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Metal Chelators as Anticancer Approach: Part I; Novel 7-Anisidine Derivatives with Multidentate at 7-8 Carbons of Fluoroquinolone Scaffold as Potential Chelator Anticancer and Antilipolytic Candidates

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ABSTRACT

Background: Cancer is one of the greatest troubling maladies currently. It is believed that it is the second reason for death following cardiovascular maladies. Owing to the multiplicity of its types, stages and genetic basis, there is no existing drug to cure all types of cancer. Resistance to present drugs and severe adverse effects are other challenges in the struggle against cancer. In such pursuit, fluoroquinolones (FQs) have the potential as antiproliferative compounds due to safety, low cost, and absence of resistance.

Aims: In this study, we aim to synthesize biologically active compounds that have dual anticancer and anti-lipase potential. Sixteen compounds were prepared, fully characterized, and studied through identification of IC_{50} values against the highly susceptible cancer cell lines.

Methods: In this work we are concerned with synthesizing biologically active compounds that belong to fluoroquinolones (FQs) with dual anti-colorectal cancer and anti-lipase activity, owing to association between cancer and obesity, conduct titration and docking experiments to validate our hypothesis.

Results: *In vitro* findings indicated that these compounds demonstrated promising anticancer activity against tested cell lines in micromolar range with a potency comparable to cisplatin. Compound **11** exhibited approximately doubled potency compared to cisplatin against SW620 colorectal cancer cell line with IC50 3.2 μM which proposes FQs as potent antiproliferative agents. The synthesized Fluoroquinolone (FQ) compounds were further screened for their *in vitro* anti-lipase potential. The findings demonstrated that all the screened compounds have demonstrated remarkable anti-lipase activity, as compared to control molecule orlistat. Compound **9** exhibited comparable activity to orlistat against pancreatic lipase with IC_{50} 0.4 μM which proposes FQs as potent pancreatic lipase inhibitors.

Conclusions: The anticancer potential of these derivatives is referred to their ability to inhibit Topo II which indicates that chelation is the mechanism of inhibition of Topo II emphasized with titration and docking experiments.

Keywords: Metal chelators, Multidentate, Fluoroquinolones, Chelator anticancer.

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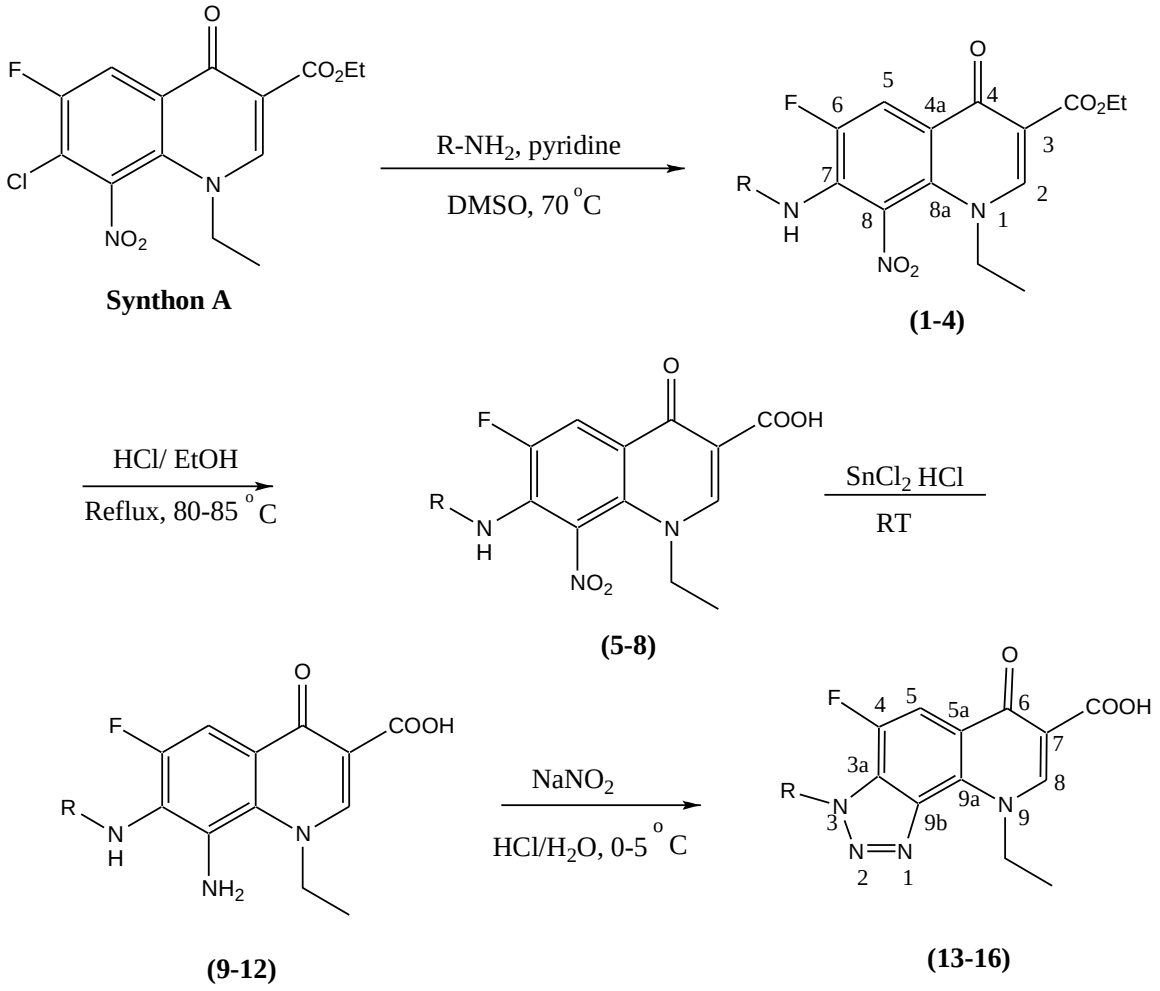
INTRODUCTION

Fluoroquinolones have been recognized for more than 40 years as one of the highest active antibacterial agents [1]. Fluoroquinolones elucidated to have other biological activities as antidiabetic[2], antimycobacterial[3], antiviral[4], anticancer[5], as well as pancreatic lipase inhibitors[6]. The ability of quinolones exemplified by Vosaroxin to target type II topoisomerase enzymes suggests them as anticancer agents. Their potential to chelate metal ions represents another way of achieving their pharmacological activities[7]. Ciprofloxacin use for the experimental adjunctive therapy of lung cancer is reported[8]. Moreover, ciprofloxacin derivatives have shown potent *in vitro* antiproliferative activity[9]. Antitumor activity for a series of N1-decyl and C7 secondary amine derivatives of fluoroquinolones was investigated. Most of the compounds were found significantly potent (IC_{50} <0.01-8.8µM). Their antiproliferative activity has been tested against four cancer cell lines: human breast carcinoma (MDA-MD-231), human pancreatic carcinoma (MIA PaCa), human cervical carcinoma (HeLa) and human neuroblastoma cells (IMR32)[10]. Derivatives of 6-fluoro-4-oxopyrido[2,3-*a*] carbazole-3-carboxylic acids were evaluated for their *in vitro* antiproliferative potential against A549 non-small cell lung cancer cells and MCF-7 breast tumor. Some compounds were very potent compared to positive control ellipticine coupled with an absence of cytotoxicity toward normal human-derm fibroblasts (HuDe)[11]. Vosaroxin (Voreloxin) is an anti-cancer quinolone that inhibits topoisomerase-II leading to cell cycle arrest and apoptosis[12]. It has shown efficacy in a range of solid organ and hematopoietic tumors *in vitro*[13]. Referring to the potential biological interest in these heterocyclic compounds[14] **,**numerous FQs derivatives have been synthesized and evaluated for anticancer potential. Many compounds were in the form of hybrid structures. Initial cytotoxicity analyses were conducted for some derivatives against MCF-7 cells, a human breast adenocarcinoma cell line[15]. Such derivatives demonstrated remarkable bio-properties such as antitumor and/or antimicrobial potential[16]. In the same vein, this work involves new FQs as potential pancreatic lipase inhibitors. Pancreatic lipase (PL) is the key enzyme for lipid absorption. It catalyzes the hydrolysis of triacylglycerides in the gastrointestinal tract and is responsible for the hydrolysis of 50–70% of total dietary fats[17,18]. Orlistat, a semisynthetic derivative of lipstatin, is a selective and potent inhibitor of PL. The success of Orlistat has encouraged research to identify newer PL inhibitors that lack unpleasant side effects like oily stool, abdominal pain, flatulence and fecal urgency[19]. Fluoroquinolone (FQ) derivatives were declared as efficacious and powerful antilipolytic agents *in vitro*[6]. Since Colorectal cancer is statistically associated with obesity, this research aims at preparing new FQs with dual inhibitory activity.

A. EXPERIMENTAL

A. Synthesis of novel title compounds (Supplementary attached)



Synthon A

(1-4)

(5-8)

(9-12)

(13-16)

Scheme 1: Synthetic pathway of target compounds (FQ targets); R: *o*-anisidine, *p*-anisidine, *m*-anisidine, 2,4-dimethoxyaniline

Substitution	Ester	Nitro Acid	Amino	Triazolo
***o*-** methoxy phenyl	**1**	**5**	**9**	**13**
***m*-** methoxy phenyl	**2**	**6**	**10**	**14**
***p*-** methoxy phenyl	**3**	**7**	**11**	**15**
2,4- methoxy phenyl	**4**	**8**	**12**	**16**

B- In vitro antiproliferative assay

Obesity associated colorectal cell lines HT29, HCT116, SW620, and SW480 were kindly provided by Dr. Rick F. Thorne (University of Newcastle, Australia) in high-glucose DMEM (Bio Whittaker, Verviers, Belgium) containing 10% FCS. cultured. The CACO2 cell line was provided by Professor Yasser Bustanji, Faculty of Pharmacy, The University of Jordan. The CACO2 cell line was treated with L-glutamine (2 mM), penicillin (100 U/ml), gentamicin (50 µg/ml), streptomycin sulfate (100 mg/ml), 10% FBS and HEPES buffer (10 mM) (Sigma, St. Louis, Missouri, USA). The cytotoxicity assay was performed using sulforhodamine

B (SRB; Santa Cruz Biotechnology, Inc. Texas, USA) A calorimetric assay to assess the mechanisms of cytotoxicity and decreased cell viability[20]. The method outlined in this work has been adapted for toxicity assessment of compounds against adherent cells in a 96-well format. After the incubation period, cell monolayers were fixed with 10% (w/v) trichloroacetic acid (TCAA), stained for 30 min, and excess pigment was removed by repeated washing with 1% (v/v) acetic acid. Protein-bound pigment is dissolved in 10 mM Tris stock solution for optical density detection at 570 nm using a microplate reader. Human periodontal fibroblasts (PDL) are the primary cell culture to confirm selective cytotoxicity with the lowest antiproliferative IC50 values achieved. Cisplatin (1–100 µg/mL, Sigma, St. Louis, MO, USA) was used as a standard antitumor reference compound for comparison[21]. Relative cell viability was recorded as the average percentage of viable cells compared to DMSO-treated cells (control). All tests were performed in triplicate and the calculated antiproliferative potencies were recorded as mean ± SD (n = 3).

D. Docking Methodology

To explore drug-likeness properties and the prospective anticancer activity of the synthesized FQ derivatives (Fig. 2) against human topoisomerase II (TopII), *in silico* computational tools were used. Docking simulations were conducted to predict FQ's-TopII binding modes as potential target. TopII has been identified as an effective target in the treatment of cancers. The enzyme induces topological changes (unwinding) to the DNA molecule through the formation of transient double-stranded breaks in the DNA double helix, which makes topoisomerase essential for cell proliferation. TopII is targeted by several chemotherapeutic agents e.g., doxorubicin; mitoxantrone; etoposide and daunorubicin[22].

D.1 Molecular Modeling

D.1.1. *In silico* determination of drug-likeness properties

The Molinspiration server[23] was used to calculate the physicochemical properties of the designed compounds and to calculate a biological activity score for the six most important drug classes: GPCR ligands, kinase inhibitors, ion channel modulators, nuclear receptors, protease inhibitors, and enzyme inhibitors.

D.1.2. Docking experiment

Two-dimensional (2D) chemical structures of synthesized FQ derivatives (1–16; Scheme 2) were sketched with MarvinSketch[24] and saved in MDL Molfile format. As a result, a collection of energetically accessible conformers was generated using the OMEGA software[25]. OMEGA builds an initial structural model by assembling fragment templates along sigma bonds. The generated conformers were saved in SDF format. A 3D structural model of TopII (PDB ID: 3QX3) was obtained from the Protein Data Bank (www.rcsb.org)[26]. TopII receptor (binding) sites were identified using the MAKE_RECEPTOR module, a "graphical utility for creating or modifying receptors". A receptor is a special file used by FRED that contains target protein structure, active site location and geometry, binding ligand structure, and docking conditions, at least two of which are optional (OEDOCKING 3.2.0.2)[27]. Hydrogen atoms were added to proteins using the Discovery Studio (DS) Protein Residue Visualizer templat[28]. The synthesized compound and intercalating anticancer drug doxorubicin were docked into the TopII receptor site using the FRED program (OEDOCKING 3.2.0.2)[27]. Protein structures and ligand conformers were treated as rigid bodies during the docking process. This involves a thorough evaluation of each possible position of the ligand in the active binding site. The highest rated pose is optimized and given a final rating (Chemgauss4 rating). Doxorubicin showed similar pattern of results compared to positive control and cisplatin as test compound (Fig. 1).

E. PL activity assay

E.1 Preparation of the test compounds and Orlistat for the in vitro PL activity assay

Orlistat (10 mg, Sigma, St. Louis, MO, USA) was

dissolved in DMSO (10 mL) to make a stock solution (1 mg/mL), which was used to quantify 6 different concentrations ranging from 0.625 to 20. We got 6 different stock solutions. µg/ml. As a result, 20 µL aliquots of each working solution were used in the reaction mixture to obtain final working concentrations ranging from 0.0125 to 0.4 µg/mL. The test compounds were then dissolved primarily in DMSO to prepare 3 stock solutions, which were diluted to give 5 stock solutions (0.01-100 mg/ml). A 20 µL aliquot of each stock solution was then used in the reaction mixture to provide the final working concentration range (0.2–2000 µg/mL). A negative control containing 2% DMSO was run in parallel.

E.2 Quantification of PL potential spectrophotometrically

Crude porcine PL type II (0.5 mg/ml) (Sigma, St. Luis, MO, USA, EC 3.1.1.3) was diluted in Tris-HCl buffer (2.5 mM, pH 7.4, Promega Corp WI, USA) to final concentration of 200 units/mL. A 100 µM solution of para-nitrophenyl butyrate (p-NPB, Sigma, St. Louis, MO, USA) in DMSO was used as the PL substrate. An aliquot (0.1 mL) of the PL solution was included in the reaction mixture and the volume was brought to a final 1 mL with Tris-HCl buffer. PLs was pre-incubated with various concentrations of test materials for at least 1 min after substrate inclusion. Reactions were kept at room temperature and initiated by the addition of 5 µl of p-NPB substrate solution. The p-nitrophenol formed during the reaction was denatured using a SpectroScan 80D UV-VIS spectrophotometer (Sedico Ltd., Nicosia, Cyprus) at 410 nm over at least 7 time points (1–4 min), contains denatured enzymes. The catalytic ability of PL was measured calorimetrically by its activity towards the hydrolysis of p-NPB to p-nitrophenol. The activity of PL in this experiment was measured by determining the increase in p-nitrophenol production rate from the slope of the even part of the absorbance vs. time profile. The percentage of PL activity remaining for each test compound compared to control incubations was determined to calculate the concentration required to inhibit PL activity by 50% (i.e., IC50). All assays were performed in triplicate and calculated activities were reported as mean ± standard deviation (n = 3). 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.

C- Results and Discussion

A. Synthesis

The most versatile position for antibacterial fluoroquinolone is secondary amine at the C-7 position[29]. However, activity has been observed for quinolones with a primary amino group substituted at the C-7 position by our group creating new lipophilic anticancer members[30,31]. The chemistry included nucleophilic aromatic substitution reaction (S_N2 AR) of the primary amino group into C-7 position of quinolone nucleus. Substituted anisidines were introduced at C-7 of 7-chloro-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic ester using DMSO and pyridine as catalyst. This step was very straightforward producing good yields of the FQs esters (1-4)[32]. Subsequent acid hydrolysis of the esters and stannous chloride reduction produced the acids (5-8) and the reduced amines (9-12) in high yields. Cyclization was carried out through diazotization reaction (acidic sodium nitrite) of the 8-amino group to produce the triazoles 13-16 in high yields. All compounds were separated and fully characterized by NMR spectra as detailed in supplementary sheets.

B. Modulation of proliferative activity by FQ in obesity associated colorectal cancer cell lines

The epidemiological indication of connotation between cancer and obesity has been officially recognized in the last 10-year report from American Institute for Cancer Research and the World Cancer Research Fund[33]. The reported potential anti-cancer activity of FQ [5, 34] is associated with anti-cancer activity against a panel of colon cancer cell lines (HT29, HCT116, SW620, CACO2, and SW480) led to cancer screening[35]. The significant antiproliferative potential

of four series of FQs evaluated against a panel of cancer cell lines was validated with IC_{50} values against Cisplatin (Table 1). All tested FQs revealed good activity against all CRC cells, with IC_{50} values below 200 μM. The reduced series which contains the 7-8 ethylene diamine bridge showed the lowest IC_{50} values. Significantly reduced derivative **11** cytotoxicity against colorectal cancer cell lines was expressed as equivalent to or superior to that of cisplatin's in 72h incubations (Table 1). Substitutions like anisidine have major contribution to activity. Notably compound **11** with IC_{50} value (±SD) of cytotoxicity in HCT116 (μM) of 35.3±3.0 vs. **cisplatin's** 38.0±0 **was** more potent than cisplatin. The active FQs **11** was pronouncedly **antineoplastic** in SW620 colorectal incubations with IC_{50} values (μM) (±SD) of 3.2±0.8 vs. cisplatin's 5.7±0.9).

Table 1. *In vitro* antiproliferative activity IC_{50} values (μg/mL; μM) of FQs and cisplatin on colorectal cancer cell lines

COMPOUND	HT29	HCT116	SW620	CACO2	SW480	Fibroblasts
(1)	61.2±0.6 (142.4±1.5)	105.1±17.0 (244.9±40)	112.1±3.3 (261.0±1.2)	87.1±0.5 (202.8±1.2)	82.3±16.1 (191.6±38)	174.6±11.9 (406.6±28)
(3)	31.7±3.0 (82.8±7.8)	51.7±2.9 (134.9±7.6)	43.1±3.7 (112.3±9.7)	56.4±6.4 (147.0±16.7)	100.9±7.0 (263.3±18)	60.1±5.4 (156.8±14)
(5)	50.1±0.2 (135.2±0.6)	21.8±2.2 (54.4±5.5)	43.6±2.3 (108.7±5.8)	69.7±15.2 (173.7±38)	15.9±1.6 (39.6±4.0)	54.8±9.2 (136.5±22.8)
(6)	1298.5±152.0 (3235.4±379)	177.3±7.1 (441.7±18)	250.6±19.7 (624.4±49.0)	NI	98.9±11.3 (246.4±28)	94.2±10.3 (234.7±25.6)
(7)	139.1±5.3 (346.6±13.2)	37.4±6.2 (93.2±15.6)	44.4±6.0 (110.7±14.9)	62.7±7.2 (156.2±18)	16.7±2.2 (41.6±5.6)	29.7±2.5 (74.0±6.2)
(8)	250.2±33.7 (580.0±78.1)	NI	341.1±65.2 (790.7±151.1)	7.3±1.4 (17.0±3.2)	96.7±11.5 (224.2±27)	625.0±37.1 (1448.8±86)
(9)	48.3±1.8 (130.0±5)	54.7±147) (147.3±4)	61.3±3.4 (165.1±9)	122.1±11.2 (328.8±30)	40.5±0.7 (109.0±1.9)	48.4±5.5 (130.3±14.8)
(10)	97.3±1.2 (261.9±3)	63.6±6.5 (171.1±17)	56.4±6.2 (151.9±17)	73.8±5.4 (198.7±14.5)	69.6±2.7 (187.3±7.3)	63.2±3.3 (170.3±8.8)
(11)	11.2±1.7 (30.3±4.5)	13.1±1.1 (**35.3±3.0**)	1.2±0.3 (**3.2±0.8**)	20.2±2.5 (54.4±6.8)	12.6±0.3 (34.0±0.7)	18.0±1.4 (48.4±3.8)
(13)	52.1±2.3 (136.2±6.0)	64.9±4.4 (169.7±12)	74±7.5 (193.5±20)	203.9±16.3 (533.2±43)	39.3±2.5 (102.8±7)	68.9±3.5 (180.2±9.2)
(14)	175.9±13.8 (460.1±36.1)	75±3 (196.2±7.7)	46.1±2.4 (120.6±6.2)	176.8±4 (462.4±10)	31±2.5 (81.1±6.6)	39.9±5.4 (104.3±14)
(15)	171±26.1 (447.1±68.1)	122.9±14.2 (321.4±37)	84.7±4.2 (221.5±11)	149.2±14.9 (390.2±39)	84.1±7.1 (220±19)	72.9±6.9 (190.8±18)
Cisplatin	2.1±0.2 (6.9±0.5)	11.4±0.02 (38.0±0.1)	1.7±0.3 (5.7±0.9)	0.4±0.06 (**1.3±0.2**)	1.6±0.2 (**5.3±0.7**)	2.1±0.2 (7.0±0.7)

Results are mean ± standard deviation (n = 3 independent replicates). IC50 values (concentration at which 50% inhibition of cell proliferation occurred compared to non-induced basal 72 h incubation) were calculated in the range 5-200 μg/ml. NI is lack of cytotoxicity.

Supported by previous clues about the mechanism of action of new FQs[36], it was decided to investigate the antiproliferative mechanism and target of these new FQs by comparing their IC_{50} with each other's and with doxorubicin against 5 CRC cell lines (Fig. 1A and B).

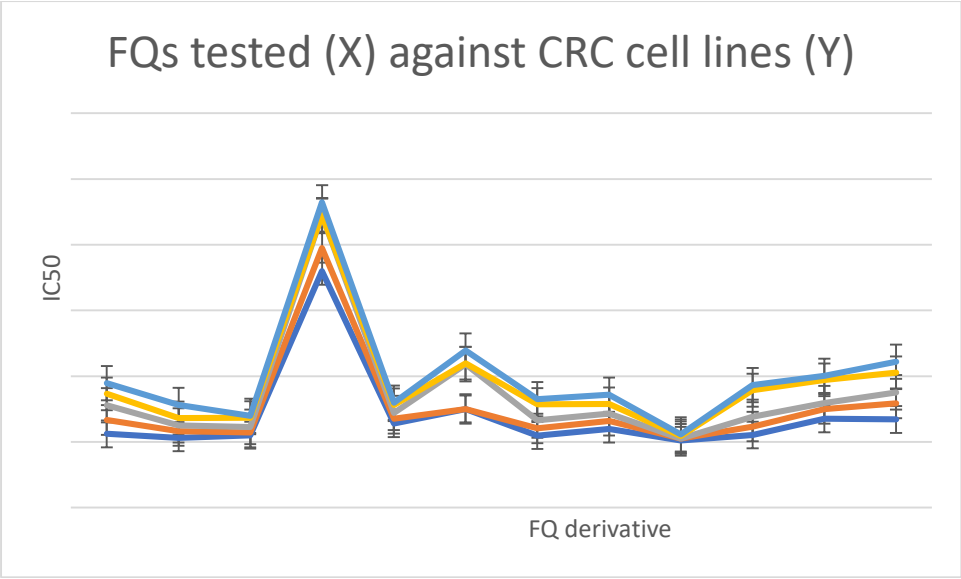


Figure 1A: IC_{50} comparison of synthesized FQs (5-16) against 5 CRC cell lines revealing similar pattern of screened FQs against CRCs.

Capriciously, the new FQs (5-16) showed a similar pattern of inhibition to each other against all CRC cell lines incriminating that they have common mechanism or target (Fig. 1A). To our surprise, such similarity in pattern was also noticed with reference drug doxorubicin against all CRC cell lines (Fig. 1B).

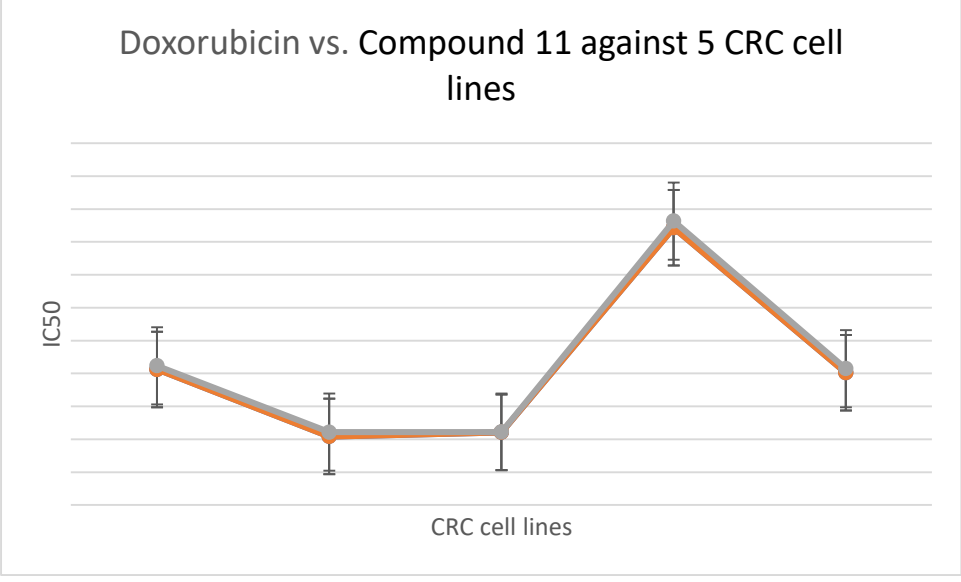


Figure 1B: IC_{50} comparison of synthesized FQs 11 and Doxorubicin against colorectal cancer cell lines.

Figure 1: IC50 A) Similarities in antiproliferative sequence and pattern of activity among tested FQ compounds B) Doxorubicin, and most active 11 against colorectal cancer cell lines (HT29 (1); HCT116 (2); SW620 (3); CACO2 (4); SW480 (5)). IC_{50} data of Doxorubicin used in this table were previously published[36] and further validated in this work

These findings point out clearly that there is structural scaffold similarity between our compounds (mainly 11) and doxorubicin. Since doxorubicin targets topoisomerase II, this means that our FQs work also on topoisomerase II similar to doxorubicin. Herein, we propose that the key structural features of eukaryotic topoisomerase II inhibitors common to our FQs are the C7-8- diamine ethylene chelator bridge serving as H-B donor/acceptor and its chelating similarity to hydroxyl groups in doxorubicin. Both FQ 11 and doxorubicin share a hydrogen bond acceptor-donor substituent, the amino group at C-8, and the isosteric OH of doxorubicin. They all feature such amino groups as part of an extended chelation system between C-7 and C-8 of compound **11** and the β-hydroxyketone of doxorubicin (Figure 2). This site/chelator system has been widely discussed in prokaryotic topoisomerase IV and gyrase inhibitors but seems to have been overlooked in all previously reported SAR scaffolds of eukaryotic topoisomerase II inhibitors. Here, we would like to emphasize the importance of C-8 amino groug in FQ 11 in the development of new anti-tumor FQ agents, and spot the light on the diamine chelate system generated in reduced FQ11 which has a major impact on the antiproliferative activity. Such findings led to explore binding sites on top. II enzyme of both compounds.

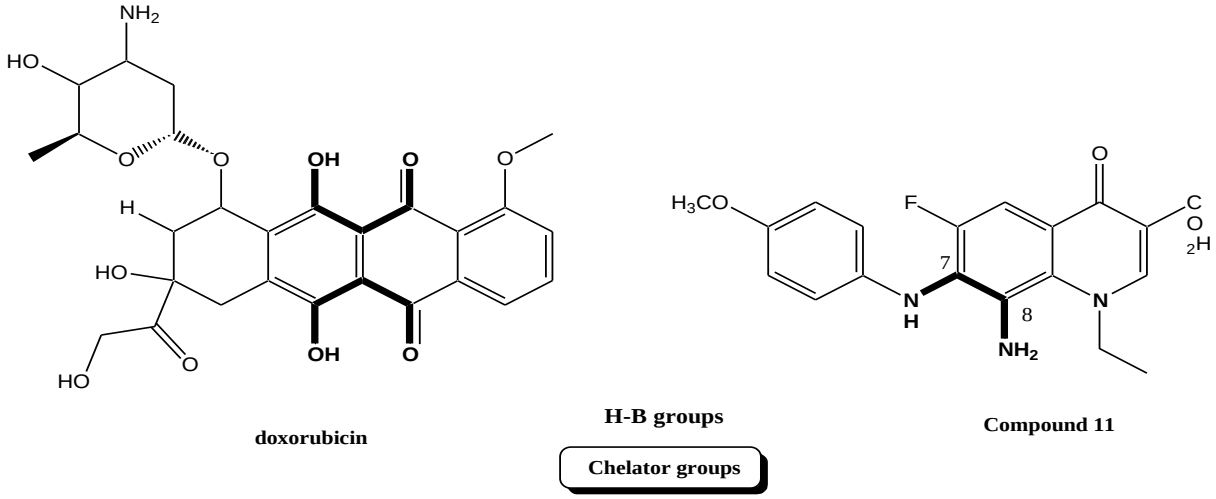


Figure 2: Proposed Chelator groups shared in both FQ 11 and doxorubicin.

D. Docking Experiment

D.1. *In silico* drug-likeliness analysis

Good oral bioavailability is an essential requirement in the identification and development of chemotherapeutic compounds. Therefore, when developing new drugs, it is very important to consider molecular properties that reduce oral bioavailability to support the development of valuable potential drug candidates. Reducing the number of experimental studies required for drug selection and development by using in silico computational tools throughout the drug discovery process to perform in silico ADMET analysis and bioactivity prediction to predict potential drug properties can save you time and money. As a result, several important physicochemical parameters. For example, Lipinski's parameters, the number of rotatable bonds, and the polar surface area (PSA) of synthesized compounds [37, 38], were identified using the computational tool Molinspiration for molecular property predictions.

Absorption rate (% ABS) was determined using the following formula: %ABS = 109 − 0.345 × PSA [10]. The results in table 2 showed that all the synthesized compounds meet Lipinski's rule of five and Veber rule with zero violations [37, 38]. The obtained results suggest that the synthesized compounds hypothetically have good oral bioavailability as suggested by the determined %ABS as shown in table 2.

Table 2. Drug-likeliness prediction of the synthesized compounds utilizing Molinspiration server.

Compound	PSA	NRB	miLogP	HBA	HBD	Mol Vol	MW	%ABS	MBS
1	106.16	6	2.12	8	1	338.92	399.38	72.40%	- 0.13
2	106.16	6	2.15	8	1	338.92	399.38	72.40%	- 0.11
3	106.16	6	2.17	8	1	338.92	399.38	72.40%	- 0.10
4	115.39	7	2.16	9	1	364.46	429.40	69.20%	- 0.12
5	126.39	6	1.80	9	2	330.37	401.35	65.40%	- 0.00
6	126.39	6	1.82	9	2	330.37	401.35	65.40%	0.01
7	126.39	6	1.85	9	2	330.37	401.35	65.40%	0.03
8	135.62	7	1.83	10	2	355.92	431.38	62.21%	- 0.01
9	86.36	5	1.65	6	3	326.87	369.40	79.21%	0.05
10	86.36	5	1.67	6	3	326.87	369.40	79.21%	0.07
11	86.36	5	1.70	6	3	326.87	369.40	79.21%	0.08
12	95.59	6	1.68	7	3	352.42	399.42	76.02%	0.04
13	79.03	4	1.13	7	0	324.23	380.38	81.73%	- 0.08
14	79.03	4	1.15	7	0	324.23	380.38	81.73%	- 0.07
15	79.03	4	0.96	7	0	324.23	380.38	81.73%	- 0.05
16	88.26	5	1.16	8	0	349.77	410.40	78.55%	- 0.07

PSA: polar surface area, NRB: number of rotatable bonds, miLogP: logarithmic partition coefficient, HBA: number of hydrogen bond acceptors, HBD: number of hydrogen bond donors, Mol Vol: molecular volume, MW: molecular weight, %ABS: absorption rate**.** MBS: enzyme inhibitor Molinspiration bioactivity score.

The drug-likeness score of synthesized molecules is ascertained by calculating the activity score for the ion channel modulator, GPCR, kinase inhibitor, enzyme inhibitor, protease inhibitor, and nuclear receptor ligands which has been applied to explore the compounds overall potential to be a good candidate for drug development. It was stated that the bigger the predicted bioactivity score, the higher the probability of specific molecule to be active as a drug. According to bioactivity score, a molecule having bioactivity score ≥ 0.00 is classified active, while classified as moderately active if the score is ≥−0.50 and less than 0.00 and if the score is less than− 0.50 it is presumed inactive[39]. The results of bioactivity data indicated that all the designed molecules are active to moderately active against all target classes and the highest activity was as enzyme inhibitors (Table 2). Interestingly, the most potent compound **11**as the highest bioactivity score as enzyme inhibitor and almost has the highest score against all classes.

D.2 Molecular docking study

Molecular docking studies were performed to focus on

the binding mode of the newly synthesized compound within the DNA binding site of Topo II (PDB code: 3QX3). Docking studies were performed using the FRED program of OEDocking suite [27]. As a validation step of the docking protocol, etoposide (a co-crystallized ligand) was first re-docked. FRED effectively predicted the pose of co-crystallized lower RMSD ligands, suggesting the effectiveness of the docking protocol. To discover the binding properties of synthetic compounds with TopII, we performed the same docking protocol on synthetic compounds within the DNA binding site and evaluated comparable binding modes and binding energies. All synthesized compounds successfully docked into the TopII binding site. A general examination of the docking results showed that the designed compounds exhibited binding patterns comparable to those of both the co-crystallized ligand (etoposide) and doxorubicin, with docking scores ranging from -13.45 to -15.74. was shown (Figure 3). The expected binding mode of compound **11** (the most potent compound) is shown in Figure 4. The planar quinolone unit exhibits pi-pi stacking and multiple hydrophobic interactions with DC-8, DT-9, DA-12, and DG-13, and with amino acids Glu477, Gly478, Ser480, Gly504. Using the action, the DNA was trapped between nucleotides. and Gln778. Moreover, both the amino group at position 8 and the NH of the 4-methoxyaniline moiety interact with Arg503 via hydrogen bonding (Fig. 3A). On the other hand, the proposed binding mode of the anticancer drug doxorubicin, with a predicted value of -14.345, is that the planar aromatic ring system of doxorubicin forms hydrophobic and aromatic stacking interactions with the DNA nucleotides DC-8, DT-9 and DA. made it clear that it has -12, DG-13, and several hydrophobic interactions with different key amino acid residues: Glu477, Gly478, Asp479, Leu502, Arg503, Gln778, Met782 like FQ75. In addition, the terminal 14-OH group of doxorubicin hydrogen bonds to Arg503 and DG-13, like FQ75 (Fig. 3B), while the OH at position 9 hydrogen bonds to his DT-9. In addition, the sugar moiety of doxorubicin was in the minor groove of DNA and hydrogen-bonded to Met782 and DC-8.

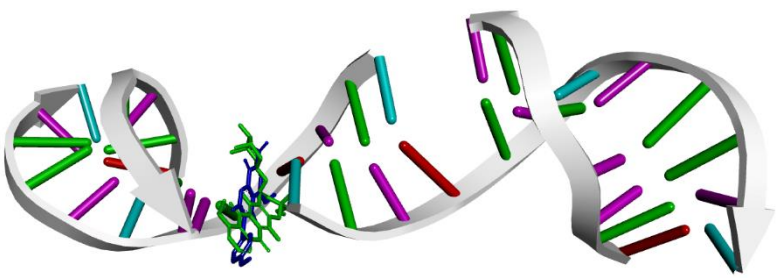


Figure 3: Diagram presenting ligand-DNA binding modes showing the overlay of docked poses of FQ11 (blue) and Doxorubicin (green) in the intercalation site (PDB ID: 3QX3).

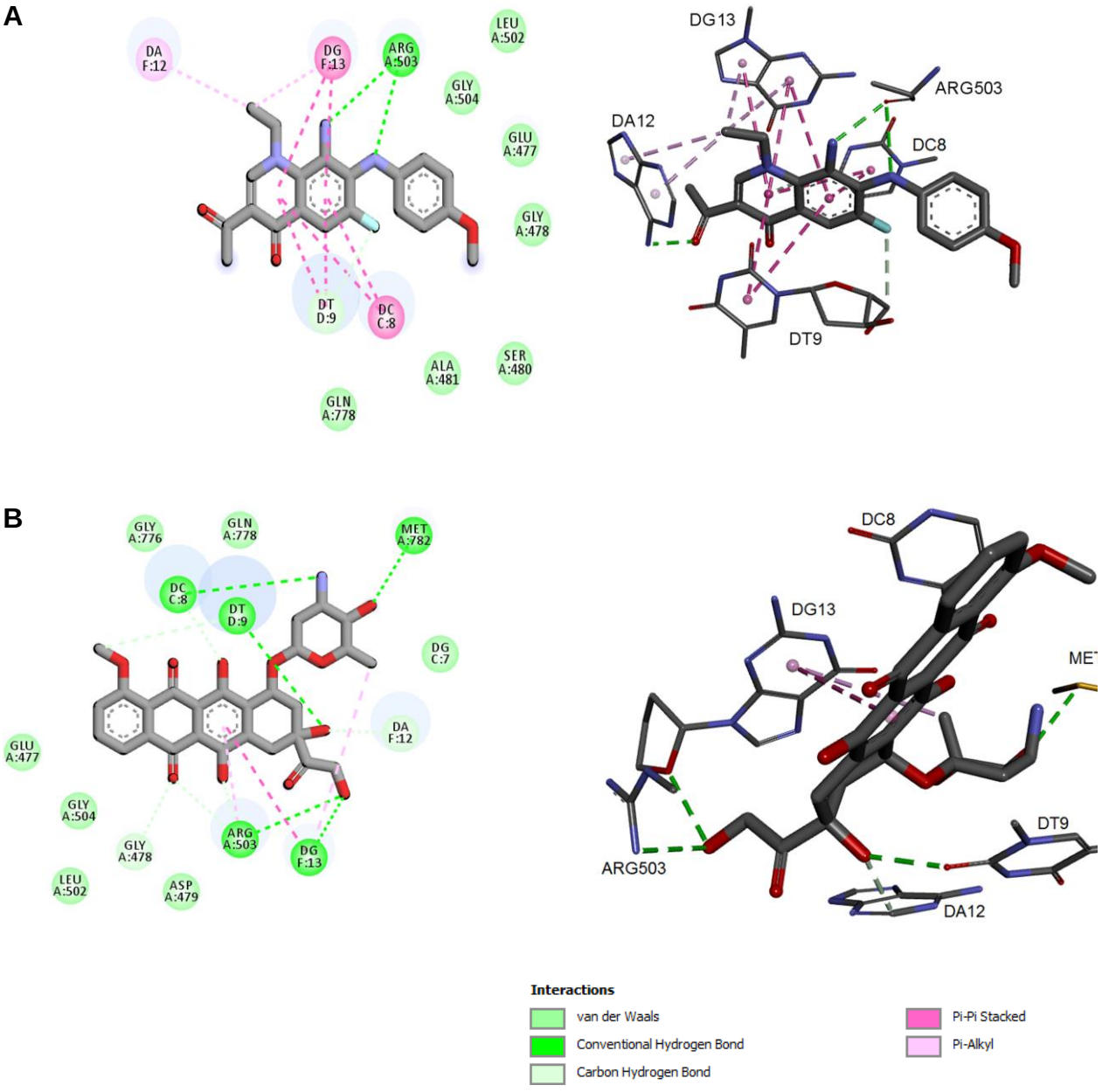


Figure 4: 2D and 3D illustration of the docking binding modes of: (**A**) designed compound FQ11, (**B**) doxorubicin in the Topoisomerase II active site. Hydrogen bonds are indicated by red dashed lines and pi-pi by purple dashed lines.

B. FQs as *In vitro* inhibitors of PL activity

Substantially based to the FQs antilipolytic activity [6, 40] and based on the fact that antilipolytic compounds can protect from Colorectal cancer; this study was directed toward

exploring dual anticancer FQs with antilipolytic effect against PL enzyme. Table 3 shows new FQs as inhibitors of pancreatic triacylglycerol lipase. These compounds have been reported to have dose-dependent anti-PL activity. IC_{50} values for both series of compounds ranged from 0.4 to 614 µM. Surprisingly, the reduced series (9-12) and the triazolo series (13-16) revealed the most potent activity which was comparable to the reference drug. Compound **9** was identified with a minimum IC50 value of 0.4 (±0.03) µM. The reference compound orlistat had an IC_{50} value of 0.2 µM, comparable to the value cited in the literature [41, 42]. Again, the antilipolytic FQs have showed the best anticancer activity exemplified by reduced series 9 -12. Although week to intermediate anti-CRC activity, this suggest primarily that such FQs might be used for such dual activity upon further investigation and optimization.

Table 3. PL-IC50 values of FQ and orlistat (µg/ml; µM)

No.	PL-IC_{50} (µg/mL)	PL-IC_{50} (µM)	No.	PL-IC_{50} (µg/mL)	PL-IC_{50} (µM)
1	16.9±2.4	44.1±6.3	10	14.0±0.7	37.6±1.8
4	21.8±2.7	52.8±6.5	11	4.6±0.5	12.3±1.3
5	9.1±0.1	22.7±0.3	12	8.3±0.2	20.6±3.0
6	4.1±0.6	10.1±1.6	**13**	**2.0±0.2**	**5.3±0.5**
7	57.3±1.2	142.8±3.0	14	12.3±1.9	32.0±5.0
8	4.4±0.3	10.2±0.6	15	56.2±1.0	147.1±2.7
9	**0.14±0.01**	**0.4±0.03**	16	243.3±17.1	614.4±43.4
Orlistat	**0.114±0.01**	**0.2±0.0**			

Results are mean ± standard deviation (n = 3 independent replicates).

Complexation studies:

To understand and confirm the chelation impact within the fluoroquinolones structure; we have decided to conduct Zn-complexation studies on PL enzyme for selected FQs. The acid CA, (7-chloro-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid) and its precursor ester CE (ethyl 7-chloro-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate) were selected such study due to availability in good amounts. Both were complexed with Zn metal (1 to 1 ratio) before adding to the PL enzyme, since FQs are well known to exhibit their antibacterial activity through metal chelation. Ciprofloxacin, the well-known antibacterial FQ, was used to confirm the idea since previously reported to have PL inhibitory activity[6, 43,44]. Then presence of excess metal ions within the test mixture of both FQs and ciprofloxacin, should neutralize the drug and the inhibitory effect would be lessened. The anti-lipase activities of CA, CE and ciprofloxacin are shown in Table 4.

Table 4.PL-IC_{50} values (µg/mL; µM) of FQs and FQs/$ZnCl_2$ complex

FQs test compounds/drugs	IC_{50} value (µg/mL)	IC_{50} value (µM)
Ciprofloxacin	23.55±1.13	71.08±4.46
Ciprofloxacin/$ZnCl_2$ complex	107.5±6.92	248.15±15.97
$ZnCl_2$	64.31±8.35	471.79±61.25
CA /$ZnCl_2$ complex	35.77±2.63	91.23±6.7
CA	5.35±5.8	16.38±1.6
CE	130±6.16	366.49±17.36

Results are mean ± standard deviation (n = 3 independent replicates)

Table 4 shows that both ciprofloxacin and CA exhibited potent inhibitory activity against PL with IC_{50} values of 71.08 and 16.38 μM, respectively. However, upon adding $ZnCl_2$ in-excess within the test mixture of both ciprofloxacin and CA showed significant decline in their inhibitory activity, with IC_{50} values of 248.15 and 91.23 μM, respectively. These data are attributed to pre-complexation of possibly the free COOH of both FQs with Zn ions and subsequently approve the role chelator groups against PL. Further assessing the CE ester derivative**,** PL-IC_{50} value was 366.49 μM (Table 4). These results indicate clearly that PL-inhibitory potential within the FQs tested can be linked to the free chelator groups beard by the molecules. Unfortunately, this study was not possible with active compounds due to low amounts, nor it was possible to conduct on CRC lines due to cell membrane barriers and metal penetration strains.

Conclusion

This work addressed the synthesis of biologically active compounds belonging to FQs with dual anticancer and anti-lipase activity, hypothesized to act via a chelation mechanism or inhibition validated by titration and docking experiments. Sixteen compounds were synthesized, fully characterized and tested by determining IC50 values against the most sensitive cancer cell lines. *In vitro* results showed that these compounds exhibited potent anticancer activity against test cell lines in the micromolar range with potency comparable pattern to doxorubicin. Compound 11 showed approximately 2-fold potency compared to cisplatin against the colorectal cancer cell line SW620 with an IC50 of 3.2 μM, suggesting that FQ is a potent anti-proliferative agent. The antiproliferative activity of these derivatives correlated well with their potential to inhibit topo II, suggesting that chelation is the mechanism of topo II inhibition, underscored by titration and docking experiments. Synthesized FQ derivatives were screened for their in vitro anti-lipase activity. The results showed that all tested compounds exhibited superior anti-lipase activity compared to the control molecule orlistat. Compound 9 exhibited comparable activity to orlistat against pancreatic lipase with an IC50 of 0.4 μM, suggesting that FQs are potent inhibitors of pancreatic lipase.

Acknowledgments

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Supplementary 1 data : Chemistry spectral data

All chemicals, solvents, and reagents were of analytical grade and used directly without further purification. The starting materials are ethyl 3- (N, N-dimethyl-amino)acrylate and ethylamine purchased from his Acros, Belgium. 2,4-Dichloro-5-fluoro-3-nitrobenzoic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). Primary aromatic amines: m-anisidine and 2,4-dimethoxyaniline were purchased from Aldrich Chemicals (UK), p-anisidine and o-anisidine from Merck (Darmstadt, Germany). The reducing agent, anhydrous tin chloride crystals ($SnCl_2$), was from Fluka (Switzerland) and the cyclizing agent, sodium nitrate, from Sigma Aldrich (St. Louis, MO, USA). Thin-layer chromatography (TLC) was performed on 10 x 10 cm aluminum plates precoated with fluorescent silica gel GF254 (ALBET, Germany) and visualized using UV light with a wavelength of 254 nm. The mobile phase combination was 94:5:1 chloroform-methanol-formic acid (CHCl3-MeOH-FA) (system 1) and 50:50 (n-hexane-ethyl acetate) (system 2). Melting points (mp) were determined on an open capillary on a Stuart Scientific Electrothermal Melting Point Apparatus (Stuart, Staffordshire, UK) and were recorded uncorrected. Nuclear magnetic resonance (NMR) spectra were obtained on a Varian Oxford-300 (300 MHz) spectrometer, a Bruker Avance DPX-300 spectrometer, a Bruker Avance-400 (400 MHz) Ultra Shield spectrometer, and a Bruker 500 MHz-Avance III (500MHz). Chemical shifts were reported in ppm relative to tetramethylsilane (TMS) as internal standard. Deuterated chloroform (CDCl3) and deuterated dimethylsulfoxide (DMSO-d6) were used as solvents for sample preparation. 1H NMR data are reported as: chemical shifts (ppm), (multiplicities, coupling constants (Hz), number of protons, corresponding protons). Low-resolution mass spectra (LRMS) were analyzed by Applied Biosystems MDS SCIEX API. High-resolution mass spectra (HRMS) were evaluated in positive ion mode using the electrospray ionization (ESI) technique with collision-induced dissociation on a Bruker APEX-4 (7 Tesla) instrument., inject the spray solution (methanol/water 1:1 v/v + 0.1 formic acid) and a syringe pump at a flow rate of 2 µL/min. External calibration was performed using the arginine cluster in the mass range m/z 175-871. Molecular masses were reported as AMU + 1 because the positive mode of ESI 1 adds his AMU to the molecular ion peak. Some compounds were documented as AMU+2 because the positive mode of ESI 1 added AMU to the molecular ion peak and ran the Ion Trap Analyzer. Infrared (IR) spectra were recorded on a Shimadzu 8400F FT-IR spectrophotometer Shimadzu, Kyoto, Japan. Samples were prepared as potassium bromide (KBr) (Sigma, St. Luis, MO, USA) discs.

B1. Synthesis of ethyl -1-ethyl-6-fluoro-7-(2-methoxy-phenylamino)-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylate (1)

3 molar equivalents of *o*-anisidine (2.7 g, 21.9 mmol) were introduced into a solution (starting synthon A, 2.5 g, 7.3 mmol) and 10 ml of dimethylsulfoxide (DMSO) as a vehicle and few drops of pyridine and then was refluxed at 65-70 °C under anhydrous conditions for (2-3) days. The reaction mixture was observed until the starting material totally reacted and then was allowed to crystallize at room temperature. The resulting product was filtered, rinsed and dried in the dark to produce orange crystals. Color of produced compound: orange. Yield ≈ 89% (2.8 g); mp = 199-200 °C; R*f* value in system 1 = 0.68 and in system 2 = 0.4. ^{1}H NMR (300 MHz, DMSO-d_6): 1.23 (2t, 6H, CH_3-1', OCH_2CH_3), 3.68 (s, 3H, OCH_3), 3.99 (q, J =7.13 Hz, 2H, NCH2-1'), 4.21 (q, J = 7.09 Hz, 2H, OCH_2CH_3), 6.82 (dd, J = 7.8 Hz, 8.2Hz, 1H, CH-5"), 6.90 (d, J = 7.81 Hz, 1H, CH-3"), 6.96 (m, 1H, CH-6"), 7.00 (m, 1H, CH-4"), 8.01 (d, ^{3}JH–F = 11.86 Hz, 1H, H-5), 8.06 (s, 1H, NH), 8.59 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6): 14.73 (C-2'),15.77 (OCH_2CH_3), 50.50 (NC-1'), 56.13 (OCH_3), 60.69 (OCH_2), 111.91 (C-3"), 112.24 (C-3), 114.77 (d, 2JC–F =

21.38 Hz, C-5), 120.52 (C-6"), 120.95 (C-4"), 124.37 (C-5"), 124.55 (d, 3JC–F = 5.78 Hz, C-4a), 130.59 (C-8a), 130.99 (C-1"), 132.43 (d, 2JC–F = 16.13 Hz, C-7), 134.47 (C-8), 150.94 (C-2"), 151.43 (C-2), 152.64 (d, ^{1}JC–F = 244.95 Hz, C-6), 164.38 (CO_2Et), 170.62 (C-4). IR (KBr): ν 3396, 3033, 2358, 1714, 1636, 1527, 1317, 1077, 980 cm^{-1}. LRMS (ES, +ve) m/z calculated for $C_{21}H_{20}FN_3O_5$ (429.13): Found 430.6 (100%, M+1), 402.2 (1%), 384.3 (15%), 356.4 (1%), 310.4 (1%), 155.5 (8%), 141.2 (55%), 124.1 (1%), 114.2 (4%), 85.2 (4%), 78.9 (1%), 64.1 (7%).

B2. Synthesis of ethyl -1-ethyl-6-fluoro-7-(3-methoxy-phenylamino)-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylate (2)

3 molar equivalents of *m*-anisidine (3.2 g, 26.3 mmol) were introduced into a solution containing (starting synthon A, 3 g, 8.75 mmol) and 10 ml of dimethylsulfoxide (DMSO) as a vehicle and drops of pyridine and then was refluxed at 65-70 °C under anhydrous conditions for (2-3) days. The reaction mixture was observed until the starting material totally reacted and then was allowed to crystallize at room temperature. The resulting product was filtered, rinsed and dried in the dark to produce dark orange crystals. Color of produced compound: dark orange. Yield ≈ 70% (2.6 g); mp = 170-171 °C; R*f* value in system 1 = 0.75 and in system 2 = 0.43. ^{1}H NMR (300 Hz, DMSO d_6): 1.24 (2t, 6H, OCH_2CH_3, CH_3-2'), 3.65 (s, 3H, OCH_3), 4.02 (q, J= 7.01 Hz, 2H, NCH_2-1'), 4.22 (q, J= 7.05 Hz, 2H, OCH_2), 6.38 (d, J= 8.14 Hz, 1H, H-6"), 6.42 (br s, 1H, H-2"), 6.46 (d, J= 8.18 Hz, 1H, H-4"), 7.07 (dd, J= 8.2 Hz, 8.04 Hz, 1H, H-5"), 8.12 (d, ^{3}JH-F= 11.1 Hz, 1H, H-5), 8.49 (br s, 1H, NH), 8.62 (s, 1H, H-2). ^{13}C NMR (75 Hz, DMSO d_6): 14.74 (C-1'), 15.98 (OCH_2CH_3), 50.29 (C-2'), 55.45 (OCH_3), 60.74 (OCH_2), 103.30 (C-4"), 107.35 (C-2"), 109.62 (C-6"), 112.06 (C-3), 115.57 (d, ^{2}JC-F= 21.45, C-5), 126.64 (d, ^{3}JC-F= 6.08, C-4a), 129.78 (d, ^{4}JC-F= 2.03, C-8a), 130.11 (d, ^{3}JC-F= 9.3, C-8), 130.28 (C-5"), 137.79 (C-7), 145.04 (d, ^{4}JC-F= 1.65, C-1"), 151.82 (C-2), 153.77 (d, ^{1}JC-F= 251.33, C-6), 160.39 (C-3"), 164.33 (CO_2Et), 170.62 (C-4). IR (KBr): ν 3387, 2978, 2255, 1722, 1615, 1522, 1452, 1322, 1060, 988 cm^{-1}. LRMS (ES, +ve) m/z calculated for $C_{21}H_{20}FN_3O_5$ (429.13): Found 430.3 (100%, M+1), 415.3 (5%), 402.2 (1%), 385.3 (39%), 373.7 (1%), 352.1 (4%), 339.2 (7%), 324.4 (8%), 310.3 (1%), 272.3 (1%), 258.2 (11%), 247.3 (1%), 186.5 (7%), 170.4 (1%), 138.4 (4%), 124.2 (19%), 94.0 (1%), 79.7 (3%).

B3. Synthesis of 1 ethyl -1-ethyl-6-fluoro-7-(4-methoxy-phenylamino)-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylate (3)

3 molar equivalents of *p*-anisidine (3.2 g, 26.3 mmol) were introduced into a solution containing (starting synthon A, 3 g, 8.75 mmol) and 10 ml of dimethylsulfoxide (DMSO) as a vehicle and drops of pyridine and then was refluxed at 65-70 °C under anhydrous conditions for (2-3) days. The reaction mixture was observed until the starting material totally reacted and then was allowed to crystallize at room temperature. The resulting product was filtered, rinsed and dried in the dark to give brick red powder. Color of produced compound: brick red. Yield ≈ 93% (3.5 g); mp= 118-119 °C; R*f* value in system 1 = 0.77 and in system 2 = 0.44. ^{1}H NMR (400 MHz, DMSO-d_6): 1.27 (2t, 6H, CH_3-1', OCH_2CH_3), 3.70 (s, 3H, OCH_3), 4.02 (q, J= 6.7 Hz, 2H, NCH_2-1'), 4.24 (q, J= 7.2 Hz, 2H, OCH_2CH_3), 6.48 (d, J= 7.89 Hz, J= 8.03 Hz, 2H, CH-2", CH-6"), 6.82 (d, J= 7.89 Hz, 2H, CH-3", CH-5"), 8.03 (d, ^{3}JH–F = 11.72 Hz, 1H, H-5), 8.53 (s, 1H, NH), 8.60 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6): 15.6 (C-2'), 15.39 (OCH_2CH_3), 52.6 (NC-1'), 55.54 (OCH_3), 61.28 (OCH_2), 110.91 (C-3), 116.9 (d, ^{2}JC-F = 16.92 Hz, C-5), 114.26 (C-4a), 114.75 (2C, C-3", C-5"), 122.6 (2C, C-2", C-6"), 130.43 (d, ^{2}JC–F = 16.35 Hz, C-7), 134.14 (C-8a), 134.77 (C-8), 138.86 (C-1"), 150.4 (d, ^{1}JC-F = 253 Hz, C-6), 151.13 (C-2), 157.31(C-4"), 164.59 (CO_2Et), 171.22 (C-4). IR (KBr): ν 3390, 3077, 2354, 1764, 1614, 1508, 1463, 1089, 928 cm^{-1}. LRMS (ES, +ve) m/z calculated for $C_{21}H_{20}FN_3O_5$ (429.13): Found 430.4 (100%, M+1), 416.1 (1%), 407.5 (5%), 385.3 (36%), 365.6

(1%), 343.3 (3%), 339.3 (4%), 324.5 (3%), 122.4 (45%), 104.2 (12%), 56.1 (1%). 3.2.2.10

B4. Synthesis of ethyl -7-(2,4-dimethoxy-phenylamino)-1-ethyl-6-fluoro-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylate (4)

3 molar equivalents of 2,4-dimethoxyaniline (3.35 g, 21.9 mmol) were introduced into a solution containing (starting synthon A, 2.5 g, 7.3 mmol) and 10 ml of dimethylsulfoxide (DMSO) as a vehicle and drops of pyridine and then was refluxed at 65-70 °C under anhydrous conditions for (2-3) days. The reaction mixture was observed until the starting material totally reacted and then was allowed to crystallize at room temperature and the resulting product was filtered, rinsed and dried in the dark to produce bright orange crystals. Color of produced compound: bright orange. Yield ≈ 80% (2.7 g); mp = 114-115 °C; R*f* value in system 1 = 0.88 and in system 2 = 0.3. ^{1}H NMR (500 MHz, DMSO-d_6): 1.2 (t, J = 7.05 Hz, 3H, CH_3-1'), 1.25 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 3.63 (s, 3H, OCH_3-2"), 3.72 (s, 3H, OCH_3-4"), 3.95 (q, J = 7.05 Hz, 2H, NCH_2-1'), 4.20 (q, J = 7.05 Hz, 2H, OCH_2CH_3), 6.43 (dd, J= 6.2, 2.45 Hz, 1H, CH-5"), 6.55 (d, J = 1.4 Hz, 1H, CH-3"), 7.00 (d, J= 8.45Hz, 1H, , CH-6"), 7.9 (d, 3JH–F = 12.5 Hz, 1H, H-5), 8.16 (s, 1H, NH), 8.54 (s, 1H, H-2). ^{13}C NMR (125 MHz, DMSO-d_6): 14.7 (C-2'),15.69 (OCH_2CH_3), 50.51 (NC-1'), 55.84 (OCH_3-2"), 56.17 (OCH_3-4"), 60.59 (OCH_2), 99.74 (C-5"), 104.84 (C-3"), 112.33 (C-3), 114.38 (d, 2JC–F = 21.06 Hz, C-5), 122.22 (d, ^{3}JC–F = 5.59 Hz, C-4a), 122.78 (C-8a), 124.79 (C-6"), 131.24 (d, ^{3}JC–F = 3.23 Hz, C-8), 131.33 (C-1"), 134.3 (d, ^{2}JC–F = 14.34 Hz, C-7), 151.05 (C-2), 151.32 (d, ^{1}JC–F = 249.89 Hz, C-6), 153.9 (C-2"), 158.14 (C-4"), 164.40 (CO2Et), 170.57 (C-4). IR (KBr): ν 3367, 3034, 2347, 1738, 1633, 1512, 1460, 1344, 1087, 960 cm^{-1}. LRMS (ES, +ve) m/z calculated for $C_{22}H_{22}FN_3O_7$ (459.14): Found 459.9; (M+1, 100%), 416.3 (11.2%), 414.4 (41.6%), 386.2 (1.1%), 367.3 (2.2%), 340.3 (5.6%), 325.3 (1.1%), 74.2 (4.5%), 57.5 (6.7%).

B5. Synthesis of 1-ethyl-6-fluoro-7-(2-methoxy-phenylamino)-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (5)

A forcefully agitated suspension of (1.2 g, 4.7 mmol) in 12 N HCl (28 mL) and ethanol (12 mL) was heated at 80-85 °C under reflux conditions. Advancement of ester hydrolysis was observed by TLC and was done in 24-36 hours. The reaction mixture was then cooled and, poured onto mashed ice (250 g) and the resultant orange precipitate was taken, washed with cold water (2 x 20 mL) and dried. Yield ≈ 70% (1.3 g). mp = 210-212 °C; R*f* value in system 1 = 0.49. ^{1}H NMR (300 MHz, DMSO-d_6): 1.26 (t, 3H, CH_3-2'), 3.64 (s, 3H, OCH_3), 4.14 (q, J = 7.1Hz, 2H, NCH_2-1'), 6.86 (dd, J = 7.42 Hz, 8.22 Hz, 1H, CH-5"),6.99 (d, J = 7.89 Hz 1H, CH-3"), 7.07 (m, 2H, CH-4",CH-6"), 7.44 (d, ^{3}JH–F = 9.54 Hz, 1H, H-5), 8.51 (s, 1H, NH), 8.88 (s, 1H, H-2), 15.11 (br s, 1H, COOH).^{13}C NMR (75 MHz, DMSO-d_6): 15.96 (C-2'), 51.50 (NC-1'), 56.13 (OCH_3), 109.62 (C-3), 112.03 (C-3"), 113.77 (d, ^{2}JC–F = 21.6 Hz, C-5), 120.59 (d, ^{3}JC–F = 7.2 Hz, C-4a), 120.88 (C-6"), 122.77 (C-4"), 125.76 (C-5"), 129.79 (C-8), 131.54 (C-1"), 132.57 (d, ^{4}JC–F = 3.9 Hz, C-8a), 134.77 (d, ^{2}JC–F = 15.08 Hz, C-7), 152.17 (C-2), 152.17 (C-2"), 152.59 (d, ^{1}JC–F = 252.45 Hz, C-6), 165.55 (CO_2Et), 175.70 (d, ^{4}JC–F = 2.63 Hz, C-4). IR (KBr): ν 3374, 2988, 2714, 2242, 1768, 1625, 1478, 1331, 1074, 936 cm^{-1}. LRMS (ES, +ve) m/z calculated for $C_{19}H_{16}FN_3O_6$ (401.10): Found 402.2 (M+1, 100%), 384.3 (45%), 357.5 (90%), 339.2 (37%), 102.1; (1.1%), 74.3 (3.4%), 57.3 (5.6%).

B6. Synthesis of 1-ethyl-6-fluoro-7-(3-methoxy-phenylamino)-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (6)

A forcefully agitated suspension of (2, 2.1 g, 4.9 mmol) in 12 N HCl (29.5 mL) and ethanol (12.5 mL) was heated at 80-85 °C under reflux conditions. Advancement of ester hydrolysis was observed by TLC and was done in 24-36 hours. The reaction mixture was then cooled and, poured onto mashed ice (250 g) and the resultant dark orange precipitate was taken, washed with cold water (2 x 20 mL)

and dried. Yield ≈ 78% (1.64 g). mp = 171-173 °C; R*f* value in system 1 = 0.43. ^{1}H NMR (300 Hz, DMSO d_6): 1.24 (m, 3H, CH_3-2'), 3.66 (s, 3H, OCH_3), 4.18 (q, J= 6.95 Hz, 2H, NCH_2-1'), 6.46 (d, J= 8.0 Hz, 1H, H-6"), 6.52 (d, J*m*= 4.2 Hz, 1H, H-2"), 6.53 (d, J*o*= 8.13 Hz, 1H, H-4"), 7.10 (dd, J*o*= 7.93 Hz, J*o*= 7.97 Hz, 1H, H-5"), 8.24 (d, ^{2}JH-F= 11.14 Hz, 1H, H-5), 8.79 (br s, 1H, NH), 8.95 (s, 1H, H-2), 14.52 (br s, 1H, COOH). ^{13}C NMR (75 Hz, DMSO d_6): 16.15 (C-1'), 51.43 (C-2'), 55.52 (OCH_3), 104.38 (C-4"), 108.35 (C-2"), 109.57 (C-6"), 110.7 (C-3), 115.01 (d, ^{2}JC-F= 21.53, C-5), 123.18 (d, ^{3}JC-F= 7.2, C-4a), 130.15 (C-5"), 130.69 (d, ^{4}JC-F= 1.8, C-8a), 132.15 (d, ^{2}JC-F= 16.35, C-7), 137.0 (C-8), 144.18 (d, ^{4}JC-F= 1.9, C-1"), 152.2 (C-2), 153.9 (d, ^{1}JC-F= 252.9, C-6), 160.32(C-3"), 165.45 (COOH), 175.81 (d, ^{4}JC-F= 2.25, C-4). IR (KBr): ν 3360, 3077, 2843, 2353, 1714, 1644, 1485, 1307, 1078, 930 cm^{-1}. LRMS (ES, +ve) m/z calculated for $C_{19}H_{16}FN_3O_5$ (401.10): Found 402.2 (100%, M+1), 385.2 (11%), 384.3 (31.5%), 357.5 (5.6%), 355.3 (1%), 337.9 (1%), 102.2 (2%), 75.3 (4.5%), 60.8 (6.7%), 57 (6.7%).

B7. Synthesis of 1-ethyl-6-fluoro-7-(4-methoxy-phenylamino)-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (7)

A forcefully agitated suspension of (3, 2.6 g, 6 mmol) in 12 N HCl (36.5 mL) and ethanol (15.5 mL) was heated at 80-85 °C under reflux conditions. Advancement of ester hydrolysis was observed by TLC and was done in 24-36 hours. The reaction mixture then was cooled, poured onto mashed ice (250 g) and the resultant yellow precipitate was taken, washed with cold water (2 x 20 mL) and dried. Yield ≈ 60% (1.45 g). mp = 250-252 °C; Rf value in system 1 = 0.47. ^{1}H NMR (300 MHz, DMSO-d_6): 1.25 (t, J=6.87 Hz, 3H, CH_3-1"), 3.69 (s, 3H, OCH_3), 4.14 (q, J= 6.95 Hz, 2H, NCH_2-1'), 6.82 (d, J= 8.86 Hz, 2H, CH-2", CH-6"), 6.99 (d, J= 8.32 Hz, 2H, CH-3", CH-5"), 8.13 (d, ^{3}JH–F = 12.02 Hz, 1H, H-5), 8.85 (s, 1H, NH), 8.91 (s, 1H, H-2), 14.16 (br s, 1H, COOH). ^{13}C NMR (75 MHz, DMSO-d_6): 16.05 (C-2'), 51.58 (NC-1'), 55.78 (OCH_3), 109.59 (C-3), 114.60 (d, ^{2}JC–F = 21.83 Hz, C-5), 114.92 (C-4a), 114.75 (2C, C-3", C-5"), 120.12 (C-7), 122.45 (2C, C-2", C-6"), 134.14 (C-8a), 134.77 (C-8), 138.81 (C-1"), 151.78 (C-2), 153.17 (d, ^{1}JC–F = 253.13 Hz, C-6), 156.47 (C-4"), 165.56 (COOH), 175.69 (C-4). IR (KBr): ν 3380, 3070, 2648, 2322, 1716, 1621, 1485, 1312, 1122, 970 cm^{-1}. LRMS (ES, +ve) m/z calculated for $C_{19}H_{16}FN_3O_5$ (401.10): Found 402.2 (100%, M+1), 385.3 (30%), 379.2 (2%), 357.6 (26%), 355 (3.4%), 339.1 (25%), 310.3 (1%), 296.4 (2%), 241.1 (1%), 127.3 (1%), 102.2 (2%), 74.3 (3.4%), 61.1 (1%), 59.2 (13.5%), 57.3 (6.7%).

B8. Synthesis of 7-(2,4-dimethoxy-phenylamino)-1-ethyl-6-fluoro-8-nitro-4-oxo-1,4- dihydro-quinoline-3-carboxylic acid (8)

A forcefully agitated suspension (4, 2 g, 4.35 mmol) in 12 N HCl (28 mL) and ethanol (12 mL) was heated at 80-85 °C under reflux conditions. Advancement of ester hydrolysis was observed by TLC and was done in 24-36 hours. The reaction mixture then was cooled, poured onto mashed ice (250 g) and the resultant orange precipitate was taken, washed with cold water (2 x 20 mL) and dried. Yield ≈1.5 g (84 %). mp = 212-215 °C; R*f* value in system 1 = 0.7. ^{1}H NMR (300 Hz, DMSO d_6): 1.25 (t, J= 7.02 Hz, 3H, CH_3-2'), 3.62 (s, 3H, OCH_3-2"), 3.72 (s, 3H, OCH_3-4"), 4.13 (q, J= 7.01 Hz, 2H, NCH_2-1'), 6.46 (dd, J= 8.48 Hz, 4.2 Hz, 1H, H-5"), 6.55 (s, 1H, H-3"), 7.07 (d, J*o*= 8.57 Hz, 1H, H-6"), 8.01 (d, JH-F= 12.48 Hz, 1H, H-5), 8.58 (br s, 1H, NH), 8.86 (s, 1H, H-2), 14.70 (br s, 1H, COOH). ^{13}C NMR (75 Hz, DMSO d_6): 15.97 (C-2'), 51.65 (NC-1'), 55.89 (OCH_3-2"), 56.21(OCH_3-4"), 99.25 (C-3"), 104.90 (C-5"), 109.56 (C-3), 113.39 (d, ^{2}JC-F= 21.5 Hz, C-5), 118.74 (d, ^{4}JC-F= 6.83, C-8a), 121.81 (C-1"), 126.14 (C-6"), 130.04 (C-8), 132.15 (C-4a), 136.22 (d, ^{2}JC-F= 13.8, C-7), 151.44 (C-2), 151.64 (d, ^{1}JC-F= 254.85, C-6), 154.62 (d, ^{5}JC-F= 2.03, C-2"), 158.81 (C-4"), 165.63 (COOH), 175.61 (d, ^{4}JC-F= 2.7, C-4). IR (KBr): ν 3392, 3060, 2843, 2274, 1776, 1633, 1474, 1319, 1120, 915 cm^{-1}. LRMS (ES, +ve) m/z calculated for $C_{20}H_{18}FN_3O_7$

(431.11): Found 432.2 (100%, M+1), 416.1 (12.5%), 415.4 (62.5%), 401.3 (6.25%), 389.7 (2%), 387.2 (89.6%), 369.3 (14.6%), 296.3 (4%), 279.4 (2%), 251.5 (2%), 233.4 (2%), 74.4 (4%), 57.3 (8%).

B9. Synthesis of 8-amino-1-ethyl-6-fluoro-7-(2-methoxy-phenylamino)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (9)

A mixture of (5, 0.65 g, 1.6 mmol) in 6.4 mL of 12 N HCl was stirred in ice bath (0-5 °C) for 15 minutes. Subsequently, the ice bath was removed and (1.2 g, 6.4 mmol) stannous chloride ($SnCl_2$) was introduced gradually and the reaction mixture stirred during the night and was observed by TLC until completion. The reaction mixture then was poured on mashed ice to yield a yellow precipitate that is collected by filtration and dried. Yield = 0.38 g (≈ 65%). mp = 215-217°C (decomposition); R*f* value in system 1 = 0.19. ^{1}H NMR (300 MHz, DMSO-d_6): 1.19 (t, 3H, CH_3-1'), 3.84 (s, 3H, OCH3), 4.81 (m, 2H, NCH_2-1'), 5.51 (br s, 2H, NH2), 6.18 (dd, J*o* = 6.73 Hz, 1H, CH-3"), 6.70 (dd, J*o* = 8.05 Hz, J*o* = 7.84 Hz, 2H, CH-4", CH-5"), 6.94 (d, J*o* = 7.79 Hz 1H, CH-6"), 7.02 (s, 1H, NH), 7.41 (d, ^{3}JH–F = 11.99 Hz, 1H, H-5), 8.88 (s, 1H, H-2), 14.60 (br s, 1H, COOH). ^{13}C NMR (75 MHz, DMSO-d_6): 16.19 (C-2'), 52.61 (NC-1'), 56.10 (OCH^3), 99.92 (d, ^{2}JC–F = 23.48 Hz, C-5),107.37 (C-3), 111.49 (C-3"), 112.75 (C-6"), 119.68 (C-4"), 121.75 (C-5"), 122.90 (d, ^{2}JC–F = 16.58 Hz, C-7), 126.21 (d, ^{3}JC–F = 9.3 Hz, C-4a), 127.25 (C-8a), 134.52 (C-8), 139.88 (C-1"), 148.18 (C-2"), 151.46 (C-2), 157.30 (d, ^{1}JC–F = 244.95 Hz, C-6), 166.55 (COOH), 177.44 (d, ^{4}JC–F = 2.85 Hz, C-4). IR (KBr): ν 3363, 3088, 2941, 1604, 1512, 1436, 1273, 1157, 1054, 1021 cm^{-1}. LRMS (ES, +ve) m/z calculated for $C_{19}H_{18}FN_3O_4$ (371.16): Found 372.4 (100%, M+1), 354.2 (51%), 339.3 (38%), 328.4 (42%), 102.2 (1%), 74.2 (2.2%), 59.2 (3.4%).

B10. Synthesis of 8-amino-1-ethyl-6-fluoro-7-(3-methoxy-phenylamino)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (10)

A mixture of (6, 1.5 g, 3.7 mmol) in 4 mL of 12 N HCl was stirred in ice bath (0-5 °C) for 15 minutes. Subsequently, the ice bath was removed and (2.8 g, 15 mmol) stannous chloride ($SnCl_2$) was added gradually and the reaction mixture stirred during the night and was observed by TLC until completion. The reaction mixture then was poured on mashed ice to yield a yellow precipitate that is collected by filtration and dried. Yield = 0.9 g (≈ 65%). mp = 295-297 °C (decomposition); R*f* value in system 1 = 0.27. ^{1}H NMR (300 Hz, DMSO d_6): 1.16 (m, 3H, CH_3-2'), 3.6 (s, 3H, OCH_3), 4.16 (q, J= 7.11 Hz, 2H, NCH_2-1'), 5.94 (br s, 2H, NH_2), 6.46 (d, J*o*= 8.0 Hz, 1H, H-6"), 6.52 (d, J*m*= 2.5 Hz, 1H, H-2"), 6.53 (d, J*o*= 8.13 Hz, 1H, H-4"), 7.10 (dd, J*o*= 7.93 Hz, J*o*= 7.97 Hz, 1H, H-5"), 8.24 (d, ^{2}JH-F= 11.14 Hz, 1H, H-5), 8.79 (br s, 1H, NH), 8.95 (s, 1H, H-2), 15.88 (br s, 1H, COOH). ^{13}C NMR (75 Hz, DMSO d_6): 16.15 (C-1'), 51.43 (C-2'), 55.52 (OCH_3), 104.38 (C-4"), 108.35 (C-2"), 109.57 (C-6"), 110.7 (C-3), 115.01 (d, ^{2}JC-F= 21.53, C-5), 123.18 (d, ^{3}JC-F= 7.2, C-4a), 130.15 (C-8), 130.69 (d, ^{4}JC-F= 1.8, C-8a), 132.15 (d, ^{2}JC-F= 16.35, C-7), 137.0 (C-5"), 144.18 (d, ^{4}JC-F= 1.9, C-1"), 152.2 (C-2), 153.9 (d, ^{1}JC-F= 252.9, C-6), 160.32(C-3"), 165.45 (COOH), 175.81 (d, ^{4}JC-F= 2.25, C-4). IR (KBr): ν 3363, 3086, 2938, 2360, 1624, 1510, 1450, 1124, 1062, 1031 cm^{-1}. LRMS (ES, +ve) m/z calculated for $C_{19}H_{18}FN_3O_4$ (371.16): Found 372.4 (100%, M+1), 354.2 (51%), 339.3 (8%), 328.4 (42%), 102.2 (1%), 74.2 (2.2%), 59.2 (3.4%).

B11. Synthesis of 8-Amino-1-ethyl-6-fluoro-7-(4-methoxy-phenylamino)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (11)

A mixture of (7, 0.4 g, 1 mmol) in 4 mL of 12 N HCl was stirred in ice bath (0-5 °C) for 15 minutes. After that, the ice bath was removed and (0.75 g, 4 mmol) stannous chloride ($SnCl_2$) was added portion wise and the reaction mixture stirred overnight and was monitored by TLC until completion. The reaction mixture then was poured on

mashed ice to precipitate to a red product that is collected by filtration and dried. Yield = 0.12 g (≈ 33%). mp = 295-297 °C (decomposition); R*f* value in system 1 = 0.25. ^{1}H NMR (300 MHz, DMSO-d_6): 1.25 (t, 3H, CH_3-1"), 3.69 (s, 3H, OCH_3), 4.59 (q, J= 6.98 Hz, 2H, NCH_2-1'), 6.23 (br s, 2H, NH_2), 6.93 (dd, J*o*= 8.79 Hz, 2H, CH-2", CH-6"), 7.22 (dd, J*o*= 8.29 Hz, 2H, CH-3", CH-5"), 7.47 (d, ^{3}JH–F = 11.53 Hz, 1H, H-5), 8.42 (s, 1H, NH), 8.72 (s, 1H, H-2), 15.46 (br s, 1H, COOH).^{13}C NMR (75 MHz, DMSO-d_6): 16.05 (C-2'), 51.58 (NC-1'), 55.78 (OCH_3), 105.59 (C-3), 106.65 (d, ^{3}JC–F = 20.33 Hz, C-5), 114.45 (2C, C-3", C-5"), 118.86 (d, ^{3}JC–F = 6.68 Hz, C-4a), 125.21 (C-8a), 129.25 (2C, C-2", C-6"), 129.96 (d, ^{2}JC–F = 16 Hz, C-7), 136.14 (C-8), 144.96 (C-1"), 147.89 (C-4"), 149.98 (C-2), 153.17 (d, ^{1}JC–F = 253.13 Hz, C-6), 166.89 (COOH), 176.96 (C-4). IR (KBr): ν 3356, 2924, 2854, 2291, 1625, 1531, 1458, 1377, 1180, 1033 cm^{-1}. LRMS (ES, +ve) m/z calculated for $C_{19}H_{18}FN_3O_4$ (371.16): Found 372.2 (4%, M+1), 368.4 (32%), 364.6 (18%), 357.5 (43%), 352.2 (100%), 339.0 (18%), 74.4 (14%), 59.1 (39%).

B12. Synthesis of 8-amino-7-(2,4-dimethoxy-phenylamino)-1-ethyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (12)

A mixture of (8, 1.35 g, 3 mmol) in 12 mL of 12 N HCl was stirred in ice bath (0-5 °C) for 15 minutes. The ice bath was then removed and (2.4 g, 12 mmol) stannous chloride ($SnCl_2$) was added gradually and the reaction mixture stirred during the night and observed by TLC until completion. Later, the reaction mixture was poured on mashed ice to yield light brown precipitate that is collected by filtration and dried. Yield = 0.22 g (≈ 18%). mp = 285-287 °C (decomposition); R*f* value in system 1 = 0.15. ^{1}H NMR (300 Hz, DMSO-d_6): 1.22 (t, J= 8.2 Hz, 3H, CH_3-2'), 3.4 (s, 3H, OCH_3-2"), 3.62 (s, 3H, OCH_3-4"), 4.3 (m, 2H, NCH_2-1'), 5.6 (br s, 2H, NH_2), 6.88 (d, J= 8 Hz, 1H, H-5"), 7.1 (s, 1H, H-3"), 7.4 (d, J= 7.2 Hz, 1H, H-6"), 8.3 (d, JH-F= 14.75 Hz, 1H, H-5), 8.6 (br s, 1H, NH), 8.82 (s, 1H, H-2), 14.96 (br s, 1H, COOH). ^{13}C NMR (75 Hz, DMSO-d_6): 14.3 (C-2'), 52.6 (C-1'), 56.2 (OCH_3-2"), 57.8(OCH3-4"), 100.6 (C-3"), 105.4 (C-5"), 110.42 (C-3"), 144.7 (d, ^{2}JC-F= 18.25 Hz, C-5), 116.2 (C-4a), 121.3 (C-1"), 125.25 (C-6"), 131.0 (C-8a), 133.6 (C-8), 135.4 (C-7), 148.6 (C-2), 150.55 (d, ^{1}JC-F= 253 Hz, C-6), 154.62 (C-2"), 160.35 (C-4"), 166.4 (COOH), 173.6 (d, ^{4}JC-F= 2 Hz, C-4). IR (KBr): ν 3351, 2933, 2854, 2279, 1604, 1527, 1470, 1410, 1375, 1038 cm^{1}. LRMS (ES, +ve) m/z calculated for $C_{20}H_{20}FN_3O_5$ (401.14): Found 401.4 (15%), 396.6 (5%), 390.7 (1%), 384.2 (3%), 382.3 (7%), 380.6 (50.6), 387.2 (100%), 374.4 (11%), 369.2 (39%), 361.4 (9%), 356.2 (8%), 343.4 (11%), 334.3 (5%), 329.4 (7%), 326.5 (1%), 310.3 (4%), 284.7 (1%), 279.4 (9%), 270.4 (5%), 256.3 (4%), 233.3 (5%), 178.4 (3%), 154.5 (3%), 141.2 (20%), 122.4 (3%), 102.2 (11%), 78.9 (3%), 74.3 (2%).

B13. Synthesis of 9-ethyl-4-fluoro-3-(2-methoxy-phenyl)-6-oxo-6,9-dihydro-3H- [1,2,3] triazolo[4,5-h] quinoline-7-carboxylic acid (13)

Compound 13 was synthesized by cyclizing the previously reduced acid. (9, 0.25 g, 1 mmol) in 20 ml HCl with stirring for 15 minutes in an ice bath(0-5 °C) for 15 minutes. $NaNO_2$ (0.07 g, 1 mmol) dissolved in 10 mL H_2O is added gradually. The reaction mixture was stirred during the night. Improvement of cyclization reaction was checked by TLC and was completed in 24 hours. After that, the reaction mixture was cooled, poured onto mashed ice (250 g) and the resulting light brown precipitate was collected, washed with cold water (2 x 20 mL) and dried. Yield= 0.14 g (≈ 37 %). mp = 219-220 °C; R*f* value in system 1= 0.32. ^{1}H NMR (300 MHz, DMSO-d_6): 1.54 (t, J = 6.47 Hz, 3H, CH_3-1'), 3.78 (s, 3H, OCH_3), 5.27 (s, 2H, NCH_2-1'), 7.22 (dd, J*o* = 7.41 Hz, J*o* = 7.50Hz, 1H, CH-5"), 7.37 (d, J*o* = 8.26 Hz, 1H, CH-3"), 7.71 (m, 2H, CH-4", CH-6"), 8.24 (d, ^{3}JH–F = 23.77 Hz, 1H, H-5), 9.17 (s, 1H, H-8), 15.18 (br s, 1H, COOH). ^{13}C NMR (75 MHz, DMSO-d_6): 16.15 (C-2'), 53.39 (NC-1'), 56.60 (OCH_3), 109.26 (d, ^{2}JC–F = 19.35 Hz, C-5), 110.31 (C-7), 113.24

(C-3"), 121.32 (C-6"), 123.51 (C-9a), 124.50 (C-5a), 128.76 (C-4"), 129.0 (d, ^{2}JC–F = 15.9 Hz, C-3a), 130.67 (C-9b), 133.20 (C-5"), 139.25 (C-1"), 146.10 (d, ^{1}JC–F = 252.53 Hz, C-4), 149.73 (C-8), 154.40 (C-2"), 166.00 (COOH), 176.46 (C-6). IR (KBr): ν 3428, 3067, 2655, 1760, 1622, 1495, 1162, 1084, 1003 cm^{-1}. LRMS (ES, +ve) m/z calculated for $C_{19}H_{15}FN_4O_4$ (382.1): Found 383.3 (23%, M+1), 365.5 (100%), 337.2 (8%), 306.2 (7%).

B14. Synthesis of 9-ethyl-4-fluoro-3-(3-methoxy-phenyl)-6-oxo-6,9-dihydro-3H- [1,2,3] triazolo[4,5-h]quinoline-7-carboxylic acid (14)

Compound 14 was synthesized by cyclizing the previously reduced acid. (10, 0.74 g, 2 mmol) in 20 ml HCl with stirring for 15 minutes in an ice bath (0-5 °C) for 15 minutes. $NaNO_2$ (0.14 g, 2 mmol) dissolved in 10 mL H_2O is added gradually. The reaction mixture was stirred during the night. Improvement of cyclization reaction was checked by TLC and was completed in 24 hours. After that, the reaction mixture was cooled, poured onto mashed ice (250 g) and the resulting light brown precipitate was collected, washed with cold water (2 x 20 mL) and dried. Yield= 0.4 g (≈ 52 %). mp = 240-242 °C; R*f* value in system 1= 0.35. ^{1}H NMR (300 Hz, DMSO d_6): 1.52 (t, J= 6.2 Hz, 3H, CH_3-2'), 3.91 (s, 3H, OCH_3), 5.26 (d, J= 5.96 Hz, 2H, NCH2-1'), 7.23 (d, J*o*= 8.08 Hz, 1H, H-6"), 7.38 (d, J*o*= 7.38 Hz, 1H, H-4"), 7.44 (d, Jm= 3.5 Hz, 1H, H-2"), 7.57 (d, J*o*= 7.92 Hz, J*o*= 7.18 Hz 1H, H-5"), 8.11 (d, ^{3}JH-F= 13.53 Hz, 1H, H-5), 9.13 (s, 1H, H-8), 12.0 (br s, 1H, COOH). ^{13}C NMR (75 Hz, DMSO d_6): 16.1 (C-2'), 53.42 (C-1'), 56.23 (OCH_3), 109.32 (d, ^{2}JC-F= 20 Hz, C-5), 110.22 (C-7), 112.08 (C-2"), 116.68 (C-4"), 118.33 (C-6"), 123.38 (d, ^{4}JC-F= 5.85 Hz, C-9a), 127.78 (d, ^{3}JC-F= 16.05 Hz, C-5a), 130.59 (C-5"), 136.96 (C-3a), 139.59 (C-9b), 144.32 (C-1"), 146.0 (d, ^{1}JC-F= 253.35 Hz, C-4), 149.77 (C-8), 160.17 (C-3"), 165.84 (COOH), 176.27 (C-6). IR (KBr): ν 3389, 3064, 2895, 1720, 1618, 1482, 1136, 1078, 1016 cm^{-1}. LRMS (ES, +ve) m/z calculated for $C_{19}H_{15}FN_4O_4$ (382.1): Found 383.3 (100%, M+1), 374.5 (3.4%), 365.5 (30%), 355.3 (1%), 339.3 (1%), 279.4 (9%), 258.3 (1%), 251.6 (1%), 233.3 (3.4%), 163.3 (9%), 102.2 (10%), 79.9 (5.6%), 74.1 (1%), 60.1 (1%).

B15. Synthesis of 9-Ethyl-4-fluoro-3-(4-methoxy-phenyl)-6-oxo-6,9-dihydro-3H- [1,2,3] triazolo[4,5-h] quinoline-7-carboxylic acid (15)

Compound 15 was synthesized by cyclizing the previously reduced acid. (11, 0.8 g, 2.1 mmol) in 20 ml aqueous HCl, stirred overnight in ice bath (0-5 °C) for 15 minutes. $NaNO_2$ (0.14 g, 2.1 mmol) dissolved in 10 mL H_2O is added gradually. The reaction mixture was stirred during the night. Improvement of cyclization reaction was checked by TLC and was completed in 24 hours. After that, the reaction mixture was cooled, poured onto mashed ice (250 g) and the resulting grayish green precipitate was collected, washed with cold water (2 x 20 mL) and dried. Yield= 0.6 g (≈ 75 %). mp = 305-307 °C; R*f* value in system 1= 0.3. ^{1}H NMR (400 MHz, DMSO-d_6): 1.24 (m, 3H, CH_3-1"), 4.02 (s, 3H, OCH_3), 5.23 (m, 2H, NCH_2-1'), 7.2 (2H, CH-3", CH-5"), 7.42-7.47 (m, 2H, CH-6", CH-2"), 8.16 (1H, H-5), 9.18 (s, 1H, H-8), 15.46 (br s, 1H, COOH). ^{13}C NMR (75 MHz, DMSO-d_6): 16.09 (C-2'), 53.21 (NC-1'), 56.83 (OCH_3), 107.25 (C-7), 109.73 (d, ^{2}JC–F = 19.58 Hz, C-5), 110.37 (2C, C-3", C-5"), 127.77 (2C, C-2", C-6"), 130.72 (C-9b), 131.04 (C-9a), 131.99 (C-5a), 138.22 (C-3a), 138.81 (C-1"), 149.87 (C-8), 151. 25 (d, ^{1}JC–F = 249 Hz, C-4), 162.32 (C-4"), 166.00 (COOH), 176.52 (C-6). IR (KBr): ν 3366, 2978, 2710, 1745, 1625, 1496, 1135, 1073, 1015 cm^{-1}. LRMS (ES, +ve) m/z calculated for $C_{19}H_{15}FN_4O_4$ (382.1): 385.2 (25%, M+3), 366.3 (25%), 364.6 (55%), 336.2 (5%), 186.6 (15%), 122.4 (100%), 104.2 (25%).

B16. Synthesis of 3-(2,4-dimethoxy-phenyl)-9-ethyl-4-fluoro-6-oxo-6,9-dihydro-3H- [1,2,3] triazolo[4,5-h] quinoline-7-carboxylic acid (16)

Compound 16 was synthesized by cyclizing the previously reduced acid (12, 0.5 g, 1.2 mmol) in 20 ml HCl

with stirring for 15 minutes in an ice bath (0-5 °C) for 15 minutes. $NaNO_2$ (0.09 g, 1.2 mmol) dissolved in 10 mL H_2O is added gradually. The reaction mixture was stirred during the night. Improvement of cyclization reaction was checked by TLC and was completed in 24 hrs. After that, the reaction mixture was cooled, poured onto mashed ice (250 g) and the resulting off-white precipitate was collected, washed with cold water (2 x 20 mL) and dried. Yield= 0.35 g (≈ 70 %). mp = 320-322 °C; R*f* value in system 1= 0.58. ^{1}H NMR (400 Hz, DMSO d_6): 1.14 (m, 3H, CH_3-2'), 3.89 (s, 6H, OCH_3-2", OCH_3-4"), 5.32 (m, 2H, NCH_2-1'), 7.23 (d, J= 8.32 Hz, 4.2 Hz, 1H, H-6"), 7.46 (s, 1H, H-3"), 7.78 (d, J*o*= 8.28 Hz, 1H, H-5"), 8.17 (d, JH-F= 10.6 Hz, 1H, H-5), 9.22 (s, 1H, H-8), 14.70 (br s, 1H, COOH). ^{13}C NMR (75 Hz, DMSO d_6): 14.50 (C-1'), 51.80 (C-2'), 54.59 (OCH_3-2"), 55.21(OCH_3-4"), 104.9 (C-5"), 109.5 (d, ^{2}JC-F= 21.5 Hz, C-5), 110.2 (C-7), 113.42 (C-3"), 118.74 (C-9b), 121.81 (C-1"), 126.16 (C-6"), 130.04 (C-9a), 132.15 (C-5a), 136.22 (d, ^{2}JC-F= 13.8, C-3a), 148.12 (C-8), 151.64 (d, ^{1}JC-F= 255, C-5), 154.62 (C-2"), 158.81 (C-4"), 165.63 (COOH), 175.61 (C-6). IR (KBr): ν 3390, 2973, 2643, 2382, 1726, 1621, 1469, 1319, 1089 cm^{-1}. LRMS (ES, +ve) m/z calculated for $C_{20}H_{17}FN_4O_5$ (412.12): Found 412.3 (24%), 411.5 (72%), 404.4 (8%), 399.3 (16%), 397.6 (1%), 390.6 (2%), 384.2 (26%), 383.3 (9.2), 369.2 (3%), 365.5 (37%), 336.3 (3%), 308.6 (2%), 282.5 (2%), 259.3 (2%), 251.5 (1%), 203.5 (2%), 195.4 (4%), 180.4 (1%), 163.3 (3%), 158.2 (9%), 149.0 (4%), 130.5 (3%), 102.2 (1%), 78.9 (2%), 74.3 (1%).

REFERENCES

(1) Appelbaum, P. C. & Hunter, P. A. The fluoroquinolone antibacterials: past, present and future perspectives. International Journal of Antimicrobial Agents, 2000; 16 (1), 5-15.

(2) Edmont, D. S., Rocher, R., Plisson, C. et al.Synthesis and evaluation of quinoline carboxyguanidines as antidiabetic agents. Bioorganic & Medicinal Chemistry Letters, 2000; 10 (16), 1831-1834.

(3) Zhao, Y.-L., Chen, Y.-L., Sheu, J.-Y.et al.Synthesis and antimycobacterial evaluation of certain fluoroquinolone derivatives. Bioorganic & Medicinal Chemistry, 2005; 13 (12), 3921-3926

(4) Lucero, B. D. A., Gomes, C. R. B., Frugulhetti, I. C. D. P. P. et al. Synthesis and anti-HSV-1 activity of quinolonic acyclovir analogues. Bioorganic & Medicinal Chemistry Letters, 2006; 16 (4), 1010-1013.

(5) Shaharyar, M., Ali, M. A. & Abdullah, M. M. 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, 2007; 16 (6), 292-299.

(6) Al-Hiari, Y. M., Kasabri, V. N., Shakya, A. K. et al. Fluoroquinolones: novel class of gastrointestinal dietary lipid digestion and absorption inhibitors. Medicinal Chemistry Research, 2014a; 23 (7), 1-11.

(7) Sissi, C. & Palumbo, M. The quinolone family: from antibacterial to anticancer agents. Current Medicinal Chemistry-Anti-Cancer Agents, 2003; 3 (6), 439-450.

(8) Kloskowski, T., Gurtowska, N., Nowak, M.et al. The influence of ciprofloxacin on viability of A549, HepG2, A375. S2, B16 and C6 cell lines in vitro. Acta Poloniae Pharmceutica, 2011; 68 859-865.

(9) Azema, J. L., Guidetti, B., Dewelle, J.et al. 7-((4-Substituted) piperazin-1-yl) derivatives of ciprofloxacin: synthesis and in vitro biological evaluation as potential antitumor agents. Bioorganic & Medicinal Chemistry, 2009; 17 (15), 5396-5407.

(10) Kumar, A. R., Lingaiah, B. P. V., Rao, P. S.et al.. Synthesis and biological evaluation of novel N1-decyl and C7-sec amine substituted fluoroquinolones as antitubercular and anticancer agents. Indian Journal of Chemistry, 2015; 54 1495-1501.

(11) Al-Trawneh, S. A., Zahra, J. A., Kamal, M. R.et al. Synthesis and biological evaluation of tetracyclic fluoroquinolones as antibacterial and anticancer agents. Bioorganic & Medicinal Chemistry, 2010; 18 (16), 5873-5884.

(12) Abbas, J. A. & Stuart, R. K. Vosaroxin: a novel antineoplastic quinolone. Expert Opinion on Investigational Drugs, 2012; 21 (8), 1223-1233.

(13) Hawtin, R. E., Stockett, D. E., Byl, J. A. W.et al. Voreloxin is an anticancer quinolone derivative that intercalates DNA and poisons topoisomerase II. PLoS One, 2010; 5 (4), e10186.

(14) Sweidan K, Sabbah DA, Bardaweel S, et al. Facile synthesis, characterization, and cytotoxicity study of new 3-(indol-2-yl) bicyclotetrazatridecahexaens. Can J Chem 2017; 95(8):858-862.

(15) Al-Hiari, Y. M., Abu-Dahab, R. & El-Abadelah, M. M. Heterocycles [h]-Fused Onto 4-Oxoquinoline-3-Carboxylic Acid, Part VIII [1]. Convenient Synthesis and Antimicrobial Properties of Substituted Hexahydro [1, 4] diazepino [2, 3-h] quinoline-9-carboxylic acid and Its Tetrahydroquino [7, 8-b] benzodiazepine Analog. Molecules, 2008; 13 (11), 2880-2893.

(16) Al-Hiari, Y. M., Shakya, A. K., Alzweiri, M. H. Synthesis and biological evaluation of substituted tetrahydro-1h-quino [7, 8-b][1, 4] benzodiazepine-3-carboxylic derivatives. FARMACIA, 2014b; 62 (3), 570-588.

(17) Winkler, F. K., D'arcy, A. & Hunziker, W. Structure of human pancreatic lipase. Nature, 1990; 343 (6260), 771-774.

(18) Lowe, M. E. Structure and function of pancreatic lipase and colipase. Annual Review of Nutrition, 1997; 17 (1), 141-158.

(19) Birari, R. B. & Bhutani, K. K. Pancreatic lipase inhibitors from natural sources: unexplored potential. Drug Discovery Today, 2007; 12 (19), 879-889.

(20) Vichai, V., and Kirtikara, K. Sulforhadamine B colorimetric assay for cytotoxicity screening. Nature Protocols, 2006; 1, 1112-1116.

(21) Kasabri, V., Afifi, F. U., Abu-Dahab, R. 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, 2014; 59 693-705.

(22) Nitiss, J. L. Targeting DNA topoisomerase II in cancer chemotherapy. Nature Reviews Cancer, 2009; 9(5), 338-350.

(23) Molinspiration server (www.molinspiration.com): Molinspiration property engine v2021.10

(24) MarvinSketch16.10.24 ChemAxon - Software Solutions and Services for Chemistry & Biology 2016.

(25) OMEGA, O. 2.5. 1.4: OpenEye Scientific Software, Santa Fe, NM. Google Scholar There is no corresponding record for this reference.

(26) Wu, C. C., Li, T. K., Farh, L.et al. Structural basis of type II topoisomerase inhibition by the anticancer drug etoposide. Science, 2011; 333(6041), 459-462.

(27) OEDOCKING 3.2.0.2: Open Eye Scientific Software, Santa Fe, NM. http://www.eyesopen.com

(28) BIOVIA, Dassault Systemes, BIOVIA Discovery Studio Visualizer, v17.2.0.16349, San Diego: Dassault systems, 2016.

(29) Mitscher, L. A. Bacterial topoisomerase inhibitors: quinolone and pyridone antibacterial agents. Chemical Reviews, 2005; 105 (2), 559-592.

(30) Al-Hiari, Y. M., Qaisia, A. M., Shuheil, M. Y. A.et al. Synthesis and Antibacterial Potency of 4-Methyl-2, 7-dioxo-1, 2, 3, 4, 7, 10-hexahydropyrido [2, 3-f] quinoxaline-8-carboxylic acid, Selected [a]-Fused Heterocycles and Acyclic Precursors. Zeitschrift fuer Naturforschung B: Chemical Sciences, 2007b ; 62 (11), 1453-1458.

(31) Al-Hiari, Y. M., Qandil, A. M., Al-Zoubi, R. M.et al. (2010), 7-(3-Chlorophenylamino)-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1, 4-dihydroquinoline-3-carboxylic Acid. Molbank, 2010 (2), M669.

(32) Arabiyat, S., Kasabri, V., Al-Hiari, Y.et al. Antilipase and antiproliferative activities of novel fluoroquinolones and triazolofluoroquinolones. Chemical Biology & Drug Design, 2017; 90(6), 1282-1294.

(33) World Cancer Research, F. & American Institute for Cancer, R. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. American Institution for Cancer Research, 2007.

(34) Kathiravan, M. K., Khilare, M. M., Nikoomanesh, K.et al. Topoisomerase as target for antibacterial and anticancer drug discovery. Journal of Enzyme Inhibition and Medicinal Chemistry, 2013; 28 (3), 419-435.

(35) Arabiyat, S., Kasabri, V., & Al-Hiari, Y. Antilipolytic-Antiproliferative Activity of Novel Antidiabesity Triazolo/Fluoroquinolones. Jordan J. of Pharm. Scie., 2020; 13(1).

(36) Khaleel, S., Al-Hiari, Y., Kasabri, V.et al. Antiproliferative Properties of 7, 8-Ethylene Diamine Chelator-Lipophilic Fluoroquinolone Derivatives Against Colorectal Cancer Cell Lines. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), 2022; 22(5), 1012-102835.

(37) Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 2001; 46(1-3):3-26

(38) Veber DF, Johnson SR, Cheng HY.et al.Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem. 2002; 45(12):2615-23.

(39) Nath, A., Kumer, A., Zaben, F.et al. Investigating the binding affinity, molecular dynamics, and ADMET properties of 2, 3-dihydrobenzofuran derivatives as an inhibitor of fungi, bacteria, and virus protein. Beni-Suef University Journal of Basic and Applied Sciences, 2021; 10(1), 1-13.

(40) Arabiyat, S., Kasabri, V., Al-Hiari, Y.et al. Dual glycation-inflammation modulation, DPP-IV and pancraetic lipase inhibitory potentials and antiproliferative activity of novel fluoroquinolones. Asian Pacific journal of cancer prevention: APJCP, 2019; 20(8), 2503.

(41) Bentley, D., Young, A.-M., Rowell, L.et al. Evidence of a drug-drug interaction linked to inhibition of ester hydrolysis by orlistat. Journal of Cardiovascular Pharmacology, 2012; 60 (4), 390-396.

(42) Habtemariam, S. The anti-obesity potential of sigmoidin A. Pharmaceutical Biology, 2012; 50 (12), 1519-1522.

(43) Arabiyat, S., Al-Hiari, Y., Bustanji, Y.et al. In Vitro modulation of pancreatic lipase and proliferation of obesity related colorectal cancer cell line panel by novel synthetic triazoloquinolones. Rev. Roum. Chim, 2016; 61(11-12), 871-879.

(44) Al-Hiari, Y., Sweileh, B., Shakya, A.et al. Synthesis of 1-Benzyl-1, 2, 3, 4-Tetrahydroisoquinoline, Part I: Grignard Synthesis of 1-(Substitutedbenzyl)-1,2,3,4-Tetrahydroisoquinoline Models with Potential Antibacterial Activity, Jordan J. of Pharm. Sci., 2009; 2(1), 1-21, 2009.

تخلب المعادن كميكانيكية لعلاج السرطان: الجزء الأول؛ مركبات جديدة من مشتقات 7- انيسيدين مع متعدد التخلب في ذرات الكربون 7-8 في نموذج حلقة الفلوروكوينولون كمضاد محتمل للسرطان ومضاد محتمل لتسكير الدهون

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ملخص

الخلفية: السرطان هو أحد أعظم الأمراض المزعجة في الوقت الحالي. يُعتقد أنه السبب الثاني للوفاة بعد أمراض القلب والأوعية الدموية. نظرا لتعدد أنواعها ومراحلها وأساسها الجيني، فلا يوجد دواء موجود لعلاج جميع أنواع السرطان. تعتبر مقاومة الأدوية الحالية والآثار الضارة الشديدة من التحديات الأخرى في مكافحة السرطان. في مثل هذا المسعى، تمتلك الفلوروكينولونات (FQs) إمكانات كمركبات مضادة للتكاثر بسبب السلامة والتكلفة المنخفضة وغياب المقاومة.

الهدف: في هذه الدراسة، نهدف إلى تصنيع مركبات نشطة بيولوجيًا لها إمكانات مزدوجة مضادة للسرطان ومضادة لليبيز. تم تحضير ستة عشر مركبًا، وتم تصنيفها بالكامل، ودراستها من خلال تحديد قيم IC50 ضد خطوط الخلايا السرطانية شديدة الحساسية.

المنهجية: في هذا العمل، نحن مهتمون بتجميع المركبات النشطة بيولوجيًا التي تنتمي إلى الفلوروكينولونات (FQs) مع نشاط مزدوج مضاد لسرطان القولون والمستقيم ومضاد لليبيز، نظرًا للارتباط بين السرطان والسمنة، وإجراء تجارب المعايرة ومحاكاة الارتباط للتحقق من صحة فرضيتنا

النتائج: أشارت النتائج في المختبر إلى أن هذه المركبات أظهرت نشاطًا واعدًا مضادًا للسرطان ضد خطوط الخلايا المختبرة في نطاق الميكرومولار بقوة مماثلة لسيسبلاتين. أظهر المركب 11 فاعلية مضاعفة تقريبًا مقارنة بالسيسبلاتين ضد خط خلايا سرطان القولون والمستقيم SW620 مع IC50 3.2 ميكرومتر والذي يقترح FQs كعوامل فعالة مضادة للتكاثر. تم فحص مركبات الفلوروكينولون المُصنَّعة (FQ) بشكل إضافي لمعرفة إمكاناتها المضادة لليباز في المختبر. أظهرت النتائج أن جميع المركبات التي تم فحصها أظهرت نشاطًا ملحوظًا مضادًا لليبيز، مقارنةً بجزيء التحكم أورليستات. أظهر المركب 9 نشاطًا مشابهًا لأورليستات ضد الليباز البنكرياس مع IC50 0.4 ميكرومتر والذي يقترح FQs كمثبطات قوية لليبيز البنكرياس

الخلاصة: تشير الإمكانات المضادة للسرطان لهذه المشتقات إلى قدرتها على تثبيط أنزيم Topo II مما يشير إلى أن عملية الاتحاد مع المعادن الثقيلة هي آلية تثبيط أنزيم Topo II التي يتم التأكيد عليها من خلال تجارب المعايرة ومحاكاة الارتباط.

الكلمات الدالة: مخلبات المعادن، متعدد التخلب، الفلوروكوينولونات، مضاد سرطاني مخلب.

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