 (1 H, d, J = 8.7 Hz), 7.85 (1 H, d, J = 8.2 Hz), 7.76 (6 H, m), 7.65 (3 H, m), 7.45 (2 H, m), 7.33 (2 H, m), 7.15 (2 H, m), 2.45 (3 H, s). – ¹³C NMR (CDCl₃): δ = 200.1, 160.2, 140.4, 138.1, 136.3, 133.7, 133.3, 131.4, 130.2, 128.8, 128.1, 127.4, 126.8, 123.9, 121.5, 121.4, 121.2, 121, 119.7, 111, 103. – IR (KBr): ν = 3182, 3051, 1662, 1627, 1604, 1585, 1536, 1523, 1446, 1397, 1384, 1304, 1290, 1288, 1266, 1250, 1149, 1122, 925, 844, 794, 740 cm⁻¹. – MS (ESI, positive mode): m/z = 380.08253 [M+Na⁺]⁺ (C₂₂H₁₅NNaO₂S requires 380.08201). – C₂₂H₁₅NO₂S: calcd. C 73.93, H 4.23, N 3.84, S 9.01.

Preparation of N-(3-benzoylphenyl)-benzothiophene-2-carboxamide (2)

Benzothiophene-2-carbonyl chloride (0.5 g, 2.8 mmol) was treated with 3-aminobenzenophenone (0.5 mmol). The mixture was heated at 150 °C for 25 min, and then the resulting residue was purified by recrystallization using CHCl₃ and methanol to afford the title compound as pale yellow solid (1.04 g, 94%). – M.p. 156 – 159 °C. – Rf = 0.9 (100% CHCl₃). – ¹H NMR (CDCl₃): δ = 12.10 (1 H, br s, NHCO), 8.30 (1 H, d, J = 8.7 Hz), 7.85 (1 H, d, J = 8.2 Hz), 7.76 (6 H, m), 7.65 (3 H, m), 7.45 (2 H, m), 7.33 (2 H, m), 7.15 (2 H, m), 2.45 (3 H, s). – ¹³C NMR (CDCl₃): δ = 200.1, 160.2, 140.4, 138.1, 136.3, 133.7, 133.3, 131.4, 130.2, 128.8, 128.1, 127.4, 126.8, 123.9, 121.5, 121.4, 121.2, 121, 119.7, 111, 103. – IR (KBr): ν = 3062, 1643, 1589, 1536, 1523, 1446, 1397, 1384, 1290, 1288, 1266, 1250, 1149, 1122, 925, 844, 794, 740 cm⁻¹. – MS (ESI, positive mode): m/z = 394.10135 [M⁺]⁺ (C₂₂H₁₅NO₂S requires 394.10135). – C₂₂H₁₅NO₂S: calcd. C 73.93, H 4.23, N 3.84, S 9.01.

Preparation of N-(4-benzoylphenyl)-benzothiophene-2-carboxamide (3)

Benzothiophene-2-carbonyl chloride (0.5 g, 2.8 mmol) was treated with 4-aminobenzenophenone (0.54 g, 2.7 mmol). The mixture was heated at 150 °C, using a hot plate, and then the resulting residue was purified by recrystallization using CHCl₃ and methanol to afford the title compound as yellow crystals (0.81 g, 81%). – M.p. 198 – 202 °C. – Rf = 0.52 (CHCl₃/CH₃OH, 90:10). – ¹H NMR (CDCl₃): δ = 10.80 (1 H, br s, NHCO), 8.30 (1 H, d, J = 8.5 Hz), 7.78 (2 H, d, J = 8.2 Hz), 7.70 (2 H, d, J = 8.2 Hz), 7.63 (1 H, d, J = 8.4 Hz), 7.54 (1 H, d, J = 8.4 Hz), 7.42 – 7.52 (5H, m). – ¹³C NMR (CDCl₃): δ = 151.14, 150.3, 138.1, 136.3, 133.7, 133.3, 131.4, 130.2, 128.8, 128.1, 127.4, 126.8, 123.9, 121.5, 121.4, 121.2, 121, 119.7, 111, 103. – IR (KBr): ν = 3406, 3055, 1924, 1639, 1593, 1527, 1500, 1404, 1315, 1284, 1242, 1184, 1149, 1122, 925, 844, 794, 740 cm⁻¹. – MS (ESI, positive mode): m/z = 380.08253 [M²⁺]⁺ (C₂₂H₁₅NNa₂O₂S requires 380.08201). – C₂₂H₁₅NO₂S: calcd. C 73.93, H 4.23, N 3.84, S 9.01.

Preparation of N-[2-(4-methylbenzoyl)phenyl]-1-benzothiophene-2-carboxamide (4)

Benzothiophene-2-carbonyl chloride (0.5 g, 2.8 mmol) was treated with 2-amino-4'-methylbenzenophenone (0.5 mmol). The mixture was heated at 150 °C for 25 min, and then the resulting residue was purified by column chromatography using CHCl₃ to give the desired compound as white solid (0.15 g, 16%). – M.p. 168 – 170 °C. – Rf = 0.65 (100% CHCl₃). – ¹H NMR (CDCl₃): δ = 10.75 (1 H, br s, NHCO), 8.40 (1 H, s), 8.16 (1 H, s), H-3 benzothiophene), 8.10 (1 H, d, J = 8.7 Hz), 7.82 – 8.05 (3 H, m), 7.65 (1 H, d, J = 8.2 Hz), 7.60 (1 H, d, J = 8.2 Hz), 7.40 – 7.56 (6 H, m). – ¹³C NMR (CDCl₃): δ = 199.2, 159.6, 149.5, 137.6, 135.8, 133.1, 136.4, 131.7, 130.4, 128.6, 128.1, 127.6, 126.2, 124.2, 121.9, 121.4, 121.1, 120.8, 120.3, 119.7, 111.6, 103.2. – IR (KBr): ν = 3363, 3062, 1666, 1643, 1589, 1546, 1427, 1315, 975, 871 cm⁻¹. – MS (ESI, positive mode): m/z = 358.08570 [M⁺]⁺ (C₂₂H₁₅NO₂S requires 358.08235). – C₂₂H₁₅NO₂S: calcd. C 73.93, H 4.23, N 3.84, S 9.01.

G. Abu Sheikha · Novel Potent Antihypertriacylglyceridemic Agents 97
Preparation of N-[2-(benzoyl-4-chlorophenyl)]-benzothiophene-2-carboxamide (5)

Benzothiophene-2-carbonyl chloride (0.5 g, 2.8 mmol) was treated with 2-amino-5-chlorobenzophenone (0.57 g, 2.4 mmol). The mixture was heated at 150 °C for 20 min directly on a hot plate. The resulting residue was dissolved in CHCl₃ and extracted from acidic water, then evaporated, and recrystallized from ethanol to afford the title compound as yellow crystals (0.76 g, 77%). – M.p. 179–181 °C. – Rₜ = 0.73 (100% CHCl₃). – ¹H NMR (CDCl₃): δ = 12.01 (1 H, br s, NHCO), 9.45 (1 H, d, J = 7.2 Hz), 8.10 (1 H, d, J = 7.2 Hz), 7.99 (1 H, s), 7.83 (1 H, d, J = 9 Hz), 7.43–7.53 (4 H, m). – ¹³C NMR (CDCl₃): δ = 199.35, 160.92, 141.62, 140.53, 139.37, 139.24, 139.19, 138.94, 134.54, 134.01, 133.31, 132.98, 128.92, 128.65, 127.55, 126.74, 125.91, 125.49, 125.05, 123.95, 122.81, 122.78. – IR (KBr): ν = 3360, 3093, 1647, 1593, 1527, 1492, 1431, 1400, 1307, 1242, 1184, 1095, 1014, 821, 790, 725 cm⁻¹. – MS (ESI, positive mode): m/z = 414.04338 [M+Na]+ (C₂₂H₁₄ClNNaO₂S requires 414.04300). – C₂₃H₁₇NO₂S: calcd. C 74.37, H 4.61, N 3.77, S 8.68. – C₂₃H₁₇NO₂S Na: calcd. C 66.04, H 4.02, N 10.93, S 12.66.

Preparation of N-(pyridin-2-yl)-benzothiophene-2-carboxamide (6)

Benzothiophene-2-carbonyl chloride (0.5 g, 2.8 mmol) was treated with 2-aminopyridine (0.24 g, 2.5 mmol). The mixture was heated on a hot plate at 150 °C for 15 min directly on a hot plate. The resulting residue was then purified by recrystallization using CHCl₃ to afford the title compound as white crystals (0.36 g, 58%). – M.p. 225–228 °C. – Rₜ = 0.49%. – M.p. 215–220 °C. – Rₜ = 0.51 (100% CHCl₃). – ¹H NMR (CDCl₃): δ = 10.70 (1 H, br s, NHCO), 8.37 (1 H, s), 8.01–8.08 (2 H, m), 7.83 (2 H, d, J = 9 Hz), 7.43–7.53 (4 H, m). – ¹³C NMR (CDCl₃): δ = 165.62, 145.78, 144.89, 144.28, 142.28, 139.36, 139.15, 138.85, 133.89, 132.83, 131.81, 130.67, 130.31, 128.08, 127.05. – IR (KBr): ν = 3360, 3093, 1647, 1593, 1527, 1492, 1431, 1400, 1307, 1242, 1184, 1095, 1014, 821, 790, 725 cm⁻¹. – MS (ESI, positive mode): m/z = 288.01716 [M+H]+ (C₁₄H₁₁N₂OS requires 288.01664). – C₁₄H₁₀N₂OS: calcd. C 62.61, H 3.50, N 4.87, S 11.22.

Preparation of N-(4-chlorophenyl)-benzothiophene-2-carboxamide (7)

Benzothiophene-2-carbonyl chloride (0.25 g, 1.4 mmol) was treated with 3-aminopyridine (0.12 g, 1.3 mmol). The mixture was heated at 130 °C for 22 min directly on a hot plate. The resulting residue was then purified by recrystallization using CHCl₃ to afford the title compound as light gray solid (0.32 g, 98%). – M.p. 265–269 °C. – Rₜ = 0.6 (CHCl₃/CH₂OH, 90:10). – ¹H NMR (CDCl₃): δ = 12.30 (1 H, br s, NHCO), 9.45 (1 H, s), 8.90 (1 H, d, J = 7.2 Hz), 8.70 (1 H, s), 8.65 (1 H, d, J = 7.2 Hz), 8.15 (1 H, d, J = 7.2 Hz), 8.05 (2 H, m), 7.50 (2 H, m). – ¹³C NMR (CDCl₃): δ = 166.51, 146.09, 144.18, 143.40, 142.70, 139.63, 139.03, 136.41, 133.21, 132.28, 132.16, 130.98, 130.50, 128.16. – IR (KBr): ν = 3363, 3062, 1666, 1643, 1589, 1546, 1427, 1315, 975, 871 cm⁻¹. – MS (ESI, positive mode): m/z = 255.05138 [M+H]+ (C₁₄H₁₁ClNOS requires 255.05159). – C₁₄H₁₀N₂OS: calcd. C 67.12, H 3.96, N 11.02, S 12.61; found C 67.04, H 4.02, N 10.93, S 12.66.

Preparation of N-(pyridin-3-yl)-benzothiophene-2-carboxamide (8)

Benzothiophene-2-carbonyl chloride (0.5 g, 2.8 mmol) was added to p-chloroaniline (0.32 g, 2.5 mmol). The mixture was heated on a hot plate at 130–140 °C for 20 min. The resulting residue was then purified by recrystallization using CHCl₃ to afford the title compound as a white solid (0.37 g, 98%). – M.p. 215–220 °C. – Rₜ = 0.49%. – M.p. 225–228 °C. – Rₜ = 0.51 (100% CHCl₃). – ¹H NMR (CDCl₃): δ = 12.55 (1 H, br s, NHCO), 8.95 (1 H, d, J = 7.2 Hz), 8.75 (1 H, s), 8.22 (2 H m), 7.95 (1 H, d, J = 7.2 Hz), 7.83 (1 H, d, J = 7.2 Hz), 7.32–7.42 (3 H, m). – ¹³C NMR (CDCl₃): δ = 168.62, 149.58, 146.07, 142.58, 140.02, 139.24, 137.37, 130.07, 127.68, 126.73, 125.29, 122.60, 119.30, 117.49. – IR (KBr): ν = 3070, 3005, 1681, 1651, 1506, 1500, 1431, 1330, 1292, 1238, 1211, 1153, 1041, 1006, 887 cm⁻¹. – MS (ESI, positive mode): m/z = 277.05138 [M+Na]+ (C₁₄H₁₀N₂NaOS requires 277.05179). – C₁₄H₁₀N₂OS: calcd. C 66.12, H 3.96, N 11.02, S 12.61; found C 66.20, H 3.90, N 11.11, S 12.68.
triethylamine (2.0 ml, 7.5 mmol). The mixture was refluxed for 48 h. DMF was removed by evaporation under reduced pressure, and the resulting residue was redissolved in CHCl₃ and extracted with acidic water. The organic layer, after drying over anhydrous Na₂SO₄, was purified by column chromatography using CHCl₃ as eluent to afford the title compound as a white solid (0.3 g, 27%).

- M.p. 262–268 °C.
- Rᵢ = 0.82 (CHCl₃/CH₃OH, 85:15).
- ¹H NMR (CDCl₃): δ = 12.00 (1 H, br s, NHCO), 10.23 (1 H, br s), 8.65 (1 H, s), 8.00 (2 H, t, J = 7.5 Hz), 7.60 (2 H, m), 7.45 (2H, m), 7.30 (2 H, m).
- ¹³C NMR (CDCl₃): δ = 163.51, 146.23, 141.82, 139.38, 139.15, 139.01, 138.96, 138.75, 129.73, 129.53, 127.75, 126.42, 125.78, 124.58, 123.47, 113.57.
- IR (KBr): v = 3344, 3093, 1639, 1597, 1516, 1462, 1431, 1346, 1276, 1249, 1219, 1180, 1157, 1099, 883 cm⁻¹.
- MS (ESI, positive mode): m/z = 316.06228 [M+Na]+ (C₁₆H₁₁N₃NaOS requires 316.06283).

Pharmacological studies

Triton WR-1339 was obtained from Sigma-Aldrich. The rest of the chemicals (fine super grade) were purchased from Acros Organics (Amman, Jordan).

Animals and treatments

Sixty four adult male Wistar rats, weighing around 180 g, bred in the animal care centre of Faculty of Pharmacy, Al-Zaytoonah Private University, Amman, Jordan, were provided ad libitum access only to tap water throughout the experimental duration (24 h). Rats were maintained in a 12-h light-dark cycle under constant humidity and (22 ± 2) °C. All experiments were performed in accordance with the Guidelines of Animal Welfare Committee of the University.

Triton WR-1339 model of hyperlipidemia

Triton WR-1339 was dissolved in DMSO and administered intraperitoneally (0.6 ml) to the rats (300 mg/kg body weight) in order to induce hyperlipidemia.

Experimental design

Overnight fasted rats were randomly divided into eight groups of eight animals each. The first group, serving as normal control group (NCG), received an intraperitoneal administration of normal saline; the second hyperlipidemic plus 4% DMSO control group (TDCG) received an intraperitoneal injection of Triton WR-1339 dissolved in 4% DMSO. In the third group (C3) animals were intraperitoneally injected with Triton WR-1339, followed by an intragastric administration of compound 3 (15 mg/kg body weight) dissolved in 4% DMSO. The rats of the fourth group (C6) were also intraperitoneally injected with Triton WR-1339, followed by an intragastric administration of compound 6 (15 mg/kg body weight) dissolved in 4% DMSO. At the same time the rats of the fifth group (C7) were also intraperitoneally injected with Triton WR-1339, followed by an intragastric administration of compound 7 (15 mg/kg body weight) dissolved in 4% DMSO. In the sixth group (C8) animals were intraperitoneally injected with Triton WR-1339, followed by an intragastric administration of compound 8 (15 mg/kg body weight) dissolved in 4% DMSO. The rats of the seventh group (C9) were also intraperitoneally injected with Triton WR-1339, followed by an intragastric administration of compound 9 (15 mg/kg body weight) dissolved in 4% DMSO. The last group (TDFG) was also intraperitoneally injected with Triton WR-1339 and intragastrically treated with bezafibrate (100 mg/kg body weight) dissolved in 4% DMSO.

After 12 h of treatments, animals were anaesthetized with diethyl ether, and blood was collected from the renal artery. The blood samples were immediately centrifuged (1500 x g for 10 min), and the plasma was used for lipid analysis by an enzymatic method with an automatic analyzer (Model Erba XL-300, Mannheim, Germany).

Statistical analysis

Results were expressed as means ± SD. Data obtained were analysed using the Student’s t-test, and differences with p < 0.05 were considered statistically significant.

Results

Induction of hyperlipidemia by Triton WR-1339

The plasma total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) levels of all groups treated for 12 h are shown.
in Fig. 1. Triton WR-1339 caused a significant increase in plasma TC, TG and LDL-C \((p < 0.0001)\) levels in the hyperlipidemic +4% DMSO control group (TDCG), 12 h after Triton WR-1339 administration in comparison with the normal control group (NCG).

In fact, the increase of plasma TC concentration in TDCG was 45% after 12 h as compared to NCG. The TG level in TDCG was also elevated by 824% after 12 h. At the same time the LDL-C level in TDCG was also elevated by 185% after 12 h as compared to NCG, while a significant \((p < 0.0001)\) decrease in the HDL-C level occurred 12 h after Triton WR-1339 injection.

Effect of compounds 3, 6, 7, 8, 9, and bezafibrate on rat plasma lipid profile

The plasma TC, TG, HDL-C, and LDL-C levels of TDFG-, compounds 3-, 7-, 8-, and 9-treated rats after 12 h are shown in Table I. Importantly, the elevated plasma TG levels produced by Triton WR-1339 administration were significantly \((p < 0.0001)\) suppressed in TDFG- by 77%, in compound 6- by 73%, in compound 7- by 49%, and in compound 9-treated rats by 81% after 12 h, with respect to the hyperlipidemic control TDCG.

The HDL-C levels were significantly increased after 12 h by 44%, 53%, and 37% \((p < 0.0001)\) in compounds 6-, 7-, and 9-treated rats, respectively, and 16% \((p < 0.01)\) in TDFG-treated rats compared to the hyperlipidemic control TDCG (Table I).

With the exception of compound 9-treated rats, none of the treated groups showed a significant reduction in plasma TC levels after 12 h (Table I). In fact, it was found that the TC level was reduced by 8% after 12 h in compound 9- compared to TDCG-treated rats.

After 12 h of treatment, LDL-C levels were lowered by 44% \((p < 0.0001)\) in compound 9-, and 13%, 10%, and 15% \((p < 0.01)\) in compounds 6-, 7-, and TDFG-treated rats, respectively, compared to the hyperlipidemic control group TDCG (Table I).

No significant differences in TC, TG, HDL-C, and LDL-C levels were observed in compounds 3- and 8-treated rats compared to TDCG-treated rats.

Discussion

The results of the current study showed the potential hypolipidemic effect of compounds 6,

Table I. Effect of the novel compounds 3, 6, 7, 8, 9 and bezafibrate on plasma lipid levels in Triton WR-1339-induced hyperlipidemic rats after 12 h.

<table>
<thead>
<tr>
<th>Lipid profile</th>
<th>TC [mg/ml]</th>
<th>TG [mg/ml]</th>
<th>HDL-C [mg/ml]</th>
<th>LDL-C [mg/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDCG</td>
<td>1.32 ± 0.08</td>
<td>5.76 ± 0.07</td>
<td>0.45 ± 0.03</td>
<td>0.66 ± 0.03</td>
</tr>
<tr>
<td>C3</td>
<td>1.29 ± 0.04</td>
<td>5.80 ± 0.09</td>
<td>0.46 ± 0.02</td>
<td>0.65 ± 0.02</td>
</tr>
<tr>
<td>C6</td>
<td>1.21 ± 0.06</td>
<td>1.56 ± 0.07</td>
<td>0.65 ± 0.03*</td>
<td>0.58 ± 0.04*</td>
</tr>
<tr>
<td>C7</td>
<td>1.35 ± 0.004</td>
<td>2.91 ± 0.05*</td>
<td>0.69 ± 0.03b</td>
<td>0.60 ± 0.04*</td>
</tr>
<tr>
<td>C8</td>
<td>1.31 ± 0.05</td>
<td>5.73 ± 0.07</td>
<td>0.44 ± 0.02</td>
<td>0.67 ± 0.02</td>
</tr>
<tr>
<td>C9</td>
<td>1.21 ± 0.03*</td>
<td>1.09 ± 0.03b</td>
<td>0.62 ± 0.03b</td>
<td>0.37 ± 0.03b</td>
</tr>
<tr>
<td>TDFG</td>
<td>1.35 ± 0.03*</td>
<td>1.33 ± 0.04b</td>
<td>0.52 ± 0.03*</td>
<td>0.56 ± 0.01*</td>
</tr>
</tbody>
</table>

Values are means ± SD from eight animals in each group.

TDCG, hyperlipidemic +4% DMSO control group; C3, compound 3 + 4% DMSO; C6, compound 6 + 4% DMSO; C7, compound 7 + 4% DMSO; C8, compound 8 + 4% DMSO; C9, compound 9 + 4% DMSO; TDFG, bezafibrate + 4% DMSO; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol.

Compounds 3, 6, 7, 8, 9 and TDFG are compared with TDCG.

\(p < 0.01; ^* p < 0.0001.\)
7, and 9 (Table 1) in Triton WR-1339-induced hyperlipidemic rats. Compounds 6, 7, and 9 significantly reduced serum TG and increased serum HDL levels.

Triton WR-1339-induced hyperlipidemic rats have been widely used as model to investigate the potential hypolipidemic effect of synthesized and naturally derived compounds (Paoletti, 1962; Kalopissis et al., 1980; Khanna et al., 1992; Frishman, 1998). It has been reported that hyperlipidemia could be induced by parenteral administration of Triton WR-1339 to adult rats. The peak plasma TC and TG levels were reached at 20 h followed by a decline to normal values (Schurr et al., 1972). In our study, the same model showed a similar pattern of lipid profile changes 12 h after Triton WR-1339 administration (Fig. 1).

Clearly, the results in our hands showed that compounds 6, 7, and 9 at a dose of 15 mg/kg body weight were able to significantly decrease serum TG levels.

Yamamoto and his colleagues (1984) reported that the large decrease in plasma HDL-C levels due to Triton WR-1339 injection results mostly from a progressive displacement of the apo A-1 protein from the HDL surface without loss of lipid. Meanwhile the large increase in plasma TG levels due to Triton administration results mostly from an increase of very low-density lipoprotein (VLDL) secretion by the liver accompanied by strong reduction of VLDL and LDL catabolism (Perez et al., 1999).

Accordingly, given that the portion of TG in VLDL is persistently higher than that of cholesterol, it is not unexpected that the hypolipidemic activity of compounds 6, 7, and 9 was significantly higher for TG than for cholesterol. This result suggests that the catabolism of B-lipoproteins could be, at least partially, restored by our compounds as hypothesized by many works with other lipid-lowering agents (Khanna et al., 2002; Malloy and Kan, 1994).

In addition, compounds 6, 7, and 9 increased the HDL level, which is known for its preventive role against atherogenesis. HDL also promotes substantial cholesterol egress from the liver by facilitating the mobilization of TG and cholesterol from plasma to liver where it undergoes catabolism and then is eliminated in the form of bile acids (Anila and Vijayalakshmi, 2002; Staels et al., 1998).

Promisingly, administration of compounds 6, 7, and 9 at a dose of 15 mg/kg body weight after 12 h of Triton injection is more significant than the reduction induced by bezafibrate administration at a dose of 100 mg/kg body weight, which in this study has been used as standard reference hypolipidemic drug. Furthermore, TC levels were not significantly changed which agrees with the mechanism of action of fibrates in that their TC-lowering activity is not strongly marked, but the TG decreasing effect of them is very impressive.
especially by stimulation of the gene expression of lipoprotein lipase (Staels et al., 1998).

The findings of the current study are compatible with our previously published data (Al-Qirim et al., 2009; Shahwan et al., 2010), which highlighted that introducing a hydrogen bond between aromatic heterocyclic rings and the target(s) is essential for the biological activity (Fig. 2). It was noticed that replacing indole and benzofuran by benzothiophene will abolish the activity even though the lipophilic moiety is conserved. On the other hand, the activity was retained when imidazole and pyridine are introduced into the compounds instead of indole and benzofuran. From these observations, we concluded that the presence of an aromatic heterocyclic ring which is able to form a hydrogen bond is crucial for the biological activity (Fig. 2).

In addition, it seems that the presence of a large lipophilic moiety is also important for the biological activity. So when benzothiophene was used to replace benzophenone the activity was maintained whereas the activity was dramatically decreased when it was replaced by small lipophilic rings. This could be explained by ring equivalency between benzothiophene and benzophenone (Fig. 2).

Overall, these preliminary observations lead us to conclude that the presence of a large lipophilic moiety, carboxamide linker along with a heterocyclic ring (able to form hydrogen bonds) are three important requirements to obtain hypolipidemic activity.

In conclusion, compounds 6, 7, and 9 were shown to improve the lipid profile in Triton-induced hyperlipidemic rats. The results are highly promising but more studies are necessary to elucidate the exact mechanism of action of these novel compounds as lipid-lowering agents and to clarify their structure-activity relationship.


Schoonjans K., Staels B., and Auwerx J. (1996), Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. J. Lipid Res. 37, 907–925.


