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IN VITRO MODULATION OF PANCREATIC LIPASE AND PROLIFERATION OF OBESITY RELATED-COLORECTAL CANCER CELL LINE PANEL BY NOVEL SYNTHETIC TRIAZOQUINOLONES**

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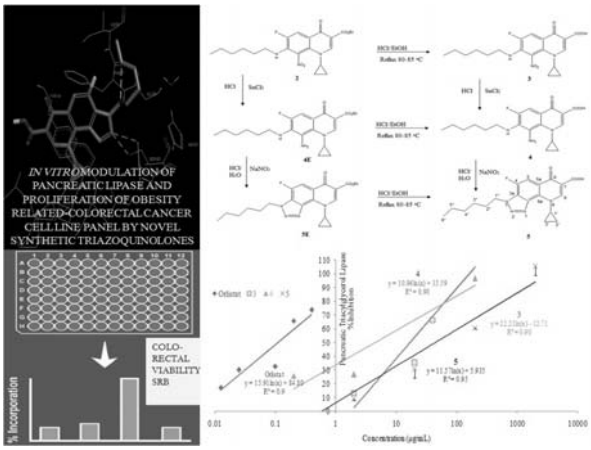
The epidemic of obesity as a disease is alarmingly escalating. Pancreatic triacylglycerol lipase (PL) inhibition is an interesting pharmacotherapy target for the management of obesity and related metabolic disorders. As fluoroquinolones (FQ) have been identified as potent inhibitors of PL; three novel FQs (**3**, **4** and **5**) were synthesized and evaluated *in vitro* with respect to their antilipolytic efficacy and potency properties. ThePL-IC_{50} values of FQs (**3**, **4** and **5**) were 45.3, 13.1 and 131.0 μM, respectively; and further supported by docking studies. Like cisplatin, their antiproliferative propensities against a panel of colorectal cancer cell lines were investigated.
Exceptionally FQ **3**'s unselective cytotoxicity against HT29, HCT116 and SW620 proved comparable to or substantially exceeding that of cisplatin, unlike FQs **4** and **5**. In conclusion, these antilipolytic FQs can advance obesity pharmacotherapy via blocking the entire gastrointestinal fat absorption.



INTRODUCTION

Potential antiobesity pharmacotherapeutic leads were intensely scrutinized as obesity was reaching alarming epidemic proportions globally.[1-2] On one approach, multiple investigations were conducted to explore multiple agents for safe and effective management of dyslipidemia and/or obesity via lipid metabolizing enzymes' inhibition with subsequent reductions in dietary lipids digestion and absorption.[3-7] Orlistat is the only FDA-approved pancreatic lipase inhibitor in US and Europe as many anti-obesity drugs were introduced and removed afterwards from the market due to various side effects.[1] Cetilistat, mainly reported as a well tolerated novel lipase inhibitor in the nanomolar range, had statistically significant slimming efficacies in obese patients with substantial improvements in HbA1c and obesity related parameters.[8-11] Similar to the naturally occurring lipase inhibitor lipstatin;[12] natural products such as alkaloids, carotenoids, glycosides, polysaccharides, saponins and terpenoids are also well studied as active inhibitors of the

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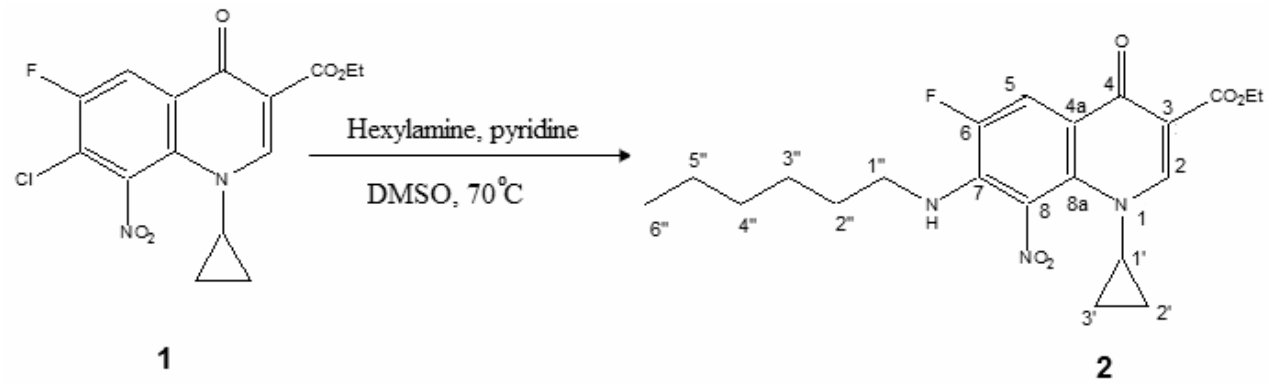
pancreatic lipase.[13,14] Additionally polyphenols, including flavonoids, hydroxycinnamic acids, hydroxybenzoic acids and lignans, were ascribed pancreatic lipase inhibitory effects.[15,16] Remarkably curcumin was recognized for its antilipolytic properties in an outstanding competitive manner.[17] Interestingly the synthesis and structure-activity relationship for a novel class of potent and selective carbamoyl-triazole based inhibitors of hormone sensitive lipase were reported.[18-20] Fluoroquinolones (FQs) derivatives were stated for their outstanding efficacious and potent antlipolytic benefits *in vitro*.[21] In effect, the purpose of present study was to investigate the inhibitory bioeffects of a novel series of FQs derivatives on pancreatic triacylglycerol lipase *in vitro*. Their alleged cytotoxicity against a panel of colorectal carcinoma cell panel (HT29, HCT116, SW620, Caco2 and SW480) was further evaluated.

RESULTS and DISCUSSION

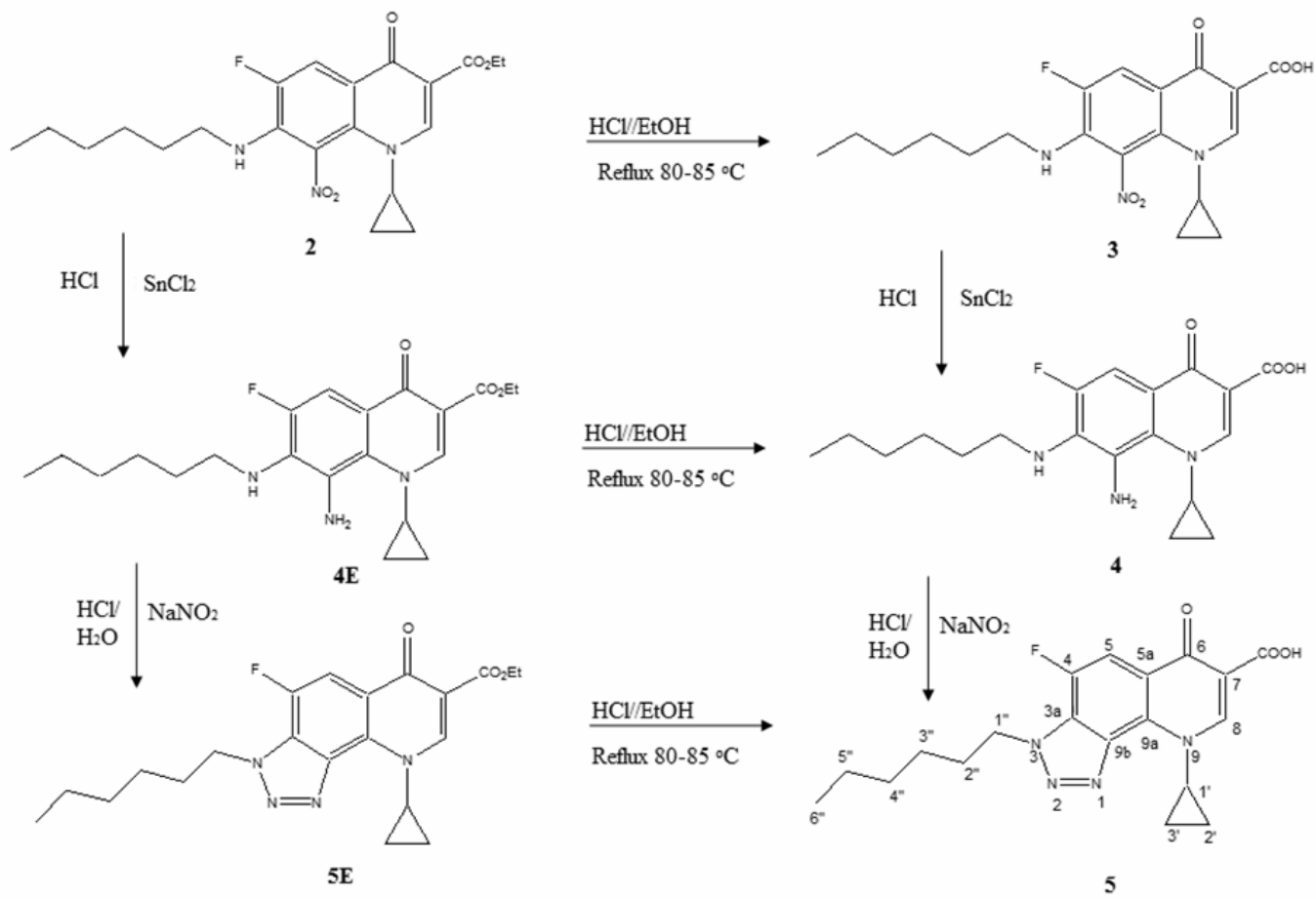
Synthesis of novel compounds

Synthesis of compound **1** was carried out following a previously reported procedure.[22] Compound **2** was synthesized by the reaction of hexylamine with compound **1**, Scheme 1. Hydrolysis of nitro ester **2** has generated nitro acid **3**. The 8-nitro derivative **3** was reduced to the corresponding amine **4** with unhydrous stannous chloride in aqueous HCl. Compound **4** was cyclized to triazoloquinolone **5** using NaNO2 in aqueous HCl via diazotization reaction. Following a second pathway, compounds **4** and **5** have been produced through hydrolysis of corresponding esters **4E** and **5E**, Scheme 2 Compound **1-5** were identified and characterized by IR, MS, and ^{1}H and ^{13}C NMR spectroscopic analyses (Supplementary). Their data is presented in the experimental part.

The ^{1}H NMR spectra of all of the synthesized compounds contained a doublet for H-5 ($J_{H\text{-}F}$=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, 3, 4** and **4E** (H-5 in compounds **5** and **5E**). The ^{1}H NMR spectra of compounds **2-5** contained two new multilplets and broad singlets in the ranges of 0.9-1.5 and 3.35–4.23 which were assigned to the aliphatic side chain and NH-aliphatic, respectively. These signals effectively confirmed that the hexylamine side chain had been effectively incorporated into compounds **2-5**. Furthermore, the appearance of a broad singlet at 4.33 ppm indicated that the reduction step had proceeded successfully to give compound **4** and **4E**. The disappearance of such broad singlet confirmed that intermediate **4E** and compound **4** had undergone the diazotization and further cyclization reactions to give intermediate **5E** and compound **5** respectively. All of the carbons belonging to the hexylamine side-chain were recognizable by their number, position and orientation in depth charts in the aliphatic region. These signals confirmed that the hexylamine chain had been successfully incorporated. The ^{13}C NMR spectra of compounds **1-5** contained a doublet ($^{1}J_{C\text{-}F}$ = 250 Hz) at ~150 ppm for C-6 (C-4 in compounds **5** and **5E**), which indicated the presence of the FQ nucleus in all of these compounds. The splitting of the neighboring carbon signals at C-5, C6a and C-7 into doublet peaks in these compounds ($^{2}J_{C\text{-}F}$ ~20 Hz) effectively confirmed that they were all vicinal to a fluorine atom.



Scheme 1 – Synthesis of compounds (**1** and **2**).



Scheme 2 – Synthesis of compounds (**3-5**).

FQs as In vitro inhibitors of PL activity

The aim of the current study is to develop new potential PL inhibitors containing FQs for dual management of obesity and diabetes. The anti-lipase activities of compounds **3-5** are shown in Table 1 and Fig. 1. These compounds showed dose-dependent anti-PL activity. The IC_{50} values of compounds **3-5** were 45.3, 13.1 and 131.0 µM, respectively. The IC_{50} value of the standard compound orlistat was 0.2 µM, which was comparable to the values cited in the literature.[23-25]

Table 1 reveals that our FQs **3-5** exhibited significant inhibitory activity against PL enzyme. It is well known that highly lipophilic ligands e.g., orlistat with ClogP > 8 do exhibit high inhibitory activity due to the lipophilic nature that perfectly suit the lipophilic catalytic site of PL. These findings are also highlighted for FQs that have been previously published by our research group.[21] Again, the activity of compounds 3-5 could be explained by their high lipophilicity. The difference in their activity could be attributed to the hydrogen bond network that might be formed on C-7 in compounds **3** and **4**/C-8 in compound **5** or COOH with lipolytic site of the enzyme, mainly Ser-152 residue. The preliminary potential *in vitro* activity of compounds 3-5 encouraged us to further conduct molecular docking studies.

Table 1

PL-IC_{50} values (µg/mL; µM) of FQs and orlistat

Treatment	PL-IC_{50} (µg/mL)	PL-IC_{50} (µM)	ClogP*	Chemguass score
3	17.7±1.2	45.3±3.2	4.8	59.1
4	4.8±0.5	13.1±1.4	3.4	54.0
5	48.8±6.2	131.0±16.7	3.8	55.1
Orlistat	0.114±0.01	0.2±0.0	8.6	

Results are mean ± SD (n = 3 independent replicates). * ClogP value was calculated using ChemDraw Ultra (V.7.0.1, 2002)

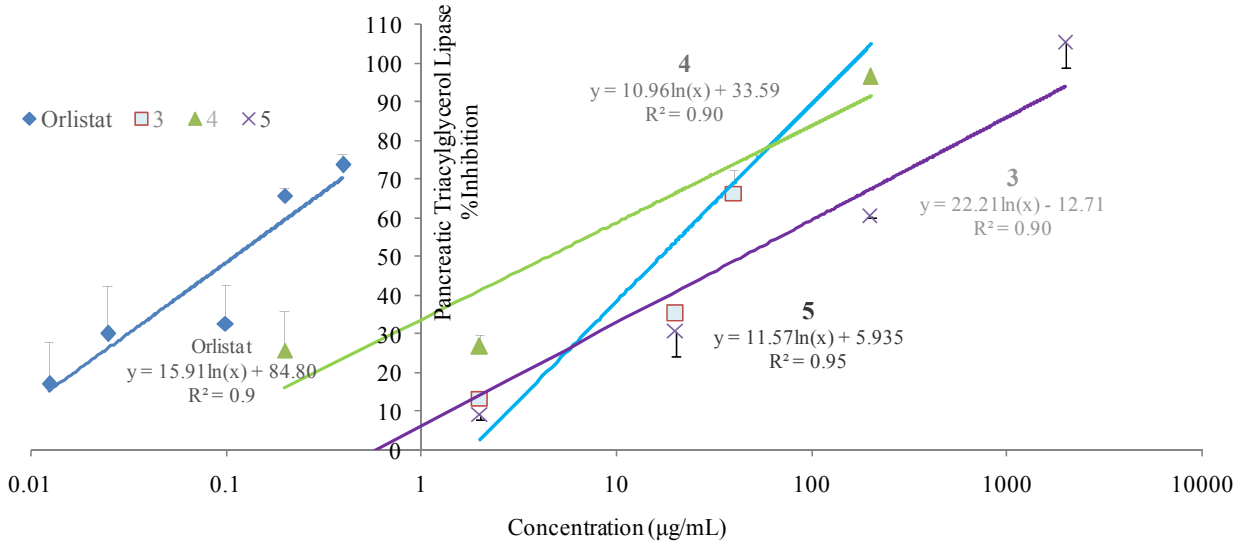


Fig. 1 – *In vitro* inhibitory effects of FQs and orlistat on Pancreatic Triacylglycerol Lipase Activity. Results are mean ± SD (n = 3 independent replicates).

Table 2

IC_{50} values (µg/mL; µM) of *in vitro* antiproliferative activity of FQs and cisplatin on colorectal cancer cell lines

Treatment	Cytotoxicity (as of %Control) IC_{50} value µg/mL (µM)					
	HT29	HCT116	SW620	CACO2	SW480	Fibroblasts
3	0.11±0.02	15.4±2.6	1.1±0.2	13.3±1.9	4.1±0.4	0.1±0.02
	(**0.3±0.1**)	(**39.3±6.7**)	(**2.8±0.5**)	(33.9±4.9)	(10.6±1.0)	(**0.3±0.0**)
4	35.9±4.3	36.2±1.3	31.1±1.5	4.9±0.8	15.9±0.4	15.5±0.8
	(99.3±12.0)	(100.3±3.6)	(86.1±4.1)	(13.7±2.2)	(43.9±1.2)	(43.0±2.3)
5	78.2±5.7	87.7±2.7	51.2±3.8	17.0±0.8	25.9±2.8	30.3±2.7
	(209.9±15.4)	(235.6±7.2)	(137.4±10.2)	(45.8±2.1)	(69.9±7.4)	(81.2±7.4)
Cisplatin	2.1±0.2	11.4±0.02	1.7±0.3	0.4±0.06	1.6±0.2	2.1±0.2
	(6.9±0.5)	(38.0±0.1)	(5.7±0.9)	(**1.3±0.2**)	(**5.3±0.7**)	(7.0±0.7)

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.

Molecular modeling and docking

We have started evaluating some newly synthesized FQs as PL inhibitors due to the reported scaffold similarities in the structures with quinolone and quinolone-based toward PL binding pocket.[21] Presently we have synthesized another new 3 compounds from FQs (**3**, **4** and **5**) and tested them against PL. The possibility of these compounds binding within pancreatic lipase (PL) binding pocket via computer-aided molecular modeling techniques was evaluated employing in-silico docking techniques. The final docked conformations are selected according to their scores. FRED docking engine were used in this study.[26] It was reported to illustrate a good overall performance, particularly in virtual high throughput screening experiments. However, the user has to provide FRED with an optimal set of parameters for the docking experiment (see Experimental). Those parameters differ according to the protein in question. For PL; we have previously identified the optimal docking configuration and scoring function by the self-docking of the co-crystallized ligand. These parameters were further validated experimentally when they were used to successfully predict the bioactivity of some natural PL inhibitors.[17, 21] Therefore, our compounds were docked into the binding pocket of PL employing the same optimal docking parameters, and predicted by simulated docking to bind within the active site of PL. The highest ranking pose for each docked compound within the binding site of PL are shown in Fig. 2. Table 1 shows the *in vitro* activity, CLogP and the estimated binding affinities of docked FQs, which had been calculated using the Chemgauss2 scoring function. On the molecular level, several significant binding interactions can be observed between our docked fluoroquinolones and the

Pancreatic Lipase (PL), as shown in Fig. 2. A comparison of the docked poses of our newly synthesized FQs within the binding site of PL (**3**, **4** and **5**, Fig. 2) with the co-crystallized ligand previously mentioned highlights the similarities in their binding profiles.[17,21] A potential hydrophobic interaction with extra aromatic π–π stacking between flouroquinoline ring and Phe-77, Tyr-114 and Phe-215 amino acid residues which collectively form a lipophilic pocket within the binding pocket of the PL is seen in the three compounds, this hydrophobic interaction was reported previously for the co-crystallized ligand MUP901(C11P). In addition to that they also undergo a hydrophobic interaction with Pro180 and Ile-209 residues. Extra hydrophobic interaction was reported for the ligand with Leu-213 is noticed for compound **3** (IC_{50} = 17.72 μg/mL).[27] The carboxylate group of compound **3**, lies within the positive electrostatic attractive field of the basic amino acid residues Ser-152 and His-263, it also forms a hydrogen bond with Phe-77 which stabilizes the ligand–protein complex and contributes to the affinity of **3** for the protein. In the structure of PL, His-263, Asp-176 and Ser-152 form a triad representing the lipolytic site. Furthermore, enzymatic activity has shown to be diminished after chemical modification of Ser-152 indicating its essential role for the catalytic activity.[26] Thus, it is unsurprising that compounds strongly bind to the catalytic triad; especially Ser-152 could inhibit the lipolytic activity. Both **4** (IC_{50} = 4.75 μg/mL) and **5** (IC_{50} = 48.8 μg/mL) forms a strong hydrogen bonding with Ser-152. The triazole moiety of compound **5** forms another hydrogen bonding with His-263 residue. Although docking studies did not explain the difference in *in vitro* catalytic activity, it has confirmed the critical importance of the hydrogen bond interaction between FQs **3-5** and Ser-152 within the lipolytic site, since all FQs do interact at this site.

FQs' modulation of proliferative activity in obesity related colorectal cancer cell lines

Significant antiproliferative effectiveness of FQs tested against a panel of cancer cell lines was demonstrated with IC_{50} values ≤ 0.11 μg/mL (Table 2). The antiproliferative efficacies of cisplatin in all colorectal carcinomas tested are further illustrated (Table 2).

Exceptionally FQ **3** cytotoxity against HT29, HCT116 and SW620 proved comparable to or substantially exceeding that of cisplatin in 72h incubations. Nevertheless, it lacked selective cytotoxicity in PDL fibroblasts wells (Table 2). Presently among the 3 FQs, FQ **4** antineoplastic efficacies in CACO2 incubations were associated with higher potency. Apart from FQ **3**, the tested FQs were not impressively antiproliferative in any of the SW480 colorectal carcinomas panel incubations.

EXPERIMENTAL

General

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. 2,4-Dichloro-5-fluoro-3-nitrobenzoic acid was purchased from Sigma-Aldrich (St. Luis, MO, USA). Ethyl 3-(N,N-dimethyl-amino) acrylate, cyclopropyl amine and n-hexyl amine were procured from Across (Geel, Belgium). Sodium bicarbonate, sodium dithionite and HCl were obtained from Sigma-Aldrich (St. Luis, MO, USA). 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 (CHCl3-MeOH-FA) (system 1) and 50:50 (*n*-hexane - Ethyl actate) (system 2). Nuclear magnetic resonance spectra (NMR) were recorded on Bruker Advance DPX-300 (300 MHz) spectrometer (Bruker, Bellirica, Massachusetts,USA) and a 400 MHz Bruker Avance Ultrashield.

The chemical shifts were reported in ppm relative to tetramethylsilane (TMS), which was used as an internal reference standard. Deuterated dimethylsulfoxide ($DMSOd_6$) 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. Low-resolution mass spectra (LRMS) were measured by Applied Biosystems-MDS SCIEX API 3200 LC/MS/MS system, employing the positive mode using an electrospray ionizer (ESI) which was operated at 5.0-5.5k V, with the capillary heater at 350 °C, sheath gas pressure 45 psi (USA) and an ion trap analyzer. Molecular weight was recorded as AMU+2 as positive mode of ESI adds 1 AMU to molecular ion peak and also does the iontrap analyzer.

Synthesis of novel title compounds

Compound 1: Ethyl-7-Chloro-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate **(1)**

Synthesis of compound (**1**) was carried out following a previously reported procedure. [22]

Compound 2: Ethyl-1-Cyclopropyl-6-fluoro-7-hexylamino-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate **(2)**

Scheme 1

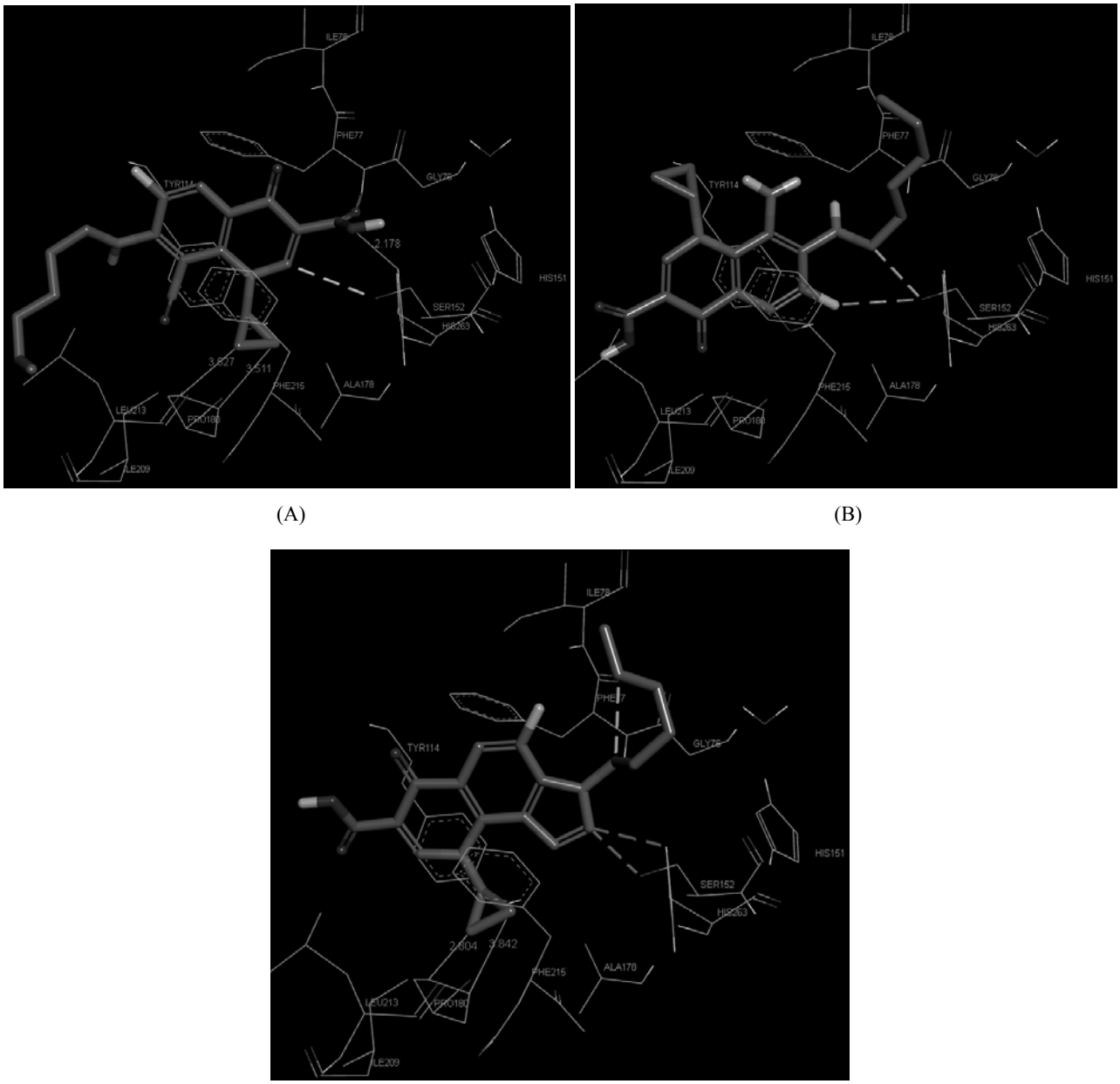


Fig. 2 – Detailed view of the docked pose of FQs derivatives and the corresponding interacting amino acids moieties within the PL-binding site (PDB code: 1LPB, **3:** 122, **4:** 123, **5:** 124).

Three molar equivalents of hexylamine (4.28g, 42.29mmol) were added into a solution containing ethyl 7-chloro-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate **1** (5 gm, 14.1mmol) and 10 ml of dimethylsulfoxide (DMSO) as a solvent and drops of pyridine and then was refluxed for 65-70°C under anhydrous conditions for (4-5) days. The reaction mixture monitored until no starting material remained then was left to crystallize at room temperature and the product was filtered and washed, left to dry in a produce dark place to give a yellow solid. Color of solid compound: yellow; yield ≈ 76% (4.5 g); mp = 111-112°C (Decomposition); R_f value in system 1 = 0.90 and in system 2 = 0.77. ^{1}H NMR (300 MHz, DMSO-d_6): 0.9 (m, 3H, CH_3-6"), 1.1 (m,15H, OCH_2CH_3, CH_2-2",3"4",5", CH_2-2',3'), 3.35 (m, 3H, NCH-1', NCH_2-2"), 4.21 (m, 2H, OCH_2CH3), 7.08 (br s, 1H, NH), 7.88 (d, $^3J_{H-F}$ = 16.98 Hz, 1H, H-5), 8.48 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6): 9.90 (2C,C-2"/3"),13.72 (C6"), 14.13 (OCH_2CH_3), 21.90 (C5"), 25.87 (C-4"), 30.11 (C-3"), 30.71 (C-2"), 38.69 (C-1'), 45.68 (CH_2-1"), 60.03 ($OCH2$), 111.60 (C-3), 114.77 (d,$^2J_{C-F}$ = 22.43 Hz, C-5), 118.56 (C-8a), 127.94 (C-4a), 134.28 (C-8), 136.86 (d, $^2J_{C-F}$ = 14.32 Hz, C-7), 150.80 (C-2),149.14 (d, $^1J_{C-F}$ = 247.87 Hz, C-6),163.56 (CO_2Et), 169.85 (C-4). IR (KBr): ν 3339, 2949, 2922, 1726, 1614, 1514, 1458, 13311, 1233, 950 cm^{-1}. LRMS (ES, +ve) m/z calc. for $C_{21}H_{26}FN_3O_5$: Found 421.4 (100%, M+2), 374.4 (10%), 364.5 (16%), 102.2 (23%), 73.3 (61%), 59.2 (18%). Anal. Calcd for $C_{21}H_{26}FN_3O_5$ (419.19): C, 60.13; H, 6.25; N, 10.02 Found C, 59.87; H, 6.05; N, 9.72**.**

Acid pathway (Scheme 2)

Synthesis of 1-Cyclopropyl-6-fluoro-7-hexylamino-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid **(3)**

A vigorously stirred suspension of ethyl 1-cyclopropyl-6-fluoro-7-hexylamino-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**2**, 2g, 4.8 mmol) 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 36-48 h. Thereafter, the reaction mixture was cooled, poured onto crushed ice (250 g) and the resulting heavy faint yellow precipitate was collected, washed with cold water (2 x 20 mL) and left to dry. Yield ≈1.8 g (90 %). mp = 167-169 °C (decomposition); R_f value in system 1 = 0.73 and in system 2 = 0.76. ^{1}H NMR (300 MHz, DMSO-d_6): 0.78 (t, J = 6.71 Hz 3H, CH_3-6"), 0.95 (m, 4H, , 2CH_2-2'/3'), 1.21 (m,6H, 3CH_2-C-3",4" and 5"), 1.53 (m, 2H, CH_2-2"), 3.47 (t, J = 8.2 Hz, 2H,NCH_2-1"), 3.67 (m, 1H, NCH-1') 7.41 (br s, 1H, N*H*), 7.98 (d, $^3J_{H-F}$ = 14.08 Hz, 1H, H-5), 8.7 (s, 1H, H-2), 14.73 (br s, COO*H*).^{13}C NMR (75 MHz, DMSO-d_6): 10.21 (2CH_2-C2'/3'), 14.31 (CH_3-6"), 22.47 (CH_2-4"), 26.10 (CH_2-5"), 30.68 (CH_2-3"), 31.26 (CH_2-2"), 37.39 (NC-1'), 46.11 (NCH_2-1''), 109.48 (C-3), 114.58 (d, $^2J_{C-F}$ = 22.80 Hz, C-5), 116.26 (C-8a), 135.82 (C-4a), 138.89 (d, $^2J_{C-F}$ = 14.10 Hz, C-7), 141.43 (C-8), 150.30 (d, $_1J_{C-F}$ = 238.28 Hz, C-6), 151.89 (C-2), 165.49 (*C*O_2H), 175.47 (C-4). IR (KBr*)*: *v* 3568, 3374, 3082, 2957, 2932, 2859, 1721, 1626, 1555, 1518, 1456, 1317, 1240, 901 cm^{-1}. LRMS (ES, +ve) m/z calc. for $C_{19}H_{22}FN_3O_5$: Found 392.4 (100%, M+1), 374.4 (14%), 347.4. (16%), 102.2 (9%), 73.2 (53%), 59.2 (16%).Anal. calcd. for $C_{19}H_{22}FN_3O_5$ (391.15): C, 58.31; H, 5.67; N, 10.74. Found C, 58.26; H, 5.41; N, 10.66.

Synthesis of 8-Amino-1-cyclopropyl-6-fluoro-7-hexylamino-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid **(4).**

A mixture of 1-Cyclopropyl-6-fluoro-7-hexylamino-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**3**, 0.2g, 0.51mmol) in 6.7 ml of 12N HCl, left stirring in ice bath (2-5°C) for 15 minutes. After that, the ice bath was removed and (0.44g, 2.3mmol) 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 product that is collected by filtration and left to dry. Yield ≈ 0.18 g (90%). mp = 130-131 °C (decomposition); R_f value in system 1 = 0.35 and in system 2 = 0.24. ^{1}H NMR (400 MHz, DMSO-d6): 0.80 (m, 4H, 2CH_2-2'/3'), 1.00 (3H, CH_3-6"), 1.17-1.52 (m, 6H, CH_2-3",4" and 5"), 3.23 (m, CH_2-2"), 4.50 (m, 1H, NH-1'), 5.14 (s, 1H, NH),5.38 (br s, 2H, NH_2), 7.33(d, d, $^3J_{H-F}$ = 11.68 Hz, 1H,H-5), 8.66 (s, 1H, H-2), 12.82 (br s, 1H, COOH) 13C NMR (75 MHz, DMSO-d_6): 10.35 (CH_3-6"), 14.10 (2CH_2-2'/3'), 22.48 (CH_2-2''), 26.21 (CH_2-3"), 30.44 (CH_2-4''), 31.28 (CH_2-5''), 39.07 (C-1'), 45.88 (C-1"), 100.32 (d, $^2J_{C-F}$ = 17.50 Hz, C-5), 105.68 (C-3), 119.41 (d, $^3J_{C-F}$ = 6.8 Hz, C-4a), 128.13(C-8a), 130.81 (d, $^2J_{C-F}$ = 10.44 Hz, C-7) 130.96 (d, $^3J_{C-F}$ = 4.98 Hz C-8), 130.78 (C-8a), 150.70 (C-2), 152.70 (d, $^1J_{C-F}$ = 240.0 Hz, C-6), 166.39 (*C*OOH), 176.90 (CO,C-4). IR (KBr*)*: *v* 3526, 3390, 3079, 2953, 2926, 2857, 2365, 1701, 1516, 1444, 1336, 1265, 1022 cm^{-1}. LRMS (ES, +ve) m/z calc. for $C_{19}H_{24}FN_3O_3$: Found 363.5 (100%, M+2), 362.2 (43%), 244.3 (34%), 102.2 (22%), 73.2 (46%), 59.2 (22%). Anal. calcd. for $C_{19}H_{24}FN_3O_3$ (361.14): C,63.14; H, 6.69; N, 11.63. Found: C,63.55; H, 7.05; N, 11.88.

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

Compound 5 was synthesized by cyclization of preceding reduced acid **4**. 8-Amino-1-cyclopropyl-6-fluoro-7-hexylamino-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid **4** (0.4g, 1.1mmol) in 20 ml aqueous HCl, left stirring in ice bath (2-5°C) for 15 minutes. NaNO2 (0.08g, 1.2mmol) dissolved in 10 mL 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 (250 g) and the resulting off-white precipitate was collected, washed with cold water (2 x 20 mL) and left to dry. Yield ≈ 0.31 g (75 %). mp = 95-97°C (Decomposition); R_f value in system 1= 1.73 and R_f value in system 2= 1.56.^{1}H NMR (300 MHz, DMSO-d_6): 0.79 (m, 4H, 2CH_2-2'/3'), 0.99 (m, 9H, CH_3-6", CH_2-5",4",3"), 1.99 (m, CH_2-2"), 4.53 (m, 1H, NCH-1'), 4.89 (m, 2H, NCH_2-1"), 7.99 (d, $^3J_{H-F}$ = 10.8 Hz, 1H, H-5), 8.86 (s, 1H, H-8), 12.72 (br s, 1H, COOH). ^{13}C NMR (75 MHz, DMSO-d_6):10.46 (2$CH_2$2'/3'),15.40 (CH_3-6"), 21.84 (C-5"), 25.46 (C-4"), 29.75 (C-3"), 30.48 (C-2"), 41.22 (NC-1'), 49.97 (NCH_2-1'), 107.25 (C-7), 108.50 (d, $^2J_{C-F}$= 18.7 Hz, C-5), 123.78 (C-5a), 126.82 (C-9b), 131.76 (C-9a), 132.89 (d, $^2J_{C-F}$= 16.4 Hz, C-3a), 147.59 (C-8), 165.39 (COOH), 172.45 (C-4) IR (KBr): *v* 3435, 3063, 2953, 2926, 2856, 1722, 1630, 1587, 1510, 1330, 1215, 1028cm^{-1} LRMS (ES, +ve) m/z calc. for $C_{19}H_{21}FN_4O_3$: Found 374.3 (100%, M+2), 355.2 (63%), 347.4 (72%), 102.2 (47%), 73.2 (46%), 59.2 (18%). Anal. calcd. for $C_{19}H_{21}FN_4O_3$ (372.39): C,61.28; H, 5.68; N, 15.05. Found: C, 61.44; H, 6.06; N, 15.38

Preparation of the test compounds and orlistat for the in vitro PL activity assay

Orlistat (10 mg, Sigma, St. Luis, MO, USA) was dissolved in DMSO (10 mL) to give a stock solution (1 mg/mL), which was used to make six different working solutions with concentrations in the range of 0.625 – 20 µg/mL. Thereafter, 20 µL aliquots of each working solution were used in the reaction mixture to give final concentrations in the range of 0.0125–0.4 µg/mL. Furthermore the test compounds (**3-5**) were initially dissolved in DMSO to give three stock solutions, which were subsequently diluted to give five different stock solutions (0.01 – 100 mg/mL). Thereafter, 20 µL aliquots of each stock solution were used in the reaction mixture to give the final concentration range (0.2 – 2000 µg/mL).

Quantification of PL activity by spectrophotometric assay

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 100 µM solution of *para*-nitrophenyl butyrate (*p*-NPB, Sigma, St. Luis, MO, USA) in DMSO was used as the PL substrate. Aliquots (0.1 mL) of the PL solution were added to the reaction mixtures and the volumes were made up to 1 mL with Tris-HCl buffer. The PL was preincubated with different concentrations of the test material for at least 1 min prior to the addition of the substrate. The reactions were maintained at 37 °C and initiated by the addition of 5 µL of the *p*-NPB substrate solution. The *p*-nitrophenol released during the reaction was measured at 410 nm using a 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 catalytic activity of PL was determined colorimetrically by measuring its activity

towards the hydrolysis of *p*-NPB to *p*-nitrophenol. 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.[26] 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.

In vitro antiproliferative assay

Obesity related colorectal cell lines HT29, HCT116, SW620 and SW480 were generously provided by Dr Rick F. Thorne (University of Newcastle, Australia) and were cultured in high glucose DMEM containing 10% FCS (Bio Whittaker, Verviers, Belgium). CACO2 cell line was a gift of Professor Yasser Bustanji, The University of Jordan, School of Pharmacy. 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). 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.[28] Human periodontal fibroblasts (PDL) are a primary cell culture for verification of selective cytotoxicity with the least antiproliferative IC_{50} value obtained. As a robust and classical antineoplastic reference agent, cisplatin (1-100 µg/mL, Sigma, St. Luis, MO, USA) was recruited for comparison purposes.[26] All of the assays were performed in triplicate and the calculated antiproliferative activities were reported as the mean values ± SD (n=3).

Docking Software

CS ChemDraw Ultra 7.01, Cambridge Soft Corp. (http://www.cambridgesoft.Com), USA. OMEGA (Version 2.5.1.4), OpenEye Scientific Software (www. eyesopen.com), USA.[29] FRED (Version 2.2.5), OpenEye Scientific Software, (www.eyesopen.com), USA.[26] BIOVIA Discovery Studio visualizer 4.5, 2015 Biovia, Accelrsy Inc. (www.accelrys.com) USA. All installed in hp Z420 workstation.

Preparation of structures

The chemical structures of the fluoroquinolone derivatives (**3**, **4** and **5**) (Scheme 2) were sketched in Chemdraw Ultra (7.01) and saved in MDL molfile format. Subsequently, an ensemble of energetically accessible conformers was generated using OMEGA2 software.[29] OMEGA builds initial models of structures by assembling fragment templates along sigma bonds. Input molecules graphs are fragmented at exocyclic sigma bonds, and carbon to heteroatom acyclic (but not exocyclic) sigma bonds. Conformations for the fragments are retrieved from pre-generated libraries within the software. Once an initial model of a structure is constructed, OMEGA generates additional models by enumerating ring conformations and invertible nitrogen atoms.[26, 29] The generated conformers are saved in SD format.

Docking experiment

The 3D coordinates of PL were retrieved from the Protein Data Bank (PDB code: 1LPB, resolution; 2.46 Å).[27] Hydrogen atoms were added to the protein using the BIOVIA Discovery Studio visualizer templates for protein residues. No energy minimization for the protein structure was done. The docking study was multiconformer database of one or more ligands, a target protein structure, a box defining the active site of the protein based on the co-crystallized ligand and several optional parameters as input. The ligand conformers and protein structure are treated as rigid entities during the docking process. All possible positions of each ligand in the active site were exhaustively scored by FRED software; this is FRED's docking strategy.[26] The OMEGA software generated conformers of our compounds (**3**, **4** and **5**) was used as input in the FRED software. We employed the docking settings that succeeded in reproducing the experimental pose of the co-crystallized ligand (MUP901 (C11P), Figure 2A).[27] Our previously optimized FRED docking simulation parameters for pancreatic lipase have been reported elsewhere.[17, 26]

CONCLUSIONS

As novel and effective therapies for dyslipidemia and atherosclerosis; the selected series of potent FQs were robustly proven for their *in vitro* antidiabesity activity. Action mechanism of FQs' enzyme inhibition maybe delineated. These synthetic compounds' propensities are warranted further *in vivo* models' testing and clinical trials for effective utilization as diabesity therapeutic and/or preventive agents.

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