Jordan Journal of Pharmaceutical Sciences, Volume 13, No. 1, 2020

Antilipolytic-Antiproliferative Activity of Novel Antidiabesity Triazolo/Fluoroquinolones

Shereen Arabiyat,[1] * Violet Kasabri,[2] Yusuf Al-Hiari[2]

[1]Salt College, Al-Balqa Applied University, Jordan

[2]School of Pharmacy, The University of Jordan , Amman, Jordan

ABSTRACT

Background & Aim: The new Fluoroquinolones (FQs) and triazolofluoroquinolones (TFQs) were synthesized and evaluated for antiproliferative – antilipolytic activity and anti-diabesity activity via DPP IV enzyme and glycation-inflammation bioassays.

Methods: new synthetic approaches to 3 fluoroquinolones (FQs) have been developed. Enzymatic bioassays with colorimetric inhibition kinetics were undertaken. Cell lines' cultures with colorimetric endpoint bioassays were utilised. Appropriate reference agents were procured.

Results: Incomparably to Diprotin A; TFQs lacked for DPPIV inhibition. The superior antiglycation activity of derivative **5** with IC_{50} (μM) value of 1.61±0.14 exceeded aminoguanidine's (AG). Compounds **3** and **4** impressively exerted a comparable protection to AG against methylglyoxal-induced carbonyl toxicity (respective IC_{50} values of 7.9 and 6.35 μM). All 3 TFQs had a moderate safety profile. All 3 TFQs incomparably exceeded the antiinflammatory indomethacin (IC_{50} value= 212 μM) efficacy against LPS-induced nitric oxide production in RAW 264.7 macrophages with minimal cytotoxicity. In their respective PL-IC_{50} values; appreciable antilipolytic activity was recognized, though less potent than orlistat. Noticeably **3** and **4** (but not **5**) could be identified for their comparable or outstanding antiprolifertaive capacities vs. cisplatin in the colorectal cancer cell lines (HT29, HCT116, SW620 CACO2 and SW480) with unselective cytotoxity.

Conclusion: FQ and TFQ derivatives may unveil new antidiabesity and anticancer agents.

Keywords: Fluoroquinolones & triazolofluoroquinolones, glycation- inflammation & DPP IV-Pancreatic Lipase; obesity-colorectal cancer.

INTRODUCTION

Fluoroquinolones (FQs) have been identified for over 40 years as one of the most clinically successful antibacterials.[1-4] FQs have other biological activities as antidiabetic, [5] antimycobacterial, [6] pancreatic lipase inhibitors [7] as well as anticancer properities.[8-10] Triazole and its various derivatives possess a great importance in medicinal chemistry with wide range of biological activities including antioxidant, analgesic, antinflammatory, antianxiety, antimicrobials and anticancer properties. [11-13] The hybrid structure triazoloquinolones (TQs) have been reported to exhibit antimicrobial, anti-infective of the urinary tract, antimycobacterial and anti-Alzheimer activities. [14] FQs have also been proven as potential pancreatic lipase inhibitors. [7] The capability of the quinolone family to target type II topoisomerases (gyrase and Topo IV) signifies its members as anticancer agents. Their ability to bind metal ions and ion cofactors represents an additional means of modulating their pharmacological responses [48]. The reported potential anticancer effect of FQs against human breast adenocarcinoma cell line [9, 10] initiated FQs′

* shereen.arabiayt@bau.edu.jo

Received on 2/12/2018 and Accepted for Publication on 19/3/2019.

anticancer testing against a panel of obesity related colorectal cancer cell lines (HT29, HCT116, SW620, CACO2 and SW480).

Nonenzymatic formation of advanced glycation end products (AGEs) takes place between reducing sugars and amino groups in proteins, lipids and nucleic acids. Recently, the accumulation of AGEs *in vivo* has been implicated as a major pathogenic process in atherosclerosis, Alzheimer's disease and normal aging. [15] Also, accelerated AGEs accumulation under hyperglycemic conditions is characteristic to type 2 diabetes mellitus (T2DM) and contributes to the development of vascular complications. [16] The interaction between AGE-modified proteins and AGE-specific receptors (RAGEs) on the cell surface induces the overproduction of reactive oxygen species (ROSs) and inflammatory mediators, which leads to cellular disorders in biological systems. [17, 18] Recently, more detrimental effects of AGEs in T2DM have emerged. AGEs interfere with the complex molecular pathway of insulin signalling, leading to insulin resistance; AGEs modify the insulin molecule, and, consequently, its function; AGEs decrease insulin secretion and insulin content. [19, 20] Thus, Inhibition of RAGE was found to effectively protect β-cells against AGE-induced apoptosis, [21] but could not reverse islet dysfunction in glucose stimulated insulin secretion (GSIS). [22] Additionally, AGEs suppression of insulin-stimulated glucose transport in adipocytes was blocked by anti-AGEs antibodies. [18] The presence of glycated insulin has been demonstrated in plasma and pancreatic islets of diabetic patients. [23, 24] The sites of monoglycated and diglycated insulin entities were identified. [25, 26] A novel triglycated form of insulin was purified lately. [27] Glucotoxicity and non-enzymatic glycation were demonstrated to be controlled *in vivo* by deglycation systems. [28] A transglycation product, glucose-cysteine, was found in human urine and its concentrations were increased in diabetes. [29] It is hypothesized that cellular supplementation with very high concentrations of scavenging nucleophilic amino acids counteract non-enzymatic glycation and adverse effects of hyperglycemia. [28] Glutathione, [30] taurine, [31] penicillamine, [32] dipeptides like carnosine and anserine, and alpha-thiolamines such as cysteine and cysteamine retard and reverse non-enzymatic glycation, acting as effective transglycation/deglycation agents. [33-35] As such, inhibition of AGE formation; especially the natural anti-AGE agents without adverse effects, represents a potential therapeutic target for the prevention of premature aging and treatment of diabetic complications. [15, 16, 31] Additionally, intense efforts by pharmaceutical industry to identify new targets for obesity-diabetes (Diabesity) pharmacological intervention has led to a number of agents developed and directed at dipeptidyl peptidase IV (DPP IV) enzyme inhibition. DPP IV is a serine aminopeptidase cleaving off dipeptides from the aminoterminus of peptides, with a preference for proline at the penultimate position. [36] Many of DPP IV substrates share a Xaa-Pro or Xaa-Ala motif at their amino-terminus. This sequence contributes to the biological activity of the peptides, and serves as a structural protection against non-specific proteolysis. DPP IV may therefore be a key modulator of the biological activity of several of these peptides. [36] Dipeptidyl peptidase-IV (DPP-IV) is involved in the inactivation of glucagon like peptide-1 (GLP-1), a potent insulinotropic peptide. Thus, DPP IV inhibition can be an effective approach to treat type 2 DM by potentiating insulin secretion. [37-39] Diprotin A is a selective reversible peptide inhibitor of DPP IV with *in vivo* antidiabetic effects. [40, 41] DPP IV Inhibitors have the capacity to repair β-cell dysfunction and insulin resistance [42] and show a safe CV profile in patients with type 2 DM.[43-45] Thus new leads for DPP IV inhibition [46] were scrutinized; among many were the FQs as in sparfloxacin. [47] FQs have also been proven as potential pancreatic lipase inhibitors. [7] The capability of the quinolone family to target type II topoisomerases (gyrase and Topo IV) signifies its members as anticancer agents.

Their ability to bind metal ions and ion cofactors represents an additional means of modulating their pharmacological responses [48]. The reported potential anticancer effect of FQs against human breast adenocarcinoma cell line [9, 10a,b] initiated FQs′ anticancer testing against a panel of obesity related colorectal cancer cell lines (HT29, HCT116, SW620, CACO2 and SW480). This work involves synthesis of novel FQs and Triazolofluoroquinolones (TFQs) and evaluation of their *in vitro* DPP IV inhibition, dual glucation inflammation modulation, antiobesity and antiproliferative capabilities.

2. EXPERIMENTAL

2.1. Materials

Generally all of the chemicals and solvents used in this study were purchased as the analytical grade, unless indicated otherwise, and used directly without further purification Ethylamine (Acros, Belgium) and *p*-hexylaniline (Sigma, St. Luis, MO, USA), stannous chloride (Fluka, Switzerland) were procured. Melting points (mp) were determined in open capillaries on a Stuart scientific electro-thermal melting point apparatus (Stuart, Staffordshire, UK) and are uncorrected. Thin layer chromatography (TLC) was performed on 10 x 10 cm^2 aluminum plates pre-coated with fluorescent silica gel GF254 (ALBET, Germany) and was visualized using UV lamp (at 254nm wave length/ short wave length/ long wavelength). Mobile phase mixtures were: 94:5:1 chloroform-methanol-formic acid ($CHCl_3$-MeOH-FA) (system 1) and 50:50 (*n*-hexane - ethyl actate) (system 2). Nuclear magnetic resonance spectra (NMR) were recorded on 400 MHz Bruker Avance Ultrashield. The chemical shifts were reported in ppm relative to tetramethylsilane (TMS). Deuterated dimethylsulfoxide (DMSO d_6) was used as the NMR solvents. Infrared (IR) spectra were recorded using Shimadzu 8400F FT-IR spectrophotometer Shimadzu, Kyoto, Japan). The samples were prepared as potassium bromide (KBr) (Merck, Darmstadt, Germany) disks. High- resolution mass spectra (HRMS) were measured in positive ion mode using electrospray ionization (ESI) technique by collision-induced dissociation on a Bruker APEX-4 (7 Tesla) instrument. The samples were dissolved in acetonitrile, diluted in spray solution (methanol/water 1:1 v/v + 0.1 formic acid) and infused using a syringe pump with a flow rate of 2µL/min. External calibration was conducted using Arginine cluster in a mass range m/z 175-871. All cell lines were procured from ATCC (USA) and seeded at passage numbers<20

2.2.Synthesis of novel title compounds; Scheme 1

2.2.1.Compound 2: Synthesis of ethyl -1-ethyl-6-fluoro-7-(4-hexyl-phenylamino)-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylate (2)

Three molar equivalents of 4-hexylaniline (4.7g, 26.3mmol) were added into a solution containing (**1**, 3g, 8.75mmol) and 10 ml of dimethylsulfoxide (DMSO) as a solvent and drops of pyridine then was refluxed at 65-70°C under anhydrous conditions for (2-3) days. The reaction mixture was monitored until no starting material remained then was left to crystallize at room temperature. The product was filtered and washed, left to dry in dark place to give orange crystals. Color of solid compound: orange; yield ≈ 90% (3.8g); mp = 100-102°C; *Rf* value in system 1 = 0.93 and in system 2 = 0.45. ^{1}H NMR (500 MHz, DMSO-d6): 0.81 (m, 3H, CH_3-6'''), 1.22-1.27 (m, 12H, CH_3-1', OCH_2CH_3, CH_2-3''', CH_2-4''', CH_2-5'''), 1.48 (m, 2H, CH_2-2'''), 3.28 (m, 2H, CH_2-1'''), 4.01 (q, J = 6.85 Hz, 2H, NCH_2-1'), 4.22 (q, J = 7 Hz, 2H, OCH_2CH_3), 6.80 (d, J = 8.05 Hz, 2H, H-2", H-6"), 7.00 (d, J = 8 Hz, 2H, CH-3", H-5"), 8.07(d, $^3J_{H-F}$ = 11.45 Hz, 1H, H-5), 8.47 (br s, 1H, NH), 8.60 (s, 1H, H-2). ^{13}C NMR (125 MHz, DMSO-d6): 14.39 (CH_3-6'''), 14.70 (CH_3-1'), 15.87 (OCH_2CH_3), 22.53 (CH_2-5'''), 28.77 (CH_2-4'''), 31.52 (CH_2-3'''), 31.58 (CH_2-2'''), 34.9 (CH_2-1'''), 50.31 (CH_2-1'), 60.66 (OCH_2), 112.12 (C-3), 115.41 (d, $^2J_{C-F}$= 21.48, C-5), 118.2 (2C, C-2",C- 6"), 125.39 (d, $^3J_{C-F}$= 5.89, C-4a), 129.02 (2C, C-3", C-5"), 130.21 (C-4"), 131.02 (d, $^2J_{C-F}$= 16.28, C-7), 136.31(C-8a), 136.47 (C-8), 140.87 (C-1"), 151.60 (C-2), 153.12 (d, $^1J_{C-F}$= 251.14 Hz, C-6), 165.31 (CO_2Et), 170.57

(C-4). IR (KBr): *v* 3376, 3044, 2358, 1767, 1628, 1513, 1443, 1317, 1076, 988 cm^{-1}. LRMS (ES, +ve) m/z calc. for $C_{26}H_{30}FN_3O_5$ (483.22): Found 485.5 (M+2, 100%), 470.9 (1.1%), 440.6 (3.4%), 439.5 (11.2%), 400.3 (32%), 391.5 (3.4%), 324.3 (4.5%), 284.7 (2.2%), 270.4 (3.4%), 242.2 (3.9%), 228.5 (3.4%), 180.5 (2.2%), 178.6 (65.2%), 170.5 (4.5%), 74.2 (1.1%), 60.9 (1.1%), 57.3 (4.5%).

2.2.2. Compound 3: Synthesis of 1-ethyl-6-fluoro-7-(4-hexyl-phenylamino)-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (3)

A vigorously stirred suspension (**2,** 2g, 4.1mmol) in 12N HCl (28 mL) and ethanol (12 mL) was heated at 80-85 °C under reflux conditions. Progress of the ester hydrolysis was monitored by TLC and was completed within 24-36h. Thereafter, the reaction mixture was cooled, poured onto crushed ice (250 g) and the resulting orange precipitate was collected, washed with cold water (2 x 20 mL) and left to dry. Yield ≈1.35 g (72 %). mp = 192-194 °C; *Rf* value in system 1 = 0.75. ^{1}H NMR (300 Hz, DMSO d_6): 0.85 (t, 3H, CH_3-6'''), 1.22-1.30 (m, 11H, CH_3-2', CH_2-2''', CH_2- 3''', CH_2-4''', CH_2-5'''), 1.48 (t, 2H, CH_2-1'''), 4.19 (q, J= 7.04 Hz, 2H, NCH_2-1'), 6.89 (d, J= 7.68 Hz, 2H, H-2", H-6"), 7.03 (d, J= 8.16 Hz, 2H, H-3", H-5"), 8.12 (d, $^3J_{H-F}$= 22.83 Hz, 1H, H-5), 8.81 (br s, 1H, NH), 8.93 (s, 1H, H-2), 14.52 (br s, 1H, COOH). ^{13}C NMR (75 Hz, DMSO d_6): 14.43 (CH_3-6'''), 16.09 (CH_3-2'), 22.56 (CH_2-5'''), 28.78 (CH_2-4'''), 31.51 (CH_2-3'''), 31.60 (CH_2-2'''), 35.00 (CH_2-1'''), 51.46 (CH_2-1'), 109.58 (C-3), 114.80 (d, $^2J_{C-F}$= 21.2 Hz, C-5), 119.50 (2C, C-2", C-6"), 121.83 (d, $^3J_{C-F}$= 7.13, C-4a), 129.04 (2C, C-3", C-5"), 131.19 (C-4"), 133.03 (d, $^2J_{C-F}$= 15.3, C-7) 137.61 (C-8a), 139.98 (C-8), 140.01 (C-1"), 152.00 (C-2), 153.23 (d, $^1J_{C-F}$= 253.85 Hz, C-6), 165.48 (COOH), 175.76 (C-4). IR (KBr): ν 3386, 2982, 2624, 2274, 1738, 1624, 1495, 1319, 1074cm^{-1}. LRMS (ES, +ve) m/z calc. for $C_{24}H_{26}FN_3O_5$ (455.19): Found 456.5 (69%, M+1), 458.4 (7%), 453.4 (1%), 448.4 (16%), 434.4 (5%), 439.3 (100%), 430.5 (12%), 412.7 (8%), 411.5 (23%), 406.7 (1%), 402.3 (3%), 393.5 (17%), 391.6 (23%), 385.2 (1%), 380.6 (60%), 377.4 (5%), 361.4 (4%), 340.7 (4%), 334.3 (8%), 326.5 (3%), 312.5 (1%), 284.6 (5%), 270.4 (3%), 176.3 (3%), 141.1 (13%).

2.2.3. Compound 4: Synthesis of 8-amino-1-ethyl-6-fluoro-7-(4-hexyl-phenylamino)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (4)

A mixture of (**3**, 0.76g, 1.7mmol) in 6.7mL of 12N HCl was left stirring in ice bath (0-5°C) for 15 minutes. After that, the ice bath was removed and (1.3g, 6.8mmol) stannous chloride ($SnCl_2$) was added portion wise and the reaction mixture left stirring overnight and monitored by TLC until completion. Then, the reaction mixture was poured on crushed ice to precipitate a light brown product that is collected by filtration and left to dry. Yield = 0.65g (≈ 90%). mp = 275-277°C (decomposition); *Rf* value in system 1 = 0.5. ^{1}H NMR (300 Hz, $CDCl_3$, 20% CD_3OD): 0.84 (m, 3H, CH_3-6'''), 1.26 (m, 7H, CH_3-2', CH_2-5''', CH_2-4'''), 1.42 (m, 4H, CH_2- 3''', CH_2-2'''), 1.54 (m, 2H, CH_2-1'''), 4.28 (m, 2H, NH_2), 4.85 (m, 2H, NCH_2-1'), 6.68 (d, J= 7.15 Hz, 2H, H-2", H-6"), 7.04 (d, J= 7.15 Hz, 2H, H-3", H-5"), 7.38 (br s, 1H, NH), 7.68 (d, J=9.4 Hz, 1H, H-5), 8.73 (s, 1H, H-2), 14.50 (br s, 1H, COOH).^{13}C NMR (75 Hz, $CDCl_3$, 20%MeOD): 16.94 (CH_3-6'''), 19.33 (CH_3-2'), 25.58 (CH_2-5'''), 31.95 (CH_2-4'''), 34.61 (CH_2-3'''), 34.71 (CH_2-2'''), 38.14 (CH_2-1'''), 56.13 (CH_2-1'), 104.82 (d, $^2J_{C-F}$= 23.55 Hz, C-5), 109.67 (C-3), 118.91 (2C, C-2", C-6"), 126.12 (C-4a), 128.28 (C-8), 131.07 (C-8a), 132.17 (2C, C-3", C-5"), 138.0 (C-7), 139.11 (C-4"), 143.19 (C-1"), 153.48 (C-2), 161.22 (d, $^1J_{C-F}$= 250.0 Hz, C-6), 171.7 (COOH), 178.7 (C-4). IR (KBr): ν 3388, 3068, 2953, 2926, 2733, 2361, 1738, 1624, 1495, 1319, 1335, 1275, 1034 cm^{-1}. LRMS (ES, +ve) m/z calc. for $C_{24}H_{28}FN_3O_5$ (425.21): Found 426.5 (100%, M+1), 411.6 (2.2%), 408.6 (9%), 262.3 (1%), 253.6 (2.2%), 178.1 (2.2%), 158.1 (3.4%), 149.3 (1%), 126.0 (4.5%), 102.1 (3.4%), 85.1 (2.2%), 74.2 (9%), 59.2 (21%).

2.2.4. *Compound 5: Synthesis of 9-ethyl-4-fluoro-3-(4-hexyl-phenyl)-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h] quinoline-7-carboxylic acid (5)*

Compound (**5**) was synthesized through cyclization of preceeding reduced acid (**4**, 0.4g, 0.94mmol) in 20ml aqueous HCl, left stirring in ice bath (0-5°C) for 15 minutes. $NaNO_2$ (0.065g, 0.94mmol) dissolved in 10mL H_2O is added drop wise. The reaction mixture was left stirring overnight. Progress of cyclization reaction was monitored by TLC and was completed within 24 hrs. Thereafter, the reaction mixture was cooled, poured onto crushed ice (250g) and the resulting off-white precipitate was collected, washed with cold water (2 x 20mL) and left to dry. Yield= 0.28g (≈ 68 %). mp = 220-222°C; *Rf* value in system 1= 0.6. ^{1}H NMR (300 Hz, $CDCl_3$, CD3OD): 0.89 (m, 3H, CH_3-6'''), 1.33-1.69 (2m, 11H, CH_3-2', CH_2-2''', CH_2- 3''', CH_2-4''', CH_2-5'''), 2.74 (m, 2H, CH_2-1'''), 5.29 (m, 2H, NCH_2-1'), 7.53 (2H, H-2'', H-6''), 7.56 (2H, H-3'', H-5''), 8.24 (d, $^3J_{H\text{-}F}$= 8.9 Hz, 1H, H-5), 8.85 (s, 1H, H-8), 14.95 (br s, 1H, COOH).^{13}C NMR (75 Hz, $CDCl_3$, CD_3OD): 14.13 (CH_3-6'''), 16.08 (CH_3-2'), 22.63 (CH_2-5'''), 28.98 (CH_2-4'''), 31.27 (CH_2-3'''), 31.34 (CH_2-2'''), 35.74 (CH_2-1'''), 54.06 (CH_2-1'), 109.96 (d, $^2J_{C\text{-}F}$= 20.4 Hz, C-5), 110.81 (C-7), 125.02 (2C, C-2'', C-6''), 127.13 (C-5a), 129.15 (C-9b), 129.49 (2C, C-3'', C-5''), 130.12 (C-9a), 133.33 (C-3a), 139.71 (C-4''), 144.15 (C-1''), 148.17 (C-8), 148.17 (d, $^1J_{C\text{-}F}$= 250.0 Hz, C-4), 166.6 (COOH), 176.6 (C-6). IR (KBr): ν 3433, 3059, 2994, 2683, 1745, 1632, 1520, 1140, 1077, 1010 cm^{-1}. LRMS (ES, +ve) m/z calc. for $C_{24}H_{25}FN_4O_3$ (436.19): Found 437.6 (87%, M+1), 419.2 (6.7%), 411.6 (1%), 361.5 (2.2%), 258.2 (1%), 202.6 (5.6%), 189.3 (100%), 178.4 (11%), 124.1 (1%), 105.2 (10%), 91.1 (50%), 74.2 (3.4%), 57.4 (5.6%).

2.3. Antiglycation determination in vitro via Metheylglyoxal induced cytotoxicity

A continuous cell line of murine macrophages cells (RAW 264.7) were routinely cultured in DMEM enriched with the following supplements (mentioned with their corresponding final concentrations): Fetal Bovine Serum (FBS) (10%), streptomycin sulfate (100 mg/mL), penicillin (100 U/mL), gentamicin (50 µg/mL), beta-mercaptoethanol (50 µM), HEPES buffer (10 mM), L-glutamine (2 mM). The cells were cultured routinely and harvested biweekly using 1% trypsin-EDTA. The cells were seeded at 10^4 cell/ well [u1]in 96-well tissue culture plates and incubated at 37°C and 5% CO_2 overnight. After 12 hr incubation, the cells were treated with the test compound (in DMSO such that the maximum final concentration of each on cells did not exceed 1%) in 10-50 µg/mL concentrations 20 minutes prior to the glycating sugar methylglyoxal (MGO; 300 µM) treatment. IC_{50} value of MGO induced cytoxicity in RAW264.7 macrophages was determined over 4 concentrations (100-400 µM). To rule out the possible interfering effects, the following controls were used: 1) Negative control: Normal RAW264.7[u2].cells: cells in culture medium only without test compound or MGO were used as a control for the natural growth/death of cells. 2) Positive control: cells with AMG at 1mM and MGO [49], 3) Test compound control: the cells with the test compound only (with no MGO) were incubated in their respective test concentration gradients to assess the effect of the test compound on the cells, 4) Co-solvent control: each co-solvent was added in its maximum concentration over non-treated cells to assess its effect on cells. The cells were then incubated for 48 hr and assessed for viability using Sulforhodamine B (SRB; Santa Cruz Biotechnology, Inc. Texas, USA) colorimetric assay for cytotoxicity screening and mechanism of reduction of cell viability as described previously [50]. The absorbance was read at 570 nm and 630 nm as a reference wavelength using absorbance plate reader (Biotek, ELx800). The activity of the test compound was assessed by calculating the percentage of viable treated cells versus negative control. Cell viability of ≤ 70% was considered as toxicity cut-off point [51].

2.4. Anti-Inflammatory (Nitrite) Determination in Vitro

As NO is considered as a pro-inflammatory mediator that induces inflammation; Murine macrophage cell line RAW 264.7 were cultured in high glucose DMEM supplemented with 10% foetal bovine serum (FBS),

penicillin (100 U/mL), streptomycin (100 µg/mL), and L-glutamate (100 µg/mL) in a 37 °C humidified atmosphere with 95% air and 5% CO2. The cells (2 x 10^5 /well) were incubated with test compounds at different concentrations (5-200 µg/mL) in the presence of lipopolysaccharide (LPS; 10 µg/mL; Sigma, St. Luis, MO, USA) for 24 h. Following overnight incubation, aliquots of 100 µL of cell culture media were mixed with 100 µL Griess reagent (50 µL of 1 % Sulfamilamide in 5 % phosphoric acid and 50 µL of 0.1 % napthylehtyllenediamine-HCL), and incubated at RT for 10 min. Absorbance at 550 nm was determined using microplate reader (Bio-Tek Instrument, USA).The concentration of nitrite was determined by comparison with sodium nitrite standard curve. SRB cytotoxity protocol was performed for evaluation of the effect of test compounds on RAW 264.7 viability [52-53] Indomethacin (25-200 µg/mL) served as the positive control [54].

2.5. Measurement of DPP IV inhibition in vitro

All chemicals of analytical grade (DPPIV from porcine kidney, Gly-pro-p-nitroanilide, Diprotin-A (Ile-Pro-Ile), and Tris-HCl Buffer were purchased from Sigma, St Louis, USA. Test compounds were dissolved in DMSO and diluted with the assay buffer (Tris, pH 7.5). The DMSO concentration was less than 1.0 % in all experiments and controls. In a 96-well titer plate reader, inhibition assay was conducted in triplicates. The assay was based on the cleavage of the chromogenic substrate Gly-pro-para-nitroanilide (GPPN) by the serine protease DPP-IV resulting in release of Paranitroaniline (pNA), a yellow colored product measured at 405nm (Bio-TEK, USA) [55]. Diprotin A or test compounds (0.01-100 µg/mL) were in the final volume of 35 µL in Tris-HCl Buffer (50mM, pH 7.5). DPP-IV enzyme (0.05U/mL; 15µL) was added and the mixture was pre-incubated for 10 minutes at 37°C. One unit enzyme activity was defined as the amount of enzyme that catalyzes the release of 1µ mol pNA from the substrate/min under assay conditions. GPPN (50 µL of 0.2mM in Tris-HCl) was added for 30 minutes incubation at 37°C. The reaction was terminated by addition of 25 µL of 25% glacial acetic acid. All values are represented as Mean ± Standard Deviation. The % inhibition = 100-((Absorbance of inhibitor/ Absorbance of control) /Absorbance of control x 100). The IC_{50} value represents the amount of inhibitor required to achieve 50% enzyme inhibition.

2.6. In vitro quantification of PL activity

Orlistat (the golden antilipolytic standard; 1 mg/mL, Sigma, St. Luis, MO, USA) was used in the reaction mixture to give final concentrations in the range of 0.0125–0.4 µg/mL. Furthermore the test compounds were used in the reaction mixture to give the final concentration range (0.2 – 2000 µg/mL). Crude porcine PL type II (0.5 mg/mL; Sigma, St. Luis, MO, USA, EC 3.1.1.3) was suspended in Tris-HCl buffer (2.5 mM, pH 7.4, Promega Corp. WI, USA) to a final concentration of 200 units/mL. A *para*-nitrophenyl butyrate solution (*p*-NPB, 100 µM; Sigma, St. Luis, MO, USA) was used as the PL substrate. The catalytic activity of PL was determined colorimetrically by measuring its activity towards the hydrolysis of *p*-NPB to *p*-nitrophenol. PL aliquots were preincubated at 37 °C with different concentrations of the test material for at least 1 min prior to the addition of the substrate. The *p*-nitrophenol released during the reaction was measured at 410 nm (SpectroScan 80D UV-VIS spectrophotometer; Sedico Ltd., Nicosia, Cyprus) over a minimum of five time points (1–5 min), against a blank of the same mixture containing the denatured enzyme. The activity of PL in this reaction was quantified by measuring the increase in the rate of the release of *p*-nitrophenol from the slope of the linear segment of the absorbance *versus* time profiles [56]. The percentage of residual PL activity was determined for all of the test compounds relative to the control compounds, to calculate the concentration required to inhibit the activity of PL by 50% (i.e., the IC_{50}). All of the assays were performed in triplicate and the calculated activities reported as the mean values ± SD (n=3). The PL inhibition values (%) were calculated according to the following formula: Inhibition (%) = 100 – [(B/A) × 100],

where A is the PL activity in the absence of an inhibitor or test compound and B is the PL activity in the presence of an inhibitor or test compound.

2.7. In vitro antiproliferative assay

The cytotoxicity measurements were determined using Sulforhodamine B (SRB; Santa Cruz Biotechnology, Inc. Texas, USA) colorimetric assay for cytotoxicity screening and mechanism of reduction of cell viability as described previously [50]. As a robust and classical antineoplastic reference agent, cisplatin (1-100 µg/mL, Sigma, St. Luis, MO, USA) was recruited for comparison purposes [57]. Obesity related colorectal cell lines HT29, HCT116, SW620 and SW480 were cultured in high glucose DMEM containing 10% FCS (Bio Whittaker, Verviers, Belgium). CACO2 cell line was cultured in RPMI 1640 containing 10% FBS, HEPES Buffer (10 mM), L-glutamine (2 mM), gentamicin (50 µg/mL), penicillin (100 U/mL), and streptomycin sulfate (100 mg/mL) (Sigma, St. Luis, MO, USA). Human periodontal ligament fibroblasts (PDL) are a primary cell culture for verification of selective cytotoxicity. All of the assays were performed in triplicate and the calculated antiproliferative activities were reported as the mean values ± SD (n=3).

3. RESULTS AND DISCUSSION

3.1. Synthesis of novel compounds

Synthesis of compounds **1** was carried out following a previously reported procedure. [62] Compound **2** was synthesized by the reaction of *p*-hexylaniline with **1**; scheme 1. Hydrolysis of nitro ester **2** has generated the respective nitro acid **3** in high yield. The 8-nitro derivatives **3** was reduced to its respective amine **4** with stannous chloride in aqueous HCl. Compound **4** was cyclized to its respective TFQ **5** using NaNO2 in aqueous HCl via diazotization reaction, scheme 1. Compound **2-5** were identified and characterized by IR, MS, EA and 1H and 13C NMR spectroscopic analyses. Their data is presented in the experimental part.

The 1H NMR spectra of all of the synthesized compounds contained a doublet for H-5 (*JH-F*=11-13 Hz) at ~8.0 ppm. The splitting of this signal was caused by the vicinal fluorine and indicated the presence of the FQ nucleus in all of these compounds. Similarly, the singlet for H-2 at ~9.0 ppm effectively confirmed that compound **1** had been formed successfully. Similar patterns were also observed for compounds **2-5** (H-5 in compounds **5**). The 1H NMR spectra of compounds **2-5** contain two new multilplets and broad singlets in the range of 0.85-2.4, which were assigned to the aliphatic side chain. These signals effectively confirmed that hexylaniline had been effectively incorporated into compounds **2-5**. Moreover, new singlets, two new doublet of doublet and broad singlets in the ranges of 6.62-7.4 and 7.8-9.1 had appeared in the1H NMR spectra of compounds **2-5** which were assigned to the aromatic side chain and NH-aromatic, respectively. Again, these signals effectively confirmed that hexylaniline had been effectively incorporated into compounds **2-5.** Furthermore, the appearance of a broad singlet at 4.2-4.5 ppm indicated that the reduction step had proceeded successfully to give compound **4.** The disappearance of such broad singlet confirmed that compound **4** had undergone the diazotization and further cyclization reactions to give its cyclized respective **5**. All of the carbons belonging to the hexylaniline sidechain were recognizable by their number, position and orientation in depth charts in the aliphatic and the aromatic regions. These signals confirmed that the hexylaniline sidechain had been successfully incorporated. The 13C NMR spectra of compounds **1-5** contained a doublet (*1JC-F* = 250 Hz) at ~150 ppm for C-6 (C-4 in compound **5**), which indicated the presence of the FQ nucleus in all of these compounds. The splitting of the neighboring carbon signals at C-5 (C-3a in compound **5**) and C-7 (C-5 in compound **5**) into doublet peaks in these compounds (*2JC-F* ~20 Hz) effectively confirmed that they were all vicinal to a fluorine atom.

3.2. Anti-glycation effects of FQs' and TFQ' on methylglyoxal induced cytotoxicity and Anti-inflammatory effects on LPS-stimulated RAW 264.7 mouse macrophages

In this newly developed screening method adapted to

96-well microtiter plate, the identification of carbonyl scavengers was based to using a rapid glycation system that proceeds independent of oxygen, and therefore, excludes identification of inhibitory compounds acting as antioxidants. MGO exerted a cytotoxicity IC_{50} value of 306.15 ±45.6 μM; henceforth it was the choice glucotoxicity concentration. Aminoguanidine (AG) was identified as a potent inhibitor of non-oxidative advanced glycation with an IC_{50} value of 3.1±0.35 μM (its unselective cytotoxicity IC_{50} value (5394.4±1345.21 μM) in absence of MGO was more than a 1000fold increment of its efficacious antiglycation range (Table 1). Comparative analyses demonstrated the superior antiglycation activity of the derivative **5** with IC_{50} (μM) value of 1.61±0.14; thus hugely exceeding AG efficacy. Compounds **3** and **4** could impressively exert a comparable protection against methylglyoxal-induced carbonyl toxicity (respective IC_{50} values of 7.9 and 6.35 μM) with a moderate safety profile among the rest, AG inclusive (Table 1). Our research qualifies FQs and TFQs as promising candidates for the development of related α-dicarbonyl scavengers as therapeutic agents to protect cells against carbonyl stress. In the ascending order, the inhibitory bioactivities of compounds **3< 4< 5** against LPS-induced nitric oxide (NO) production in RAW 264.7 macrophages were examined using the Griess assay. All 3 of them incomparably exceeded the positive control drug indomethacin (IC_{50} value= 212 μM) efficacy with minimal cytotoxicity against RAW 264.7 macrophages (Table 1).

3.3. FQs' and TFQ' as In vitro inhibitors of PL activity but not DPP IV and modulators of proliferative activity in obesity related colorectal cancer cell lines

Diprotin A is a selective reversible peptide inhibitor of DPP IV with in vivo antidiabetic effects. [40, 41] DPP IV is involved in the inactivation of GLP-1, a potent insulinotropic peptide. Thus, DPP IV inhibition can be an effective approach to treat type 2 DM by potentiating insulin secretion. [37, 38] Nevertheless and highly unlike Diprotin A dose dependent inhibition of DPP IV with an IC50 value of 5.14±0.75 μM, none of the tested synthetic compounds could perform equally effectively (Table 1). Substantially based to the antilipolytic activity of newly synthesized antimicrobial agents; [7] this study aimed is to examine new potential PL inhibitors for dual management of obesity and diabetes. The IC_{50} value of the standard compound orlistat was 0.2 μM, which was comparable to the values cited in the literature [63-64]. Currently the tested compounds were recognized for their dose-dependent anti-PL activity with their IC_{50} values (in an ascending order **4<5<3**) displayed in Table 2. Appreciable antiproliferative effectiveness of tested compounds **3** and **4** (but not **5**) against a panel of cancer cell lines was demonstrated with respective IC_{50} values (Table 2). Cisplatin antiproliferative efficacies in all colorectal carcinomas are further illustrated (the same Table). Noticeably among these present bioactive compounds; **3** and **4** could be identified for their comparable or outstanding antiprolifertaive capacities vs. cisplatin in the whole panel of colorectal cancer cell lines. Nevertheless, incomparable to cisplatin unselective cytoxicity in noncancerous periodontal ligament fibroblasts; tested compounds **3, 4** and **5** could be ascribed a high safety profile (Table 2). Recently, the accumulation of advanced glycation end products (AGEs) *in vivo* has been implicated as a major pathogenic process in atherosclerosis, Alzheimer's disease and normal aging. Also, accelerated AGEs build up under hyperglycaemic conditions is a characteristic of T2DM and contributes to the development of vascular complications. As such, inhibition of AGE formation represents a potential therapeutic target for the prevention of premature aging and treatment of diabetic complications. Methylglyoxal, a metabolite increased in diabetes, induces cell death through endoplasmic reticulum stress-associated ROS production and mitochondrial dysfunction [65]. It is associated with insulin resistance, vascular dysfunction and neuropathies [66]. Naturally occurring polyphenols were confirmed for their physiologically regulated antiglycation action mechanisms [67-68]. The antioxidative-antiinflammtory troxerutin (a flavonol derivative of rutin) was proven for its antiglycation potential in preventing the postglycation aggregation of

albumin [69]. Glycated albumin was found proinflammtory eliciting the activation and secretion of cytokines [70]. Besides Non-enzymatic glycosylation of human serum albumin manifests immunological complications in diabetes mellitus due to change in its structure that enhances neo-epitopes generation with high autoantibodies titre [71-72]. Recently, anti-inflammatory molecules that inhibit AGEs have been shown to be good candidates for ameliorating diabetic complications as well as degenerative diseases [73]. Scaffold similarities among the structures of polyphenols and FQs attracted our attention to investigate the inhibitory activities of the synthetic FQs and TFQs **3-5** as dual glycation-inflammation inhibitors. Our selection was based entirely on the simple postulation that similar chemical structures could have similar biological activities [74]. Further work is still necessary to enhance its efficacies via optimizing their structure activity relationship (SAR). Intense efforts by pharmaceutical industry to identify new targets for obesity-diabetes (Diabesity) pharmacological intervention has led to a number of agents developed and directed at the antilipase activity based mainly to 4-oxo-pyridine-3-carboxylic acid.

CONCLUSION

In comparison to Arabiyat et al.[75]; Six newly synthesized FQs and TFQs were tested with respect to their *in vitro* dual glycation inflammation modulation, DPP IV inhibitory activity, antilipase and antiproliferative activities. Further structural modification and optimization is required to improve inhibitory activities of this novel series of FQs and TFQs. Action mechanistic studies for enzyme inhibition and apoptogenic antiproliferative efficacies need to be undertaken. The active compounds with proven dual antiglycation-antiinflammatory propensities correlate well with its proposed success at retarding and reversing development and progression of diabetic complications and aging, thus providing templates for drug design with high safety and efficacy profile. Novel antidiabesity strategies may be formulated thereby translating active compounds inclusion in healthy diet to the clinical practice for treatment/prevention of diabesity-related chronic diseases. Further assessment and treatment of the diabesity patients should address overall cardiovascular disease risk mainly attributable to adiposity; where closely monitored clinical trials can identify the most effective drug therapies for reducing cardiovascular disease in obese patients.

Financial & competing interests disclosure We are grateful for the Scientific Research Fund at the Ministry of Higher Education [grant MPH/1/05/2014] and the Deanship of Academic Research & Quality Assurance at the University of Jordan for research funds [grant 49/2015-2016 (1850)]. The authors report no conflicts of interest.

Scheme 1. Synthesis of target compounds (R= hexyl)

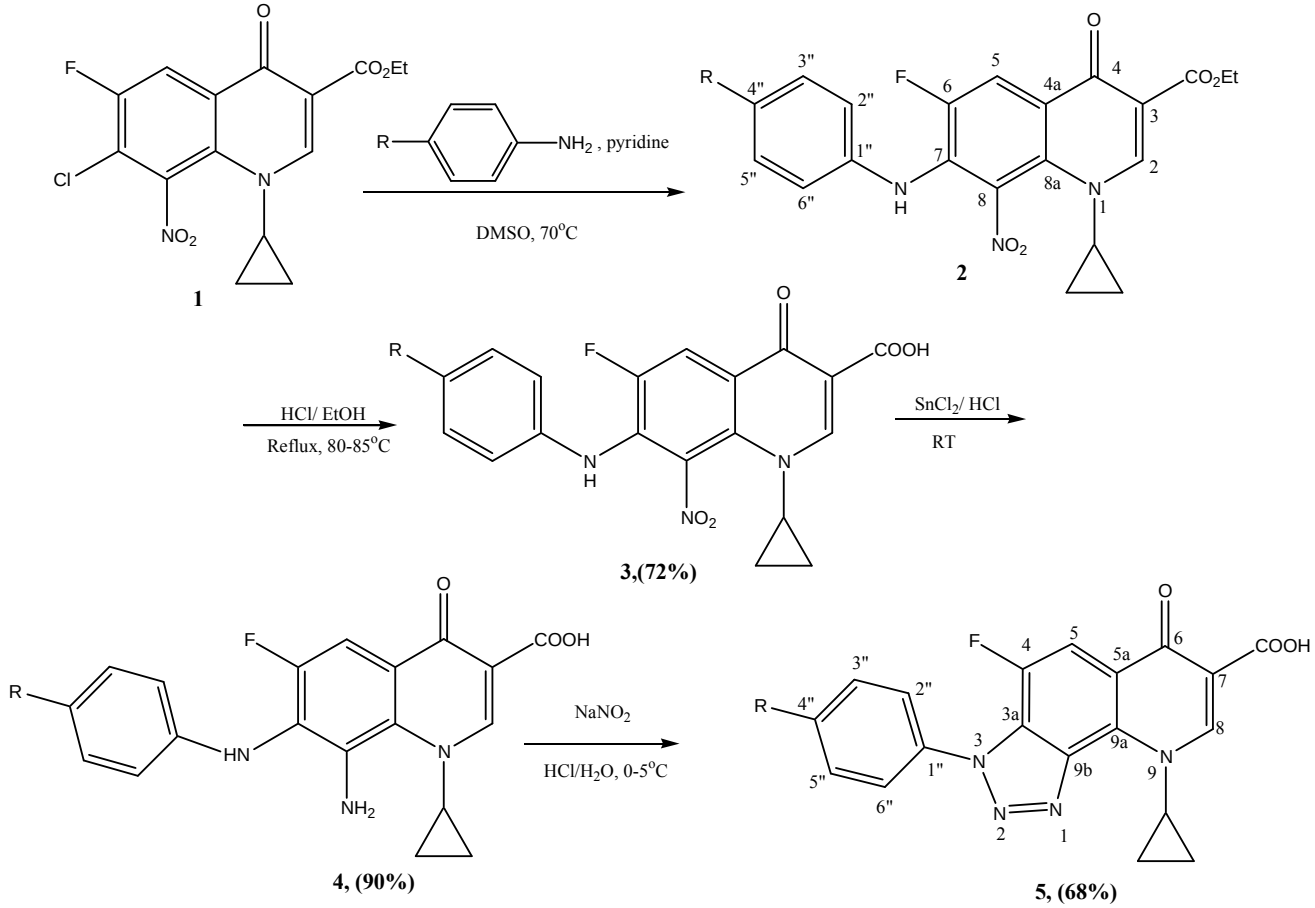

Table 1

IC_{50} values (µg/mL; µM) of In vitro antiinflammatory activities of tested compounds and reference drugs on LPS-induced Nitric Oxide production and methylglyoxal induced cytotoxicity in RAW264.7 murine macrophages

Treatment	Glycation-IC_{50} value µM (µg/mL)	Glycation related Cytotox.-IC_{50} value µM (µg/mL)	NOS-IC_{50} value µM (µg/mL)	NOS related Cytotox.-IC_{50} value µM (µg/mL)	DPP4-IC_{50} value µM (µg/mL)
HXANCETA (3)	7.9±0.71 (3.6±0.32)	21.51±1.94 (9.8±0.88)	**6.31±0.35** (2.87±0.16)	1613.6±242 (735±110.3)	NI
RED-HXANCETA (4)	6.35±0.57 (2.7±0.24)	9.4±0.85 (4±0.36)	**23.78±2.82** (10.12±1.2)	250.1±8.96 (106.4±3.81)	NI
T-HXANCETA (5)	**1.61±0.14** (0.7±0.06)	34.21±3.08 (14.9±1.34)	**67.62±12.17** (29.45±5.3)	NI	NI
Reference Drug	Aminogaunidine 3.1±0.35 µM (0.34±0.0 µg/mL)	Aminogaunidine 5394.4±1345.21 µM (596.62±148.8 µg/mL)	Indomethacin 212±8 µM (75.9±4.7 µg/mL)	NI	Diprotin-A (Ile-Pro-Ile) 5.14±0.75 µM (1.76±0.26 µg/mL)

Results are mean ± SD (n = 3-4 independent replicates). IC_{50} values (concentration at which 50% inhibition of bioactivity determined in comparison to non-induced basal incubations) were calculated within dosage range (µg/mL). **Bolded** numerals stand out as the least IC_{50} values (most active) among others enlisted. NI: Non Inhibitory in the tested range of concentrations

Table 2

IC_{50} values (µM; µg/mL) of In vitro antiproliferative and antilipolytic activities of tested compounds and reference drugs on colorectal cancer cell lines and pancreatic triacylglycerol lipase bioassay

	Cytotoxicity (as of %Control) IC_{50} value µM (µg/mL)						PL-IC_{50} value
Treatment	**HT29**	**HCT116**	**SW620**	**CACO2**	**SW480**	**Fibroblasts**	**µM (µg/mL)**
HXANCETA (3)	**3.3±0.1 (7.2±0.1)**	**3.1±0.4 (6.8±0.8)**	**2.5±0.3 (5.6±0.7)**	**4.9±0.2 (10.7±0.4)**	**1.3±0.1 (2.8±0.1)**	**1.6±0.2 (3.5±0.4)**	70.5±5.6 (32.1±2.6)
RED-HXANCETA (4)	**2.0±0.2 (4.7±0.5)**	**1.4±0.6 (3.4±1.4)**	**4.03±0.3 (9.5±0.8)**	**3.8±0.5 (9.0±1.2)**	**0.1±0.0 (0.3±0.0)**	**0.7±0.1 (1.7±0.3)**	19.1±2.6 (8.2±1.1)
T-HXANCETA (5)	26.7±2.7 (61.2±6.2)	33.8±4.6 (77.4±10.6)	42.6±3.4 (97.7±7.9)	33±1.4 (75.4±3.3)	33.8±1.9 (77.4±4.4)	41.4±4.9 (94.8±11.3)	29.5±0.8 (12.9±0.3)
Reference Drug	Cisplatin 2.1±0.2 (6.9±0.5)	Cisplatin 11.4±0.02 (38.0±0.1)	Cisplatin 1.7±0.3 (5.7±0.9)	Cisplatin 0.4±0.06 (**1.3±0.2**)	Cisplatin 1.6±0.2 (**5.3±0.7**)	Cisplatin 2.1±0.2 (7.0±0.7)	Orlistat 0.2±0.0 (0.11±0.01)

Results are mean ± SD (n = 3-4 independent replicates). IC_{50} values (concentration at which 50% inhibition of cell proliferation took place in comparison to non-induced basal 72 h incubations) were calculated within 0.1-200 µg/mL range. **Bolded** numerals stand out as the least IC_{50} values (most active) among others enlisted in the same tested colorectal cell line. NI: Non Inhibitory in the tested range of concentrations.

REFERENCES

(1) Appelbaum PC, Hunter PA. (2000).The fluoroquinolone antibacterials: past, present and future perspectives. *International Journal of Antimicrobial Agents* 16, 5-15

(2) Ball P. (2000). Quinolone generations: natural history or natural selection? *Journal of Antimicrobial Chemotherapy* 46, 17-24

(3) Langer O, Mitterhauser M, Wadsak W, Brunner M, Muller U, Kletter K, Muller M. (2003). A general method for the fluorine-18 labelling of fluoroquinolone antibiotics. *Journal of Labelled Compounds and Radiopharmacy* 46, 715-727

(4) Bolon MK. (2011). The newer fluoroquinolones. *Medicinal & Clinical Northern America* 95, 793-817

(5) Edmont DS, Rocher R, Plisson C, Chenault J. (2000).Synthesis and evaluation of quinoline carboxyguanidines as antidiabetic agents. *Bioorganic Medicinal Chemistry Letters* 10,1831-1834

(6) Zhao Y-L, Chen Y-L, Sheu J-Y, Chen IL, Wang T-C, Tzeng C-C. (2005). Synthesis and antimycobacterial evaluation of certain fluoroquinolone derivatives. *Bioorganic Medicinal Chemistry* 13, 3921-3926

(7) Al-Hiari YM, Kasabri VN, Shakya AK, Alzweiri MH, Afifi FU, Bustanji YK, Al-Masri IM. Fluoroquinolones: novel class of gastrointestinal dietary lipid digestion and absorption inhibitors. *Medicinal Chemistry Research* 23, 1-11(2014).

(8) El-Rayes BF, Grignon R, Aslam N, Aranha O, Sarkar FH. (2002). Ciprofloxacin inhibits cell growth and synergises the effect of etoposide in hormone resistant prostate cancer cells. *International Journal of Oncology* 21, 207-211

(9) Shaharyar M, Ali MA, Abdullah MM. (2007). Synthesis and antiproliferative activity of 1-[(sub)]-6-fluoro-3-[(sub)]-1, 3, 4-oxadiazol-2-yl-7-piperazino-1, 4-dihydro-4-quinolinone derivatives. *Medicinal Chemistry Research* 16, 292-299

(10) Kathiravan MK, Khilare MM, Nikoomanesh K, Chothe AS, Jain KS. (2013). Topoisomerase as target for antibacterial and anticancer drug discovery. *Journal of Enzyme Inhibition & Medicinal Chemistry* 28, 419-435

(11) Asif M. (2014). A Brief Review on Antitubercular Activity of Pharmacological Active Some Triazole Analogues. *Global Journal of Research & Reviews* 1, 51-58

(12) Haider S, Alam MS, Hamid H. (2014). 1, 2, 3-triazoles: Scaffold with medicinal significance**.** ***Inflammatory Cell Signalling*** 1, e95

(13) Asif M. Anti-neuropathic and anticonvulsant activities of various substituted triazoles analogues. ***Chemistry Internal*** 1, 174-183 (2015).

(14) Carta A, Piras S, Palomba M, Jabes D, Molicotti P, Zanetti S. (2008). Anti-mycobacterial activity of quinolones. Triazoloquinolones a new class of potent anti-mycobacterial agents. ***Anti-Infectious Agents in Medicinal Chemistry*** 7, 134-147

(15) Peng X, Ma J, Chen F, Wang M. (2011). Naturally occurring inhibitors against the formation of advanced glycation end-products. ***Food & Function*** 2, 289-2301

(16) Harris CS, Beaulieu L-P, Fraser M-HlN, McIntyre KL, Owen PL, Martineau LC, Cuerrier A, Johns T, Haddad PS, Bennett SAL. (2011). Inhibition of advanced glycation end product formation by medicinal plant extracts correlates with phenolic metabolites and antioxidant activity. *Planta Medica* 77, 196-204

(17) Wu C-H, Huang S-M, Lin J-A. Yen G-C. (2011). Inhibition of advanced glycation end product formation by foodstuffs. *Food & Function* 22, 24-234

(18) Wu C-H, Huang H-W, Huang S-M, Lin J-A, Yeh C-T, Yen G-C. (2011). AGE-induced interference of glucose uptake and transport as a possible cause of insulin resistance in adipocytes. *Journal of Agriculture & Food Chemistry* 59, 7978-7984

(19) Coughlan MT, Yap FYT, Tong DCK, Andrikopoulos S, Gasser A, Thallas-Bonke V, Webster DE, Miyazaki J-I, Kay TW, Slattery RM. (2011). Advanced glycation end products are direct modulators of ß-cell function. *Diabetes***.** 60, 2523-2532

(20) Puddu AL, Viviani G. (2011). Advanced glycation

endproducts and diabetes. Beyond vascular complications. *Endocrine, Metabolism and Immunity Disorders* 11, 132-140

(21) Zhu Y, Shu T, Lin Y, Wang H, Yang J, Shi Y, Han X. (2011). Inhibition of the receptor for advanced glycation end products (RAGE) protects pancreatic -cells. *Biochemistry & Biophysics Research Communications* 404, 159-65

(22) Shu T, Zhu Y, Wang H, Lin Y, Ma Z, Han X. (2011). AGEs decrease insulin synthesis in pancreatic β-cell by repressing Pdx-1 protein expression at the post-translational level. ***PLoS One.*** 6, e18782

(23) McKillop AM, McCluskey JT, Boyd AC, Mooney MH, Flatt PR, O'Harte FP. (2000). Production and characterization of specific antibodies for evaluation of glycated insulin in plasma and biological tissues. ***Journal of Endocrinology*** 167, 153-163

(24) Hunter SJ, Boyd AC, O'Harte FPM, McKillop AM, Wiggam MI, Mooney MH, McCluskey JT, Lindsay JR, Ennis CN, Gamble R. (2003). Demonstration of glycated insulin in human diabetic plasma and decreased biological activity assessed by euglycemic-hyperinsulinemic clamp technique in humans. ***Diabetes***. 52, 492-498

(25) O'Harte FPM, H[ ]jrup P, Barnett CR, Flatt PR. (1996). Identification of the site of glycation of human insulin. ***Peptides***. 17, 1323-1330

(26) O'Harte FPM, Boyd AC, McKillop AM, Abdel-Wahab YHA, McNulty H, Barnett CR, Conlon JM, Højrup P, Flatt PR. (2000). Structure, antihyperglycemic activity and cellular actions of a novel diglycated human insulin. ***Peptides***. 21, 1519-1526

(27) Guedes S, Vitorino R, Domingues MRrM, Amado F, Domingues P. (2009). Mass spectrometry characterization of the glycation sites of bovine insulin by tandem mass spectrometry. ***Journal of American Society of Mass Spectroscopy*** 20, 1319-1326

(28) Szwergold BS. Intrinsic toxicity of glucose, due to non-enzymatic glycation, is controlled in-vivo by deglycation systems including: FN3K-mediated deglycation of fructosamines and transglycation of aldosamines. *Med Hypoth* 65, 337-348 (2005).

(29) Szwergold BS, Howell SK, Beisswenger PJ. (2005). Transglycation—A Potential New Mechanism for Deglycation of Schiff's Bases. *Annals of New York Academy of Science* 1043, 845-864

(30) Ramamurthy B, Jones AD, Larsson L. (2003). Glutathione reverses early effects of glycation on myosin function. *American Journal of Cell Physiology* 285, C419-C424

(31) di Wu Q, Wang JH, Fennessy F, Redmond HP, Bouchier-Hayes D. (1999). Taurine prevents high-glucose-induced human vascular endothelial cell apoptosis. *American Journal of Cell Physiology* 277, C1229-C1238

(32) Keita Y, Michailova M, Kratzer W, Wrner G, Wrner W, Rietbrock N. (1992). Influence of penicillamine on the formation of early non-enzymatic glycation products of human serum proteins. *International Journal of Clinical Pharmacy & Therapeutic Toxicology* 30, 441

(33) Argirova M, Argirov O. (2003). Inhibition of ascorbic acid-induced modifications in lens proteins by peptides. *Journal of Peptide Science* 9, 170-176

(34) Szwergold BS. (2005). Carnosine and anserine act as effective transglycating agents in decomposition of aldose-derived Schiff bases. *Biochemical & Biophysical Research Communications* 336, 36-41

(35) Szwergold BS. (2006). Alpha-Thiolamines such as cysteine and cysteamine act as effective transglycating agents due to formation of irreversible thiazolidine derivatives. *Medical Hypothesis* 66, 698-707

(36) Vanhoof G, De Meester I, Van Sande M, Scharpe, S, Yaron A. (1992). Distribution of proline-specific aminopeptidases in human tissues and body fluids. *Clinical Chemistry & Laboratory Medicine* 30, 333-338

(37) Hsu K-C, Tung Y-S, Huang S-L, Jao C-L. (2013). Dipeptidyl peptidase-IV inhibitory activity of peptides in porcine skin gelatin hydrolysates. Bioactive food peptides in health and disease: *InTechnology*.

(38) Singh A-K, Jatwa R, Purohit A, Ram H. (2017). Synthetic and phytocompounds based dipeptidyl peptidase-IV (DPP-IV) inhibitors for therapeutics of diabetes. *Journal of Asian Natural Product Research* 1-10

(39) Gorrell MD. (2005). Dipeptidyl peptidase IV and related enzymes in cell biology and liver disorders. *Clinical Science* 108, 277-292

(40) Jao C-L, Y-S C-C, Hung Tung, Lin P-Y, Chen M-C, Hsu K-C. (2015).The development of bioactive peptides from dietary proteins as a dipeptidyl peptidase IV inhibitor for the management of type 2 diabetes. *Biomedicine***.** 2015, 5

(41) Zhang Y, Chen R, Ma H, Chen S. (2015). Isolation and identification of dipeptidyl peptidase IV-inhibitory peptides from trypsin/chymotrypsin-treated goat milk casein hydrolysates by 2D-TLC and LC-MS/MS. *Journal of Agriculture & Food Chemistry* 63, 8819-8828

(42) Horie A, Tokuyama Y, Ishizuka T, Suzuki Y, Marumo K, Oshikiri K, Ide K, Sunaga M, Kanatsuka A. (2014). The dipeptidyl peptidase-4 inhibitor vildagliptin has the capacity to repair β-cell dysfunction and insulin resistance. *Hormone & Metabolism Research* 46, 814-818

(43) Kawalec P, Mikrut A, Łopuch S. (2014).The safety of dipeptidyl peptidase-4 (DPP-4) inhibitors or sodium-glucose cotransporter 2 (SGLT-2) inhibitors added to metformin background therapy in patients with type 2 diabetes mellitus: a systematic review and meta-analysis. *Diabetes/Metabolism Research & Reviews* 30, 269-283

(44) Savarese G, D'Amore C, Federici M, De Martino F, Dellegrottaglie S, Marciano C, Ferrazzano F, Losco T, Lund LH, Trimarco B. (2016). Effects of dipeptidyl peptidase 4 inhibitors and sodium-glucose linked cotransporter-2 inhibitors on cardiovascular events in patients with type 2 diabetes mellitus: a meta-analysis. *International Journal of Cardiology*. 220, 595-601

(45) Gómez-Peralta F, Abreu C, Mora-Navarro G, López-Morandeira P, Pérez-Gutierrez E, Cordero-Garcí a B, Brito-Sanfiel M, Giugliano D, Maiorino MI, Bellastella G. (2011). Switching from Premixed Insulin To Basal Insulin Analogue For Type 2 Diabetes and Role of Dipeptidyl Peptidase-4 Inhibitors. *Experimental & Clinical Endocrinology & Diabetes*. 92, 1-10

(46) Almasri IM, Taha MO, Mohammad MK. (2013). New leads for DPP IV inhibition: structure-based pharmacophore mapping and virtual screening study. *Archives of Pharmaceutical Research*. 36, 1326-1337

(47) Saleh S, Mohammad M, Mashallah SH, Elayeh E, AlMasri I, AlKhatib HS, Fararjeh M, Bustanji Y. (2012). Inhibition of DPP IV is a suggested mechanism of the immunomodulatory effects of sparfloxacin. *Scientific Research Essays* 7, 310-317

(48) Sissi C, Palumbo M. (2003).The quinolone family: from antibacterial to anticancer agents. *Current Medicinal Chemistry* 3, 439-450

(49) Wondrak GT, Cervantes-Laurean D, Roberts MJ, Qasem JG, Kim M, Jacobson EL, Jacobson MK. (2002). Identification of α-dicarbonyl scavengers for cellular protection against carbonyl stress. *Biochemical. Pharmacy* 63, 361-73

(50) Vichai V, Kirtikara K. (2006).Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nature Protocols* 1, 1112-1116

(51) Farsalinos KE, Romagna G, Tsiapras D, Kyrzopoulos S, Voudris V. (2013). Evaluating nicotine levels selection and patterns of electronic cigarette use in a group of â€œvapersâ€ who had achieved complete substitution of smoking. *Substance Abuse: Research & Treatment***.** 7,139

(52) Ghimeray AK, Lee HY, Kim YH, Ryu EK, Chang MS. (2015). Evaluation of Antioxidant and Anti-Inflammatory Effect of *Rhododendron brachycarpum* Extract Used in Skin Care Product by in Vitro and in Vivo Test. *Technology & Investment* 6, 105

(53) Assanga SBI, Luj LML, Gil-Salido AA, Espinoza CLL, Angulo DF, Rubio-Pino JL, Riera RB. (2017). Anti-inflammatory activity and modulate oxidative stress of *Bucida buceras* in lipopolysaccharide-stimulated RAW 264.7 macrophages and Carrageenan-induced acute paw edema in rats. *Journal of Medicinal Plants Research*. 11,

239-252

(54) Huang J, Wang Y, Li C, Wang X, He X. (2016). Anti-Inflammatory Oleanolic Triterpenes from Chinese Acorns. *Molecules***.** 21, 669

(55) Chakrabarti R, Bhavtaran S, Narendra P, Varghese N, Vanchhawng L, Mohamed Sham Shihabudeen H, Thirumurgan K. (2011). Dipeptidyl peptidase-IV inhibitory activity of *Berberis aristata*. *J Nat Prod*. 4,158-163

(56) Arabiyat S, Al-Rabi'ee A, Zalloum H, Hudaib M, Mohammad M, Bustanji Y. (2016). Antilipolytic and hypotriglyceridemic effects of dietary *Salvia triloba* Lf (Lamiaceae) in experimental rats. *Tropical Journal of Pharmaceutical Research.* 15, 723-728

(57) Kasabri V, Afifi FU, Abu-Dahab R, Mhaidat N, Bustanji YK, Abaza IF, Mashallah S. (2014). In vitro modulation of metabolic syndrome enzymes and proliferation of obesity related-colorectal cancer cell line panel by *Salvia* species from Jordan. *Revue Roumaine de Chimie*. 59, 693-705

(58) MarvinSketch (16.10.24), (2016). ChemAxon. (http://www.ChemAxon. Com)

(59) OMEGA (2.5.1.4) (2013). OpenEye Scientific Software, Santa Fe, NM. http://www.eyesopen.com

(60) Egloff MP, Marguet F, Buono G, Verger R, Cambillau C, van Tilbeurgh H. (1995). The 2.46 A resolution structure of the pancreatic lipase-colipase complex inhibited by a C11 alkyl phosphonate. *Biochemistry***.** 34(9), 2751-2762

(61) OEDOCKING (3.2.0.2) (2015).OpenEye Scientific Software, Santa Fe, NM. (http://www.eyesopen.com)

(62) Al-Hiari YM, Al-Mazari IS, Shakya AK, Darwish RM, Abu-Dahab R. (2007). Synthesis and antibacterial properties of new 8-nitrofluoroquinolone derivatives. *Molecules***.** 12, 1240-1258

(63) Bentley D, Young A-M, Rowell L, Gross Tardio G, Carlile JD. (2012).Evidence of a drug-drug interaction linked to inhibition of ester hydrolysis by orlistat. *Journal of Cardiovascular Pharmacology***.** 60, 390-396

(64) Habtemariam S. (2012). The anti-obesity potential of sigmoidin A. *Pharmaceutical Biology* 50, 1519-1522

(65) Chan C-M, Huang D-Y, S-H Y-P, Huang Hsu, L-Y, Kang Shen C-M, Lin W-W. (2016). Methylglyoxal induces cell death through endoplasmic reticulum stress - associated ROS production and mitochondrial dysfunction. *Journal of Cell Molecular Medicine* 20,1749-1760

(66) Shamsaldeen YA, Mackenzie LS, Lione LA, Benham CD. (2016). Methylglyoxal, a metabolite increased in diabetes is associated with insulin resistance, vascular dysfunction and neuropathies. *Current Drug* ***Metabolism*** 17, 359-367

(67) Valavala VK, Vangipurapu RK, Banam VR, Pulukurthi UMA, Reddy M, Turlapati NR. Effect of mustard (*Brassica juncea*) leaf extract on streptozotocin induced diabetic cataract in wistar rats. *J Food Biochem* 35,109-124 (2011).

(68) Yeh W-J, Hsia S-M, Lee W-H, Wu C-H. (2017). Polyphenols with antiglycation activity and mechanisms of action: A review of recent findings. *J Food Drug Ana*l 25, 84-92

(69) Awasthi S, Ravi A, Saraswathi NT. (2017). Troxerutin imparts preservative effects on albumin by preventing Maillard reaction-mediated early and advanced glycation modification. *Journal of Bio-molecule Structural Dynamics* 35, 2681-2687

(70) IbrahFim AS, El-Remessy AB, Matragoon S, Zhang W, Patel Y, Khan S, Al-Gayyar M.M, El-Shishtawy MM, Liou GI. (2011).Retinal microglial activation and inflammation induced by amadori-glycated albumin in a rat model of diabetes. *Diabetes*. 60,1122-1133

(71) Raghav A, Ahmad J, Alam K. (2016). Impact of glycation on structural and antioxidant function of human serum albumin: relevance in diabetic complications. *Diabetes Metabolic Syndrome Clinical Research & Reviews* 10, 96-101

(72) Raghav A, Ahmad J, Alam K. (2017). Nonenzymatic glycosylation of human serum albumin and its effect on antibodies profile in patients with

diabetes mellitus. *PLoS One*. 12, e0176970

(73) Byun K, Yoo Y, Son M, Lee J, Jeong G-B, Park YM, Salekdeh GH, Lee B. (2017). Advanced glycation end-products produced systemically and by macrophages: A common contributor to inflammation and degenerative diseases. *Pharmacological Therapy* 177, 44-55

(74) Polur H, Joshi T, Workman CT, Lavekar G, Kouskoumvekaki I. (2011). Back to the roots: prediction of biologically active natural products from Ayurveda traditional medicine. *Molecular Informatics* 30, 181-187

(75) Arabiyat S, Kasabi V, Al-Hiari Y, Albashiti R, Almasri I, Sabbah DA (2017). Antilipase and antiproliferative activities of novel fluoroquinolones and triazolofluoroquinolones *Chemical Biology & Drug Design* 90 (6), 1282-1294

مركبات جديدة من الفلوروكولينولونات والترياز واوفلوروكوينولونات كمضادات السمنة-السكري ومضادات الدهنيات ومضادات السرطان

شيرين عربيات[1] ، فيوليت كسابري [2]، يوسف الحياري [2]

[1] كلية السلط، جامعة البلقاء التطبيقية السلط ، الأردن

[2] كلية الصيدلة، الجامعة الأردنية، عمان، الأردن

ملخص

الخلفية والهدف: تم تخليق وتقييم مركبات جديدة من الفلوروكولينولونات والترياز واوفلوروكوينولونات لفعاليتها كمضادات للسرطان ومضادات للدهنيات من خلال قياس فعاليتها ضد انزيم DPP IVو قياس التسكر – الالتهاب

الطريقة:طرق تخليق جديدة تم تصميمها لتصنيع ثلاثة مركبات جديدة من الفلوروكولينولونات . تم استخدام طرق قياس فعالية أنزيمات تعتمد حرائك التثبيط اللوني. تم زراعة خطوط خلايا بنهايات لونية. وبالتالي تم استخدام مرجعيات تتسق مع ذلك.

النتائج: على عكس مركب Diprotin A ، افتقرت مركبات الترياز واوفلوروكوينولونات إلى الفعالية ضد أنزيم DPP IV. بالمقابل كانت فعالية مركب 5 المضادة للتسكر بقيمة µM IC50= 1.61±0.14 كانت تتفوق على فعالية aminoguanidine. مركبات 3 و 4 ثبطت بفعالية مقاربة كمضادة للتسكر بفعاليات (7.9µM و 6.35µM) على الترتيب. تعتبر المركبات الثلاثة متوسطة الأمان. الترتيب التصاعدي لها 3>4>5.فعالية المركبات الثلاثة تجاوزت فعالية مضاد الالتهاب للدواء المرجع اندوميثاسين IC50=212 (µM) ضد إنتاج أكسيد النيتريك. في خلايا RAW 264.7 الخلايا الأكولة مع سمية قليلة. تم التعرف على فعالية المركبات الثلاثه كمضادات الدهنيات بالترتيب التصاعدي 4>5>3 مع أنها اقل فعالية من الاورليستات. الجدير بالذكر أن مركبات 3و 4(و لكن ليس 5) لها فعالية مضادة للسرطان مقارنة بالدواء المرجع سيسبلاتين ضد في خطوط خلايا سرطان القولون (HT29, HCT116, SW620, SW480)

الخلاصة : تم الكشف عن فعالية الفلوروكولينولونات والترياز واوفلوروكوينولونات كمضادات للسكري–السمنة ومضادات للسرطان

الكلمات الدالة: الفلوروكولينولونات والترياز واوفلوروكوينولونات، التسكر–الالتهاب، DPP IV، بنكرياتك ليبيز ، سرطان القولون المرتبط بالسمنة.

تاريخ استلام البحث 2018/12/2 وتاريخ قبوله للنشر 2019/3/19.