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Full Paper

Pharmacological Evaluation of Novel Isonicotinic Carboxamide Derivatives as Potential Anti-Hyperlipidemic and Antioxidant Agents

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Hyperlipidemia and oxidative stress have been implicated as contributing factors to the development of atherosclerosis and cardiovascular diseases (CVDs). Currently, a large number of antihyperlipidemic medications are conveniently available in the market. Nonetheless, the majority of antihyperlipidemics lack the desired safety and efficacy. Thus, the present study was undertaken to evaluate the potential effect of novel *N*-(benzoylphenyl)pyridine-4-carboxamide and *N*-(9,10-dioxo-9,10-dihydroanthracenyl)pyridine-4-carboxamide derivatives in controlling hyperlipidemia and oxidative stress using the Triton WR-1339-induced hyperlipidemic rat model for antihyperlipidemic activity and the DPPH radical scavenging assay for antioxidant activity. This study revealed the antihyperlipidemic activities of some of the newly synthesized, novel carboxamide derivatives, mainly **C4** and **C12** ($p < 0.05$). The majority of the compounds displayed a relatively low or no DPPH radical scavenging effect, with **C20** possessing the best radical scavenging effect (22%) among all. This research opens the door for new potential antihyperlipidemic compounds derived from isonicotinic acid. *N*-(3-Benzoylphenyl)pyridine-4-carboxamide (**C4**) was found to have promising lipid-lowering and antioxidant effects, which may create a protective effect against CVDs, by reducing the LDL-C levels and diminishing the generation of reactive oxygen species.

Keywords: Antihyperlipidemic / Antioxidant / Isonicotinic acid / Triton WR-1339

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Introduction

Hyperlipidemias have been implicated as contributing factors for the development of atherosclerosis and cardiovascular diseases (CVDs) [1, 2]. Elevated blood total cholesterol (TC), elevated low-density lipoprotein cholesterol (LDL-C), and elevated triglyceride (TG) levels have been reported by the World Health Organization (WHO) to contribute to approximately 56% of coronary heart diseases (CHDs) and 18% of cerebrovascular diseases worldwide, which consequently lead to 4.4 million deaths each year [3].

The oxidative modification hypothesis of atherosclerosis predicts that LDL-C particles must be oxidized before they become a contributing factor for atherosclerosis [4–6]. LDL-C particles oxidation and retention in the subendothelial space of blood vessel is considered to be the initiating factor of atherosclerotic lesion development [4–6]. Thus, in the development of a new novel antihyperlipidmic agent, an important area of interest is to search for an agent with antioxidant activity that will protect endothelial and myocardial functions and may serve as a better anti-atherosclerotic agent.

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Currently, a large number of antihyperlipidemic medications are conveniently available in the market. Nonetheless, the majority of antihyperlipidemics lack the desired safety and efficacy, which limits their long-term use. Although nicotinic acid has been historically used for its lipid-lowering and antioxidant effect to prevent cardiovascular diseases and atherosclerosis, the associated multiple side effects have marred its popularity [7–11]. Therefore, tremendous efforts were employed to synthesize and evaluate different nicotinic acid derivatives for the treatment of hyperlipidemia with improved anti-atherogenic effect and with less adverse events [12–16].

Previously, our laboratory has reported the synthesis and evaluation for antihyperlipidemic activity of nicotinic acid derivatives, *N*-(benzoylphenyl)pyridine-3-carboxamide analogs [16]. In this study, isonicotinic acid (which is an isomer of nicotinic acid) was used as our starting point to synthesize novel isonicotinic carboxamide derivatives bearing the benzophenone unit of the fenofibrate drug (Fig. 1), as well as to synthesize other carboxamide derivatives bearing the anthraquinone unit to increase the rigidity of the compounds. The potential lipid-lowering and antioxidant effects of these compounds were evaluated using Triton WR-1339 hyperlipidemic rat model and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, respectively. We are proposing that this combination may produce a promising candidate for the treatment of hyperlipidemia with potentially higher antioxidant effect.

Results

Chemistry

In this study, the target carboxamide derivatives were prepared using the acid chloride pathway, which gave relatively good yields (range 27.7–58.5%) (Schemes 1 and 2) [17]. Isonicotinic acid (**C1**) was treated with $SOCl_2$ in dry benzene, and the mixture was refluxed at 70°C for 48 h till the formation of the corresponding isonicotinyl chloride (**C2**). Excess $SOCl_2$ and benzene were then distilled out from the reaction mixture. Next, aminobenzophenone or aminoanthraquinone amine was added to the acid chloride (**C2**) and the reaction mixture was mixed and refluxed over 18 h at 70°C, followed by the addition of 1,4-dioxane as a solvent and the reaction mixture was stirred for additional 24 h at room temperature. Finally, the final product was precipitated by the addition of crushed ice water, which was then filtered to afford the corresponding amide. Structure elucidation and confirmation of the target compounds was performed using ^{1}H-NMR, ^{13}C-NMR, IR, and HRMS analysis.

Biology

Induction of hyperlipidemia in experimental rats

As shown in Fig. 2, induction of hyperlipidemia in experimental rats (hyperlipidemic group, HG) using a single dose of 300 mg/kg Triton WR-1339 showed significant increase in serum levels of LDL-C, TG, and TC compared with control group (CG) after 18 h of Triton WR-1339 administration ($p < 0.05$). These results were consistent with previously published literature on the suitability of using this model in the evaluation of hypolipidemic effect of different chemical and natural compounds [18–21].

Acute toxicity study and selection of the experimental dose

Based on the previous acute toxicity study for a related compounds (*N*-(benzoylphenyl)pyridine-3-carboxamide derivatives), doses of 20–100 mg/kg were considered to be safe for the evaluation of antihyperlipidemic effect [16]. In this study, a dose–response curve was established at five different doses for **C6** compound (20, 30, 50, and 100 mg/kg) to evaluate the potential antihypelipidemic effect of each dose. Results showed that LDL-C, TC, and TG were significantly suppressed compared to Triton WR-1339 group at all doses except for the 20 mg/dL

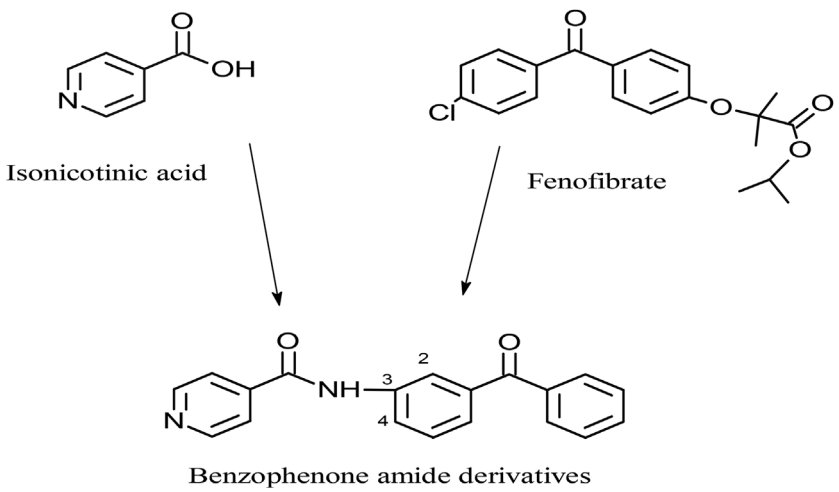

Figure 1. Schematic representation of the rationale of the study.

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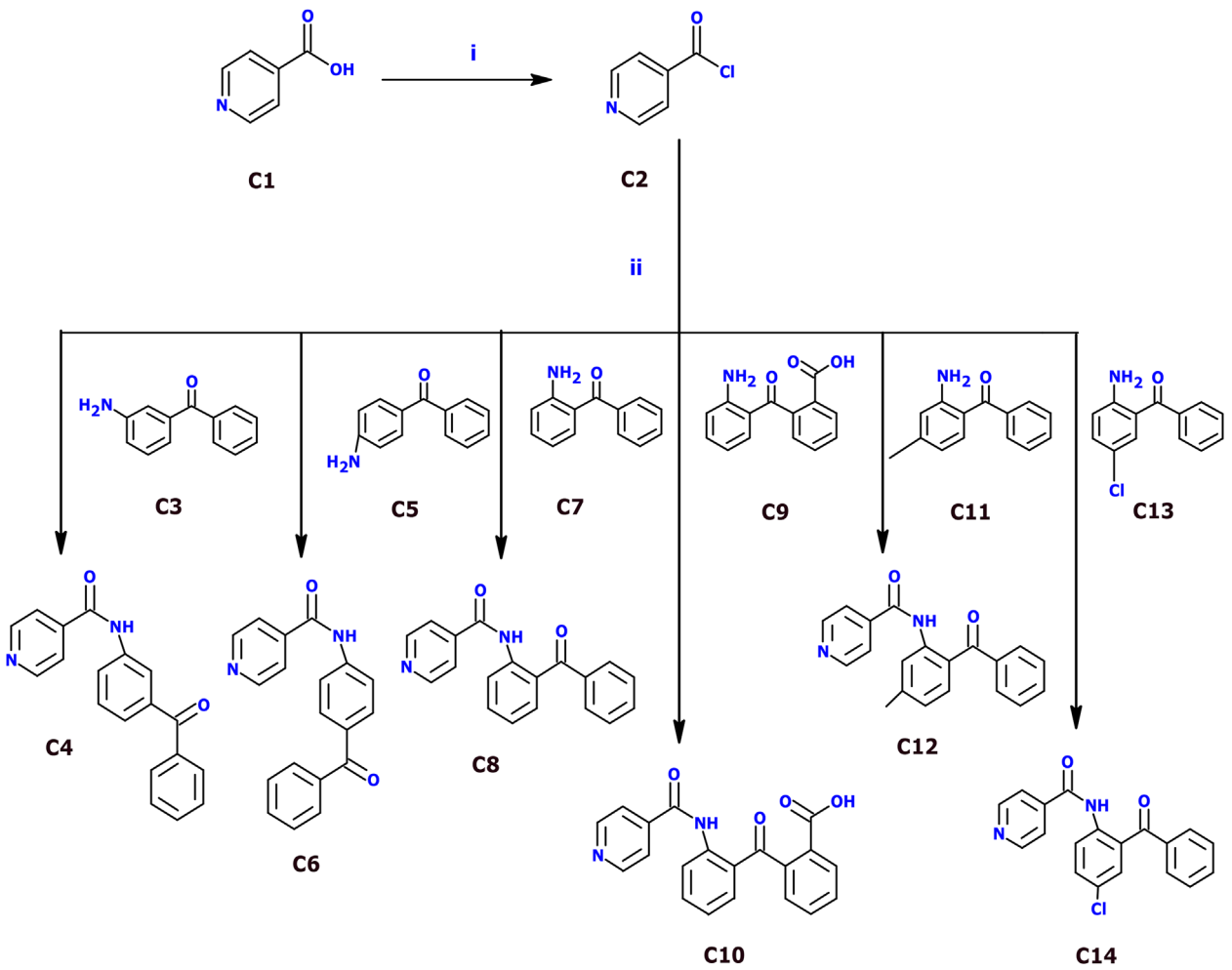

Scheme 1. Preparation of *N*-(benzoylphenyl)pyridine-4-carboxamide derivatives (**C4**, **C6**, **C8**, **C10**, **C12**, and **C14**): (i) $SOCl_2$, dry benzene, refluxed at 70°C for 48 h, distillation to get rid of excess $SOCl_2$ and dry benzene; (ii) refluxed at 70°C for 18 h, followed by the addition of 1,4-dioxan and stirring for 24 h.

($p < 0.05$). So a dose of 30 mg/kg was considered to be suitable for the assessment of antihyperlipidemic activity for all compounds.

Evaluation of antihyperlipidemic effect of novel isonicotinic carboxamide derivatives

The effects of pretreatment with 30 mg/kg of newly synthesized novel *N*-(benzoylphenyl)pyridine-4-carboxamide derivatives (**C4**, **C6**, **C8**, **C10**, **C12**, and **C14**), *N*-(9,10-dioxo-9,10-dihydroanthracenyl)pyridine-4-carboxamide derivatives (**C16**, **C18**, and **C20**), 30 mg/kg nicotinic acid, and 65 mg/kg fenofibrate on serum lipid components are presented in Table 1.

At the recommended doses of nicotinic acid (30 mg/kg) and fenofibrate (65 mg/kg) [22–25], nicotinic acid did not show any significant differences in TG or TC compared to the HG ($p \geq 0.05$), while fenofibrate showed a significant suppression in the elevated plasma level of both TG and TC compared to the HG ($p < 0.05$), which is consistent with its reported effects [26].

The effect of acute administration of Triton WR-1339 on the elevation of serum LDL-C level was evident with the coadministration of **C8**, **C10**, **C14**, **C16**, **C18**, and **C20**, while pretreatment with **C4**, **C6**, and **C12** was associated with lower LDL-C values (38.7 ± 2.8, 124.2 ± 15.1, and 34.6 ± 3.1 mg/dL respectively) compared to the HG (202.6 ± 8.7 mg/dL) ($p < 0.05$). **C4** and **C12** were the most potent among them.

TG levels were significantly suppressed in a similar pattern to that of LDL-C by **C4**, **C6**, and **C12** (78.5 ± 4.0, 1899.8 ± 148.3, and 49.3 ± 4.2 mg/dL, respectively) compared to the HG (2317.8 ± 13.7 mg/dL) ($p < 0.05$). While **C8**, **C10**, **C14**, **C16**, **C18**, and **C20** showed almost similar elevation in TG serum level compared to the HG.

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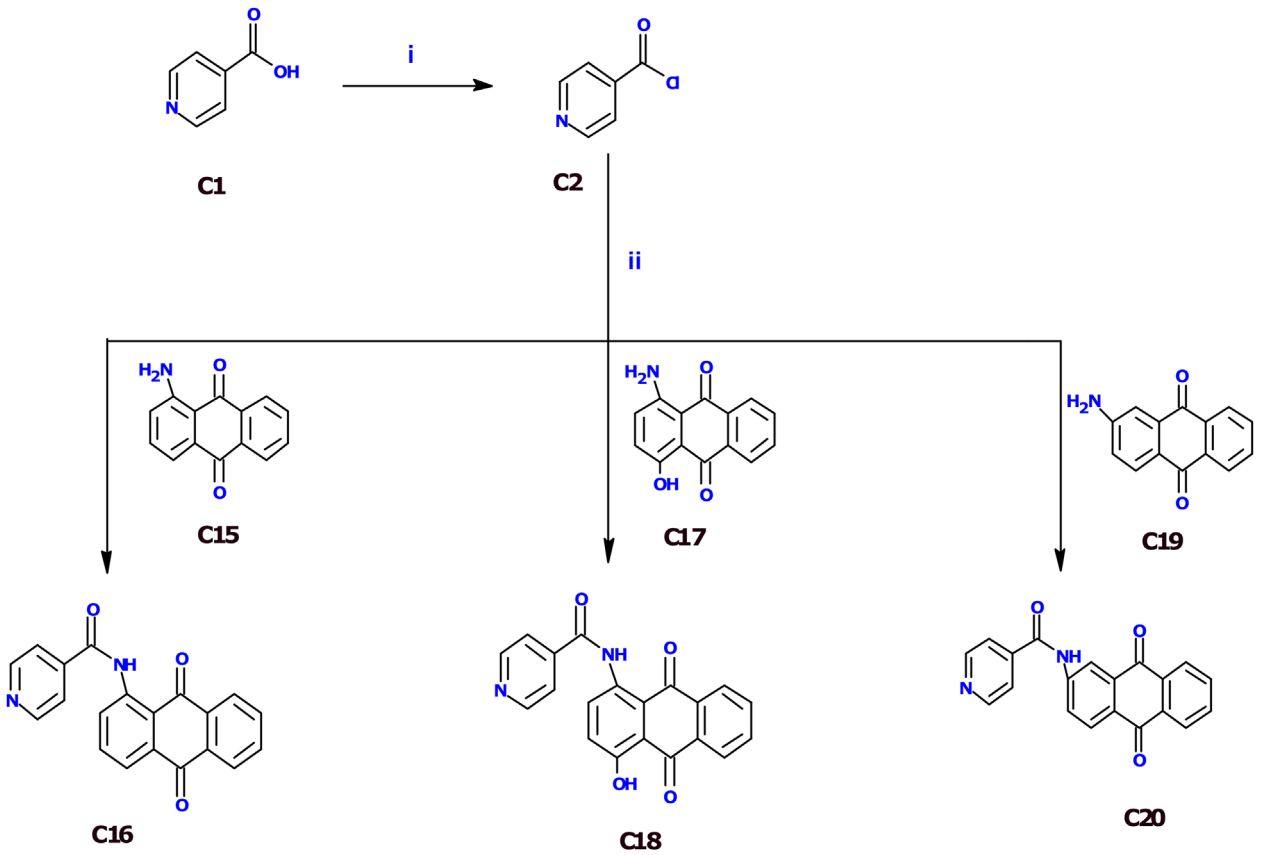

Scheme 2. Preparation of *N*-(9,10-dioxo-9,10-dihydroanthracenyl)pyridine-4-carboxamide derivatives (**C16**, **C18**, and **C20**): (i) $SOCl_2$, dry benzene, refluxed at 70°C for 48 h, distillation to get rid of excess $SOCl_2$ and dry benzene; (ii) refluxed at 100°C for 18 h, followed by the addition of 1,4-dioxan and stirring for 24 h.

Regarding TC levels, **C8**, **C10**, **C16**, **C18**, and **C20** did not show any significant changes from the HG, where **C4** and **C12** showed pronounced activities in lowering serum TC level (77.0 ± 4.2 and 51.5 ± 5.2 mg/dL, respectively) in comparison to the HG (398.8 ± 17.3 mg/dL) ($p < 0.05$). Even though **C6** and **C14** showed lower potency compared to **C4** and **C12**, still they showed a significant reduction in serum TG levels (226.0 ± 24.9 and 318.5 ± 13.2 mg/dL, respectively) compared with the Triton WR-1339-induced HG (398.8 ± 17.3 mg/dL) ($p < 0.05$).

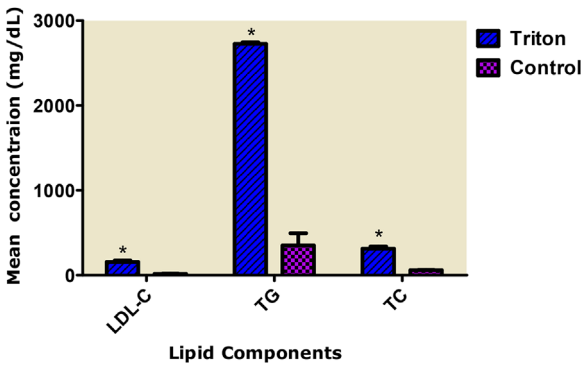

Figure 2. Effect of Triton WR-1339 on lipid profile after 18 h. Values are means ± SEM from six rats in each group. TG: triglyceride; TC: total cholesterol. *$p < 0.01$ using Mann–Whitney U-test.

Evaluation of antioxidant effect of novel N-(benzoylphenyl)-pyridine-4-carboxamide derivatives

Under experimental conditions, reference ascorbic acid was tested at six different concentrations and showed a potent scavenging effects on DPPH radicals with $IC_{50} = 7.2$ μg/mL (Fig. 3).

The scavenging effects of the synthesized *N*-(benzoylphenyl)pyridine-4-carboxamide and *N*-(9,10-dioxo-9,10-dihydroanthracenyl)pyridine-4-carboxamide derivatives on DPPH radicals were tested and are presented in Table 2. These data represent a preliminary screening on a single concentration (100 μg/mL). Results showed that the majority of the tested compounds displayed a relatively low or no DPPH radical scavenging effect, with most of the compounds achieving less than 22% DPPH radical scavenging effect, with **C20** and **C4** being the most potent among them.

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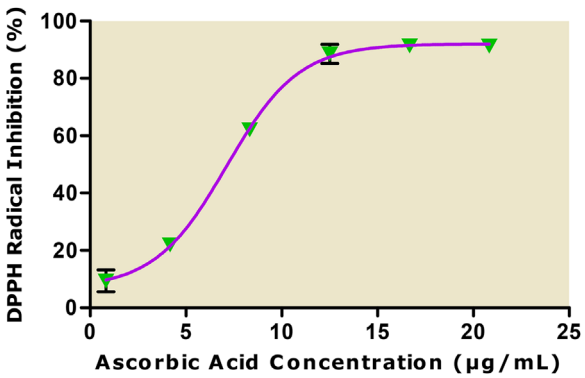

Figure 3. DPPH radical inhibition by ascorbic acid. Results represent the average of three replicates.

Discussion

Hyperlipidemia and oxidative stress have been implicated as essential contributing factors for the development of atherosclerosis and CVDs such as hypertension, myocardial infarction, peripheral vascular diseases, and CHDs [1, 2]. The risk of CVDs can be reduced by using drugs with both hypolipidemic and antioxidant effects. Despite the availability of many drugs for treating hyperlipidemia, unfortunately, most of them lack the desired safety. Thus, the present study was undertaken to evaluate the potential effect of novel *N*-(benzoylphenyl)pyridine-4-carboxamide and *N*-(9,10-dioxo-9,10-dihydroanthracenyl)pyridine-4-carboxamide derivatives in controlling hyperlipidemia and oxidative stress using hyperlipidemic rat model and DPPH radical scavenging assay, respectively.

Triton WR-1339-induced hyperlipidemia in rats is a widely accepted animal model [20, 21, 27–29], successfully used for screening lipid-lowering activities of natural products and chemical entities, since it is simple to handle and requires shorter time to induce hyperlipidemia [30]. The administration of single intraperitoneal dose of Triton WR-1339 to adult rats has been reported to increase TC, LDL-C, and TGs to reach a maximum value in about 20 h and return to normal thereafter [30].

The antihyperlipidemic activities of different isonicotinic acid carboxamide derivatives, mainly **C4**, **C6**, **C8**, **C10**, **C12**, **C14**, **C16**, **C18**, and **C20**, were evaluated in Triton WR-1339-induced hyperlipidemic rats. In the current study, Triton WR-1339 (300 mg/kg, i.p.) administration caused alterations in the lipid metabolism in about 18 h, which resulted in severe hyperlipidemia. The maximum effects on lipid components in our model revealed similar pattern to previous report, where LDL-C, TG, and TC peaked after 18 h from Triton WR-1339 administration [30].

Treatment with benzophenone amide derivatives **C4** and **C12** (at a dose of 30 mg/kg) offered significant protection against Triton WR-1339-induced hyperlipidemia by maintaining serum LDL-C, TG, and TC nearly to normal levels compared to Triton WR-1339 only treated group ($p < 0.05$). The activity

Table 1. Effect of the novel C4, C6, C8, C10, C12, C14, C16, C18, C20, fenofibrate (FF), and nicotinic acid (NA) on plasma lipid levels in Triton WR-1339-induced hyperlipidemic rats after 18 h.

Groups	Lipid profile components		
	LDL-C (mg/dL)	TG (mg/dL)	TC (mg/dL)
CG	44.0 ± 3.2*	131.0 ± 18.1*	86.1 ± 6.5*
HG	202.6 ± 8.7	2317.8 ± 13.7	398.8 ± 17.3
C4	38.7 ± 2.8*	78.5 ± 4.0*	77.0 ± 4.2*
C6	124.2 ± 15.1*	1899.8 ± 148.3*	226.0 ± 24.9*
C8	227.0 ± 7.8	2369.0 ± 7.2	381.5 ± 14.9
C10	191.4 ± 16.8	2349.0 ± 41.0	339.2 ± 31.7
C12	34.6 ± 3.1*	49.3 ± 4.2*	51.5 ± 5.2*
C14	182.8 ± 9.6	2266.3 ± 39.6	318.5 ± 13.2*
C16	240.4 ± 8.0	2406.2 ± 66.8	418.9 ± 25.2
C18	200.2 ± 16.5	2384.1 ± 7.4	342.8 ± 25.1
C20	200.4 ± 7.5	2358.2 ± 8.6	360.6 ± 19.8
FF	213.8 ± 9.1	1540 ± 85.2*	271.2 ± 8.3*
NA	2272.4 ± 2.74	392.5 ± 39.1	392.5 ± 39.1

Values are expressed as means ± SEM from six rats in each group. All groups received 300 mg/kg Triton WR-1339 i.p. injection except for CG (control group), which received water as i.p. injection. Regarding oral gavage, CG and HG (hyperlipidemic group) received: 6% DMSO/corn oil; **C4**: **C4** + 6% DMSO/corn oil; **C6**: **C6** + 6% DMSO/corn oil; **C8**: **C8** + 6% DMSO/corn oil; **C10**: **C10** + 6% DMSO/corn oil; **C12**: **C12** + 6% DMSO/corn oil; **C14**: **C14** + 6% DMSO/corn oil; **C16**: **C16** + 6% DMSO/corn oil; **C18**: **C18** + 6% DMSO/corn oil; **C20**: **C20** + 6% DMSO/corn oil; FF: fenofibrate + 6% DMSO/corn oil; NA: nicotinic acid + 6% DMSO/corn oil. TG: triglyceride; TC: total cholesterol. **C4, C6, C8, C10, C12, C14,** FF, and NA were compared with the HG. *p-value < 0.05 using Mann–Whitney U-test.

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Table 2. *In vitro* antioxidant activity of synthesized *N*-(benzoylphenyl)pyridine-4-carboxamide and *N*-(9,10-dioxo-9,10-dihydroanthracenyl)pyridine-4-carboxamide derivatives using DPPH radical scavenging assay.

Compounds	DPPH radical inhibition (%)[a)]
C4	12.80 ± 4.3
C6	8.90 ± 2.5
C8	5.40 ± 6.5
C10	0.10 ± 2.4
C12	1.40 ± 0.8
C14	NA
C16	1.20 ± 2.3
C18	NA
C20	21.90 ± 0.7

Concentration used for each compound = (100 μg/mL). NA: not active.
[a)]Results represent the average of three replicates, data are expressed as mean ± SD.

was reduced for **C6** and it was almost abolished for **C10**, **C12**, **C14**, **C16**, **C18**, and **C20**. Thus, **C4** and **C12** may represent promising leads for the treatment of hyperlipidemia.

Since the active compounds **C4**, **C6**, and **C12** showed a significant reduction in TG, LDL-C, and TC levels, it is suggested that those compounds may antagonize the activity of Triton WR-1339 by stimulating lipoprotein lipase enzyme, which is the main target in Triton WR-1339-induced hyperlipidemic rat model.

Activities were shown to be mainly associated with benzophenone derivatives, while being concealed with anthraquinone derivatives. Rigidity of the compounds seems to play an important role in compounds activities, where the more rigid anthraquinone derivatives were not active compared to benzophenone derivatives.

Regarding benzophenone derivatives, it is important to highlight that the three-dimensional energy-minimized structures of these compounds may play a role in their hypolipidemic activities, where an extended linear structure is found to be active, while the non-extended form is less active/inactive. This is related to the attachment point of the amide linker where compounds with amide nitrogen near to the carbonyl moiety (at position 2 in benzophenone) were less active/inactive (with the exception of **C12**). This position may create intramolecular hydrogen bonding between the carbonyl and the amide nitrogen, thus contributing to the non-extended structure and also preventing those two moieties from participating in H-bonding with the amino acid residue of the receptor. **C12** lacks such interaction due to steric 3-methyl substituent. These suggestions are in concordance with previously obtained results on *N*-(benzoylphenyl)pyridine-3-carboxamide [16], where activities were decreased in general by switching the amide linker from positions 3 or 4 to position 2.

Regarding the antioxidant activities, our results showed that the majority of the tested compounds displayed a relatively low or no DPPH radical scavenging effect, with most of the compounds achieving less than 22% DPPH radical scavenging effect. This finding was comparable to results obtained previously by Ogata et al. [14], who have studied the scavenging capacity of 23 kinds of nicotinic and isoncotinic acid-related compounds using DPPH radical scavenging assay.

Ogata et al. [14] found that nicotinic acid and isonicotinic acids as a nucleus have scavenging rates of 27.4% and 40.9%, respectively. The structural modification of both kinds of nucleus resulted in the reduction of their antioxidant capacity to less than 20% in all cases, except for the hydrazide derivative (nicotinic hydrazide and isonicotinic hydrazide), which have achieved 100% DPPH radical inhibition ability.

Also mild antioxidant capacities of different nicotinic acid derivatives, namely thionicotinic acid, thioamide, and thionitrile, were reported [31], with thionicotinic acid showing the most potent antioxidant radical scavenging activity (33.20%) while the other two compounds exerted almost no activities [31].

In spite that the scavenging rates for most of our compounds were not significant, **C4** demonstrated a mild scavenging effect of 12.8%. This makes **C4** a distinct compound among all compounds, because of its wide range of biological activities including antioxidant and antihyperlipidemic effects. These dual activities will create a protective effect against CVDs, by reducing LDL-C levels and diminishing the generation of reactive oxygen species. This finding specifically makes **C4** a promising lipid lowering and antioxidant agent.

Finally, since our experiments were conducted only on a single dose of the compounds (30 mg/kg for hypolipidemic evaluation and 100 μg/mL for antioxidant evaluation), it is recommended to conduct further studies at higher doses, and to perform an extensive investigation of the effect of different functional groups or substitutions (either on pyridine or benzophenone by using different linkers instead of amide linker) on the hypolipidemic and antioxidant activities.

Based on the results obtained from the present study, this study revealed the antihyperlipidemic activities of some novel isonicotinic carboxamide derivatives. It is recommended to perform extensive investigations of the effect of different functional groups or substitutions on the biological activities, as well as to investigate the potential mechanism of action for these compounds. This could provide a basis for the discovery and development of new promising leads as potential antihyperlipidemic agents.

Experimental

Chemistry

General

All chemicals solvents, and reagents were purchased from Acros Organics (United Kingdom) and Aldrich Chemicals

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(United Kingdom) and were utilized without any purification. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker-500 (500 MHz) at the University of Jordan. Deuteriated dimethylsulfoxide (DMSO-d_6) was used as solvent in sample preparation. Infrared (IR) spectra were recorded using Shimadzu 8400F FT-IR spectrophotometer at the University of Jordan. The samples were prepared by mixing 198 mg KBr with 2 mg of the target compound and then analyzed as thin solid films (KBr discs). Melting points were determined in open capillaries on a Stuart scientific electrothermal melting point apparatus (United Kingdom). High-resolution mass spectra (HRMS) were measured in negative or positive ion mode using electrospray ionization (ESI) technique by collision-induced dissociation on a Bruker APEX-IV (7 T) instrument at the University of Jordan. The samples were dissolved in chloroform. Plate Reader Biotek ELX800 with BioTek's Gen5^{TM} (USA) was used for measuring absorbance for antioxidant experiment.

The InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

General synthesis procedure

A mixture of isonicotinic acid (**C1**, 1 g, Mwt 123.11, 8.1 mmol) and $SOCl_2$ (1 mL, Mwt 118.97, 13.78 mmol) was placed in a round bottomed flask with 10 mL of dry benzene. The reaction mixture was refluxed for 48 h at 70°C until the reaction was completed. Dry benzene and $SOCl_2$ solvents were distilled out from the reaction mixture to afford isonicotinoyl chloride (**C2,** 0.6 g liquid, Mwt 141.56, 51.8%), as colorless liquid (Scheme 1).

Later, the corresponding amine (3.2 mmol) was added to isonicotinoyl chloride (**C2**) (0.50 g, Mwt 141.56, 3.5 mmol) and the reaction mixture was refluxed at 70°C for 18 h in air condenser. Fifteen milliliters of 1,4-dioxane was then added and the reaction mixture was stirred for additional 24 h at room temperature. Next, 200 mL crushed ice was added to the reaction mixture, followed by suction filtration to afford the final product.

Synthesis of the N-(benzoylphenyl)pyridine-4-carboxamide derivatives (Scheme 1)

*N-(3-Benzoylphenyl)pyridine-4-carboxamide (**C4**):* A mixture of isnicotinoyl chloride (**C2**, 0.50 g, Mwt 141.56, 3.5 mmol) and 3-amino-benzophenone (**C3**, 0.63 g, Mwt 197.24, 3.2 mmol) was used to produce *N*-(3-benzoylphenyl)pyridine-4-carboxamide as pale yellow solid (**C4**, 0.50 g, Mwt 302.3, 51.7%); Rf: 0.11 ($CHCl_3$/MeOH, 96:4); Melting point: 134–136°C; ^{1}H-NMR (500 MHz, DMSO-d_6): $\delta = 10.72$ (s, 1H, NH amide), 8.80 (d, $J = 5.7$ Hz, 2H, Ar-H), 8.21 (m, 1H, Ar-H), 8.13 (d, $J = 8.1$ Hz, 1H, Ar-H), 7.88 (d, $J = 5.9$ Hz, 2H, Ar-H), 7.78 (d, $J = 7.2$ Hz, 2H, Ar-H), 7.71 (dd, $J = 7.5$, 7.4 Hz, 1H, Ar-H), 7.59 (dd, $J = 7.9$, 8.2 Hz, 3H, Ar-H), 7.52 (d, $J = 7.7$ Hz, 1H, Ar-H) ppm. ^{13}C-NMR (125 MHz, DMSO-d_6): $\delta = 196.01$ (ketone carbonyl), 164.73 (amide carbonyl), 150.78 (2 Ar-CH), 148.70 (Ar-C Quaternary), 142.06 (Ar-C Quaternary), 139.27 (Ar-C Quaternary), 137.94 (Ar-C Quaternary), 137.44 (Ar-C Quaternary), 133.23 (Ar-CH), 130.10 (2 Ar-CH), 129.60 (Ar-CH), 129.08 (2 Ar-CH), 125.79 (Ar-CH), 124.75 (Ar-CH), 122.04 (2 Ar-CH), 121.86 (Ar-CH) ppm. IR (KBr disk): $\nu = 3325$ (NH amide), 3055, 1944, 1874, 1828, 1751, 1658 (ketone carbonyl), 1604 (amide carbonyl), 1550, 1481, 848, 717 cm^{-1}. HRMS (ESI, negative mode): *m/z* [M−H$^+$] 301.10570 ($C_{19}H_{13}N_2O_2$ requires 301.09715).

*N-(4-Benzoylphenyl)pyridine-4-carboxamide (**C6**):* A mixture of isonicotinoyl chloride (**C2**, 0.50 g, Mwt 141.56, 3.5 mmol) and 4-amino-benzophenone (**C5**, 0.63 g, Mwt 197.24, 3.2 mmol) was used to produce *N*-(4-benzoylphenyl)pyridine-4-carboxamide as pale yellow solid (**C6**, 0.566 g, Mwt 302.3, 58.5%). Rf: 0.39 ($CHCl_3$/MeOH, 96:4); Melting point: 150–152°C. ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 10.94$ (s, 1H, NH amide), 8.84 (m, 2H, Ar-H), 7.57–7.98 (m, 11H, Ar-H) ppm. ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta = 194.64$ (ketone carbonyl), 164.27 (amide carbonyl), 149.53 (2 Ar-CH), 142.67 (Ar-C Quaternary), 142.36 (Ar-C Quaternary), 137.35 (Ar-C Quaternary), 132.37 (Ar-CH), 132.24 (Ar-C Quaternary), 130.98 (2 CH-Ar), 129.42 (2 Ar-CH), 128.51 (2 Ar-CH), 122.05 (2 Ar-CH), 119.64 (2 Ar-CH) ppm. IR (KBr disk): $\nu = 3363$ (NH amide), 3039, 1936, 1851, 1820, 1782, 1681 (ketone carbonyl), 1643 (amide carbonyl), 1597, 1527, 1404, 848, 740, 702 cm^{-1}. HRMS (ESI, negative mode): *m/z* [M−H$^+$] 301.10357 ($C_{19}H_{13}N_2O_2$ requires 301.09715).

*N-(2-Benzoylphenyl)pyridine-4-carboxamide (**C8**):* A mixture of isonicotinoyl chloride (**C2**, 0.50 g, Mwt 141.56, 3.5 mmol) and 2-amino-benzophenone (**C7**, 0.63 g, Mwt 197.24, 3.2 mmol) was used to produce *N*-(2-benzoylphenyl)pyridine-4-carboxamide as pale yellow solid (**C8**, 0.317 g, Mwt 302.3, 32.7%). Rf: 0.50 ($CHCl_3$/MeOH, 96:4); Melting point: 115–117°C; ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 10.83$ (s, 1H, NH amide), 8.69 (d, $J = 4.8$ Hz, 2H, Ar-H), 7.36–7.73 (m, 11 H, Ar-H) ppm. ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta = 195.33$ (ketone carbonyl), 163.82 (amide carbonyl), 150.18 (2 Ar-CH), 141.04 (Ar-C Quaternary), 137.15 (Ar-C Quaternary), 135.96 (Ar-C Quaternary), 132.59 (Ar-CH), 132.59 (Ar-CH), 131.45 (Ar-C Quaternary), 130.32 (Ar-CH), 129.47 (2 Ar-CH), 128.19 (2 Ar-CH), 125.19 (Ar-CH), 124.61 (Ar-CH), 121.08 (2 Ar-CH) ppm. IR (KBr disk): $\nu = 3271$ (NH amide), 3063, 1982, 1944, 1836, 1805, 1681 (ketone carbonyl), 1620 (amide carbonyl), 1581, 1535, 1442, 925, 756, 702 cm^{-1}. HRMS (ESI, negative mode): *m/z* [M−H$^+$] 301.09653 ($C_{19}H_{13}N_2O_2$ requires 301.09715).

*2-(2-[(Pyridin-4-ylcarbonyl)amino]benzoyl)benzoic acid (**C10**):* A mixture of isonicotinoyl chloride (**C2**, 0.50 g, Mwt 141.56, 3.5 mmol) and 2-amino-benzophenone-2′-carboxylic acid (**C9**, 0.77 g, Mwt 241.24, 3.2 mmol) was used to produce 2-(2-[(pyridin-4-ylcarbonyl)amino]benzoyl)benzoic acid as pale yellow solid (**C10**, 0.307 g, Mwt 346.3, 27.7%). Rf: 0.58 ($CHCl_3$/MeOH, 96:4); Melting point: 246–248°C; ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 12.28$ (s, 1H, COOH), 11.12 (s, 1H, NH amide), 8.13 (m, 1H, Ar-H), 7.50–7.83 (m, 4H, Ar-H), 7.31 (m, 2H, Ar-H), 7.21 (m, 1H, Ar-H) 7.17 (m, 1H, Ar-H), 7.14 (m, 2H, Ar-H) ppm. ^{13}C-NMR (125 MHz, DMSO-d_6): $\delta = 192.93$ (ketone

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carbonyl), 165.93 (COOH), 163.90 (amide carbonyl), 151.63 (2 Ar-CH), 138.68 (Ar-C Quaternary), 137.09 (Ar-C Quaternary), 134.30 (Ar-CH), 133.60 (Ar-CH), 133.43 (Ar-CH), 131.62 (Ar-CH), 130.61 (Ar-C Quaternary), 130.29 (Ar-C Quaternary), 130.04 (Ar-C Quaternary), 129.70 (Ar-CH), 128.61 (Ar-CH), 124.26 (Ar-CH), 121.32 (Ar-CH), 120.90 (Ar-CH) ppm. IR (KBr disk): $\nu = 3302$ (NH amide), 2607–3263 (OH), 3101, 3032, 1959, 1928, 1898, 1851, 1643 (ketone carbonyl), 1589 (acid carbonyl), 1543 (amide carbonyl), 1481, 1435, 1396, 1234 (C-O), 763, 702 cm^{-1}. HRMS (ESI, negative mode): *m/z* [M−H$^+$] 345.08426 ($C_{20}H_{13}N_2O_4$ requires 345.08698).

*N-(2-Benzoyl-5-methylphenyl)pyridine-4-carboxamide (**C12**):* A mixture of isonicotinoyl chloride (**C2**, 0.50 g, Mwt 141.56, 3.5 mmol) and 2-amino-4-methylbenzophenone**C11**, 1.56 g, Mwt 211.26, 7.4 mmol) was used to produce *N*-(2-benzoyl-5-methylphenyl)pyridine-4-carboxamide obtained as pale yellow solid (**C12**, 0.541 g, Mwt 316.6, 53.4%). Rf: 0.34 ($CHCl_3$/MeOH, 96:4); Melting point: 130–132°C; ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 10.80$ (s, 1H, NH amide), 8.69 (m, 2H, Ar-H), 7.48–7.70 (m, 7H, Ar-H), 7.38 (d, $J = 6.3$ Hz, 1H, Ar-H), 7.27 (d, $J = 7.2$ Hz, 2H, Ar-H), 2.33 (s, 3H, CH_3) ppm. ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta = 195.07$ (ketone carbonyl), 163.88 (amide carbonyl), 150.17 (2 Ar-CH), 143.01 (Ar-C Quaternary), 140.73 (Ar-C Quaternary), 135.99 (Ar-C Quaternary), 134.57 (Ar-C Quaternary), 131.88 (Ar-CH), 131.55 (Ar-CH), 130.28 (2 Ar-CH), 129.70 (2 Ar-CH), 128.74 (Ar-C Quaternary), 125.08 (Ar-CH), 124.62 (Ar-CH), 121.09 (2 Ar-CH), 21.06 (CH_3) ppm. IR (KBr disk): $\nu = 3232$ (NH amide), 3070, 2924, 2862, 1990, 1944, 1836, 1797, 1681 (ketone carbonyl), 1620 (amide carbonyl), 1573, 1535, 1442, 933, 840, 763 cm^{-1}. HRMS (ESI, negative mode): *m/z* [M−H$^+$] 315.11281 ($C_{20}H_{15}N_2O_2$ requires 315.11280).

*N-(2-Benzoyl-4-chlorophenyl)pyridine-4-carboxamide (**C14**):* A mixture of isonicotinoyl chloride (**C2**, 0.50 g, Mwt 141.56, 3.5 mmol) and 2-amino-5-chlorobenzophenone (**C13**, 0.68 g, Mwt 213.68, 3.2 mmol) was used to produce *N*-(2-benzoyl-4-chlorophenyl)pyridine-4-carboxamide as pale yellow solid (**C14**, 0.437 g, Mwt 336.8, 40.5%). Rf: 0.73 ($CHCl_3$/MeOH, 96:4); Melting point: 154–156°C; ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 10.79$ (s, 1H, NH amide), 8.66 (m, 2H, Ar-H), 7.47–7.67 (m, 10H, Ar-H) ppm. ^{13}C-NMR (300 MHz, DMSO-d_6): $\delta = 193.38$ (ketone carbonyl), 163.95 (amide carbonyl), 150.15 (2 CH-Ar), 140.68 (Ar-C Quaternary), 136.39 (Ar-C Quaternary), 134.43 (2 Ar-C Quaternary), 133.78 (Ar-C Quaternary), 132.93 (Ar-CH), 131.58 (Ar-CH), 129.46 (2 Ar-CH), 129.33 (Ar-CH), 128.29 (2 Ar-CH), 126.86 (Ar-CH), 121.09 (2 Ar-CH) ppm. IR (KBr disk): $\nu = 3236$ (NH amide), 3124, 3063, 1936, 1851, 1805, 1797, 1689 (ketone carbonyl), 1620 (amide carbonyl), 1581, 1519, 1396, 949, 833, 748, 686 cm^{-1}. HRMS (ESI, negative mode): *m/z* [M−H$^+$] 335.05652 ($C_{19}H_{12}ClN_2O_2$ requires 335.05818).

Synthesis of the N-(9,10-dioxo-9,10-dihydroanthracenyl)-pyridine-4-carboxamide derivatives (Scheme 2)

*N-(9,10-Dioxo-9,10-dihydroanthracen-1-yl)pyridine-4-carboxamide (**C16**):* A mixture of isonicotinoyl chloride (**C2**, 0.50 g, Mwt 141.56, 3.5 mmol) and 1-amino-anthraquinone (**C15**, 0.71 g, Mwt 223.23, 3.2 mmol) was used to produce *N*-(9,10-dioxo-9,10-dihydroanthracen-1-yl)pyridine-4-carboxamide as orange solid (**C16**, 0.478 g, Mwt 328.3, 45.5%), Rf: 0.22 ($CHCl_3$/MeOH, 96:4); Melting point: 258–260°C. ^{1}H-NMR (500 MHz, DMSO-d_6): $\delta = 13.10$ (s, 1H, NH amide), 9.12 (d, $J = 6.4$ Hz, 1H, Ar-H), 8.94 (d, $J = 5.7$ Hz, 2H, Ar-H), 8.30 (m, 1H, Ar-H), 8.21 (m, 1H, Ar-H), 8.06 (d, $J = 7.4$ Hz, 2H, Ar-H), 7.97–8.02 (m, 4H, Ar-H) ppm. ^{13}C-NMR (125 MHz, DMSO-d_6): $\delta = 187.53$ (ketone carbonyl), 182.54 (ketone carbonyl), 164.52 (amide carbonyl), 151.53 (2 Ar-CH), 141.68 (Ar-C Quaternary), 141.02 (Ar-C Quaternary), 136.50 (Ar-CH), 135.42 (Ar-CH), 135.33 (Ar-CH), 134.40 (Ar-C Quaternary), 134.10 (Ar-C Quaternary), 132.78 (Ar-C Quaternary), 127.73 (Ar-CH), 127.05 (Ar-CH), 126.14 (Ar-CH), 123.18 (Ar-CH), 121.42 (2 Ar-CH), 119.18 (Ar-C Quaternary) ppm. IR (KBr disk): $\nu = 3194$ (NH amide), 3109, 3070, 1994, 1851, 1782, 1689 (ketone carbonyl), 1666 (ketone carbonyl), 1635 (amide carbonyl), 1581, 1519, 1404, 709 cm^{-1}. HRMS (ESI, positive mode): *m/z* [M+H$^+$] 329.09195 ($C_{20}H_{13}N_2O_3$ requires 329.09207).

*N-(4-Hydroxy-9,10-dioxo-9,10-dihydroanthracen-1-yl)pyridine-4-carboxamide (**C18**):* A mixture of isonicotinoyl chloride (**C2**, 0.50 g, Mwt 141.56, 3.5 mmol) and 1-amino-4-hydroxyanthraquinone (**C17**, 0.77 g, Mwt 239.23, 3.2 mmol) was used to produce *N*-(4-hydroxy-9,10-dioxo-9,10-dihydroanthracen-1-yl)pyridine-4-carboxamide as orange-red solid (**C18**, 0.238 g, Mwt 344.3, 21.6%), Rf: 0.14 ($CHCl_3$/MeOH, 96:4); melting point: 254–256°C. ^{1}H-NMR (500 MHz, DMSO-d_6): $\delta = 13.03$ (s, 1H, NH amide), 9.05 (d, $J = 9.4$ Hz, 1H, Ar-H), 8.93 (d, $J = 3.2$ Hz, 2H, Ar-H), 8.30 (dd, $J = 4.1$, 6.7 Hz, 2H, Ar-H), 8.27 (dd, $J = 3.5$, 5.1 Hz, 1H, Ar-H), 7.99 (d, $J = 4.1$ Hz, 2H, Ar-H), 7.95 (d, $J = 4.2$ Hz, 2H, Ar-H), 7.58 (d, $J = 9.4$ Hz, 1H, Ar-H) ppm. ^{13}C-NMR (125 MHz, DMSO-d_6): $\delta = 188.42$ (ketone carbonyl), 186.42 (ketone carbonyl), 164.42 (amide carbonyl), 159.41 (Ar-C Quaternary C-OH), 151.49 (2 Ar-CH), 141.70 (Ar-C Quaternary), 135.85 (Ar-CH), 135.49 (Ar-CH), 135.27 (Ar-C Quaternary), 134.03 (Ar-C Quaternary), 132.60 (Ar-C Quaternary), 131.06 (Ar-CH), 127.83 (Ar-CH), 127.57 (Ar-CH), 126.93 (Ar-CH), 121.43 (2 Ar-CH), 117.77 (Ar-C Quaternary), 115.11 (Ar-C Quaternary) ppm. IR (KBr disk): $\nu = 3433$ (NH amide), 3171–3294 (OH), 3101, 3032, 1952, 1689 (ketone carbonyl), 1627 (ketone carbonyl), 1581 (amide carbonyl), 1489, 1465, 786, 748, 725 cm^{-1}. HRMS (ESI, negative mode): *m/z* [M−H$^+$] 343.07151 ($C_{20}H_{11}N_2O_4$ requires 343.07133).

*N-(9,10-Dioxo-9,10-dihydroanthracen-2-yl)pyridine-4-carboxamide (**C20**):* A mixture of isonicotinoyl chloride (**C2**, 0.50 g, Mwt 141.56, 3.5 mmol) and 2-amino-anthraquinone (**C19**, 0.71 g, Mwt 223.23, 3.2 mmol) was used to produce *N*-(9,10-dioxo-9,10-dihydroanthracen-2-yl)pyridine-4-carboxamide as green solid (**C20**, 0.835 g, Mwt 328.3, 79.5%), Rf: 0.15 ($CHCl_3$/MeOH, 96:4); Melting point: 300–302°C. ^{1}H-NMR (500 MHz, DMSO-d_6): $\delta = 11.41$ (s, 1H, NH amide), 9.14 (m, 1H, Ar-H), 8.68 (m, 1H, Ar-H), 8.18–8.41 (m, 6H, Ar-H), 7.90 (d, $J = 3.7$ Hz, 2H, Ar-H) ppm. ^{13}C-NMR (125 MHz, DMSO-d_6): $\delta = 182.18$ (ketone

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carbonyl), 181.39 (ketone carbonyl), 163.30 (amide carbonyl), 145.32 (Ar-C Quaternary), 143.83 (Ar-C Quaternary), 134.58 (Ar-CH), 134.29 (2 Ar-CH), 133.96 (Ar-C Quaternary), 133.04 (2 Ar-C Quaternary), 128.82 (Ar-C Quaternary), 128.30 (2 Ar-CH), 126.73 (2 Ar-CH), 126.65 (2 Ar-CH), 125.06 (Ar-CH), 117.30 (Ar-CH) ppm. IR (KBr disk): $\nu = 3433$ (NH amide), 3063, 1898, 1674 (ketone carbonyl), 1635 (ketone carbonyl), 1589 (amide carbonyl), 1534, 1496, 1419, 717 cm^{-1}. HRMS (ESI, negative mode): *m/z* [$M-H^+$] 327.08375 ($C_{20}H_{11}N_2O_3$ requires 327.07642).

Biological assays

Animals

Adult healthy male Wistar albino rats weighing 180–200 g were procured from the Applied Science University animal house. Rats were housed in polypropylene cages in room at a temperature of 25°C with a 12-h light-dark cycle. Animals were allowed to acclimatize for 7 days prior to any experimentation, and they received water and a standard rodent diet *ad libitum*. The study protocol was approved by the ethical and graduates studies council at the Faculty of Pharmacy/the University of Jordan (Ref. No. 80/2014/165).

Induction of hyperlipidemia in experimental rats

Hyperlipidemia was induced experimentally using a single intraperitoneal (i.p.) injection of 300 mg/kg Triton WR-1339 dissolved in water. Dose was selected based on literature recommendations where Triton WR-1339 has been reported to be used in doses ranging from 200 mg/kg to 400 mg/kg intraperitoneally for hyperlipidemia induction [18–21, 27–29, 32, 33]. Triton WR-1339 group was compared with a control group of six healthy rats receiving i.p. water injection only.

Acute toxicity study and selection of the experimental dose

The selection of the experimental dose was based on previous acute toxicity study that was conducted by the same research team on related compounds "*N*-(benzoylphenyl)pyridine-3-carboxamide derivatives" at doses from 200 mg/kg to 1000 mg/kg on Balb/c mice [16].

Due to similarity in structure between these compounds and our carboxamide derivatives, a dose–response curve was established for **C6** compound at four different doses (20, 30, 50, and 100 mg/kg). This was conducted to select the dose with significant efficacy to be used in the subsequent experimentation.

Anti-hyperlipidemic activity of N-(benzoylphenyl)-pyridine-4-carboxamide derivatives

The experimental rats were randomly divided into 13 groups (each consisting of six rats). All groups except the control group (CG) were injected intarperitonially with 300 mg/kg Triton WR-1339 dissolved in water, while rats in control group received i.p. water injection.

Animals in the CG and the hyperlipidemic group (HG) were gavaged with 6% DMSO/corn oil, while animals in the remaining 11 groups received an intragastric administration of different target compounds (30 mg/kg of **C4**, **C6**, **C8**, **C10**, **C12**, **C14**, **C16**, **C18**, **C20,** nicotinic acid [NA] and 65 mg/kg fenofibrate [FF]) dissolved in 6% DMSO/corn oil.

Blood sample collection and biochemical evaluation

Eighteen hours following drug administration, 2-mL blood sample was collected by retro-orbital sinus puncture from each rat under mild anesthesia using inhaled diethyl ether [30]. Blood samples were collected in plain tubes and serum was separated by centrifugation at 4000 rpm for 10 min and stored at 4°C for biochemical evaluation.

Since TG level exceeded 400 mg/dL in this model, we cannot rely on Friedewald formula in calculating LDL-C level [34, 35]. So serum TC, LDL-C, and TG were measured directly using commercially available enzymatic colorimetric assay kits by the automatic analyzer (Model Erba XL-300, Germany, Mannheim, Germany) at Alzaytoonah University of Jordan.

Antioxidant activity

The DPPH radical scavenging assay was used to measure the ability of the compounds to scavenge the stable free radical of DPPH spectrophotometrically [36, 37]. DPPH radical (1 mM) solution in methanol was prepared. Stock solutions were prepared for all test compounds (2 mg/mL in DMSO/methanol [1:1]). In 96-well plates, 15 μL of each compound was added in triplicate, followed by the addition of 41.7 μL of DPPH radical and 243.3 μL of methanol to complete the volume up to 300 μL. A final concentration of 100 μg/mL of each compound was obtained within each well. The blank used was 15 μL of methanol and DMSO (1:1) with 41.7 μL of DPPH radical solution and 243.3 μL methanol. After 30 min of incubation in the dark at room temperature, the absorbance was recorded at 517 nm using Biotek ELX800 plate reader (USA). Lower absorbance values of reaction mixture indicate higher free radical scavenging activities. Ascorbic acid was used as the standard reference at six different concentrations (0.8, 4.2, 8.3, 12.5, 16.7, 20.8 μg/mL) to calculate its half maximal inhibitory concentration (IC_{50}) value.

The capability of scavenging the DPPH radical was calculated by using the following formula:

$$\% \text{ Inhibition} = [(AB - AA)/AB] \times 100$$

where AB is the absorption of the blank and AA is the absorption of the test compounds. All the tests were performed in triplicates and the results were averaged.

Statistical analysis

All data were entered and analyzed using SPSS© 19 (SPSS, Inc., Chicago, IL, USA) to evaluate the potential antihyperlipidemic effect of the studied compounds. Results were presented as means ± standard error of the mean (SEM). To investigate any differences between groups, non-parametric Mann–Whitney U-test was used. For all statistical analysis, a *p*-value of less than 0.05 was considered statistically significant. All tests were two tailed.

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Regarding the antioxidant experiment, all data were entered in Excel to calculate the DPPH radical scavenging effect for each compound. *XY* plot was constructed using GraphPad Prism-5 between concentration of ascorbic acid in μg/mL (*X* axis) and % DPPH radical inhibition (*Y* axis), then non-linear regression analysis was used to calculate IC_{50} of ascorbic acid.

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