

Synthesis and *In Vivo* Hypolipidemic Effect of Some *N*-(Benzoylphenyl)-Carboxamide Derivatives in Triton WR-1339-Induced Hyperlipidemic Rats

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A series of *N*-(benzoylphenyl)-carboxamide derivatives (**2a**, **2b**, **3a**, **3b**, **4a**, **4b**, **5a**, **5b**, **6a** and **6b**) was prepared with good yields by reacting the corresponding carbonyl chlorides with aminobenzophenones at room temperature. This was followed by evaluating the hypotriglyceridemic and hypocholesterolemic effects of **3b**, **5a** and **5b**. Triton WR-1339 (300 mg/kg) was intraperitoneally administered to overnight-fasted rats to induce hyperlipidemia. Rats were divided into six groups: control, hyperlipidemic, hyperlipidemic plus compounds **3b**, **5a** and **5b** and hyperlipidemic plus bezafibrate. Results showed that after 18 h of treatment at a dose of 15 mg/kg body weight of each of the test compounds, the elevated plasma levels of triglycerides (TG) and total cholesterol (TC) were significantly lowered by compounds **5b** and **3b** ($p < 0.001$) and by **5a** ($p < 0.0001$), compared to the hyperlipidemic control group. Compounds **3b** and **5a** significantly increased levels of high-density lipoprotein cholesterol (HDL-C) by 58 and 71%, respectively. In addition, compounds **3b** and **5a** caused significant reduction ($p < 0.0001$) of low-density lipoprotein cholesterol (LDL-C) levels compared to the control group. These results suggest a promising potential for compounds **3b**, **5a** and **5b** as lipid-lowering agents, which may contribute to reducing the risk of atherosclerosis and cardiovascular disease.

Keywords: *N*-(Benzoylphenyl)-carboxamides. Triton WR-1339-induced hyperlipidemic rats. Anti-hypertriglyceridemic. Anti-hypercholesterolemic.

INTRODUCTION

There is a great deal of experimental and clinical evidence suggesting that hyperlipidemia is currently one of the main risk factors for atherosclerosis and cardiovascular diseases (CVDs) (Saleem *et al.*, 2019). Consequently, decreasing any elevated plasma lipid

levels could be a rational approach to the treatment and prevention of CVDs (Austin, Hokanson, Edwards, 1998; Gingham, Iliescu, 2011; Harb, Bustanji, Abdallah, 2018; Rohilla *et al.*, 2012; Stapleton *et al.*, 2010).

Hyperlipidemia is normally treated with different groups of drugs including fibrates, among which is the well-known commercially available drug bezafibrate. In this context, treatment with fibrates results in a significant decrease in the level of plasma triglycerides (TG) in addition to a modest decrease in low-density lipoprotein (LDL)-cholesterol and an increase in high-density lipoprotein (HDL)-cholesterol (Bkhaitan, 2017; Staels *et al.*, 1998; Stancu, Sima, 2001). Fibrates,

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including bezafibrate, exert their effect through the activation of peroxisome proliferator-activated receptors (PPARs). PPAR- α induces apoA-I and apoA-II and lowers hepatic apoC-III production, which reduces the amounts of circulating triglyceride-rich lipoproteins (Hamadneh *et al.*, 2017; Rubins *et al.*, 1999; Shattat, 2014; Staels *et al.*, 1998). Based on the correlation between hyperlipidemia and heart disease, development of new lipid-lowering agents is considered extremely important to overcome the adverse effects of current medications (Razali *et al.*, 2018). In this respect, attention has been given to research focusing on the synthesis of *N*-(benzoylphenyl)-carboxamide derivatives with substantial antihyperlipidemic activity. In this class of

compounds, studies have demonstrated the importance of a very large lipophilic aromatic ring represented by the benzoylphenyl moiety linked through a carboxamide to various heterocyclic rings such as furan, benzofuran, pyridine and others (Abu Farha *et al.*, 2016; Al-Qirim *et al.*, 2012; Hikmat *et al.*, 2017; Jasim *et al.*, 2018; Shattat *et al.*, 2010).

Figure 1 illustrates the common structural features between most of the proposed compounds (**2-5, a, b**), our previous imidazole derivatives (compounds **I**, (Jasim *et al.*, 2018), and bezafibrate. All these compounds have an aryl carboxamide linkage in their structure; however, compounds **I** and the proposed ones in this work vary in aromatic heterocyclic rings.

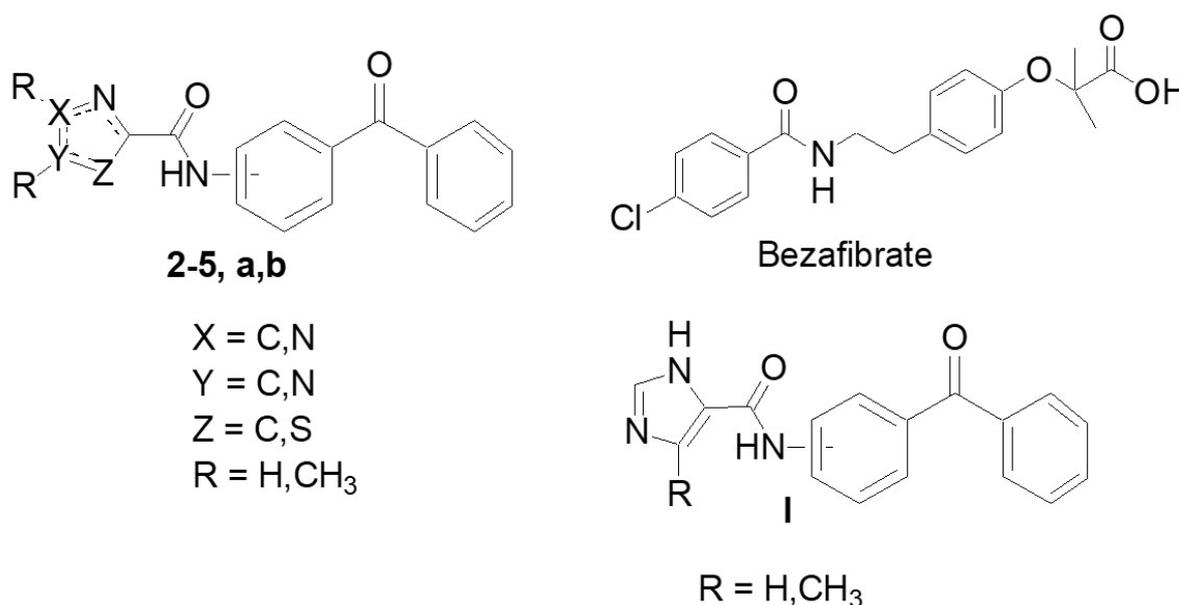


FIGURE 1 - Representative chemical structures of compounds **2-5, (a, b)**, bezafibrate and imidazole derivatives (**I**).

Triton WR-1339 is a globally accepted model for inducing acute hyperlipidemia in animal models to evaluate potential hypolipidemic drugs (Hayashi *et al.*, 1981; Otway, Robinson, 1967). Published research showed that a single intraperitoneal administration of Triton WR-1339 to adult rats produced hyperlipidemia, where levels of cholesterol, triglycerides and phospholipids increased over 20 h and decreased thereafter (Al-Najdawi *et al.*, 2014; Friedman, Byers, 1957; Schurr, Schultz, Parkinson,

1972; Sheikha *et al.*, 2018). Triton WR-1339 causes an elevated plasma lipid profile by inhibiting the uptake of circulating lipoproteins by extrahepatic tissues, resulting in increased blood lipoprotein levels (Da Rocha *et al.*, 2009; Schotz *et al.*, 1957).

Accordingly, in continuation of our work in exploring the effects of different heterocyclic rings on improving the antihyperlipidemic kinetics and efficacy of new *N*-(benzoylphenyl)-carboxamide

derivatives, we report herein on the synthesis of such derivatives containing 1,5-dimethyl-1*H*-pyrazole, 2,5-dimethyloxazole, imidazole, thiazole and 1-methylindole rings. We also evaluated the pharmacological properties of the synthesized compounds on Triton WR-1339-induced hyperlipidemic rats used as a model. In addition, we only analyzed 3 compounds, specifically **3b** and **5a** and **5b**, as potential antihyperlipidemic agents. The selection of the three compounds to test was based on the study of the effect of the size of the heteroatom in the ring and the steric hindrance effect on the biological activity.

MATERIAL AND METHODS

The following chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification: 3-aminobenzophenone 97%, 4-aminobenzophenone 97%, sodium bicarbonate and oxalyl chloride 98%, *N*-methylindole-2-carboxylic acid, 4-imidazolecarboxylic acid, 2,5-dimethyl-1,3-oxazole-4-carbonyl chloride (**8**), 1,3-thiazole-2-carbonyl chloride (**10**) and 1,5-dimethylpyrazole-4-carbonyl chloride (**7**), *N,N*-Dimethylformamide (DMF) (HPLC grade) and triethylamine were obtained from (Tedia). Ethyl acetate, *n*-hexane, acetone, and diethylether were acquired from AZ Chem., whereas chloroform was obtained from Labchem; these solvents were purified by stirring with anhydrous sodium sulfate overnight and then distilled.

NMR spectra were obtained using Bruker-Avance III 500 MHz spectrometers with DMSO-*d*₆ as solvent and TMS as the internal standard. Chemical shifts (δ) are expressed in ppm units. High-resolution mass spectra (HRMS) were acquired (in positive or negative ion mode) using electrospray ion trap (ESI) technique by collision-induced dissociation on a BrukerAPEX-4 (7 Tesla) instrument. Thin layer chromatography (TLC) was performed using Merck aluminum plates pre-coated with silica gel PF254 (20 cm x 20 cm x 0.25 mm), and the plate was examined under a UV lamp ($\lambda = 254$ nm). Melting points were measured with SMP 10 Stuart apparatus and are uncorrected.

Synthesis of the targeted compounds

General procedure for the synthesis of 4-imidazole carbonyl chloride (9) and N-methylindole-2-carbonyl chloride (11)

Oxalyl chloride (20 ml) and 0.05 mL DMF were added to a solution of 4-imidazole carboxylic acid (10 mmol) or *N*-methylindole-2-carboxylic acid (10 mmol) at 0 °C. The mixture was stirred for 45 min and then refluxed for 90 min. After cooling, the excess oxalyl chloride was evaporated under reduced pressure, and the pale brownish residue was washed twice with dry toluene (15 ml) and then used for the next step without further purification.

General procedure for the synthesis of carboxamide derivatives (2-6, a-b)

To a cold stirred solution of a specified quantity of carbonyl chloride in 15 ml of chloroform, a suspended solution of aminobenzophenone and triethylamine (2 ml, 14 mmol), in 5 ml of chloroform was added, and the mixture was stirred for 48 h at room temperature. Next, organic liquids were evaporated under vacuum to dryness. Thereafter, 15 ml of dichloromethane were added, followed by extraction twice with a saturated solution of sodium bicarbonate (10 ml) and water (15 ml), sequentially. The organic layer was dried using anhydrous sodium sulfate and then evaporated under reduced pressure to obtain a crude material, which was purified by silica gel plate chromatography.

***N*-(3-benzoylphenyl)-1,5-dimethyl-1*H*-pyrazole-3-carboxamide (2a)**

Reaction of **7** (1.26 mmol, 0.20 g) with 3-aminobenzophenone (2.48 mmol, 0.49 g) afforded compound **2a** (72% yield, 0.29 g) as a pale yellow solid, m.p. 158–160 °C, chloroform:ethyl acetate (7:3) R_f: 0.58. The product was further purified on silica gel TLC plates using ethyl acetate:ether:hexane (2:3:7 (v/v) as the developing solvent mixture. ¹H-NMR (DMSO-*d*₆): δ 2.26 (s, 3H), 3.79 (s, 3H), 6.52 (s, 1H),

7.39 (m, 1H), 7.45 (d, $J = 8.0$ Hz, 1H), 7.51 (m, 2H), 7.64 (t, $J = 7.5$ Hz, 1H), 7.72 (d, $J = 7.5$ Hz, 2H), 8.05 (d, $J = 8.0$, 1H), 8.24 (s, 1H), 10.19 (s, 1H, NH). $^{13}\text{C-NMR}$ (DMSO- d_6): δ 11.2 (CH₃), 37.1 (CH₃), 106.6 (C), 121.7 (C), 124.6 (C), 124.9 (C), 129.0 (2C), 129.3 (2C), 130.1 (C), 133.1 (C), 137.5 (C), 137.8 (C), 139.7 (C), 141.2 (C), 144.9 (C), 161.0 (CON), 196.2 (CO). HRMS (ESI) m/z : calculated for C₁₉H₁₆N₃O₂ [M – H][–] 318.12425, found 318.12480.

***N*-(4-benzoylphenyl)-1,5-dimethyl-1H-pyrazole-3-carboxamide (2b)**

Reaction of **7** (1.26 mmol, 0.20 g) with 4-aminobenzophenone (2.48 mmol, 0.49 g) yielded compound **2b** (79% yield, 0.32 g) as a pale yellow solid, m.p. 162–165 °C, chloroform:ethyl acetate (7:3) Rf: 0.65. The resulting product was purified on silica gel TLC plates using ethyl acetate as the developing solvent mixture. $^1\text{H-NMR}$ (DMSO- d_6): δ 2.28 (s, 3H), 3.81 (s, 3H), 6.56 (s, 1H), 7.52 (t, $J = 7.3$ Hz, 2H), 7.62 (t, $J = 7.3$ Hz, 2H), 7.67–7.70 (m, 3H), 7.98 (d, $J = 8.3$ Hz, 2H), 10.29 (s, 1H, NH). $^{13}\text{C-NMR}$ (DMSO- d_6): δ 11.2 (CH₃), 37.0 (CH₃), 106.7 (C), 119.7 (2C), 129.0 (2C), 129.8 (2C), 130.5 (C), 131.8 (2C), 132.7 (C), 137.1 (C), 138.0 (C), 141.4 (C), 144.8 (C), 161.0 (CON), 195.1 (CO). HRMS (ESI) m/z : calculated for C₁₉H₁₇N₃O₂Na [M + Na]⁺ 342.12185, found 342.12130.

***N*-(3-benzoylphenyl)-2,5-dimethyloxazole-4-carboxamide (3a)**

Compound **3a** (66% yield, 0.25 g) was obtained as pale yellow solid, m.p. 171–174 °C, chloroform:ethyl acetate (6:2) Rf: 0.87 from the reaction of **8** (1.19 mmol, 0.19 g) with 3-aminobenzophenone (2.38 mmol, 0.47 g) afforded. The product was purified on silica gel TLC plates using chloroform:ether (2: 1 (v/v) as the developing solvent mixture. $^1\text{H-NMR}$ (DMSO- d_6): δ 2.41 (s, 3H), 2.53 (s, 3H), 7.41 (m, 1H), 7.48 (d, $J = 8.0$, 1H), 7.51 (m, 2H), 7.65 (t, $J = 7.5$ Hz, 1H), 7.72 (d, $J = 7.5$ Hz, 2H), 8.05 (d, $J = 8.0$, 1H), 8.27 (s, 1H), 10.21 (s, 1H, NH). $^{13}\text{C-NMR}$ (DMSO- d_6): δ 11.9 (CH₃), 13.7 (CH₃), 121.9 (C), 124.8 (C), 125.2 (C), 129.0 (2C), 129.3 (2C), 129.5 (C), 130.1

(C), 133.2 (C), 137.5 (C), 137.8 (C), 139.2 (C), 153.9 (C), 158.9 (C), 160.8 (CON), 196.1 (CO). HRMS (ESI) m/z : calculated for C₁₉H₁₆N₂O₃Na [M + Na]⁺ 343.10586, found 343.10529.

***N*-(4-benzoylphenyl)-2,5-dimethyloxazole-4-carboxamide (3b)**

Reaction of **8** (1.19 mmol, 0.19 g) with 4-aminobenzophenone (2.38 mmol, 0.47 g) afforded compound **3b** (73% yield, 0.28 g) as a pale yellow solid, m.p. 180–183 °C, chloroform:ethyl acetate (6: 2) Rf: 0.86. The product was separated on silica gel TLC plates using chloroform:ether (2:1 (v/v) as the developing solvent mixture. $^1\text{H-NMR}$ (DMSO- d_6): δ 2.43 (s, 3H, CH₃), 2.56 (s, 3H, CH₃), 7.51 (t, $J = 7.5$ Hz, 2H), 7.62 (t, $J = 7.5$ Hz, 2H), 7.67–7.70 (m, 3H), 8.01 (d, $J = 8.6$ Hz, 2H), 10.29 (s, 1H, NH). $^{13}\text{C-NMR}$ (DMSO- d_6): δ 11.9 (CH₃), 13.7 (CH₃), 120.0 (C₂), 129.2 (2C), 129.3 (C₄), 129.9 (2C), 130.3 (C), 131.3 (2C), 133.0 (C), 137.9 (C), 138.0 (C), 154.3 (C), 159.0 (C), 160.9 (CON), 195.1 (CO). HRMS (ESI) m/z : calculated for C₁₉H₁₆N₂O₃Na [M + Na]⁺ 343.10586, found 343.10531.

***N*-(3-benzoylphenyl)-1H-imidazole-4-carboxamide (4a)**

Reaction of **9** (2.75 mmol, 0.36 g) with 3-aminobenzophenone (5.50 mmol, 1.08 g) yielded compound **4a** (80% yield, 0.64 g) as a pale yellow solid, m.p. 221–223 °C (decom.), acetone:hexane:ethyl acetate (1:4:2) Rf: 0.38. The resulting product was separated on silica gel TLC plates using acetone:hexane:ethyl acetate (6:1:3 (v/v) as the developing solvent mixture. $^1\text{H-NMR}$ (DMSO- d_6): δ 7.38 (m, 1H), 7.47 (d, $J = 8.0$, 1H), 7.54 (m, 2H), 7.65 (t, $J = 7.5$ Hz, 1H), 7.72 (d, $J = 7.5$ Hz, 2H), 7.79 (d, $J = 7.9$, 1H), 8.06 (s, 1H), 8.19 (s, 1H), 8.28 (s, 1H), 10.41 (s, 1H, NH), 12.67 (s, 1H, NH). $^{13}\text{C-NMR}$ (DMSO- d_6): δ 116.1 (C), 121.5 (C), 124.4 (C), 124.7 (C₄), 129.0 (2C), 129.3 (C), 130.1 (C), 133.1 (C), 136.5 (C), 137.6 (C), 137.8 (C), 139.5 (C), 139.7 (C), 160.7 (CON), 196.2 (CO). HRMS (ESI) m/z : calculated for C₁₇H₁₃N₃O₂Na [M + Na]⁺ 314.09055, found 314.09000.

N-(4-benzoylphenyl)-1H-imidazole-4-carboxamide (4b)

Reaction of **9** (2.75 mmol, 0.36 g) with 4-aminobenzophenone (5.50 mmol, 1.08 g) afforded compound **4b** (77% yield, 0.62 g) as pale yellow solid, m.p. 189–191 °C, acetone:hexane:ethyl acetate (1:4:2) Rf: 0.22. The resulting product was separated on silica gel TLC plates using acetone:hexane:ethyl acetate (6:2:3 (v/v) as the developing solvent mixture. ¹H-NMR (DMSO-*d*₆): δ 7.51 (t, *J* = 7.4 Hz, 2H), 7.62 (t, *J* = 7.3 Hz, 2H), 7.68–7.73 (m, 3H), 8.01 (d, *J* = 8.3 Hz, 2H), 8.11 (s, 1H), 8.19 (s, 1H), 10.55 (s, 1H, NH), 12.51 (s, 1H, NH). ¹³C-NMR (DMSO-*d*₆): δ 116.1 (C), 119.6 (2C), 129.0 (2), 129.9 (2C), 130.5 (C), 131.4 (2C), 132.8 (C), 136.6 (C), 137.0 (C), 138.0 (C), 139.4 (C), 160.2 (CON), 195.1 (CO). HRMS (ESI) *m/z*: calculated for C₁₇H₁₃N₃O₂Na [M + Na]⁺ 314.09055, found 314.09000.

N-(3-benzoylphenyl)-thiazole-2-carboxamide (5a)

Reaction of **10** (0.75 mmol, 0.11 g) with 3-aminobenzophenone (1.50 mmol, 0.29 g) afforded compound **5a** (78% yield, 0.18 g) as orange solid, m.p. 181–183 °C, chloroform:hexane:ethyl acetate (6:2:1) Rf: 0.73. The product was further purified on silica gel TLC plates using acetone:hexane:ethyl acetate (6:1:3 (v/v) as the developing solvent mixture. ¹H-NMR (DMSO-*d*₆): δ 7.33 (m, 1H), 7.39 (d, *J* = 8.0 Hz, 1H), 7.50 (m, 2H), 7.61 (t, *J* = 7.5 Hz, 1H), 7.70 (d, *J* = 7.5 Hz, 2H), 8.01 (d, *J* = 8.0 Hz, 1H), 8.14 (s, 1H), 8.20 (s, 1H), 8.29 (s, 1H), 10.92 (s, 1H, NH). ¹³C-NMR (DMSO-*d*₆): δ 121.4 (C), 124.4 (C), 124.6 (C), 129.1 (2C), 129.3 (2C), 130.0 (C), 132.9 (C), 133.1 (C), 137.7 (C), 137.9 (C), 139.8 (C), 144.6 (C), 158.7 (C), 160.7 (CON), 196.1 (CO). HRMS (ESI) *m/z*: calculated for C₁₇H₁₃N₂O₂S [M + H]⁺ 309.06977, found 309.06922.

N-(4-benzoylphenyl)-thiazole-2-carboxamide (5b)

Reaction of **10** (0.75 mmol, 0.11 g) with 4-aminobenzophenone (1.50 mmol, 0.29 g) afforded compound **5b** (74% yield, 0.17 g) as orange solid, m.p. 172–175 °C, chloroform:hexane:ethyl acetate (6:2:1) Rf: 0.65. The resulting product was separated on

silica gel TLC plates using acetone:hexane:ethyl acetate (6:1:3 (v/v) as the developing solvent mixture. ¹H-NMR (DMSO-*d*₆): δ 7.53 (t, *J* = 7.3 Hz, 2H), 7.63 (t, *J* = 7.3 Hz, 2H), 7.69–7.75 (m, 3H), 8.01 (d, *J* = 8.3 Hz, 2H), 8.27 (s, 1H, C), 8.34 (s, 1H, C), 11.12 (s, 1H, NH). ¹³C-NMR (DMSO-*d*₆): δ 120.4 (2C), 129.0 (2C), 129.9 (2C), 130.4 (C), 131.3 (2C), 132.8 (C), 132.9 (C), 137.8 (C), 142.6 (C), 144.6 (C), 158.7 (C), 163.8 (CON), 195.1 (CO). HRMS (ESI) *m/z*: calculated for C₁₇H₁₂N₂O₂Na [M + Na]⁺ 331.05172, found 331.05117.

N-(3-benzoylphenyl)-1-methyl-1H-indole-2-carboxamide (6a)

Reaction of **11** (2.63 mmol, 0.51 g) with 3-aminobenzophenone (5.27 mmol, 1.04 g) gave compound **6a** (67% yield, 0.62 g) as off-white solid, m.p. 161–163 °C, chloroform:hexane:ethyl acetate (6:2:1) Rf: 0.79. The resulting product was separated on silica gel TLC plates using chloroform:hexane:ethyl acetate (6:2:1 (v/v) as the developing solvent mixture. ¹H-NMR (DMSO-*d*₆): δ 3.98 (s, 3H, CH₃), 7.08 (t, *J* = 7.3 Hz, 1H), 7.28 (t, *J* = 7.4 Hz, 1H), 7.34 (s, 1H), 7.43 (t, *J* = 7.2 Hz, 2H), 7.50–7.57 (m, 4H), 7.65–7.68 (m, 2H), 7.74 (d, *J* = 8.3 Hz, 2H), 8.10 (d, *J* = 8.1 Hz, 2H), 8.29 (s, 1H), 10.53 (s, 1H, NH). ¹³C-NMR (DMSO-*d*₆): δ 32.0 (CH₃), 106.5 (C), 111.1 (C), 120.8 (C), 121.5 (C), 121.7 (C), 122.3 (C), 124.5 (C), 124.6 (C), 125.9 (C), 129.1 (2C), 129.5 (2C), 130.1 (C), 132.1 (C), 133.2 (C), 137.5 (C), 137.9 (C), 139.2 (C), 139.7 (C), 161.2 (CON), 196.1 (CO). HRMS (ESI) *m/z*: calculated for C₂₃H₁₈N₂NaO₂ [M + Na]⁺ 377.12600, found 377.12605.

N-(4-benzoylphenyl)-1-methyl-1H-indole-2-carboxamide (6b)

Reaction of **11** (2.63 mmol, 0.51 g) with 4-aminobenzophenone (5.27 mmol, 1.04 g) afforded compound **6b** (74% yield, 0.69 g) as off-white solid, m.p. 151–153 °C, chloroform:hexane:ethyl acetate (6:2:1) Rf: 0.71. The resulting product was separated on silica gel TLC plates using chloroform:hexane:ethyl acetate (6:2:1 (v/v) as the developing solvent mixture. ¹H-NMR (DMSO-*d*₆): δ 3.99 (s, 3H, CH₃), 7.11 (t, *J* =

7.5 Hz, 1H), 7.29 (t, $J = 7.5$ Hz, 1H), 7.37 (s, 1H), 7.53 (t, $J = 7.3$ Hz, 2H), 7.63 (t, $J = 7.3$ Hz, 2H), 7.64-7.69 (m, 3H), 7.76 (d, $J = 8.5$ Hz, 2H), 10.59 (s, 1H, NH). ^{13}C -NMR (DMSO- d_6): δ 32.0 (CH₃), 106.5 (C), 111.1 (C), 120.8 (C), 121.5 (C), 121.7 (C), 122.3 (C), 124.5 (C), 124.6 (C), 125.9 (C), 129.1 (2C), 129.5 (2C), 130.1 (C), 132.1 (C), 133.2 (C), 137.5 (C), 137.9 (C), 139.2 (C), 139.7 (C), 161.2 (CON), 196.1 (CO). HRMS (ESI) m/z : calculated for C₂₃H₁₇N₂O₂ [M – H][–] 353.12900, found 353.12955.

Animals and treatment

For the *in vivo* study, forty-eight 2-month-old male Wistar rats weighing 180–200 g, bred in the animal care center of the Faculty of Pharmacy, Al-Zaytoonah Private University, Amman, Jordan were used throughout this investigation. They were given free access to tap water *ad libitum* throughout the experimental duration (18 h). Rats were housed under standard conditions of 12 h light-dark cycle and under constant relative humidity ($55 \pm 15\%$) and temperature (22 ± 2 °C). All experiments were performed in accordance with the Guidelines for Animal Welfare Committee of Al-Zaytoonah University.

Induction of hyperlipidemia with Triton WR-1339

Acute hyperlipidemia was induced in animals by intraperitoneal administration of Triton WR- 1339 (Sigma-Aldrich, St. Louis, MO, USA) at a dose of 300 mg/kg (Schurr, Schultz, Parkinson, 1972) (Shattat *et al.*, 2013).

Pharmacological experimental design

Rats were fasted overnight and were randomly divided into six groups of eight animals each. The first group, serving as the normal control group (NC), received an intraperitoneal administration of normal saline; the second, hyperlipidemic control group (HC), received an intraperitoneal injection of Triton WR-1339 (300 mg/kg) dissolved in distilled water. Rats in the third, fourth and fifth groups were intraperitoneally injected with Triton, followed by an intragastric administration (15 mg/kg) of compounds **3a**, **5a** and **5b**, respectively. In the last group (BF), rats were also intraperitoneally injected with Triton

and intragastrically treated with bezafibrate (100 mg/kg) (Mori *et al.*, 2004; Nakajima *et al.*, 2009). After 18 h of Triton administration, animals were anesthetized, and blood was collected from the renal artery. The blood samples were immediately centrifuged (3000 rpm for 10 min) and the plasma was used for lipid analysis using an enzymatic method with an automatic analyzer (Model Erba XL-300, Mannheim, Germany).

Statistical analysis

All antihyperlipidemic activity data are presented as the mean \pm standard error of the mean (SEM) ($n = 8$). The data were analyzed using Student's t-test, and $p < 0.05$ was considered statistically significant (SPSS version 2015, IBM SPSS Statistics).

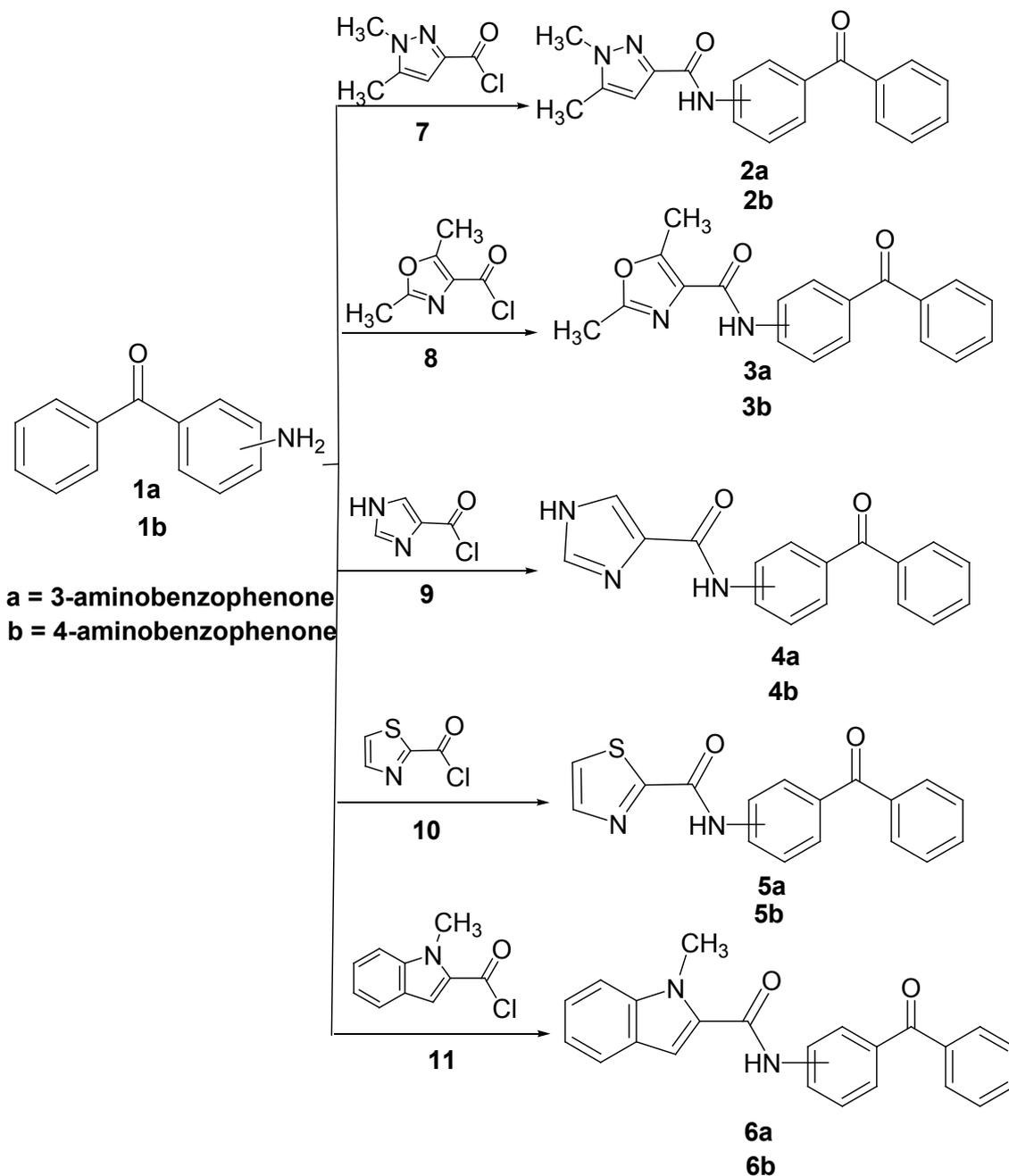
RESULTS AND DISCUSSION

Chemistry

New carboxamide derivatives of heterocyclic compounds (**2–6 a, b**) were prepared in good yield (Scheme 1). 4-Imidazole carbonyl chlorides (**9**) and 1-methylindole-2-carbonyl chloride (**11**) were synthesized by reaction of their carboxylic acids with oxalyl chloride under reflux, whereas other carbonyl chloride derivatives were obtained from commercial sources. A large amount of oxalyl chloride was used since it acts as a solvent and chlorinating agent in the presence of one drop of DMF as catalyst. The synthesis of products **2–6 a, b** was conducted by coupling of the corresponding carbonyl compound with aminobenzophenones, which are weak nucleophiles due to the delocalization of a lone pair of electrons (due to the resonance) at the nitrogen atom. To overcome this problem, triethylamine (weak organic base) was added to substitute the chlorine atom in carbonyl chlorides with trimethylamine, which would carry a positive charge *via* the nitrogen atom and consequently enhance the electrophilicity of the carbon atom of the carbonyl group. In addition, it enhances the nucleophilicity of the aromatic amine by abstracting a proton to attack the carbonyl group to give the products and triethylammonium chloride. It is worth

mentioning that the present experimental procedure has advantages over a previously published one in terms of yield and reaction conditions for preparing compounds **4a** and **4b** (Jasim *et al.*, 2018). In addition, compound **2a** was originally prepared by converting 1,5-dimethyl-1*H*-pyrazole-3-carboxylic acid to the corresponding anhydride using polymer-bound 1-ethyl-

3-[3-(dimethylamino)-propyl]carbodiimide, followed by reaction with 3-aminobenzophenone (Parlow, Mischke, Woodard 1997). Progress of the reaction was monitored by TLC, which is based on the disappearance of the heterocyclic acyl chloride spot (limiting reactant). All target products were well-characterized by means of HRMS and NMR (^1H , ^{13}C , DEPT) techniques.



Scheme 1 - Synthesis of the target compounds 2-6 (a, b)

Lipid-lowering activity

Induction of hyperlipidemia with Triton WR-1339

Shown in Figure 2 are the results pertaining to the plasma levels of TC, low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C) and triglycerides (TG) of all groups treated for 18 h. Triton WR-1339 caused a significant elevation

in plasma TC, TG and LDL-C ($p < 0.0001$) levels in the HC group in comparison with the NC group ($n = 8$). Our results showed that the increase in plasma TC concentration in HC was 122% as compared to NC, whereas the LDL-C level in HC was elevated by 73% as compared to NC. At the same time, the TG level in HC was increased by 791%, while a significant ($p < 0.0001$) decrease of 45% in the HDL-C level occurred after Triton WR-1339 injection.

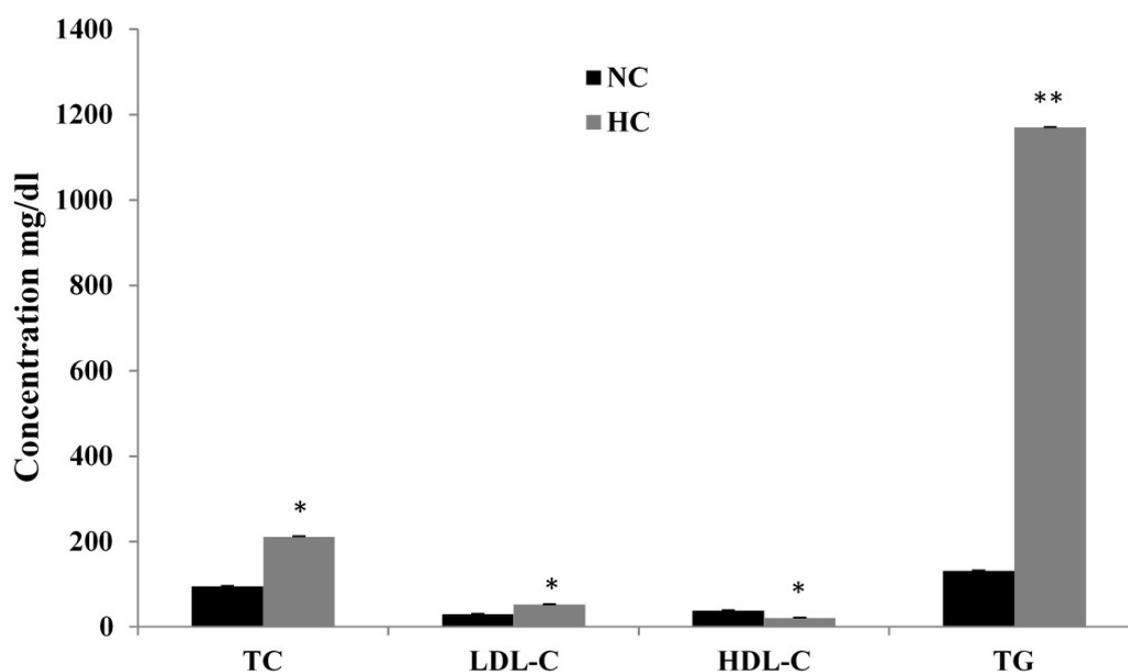


FIGURE 2 - Effect of Triton WR-1339 on plasma lipid levels after 18 h. Values are presented as the mean \pm SEM ($n = 8$) in each group. NC, normal control group; HC, hyperlipidemic control group; TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; TG, triglycerides; HC is compared to NC. * $p < 0.001$, ** $p < 0.0001$.

Effect of compounds **3b**, **5a**, **5b** and bezafibrate on rats' plasma lipid profile

The effect of compounds **3b**, **5a**, **5b** and **BF** on plasma lipid levels (TC, LDL-C, HDL-C and TG) of Triton WR-1339-treated rats after 18 h are shown in

Table 1. The results revealed that the elevated plasma TG levels produced by Triton WR-1339 administration were significantly ($p < 0.0001$) reduced by 77, 88, 68 and 23% ($p < 0.001$) in rats treated with compound **3b**, **5a**, **BF** and **5b**, respectively, as compared to the HC group.

TABLE I - Results of plasma lipid levels for compounds **3b**, **5a**, **5b** and **BF** in Triton WR 1339 induced hyperlipidemic rats after 18 h

Compounds	TC [mg/dl]	LDL-C [mg/dl]	HDL-C[mg/dl]	TG [mg/dl]
NC	95.0 ± 2.6	30.0 ± 1.0	38.0 ± 1.6	131.0 ± 6.0
HC	211.1 ± 3.2*	52.1 ± 0.5*	21.0 ± 0.3*	1170.1 ± 4.2**
3b	97.2 ± 2.1**	42.2 ± 0.8**	33.2 ± 0.4**	150.2 ± 2.2**
5a	92.1 ± 2.0**	36.4 ± 0.8**	36.0 ± 1.1**	135.2 ± 3.3**
5b	157.3 ± 4.0*	46.1 ± 1.0	23.6 ± 0.5	899.0 ± 5.0*
BF	221.3 ± 2.2	48.6 ± 2.0	36.3 ± 0.9*	371.0 ± 4.5**

Values are means ± SEM ($n=8$) in each group. NC: normal control group; HC: hyperlipidemic control group. TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglyceride. HC is compared to NC; **3b**, **5a**, **5b** and **BF** are compared with HC.

* $p < 0.001$, ** $p < 0.0001$.

Values are expressed as the mean ± standard error of the mean (SEM) ($n = 8$, to get representative results in terms of statistical analysis) in each group. NC, normal control group; HC, hyperlipidemic control group; **3b**, compound **3b**; **5a**, compound **5a**; **5b**, compound **5b**; **BF**, bezafibrate; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides. **3b**, **5a**, **5b** and **BF** are compared with HC ^a $p < 0.001$, ^b $p < 0.0001$.

After Triton administration, TC levels were significantly ($p < 0.0001$) decreased by compounds **3b** and **5a** by 54 and 56%, respectively, and reduced by 25% by **5b** ($p < 0.001$). Compounds **3b** and **5a** significantly reduced the elevated LDL-C levels by 19 and 30% ($p < 0.0001$) compared to HC-treated rats (Table I). No significant differences in LDL-C levels were observed with compounds **5b** and **BF** compared to HC-treated rats.

All tested compounds, except compound **5b**, significantly increased HDL-C levels. In fact, compounds **3b** and **5a** and **BF** significantly elevated HDL-C levels by 58, 71 and 73%, respectively ($p < 0.0001$), as compared to HC. To be more specific, the order of the hypolipidemic activity was **5a** > **3b** > **5b**.

In this Investigation, we determined the antihyperlipidemic activity of aminobenzophenone thiazole derivatives and aminobenzophenone oxazole

derivative. Our findings demonstrated that the replacement of the oxygen atom in the nucleus ring with sulfur significantly affected the biological activity of the compounds. Previous works have shown the importance of the heteroaromatic ring for biological activity. We suggested that the heteroaromatic ring interacts with the proper target(s) through hydrogen bonding. Our results confirmed that the heterocyclic ring plays a critical role in activity. Our results also showed that compound **5a** is more active than **5b** even though both contain the aminothiazole ring, but they differ in the position of the benzophenone carboxamide linkage. Apparently, the para derivative, compound **5b**, allows alignment extension of the molecule with the proper target in relation to the different orientation obtained from the meta isomer **5a**. Therefore, the distance between the heteroatoms in the nucleus and the points of interaction in the proper target would be affected. In case of compound **5a**, the meta derivative allows orientation of the nitrogen atom in the thiazole nucleus to accept an H-bond while the para derivative should not allow an exact orientation to fit with the target losing the feature of H-bond acceptance.

On the other hand, the activity of dimethylloxazole benzophenone carboxamide derivative (compound **3b**) would not be affected by the influence of the position of the benzophenone carboxamide linkage, as

both heteroatoms of the ring are capable of hydrogen bond interaction with the proper target(s). The effect of the dimethyl groups shows no significant variation in biological activity in comparison with substituted heteroaromatic rings in previous published works, which support our findings pertaining to the chemical nature of the heterocyclic ring.

In addition, it is obvious that the presence of the two methyl groups in the oxazole ring did not provide the expected benefit to the compound, producing almost the same efficacy as compound **5a**. Moreover, the results clearly showed that a small heterocyclic ring provides the same, if not better, pharmacological activity as bigger and more substituted heterocyclic derivatives. Additionally, our results confirmed that bioisosteric replacement is possible and potentially useful in the benzoylphenyl carboxamide class. As has been previously reported, benzoylphenyl carboxamides containing a substituted and unsubstituted imidazole ring reduce TG levels by 54–65% (Jasim *et al.*, 2018), whereas benzoylphenyl carboxamides containing a furan ring reduce TG by almost by 70% (Hikmat *et al.*, 2017). On the other hand, benzoylphenyl carboxamides containing an indole moiety reduced triglycerides by almost 70% and benzoylphenyl carboxamides containing 5-fluoro-1H-indol reduced triglycerides by almost 90%. (Al-Najdawi *et al.*, 2014; Hamadneh *et al.*, 2017). All these findings confirm that the nature of the heterocyclic ring attached to the benzoylphenyl carboxamide moiety is essential for antihyperlipidemic activity.

The observed increase in plasma TG in Triton-treated rats was mainly due to an increase in the secretion of very-low density lipoproteins (VLDL) by the liver, in addition to a reduction of VLDL and LDL catabolism. Furthermore, the significant decrease in plasma HDL-C levels in a Triton-treated animal results mostly from movement of the apo A-1 protein from the HDL surface (Friedman, Byers, 1957). Accordingly, while most of the lipids in VLDL are TG and while cholesterol represents a minor proportion of VLDL, it is not surprising that the anti-hypertriglyceridemic activity of compounds **3b**, **5a** and **5b** was significantly higher than the anti-hypercholesterolemic activity. This observation suggests that our compounds are able to restore, at least partially,

the catabolism of β -lipoproteins as hypothesized by many researchers with other antihyperlipidemic agents (Pérez *et al.*, 2000, 1999)

CONCLUSIONS

In summary, new *N*-(benzoylphenyl)-carboxamide derivatives were successfully prepared and characterized using NMR and HRMS data. The lipid-lowering activities of compounds **3b**, **5a** and **5b** were evaluated using Triton WR-1339-induced hyperlipidemic rats. Interestingly, compounds **3b**, **5a** and **5b** caused improvement in the lipid profile such as reducing hypercholesterolemia and hypertriglyceridemia. At the same time, compounds **3b** and **5a** significantly elevated HDL levels in Triton-induced hyperlipidemic rats, suggesting that these compounds could be possible candidates in the management of patients with lipid abnormalities. These findings are consistent with our previous published data, which confirm that compounds possessing *N*-(benzoylphenyl)-carboxamide nuclei linked to the heterocyclic core have antihyperlipidemic activity. The results of this study and previous ones are very encouraging and advance the need to determine the agents with the best pharmacokinetic profile to move ahead with their clinical application.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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