Introduction

Hyperlipidemia is an elevation of one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters and phospholipids (Raasch, 1988). This pathological condition has been ranked as one of the most important risk factors contributing to the prevalence and severity of coronary heart diseases (Goldstein et al., 1973; Frishman, 1998). These diseases along with stroke, atherosclerosis, and hypertension are found to be one of the main causes of death worldwide (Smith et al., 2004; Braunwald, 1997).

Previous studies showed that a single parenteral administration of Triton WR-1339 to adult rats produces hyperlipidemia in which cholesterol, triglycerides, and phospholipid levels increase to a maximum within about 20 h 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). Therefore Triton WR-1339-induced hyperlipidemic rats are widely used as a model to screen for or to differentiate the mechanism of action of potential hypolipidemic agents (Paoletti, 1962; Kalopissis et al., 1980).

Fibrates and their derivatives are a group of drugs, which have been widely used for a long time to treat hyperlipoproteinemia, among which is the well-known commercially available drug bezafibrate (Frick et al., 1987). Fibric acids enhance the fatty acid catabolism and accordingly reduce the 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).

Some indole derivatives are well known for their diverse pharmacological effects including a hypolipidemic effect (Al-Qirim et al., 2009; Bosies et al., 1980; Sher and Ellsworth, 2004; Kopin et al., 2006; Dasseux and Oniciu, 2002). But to the best of our knowledge N-(benzoylphenyl)-1H-indole-2-carboxamide derivatives have not been...
investigated as potential lipid-lowering agents, although some studies showed the potential role of indole-2-carboxamide derivatives as antiallergics (Robichaud et al., 1987) and antioxidants (Olgen and Coban, 2002), as well as their inhibitory mode against HPLGa (Liu et al., 2004).

Taking into consideration the importance to maintain normal plasma lipid concentrations as a protection measure against coronary heart diseases, this study aimed to evaluate the possible lipid-lowering activity of novel N-(benzoylphenyl)-1H-indole-2-carboxamide derivatives.

**Material and Methods**

**Chemical studies**

A novel series of the N-(benzoylphenyl)-1H-indole-2-carboxamides 1–6 were prepared in the course of this work. The target compounds 1–6 were synthesized in one step by the coupling reaction between ethyl-1H-indole-2-carboxylate (7) and the corresponding aminobenzophenones 8–13 as shown in Schemes 1 and 2. Ethyl-1H-indole-2-carboxylate was treated with an excess of aminobenzophenones in the presence of sodium ethoxide in DMF at 100–150 °C. The reaction mixtures were purified using column chromatography to afford the desired compounds.

**Preparation of N-(2-benzoylphenyl)-1H-indole-2-carboxamide (1)**

Ethyl-1H-indole-2-carboxylate (7) (0.55 g, 2.9 mmol) was treated with 2-aminobenzophenone (8) (1.54 g, 7.8 mmol) in the presence of sodium ethoxide, DMF, 100–150 °C.
ethoxide (0.16 g, 2.4 mmol) and dimethylformamide (DMF) (5 ml) (Scheme 1). The mixture was refluxed for 4 d at 100 °C and then filtered. DMF was removed by evaporation under reduced pressure, and the residue was purified by column chromatography using CHCl₃/EtOH (99:1) as eluent to afford the title compound as a yellow solid (0.16 g, 16%). – M.p. 221 – 222 °C. – \( R_f = 0.84 \) (CHCl₃/MeOH, 98:2). – ¹H NMR (CDCl₃): \( \delta = 7.12 \) (2H, m), 7.25 (2H, m), 7.45 (2H, m), 7.63 (5H, m), 7.78 (2H, d, \( J = 8.1 \) Hz), 8.82 (1H, d, \( J = 8.7 \) Hz, H-3 indole), 9.7 (1H, br s, H-1 indole), 12.2 (1H, br s, NHCO). – ¹³C NMR (CDCl₃): \( \delta = 103, 111, 119.7, 120, 121, 121.2, 121.4, 121.5, 123.9, 126.8, 127.4, 128.1, 128.8, 130.2, 131.4, 133.3, 133.7, 135.9, 137.8, 140, 159.2, 199.4. – IR (thin film): \( \nu = 3302, 3062, 2924, 1654, 1589, 1546, 1485, 1431, 1319, 1284, 1261 \) cm⁻¹. – MS: \( m/z = 341.12845 \) [M+H]⁺; calcld. 341.12453. – C22H16N2O2: calcld. C 77.63, H 4.74, N 8.23; found C 77.69, H 4.66, N 8.27.

Preparation of N-(3-benzoylphenyl)-1H-indole-2-carboxamide (2)

Ethyl-1H-indole-2-carboxylate (7) (0.44 g, 2.3 mmol) was treated with 3-aminobenzophenone (9) (1.54 g, 7.8 mmol) in the presence of sodium ethoxide (0.16 g, 2.4 mmol) and DMF (5 ml) (Scheme 1). The mixture was refluxed for 24 h at 150 °C and then filtered. DMF was removed by evaporation under reduced pressure, and the residue was purified by column chromatography using CHCl₃/Methanol/formic acid (99:1:0.1) as eluent to afford the title compound as a pale brown oil (0.16 g, 20%). – \( R_f = 0.55 \) (CHCl₃/MeOH, 96:4). – ¹H NMR (CDCl₃): \( \delta = 7.40 – 7.60 \) (5H, m), 7.78 (4H, m), 8.01 (3H, m), 8.43 (1H, s), 8.82 (1H, d, \( J = 8.7 \) Hz, H-3 indole), 9.6 (1H, br s, H-1 indole), 11.9 (1H, br s, NHCO). – ¹³C NMR (CDCl₃): \( \delta = 103.2, 111.2, 119.7, 120, 121, 121.2, 121.5, 123.9, 126.8, 127.4, 128.1, 128.8, 130.2, 131.4, 133.3, 133.7, 135.9, 137.8, 140, 159.7, 199.8. – IR (thin film): \( \nu = 3309, 3062, 2924, 1654, 1589, 1546, 1485, 1431, 1319, 1284, 1261 \) cm⁻¹. – MS: \( m/z = 341.12845 \) [M+H]⁺; calcld. 341.12453. – C22H16N2O2: calcld. C 77.63, H 4.74, N 8.23; found C 77.57, H 4.78, N 8.26.

Preparation of N-(4-benzoylphenyl)-1H-indole-2-carboxamide (3)

Ethyl-1H-indole-2-carboxylate (7) (0.48 g, 2.5 mmol) was treated with 4-aminobenzophenone
Preparation of N-(2-(2-carboxybenzoylphenyl))-1H-indole-2-carboxamide (4)

Ethyl-1H-indole-2-carboxylate (7) (0.26 g, 1.4 mmol) was treated with 2-aminobenzoyl-2-benzoic acid (11) (1.0 g, 4.2 mmol) in the presence of sodium ethoxide (0.09 g, 1.4 mmol) and DMF (4 ml) (Scheme 1). The mixture was refluxed for 24 h at 150 °C and then filtered. DMF was removed by evaporation under reduced pressure, and the residue was purified by column chromatography using CHCl₃/MeOH/formic acid (99:1:0.1) as eluent to afford the title compound as a yellow solid (0.11 g, 12%). – M.p. 236–237 °C. – Rᵣ = 0.75 (CHCl₃/MeOH, 98:2). – ¹H NMR (CDCl₃): δ = 5.7 Hz, H-3 indole), 8.42 (1H, d, J = 8.4 Hz), 7.61–7.82 (4H, m), 8.95 (1H, d, 7.78 Hz, H-3 indole), 9.2 (1H, br s, H-1 indole), 12.1 (1H, br s, NHCO). – ¹³C NMR (CDCl₃): δ = 103, 111, 119.7, 120, 121, 121.2, 121.4, 121.5, 123.9, 126.8, 127.4, 128, 128.8, 130.2, 131.4, 133.3, 133.7, 136.3, 138.1, 140.4, 160.2, 200.1. – IR (thin film): ν = 3298, 2962, 1654, 1639, 1319, 1261 cm⁻¹. – MS: m/z = 363.11040 [M+Na]⁺; calced. 364.12453. – C₂₃H₁₈N₂O₄: calcd. C 71.81, H 4.28, N 7.22.

Preparation of N-[2-(4-tolylphenyl)]-1H-indole-2-carboxamide (5)

Ethyl-1H-indole-2-carboxylate (7) (0.3 g, 1.6 mmol) was treated with 2-aminophenyl-4-tolylmethanone (12) (1.0 g, 4.8 mmol) in the presence of sodium ethoxide (0.1 g, 1.6 mmol) and DMF (3 ml) (Scheme 2). The mixture was refluxed at 150 °C for 3 d and then filtered. DMF was removed by evaporation under reduced pressure and the residue was purified by column chromatography using cyclohexane/EtOAc (85:15) as eluent to afford the title compound as a yellow solid (0.12 g, 21%). – M.p. 236–237 °C. – Rᵣ = 0.75 (CHCl₃/MeOH, 98:2). – ¹H NMR (CDCl₃): δ = 2.51 (3H, s), 7.15 (4H, m), 7.25 (3H, m), 7.42 (1H, d, J = 8.4 Hz), 7.61–7.82 (4H, m), 8.95 (1H, d, J = 7.78 Hz, H-3 indole), 9.2 (1H, br s, H-1 indole), 12.1 (1H, br s, NHCO). – ¹³C NMR (CDCl₃): δ = 28.7, 103, 110, 119.8, 120, 121, 121.2, 121.4, 121.9, 123.9, 126.4, 127.1, 128, 128.5, 129.1, 131.2, 133, 133.4, 135.7, 137.5, 139.7, 158.5, 199. – IR (thin film): ν = 3305, 1658, 1627, 1535, 1446, 1315, 1265 cm⁻¹. – MS: m/z = 377.12605 [M+Na]⁺; calced. 378.14018. – C₂₃H₁₈N₂O₂: calcd. C 77.95, H 5.12, N 7.90; found C 77.88, H 5.18, N 7.82.

Preparation of N-[2-(benzoyl-4-chlorophenyl)]-1H-indole-2-carboxamide (6)

Ethyl-1H-indole-2-carboxylate (7) (1.0 g, 5.3 mmol) was treated with 2-aminobenzoyl-2-benzoic acid (11) (1.0 g, 4.2 mmol) in the presence of sodium ethoxide (0.36 g, 5.3 mmol) and DMF (5 ml) (Scheme 2). The mixture was refluxed for 24 h at 150 °C and then filtered. DMF was removed by evaporation under reduced pressure, and the residue was purified by column chromatography using cyclohexane/EtOAc (9:1) as eluent to afford the title compound as a yellow oil (0.32 g, 18%). – M.p. 236–237 °C. – Rᵣ = 0.75 (CHCl₃/MeOH, 98:2). – ¹H NMR (CDCl₃): δ = 7.22–7.31 (2H, m), 7.65 (5H, m), 7.65–7.82 (5H, m), 7.81 (1H, s), 9.8 (1H, br s, H-1 indole), 11.1 (1H, br s, NHCO). – ¹³C NMR (CDCl₃): δ = 119.7, 121, 121.2, 121.4, 121.5, 123.4, 126.8, 127.6, 127.9, 128.3, 128.6, 129, 129.2, 130.6, 131.7, 132.3, 132.8, 135, 137.9, 140, 165.7, 191.7, 199.5. – IR (thin film): ν = 3305, 3182, 3035, 2978, 2927, 1647, 1585, 1570, 1481, 1435, 1396, 1307, 1238 cm⁻¹. – MS: m/z = 358.11485 [M+H]⁺; calced. 385.11436. – C₂₃H₁₈N₂O₂: calcd. C 71.87, H 4.20, N 7.29; found C 71.81, H 4.28, N 7.22.
Pharmacological studies

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

Animals and treatments

54 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 access to tap water ad libitum throughout the experimental duration (24 h). Rats were maintained in a 12 h light-dark cycle under constant humidity and (22 ± 2) ºC. 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 to the rats (300 mg/kg body weight) in order to induce hyperlipidemia.

Experimental design

Overnight fasted rats were randomly divided into five groups of six animals each. The first group, serving as normal control group (NCG), received an intraperitoneal administration of normal saline; the second hyperlipidemic plus DMSO control group (TDCG) received an intraperitoneal injection of Triton WR-1339 and was gavaged with 4% DMSO (in distilled water). In the third group (C2) animals were intraperitoneally injected with Triton WR-1339, followed by an intragastric administration of compound 2 (15 mg/kg body weight) dissolved in 4% DMSO. The rats of the fourth group (C3) were also intraperitoneally injected with Triton WR-1339, followed by an intragastric administration of compound 3 (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 7 h and 24 h of treatments, animals were anaesthetized with diethyl ether, and blood was collected. The blood samples were immediately centrifuged (1500 × 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 ± SEM. Data obtained were analyzed 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 7 h and 24 h are shown in Table I. Triton WR-1339 caused a significant increase in plasma TC (p < 0.001), TG, and LDL-C (p < 0.0001) levels in the hyperlipidemic + DMSO control group (TDCG), at both 7 h and
24 h after Triton WR-1339 administration in comparison with the normal control group (NCG). In fact, the increases of plasma TC concentrations in the TDCG were 32% and 72% after 7 h and 24 h, respectively, as compared to the NCG. The TG levels in the TDCG were also elevated by 742% and 670% after 7 h and 24 h, respectively. At the same time the LDL-C levels in TDCG were also elevated by 314% and 328% after 7 h and 24 h, respectively, as compared to the NCG, while a significant ($p < 0.001$) decrease in the HDL-C levels occurred at 7 h and were maintained until 24 h after Triton WR-1339 injection.

**Effect of 2, 3, and bezafibrate on rat plasma lipid profile**

The plasma TC, TG, HDL-C, and LDL-C levels of TDFG-, 2- and 3-treated rats after 7 h and 24 h are shown in Fig. 1A and B, respectively. Importantly, the elevated plasma TG levels produced by Triton WR-1339 administration were significantly ($p < 0.0001$) suppressed in TDFG-treated rats by 75% and 67%, in 2-treated rats by 91% and 66%, and in the 3-treated rats by 72% and 64% after 7 h and 24 h, respectively, with respect to the hyperlipidemic control (TDCG).

![Graph showing plasma lipid levels](image)

Fig. 1. Effect of 2 and 3 on plasma lipid levels in Triton WR-1339-induced hyperlipidemic rats after (A) 7 h and (B) 24 h. Values are means ± SEM from six animals in each group. TDCG, hyperlipidemic + 4% DMSO control group; TDFG, bezafibrate + 4% DMSO group; C2, 2 + 4% DMSO group; C3, 3 + 4% DMSO group. 2, 3, and TDFG are compared with TDCG. * $p < 0.01$; ** $p < 0.001$; *** $p < 0.0001$. 

The HDL-C levels were significantly increased after 7 h by 24%, 24% \((p < 0.01)\), and 40% \((p < 0.001)\) in TDFG-, 2- and 3-treated rats, respectively, compared to TDCG-treated rats, while the HDL-C levels after 24 h were increased by 30%, 34% \((p < 0.001)\), and 42% \((p < 0.0001)\) in TDFG-, 2- and 3-treated rats, respectively, compared to TDCG-treated rats, which were considered highly significant.

After 7 h of treatment, no significant differences in the plasma TC levels between any treated group (TDFG, 2 and 3) were observed (Fig. 1A). In contrast, 24 h after treatment, in 2- and 3-treated rats the plasma TC levels was reduced significantly by 17% and 27% \((p < 0.0001)\), respectively.

Neither after 7 h nor after 24 h, in TDFG- and 2-treated groups the LDL-C level significantly decreased compared to the TDCG. However, in the 3-treated group the LDL-C levels were significantly \((p < 0.001)\) reduced by 75% and 40% after 7 h and 24 h, respectively.

Discussion

In the present study, the hypolipidemic activity of two novel indole-2-carboxamide derivatives, 2 and 3, was tested using Triton WR-1339-induced rats, which have been widely used as a model for screening the lipid lowering potential (Paoletti, 1962; Kalopissis et al., 1980).

Our results showed that the hyperlipidemia induced by Triton WR-1339 was significantly suppressed using 2, 3, and bezafibrate in comparison with the control group after 7 and 24 h, respectively, observing that the hypolipidemic action of 2 and 3 was markedly higher for triglycerides than for cholesterol. This could be explained by taking into consideration that the large increase in plasma cholesterol and triglycerides due to Triton WR-1339 administration results mostly from an increase of very low-density lipoprotein (VLDL, in which the triglycerides portion is several times greater than that of cholesterol) secretion by the liver accompanied by a strong reduction of VLDL and LDL catabolism (Otway and Robinson, 1967). This result suggests that the compounds are able to restore, at least partially, the catabolism of lipoproteins.

In addition, a noticeable reduction in plasma total cholesterol was observed with the 2- and 3-treated groups. This reduction was associated with a decrease in the LDL fraction, which equates to a higher risk factor for coronary heart disease. This result suggests that the cholesterol-lowering activity of these novel compounds can be a result from the enhancement of the LDL catabolism through the hepatic receptor (Khanna et al., 2002).

In addition, 2 and 3 significantly increased the HDL-C levels after 24 h of Triton WR-1339 administration, which have a preventive function against atherogenesis (Malloy and Kan, 1994). HDL facilitates the mobilization of triglycerides and cholesterol from plasma to liver where it is catabolized and eliminated in the form of bile acids (Anila and Vijayalakshmi, 2002).

Acknowledgement

The authors wish to express their sincere appreciation to Al-Zaytoonah Private University of Jordan for financial support and to Sameer Al-kouz and Al-zahra’a Al-Ghazzawi for technical support.


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