Synthesis and Pharmacological Evaluation of Novel Unsubstituted Indole-Anthraquinone Carboxamide Derivatives as Potent Antihyperlipidemic Agents

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Five novel derivatives of *N*-(9,10-dihydro-9,10-dioxoanthracenyl)-1*H*-indole-2-carboxamide were synthesized and their lipid-lowering effects studied in hyperlipidemic rats. Fusion of the anthraquinone derivatives at high temperature with 5-indole-2-carbonyl chloride, followed by recrystallization from chloroform/methanol gave the desired compounds in excellent yields. Compounds **1** to **5** at a non-toxic dose (1 ml of 57 μM solutions) and bezafibrate as positive control were administered to rats that were hyperlipidemic due to treatment with Triton WR-1339. A decrease in the plasma levels of triglyceride (TG) and low-density lipoprotein-cholesterol (LDL-C) and an increase in the plasma level of high-density lipoprotein-cholesterol (HDL-C) were observed with compounds **1**, **3**, **4**, and **5**. Compounds **1**, **4**, and **5** significantly reduced total cholesterol (TC) levels as well. These compounds may provide agents for targeting dyslipidemia and cardiovascular disease.

Key words: *N*-(9,10-Dihydro-9,10-dioxoanthracenyl)-1*H*-indole-2-carboxamide, High-Density Lipoprotein, Cholesterol Elevation, Triglyceride Reduction

Introduction

Hyperlipidemia and thereby atherosclerosis are the leading causes of cardiac illness and death (Farnier and Davignon, 1998). Data are now accumulating on the effects of lowering serum triglyceride (TG) levels in improving coronary risk. High serum low-density lipoprotein-cholesterol (LDL-C) and elevated total cholesterol (TC) levels are the most prevalent indicators of susceptibility to atherosclerotic heart disease (Fruchart *et al*., 2008). To reduce the rate of mortality, it is recommended to undergo a diet or/and drug therapy to lower lipid levels to the normal range. Allopathic hyperlipidemia drugs are available at large scale in the market; these drugs have different mechanisms of action and variable efficacy depending on the lipid profile of an individual (Batra *et al*., 2000).

Triton WR-1339 (tyloxapol), a non-ionic detergent (oxyethylated tertiary octyl phenol formaldehyde polymer), has been widely used to block clearance of TG-rich lipoproteins to induce acute hyperlipidemia in several animal species. This model is widely used for a number of different aims; particularly in rats it has been used for screening natural or synthetic hypolipidemic drugs. A single parenteral administration of Triton WR-1339 to adult rats produces hyperlipidemia in which the levels of cholesterol, TG, and phospholipids increase to a maximum within about 20 h and decrease thereafter (Harnafi *et al*., 2008; Schurr *et al.*, 1972). The accumulation of plasma lipids in response to Triton WR-1339 appears to be due to inhibition of the activities of lipoprotein lipase (LPL) and hepatic lipases (HL). In addition to the inhibition of LPL, Triton WR-1339 exerts a number of other physiological effects related to lipoprotein, *e. g.* it has been shown to cause dissociation of apo A-I and apo C-II from high-density lipoprotein-cholesterol (HDL-C) (Agren *et al.*, 2005).

Several different classes of drugs are used in the treatment of hyperlipidemia. These classes differ not only in their mechanisms of action but also in the type of lipids they reduce and the magnitude of the reduc-

tion. Statins, the most common group of antihyperlipidemic drugs lower the level of cholesterol by interrupting the cholesterol biosynthetic pathway (Yoshihisa and Yuichi, 2006). On the other hand, the fibrate group decreases fatty acid and TG levels by stimulating the peroxisomal β-oxidation pathway (Jun *et al*., 2010).

Indoles are considered versatile structural motifs, due to their ability to bind to many receptors within the body. As a result, much research has been dedicated to incorporating the indole functionality in the design and synthesis of antihyperlipidemic compounds for the treatment of hyperlipidemia. Some indole derivatives are well known for their diverse pharmacological effects, including a hypolipidemic effect (Al-Qirim *et al*., 2009; Sher and Ellsworth, 2004; Kopin *et al*., 2006). But *N*-(9,10-dihydro-9,10-dioxoanthracenyl)-1*H*-indole-2-carboxamide derivatives have not been investigated as potential lipid-lowering agents, although some studies have revealed the usefulness of these derivatives in the treatment of diabetes, insulin resistance, diabetic neuropathy, diabetic retinopathy, hypertension, and atherosclerosis (Bussolotti and Gammill, 2004), furthermore as selective inhibitors of monoamine oxidases (MAO) (La Regina *et al*., 2008), antiallergics (Robichaud *et al*., 1987), antioxidants (Olgen and Coban, 2002), and inhibitors of human liver glycogen phosphorylase a (HLGPa) (Liu *et al*., 2004; Gammill, 2003).

The present work reveals a class of novel heterocyclic carboxamide compounds as lipid-lowering agents, which are promising candidates for the treatment of hyperlipidemia. This work is a continuation of our previous work which started with the 5-methoxy-indole-2-carboxamide derivatives of aminobenzophenone and aminoanthraquinone (Al-Qirim *et al*., 2009) and their bioisoster benzofuran derivatives (Shattat *et al*., 2010). Therefore, this study aimed to evaluate the possible lipid-lowering activity of novel *N*-(9,10-dihydro-9,10-dioxoanthracenyl)-1*H*-indole-2-carboxamide derivatives.

Materials and Methods

General synthetic procedure

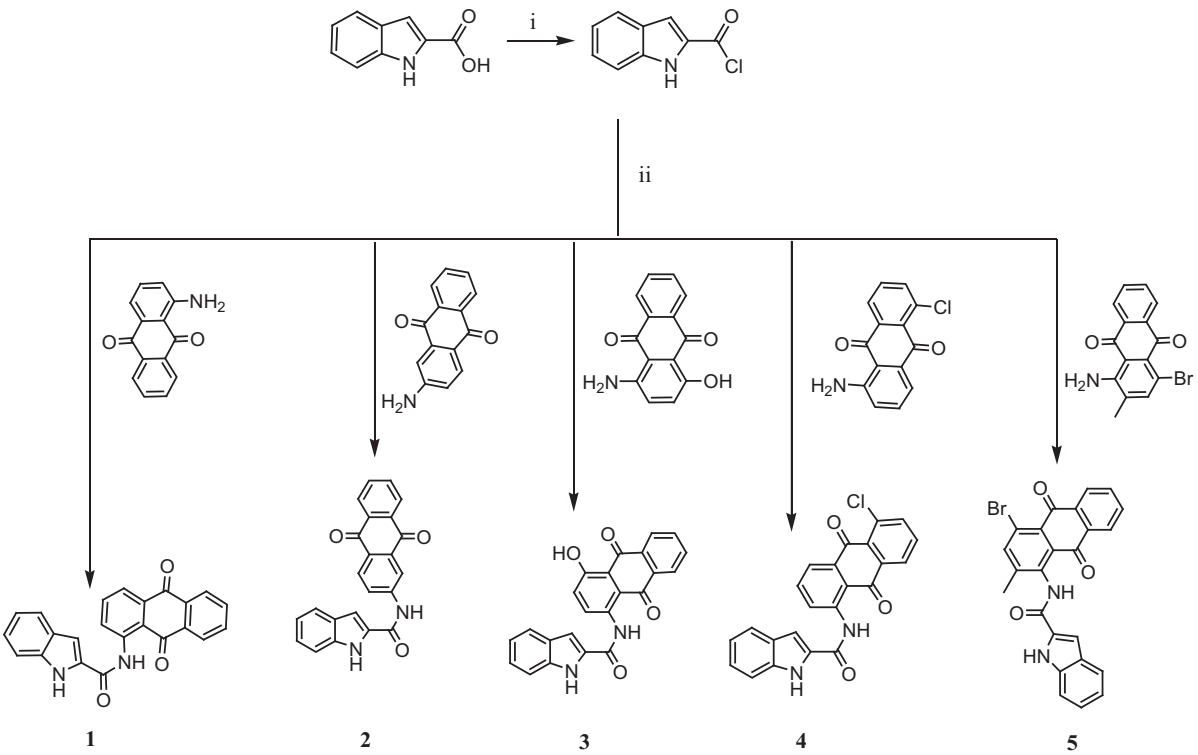
Five novel derivatives of *N*-(9,10-dihydro-9,10-dioxoanthracenyl)-1*H*-indole-2-carboxamide were prepared. They were synthesized by fusion at high temperature, by which indole-2-carbonyl chloride was thoroughly mixed with aminoanthraquinone derivatives and refluxed at 120 °C for 18 h in an air condenser. Then 1,4-dioxane was added and the mixture stirred for additional 24 h at room temperature (Gouda *et al*., 2010; procedure adopted). The resulting products were filtered and recrystallized to give the desired compounds **1** – **5** (Scheme 1). ^{1}H NMR, ^{13}C NMR, IR, elemental, and MS analyses were used for the confirmation of the structures of the target compounds.

Analytical procedures

Melting points were determined in open capillaries using a Stuart scientific electrothermal melting point apparatus (Stone, Staffordshire, UK) and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were collected on a Varian Oxford NMR300 spectrometer (Santa Clara, CA, 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 (HR) mass spectra were measured in the negative 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 part formic acid), and infused using a syringe pump with a flow rate of 2 μl/min. External calibration was done using an arginine cluster in the mass range m/z 175 – 871.

Infrared (IR) spectra were recorded on a Shimadzu 8400F FT-IR spectrophotometer (Kyoto, Japan). The samples were dissolved in $CHCl_3$ and analysed as thin solid films using NaCl plates or KBr discs (Merck, Darmstadt, Germany). Thin-layer chromatography (TLC) was performed on aluminum plates pre-coated with fluorescent silica gel, and the spots were visualized by UV light at 254 and/or 360 nm. Elemental analyses (EA) of C, H, and N were performed using an EuroVector elemental analyzer (Milan, Italy).

N-(9,10-Dihydro-9,10-dioxoanthracen-1-yl)-1H-indole-2-carboxamide (**1**): 1-Aminoanthraquinone (0.40 g, 1.8 mmol) and indole-2-carbonyl chloride (0.40 g, 2.2 mmol) were processed as described above for the general procedure. Recrystallization afforded a brown-green solid (0.30 g, 45%). – M.p. > 350 °C (decomposition). – R_f = 0.59 (cyclohexane/ethyl acetate, 6:4). – ^{1}H NMR ($CDCl_3$): δ = 13.23 (1H, br s, NH-indole), 12.08 (1H, s, NHCO), 9.15 + 9.13 (1H, 2s, Ar-H), 8.50 – 7.76 (5H, m, Ar-H), 7.62 – 6.89 (5H, m, Ar-H). – ^{13}C NMR ($CDCl_3$): δ = 182.60 (CO-ketone), 176.31 (CO-ketone), 163.21 (CONH), 134.37 (CH-Ar), 133.90 (CH-Ar), 132.90, 131.68,



Scheme 1. Preparation of indole-anthraquinone-2-carboxamide derivatives **1** – **5** starting from indole-2-carboxylic acid. (i) $SOCl_2$, 70 – 80 °C, $CHCl_3$; (ii) reflux at 120 °C.

129.37, 128.06 (CH-Ar), 127.69, 127.13, 126.48 (CH-Ar), 125.25, 124.47, 123.85 (CH-Ar), 122.37 (CH-Ar), 121.83, 120.90 (CH-Ar), 118.16 (CH-Ar), 116.20 (CH-Ar), 115.24 (CH-Ar), 112.94 (CH-Ar), 104.18 (CH-Ar). – IR (KBr): $\nu = 3314$ (NH-indole), 3213 (NHCO), 3125, 1667 (br, CO), 1636 (CONH), 1578, 1531, 1412, 1312, 1269, 1238, 1173, 1015, 806, 741, 705 cm^{-1}. – HRMS (ESI, negative mode): $m/z = 365.09317$ [M−H$^+$]. – $C_{23}H_{14}N_2O_3$ (366.09262): calcd. C 75.40, H 3.85, N 7.65; found C 75.19, H 4.01, N 7.96.

N-(9,10-Dihydro-9,10-dioxoanthracen-2-yl)-1H-indole-2-carboxamide (**2**): 2-Aminoanthraquinone (0.45 g, 2.0 mmol) and indole-2-carbonyl chloride (0.50 g, 2.8 mmol) were processed as described above for the general procedure. Recrystallization afforded a pale green solid (0.46 g, 63%). – M.p. > 350 °C (decomposition). – $R_f = 0.41$ (cyclohexane/ethyl acetate, 6:4). – ^{1}H NMR ($CDCl_3$): $\delta = 11.88$ (1H, br s, NH-indole), 10.79 (1H, s, NHCO), 8.68 (1H, d, $J = 3.0$ Hz, Ar-H), 8.39 (1H, d, $J = 9.0$ Hz, Ar-H), 8.31 – 8.11 (3H, m, Ar-H), 8.0 – 7.83 (2H, m, Ar-H), 7.75 (1H, d, $J = 6.0$ Hz, Ar-H), 7.56 (1H, s, Ar-H), 7.46 (1H, d, $J = 6.0$ Hz, Ar-H), 7.37 – 7.03 (2H, m, Ar-H). – ^{13}C NMR ($CDCl_3$): $\delta = 183.0$ (CO-ketone), 181.86 (CO-ketone), 160.67 (CONH), 145.19, 137.65, 135.03 (CH-Ar), 134.68 (CH-Ar), 134.58, 133.67, 131.20, 128.89 (CH-Ar), 128.50, 127.43, 127.21 (CH-Ar), 127.13 (CH-Ar), 124.94 (CH-Ar), 124.77 (CH-Ar), 122.48 (CH-Ar), 120.58 (CH-Ar), 117.21 (CH-Ar), 112.96 (CH-Ar), 105.59 (CH-Ar). – IR (KBr): $\nu = 3402$ (NH-indole), 3321 (NHCO), 3117, 3067, 1667 (br, CO), 1651 (CONH), 1589, 1535, 1416, 1331, 1292, 1238, 954, 840, 755, 710 cm^{-1}. – HRMS (ESI, negative mode): $m/z = 365.09317$ [M−H$^+$]. – $C_{23}H_{14}N_2O_3$ (366.09262): calcd. C 75.40, H 3.85, N 7.65; found C 75.72, H 3.74, N 7.87.

N-(1-Hydroxy-9,10-dihydro-9,10-dioxoanthracen-4-yl)-1H-indole-2-carboxamide (**3**): 1-Amino-4-hydroxy-anthraquinone (0.40 g, 1.7 mmol) and indole-2-carbonyl chloride (0.40 g, 2.2 mmol) were processed as described above for the general procedure. Re-

crystallization afforded a black solid (0.32 g, 49 %). – M.p. > 350 °C (decomposition). – R_f = 0.80 ($CHCl_3$/MeOH/acetic acid, 94:5:1). – IR (KBr): ν = 2500 − 3450 (OH), 3310 (NH-indole), 1659 (br, CO), 1624 (CONH), 1574, 1531, 1501, 1466, 1250, 1165, 1065, 741 cm^{-1}. – HRMS (ESI, negative mode): m/z = 381.08808 [M−H^+]. – $C_{23}H_{14}N_2O_4$ (382.08753): calcd. C 72.52, H 3.69, N 7.33; found C 72.20, H 3.48, N 7.52. – An NMR analysis could not be performed because of solubility problems.

N-(1-Chloro-9,10-dihydro-9,10-dioxoanthracen-5-yl)-1H-indole-2-carboxamide (**4**): 1-Amino-5-chloroanthraquinone (0.45 g, 1.7 mmol) and indole-2-carbonyl chloride (0.45 g, 2.5 mmol) were processed as described above for the general procedure. Recrystallization afforded an orange solid (0.45 g, 65 %). – M.p. > 350 °C (decomposition). – R_f = 0.48 (cyclohexane/ethyl acetate, 6:4). – ^{1}H NMR ($CDCl_3$): δ = 13.03, 12.89 (1H, 2br s, NH-indole), 12.08, 11.72 (1H, 2s, NHCO), 9.12, 9.15 (1H, 2s, Ar-H), 8.38, 8.40 (1H, 2s, Ar-H), 8.21 − 7.78 (5H, m, Ar-H), 7.71 − 7.01 (4H, m, Ar-H). – ^{13}C NMR ($CDCl_3$): δ = 184.21 (CO-ketone), 179.10 (CO-ketone), 174.25 + 169.95 (CONH), 145.40, 138.13, 136.71, 135.10, 132.41, 127.49, 125.94, 125.10, 124.22, 123.77, 122.61, 122.0, 121.85, 121.0, 118.33, 113.21, 104.19. – IR (KBr): ν = 3310 (NH-indole), 3213 (NHCO), 3071, 2986, 1674 (CO), 1636 (CONH), 1578, 1528, 1431, 1412, 1312, 1261, 1022, 806, 745 cm^{-1}. – HRMS (ESI, negative mode): m/z = 399.05419 [M−H^+]. – $C_{23}H_{13}ClN_2O_3$ (400.5364): calcd. C 68.92, H 3.27, N 6.99; found C 69.02, H 3.28, N 7.03.

N-(1-Bromo-3-methyl-9,10-dihydro-9,10-dioxoanthracen-4-yl)-1H-indole-2-carboxamide (**5**): 1-Amino-4-bromo-2-methyl-anthraquinone (0.40 g, 1.3 mmol) and indole-2-carbonyl chloride (0.40 g, 2.2 mmol) were processed as described above for the general procedure. Recrystallization afforded a black solid (90 mg, 16 %). – M.p. > 350 °C (decomposition). – R_f = 0.77 ($CHCl_3$/MeOH/acetic acid, 94:5:1). – ^{1}H NMR ($CDCl_3$): δ = 12.90 (1H, br s, NH-indole), 11.85, 11.79 (1H, 2s, NHCO), 10.64 (1H, s, Ar-H), 8.15 (1H, s, Ar-H), 8.12 − 7.05 (8H, m, Ar-H), 2.38 (3H, s, CH_3). – ^{13}C NMR ($CDCl_3$): δ = 184.41 + 184.34 (CO-ketone), 181.85 + 181.79 (CO-ketone), 176.30, 163.30 + 160.16 (CONH), 142.49, 141.76, 139.04, 134.77, 126.76, 126.46, 125.91, 124.73, 124.42, 122.40, 121.81, 120.55, 120.41, 114.42, 112.90, 107.75, 104.94, 18.99 (CH_3). – IR (KBr): ν = 3287 (NH-indole), 3179 (NHCO), 3063, 2920, 2855, 1678 (br, CO), 1639 (CONH), 1524, 1489, 1458, 1420, 1308, 1258, 1238, 1119, 1076, 872, 818, 748, 718 cm^{-1}. – HRMS (ESI, negative mode): m/z = 457.01933 [M−H^+]. – $C_{24}H_{15}BrN_2O_3$ (456.01878): calcd. C 62.76, H 3.29, N 6.10; found C 62.93, H 3.17, N 6.35.

Pharmacological studies

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

Animals and treatments

Adult male Wistar rats, weighing around 250 g, bred in the animal care centre of the Faculty of Pharmacy, Al-Zaytoonah Private University, Amman, Jordan, were given *ad libitum* access only to tap water throughout the experimental duration (24 h). Rats were maintained in a 12-h light/12-h dark cycle under constant humidity and at (22 ± 2) °C. All experiments had been approved by and were performed in accordance with the guidelines of the Animal Welfare Committee of Al-Zaytoonah Private University.

Triton WR-1339 model of hyperlipidemia

Triton WR-1339 (tyloxapol; Sigma-Aldrich) was dissolved in normal saline (pH 7.4) and administered intraperitoneally (i.p.) to the rats [250 mg/kg body weight (BW)] for induction of hyperlipidemia.

Experimental design

Overnight fasted rats were randomly divided into five groups of six animals each. The first group, serving as a negative normal control group (CG), received an i.p. administration of 1 ml normal saline. The second group, serving as a positive control group (PCG), received an i.p. administration of 4 % DMSO. The third hyperlipidemic group (HG) received an i.p. injection of TritonWR-1339 and was gavaged with 4 % DMSO (in distilled water). In the fourth group, rats were i.p. injected with Triton WR-1339, followed by intragastric administration of 1 ml of a 57-μM solution of the target compounds dissolved in 4 % DMSO. The last group was i.p. injected with Triton WR-1339 and intragastrically treated with bezafibrate (BF) (100 mg/kg BW)

dissolved in 4% DMSO as a positive reference compound (Nakajima *et al*., 2008).

After 8 h of treatment, animals were anaesthetized with diethyl ether, and blood was collected. The blood samples were immediately centrifuged ($1500 \times g$ for 10 min), and the plasma was used for lipid analysis by an enzymatic method with an automatic analyzer (Model Erba XL-300; ERBA Diagnostics, Mannheim, Germany).

Statistical analysis

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

Results

Dose selection

The lethal dose (LD_{50}) of the target compound **1** was determined by oral administration of six different concentrations of the sample (200, 400, 600, 800, 1000, and 1,200 mg/kg BW), to each group of six rats. Survival rates, recorded daily for 3 days (Jia *et al.*, 2001), were 100, 100, 83, 50, 17, and 0%, respectively. The approximate LD_{50} value of 800 mg/kg BW is equivalent to ≈ 200 mg for a rat weighing 250 g.

For assessment of the biological activities, 1 ml of the respective target compound (57 μM in 4% DMSO, corresponding to 0.021 mg/rat of 250 g for compounds **1** and **2**, and 0.022, 0.023, and 0.026 mg/rat of 250 g for compounds **3**, **4**, and **5**, respectively) was administered intragastrically to rats. The positive reference compound bezafibrate was administered at 1 ml of 276 mM based on literature recommendations (Nakajima *et al*., 2008).

Induction of hyperlipidemia by Triton WR-1339

Levels of plasma total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) in all groups treated for 8 h are shown in Table I. Eight h after the induction of hyperlipidemia with Triton WR-1339 at the dose of 250 mg/kg BW (i.p.), there was a marked increase in TC ($p < 0.0001$) and TG concentrations ($p < 0.0001$).

The plasma TC level increased more than two-fold above the normal level, and the TG level more than 11-fold. A significant increase in LDL-C levels ($p < 0.0001$) was also observed in rats treated with Triton WR-1339 alone, while a drastic reduction ($p < 0.0001$) in HDL-C levels was observed in the hyperlipidemic control group (HG) as compared to the normal control group (CG).

*Effect of compounds **1**–**5** and BF on rat plasma lipid profile*

The levels of plasma TC, TG, HDL-C, and LDL-C of the bezafibrate-treated group (BF) and unsubstituted indole-anthraquinone carboxamide compounds-treated rats 8 h after Triton WR-1339 administration are shown in Table I.

Table I. Effect of the novel unsubstituted indole-anthraquinone carboxamides on plasma lipid levels in Triton WR-1339-induced hyperlipidemic rats after 8 h.

Lipid profile	TC [mg/ml]	TG [mg/ml]	HDL-C [mg/ml]	LDL-C [mg/ml]
CG	1.07 ± 0.02	1.27 ± 0.03	0.49 ± 0.01	0.31 ± 0.01
HG	2.23 ± 0.07[c]	15.11 ± 0.22[c]	0.31 ± 0.03[c]	0.63 ± 0.02[c]
C1	1.79 ± 0.05[b]	5.29 ± 0.09[c]	0.53 ± 0.01[c]	0.48 ± 0.01[c]
C2	2.72 ± 0.02	15.23 ± 0.22	0.28 ± 0.03	0.65 ± 0.01
C3	2.28 ± 0.09	4.56 ± 0.23[c]	0.52 ± 0.01[c]	0.45 ± 0.02[b]
C4	1.80 ± 0.02[c]	4.52 ± 0.22[c]	0.52 ± 0.02[b]	0.45 ± 0.02[b]
C5	1.87 ± 0.03[c]	4.41 ± 0.32[c]	0.52 ± 0.01[b]	0.46 ± 0.01[b]
BF	2.18 ± 0.06	5.65 ± 0.18[c]	0.43 ± 0.01[c]	0.63 ± 0.02

CG, normal control group; HG, hyperlipidemic + 4% DMSO control group; C1, compound **1** + 4% DMSO group; C2, compound **2** + 4% DMSO group; C3, compound **3** + 4% DMSO group; C4, compound **4** + 4% DMSO group; C5, compound **5** + 4% DMSO group; BF, bezafibrate + 4% DMSO group; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol.

Values are means ± SEM from six rats in each group. HG is compared to CG. C1, C3, C4, C5, C2, and BF are compared with HG.

[b] $p < 0.001$; [c] $p < 0.0001$.

Cholesterol levels

The plasma TC levels decreased by 20% ($p < 0.001$) in rats treated with C1, and 20% and 17% ($p < 0.0001$) in those treated with C4 and C5, respectively, compared to the hyperlipidemic control group (HG). No significant differences were observed in rats treated with C2, C3, or BF compared to the HG-treated rats.

Triglyceride levels

The elevated plasma TG levels resulting from Triton WR-1339 administration were significantly suppressed by 62, 65, 70, 70, and 71% ($p < 0.0001$) in rats treated with BF, C1, C3, C4, or C5, respectively, compared to the hyperlipidemic control group. No significant difference in the TG level was observed in rats treated with C2.

HDL-C levels

The HDL-C levels were increased by 60% ($p < 0.001$) by C3, C4, and C5, 62% ($p < 0.0001$) by both C1, and 28% ($p < 0.0001$) by BF, respectively, compared to the hyperlipidemic control group. No significant difference in the HDL-C level was observed in response to C2.

LDL-C levels

The elevated plasma LDL-C levels produced by Triton WR-1339 administration were significantly suppressed by 24% ($p < 0.0001$) in rats treated with C1, and 29, 29, and 27% ($p < 0.001$) by those treated with C3, C4, and C5, respectively, compared to the hyperlipidemic control group. No significant differences in LDL-C levels were observed in response to C2 and BF.

In summary, significant decreases in TG and LDL-C and an increase in HDL-C plasma levels were observed with C1, C3, C4, and C5, while only C1, C4, and C5 reduced TC levels as well.

Discussion

The lipid-lowering effect of the novel derivatives **1** to **5** of *N*-(9,10-dihydro-9,10-dioxoanthracenyl)-1*H*-indole-2-carboxamide was tested in Triton WR-1339-induced hyperlipidemic rats, which have been widely used as a model in screening for compounds with a lipid-lowering potential.

The large increase in the plasma TG levels due to Triton WR-1339 administration results mostly from an increase of very low-density lipoprotein (VLDL) secretion by the liver, accompanied by a strong reduction of the catabolism of VLDL and LDL-C (Yamamoto *et al*., 1984).

The reduction of the TC levels by compounds **1**, **4**, and **5** was associated with a decrease of the LDL-C level, which is the target of several hypolipidemic drugs. This suggests that the cholesterol-lowering activity of these compounds may result from the rapid catabolism of LDL-C through its hepatic receptors for final elimination in the form of bile acids (Venkateswarulu *et al*., 2010).

Compounds **1**, **3**, **4**, and **5**, administered at a dose of 1 ml of a 57-μM solution, were able to significantly decrease the serum TG levels. Thus, since the portion of TG in VLDL is many times higher than that of cholesterol, it is not surprising that the hypolipidemic activities of these compounds were significantly higher for TG than for cholesterol. This result suggests that our compounds are able to restore, at least partially, catabolism of apo-B lipoproteins, as hypothesized by many studies with other lipid-lowering agents (Gotto, 2002).

In addition, compounds **1**, **3**, **4**, and **5** increased the level of HDL-C, which is known for its preventive role against atherogenesis. HDL-C plays an important role in facilitating the mobilization of TG and cholesterol from plasma to liver, where it undergoes catabolism and is then eliminated in the form of bile acids (Beck *et al*., 2004).

It has been hypothesized that bezafibrate enhances the catabolism of TG, and this could be due to an increase in the activity of lipoprotein lipase (LPL) (Venkateswarulu *et al*., 2010). The large decrease in the 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-C surface, without loss of lipid (Beyer *et al*., 2008).

Furthermore, several studies revealed that fibrates, like bezafibrate, are not selective agonists of the peroxisomal proliferator-activated receptor-α (PPARα), and that their effects on other isoforms of PPARs are rather complex. Therefore our compounds are more likely to act as selective PPARα agonists (Koyama *et al*., 2005). This interpretation is supported by their differential effect on HDL-C compared to BF, but this needs to be further investigated.

Promisingly, the reduction of the lipid level by compounds **1**, **3**, **4**, and **5** (1 ml of 57 μM) after 8 h of Tri-

ton WR-1339 injection was higher than that induced by bezafibrate (1 ml of 276 mM), which was used in this study as reference hypolipidemic drug. Furthermore, the results of this study show that our compounds did not significantly change the TC levels, which agrees with the mechanism of action of fibrates in that their TC-lowering activity is not strongly marked, while their strong TG-decreasing effect is brought about by the stimulation of LPL expression (Alper *et al.*, 2009).

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