***Regular Article***

# Synthesis and *in Vivo* Lipid-Lowering Activity of Novel Imidazoles-5-carboxamide Derivatives in Triton-WR-1339-Induced Hyperlipidemic Wistar Rats

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**A new series of imidazole-5-carboxamide derivatives were prepared and tested for their anti-hyperlipidemic activity in Triton-WR-1339-induced hyperlipidemic Wistar rats. The purpose of this research was to improve benzophenone carboxamides water solubility maintaining at the same time the antihyperlipidemic activity. Compounds 4, 6, 10, and 11 were synthesized through a coupling reaction between imidazoles-5-carbonyl chloride and amino benzophenones. The tested animals ($n$=48) were divided into six groups: the first group (hyperlipidemic control group; HCG) received an intraperitoneal injection (i.p.) of (300 mg/kg) Triton WR-1339. The second group received i.p. injection of Triton WR-1339 followed by an intra-gastric administration of bezafibrate (100 mg/kg) (bezafibrate; BF). The third, fourth, fifth, and sixth groups received i.p. injection of Triton WR-1339 followed by an intra-gastric administration of (30 mg/kg) of compounds 4, 6, 10, and 11, respectively. At a dose of 30 mg/kg body weight compounds 4, 6, 10, and 11 significantly ($p$<0.0001) decreased the plasma level of triglyceride (TG), low-density lipoprotein (LDL) and total cholesterol (TC) levels after 18 h of treatment. Additionally, compounds 4, 6, 11 and bezafibrate (100 mg/kg) significantly ($p$<0.0001) increased the plasma level of high-density lipoprotein (HDL) levels, which is known for its preventive role against atherogenesis. These results demonstrate the possibility of pharmacokinetic properties improvement maintaining the biological and pharmacological profile of these compounds.**

**Key words** hypolipidemic activity; imidazolecarboxamide; Triton WR-1339; cardiovascular disease

Cardiovascular diseases (CVDs) are considered among the primary causes of pathological conditions leading to death.[1)] Hyperlipidemia plays a central role by being one of the main risk factors of CVDs.[2)] Various carboxamide derivatives containing different aromatic heterocyclic rings were documented to possess appreciable hypolipidemic effect and thus are intensively investigated for the treatment of hyperlipidemia[3,4)]

Triton WR-1339-induced hyperlipidemic rats model is used as an animal model to examine potential lipid-lowering agents. Triton WR-1339 (a nonionic surfactant) causes a significant increase in plasma lipids by inhibiting the uptake of lipoprotein from the circulation by extrahepatic tissues, producing an increase in the levels of circulatory lipoproteins, this effect lasts 48 h subsequently to Triton administration.[5,6)]

Our previous published data has shown that many of the synthesized carboxamide derivatives exhibited noticeable antihyperlipidemic activity.[7–12)]

It was postulated that the hypolipidemic activity of the carboxamides is correlated with the presence of three essential parts: a large lipophilic moiety, an aromatic carboxamide linker along with a heterocyclic ring.[11)]

Although the significant biological activity, almost all earlier developed carboxamides suffered from water solubility deficiencies hindering biological testing and affecting negatively pharmacological activity. These observed deficiencies inspired the design and synthesis of novel compounds with improved water solubility profile. In a first attempt there was the design of benzimidazole-2-carboxamide derivatives that showed improved water solubility over all previous derivatives providing both feasible pharmacological testing due to easier solubilization and comparable pharmacological profile,[13)] this has led to the introduction of the imidazole-5-carboxamides possessing an imidazole ring which is characterized by a low p$K_a$ value (p$K_a$=6.9) in comparison with the p$K_a$ values of the previous aromatic heterocyclic rings (Benzimidazole p$K_a$=16.4, indole p$K_a$=21, furan p$K_a$=35.6, and thiophene p$K_a$=33) present on the previously published carboxamide derivatives[6–10,12–14)] (Fig. 1). In the present study, the aromatic heterocyclic nucleus was replaced with imidazole and 4-methylimidazole nuclei to enhance both water solubility and pharmacological activity.

## Experimental

**Chemistry** All chemicals, reagents, and solvents used in this experiment were available commercially and of analytical grade. They were used directly without extra purification: dimethylformamide (DMF), chloroform, methyl alcohol, absolute ethanol, triethylamine (TEA), *n*-hexane, ethyl acetate, and glacial acetic acid were purchased from (Fisher Scientific, U.K.). Oxalyl chloride, sodium hydroxide (NaOH), sodium hydride (NaH), 3-aminobenzophenone, 4-aminobenzophenone, ethyl-4-methyl-5-imidazole carboxylate, 1*H*-imidazole-5-carboxylic acid, and Triton WR-1339 were purchased from

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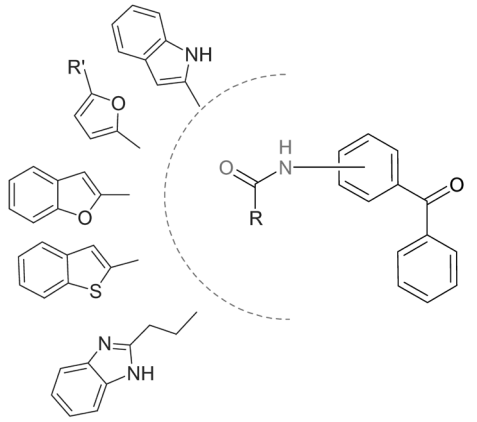


Fig. 1. Benzophenone Carboxamide Derivatives

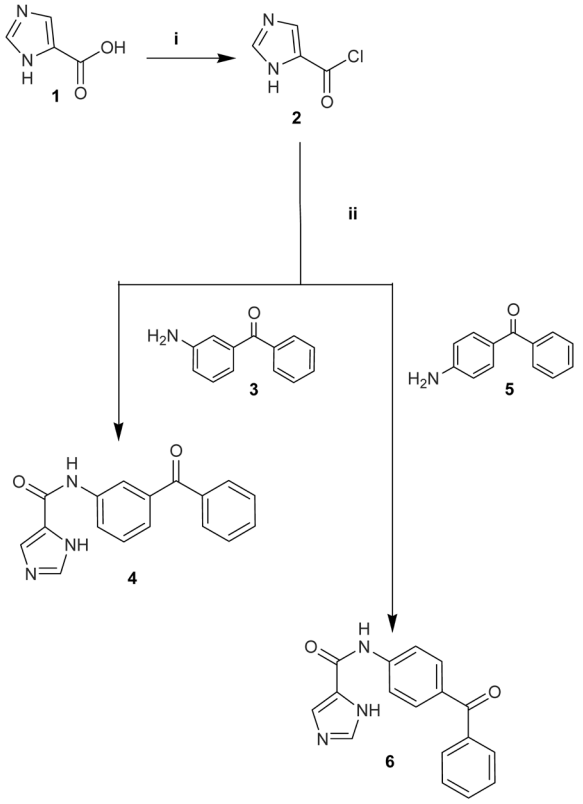
(Sigma-Aldrich, St. Louis, MO, U.S.A.). NMR spectra were executed in The Hashemite University using BRUKER Ascend 300. Chemical shifts are recorded in ppm in correlation to tetramethylsilane (TMS), internal standard. Deuterated dimethyl sulfoxide (DMSO-$d_6$) was used as a solvent for all derivatives unless mentioned otherwise. IR spectra were recorded using Shimadzu 8400 FT-IR spectrophotometer (Japan) at Al Zayotoonah Private University. All tested compounds were mixed with potassium bromide (KBr) and compressed into thin film discs (Acros, Belgium). The melting points (mp) were measured using Gallenkamp melting point apparatus (Gallenkamp, U.K.). TLC was performed on 20×20 cm aluminum plates pre-coated fluorescent silica gel GF254 (ALBET, Germany), the TLC was visualized under UV lamp,, Model CX-20 (U.S.A.), at 254 and/or 360 nm rotavapor model R-114 (Buchi, Switzerland) was used for the efficient removal of solvents from the samples.

**Synthesis of the Targeted Compounds**

*N*-(3-Benzoylphenyl)-1*H*-imidazole-5-carboxamide (**4**)

1-*H* Imidazole-5-carboxylic acid (**1**, 0.5 g, 4.5 mmol) was added to (7.4 g, 58.0 mmol) of oxalyl chloride at room temperature, followed by addition of few drops of DMF. The mixture was refluxed under stirring for 90 min and then cooled to room temperature. Removal of the solvent by evaporation was achieved to give 0.7 g of 1*H*-imidazole-5-carbonyl chloride (**2**) as a yellow solid.

Next, NaH (0.1 g, 4.6 mmol) was added to 1*H*-imidazole-5-carbonyl chloride (**2**, 0.6 g, 4.6 mmol) and 3-aminobenzophenone (**3**, 1.1 g, 5.5 mmol). The reaction mixture was dissolved in DMF and stirred for 18 h at 90°C (Chart 1). The formed precipitate was removed by filtration, then TEA was added to the filtrate to neutralize and to remove any excess of HCl. The resulted filtrate was evaporated to dryness under reduced pressure and the crude mixture was purified by using column chromatography on silica gel started with *n*-hexane–ethyl acetate (70 : 30) and gradually increased polarity until *n*-hexane–ethyl acetate (40 : 60) was reached to give the title compound (**4**) as a beige powder (0.16 g, 12%); mp 216°C with decomposition; *Rf*=0.27 in (chloroform–methanol, 95 : 5); $^1$H-NMR (400 Hz, DMSO-$d_6$) $\delta$: 11.21 (s, 1H, NH-imidazole), 9.00 (s, 1H, NH-amide), 8.65 (s, 1H, imidazole-H), 8.31 (s, 1H, imidazole-H), 8.25 (s, 1H, Ar-H), 8.16 (d, *J*=5.55 Hz, 1H, Ar-H), 7.77 (d, *J*=5.10 Hz, 2H, Ar-H), 7.69 (t, *J*=5.54 Hz, 1H, Ar-H), 7.54 (m, 3H, Ar-H), 7.48 (d, *J*=5.76 Hz, 1H, Ar-H) ppm; $^{13}$C-NMR (DMSO-$d_6$) $\delta$: 195.9 (1C), 160.1 (1C), 157.2 (1C), 139.0 (1C), 137.9 (1C), 137.4 (1C), 136.5 (1C), 133.2 (1C), 130.1 (2C), 129.5 (1C), 129.0 (2C), 125.6 (1C), 124.6 (1C), 122.3 (1C), 121.6 (1C) ppm; IR (KBr disc): $v$=3240.41 (NH-amide), 1674.21 (CO-ketone), 1589.39 (CO-amide) cm$^{-1}$.

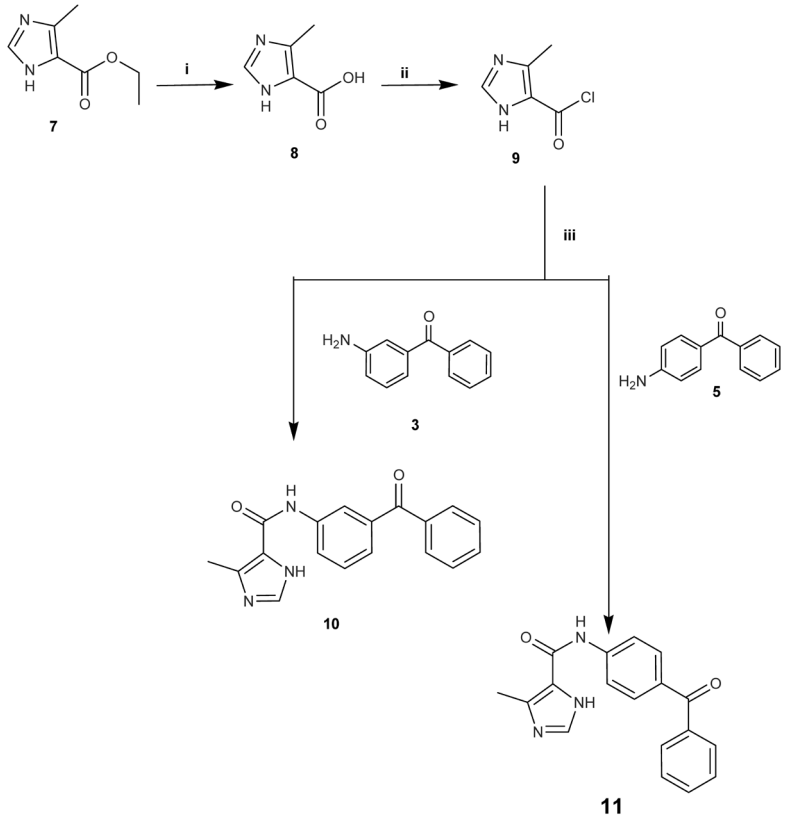


(i) Oxalyl chloride, (ii) DMF, 90°C, 18 h.

Chart 1. Synthesis of Imidazole Carboxamide Benzophenone Derivatives **4** and **6**

*N*-(4-Benzoylphenyl)-1*H*-imidazole-5-carboxamide (**6**)

NaH (0.1 g, 4.6 mmol) was added to 1*H*-imidazole-5-carbonyl chloride (**3**, 0.6 g, 4.6 mmol) which was prepared in the previous step, and 4-aminobenzophenone (**5**, 1.1 g, 5.5 mmol). The reaction mixture was dissolved in DMF and stirred for 18 h at 90°C (Chart 1). The formed precipitate was removed by filtration, then TEA was added to the filtrate to neutralize and to remove any excess of HCl. The resulted filtrate was evaporated to dryness under reduced pressure and the crude mixture was purified by using column chromatography on silica gel started with *n*-hexane–ethyl acetate (70 : 30) and gradually increased polarity until *n*-hexane–ethyl acetate (40 : 60) was reached to give the desired compound (**6**) as a pale orange powder (0.26 g, 19%); mp 180°C; *Rf*=0.25 in (chloroform–methanol, 95 : 5); $^1$H-NMR (400 Hz, DMSO-$d_6$) $\delta$: 12.72 (s, 1H, NH-imidazole), 10.23 (s, 1H, NH-amide), 8.05 (d, *J*=6.12 Hz, 2H, Ar-H), 7.88 (s, 1H, imidazole-H), 7.86 (s, 1H, imidazole-H), 7.73 (m, 4H, Ar-H), 7.66 (t, *J*=4.68 Hz, 1H, Ar-H), 7.56 (t, *J*=5.60 Hz, 2H, Ar-H) ppm; $^{13}$C-NMR (DMSO-$d_6$) $\delta$: 195.0 (1C), 161.7 (1C), 143.8 (1C), 138.0 (1C), 136.4 (1C), 132.6 (1C), 131.6 (1C), 131.4 (2C), 129.8 (3C), 128.9 (2C), 121.4 (1C), 119.5 (2C) ppm; IR (KBr disc): $v$=3610.74 (NH-amide), 1674.21 (CO-ketone), 1589.34 (CO-amide) cm$^{-1}$.




(i) NaOH 2 N, (ii) oxalyl chloride, (iii) DMF, 90°C, 18 h.

Chart 2. Synthesis of 4-Methylimidazole Carboxamide Benzophenone Derivatives **10** and **11**

*N*-(3-Benzoylphenyl)-4-methyl-1*H*-imidazole-5-carboxamide (**10**)

Hydrolysis of ethyl-4-methyl-1*H*-imidazole carboxylate (**7**, 3.0 g, 19.5 mmol) was carried out in a basic medium of freshly prepared 2 M NaOH solution for 3 h. Neutralization with glacial acetic acid was performed after the completion of the reaction. The aqueous solvent was evaporated under reduced pressure and 10 mL of absolute ethanol was used to re-dissolve the white solid product. The reaction mixture was filtered off and the precipitate was dissolved several times with absolute ethanol and co-evaporation was performed to remove water traces. 4-Methyl-1*H*-imidazole-5-carboxylic acid (**8**) was obtained as a white solid with a yield of 87.8%.

Next, the activation of 4-methyl-1*H*-imidazole-5-carboxylic acid (**8**, 2.1 g, 17.1 mmol) was achieved by adding (19.6 g, 232 mmol) of oxalyl chloride at room temperature, followed by addition of 1 drop of DMF. The mixture was stirred and refluxed for 90 min, then cooled to room temperature, followed by solvent removal by evaporation, 4-methyl-1*H*-imidazole-5-carbonyl chloride (**9**) was produced with a yield of 95%. (0.8 g, 5.5 mmol).

Finally, a mixture of 4-methyl-1*H*-imidazole-5-carbonyl chloride **9** with (0.1 g, 5.5 mmol) of NaH and (**3**, 1.3 g, 6.6 mmol) of 3-aminobenzophenone were dissolved in DMF and stirred for 24 h at 90°C (Chart 2). After the removal of the precipitate by filtration, the filtrate was neutralized with TEA to remove any excess HCl, concentrated to dryness by evaporation under reduced pressure. The crude mixture was purified by using column chromatography on silica gel started with *n*-hexane–ethyl acetate (70 : 30) and gradually increased polarity until *n*-hexane–ethyl acetate (40 : 60) was reached to afford the targeted compound (**10**) as a pale brown powder (0.4 g, 24%); mp 178°C; *Rf*=0.30 in (chloroform–methanol, 95 : 5); $^{1}$H-NMR (400 Hz, DMSO-$d_6$) $\delta$: 12.46 (s, 1H, NH-imidazole), 10.00 (s, 1H, NH-amide), 8.34 (s, 1H, imidazole-H), 8.05 (d, *J*=6.12 Hz, 1H, Ar-H), 7.76 (d, *J*=5.28 Hz, 2H, Ar-H), 7.68 (m, 2H, Ar-H), 7.54 (m, 3H, Ar-H), 7.39 (d, *J*=5.76 Hz, 1H, Ar-H), 2.49 (s, 3H, $CH_3$) ppm; $^{13}$C-NMR (DMSO-$d_6$) $\delta$: 196.2 (1C), 162.7 (1C), 139.8 (1C), 137.8 (1C), 137.5 (1C), 133.9 (1C), 133.1 (1C), 132.7 (1C), 130.2 (1C), 130.1 (2C), 129.2 (1C), 129.0 (2C), 124.5 (1C), 124.2 (1C), 121.1 (1C), 11.1 (1C) ppm; IR (KBr disc): $\nu$=3340.71 (NH-amide), 1666.5 (CO-ketone), 1589.34 (CO-amide) cm$^{-1}$.

*N*-(4-Benzoylphenyl)-4-methyl-1*H*-imidazole-5-carboxamide (**11**)

Hydrolysis of ethyl-4-methyl-1*H*-imidazole carboxylate (**7**, 3.0 g, 19.5 mmol) was carried out in a basic medium of freshly prepared 2 M NaOH solution for 3 h. Neutralization with glacial acetic acid was performed after completion of the reaction. Water solvent was evaporated under reduced pressure. 10 mL of absolute ethanol was used to re-dissolve the white solid product. The reaction was filtered off and the precipitate was evaporated several times with absolute ethanol to remove water traces. White solid of 4-methyl-1*H*-imidazole-5-carboxylic acid (**8**) with a yield of 87.8% was produced.

Next, the activation of 4-methyl-1*H*-imidazole-5-carboxylic acid (**8**, 2.1 g, 17.1 mmol) by adding (19.6 g, 232 mmol) of oxalyl chloride at room temperature, followed by 1 drop DMF. The mixture was stirred and refluxed for 90 min, then cooled to room temperature, following removal of the solvent by

evaporation, 4-methyl-1*H*-imidazole-5-carbonyl chloride (**9**) was produced with a yield of 95%.

Finally, a mixture of (**9**, 0.8 g, 5.5 mmol) of 4-methyl-1*H*-imidazole-5-carbonyl chloride with (0.1 g, 5.5 mmol) of NaH and (**4**, 1.3 g, 6.6 mmol) of 4-aminobenzophenone were dissolved in DMF and stirred for 24 h at 90°C (Chart 2). Following the removal of the precipitate by filtration, the filtrate was neutralized with TEA to remove any excess of HCl, concentrated to dryness by evaporation under reduced pressure, then, the crude mixture was purified by using column chromatography on silica gel started with *n*-hexane–ethyl acetate (70 : 30) and gradually increased polarity until *n*-hexane–ethyl acetate (40 : 60) was reached to give the final compound (**11**) as a pale orange powder (0.3 g, 16%); mp 163°C; *Rf*=0.35 in mobile phase (chloroform–methanol, 95 : 5); $^{1}$H-NMR (400 Hz, DMSO-$d_6$) $\delta$: 12.51 (s, 1H, NH-imidazole), 10.09 (s, 1H, NH-amide), 8.04 (d, $J$=6.57 Hz, 2H, Ar-H), 7.74 (s, 1H, imidazole-H), 7.71 (m, 4H, Ar-H), 7.64 (t, $J$=4.68 Hz, 1H, Ar-H), 7.56 (t, $J$=5.76 Hz, 2H, Ar-H), 2.50 (s, 3H, $CH_3$) ppm; $^{13}$C-NMR (DMSO-$d_6$) $\delta$: 195.0 (1C), 162.7 (1C), 143.9 (1C), 138.1 (1C), 134.0 (1C), 133.2 (1C), 132.6 (1C), 131.4 (2C), 130.1 (1C), 129.8 (3C), 128.9 (2C), 119.2 (2C), 11.1 (1C) ppm; IR (KBr disc): $v$=3363.86 (NH-amide), 1681.93 (CO-ketone), 1627.92 (CO-amide) cm$^{-1}$.

**Water Solubility Test** Ten milligrams from each of compounds **4**, **6**, **10**, and **11** were dissolved separately in 5 mL of distilled water. All tested compounds showed complete dissolving forming clear solutions. The same test was performed on indole, and benzothiophene carboxamide benzophenone derivatives[9,11] that showed only partial dissolution forming turbid solutions, particularly the benzothiophene derivatives.

**Pharmacology**

Animals and Treatment

For *in vivo* study, forty-eight male Wistar rats, weighing around 180–200 g, bred, cared in the animal house of Faculty of Pharmacy, Al-Zaytoonah University of Jordan, Amman, Jordan, were accessed only to tap water throughout the experimental duration (18 h). Rats were maintained in a 12 h light–dark cycle under constant humidity and (22±02°C). All animal experiments were carried out in accordance with the guidelines of Animal Welfare Committee of the University.

Induction of Hyperlipidemia by Triton WR-1339

To screen the hypolipidemic effect of natural or chemical drugs, Triton WR-1339 (Sigma-Aldrich, St. Louis, MO, U.S.A.) has been widely used to induce acute hyperlipidemia in animal models in doses ranging from 200 to 400 mg/kg.[15] In this study a dose of 300 mg/kg of Triton WR-1339 dissolved in water was given intraperitoneally to the rats.[16]

Pharmacological Experimental Design

Overnight forty-eight fasted rats were randomly divided into six groups each consisting of eight animals. The first group served as hyperlipidemic control group (HCG) receiving an intraperitoneal injection of (300 mg/kg body weight (BW)) Triton WR-1339 dissolved in distilled water. The second group was the standard control group (BF) and received an intraperitoneal injection of Triton WR-1339 followed by an intra-gastric administration of bezafibrate (100 mg/kg BW) dissolved in 4% DMSO/corn oil.[17,18] The third, fourth, fifth, and sixth groups received an intraperitoneal injection of Triton WR-1339 followed by an intra-gastric administration of (30 mg/kg BW) of compounds **4**, **6**, **10**, and **11** dissolved in 4% DMSO/corn oil respectively. After 18 h. of Triton administration, animals were anesthetized with diethyl ether and blood was collected from the renal artery. The blood samples were immediately centrifuged (3000 rpm for 10 min) and the serum was used for lipid analysis using the relevant profile kits for total cholesterol (TC), high density lipoprotein (HDL), TG, and low density lipoprotein (LDL) were measured directly using commercially available enzymatic colorimetric assay kits by the automatic analyzer (Model Erba XL-300, Mannheim, Germany) at Al-Zaytoonah University of Jordan.

**Toxicity Study Test** Forty-eight normal rats were divided into eight groups (6 animals per each group). The first, second, third and fourth groups received an intra-gastric administration of (30 mg/kg BW) of compounds **4**, **6**, **10** and **11** dissolved in 4% DMSO/corn oil respectively. While, the fifth, sixth, seventh and eighth groups received an intra-gastric administration of the double initial dose (60 mg/kg BW) of the same compounds. The tested animals were left under observation for three weeks. None of the tested animals died even the ones who received the double dose.

**Statistical Analysis** The results were expressed as mean±standard error of the mean (S.E.M.). Statistical analysis was carried out by Student's *t*-test, using Graph Pad Prism software version 7.00 (2016). $p$ values $<0.05$ were considered as statistically significant.

## Results

**Synthesis** Activation of both 1*H*-imidazole-5-carboxylic acid (**1**) and 4-methyl-1*H*-imidazole-5-carboxylic acid (**8**) in to the acyl chloride was obtained by using oxalyl chloride in excess in the presence of few drops of DMF. The mixture was refluxed under stirring for 90 min to give the corresponding imidazole carbonyl chloride (Charts 1, 2) according to a procedure reported by Berger.[19] A solution of different amino benzophenones in DMF was then added and stirred for 24 h at 90°C. The amide formation was reached reacting 1*H*-imidazole-5-carbonyl chloride with various amines as adapted by Seo and Chang.[20] The corresponding amides were then isolated by column chromatography and characterized by using IR, $^{1}$H-NMR, and $^{13}$C-NMR.

**Lipid-Lowering Activity**

Acute Induction of Hyperlipidemia by Triton WR-1339 Model

In comparison with the normal control group (NCG) tested animals ($n$=8), which received an intraperitoneal injection of normal saline, Triton WR-1339 caused a significant decrease in plasma HDL (26%, $p<0.001$) and a significant increase in TG, LDL, and TC (2053, 203, 245%, respectively, $p<0.0001$) levels in hyperlipidemic control group (HCG) after 18 h of Triton WR-1339 (300 mg/kg single dose) administration (Fig. 2).

Effects of Novel Imidazole Carboxamide Derivatives and Bezafibrate (BF) on Plasma Lipid Levels

The effects of novel imidazole carboxamide derivatives **4**, **6**, **10**, **11**, and BF on plasma lipid levels (TG, HDL, LDL, and TC) on Triton WR 1339 treated rats after 18 h. were reported (Table 1). Results indicate that rats treated with 30 mg/kg of the tested compounds **4**, **6**, **10**, and **11** suppressed significantly Triton WR- 1339-induced elevation in TG levels. Notably, the elevated plasma TG levels after a single injection of Triton WR-1339 administration were significantly ($p<0.0001$) reduced in compounds **4**, **6**, **10**, **11**, and BF by 80, 86, 73, 79,

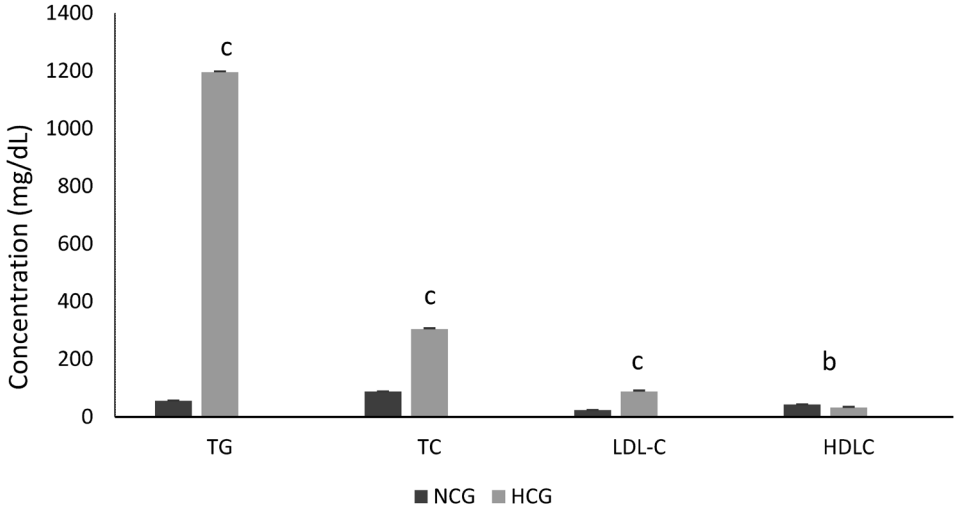



Fig. 2. Effect of Triton WR-1339 on Plasma Lipid Profile after 18 h

Values are means±S.E.M. from eight animals in each group. NCG, control group; HCG, hyperlipidemic control group, TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol. HCG is compared to NCG. [a]$p<0.01$, [b]$p<0.001$, [c]$p<0.0001$.

Table 1. Effect of the Novel Compounds **4**, **6**, **10**, **11** and Bezafibrate on Plasma Lipid Levels in Triton WR-1339-Induced Hyperlipidemic Rats after 18 h

| Groups | Lipid profile | | | |
|---|---|---|---|---|
| | TG (mg/dL) | TC (mg/dL) | LDL-C (mg/dL) | HDLC (mg/dL) |
| HCG | 1195.0±17.46 | 304.0±2.64[c] | 88.1±2.93[c] | 31.4±1.21[c] |
| BF | 411.0±3.00[c] | 235.0±2.00[a] | 52.0±1.60[b] | 56.0±5.00[c] |
| **4** | 234.7±12.30[c] | 105.3±10.65[c] | 37.4±5.35[c] | 57.6±9.11[c] |
| **6** | 163.0±17.00[c] | 109.0±10.00[c] | 34.7±7.67[c] | 49.1±11.50[c] |
| **10** | 322.5±38.50[c] | 133.5±0.50[c] | 44.2±6.50[c] | 37.8±0.70[a] |
| **11** | 249.5±2.50[c] | 140.5±28.50[c] | 51.1±8.35[b] | 48.6±7.60[c] |

Values are means±S.E.M. ($n=8$ in each group). HCG: hyperlipidemic control group; 4: compound **4**; 6: compound **6**; 10: compound **10**; 11: compound **11**; BF: bezafibrate; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; compounds **4**, **6**, **10**, **11** and BF are compared to HCG. [a]$p<0.01$, [b]$p<0.001$, [c]$p<0.0001$.

and 66%, respectively, compared to HCG (Table 1).

After 18 h. of Triton administration TC levels were significantly ($p<0.0001$) reduced in compounds **4**, **6**, **10**, **11** by 65, 64, 56, 54%, respectively, and reduced by 23% in BF ($p<0.01$). All tested compounds significantly reduced elevated LDL levels. In fact, compounds **4**, **6**, **10** significantly reduced LDL levels by (58, 61, 50%, respectively, $p<0.0001$) and by (42, 41%, $p<0.001$) in compound **11** and BF, respectively, compared to HCG.

The HDL-C levels were significantly increased after 18 h of Triton administration, +83, +56, +55, +78% ($p<0.0001$), and +20% ($p<0.01$) in compounds **4**, **6**, **11**, BF, and **10**, respectively (Table 1).

## Discussion

In the current study, the lipid-lowering activity of four novel imidazole-5-carboxamide derivatives, compounds **4**, **6**, **10** and **11**, was tested using Triton WR-1339-treated hyperlipidemic rats, a method that has been widely used as a model for screening the lipid-lowering potential.

As reported in the literature, the maximum plasma TC and TG levels were reached after 20 h. of Triton WR-1339 treatment followed by a decline to the standard values. In our study, the results of tested compounds were analyzed after 18 h. of treatment and Triton WR-1339 model gave similar pattern in lipid profile changes compared to published data[21–23] (Table 1).

All novel imidazole carboxamide benzophenone derivatives **4**, **6**, **10**, and **11** were found to possess significant anti-hyperlipidemic activity. They were found to produce a statistically significant decrease in TG, LDL, and TC levels after 18 h. of Triton WR-1339 administration. In addition, all tested compounds resulted in a significant increase in HDL levels, which is known for its preventive role against atherogenesis, compared to the hyperlipidemic group. The resulting anti-hyperlipidemia was due to the effects of the tested compounds **4**, **6**, **10**, and **11** on the hyperlipidemic rats and not to toxic effect of the above tested compounds as shown from the toxicity study test performed on the normal rats. All prepared compounds have shown comparable or even better activity than bezafibrate.

This could be explained by the fact that most of the overexpressed genes of potential interest by Triton WR-1339 including Apoc3, Apob, Hmgcs2, Apoa1, Apoe, Apof, acs11, and Decr1, are downregulated by the carboxamide derivatives producing significant decrease in Apoc3, Apob, Acaa2, Acs11, and Slc247a5 gene expression levels as shown by a previous study.[24]

The biological activity of compounds **4**, **6**, **10**, and **11** is surly maintained in the presence of the imidazole or methylimidazole groups attached to the benzophenone moiety through a carboxamide linkage. These findings are in accordance with formerly published data.[11] In addition, the replacement of the heterocyclic aromatic rings with imidazole nucleus has led to compounds with improved water solubility in comparison with those already reported in the literature[7–10,12–15] as shown in the water solubility study test. The enhanced water solubility possibly contributes to the pharmacological activity improvement.

These promising results indicate a good potential for the new series of imidazole-5-carboxamides (compounds **4**, **6**, **10**, and **11**) as anti-hyperlipidemic agents that may contribute to atherosclerosis risk reduction.

## Conclusion

Differently substituted carboxamide benzophenones demonstrated appreciable anti-hyperlipidemic activity, lowering TC, TG, and LDL as well as increasing HDL for all tested compounds, however, the biological evaluation was sometimes hindered by erratic water-solubility. This problem was addressed first by the introduction of benzimidazole-2-carboxamide derivatives, and now by even more water-soluble imidazole-5-carboxamide derivatives. Both these series, and in particular, the imidazole series demonstrated improved water solubility maintaining and sometimes even improving the pharmacological profile.

These results demonstrate the possibility of pharmacokinetic properties improvement maintaining the biological and pharmacological properties of carboxamide benzophenones as potential antihyperlipidemic agents.

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**Conflict of Interest** The authors declare no conflict of interest.

**Supplementary Materials** The online version of this article contains supplementary materials.

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