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Original Research

Novel C7 anisidinoquinolones with advantageous antibacterial activity in nanoscale concentrations against standard and resistant bacterial strains

Somia M. ElSheikhi, Rula M. Darwish, Yusuf Al-Hiari, Violet Kasabri, Mohammed A.F. Salih

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Abstract

Background The extensive clinical use of Fluoroquinolones (FQs) led to the development of bacterial resistance against these agents. In this work, new lipophilic FQs were designed, prepared and screened against standard and resistant bacteria. A series of novel 7-substituted anilino-8-nitroFQ acids **3** (**a-e**), their reduced derivatives **4** (**a-e**) and their triazolo **5** (**a-c**) were successfully prepared, identified and characterized using NMR, and MS. FQs **3-5(a-e)** were then evaluated for the *in vitro* antibacterial activity against standard and resistant gram-positive and gram-negative strains using serial dilution method. Combination between the new FQs and different classes of antibiotics were also tested for possible synergistic effect using checkerboard technique. **Results** The outcomes of the new FQs showed comparable and superior activity against both the standard strains *S.aureus* and *E.coli* with remarkable activity against the standard *S.aureus* strain. The reduced series **4** were the most active group among all derivatives with nanogram concentrations for **4d (**60 ng/mL**)** and **4e** (15 ng/mL) against the standard gram positive strain. Our compounds revealed appreciable or comparable MIC mean values to standard against resistant gram-positive strains (*MRSA)* with no inhibitory activity against gram-negative strains (MDR *E.coli)*. The hydroxyl derivatives **6** have showed the strongest MIC mean values among all compounds. Combination of these compounds with bacitracin, ampicillin and cephalexin showed synergistic effect with fractional inhibitory concentration (FIC) index between 0.185-0.375. While combinations with erythromycin, neomycin and tetracycline showed indifference effect with FIC index 2. **Conclusions** Evidently increasing number of hydrogen bond acceptor/donor leads to significant increase in activity presented by compounds **4e** and **6e**. These findings would open the floor for these novel antibacterial agents to be used alone or in combinations with conventional antibiotics for treatment of pathogenic bacteria.

Keywords: fluoroquinolones, synergism, *Staphylococcus aureus, MRSA, Escherichia coli*

INTRODUCTION

Hospital-acquired infections (HAIs) are a global issue, causing significant morbidity rates of nearly 60% among hospitalized patients.[1] The emergence of bacterial resistance has posed a formidable challenge for healthcare teams in combating these infections. *Staphylococcus aureus*, an extensively colonizing pathogen, involves a wide spectrum of infections and poses a particularly massive challenge due to its multidrug-resistant variant, methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA infections are associated with elevated rates of morbidity and mortality, warranting attention in the medical field.[2] The prevalence of MRSA exhibits regional variances, with the Middle East showcasing a considerable burden. Comprehending the epidemiology and impact of MRSA colonization and infection is necessary for effective management preventive strategies.[3] *Escherichia coli* (*E.coli*), a gram-negative opportunistic pathogen, represent a predominant pathogen responsible for various infections in humans. Its prevalence, notably in the gastrointestinal tract, underscores its role as a potential reservoir for infections such as urinary tract infections, septicemia, and gastroenteritis.[4] Furthermore, the emergence of antimicrobial resistance, particularly involving extended-spectrum beta-lactamase (ESBL) and AmpC beta-lactamase, presents significant therapeutic challenges in managing *E. coli* infections.[5] Mitigating the rising flow of antimicrobial resistance necessitates the exploration of novel therapeutic agents and formulations. FQs; as a class of orally absorbed antimicrobial drugs, have emerged as promising candidates.[6] However, their extensive utilization, coupled with inappropriate practices, has expedited the development of resistance. Understanding the pharmacological attributes and potential strategies to address resistance patterns surrounding FQs is essential for effective intervention against resilient bacterial strains.[7]

Given that MRSA is a gram-positive bacterium, it is crucial for any compound to possess enhanced lipophilic characteristics

Somia M ELSHEIKHI. School of Pharmacy, University of Jordan, Queen Ranya Street, Amman 11942, JORDAN. somia.elsheikhi@omu.edu.ly
Rula M. DARWISH*. School of Pharmacy, University of Jordan, Queen Ranya Street, Amman 11942, JORDAN. rulad@ju.edu.jo
Yusuf AL-HIARI. School of Pharmacy, University of Jordan, Queen Ranya Street, Amman 11942, JORDAN. hiary@ju.edu.jo
Violet KASABRI. School of Pharmacy, University of Jordan, Queen Ranya Street, Amman 11942, JORDAN.v.kasabri@ju.edu.jo
Mohammed A.F. SALIH. School of Pharmacy, University of Jordan, Queen Ranya Street, Amman 11942, JORDAN. mohammedalkubaisi4@gmail.com

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in order to effectively penetrate the microorganism's cell membrane. Previous research has extensively demonstrated that more lipophilic FQs exhibit greater activity against both standard and resistant strains.[8-9] Additionally we have discovered that the presence of a hydrogen bond donor on the C-7 benzene (such as aniline or anisidine) may augment the gram-positive activity. This study involves the synthesis of FQs with increased lipophilicity, achieved by introducing methoxy groups on the C-7 benzene. The lipophilic nature of the compounds was further enhanced by incorporating anisidine groups at C-7 (Figure 1: structure A).We hypothesized that enhancing lipophilicity and introducing a hydrogen bond donor would lead to an increase in gram-positive activity, consequently improving efficacy against resistant strains. Additionally, we explored the preparation of a trizolo-system to investigate the impact of a cyclic structure on C7-8 (Figure 1: structure B).

METHODS

Materials and instruments

Reagents and chemicals use in this work were purchased from Sigma -Aldrich (Dorset, UK); and Acros (Belgium) unless otherwise stated. All chemicals, reagents and solvents of analytical grade were used without further purification unless stated p-Anisidine, 2,4-Dimethoxy, and aniline 3,4,5-trimethoxy anisidines distilled before use due to oxidation. Starting materials involved in the preparation are. : 2, 4-dichloro-5-fluoro-3-nitrobenzoic acid was purchased from Sigma-Aldrich (St. Luis, MO, USA). Ethyl 3-(*N*, *N*-dimethyl-amino) acrylate and butyl amine were from Acros, Belgium. Aromatic amines; *o*-anisidine, *m*-anisidine, and *p*-anisidine, 2,4-Dimethoxy aniline and 3,4,5-trimethoxy aniline are from Sigma -Aldrich (Dorset, UK); and Acros (Belgium). Reducing agent, anhydrous stannous chloride crystals, was purchased from Fluka, (Switzerland). Sodium nitrite was purchased from Sigma Aldrich (St. Luis, MO, USA), Melting points (mp) were determined in open capillaries on a Stuart scientific electro-thermal melting point digital apparatus (Stuart, Smp3, and Staffordshire, UK).

Thin layer chromatography (TLC) was performed on 10 x 10 cm^2 aluminum plates pre-coated with fluorescent silica gel GF254 (Machevey-Nagel, Vived company, Germany) and was visualized using UV lamp fluorescence analysis cabinet (at 254nm wave length/ short wave length/ long wavelength, Spectroline model CX-20). Mobile phase mixtures were: 80:20 chloroform-ethanol ($CHCl_3$: EtOH) (system 1) and 50:50 (*n*-hexane: Ethylactate) (system 2).

Nuclear Magnetic Resonance Spectra (NMR) were recorded on Bruker 500 MHz-Avance III (500 MHz) at Hamdi Mango Center at University of Jordan. Chemical shifts are reported in ppm related to tetramethylsilane (TMS) as the internal standard. Deuteriated dimethylsulfoxide (DMSO-d_6) and deuteriated chloroform ($CDCl_3$) were used as solvents in sample preparation, otherwise is indicated. In special occasions (NaOD) was used to improve solubility. Analysis on room temperature or below was utilized to limit oxidation/degradation upon analysis, especially with reduced series (4).

^{1}H NMR data are reported in the following way; chemical shift (ppm), (multiplicity, coupling constant (Hz), number of protons, the corresponding proton(s). High resolution mass spectra (HRMS) were measured in positive ion mode using electrospray ionization (ESI) technique by collision-induced dissociation on a Bruker APEX-4 (7 Tesla) instrument at chemistry department/ the university of Jordan.

The samples were dissolved in acetonitrile, diluted in spray solution (methanol/water 1:1 v/v + 0.1 formic acid) and infused using a syringe pump with a flow rate of 2µL/min. External calibration was conducted using Arginine cluster in a mass range m/z 175-871. LR/HRMS were employed using an electrospray ionizer (ESI), molecular weight was recorded as AMU+1 in case of positive model of ESI or AMU-1 in case of negative mode of ESI.

Microorganisms and their counterpart resistant clinical isolates

Staphylococcus aureus (ATCC 6538); Escherichia coli (ATCC 4157); Methicillin Resistant *Staphylococcus aureus* (ATCC 33591) *MRSA*; *Staphylococcus epidermidis* (ATCC22105); *Bacillus subtilis* (ATCC6634); *Streptococcus oralis* (ATCC 35037); *Multi-drug resistant (MDR) Escherichia coli* isolated from hospitalized patients from the Jordan University Hospital and its identity confirmed by biochemical tests.

Synthesis of synthon 1acid and ester (*supplementary*)

Synthons**1E**; Ethyl 7-chloro-1-butyl-6-fluoro-8-nitro-4-oxo-1, 4-dihydroquinoline-3-carboxylate and its acid **1A**; were reported previously by our group and alike procedure was

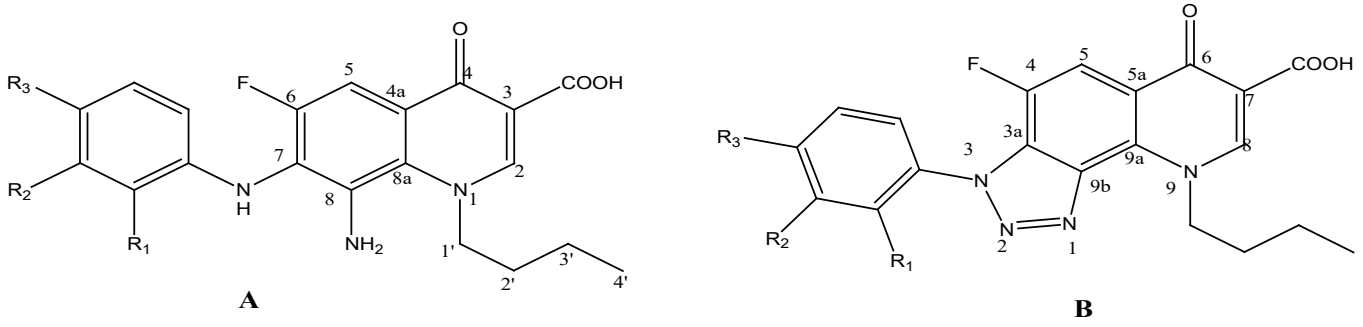


Figure 1. Synthetic scaffolds of novel FQs

used to prepare both for this work (Al Abbsi *et al*., 2018). Minor modification was adopted to prepare 50g of **1E** (with good yields) as starting point for preparation of our targets FQs **2-5 (a-c)**. All intermediates and final synthons **1A** and **1E** were fully characterized and their experimental data were exact match with reported data. Synthon **1A** (7-Chloro-1-butyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid) was obtained from acid hydrolysis **of 1E** and was prepared for antimicrobial testing only.

Bacterial broth culture

10 mL of freshly prepared sterile nutrient broth was transferred to a sterile 15 mL falcon tube and 150 µL of Bacteria strains; the tube was vortexed and incubated for 24 hours in shaking incubator (DK-SI010) at 37°C. The turbidity of the cell suspension was measured by the spectrophotometric method at 630 nm and adjusted by using sterile distilled water as needed to produce a bacteria suspension of 1–2× 10^8 CFU/mL. This was standardized according to 0.5 McFarland suspensions. The final inoculum suspension was made by a dilution of 1:100 of the suspension in the broth medium used which resulted in 0.5 x 10^6 CFU/mL (cells per mL).

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (µg/mL) of tested compounds were NCCLS broth microdilution reference method.[10] The MIC was calculated as the average of two successive concentrations of the tested compound showing no growth and growth. Aliquots of 40.96 mg of the synthesized FQ compounds were dissolved in 0.25%DMSO. The 96 wells were filled with 100 µL of broth media (1-8) Then, the diluted drug was added to the first well containing to give a concentration of 1024 µg/mL, add. Next, serial two-fold dilution was made from well 1 to well 8 (the final 100 µL from well 8 were discarded). A 100µL of a diluted culture (was made by a dilution of 1:100 of the suspension in the broth. The final inoculum suspension medium used which resulted in 0.5 x 10^6 to cells per mL) were added to each well. Negative control of experiment in well number 9 =200 µL of Broth., negative control antimicrobial agent of experiment in well number 10=100 µL of broth +5µL of antimicrobial agent, positive control in well number11=100 µL broth+100 µL of Bacteria Broth and solvent control in well number 12=1 µL DMSO+99 µL broth+100 of Bacteria broth. After that, the plate was incubated for 24 hours at 37°C in the SELECTA® incubator. Each experiment for each compound was performed in triplicate.

***In-vitro* determination of the effect of the combination on the activity**

Checkerboard microdilution assay for combinations studies and was applied for combinations of the FQs and different classes of antibiotics on different bacterial strains representing Gram positive , Gram negative and resistant bacteria . The microtiter plate was incubated aerobically for 48 hours at 37°C. Each experiment was conducted in triplicate. The lowest concentration of both drugs studied for synergism with no visible growth was recorded to be the specific MIC value. The mode of interaction between the two drugs was classified according to fractional inhibitory concentration index (FICI) values of synergism, additive, indifference, and antagonism for 24–48 hours. The FICI was calculated by the following equation:

∑FICindex = FICindexofDrugA + FICindexofDrugB

FIClogDrugA = (MIC of Drug A in Combination)/ (MIC of Drug A Alone)

FIClogDrugB = (MIC of Drug B in Combination)/ (MIC of Drug B Alone)

The nature of interaction values according to the American Society of Microbiology was assessed as: Synergism ≤0.5, additive >0.5 but <1, indifference >0.5 but ≤4, antagonism >4.[11] Each experiment was conducted in triplicate, and mean values were reported.

RESULTS

Chemistry

Synthesis of synthon 1acid and 1ester

More than 50gm of 1E was prepared, for this work and characterized.

Synthesis of novel compounds 2-5 (a-e) (scheme 1)

Synthons **2-5(a-e)** were prepared and successfully following scheme 1. Similar procedure was used to **2a-5a** prepared previously[12] were involved in preparing **2**-**5**(**b**-**e**). Enough amounts were prepared from each for full characterization and biological screening. Compounds **5d** and **5e** could not be prepared in this work due to low amount of their precursors **4d** and **e**. **4** series suffered harsh hydrolysis and oxidation upon purification and analysis (table 1). High temperature and solvents analysis produced many peaks over analysis of **4d** and **e**.

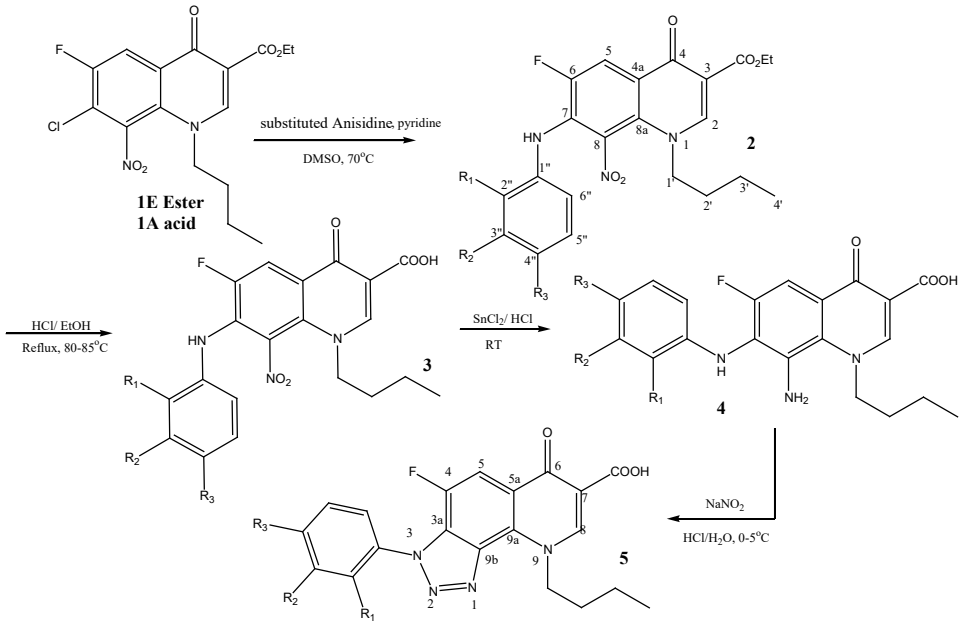
Attempts for synthesis of hydroxy compounds 6 and 7 (scheme 2)

An attempts were made to hydrolyse the methoxy ether groups of anisidine derivatives **2 b, d, e** into **6 b, d, e** and **7 b, d, e**. HBr hydrolysis at high temperature and reflux produced a mixture of these products in which the product was major in some them. Many side products in addition to starting were obtained and the number of these side products increased where the number of methoxy groups increased (table 2). Due to difficulty in isolation /separation of pure compounds of 6 series, we reported either major compounds NMR or the mass spectral data in the experimental part. A low amount of **6** has prevented preparation of reduced **7** series.

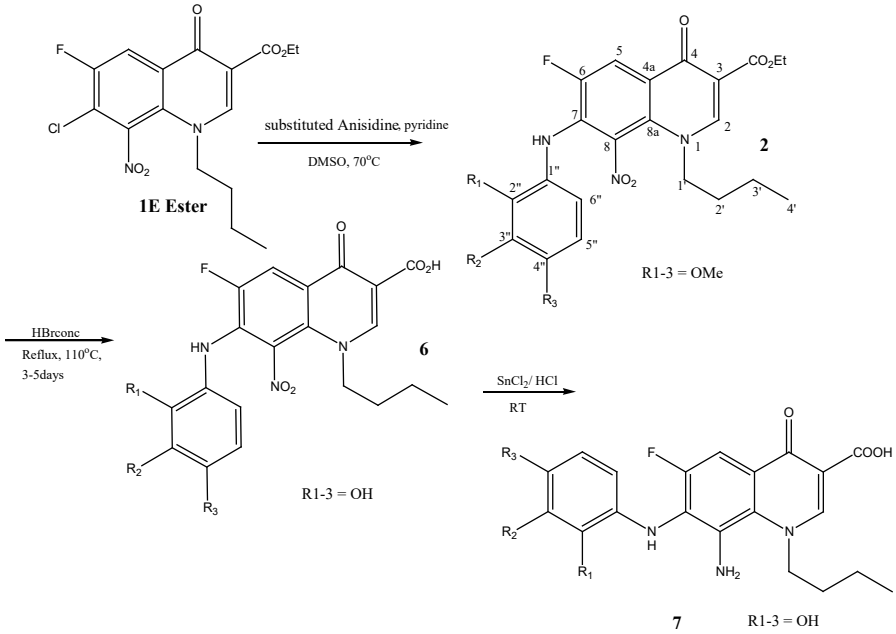
Biology

MIC determination assays (table 3)

MIC results for all compounds against gram-positive and gram-negative (standard and resistant strains) are shown in Table 3 below. All compounds showed comparable or superior activity against both standard strains with remarkable activity against standard *S.aureus* and *E.coli* strains. The reduced series **4** were the most active group among all derivatives. Our compounds



Scheme 1. Targeted compounds 2-5 (a-e)



Scheme 2. Targeted hydroxyl compounds 6 and 7 (b,d,e)

Table 1. Structure of Synthon 1-5 (a-e)				
Compound 2-5	**Anisidine derivative**	**R_1**	**R_2**	**R_3**
a	2-Methoxy aniline	OMe	H	H
b	3-Methoxy aniline	H	OMe	H
c	4-Methoxy aniline	H	H	OMe
d	2,4-Dimethoxy aniline	OMe	H	OMe
e	3,4,5-Trimethoxy aniline	OMe	OMe	OMe

Table 2. Structure of Synthon 6-7 (b, d, e)				
Compound 6 -7	**Hydroxy derivative**	**R_1**	**R_2**	**R_3**
b	3-Hydroxy aniline	H	OH	H
d	2,4-Dihydroxy aniline	OH	H	OH
e	3,4,5-Trihydroxy aniline	OH	OH	OH

Table 3. Mean MIC Values of all compounds (µg/mL) against standard and resistant gram positive and negative strains: S. *aureus* (ATCC6538), *S. aureus* (ATCC33591) *MRSA, E. coli* (ATCC4157), MDR *E.coli, S. oralis* (ATCC35037), *S. epidermidus (*ATCC22105) and *B. subtilis (*ATCC6634)

Compounds	*S.aureus* (ATCC6538)	*S.aureus* (ATCC33591) *MRSA*	*E.coli* (ATCC4157)	*MDR E. coli*	*S. oralis* (ATCC35037)	*S. epidermidus* (ATCC22105)	*B. subtilis* (ATCC6634)
1E	16	64	32	ND	16	16	16
1A	**0.5**	8	2	ND	2	4	2
3a	1	16	1	ND	1	1	**0.5**
3b	1	8	1	ND	1	1	**0.5**
3c	1	8	1	ND	1	**0.5**	**0.5**
3d	**0.25**	8	**0.25**	ND	**0.25**	**0.06**	**0.125**
3e	**0.06**	8	**0.25**	ND	**0.125**	**0.125**	**0.125**
4a	**0.5**	8	1	ND	1	**0.5**	**0.5**
4b	**0.25**	8	**0.5**	ND	**0.5**	**0.25**	**0.125**
4c	**0.25**	8	**0.5**	ND	1	**0.25**	**0.125**
4d	**0.06**	8	**0.25**	ND	1	1	**0.5**
4e	**0.015**	**4**	**0.25**	ND	**0.06**	**0.06**	**0.06**
5a	1	16	2	ND	0.125	**0.125**	**0.125**
5b	**0.5**	8	1	ND	**0.06**	**0.125**	**0.06**
5c	1	8	2	ND	**0.125**	**0.125**	**0.12**
6b	**0.03**	8	**0.25**	ND	**0.25**	**0.06**	**0.125**
6d	**0.015**	8	**0.125**	ND	**0.125**	**0.125**	**0.125**
6e	**0.0075**	4	**0.125**	ND	**0.06**	**0.06**	**0.06**
Ciprofloxacin	**0.5**	8	2	ND	1	**0.5**	**0.5**
C-7 aniline [9]	**0.639**	R	-	ND	-	-	-
Bacitracin	16	32	16	ND	-	-	-
Ampicillin	16	32	16	ND	-	-	-
Cephalexin	16	32	128	ND	-	-	-
Erythromycin		64	16	ND	-	-	-
Neomycin	32	64	32	ND	-	-	-
Tetracycline		256	128	ND	-	-	-

ND: MIC was not detected as there was no activity of the compounds against MDR *E. coli*
''-'': Untested and hence not included in combination treatments against these microorganisms

revealed acceptable or comparable MIC values against resistant gram-positive strains (*MRSA)* with no inhibitory activity against gram-negative strains (MDR *E.coli*). Although not very pure, the hydroxyl derivatives **6** have showed the strongest MIC values among all compounds. Increasing the number of methoxy groups or hydroxy groups have led to the most active derivatives in all series **e** and **d**. In fact the trimethoxy and dimethoxy **4e** and **4d** have revealed nanogram level MIC values with (0.015 and 0.06) µg/mL respectively against *S.aureus* standard strain. Although not reliable, it can be deduced clearly that increasing the number of hydroxyl groups amplified the antibacterial activity. The di- and tri- hydroxyl derivatives **6d** and **e** showed increased activity with lower MIC values.

Similar pattern of activity is revealed by our derivatives since all showed excellent and superior activity against all gram positive strains. Again increasing the number of methoxy and hydroxl groups improved the activity significantly. All compounds were active with range of MIC (0.03 µg/mL to 1µg/mL) and the most effective compounds were the reduced forms of compound series **4** and **5.**

Checkerboard combination assay (table 4)

For mechanistic studies, a combination between ciprofloxacin and all compounds and FICI mean was determined. The FICI was (0.5 to 1) indicating the additive effect (Table 4). Furthermore, we evaluated other representative common antibiotics with our compounds of with regard to their antibacterial mechanism of action (Table 4). Our FQs revealed superior activity to all commercial antibiotics tested. This indicated different mechanism of action. Table 4 also shows mean FICIs values between combination of reduced and hydroxyl compounds series **4** and **6** with 6 antibiotics. Bacitracin, ampicillin, and cephalexin displayed Synergistic effect with standard gram positive strain *S.auerus (*ATCC6538) and *MRSA*, with the mean FICIs values ranging from (0.185 to 0.49). While Combination with erythromycin, neomycin, and tetracycline showed

indifference effect with the mean FICIs values (2) as shown in Table 4. Table 5 comprehensively details the Molecular descriptors of the new novel FQs.

DISCUSSION

Given the broadly versatile prevalence of antimicrobial resistance;[13-15] the aim of this work was to investigate the antibacterial efficacies against both gram positive and negative strains along with their clinically resistant isolates of newly prepared and characterized lipophilic FQ' derivatives with substituted C7-methoxy functionalities (anisidines). Principally FQs are broad spectrum antibiotics, with pronounced activity against gram-negative bacteria. The weak gram-positive antibacterial activity of these commercial drugs restricted their use for fighting resistance gram-positive strains such as MRSA. Previous work of our group disclosed new lipophilic FQs as strong antibacterial compounds against standard gram-positive strains. We presumed that increasing lipophilicity could increase gram-positive activity and consequently antibacterial activity against resistant strains, since lipophilic compounds could penetrate better the cell wall of gram-positive bacteria. To further fortify their activity, the number of hydrogen bond acceptor and donor groups was increased since these will provide extra H-B with the receptor.[16-17] It was shown that the acid derivative **1A** was much more potent than the ester **1E** against all strains tested. This suggested that the 4-oxo-3-COOH group is essential for antimicrobial effect and our FQs. It is well documented that free acidic 4-oxo-3-COOH chelation with divalent metals is essential for antimicrobial activity of FQs. Therefore, we decided to tests the acids only **3, 4, 5**. Due to difficulty in isolating pure compounds, we reported either major compounds proton NMR or the mass spectral data in the experimental part (**Supplementary**).

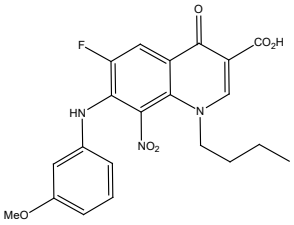
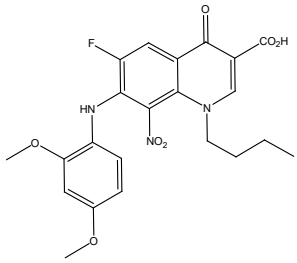
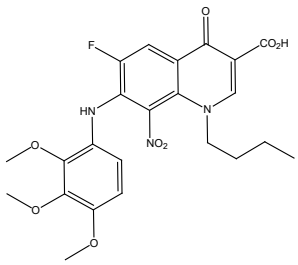
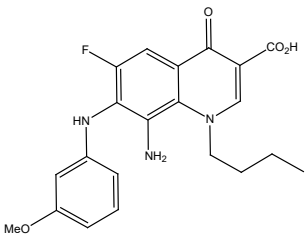
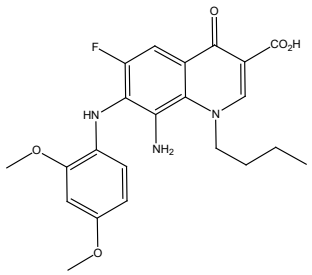
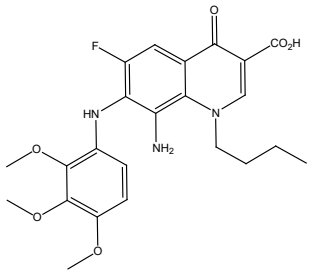
Table 4. FICIs values between combination of reduced and hydroxyl compounds series 4 and 6 with 6 antibiotics

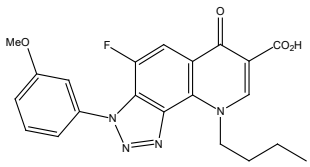
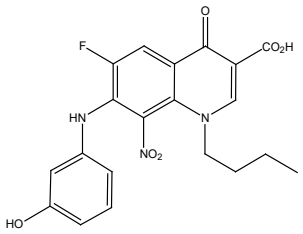
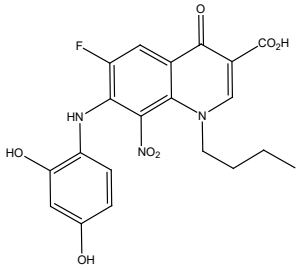
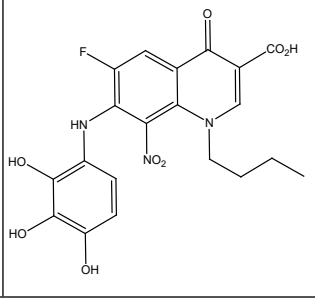
Combinations	Mean values of FIC of *S.aureus* (ATCC6538)	Mean values of FIC of *MRSA* (33591)	Mean values of FIC of *E.coli* (ATTCC4157)	FIC Index
Bacitracin+4a	0.37	0.25	-	Synergistic
Bacitracin+4b	0.49	0.25	-	Synergistic
Bacitracin+4c	0.37	0.25	-	Synergistic
Bacitracin+4d	0.375	0.185	-	Synergistic
Bacutracin+4e	0.375	0.375	-	Synergistic
Bacitracin+6b	0.325	0.25	-	Synergistic
Bacitracin+6d	0.325	0.25	-	Synergistic
Bacitracin+6e	0.325	0.25	-	Synergistic
Ampicillin+4a	0.32	0.375	-	Synergistic
Ampicillin+4b	0.36	0.25	-	Synergistic
Ampicillin+4c	0.245	0.375	-	Synergistic
Ampicillin+4d	0.375	0.25	-	Synergistic
Ampicillin+4e	0.375	0.375	-	Synergistic
Ampicillin+6b	0.325	0.25	-	Synergistic
Ampicillin+6d	0.325	0.25	-	Synergistic
Ampicillin+6e	0.325	0.25	-	Synergistic
Cephalexin+4a	0.32	0.25	-	Synergistic
Cephalexin+4b	0.36	0.25	-	Synergistic
Cephalexin+4c	0.245	0.25	-	Synergistic
Cephalexin+4d	0.375	0.185	-	Synergistic
Cephalexin+4e	0.375	0.375	-	Synergistic
Cephalexin+6b	0.325	0.25	-	Synergistic
Cephalexin+6d	0.325	0.25	-	Synergistic
Cephalexin+6e	0.325	0.25	-	Synergistic
Erythromycin+4a	2	2	-	Indifference
Erythromycin+4b	2	2	-	Indifference
Erythromycin+4c	2	2	-	Indifference
Erythromycin+4d	2	2	-	Indifference

Erythromycin+4e	2	2	-	Indifference
Erythromycin+6b	2	2	-	Indifference
Erythromycin+6d	2	2	-	Indifference
Erythromycin+6e	2	2	-	Indifference
Neomycin+4a	2	2	-	Indifference
Neomycin +4b	2	2	-	Indifference
Neomycin +4c	2	2	-	Indifference
Neomycin +4d	2	2	-	Indifference
Neomycin +4e	2	2	-	Indifference
Neomycin +6b	2	2	-	Indifference
Neomycin +6d	2	2	-	Indifference
Neomycin +6e	2	2	-	Indifference
Tetracycline+4a	2	2	-	Indifference
Tetracycline +4b	2	2	-	Indifference
Tetracycline +4c	2	2	-	Indifference
Tetracycline +4d	2	2	-	Indifference
Tetracycline +4e	2	2	-	Indifference
Tetracycline +6b	2	2	-	Indifference
Tetracycline +6d	2	2	-	Indifference
Tetracycline +6e	2	2	-	Indifference
Ciprofloxacin+3a	0.75	1	0.5	**Additive**
Ciprofloxacin+3b	0.75	1	0.75	**Additive**
Ciprofloxacin+3c	0.51	1	0.75	**Additive**
Ciprofloxacin+3d	0.62	1	1	**Additive**
Ciprofloxacin+3e	0.78	1	1	**Additive**
Ciprofloxacin+4a	0.5	1	0.51	**Additive**
Ciprofloxacin+4b	0.5	1	0.51	**Additive**
Ciprofloxacin+4c	0.75	0.77	0.75	**Additive**
Ciprofloxacin+4d	0.76	1	1	**Additive**
Ciprofloxacin+4e	0.76	1	0.75	**Additive**
Ciprofloxacin+5a	0.75	1	0.5	**Additive**
Ciprofloxacin+5b	0.75	1	0.5	**Additive**
Ciprofloxacin+5c	0.75	1	0.5	**Additive**
Ciprofloxacin+6b	1	1	0.75	**Additive**
Ciprofloxacin+6d	1	1	1	**Additive**
Ciprofloxacin+6e	0.5	1	0.5	**Additive**

FIC =MIC of drug A in combination /MIC of drug A alone + MIC of drug B in combination /MIC of drug B alone. The FIC indices :< 0.5 Synergy, 0.5-1 Additive, 1-4.0 Indifference, > 4 antagonism.
''-'': Untested and hence not included in combination treatments against these microorganisms

Table 5. Molecular descriptors of the novel FQs

Name	Structural Formula	Chemical Formula	MWt	Henry's law	tPSA (Å²)	C log P	CMR	HB Donor	HB acceptor
3b		$C_{21}H_{20}FN_3O_6$	429.4	1.56	130.68	4.93	11.01	2	8
3d		$C_{22}H_{22}FN_3O_7$	459.42	1.56	139.91	4.90	11.62	2	9
3e		$C_{23}H_{24}FN_3O_8$	489.45	1.56	149.14	4.14	12.24	2	10
4b		$C_{21}H_{22}FN_3O_4$	399.16	4.64	104.89	3.61	10.76	4	9
4d		$C_{22}H_{24}FN_3O_5$	429.44	4.64	114.12	3.58	11.38	4	10
4e		$C_{23}H_{26}FN_3O_6$	459.47	4.64	123.35	2.82	12.00	4	11

5b		$C_{21}H_{19}FN_4O_4$	410.4	5.53	94.80	4.39	10.72	1	9
6b		$C_{20}H_{18}FN_3O_6$	415.37	1.56	141.68	4.34	10.54	3	9
6d		$C_{20}H_{18}FN_3O_7$	431.11	1.56	161.91	3.67	10.69	4	10
6e		$C_{20}H_{18}FN_3O_8$	447.37	1.56	182.14	3.08	10.85	5	11

In fact, low yields and poor solubility of some derivatives were major limitation in NMR analysis. Although derivatives 6 and 7 not very pure, the obtained derivatives were tested against selected microorganism as mixture to prove the concept of increased antimicrobial activity with more H-B Acceptor/Donor groups. Many of the tested FQs have presented nanogram MIC mean values, which were much lower than the reference. Furthermore, the antimicrobial activity was more pronounced against standard gram-positive and standard gram-negative strains, with more superior potency against standard gram-positive in particular. Although stronger or equal to ciprofloxacin, our FQs showed weaker MIC values against resistant gram-positive MRSA compared to standard gram-positive strains. All FQs tested and ciprofloxacin reference exhibited no activity against resistant *E.coli* MDR strain. The data reveals that all derivatives of reduced series **4** were most active with nonogram MIC values and superior to reference on gram-positive standard *S.aureus*. Similar notice can be deduced for standard gram-negative strains with super activity against gram-positive strains. The activity of the nitro series **3** was less than reduced and the triazolo series **5** were the weakest, This pattern of order was also repeated for FQs series **3, 4, 5** against gram negative *E.coli*.

Worthy enough to notice that dimethoxy (**d**) and trimethoxy derivatives **(e)** unveiled most active derivatives in all series **3, 4** and **5,** Again**,** the reduced **4**d and **4e** were most active derivatives. Compound **4d** showed **4** folds increased activity than ciprofloxacin whereas **4e** was **6** folds stronger against standard gram-positive *S.aureus.* Increasing the number of methoxy groups in most active **4d** and **e** compared to monomethoxy **4 a-c** compounds suggests clearly that increasing the number of methoxy groups led to substantial increase on antimicrobial activity in both standard strains. Our compounds showed equipotent or stronger activity against resistant gram-positive *MRSA* but not against multi-drug resistant *E.coli.* Only the reduced **4e** has exhibited increased activity against MRSA compared to the reference, indicating that more electron accepting groups have a role to play against resistant strains. Antimicrobial screening results exposed that increasing number of hydrogen bond acceptor H-B A (provided by methoxy groups) lead to increase antibacterial activity as proven with increased methoxy groups in derivatives **d** and **e**. It was further anticipated that increasing the number of hydrogen bond donor (H-B D) can further contributes to extra

H-B with the receptor and further increases the antibacterial activity. This idea was conducted through hydrolyzing the methoxy groups to hydroxy groups in **6b**, **d** and **e** derivatives as models. The number of H-B A/D from trimethoxy aniline group will increase 3 folds from (0:3) in **3e** to (3:3) in **6e**.

Although compound 7 and its derivatives could not be prepared due to low amount of 6, the hydroxyl derivatives **6b, d,e** showed supra activity against standard gram-positive and standard gram-negative strains. All three derivatives showed nanogram MIC mean values that were ahead of the reference. Indeed, the antimicrobial activity increased significant with increased number of hydroxyl groups; the tri-hydroxy **6e** was stronger than di-hydroxy **6d** which was also stronger than mono-hydroxy derivatives **6b**. The antimicrobial activity of the hydroxyl derivatives **6** was more manifested against gram-positive strains. Similar to gram-positive *S.aureus,* the antimicrobial activity increased clearly with increased number of hydroxyl groups against *E.coli*. This further validate that in optimal antibacterial FQ compound, you need to increase total number of H-B A/D.

To evaluate whether the mechanism of actions of new lipophilic FQs are the same mechanism of ciprofloxacin FQs **3-6 a-e** and ciprofloxacin share the same mechanism of action depending on the FICI mean values. The FICI mean values were between (0.5-1 µg/mL) indicating additive effect against standard and resistant strains of gram-positive *S.aureus* and *MRSA* and gram-negative strain *E.coli.* Since our FQs have same structural scaffold of ciprofloxacin, it was expected that the new FQs will work on the same target which is prokaryotic topoisomerase. They only differ in their lipophilicity and possibly the mechanism by which they enter the bacterial cell wall. We do believe that the new FQs cross cell wall in a passive way since they are lipophilic, whereas the commercial FQs pass through active process since they are hydrophilic. The extra-lipophilicity and H-B A/D groups of FQs **3-6** provided the extra H-bonds explaining their unmatched activity.

In Figures 2A-D; 4e Compound have unmatched antibacterial activity compared to **6** commercial antibiotics indicating different mechanism again from all **6**. This was further confirmed by comparing combination between the **6** commercial antibiotics and most active FQs, Combination between most active compounds (series **4** and **6**) and different classes of antibiotic (bacitracin, ampicillin, cephalexin, erythromycin, neomycin, and tetracycline) with different mechanism of actions from FQs class showed best results.

Combination of our FQs **4** and **6** with bacitracin, ampicillin and cephalexin reveals synergistic effects and indifference effects with erythromycin, neomycin, and tetracycline. Both effects indicates different mechanism from our compounds for all antibiotics and its totally acceptable to prescribe our FQs with antibiotics (bacitracin, ampicillin, and cephalexin) in one prescription in the future (**Figures 2A-D)**.

All FQs have high clog P and the most active ones are the reduced series **4** have values between 2.82 to 3.61. This suggests that for anti gram-positive FQs, a hydrophilic/lipophilic balance is needed rather than highly lipophilic compounds. The nitro and triazolo series (**3** and **5**) have higher C log p values but less active. These results also fortify our previous assumption that for good antibacterial FQ, added lipophilicity to the FQs derivative direct activity against mainly gram-positive strains but further optimization hydrophilic/lipophilic balance is

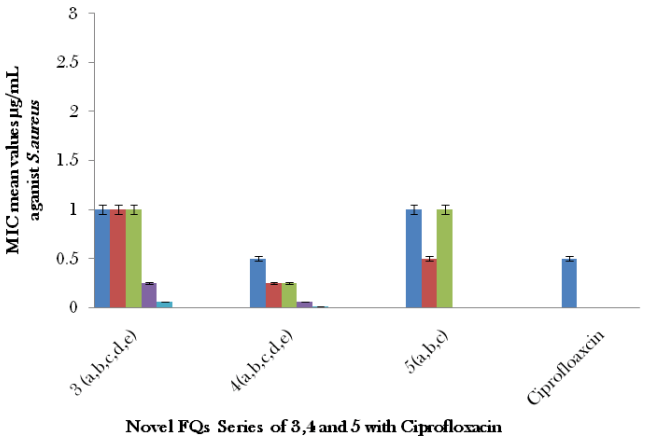


Figure 2A. MIC mean values (µg/mL) of series 3, 4 and 5 with MIC mean values (µg/mL) of ciprofloxacin against *S.aureus* (ATCC6538)

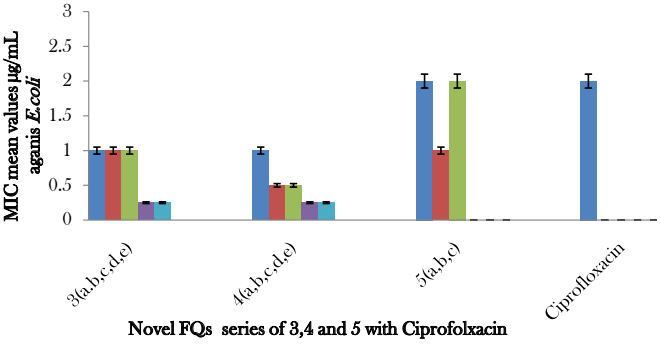


Figure 2B. MIC mean values (µg/mL) of series 3,4 and 5 with MIC mean values(µg/mL) of ciprofloxacin against *E. coli* (ATCC4157)

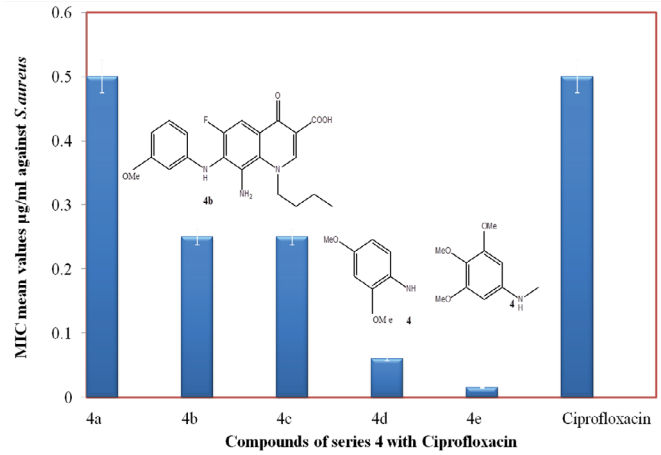


Figure 2C. MIC mean values (µg/mL) of reduced series 4 (a-e) with MIC mean values (µg/mL) of ciprofloxacin against *S.aureues* (ATCC6538)

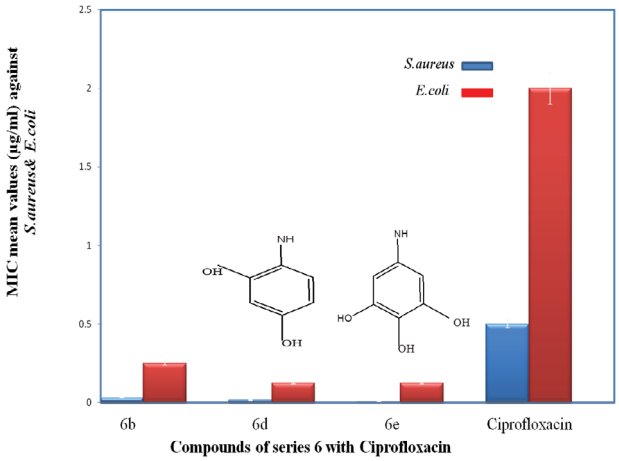


Figure 2D. MIC mean values (µg/mL) of hyroxy series 6 (b, d and e) with MIC mean values (µg/mL) of ciprofloxacin against *S.aureus* (ATCC6538) and *E.coli* (ATCC4157)

needed for strong antibacterial activity. The size and flatness seems also to be another factor that affects SAR of active FQ. Most FQs have a molecular weight above 400. The ionization and polar groups also contribute to activity. The most active which is reduced series **4** displayed with topological polar surface area tPSA of 104.89 to 123.35 (Table 5). Other FQs which showed reasonable activity have tPSA value above 100.

In Figures **4A-D**; increasing number of both hydrogen bond donor and acceptor is a major requirement for active FQ. Moreover increased values of donor are additionally important. This was indistinct with compounds 4b, **4d**, **4e**, **6d**, and **6e** that exhibited higher H-B donor compared to **3b**, **3d**, **3e**, and **5b**. The increased number of H-B acceptor can for sure increase number of H-B interaction with target receptor and fortified by increasing activity as moving from **4a, b, c** to **4d** and **4e**. The C-8 amino has contributed to both H-B donor/acceptor augmentation additions which spot the light on the importance of such position and such group in antibacterial activity. All active FQs do have the C8-amino substituent and did provide 2 H bond donor atoms and 1 H bond acceptor. This means that 8-amino (C-8-C7 diethylene amino bridge) is essential for activity as the case in all reduced and provides good vicinity for enzyme interaction with FQ through the freely accessible interfacing H-B. One further justification for the super activity of reduced **4** series comes also from the role of 8-amino group as a chelator group and as antioxidant group, Moreover, the aromatic amine of C-8 arranges for stability of free ionizable electron radical through resonance which makes excellent antioxidant group. Moreover, the loosing of a free acidic COOH group may lead lo loss of H-B donor group which lead to low activity in case of esters. The free acidic COOH group altogether with the 4-Oxo neighboring group does chelate with divalent metals as part of their antibacterial mechanism. Loosing such ionization can lead to low or lack of activity. Although not confirmed, the rigidity of the structure seems to play significant role in activity. The triazolo series **5** showed appreciable activities and some of them were even stronger than reduced series against other gram-positive microorganisms tested in Table 3. This rigidity was provided from triazolo ring in series **5** and pseudo ring in series 4, (**Figure 3)**.

CONCLUSION AND CLINICAL SIGNIFICANCE

This research produced more than 25 novel FQs **2-5 a-e.** Compounds **3-5 a-e** revealed excellent and comparable MIC values to reference ciprofloxacin against gram-positive

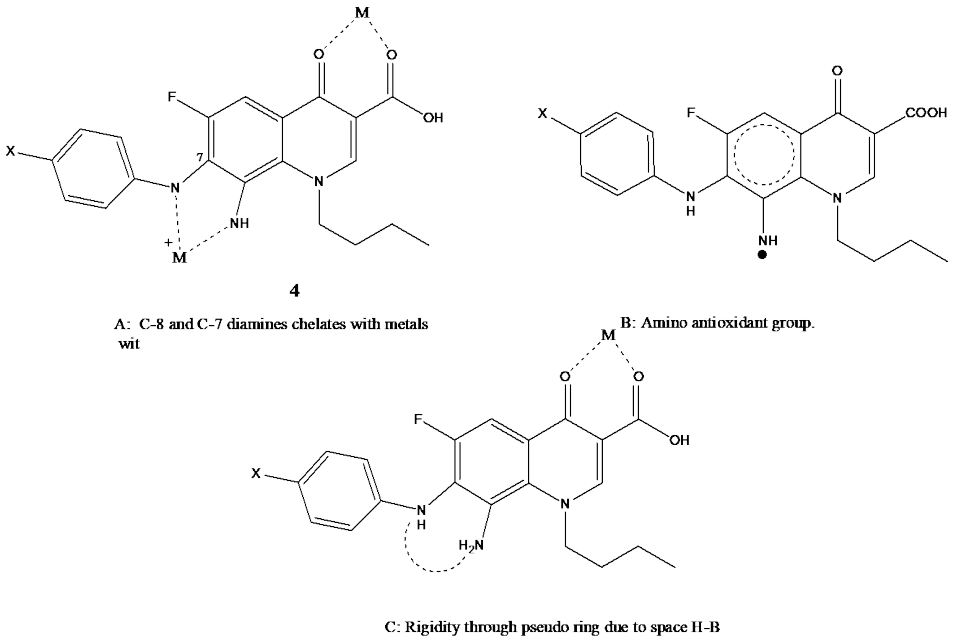


Figure 3. Potential role of C-8 amino group in increasing antibacterial activity of series 4)

standard strains. The reduced series **4** showed the activity that reached to nanogram values. Increasing number of methoxy group lead to super activity against standard and resistant gram-positive strain (MRSA). Lipophilicity and H-B D/A were the most essential requirement in active FQs. Potentially new FQs can be used to modify the activity against pathogenic resistant strains of MRSA. Acceptability to prescribe our FQs with antibiotics (bacitracin, ampicillin and cephalexin) in one prescription can be a possibility in the future leading to reduce the concentration of both and decrease the side effects.

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Authors contributed equally towards conceptualization, data collection, manuscript composition and proofreading

DECLARATION OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTION

All authors contributed equally towards to and approved the manuscript.

DATA AVAILABILITY STATEMENT

Materials supporting the findings are available from the corresponding author on reasonable request.

References

1 (a).Snow LC, Warner RG, Cheney T, et al. Risk factors associated with extended spectrum beta-lactamase *Escherichia coli* (CTX-M) on dairy farms in North West England and North Wales. Preventive Veterinary Medicine 2012; 106(3-4):225-234. http://doi.org/10.1016/j.prevetmed.2012.03.009; (b).Tokajian S. New epidemiology of *Staphylococcus aureus* infections in the Middle East. Clinical Microbiology and Infection 2014; 20(7): 624–628. http://doi.org/10.1111/1469-0691.1269; (c). Wardyn SE, Forshey BM, Farina SA, et al. Swine Farming Is a Risk Factor for Infection With and High Prevalence of Carriage of Multidrug-Resistant *Staphylococcus aureus*. Clinical Infectious Diseases 2015; 61(1):59-66. http://doi.org/10.1093/cid/civ234; (d). Santman-Berends IMGA, Gonggrijp MA, Hage JJ, et al. Prevalence and risk factors for extended-spectrum β-lactamase or AMPC-producing *Escherichia coli* in organic dairy herds in the Netherlands. Journal of Dairy Science 2017; 100(1):562–571. http://doi.org/10.3168/jds.2016-11839; (e). Sreeramoju P, Porbandarwalla NS, Arango J, et al. Recurrent skin and soft tissue infections due to methicillin-resistant *Staphylococcus aureus* requiring operative debridement. American Journal of Surgery 2011; 201(2):216-220. http://doi.org/ 10.1016/ j.amjsurg.2009.12.024; (f). Wu G, Day MJ, Mafura MT, et al. Comparative Analysis of ESBL-Positive *Escherichia coli* isolates from Animals and Humans from the UK, The Netherlands and Germany. PLoS ONE 2013; 8(9): e75392. http://doi.org/ 10.1371/ journal.pone.0075392

2 (a). Huang Y, Ogutu JO, Gu J, et al. Comparative analysis of quinolone resistance in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* from Chinese children and adults. BioMed Research International 2015; 2015:168292. http://doi.org/10.1155/2015/168292; (b). Klein E, Smith DL, Laxminarayan R. Hospitalizations and deaths caused by methicillin-resistant *Staphylococcus aureus,* United States, 1999-2005. Emerging Infectious Diseases 2007; 13(12):1840-1846. http://doi.org/10.3201/eid1312.070629; (c). Klein EY, Sun L, Smith DL, Laxminarayan R. The changing epidemiology of methicillin-resistant *Staphylococcus aureus* in the United States: a national observational study. American Journal of Epidemiology 2013; 177(7):666-674. http://doi.org/10.1093/aje/kws273.

3 (a). Zigmond J, Pecen L, Hajek P, et al. MRSA infection and colonization rates in Africa and the Middle East: a systematic review and meta-analysis. F1000Posters 5:170; International Journal of Infectious Diseases 2014; 21(S1):391. https://doi.org/10.1016/j.ijid.2014.03.1227; (b). Goyal N, Miller A, Tripathi M, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA). Bone Joint Journal 2013; 95-B(1):4-9. http://doi.org/ 10.1302/0301-620X.95B1.27973; (c). Leibler JH, León C, Cardoso LJP, et al. Prevalence and risk factors for MRSA nasal colonization among persons experiencing homelessness in Boston, MA. Journal of Medical Microbiology 2017; 66(8):1183-1188. http://doi.org/10.1099/ jmm.0.000552.

4 (a). Dorado-García A, Mevius DJ, Jacobs JJ, et al. Quantitative assessment of antimicrobial resistance in livestock during the course of a nationwide antimicrobial use reduction in the Netherlands. Journal of Antimicrobial Chemotherapy 2016; 71(12): 3607-3619. http://doi.org/10.1093/jac/dkw308; (b). Gonggrijp MA, Santman-Berends IMGA, Heuvelink AE, et al. Prevalence and risk factors for extended-spectrum β-lactamase- and AMPC-producing *Escherichia coli* in dairy farms. Journal of Dairy Science **2016;** 99(11): 9001-9013. http://doi.org/10.3168/jds.2016-11134; (c). Carmo LP, Nielsen LR, da Costa PM, et al. Exposure assessment of extended-spectrum beta-lactamases/AmpC beta-lactamases-producing *Escherichia coli* in meat in Denmark. Infection Ecology & Epidemiology 2014; 4. http://doi.org/10.3402/iee.v4.22924

5 (a). Odds FC. Synergy, antagonism, and what the chequer board puts between them. Journal of Antimicrobial Chemotherapy 2003; 52(1):1. http://doi.org/10.1093/jac/dkg301; (b). McGowan JE. Jr. Resistance in nonfermenting gram-negative bacteria: multidrug resistance to the maximum. American Journal of Medicine 2006; 119(6):S29-S36. http://doi.org/10.1016/j.amjmed.2006.03.014; (c). Schmid A, Hörmansdorfer S, Messelhäusser U, Käsbohrer et al. Prevalence of extended-spectrum β-lactamase-producing *Escherichia coli* on Bavarian dairy and beef cattle farms. Applied and Environmental Microbiology

2013; 79(9):3027-3032. http://doi.org/10.1128/AEM.00204-13.

6 Bolon MK. The newer Fluoroquinolones. Infectious Disease Clinics of North America 2009; 23:1027-1051. http://doi.org/10.1016/ j.idc.2009. 06.003.

7 (a). Abu-Sini M, Mayyas A, Al-Karablieh N, et al. Synthesis of 1,2,3-Triazolo[4,5-h]quinolone Derivatives with Novel Anti-Microbial Properties against Metronidazole Resistant *Helicobacter pylori*. Molecules 2017**;** 22(5): 841. http://doi.org/10.3390/molecules22050841; (b). Abu-Qatouseh L, Abu-Sini M, Mayyas A, et al. Synthesis of New Nitrofluoroquinolone Derivatives with Novel Anti-Microbial Properties against Metronidazole Resistant *H. pylori*. Molecules 2017**;** 22(1): 71. http://doi.org/10.3390/molecules22010071; (c). Al-Hiari YM, Shakya AK, Al-Rajab WJ, et al. Antimicrobial screening of novel N-4- fluorophenylquino-[7,8-b] [1 ,4]- benzodiazepin-3 carboxylic acid derivatives. Revue Roumaine De Chimie 2015**;** 60(9):899 -905; (d)Laxminarayan R, Duse A, Wattal C, et al. Antibiotic resistance-the need for global solutions. Lancet Infectious Diseases 2013 and 2014**;** 13(12):1057-1098. http://doi.org/ 10.1016/S1473-3099(13)70318-9. Erratums 14(1):11 and 14(3): 182

8 (a). Shoaa AR. Compounds with Antibacterial and Antiparasitic Properties**.** *PCT* International Application WO 2001036408 A1, CAN 2001**;** 135:5534; (b). Stahlmann R, Lode H. Fluoroquinolones in the elderly: safety considerations. Drugs Aging 2003**;** 20(4): 289-302. http://doi.org/ 10.2165/00002512-200320040-00005

9 (a). Qaisi AMA, Al-Hiari YMA, Zahra JAY, et al. Preparation of quinolones and pyridoquinoxalinones as antibacterials, European Patent Application No. 06 252 509.2, University of Jordan and European patent office; 45pp. Published on Scifinder 2006 (EP 1721898 A1 20061115); (b). Al-Hiari Y, Al-Mazari I, Shakya A, et al. Synthesis and Antibacterial Properties of New 8-Nitro fluoroquinolones Derivatives. Molecules 2007**;** 12:1240-1258**.** http://doi.org/10.3390/12061240; (c). Al-Hiari Y, Quandil A, Al-Zoubi R, et al. 7- (3-Chlorophenylamino)-1- cyclopropyl-6-fluoro-8-nitro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid. Molbank 2010**;** 699:1-3. http://doi.org/10.3390/ M669; (d). Al-Hiari YM, Shakya AK, Alzweiri MH, et al. Synthesis and antibacterial properties of new N4-acetylated hexahydro-2, 7-dioxopyrido [2, 3-f] quinoxaline-8-carboxylic acids. Journal of Enzyme Inhibition and Medicinal Chemistry 2011**;** 26(5): 649-656; http://doi.org/10.3109/14756366.2010.543421; (e). Al-Hiari YM, Qandil AM, Al-Zoubi RM, et al. Synthesis and antibacterial activity of novel 7-halo anilino-8-nitrofluoroquinolone derivatives. Medicinal Chemistry Research 2012; 21(8):1734-1740. https://doi.org/10.1007/s00044-011-9692-3

10 National Committee for Clinical Laboratory Standards (NCCLS): Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard, 5th edn. M7-A5; **2000;** 20(2): replaces M7-A4; 17(2)

11 Meletiadis J, Pournaras S, Roilides E, et al. Defining fractional inhibitory concentration index cutoffs for additive interactions based on self-drug additive combinations, Monte Carlo simulation analysis, and in vitro-in vivo correlation data for antifungal drug combinations against *Aspergillus fumigatus*. Antimicrobial Agents and Chemotherapy 2010; 54: 602–609. http://doi.org/10.1128/AAC.00999-09

12 AlAbsi Y, AlHiari Y, Kasabri V, et al. In vitro modulation of pancreatic lipase and proliferation of obesity related colotrectal cancer cell line panel by novel synthetic fluoroquinolones. Revue Roumaine De Chimie 2018; 63(12):1123-1134

13 (a). Chattaway MA, Jenkins C, Ciesielczuk H, et al. Evidence of evolving extraintestinal enteroaggregative *Escherichia coli* ST38 clone. Emerging Infectious Diseases 2014; 20(11): 1935-1937. http://doi.org/10.3201/eid2011.131845; (b). Shehadeh M, Suaifan G, Darwish RM, et al. Knowledge, attitudes and behavior regarding antibiotics use and misuse among adults in the community of Jordan. A pilot study. Saudi Pharmaceutical Journal 2012; 20(2):125-133. http://doi.org/ 10.1016/j.jsps.2011.11.005

14 (a). Hooper DC, Jacoby GA. Mechanisms of drug resistance: quinolone resistance. Annals *of* the New York Academy *of* Sciences 2015*;* 1354(1): 12-31. http://doi.org/10.1111/nyas.12830; (b). Hordijk J, Wagenaar JA, Kant A, et al. Cross-Sectional Study on Prevalence and Molecular Characteristics of Plasmid Mediated ESBL/AmpC-Producing *Escherichia coli* Isolated from Veal Calves at Slaughter. PLoS ONE 2013; 8(5):e65681. http://doi.org/10.1371 /journal.pone.0065681

15 (a). Darwish RM, Aburjai T, Al-Khalil S, et al. Screening of antibiotic resistant inhibitors from local plant materials against two different strains of *Staphylococcus aureus*. Journal of Ethnopharmacology 2002; 79(3):359-364. http://doi.org/10.1016/s0378-8741(01)00411-1; (b). Darwish RM, Aburjai TA. Effect of ethnomedicinal plants used in folklore medicine in Jordan as antibiotic resistant inhibitors on *Escherichia coli.* BMC Complementary and Alternative Medicine 2010**;** 10:9. http://doi.org/10.1186/1472-6882-10-9; (c). Koga H, Itoh A, Murayama S, Suzue S, et al. Structure-activity relationships of antibacterial 6,7- and 7,8-disubstituted 1-alkyl-1,4-dihydro-4-oxoquinoline-3-carboxylic acids. Journal of Medicinal Chemistry 1980; 23(12):1358-1363. http://doi.org/10.1021/jm00186a014

16 (a). Mitscher LA. Bacterial Topoisomerase Inhibitors: Quinolone and Pyridone Antibacterial Agents. *Chemical* Reviews 2005; 105:559-592. http://doi.org/10.1021/cr030101q; (b). Chai Y, Wan Z-L, Wang B, et al. Synthesis and in vitro antibacterial activity of 7-(4-alkoxyimino-3-amino-3-methylpiperidin-1-yl) fluoroquinolones derivatives. European Journal of Medicinal Chemistry 2009; 44:4063- 4069. http://doi.org/ 10.1016/j.ejmech.2009.04.041

17 (a). Da Silva AD, Almeida MV, Souza MVN, et al. Biological Activity and Synthetic Methodologies for the Preparation of Fluoroquinolones, A class of Potent Antibacterial Agents. *Current Medicinal Chemistry* 2003; 10:21-39. http://doi.org/10.2174/ 09298670 33368637; (b). Foroumadi A, Emami S, Mehni M, et al. Synthesis and antibacterial activity of N-[2-(5-bromothiophen-yl)-2-oxoethyl] and N-[(2-5 bromothiophen-2-yl)-2-Oximinoethyl] derivatives of piperazinyl quinolones. Bioorganic & Medicinal Chemistry Letters 2005a; 15(20): 4536-4539. http://doi.org/10.1016/ j.bmcl. 2005.07.005; (c). Foroumadi A, Emami S, Mehni M, et al. Synthesis and antibacterial activity of N-[2-(5-bromothiophen-2-yl)-2-oxoethyl] and

N-[(2-5-bromothiophen-2-yl)-2-oximinoethyl] derivatives of piperazinyl quinolones. Bioorganic & Medicinal Chemistry Letters 2005b; 15(20): 4536-4539. http://doi.org/ 10.1016/j.bmcl.2005.07.005; (d). Foroumadi A, Ghodsi S, Emami S, et al. Synthesis and antibacterial activity of new fluoroquinolones containing a substituted N-(phenethyl)piperazine moiety. Bioorganic & Medicinal Chemistry Letters 2006; 16(13): 3499-503. http://doi.org/10.1016/j.bmcl.2006.03.103.

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SUPPLEMENTARY

Compound 2a: Synthesis of ethyl-1-butyl-6-fluoro-7-(2-methoxy-phenylamino)-8-nitro-4-oxo-1, 4-dihydro-quinoline-3-carboxylate (2a)

Excess of *o*-anisidine (3M, 2.99g, 16.24 mmol) was added into a solution of(**1E,** 3g, 5.40 mmol), and 20 ml of dimethylsulfoxide (DMSO) as a solvent and few drops of pyridine was used as catalyst. The mixture was refluxed at 60-70°C under anhydrous conditions for 36 hrs. The reaction mixture was monitored by TLC until no starting material remained then was left to crystallize at room temperature. The product was filtered and washed, left to dry in dark place to give bright orange crystals; yield ≈ 86% (3.2g); mp=199-200; *Rf* value in system 1 = 0.65. The ester intermediate **(2a)** was confirmed by 1H-**NMR** and used directly to prepare the acids. **^{1}H-NMR** (500MHz, DMSO-d_6): *δ* 0.85 (t, J=7.5 Hz, 3H, CH_3-4'), 1.13 (m, J=7.5 Hz, 2H, CH_2-3'), 1.30 (m, J=7.00 Hz, 3H, $OCH_2\underline{CH_3}$), 1.61 (m, J=7.5Hz, 2H, CH_2-2'), 3.71 (s, 3H, Ar-OCH_3), 4.00 (m, J=7.45 Hz, 2H, N-CH_2-1'), 4.22 (q, J=7.05 Hz, 2H, $O\underline{CH_2}CH_3$), 6.85 (d,d, J=7.23, 7.40 Hz, 1H, ArCH-5''),6.90 (d, J=7.60, Hz, 1H, ArCH-3''), 6.99-7.08 (m, 2H, ArCH-4'', ArCH-6''),8.06 (d,$^3J_{H\text{-}F}$=11.5 Hz, 1H, H-5),8.15 (s, 1H,Ar-NH), 8.65 (s, 1H, H-2).

Compound 2b: Synthesis of ethyl-1-butyl-6-fluoro-7-(3-methoxy-phenylamino)-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylate (2b)

Excess of *m*-anisidine (3M, 2g, 16.24 mmol) was added into a solution of (**1E,** 2g, 5.40 mmol) and 20 mL of dimethylsulfoxide (DMSO) as a solvent. Pyridine was used as catalyst and few drops were added. The mixture was refluxed at 60-70°C under anhydrous conditions for 36 hrs. The reaction mixture was monitored by TLC for 2 days, and then was left to crystallize at room temperature. The product was filtered and washed, left to dry in dark place to give bright orange crystals; yield ≈ 50% (1g); *Rf* value in system 1 = 0.56. The ester intermediate **(2b)** was confirmed by 1H-**NMR** and used directly to prepare the acids.

^{1}H-NMR (500MHz, DMSO-d_6):*δ* 0.82 (m, *J*=7.3 Hz, 3H, CH_3-4'), 1.15 (m, *J*=7.5 Hz, 2H, CH_2-3'), 1.33 (t, 3H, $OCH_2\underline{CH_3}$) , 1.55 (m, *J*=7.6 Hz, 2H, CH_2-2), 3.71(s, 3H, OCH_3), 4.18 (m, 2H, N-CH_2-1'), 4.26 (q, 2H, $O\underline{CH_2}CH_3$), 6.46 (d, *J*=7.5 Hz, 1H, H-6''), 6.50-6.55 (m, 2H, H-2''&H-4''), 7.12 (d,d, 1H, *J*=8.5Hz, *J*=8.55 Hz, H-5''), 8.24 (d,$^3J_{H\text{-}F}$=11.52 Hz, 1H, H-5), 8.54 (brs, 1H, NH), 8.92 (s, 1H, H-2).

Compound 2c: Synthesis of ethyl-1-butyl-6-fluoro-7-(4-methoxy-phenylamino)-8-nitro-4-oxo-1, 4-dihydro-quinoline-3-carboxylate (2c)

Excess of *p*-anisidine (3M, 2g, 16.24 mmol) was added into a solution of (**1E,** 2g, 5.40 mmol) and 20 mL of dimethylsulfoxide (DMSO) as a solvent. Pyridine was used as catalyst and few drops was added. The mixture was refluxed at 60-70°C under anhydrous conditions for 36 hrs. The reaction mixture was monitored by TLC until no starting material remained then was left to crystallize at room temperature. The product was filtered and washed, left to dry in dark place to give bright orange crystals; yield ≈ 50% (1g); °; *Rf* value insystem 2 = 0.59.

The ester intermediate was confirmed by 1H-**NMR** and used directly to prepare the acids.

^{1}H-NMR (500MHz, DMSO-d_6):*δ* 0.85 (m *J*=6.9 Hz, 3H, CH_3-4'), 1.15 (m, *J*=7.20Hz, 2H, CH_2-3'), 1.33 (m, 3H, $OCH_2\underline{CH_3}$), 1.61 (m, *J*=7.5 Hz, 2H, CH_2-2'), 3.66 (s, 3H, OCH_3), 4.11 (t, *J*=7.35 Hz, 2H, N-CH_2-1'), 4.23 (q, 2H, $O\underline{CH_2}CH_3$), 6.82 (d, *J*=8.5Hz, 2H, H-2''&H-6''), 7.10 (d, *J*=8.32Hz, 2H, H-3''&H-5''), 8.10(d, $^3J_{H\text{-}F}$=12.22 Hz, 1H, H-5), 8.89 (brs, 1H, NH), 8.98 (s, 1H, H-2).

Compound 2d: Synthesis of 1-Butyl-7-(2,4-dimethoxy phenylamino) 6-fluoro--8-nitro-4-oxo-1, 4-dihydroquinoline-3-carboxylate (2d)

Excess of 2, 4-Dimetoxyaniline (3M, 2g, 13.05mmol) was added into a solution of (**1E,** 2g, 5.40 mmol) and 20 mL of dimethylsulfoxide (DMSO) as a solvent. Pyridine was used as catalyst and few drops were added. The mixture was refluxed at 60-70°C under anhydrous conditions for 36 hrs. The reaction mixture was monitored by TLC until no starting material remained then was left to crystallize at room temperature. The product was filtered and washed, left to dry in dark place to give bright orange crystals; yield ≈ 50% (1g); °; *Rf* value in system 2 = 0.55

The ester intermediate was confirmed by 1H-**NMR** and used directly to prepare the acids.

^{1}H-NMR (500MHz, DMSO-d_6): *δ* 0.85 (t, *J*=7.52 Hz, 3H, CH_3-4'), 1.19 (m, *J*=7.5 Hz, 2H, CH_2-3'), 1.33 (t, 3H, $OCH_2\underline{CH_3}$), 1.62 (m, *J*=7.8 Hz, 2H, CH_2-2'), 3.65 (s, 3H, OCH_3), 3.76(s, 3H, OCH_3), 4.12(m, *J*=7.3 Hz, 2H, N-CH_2-1'), 4.26 (m, 2H, $O\underline{CH_2}CH_3$), 6.55(d,d, *J*=2.51Hz, 8.52Hz, 1H, H-5''), 6.65 (d, *J*=2.53Hz, 1H, H-3''), 7.16 (d, *J*=8.5Hz, 1H, H-6''), 8.12 (d,$^3J_{H\text{-}F=}$13.25Hz,1H, H-5), 8.62 (brs, 1H, NH, exch.), 8.99 (s, 1H, H-2).

Compound 2e: Synthesis of 1-Butyl-7-(3,4, 5-Trimethoxy phenylamino) -6-fluoro-8- nitro-4-oxo-1, 4-dihydroquinoline-3-carboxylate (2e)

Excess of 3,4,5-Trimetoxyaniline(3M, 2g, 10.91 mmol) was added into a solution of (**1E,** 2g, 5.40 mmol) and 20 mL of dimethylsulfoxide (DMSO) as a solvent. Pyridine was used as catalyst and few drops were added. The mixture was refluxed at 60-70°C under anhydrous conditions for 36 hrs. The reaction mixture was monitored by TLC until no starting material remained then

was left to crystallize at room temperature. The product was filtered and washed, left to dry in dark place to give bright orange crystals; yield ≈ 50% (1g); °; *Rf* value in system 2 = 0.60

The ester intermediate was confirmed by 1H-**NMR** and used directly to prepare the acids.

^{1}H-NMR (500MHz, DMSO-d_6):*δ* 0.84 (m, *J*=7.5 Hz, 3H, CH_3-4'), 1.20 (m, *J*=8.2 Hz, 2H, CH_2-3'), 1.36(t, 3H, $OCH_2\underline{CH_3}$), 1.66 (m, *J*=7.3 Hz, 2H, CH_2-2'), 3.65 (s, 3H, OCH_3, 4''), 3.71 (brs, 6H, 2OCH_3, 3''& 5''), 4.15 (m, *J*=8.2 Hz, 2H, CH_2-1'), 4.19 (m, 2H, $O\underline{CH_2}CH_3$), 6. 42 (s, 2H, 2''& 6''), 8.22 (d, $^3J_{H\text{-}F}$ = 11.55Hz, 1H, H-5), 9.06 (s, 1H, NH), 8.99 (s, 1H, H-2).

Compound 3a: Synthesis of 1-butyl-6-fluoro-7-(2-methoxy-phenylamino)-8-nitro-4-oxo-1, 4-dihydro-quinoline-3-carboxylic acid (3a)

A vigorously stirred suspension of **2a** 2.0g, 4.37mmol) in 12N HCl (30 mL) and ethanol (15 mL) was heated at 80-85 °C under reflux conditions. Progress of the ester hydrolysis was monitored by TLC and was completed within 36h. Thereafter, the reaction mixture was cooled, poured onto crushed ice (250 g) and the resulting pale orange precipitate was collected, washed with cold water (2 x 25 mL) and left to dry. Yield ≈ 80% (1.5 g). Mp = 206-208 °C; *Rf* value in system 1 = 0.26.

^{1}H-NMR (500MHz, DMSO-D_6):*δ* 0.77 (t, J=7.3 Hz, 3H, CH_3-4'), 1.09 (q, J=7.2 Hz, 2H, CH_2-3'), 1.57 (q, J=6.9 Hz, 2H, CH_2-2'), 3.63 (s, 3H, OCH_3), 4.14 (q, J=7.15 Hz,2H, N-CH_2-1'), 6.85 (dd, J=7.40 Hz 7.40 Hz, 1H, ArH-5''), 6.97 (d, J=7.98 Hz, 1H, ArH-3''), 7.06 (m, 2H, ArH-4'' and ArH-6''), 8.08 (d,$^3J_{H\text{-}F}$=11.8 Hz, 1H, H-5), 8.49 (s, 1H, NH), 8.92 (s, 1H, H-2), 14.61(brs, 1H, COOH).

^{13}C –NMR (125 MHZ, DMSO-D6): *δ* 13.42 (CH_3-4'), 19.13 (CH_2-3'), 32.04 (CH_2-2'), 55.85 (N-CH_2-1'), 55.91 (OCH_3), 109.41 (C-3), 111.77 (C-5''), 113.52 (d, $^2J_{C\text{-}F}$=21.6 Hz, C-5), 120.52 (d, $^3J_{C\text{-}F}$=7.5 Hz, C-4a), 120.61 (C-3''), 122.59 (C-6''), 125.53 (C-4''), 129.68 (C-8), 131.42 (C-1''), 132.48 (C-8a), 134.71 (d, $^2J_{C\text{-}F}$=15.13 Hz, C-7), 152.21 (C-2), 152.23 (C-2''),152.57 (d, $^1J_{C\text{-}F}$=253.18 Hz, C-6), 165.48 (COOH), 175.63 (C-4).

HRMS (ESI, -ve): calculated for C21H19FN3O6 [M^+-1] $^+$(428.12579) Found (428.13675); LRMS (ES, -ve) m/z calc. for C21H19FN3O6 (429): Found 429.13 (M+, 16%), 428.137 (14%), 393.287 (27%), 365.255 (26%), 299.136; (13%), 283.270 (40%), 281.256 (23%), 281.256 (23%), 255.238 (100%), 253.223 (32%).

Compound 3b: Synthesis of 1-Butyl-6-fluoro-7-(3-methoxy phenylamino)-8-nitro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (3b)

A mixture of the ester **3b** (1.5 g, 3.49 mmol) in 12N HCl (40 mL) and ethanol (40 mL) was refluxed at 80-85 °C. Ester hydrolysis was followed by TLC and completed within 2 days. Then, the reaction mixture was cooled, poured onto crushed ice 250 g) and the resulting orange precipitate was collected, washed with cold water (2 x 30 mL) and left to dry. Yield ≈ 70% (1g). Mp = 210-213 °C; *Rf* value in system 1 = 0.24.

^{1}H-NMR (500MHz, DMSO-d_6):*δ* 0.77 (t, *J*=7.3 Hz, 3H, CH_3-4'), 1.12 (m, *J*=7.4 Hz, 2H, CH_2-3'), 1.59 (m, *J*=7.7 Hz, 2H, CH_2-2), 3.65 (s, 3H, OCH_3), 4.13 (m, 2H, N-CH_2-1'), 6.41 (d, *J*=7.8 Hz, 1H, H-6''), 6.51-6.53 (m, 2H, H-2''&H-4''), 7.09 (d,d, 1H, *J*=8.1Hz, *J*=8.45 Hz, H-5''), 8.21 (d,$^3J_{H\text{-}F}$=11.05 Hz, 1H, H-5), 8.49 (brs, 1H, NH), 8.96 (s, 1H, H-2), 14.38 (brs, 1H, COOH).

^{13}C –NMR (125 MHz, DMSO-d_6): 13.69 (CH_3-4'), 19.38 (CH_2-3'), 32.51 (CH_2-2'), 55.48 (OCH_3), 55.98 (N-CH_2-1'), 104.49 (C-3), 108.40 (C- 2''), 109.35 (C-6''), 110.81 (C-4''). 114.96 (d, $^2J_{C\text{-}F}$ =21.1Hz, C-5), 123.01 (C-4a), 130.08 (C-5''), 130.62 (C-8a), 132.13 (d, $^2J_{C\text{-}F}$=16.15Hz, C-7*)*, 136.41 (C-8), 144.01 (C-1''), 152.65(C-2), 153. 67 (d, $^1J_{C\text{-}F}$=253.8Hz, C-6), 160.28 (C-3''), 165.38 (COOH), 175.74 (C-4).

HRMS (ESI, +ve): *m/z* calculated for $C_{21}H_{21}FN_3NaO_6$ $[M+1+Na]^+$ 453.13121, Found 453.18769.LRMS (ESI, +ve): *m/z* calculated for $C_{21}H_{21}FN_3NaO_6$: 453(16%, M+1+Na), 449 (35%), 434 (22%), 433 (100%), 393 (9%), 318 (11%), 301 (16%).

Compound 3c: Synthesis of 1-Butyl-6-flouro-7-(4-methoxy phenylamino)-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3c)

The ester **3c** 2g, 4.65 mmol), was placed in 100 mL RBF. Next, 12N HCl (40 mL) and ethanol (40 mL) were added and the mixture was refluxed at 80-85 °C. Hydrolysis was completed within 2 days. Then, the reaction mixture was cooled, poured onto crushed ice (100 g) and the resulting orange precipitate was collected, washed with cold water (2 x 30 mL) and left to dry. Yield ≈ 75% (1.5g). Mp = 233-235.5 °C; *Rf*value in system 1 = 0.28.

^{1}H-NMR (500MHz, DMSO-d_6):*δ* 0.77 (t, *J*=6.7 Hz, 3H, CH_3-4'), 1.11 (m, *J*=6.95Hz, 2H, CH_2-3'), 1.56 (m, *J*=7.7 Hz, 2H, CH_2-2), 3.68 (s, 3H, OCH_3), 4.10 (t, *J*=7.3 Hz, 2H, N-CH_2-1'), 6.81 (d, *J*=7.8Hz, 2H, H-2''&H-6''), 6.98 (d, *J*=8.1Hz, 2H, H-3''&H-5''), 8.09(d, $^3J_{H\text{-}F}$=11.85 Hz, 1H, H-5), 8.85 (brs, 1H, NH), 8.92 (s, 1H, H-2), 14.35(brs, 1H, COOH).

^{13}C –NMR (125 MHz, DMSO-d_6): 13.69 (CH_3-4'), 19.38 (CH_2-3'), 32.40 (CH_2-2'), 55.72 (OCH_3), 56.10 (N-CH_2-1'), 109.35 (C-3), 114.40 (C-2''&C-6''), 114.66 (d, $^3J_{C\text{-}F}$=22Hz,C-5), 122.53(C-3''&C-5''), 125.6 (C-4a), 130.51 (C-8a), 133.01(C-8), 134.59(C-7), 142.25 (C-1''), 152.26 (C-2),153.22 (d, $^1J_{C\text{-}F=}$248Hz, C-6), 156. 49 (C-4''), 165.49 (COOH), 175.63 (C-4).

HRMS (ESI, -ve): *m/z* calculated for $C_{21}H_{19}FN_3O_6$ [M-1]$^+$ 428.12579, Found 428.13784

Compound 3d: Synthesis of 1-Butyl-7-(2,4-dimethoxy phenylamino) 6-fluoro--8-nitro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (3d)

A mixture of the ester **3d** (2g, 4.35 mmol) in 12N HCl (40 mL) and ethanol (40 mL) was refluxed at 80-85 °C. Ester hydrolysis was followed by TLC and completed within 36h. Then, the reaction mixture was cooled, poured onto crushed ice (250 g) and the resulting orange precipitate was collected, washed with cold water (2 x 30 mL) and left to dry. Yield ≈ 75% (1.5g). Mp = 174.3-176.5 °C; *Rf* value in system 2 = 0.27

^{1}H-NMR (500MHz, DMSO-d_6):*δ* 0.84 (t, *J*=7.81 Hz, 3H, CH_3-4'), 1.11 (m, *J*=7.4 Hz, 2H, CH_2-3'), 1.61 (m, *J*=7.7 Hz, 2H, CH_2-2'), 3.66 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 4.13 (t, *J*=7.3 Hz, 2H, N-CH_2-1'), 6.51 (d,d, *J*=2.45 Hz, 8.65Hz, 1H, H-5''), 6.60 (d, *J*=2.35Hz, 1H, H-3''), 7.11 (d, *J*=8.6Hz, 1H, H-6''), 8.05 (d,$^3J_{H\text{-}F=}$12.35Hz,1H, H-5), 8.58 (brs, 1H, NH), 8.93 (s, 1H, H-2), 14.38 (brs, 1H, COOH).

^{13}C –NMR (125 MHz, DMSO-d_6): 13.69 (CH_3-4'), 19.38 (CH_2-3'), 32.33 (CH_2-2'), 55.63 (OCH_3), 55.64 (N-CH_2-1'), 55.95 (OCH3), 99.01(C-5''), 104.72 (C-3''),125.86 (C-6''), 113.85 (d, $^2J_{C\text{-}F}$=21.75Hz, C-5), 118.81 (C-7), 121.80 (C-4a), 130.10 (C-8a), 132.04 (C-8), 136.20 (C-1''), 151.68 (C-2), 151.90 (d, $^1J_{C\text{-}F}$= 252Hz, C-6), 154.60 (C2''-OCH_3), 158.82 (C4''-OCH_3), 165.54 (COOH), 175.74 (C-4).

HRMS (ESI, -ve): *m/z* calculated for $C_{22}H_{21}FN_3O_7$ [M-1]$^+$ 458.13636, Found 458.14753.LRMS (ESI, +ve): *m/z* calculated for $C_{22}H_{23}FN_3O_7$: 460.17 (35%, M+1), 453.18 (100%), 437.21 (76%), 420.23 (25%), 413.27 (25%), 391.29 (24%), 353.27 (22%), 318.31 (30%), 287.26 (41%).

Compound 3e: Synthesis of 1-Butyl-7-(3,4,5-Trimethoxy phenylamino) -6-fluoro-8- nitro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (3e)

A mixture of the ester **2e** (1.25g, 2.5 mmol) in 12N HCl (40 mL) and ethanol (40 mL) was refluxed at 80-85 °C. Ester hydrolysis was followed by TLC and completed within 3 days. Then, the reaction mixture was cooled, poured onto crushed ice (250 g) and the resulting orange precipitate was collected, washed with cold water (2 x 30 mL) and left to dry. Yield ≈ 60% (0.75g). Mp = 200.5-203°C; *Rf* value in system 2 = 0.26

^{1}H-NMR (500MHz, DMSO-d_6,rotamers):*δ* 0.85 (m, *J*=7.1 Hz, 3H, CH_3-4'), 1.19 (m, *J*=7.5 Hz, 2H, CH_2-3'), 1.64 (m, *J*=6.2 Hz, 2H, CH_2-2'), 3.63 (s, 3H, OCH_3, 4''), 3.70 (brs, 6H, 2OCH_3, 3''& 5'') 4.19 (m, *J*=8.2 Hz, 2H, CH_2-1'), 6. 38 (brs, 2H, 2''& 6''), 8.22, 8.53 (2d, $^3J_{H\text{-}F}$ = 11.25, 8.1 Hz, 1H, H-5, rotamers), 8.87, 9.06 (2s, 1H, NH, rotamers), 8.99 (s, 1H, H-2), 14.45(brs, 1H, COOH).

^{13}C –NMR (125 MHz, DMSO-d_6): 13.40 (CH_3-4'), 19.06 (CH_2-3'), 32.19 (CH_2-2'), 55.47 (CH_2-1''), 55.84 (2OCH_3, 3'', 5'') , 60.34 (OCH_3, 4''), 97.58 (C-2''& 6'', ArCH),109.39 (C-3), 114.63 (d, $^3J_{C\text{-}F}$=21.4Hz, C-5), 115.46 (d, $^2J_{C\text{-}F}$= 22.7Hz, C-7), 121.90 (C-4a), 131.21 (C-8a), 134.22 (C-8), 138.01 (C-1''), 152.16 (C-2), 152.25 (C-4''), 153.53, 153.69 (C-3''& C-5''), 154.51 (d, *J*=251Hz, C-6), 165.38 (COOH), 175.68 (C-4).

HRMS (ESI, +ve): *m/z* calculated for $C_{22}H_{21}FN_3O$ [M-1, -OCH_3]$^+$ 458.13636, Found 458.18396.

Compound 4a: Synthesis of 8-amino-1-butyl-6-fluoro-7-(2-methoxy-phenylamino)-4-oxo-1, 4-dihydro-quinoline-3-carboxylic acid (4a)

A mixture of **3a** (1.0g, 2.3mmol) in 6.5 mL of 12N HCl was left stirring in ice bath (0-5°C) for 15 minutes. After that, the ice bath was removed and (1.77g, 9.3mmol, 4Mexcess) stannous chloride ($SnCl_2$) was added portion wise and the reaction mixture left stirring overnight and was monitored by TLC until completion. Then, the reaction mixture was poured on crushed ice to precipitate a brown-orange product that is collected by filtration and left to dry. Yield =0.62g (≈ 66%). mp = 201-203°C (decomposition); *Rf* value in system 1 = 0.14.

^{1}H NMR(500 MHz, DMSO-d6):*δ* 0.88 (t, J=7.4 Hz, 3H, CH_3-4'), 1.06 (m, J=7.3 Hz, 2H, CH_2-3'), 1.53 (m, J=7.2 Hz, 2H, CH_2-2'), 3.88 (s, 3H, OCH_3), 4.82 (t, J=7.0 Hz, 2H, N-CH_2-1'),5.56 (brs, 2H, NH_2),6.20 (d, J=7.65 Hz, 1H, ArH-3''), 6.70 (dd, J=7.60 Hz 7.90 Hz, 1H, ArH-5''), 6.77 (dd, J=7.55 Hz, 7.75 Hz, 1H, ArH-4''), 6.98 (d, J=7.9 Hz, 1H, ArH-6''), 7.16 (brs, 1H, NH, exchangeable),7.47 (d,$^3J_{H\text{-}F}$=9.6 Hz, 1H, H-5), 8.94 (s, 1H, H-2), 14.65(brs, 1H, COOH, exchangeable).

^{13}C –NMR (125 MHZ, DMSO-D6):*δ* 13.66 (CH_3-4'), 19.08 (CH_2-3'), 32.27 (CH_2-2'), 55.82 (OCH_3), 56.46 (N-CH_2-1'),99.81 (d, $^2J_{C\text{-}F}$=23.31 Hz, C-5), 107.02 (C-3), 111.23 (C-5''),112.39 (C-3''), 119.44 (C-4''), 120.77 (C-6''), 122.72 (d, $^2J_{C\text{-}F}$=15.40 Hz, C-7), 126.98 (d, $^3J_{C\text{-}F}$=9.34 Hz, C-4a), 127.25 (C-8a), 134.35 (C-8), 139.86 (C-1''),148.20 (C-2''),151.47 (C-2), 157.23 (d, $^1J_{C\text{-}F}$=244.88 Hz, C-6), 166.55 (COOH), 177.38 (C-4).

HRMS ((+ve)-ESI): *m/z* calculated for $C_{21}H_{22}FN_3NaO_4$ [M+Na]$^+$: 422.14920, found: 422.14866.

Compound 4b: Synthesis of 8-Amino-1-butyl-6-fluoro-7-(3-methoxy phenylamino)-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (4b)

A mixture of **3b** (1.25g, 3.12mmol) and 6.5 mL of 12N HCl in ice bath (0-5°C) was stirred for 15 minutes. Next,(4g, 21mmol) of

stannous chloride ($SnCl_2$) was added portion wise and the reaction mixture left stirring 24h. After that, the progress was monitored by TLC until completion. Then, the reaction mixture was poured on crushed ice to precipitate an orange product that is collected by suction filtration and left to dry. Yield =0.53g (≈ 40%). mp = 164-166.5°C;*Rf* value in system 1 = 0.18. This compound faced sever degradation **/**oxidation upon heating while analysis.

^{1}H-NMR (500MHz, DMSO-d_6):*δ* 0.74 (t, *J*=7.4 Hz, 3H, CH_3-4'), 1.02 (m, *J*=7.45 Hz, 2H, CH_2-3'), 1.47 (m, *J*=7.2 Hz, 2H, CH_2-2'), 3.60 (s, 3H, OCH_3), 4.77 (t, *J*=7.05Hz, 2H, N-CH_2-1'), 5.42 (brs, 2H, NH_2), 6.13 (brs, 1H, H-2''), 6.20 (d, *J*=8Hz, H-6''), 6.32 (d,d, *J*=8.1Hz,1.8Hz, H-4''), 7.01(d,d, *J*=8.1Hz,8.1Hz, 1H, H-5''), 7.45 (d,$^3J_{H\text{-}F}$=9.55Hz, 1H, H-5), 7.96 (brs, 1H, NH), 8.91 (s, 1H, H-2), 14.20 (brs, 1H, COOH).

^{13}C –NMR (125 MHz, DMSO-d_6): *δ* 13.84 (CH_3-4'), 19.38 (CH_2-3'), 32.56 (CH_2-2'), 55.25 (OCH_3), 56.89 (N-CH_2-1'),99.86 (d, $^2J_{C\text{-}F}$=23.2Hz, H-5), 100.65 (C-2''), 104.80 (C-6''), 107.09 (C-3), 107.46 (C-4''), 122.18 (d, $^2J_{C\text{-}F}$=16.88 Hz, C-7), 125.96 (C-4a), 127.28 (C-8a), 130.15 (C-5''), 139.57 (C-8), 146.37 (C-1''), 151.82 (C-2), 157.09 (d, $^1J_{C\text{-}F}$=244.76Hz, C-6), 160.65(C-3''), 166.54 (COOH), 177.41 (C-4) .

HRMS (ESI, -ve): *m/z* calculated for $C_{21}H_{20}FN_3O_4$ $[M\text{-}2]^+$ 397.14379, Found 397.23679.LRMS (ESI, +ve): *m/z* calculated for $C_{21}H_{21}FN_3NaO_6$: 318(17), 302 (18%), 301 (38%), 275 (18%), 274(100%), 262 (16%), 246 (21%), 242 (28%), 230 (17%), 218(23%).

Compound 4b: Synthesis of 8-Amino-1-butyl-6-fluoro-7-(4-methoxy phenylamino)-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (4c)

A mixture of **3c** (1g, 2.5mmol) and 6.5 mL of 12N HCl in ice bath (0-5°C) was stirred for 15 minutes. Next,(4g, 21mmol) of stannous chloride ($SnCl_2$) was added portion wise and the reaction mixture left stirring 24h. After that, the progress was monitored by TLC until completion.. Then, the reaction mixture was cooled, poured onto crushed ice 150 g) and the resulting orange precipitate was collected, washed with cold water (2 x 30 mL) and left to dry. This compound faced sever degradation **/** oxidation upon heating while analysis. Yield ≈ 50% (0.5g). Mp = 163-165 °C; *Rf* value in system 1 = 0.20.

^{1}H-NMR (500MHz, DMSO-d_6):*δ* 0.75 (t, *J*=7.15 Hz, 3H, CH_3-4'), 1.04 (m, Hz, 2H, CH_2-3'), 1.51 (m, *J*=6.85 Hz, 2H, CH_2-2'), 3.76(s, 3H, OCH_3), 4.16 (m, 2H, N-CH_2-1'), 6.70-6.87 (d, 2H, *J*=7.8 Hz, H-2''&H-6''), 6.91 (d, *J*=8.1Hz, H-3''& H-5''), 8.13 (d,$^3J_{H\text{-}F}$=9.25 Hz, 1H, H-5), 8.66 (brs, 1H, NH), 8.89 (s, 1H, H-2), 11.38 (brs, 1H, COOH).

^{13}C –NMR (125 MHz, DMSO-d_6): 13.66 (CH_3-4'), 19.09 (CH_2-3'), 32.42 (CH_2-2'), 55.48 (N-CH_2-1'), 55.51(OCH_3), 106.93 (C-3),), 114.15 (C-5), 116.18 (C-2'' & C-6''), 116.43 (C-8a), 127.41(C-4a), 128.92 (C-3''& C-5''), 129.17 (C-8), 137.21 (C-7),146.22 (C-1''), 151.70 (d, $^1J_{C\text{-}F}$=243.7Hz, C-6), 152.65(C-2), 158.52 (C-4''), 166.71 (COOH), 176.72 (C-4).

HRMS (ESI, -ve): *m/z* calculated for $C_{21}H_{21}FN_3O_4$ $[M\text{-}1]^+$ 398.15161, Found 398.16321.

Compound 4d: Synthesis of 8-Amino-1-butyl-6-fluoro-7-(2,4-dimethoxy phenylamino)-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (4d)

A mixture of the ester **3d**, (1.5 g, 9.79mmol) in 12N HCl (40 mL) and ethanol (40 mL) in ice bath (0-5°C) was stirred for 15 minutes. Next,(4g, 21mmol) of stannous chloride ($SnCl_2$) was added portion wise and the reaction mixture left stirring 24h.Then, the reaction mixture was cooled, poured onto crushed ice 150 g) and the resulting orange precipitate was collected, washed with cold water (2 x 30 mL) and left to dry. This compound faced sever degradation **/** oxidation upon heating while analysis. Yield ≈ 53% (0.53g). Mp = 204-205°C; *Rf* value in system 2 = 0.20.

^{1}H-NMR (500MHz, DMSO-d_6):*δ* 0.76 (t, *J*=7.45 Hz, 3H, CH_3-4'), 1.08 (m, Hz, 2H, CH_2-3'), 1.54 (m, , 2H, CH_2-2'), 3.64 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 4.82 (t, *J*=7.78 Hz, 2H, N-CH_2-1'), 5.55 (brs,2H, NH_2), 6.20 (d, *J*=2.35Hz, 1H, H-3''), 6.98 (d,d, *J* =8.20 Hz, 2.10Hz,1H, H-5''), 7.21 (d, *J* =8.0 Hz, , 1H, H-6''), 7.42 (d, $^3J_{H\text{-}F}$=12.35 Hz,1H, H-5), 7.58 (brs, 1H, NH, exch.), 8.94 (s, 1H, H-2), 15.23 (brs, 1H, COOH).

HRMS (ESI, +ve): *m/z* calculated for $C_{22}H_{25}FN_3NaO_5$ $[M+Na1]^+$ 453.16759, Found 453.1638.HRMS (ESI, -ve): *m/z* calculated for $C_{21}H_{23}FN_3O_3$ $[M\text{-}COOH1]^+$ 384.17235, Found 384.13953 (100%, base peak).

Compound 4e: Synthesis of 8-Amino-1-butyl-6-fluoro-7-(3,4,5-Trimethoxy phenylamino)-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (4e)

A mixture of the ester **3e** (1 g, 2.25 mmol) in 12N HCl (40 mL) and ethanol (40 mL) in ice bath (0-5°C) was stirred for 15 minutes. Next,(4g, 21mmol) of stannous chloride ($SnCl_2$) was added portion wise and the reaction mixture left stirring 24h.Then, the reaction mixture was cooled, poured onto crushed ice 150 g) and the resulting orange precipitate was collected, washed with cold water (2 x 30 mL) and left to dry. This compound faced sever degradation **/** oxidation upon heating while analysis. Yield ≈ 53% (0.53g). Mp = 173-174.5°C; *Rf* value in system 2 = 0.23.

^{1}H-NMR (500MHz, DMSO-d_6, rotamers):*δ* 0.82 (t, 3H, CH_3-4'), 1.07 (m, *J*=7.15 Hz, 2H, CH_2-3'), 1.47, 1.58 (2m, *J*=7.25, *J*=7.82 Hz, 2H, CH_2-2', rotamers), 3.52 (s, 3H, OCH_3) 3.59 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 4.81 (m, 2H, N-CH_2-1'), 5.72 (brs, 2H, NH_2), 5.97 (s, 2H, H-2''&H-6''), 7.51, 7.94 (2d, *J* =12.35, 10.5 Hz, 1H, H-5, rotamers), 8.77, 9.27 (2s, 1H,NH, rotamers), 8.94 (s, 1H, H-2), 14.54 (brs,

1H, COOH).

^{13}C –NMR (125 MHz, DMSO-d_6): 16.22 (CH_3-4'), 19.06 (CH_2-3'), 32.63 (CH_2-2'), 55.84 (N-CH_2-1'),55.53 (2 OCH_3), 60.35 (OCH_3), 92.55 (C-2''& C-6''), 99.31 (C-5), 90.99 (C-3), 106.88 (C-7),116.77 (C-7),122.53(C-4a),127.41 (C-8), 131.65 (C-8a), 141.37 (C-1''), 151.18 (OCH_3, 4'') 151.84 (OCH_3, 3''), 153.37 (OCH_3, 5'') 151.24(d, $^1J_{C-F}$=253 Hz, C-6),152.58 (C-2), 156.03 (C-4''), 166.63(COOH), 177.40 (C-4).

HRMS (ESI, -ve): *m/z* calculated for $C_{23}H_{25}FN_3O_6$ [M-1]$^+$ 458.17274, Found 458.14694.HRMS (ESI, -ve): *m/z* calculated for $C_{23}H_{26}FN_{3Na}O_6$ [M+, Na]$^+$ 482.17033, Found 482.33052.HRMS (ESI, -ve): *m/z* calculated for $C_{23}H_{25}FN_{3Na}O_6$ [M-1,Na]$^+$ 481.16251, Found 481.32714.

Compound 5a: Synthesis of 9-butyl-4-fluoro-3-(2-methoxy-phenyl)-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid (5a)

Compound **5a** was synthesized through cyclization/diazotization of preceding reduced acid **4a**. Compound **4a**(0.50g, 1.25mmol) was placed in RBF, then 20 mL aqueous HCl was added and the mixture left stirring in ice bath (0-5°C) for 15 minutes. $NaNO_2$ (0.086g, 1mmol) dissolved in 10mL H_2O was added drop wise. The reaction mixture was left stirring overnight. Progress of cyclization reaction was monitored by TLC and was completed within 24 hrs. Thereafter, the reaction mixture was cooled, poured onto crushed ice (250g) and the resulting brown precipitate was collected, washed with cold water (2 x 20mL) and left to dry. Yield= 0.21g (≈ 42 %). mp = 186-189°C; *Rf*value in system 1= 0.38.

^{1}H NMR (500 MHz, DMSO-d6): δ 0.97 (t, J=7.6 Hz, 3H, CH_3-4'), 1.47 (m, 2H, CH_2-3'), 1.94 (m, 2H, CH_2-2'), 3.78 (s, 3H, OCH_3), 5.26 (brs, 2H, N-CH_2-1'), 7.26 (dd, J = 7.42 Hz, J = 7.45 Hz, 1H, CH-5''), 7.41 (d, J= 8.41 Hz, 1H, CH-3''), 7.71 (m, 1H, CH-4'', CH-6''), 7.77 (d, J=7.05 Hz, 1H, CH-6''), 8.13 (d, $^3J_{H-F}$= 9.70 Hz, 1H, H-5), 9.17 (s, 1H, H-8), 15.17 (br s, 1H, COOH).

^{13}C NMR (75 MHz, DMSO-d6): 13.76 (CH_3-4'), 19.21 (CH_2-3'), 31.95 (CH_2-2'), 56.31 (OCH_3), 57.34 (N-CH_2-1'), 109.01 (d, $^2J_{C-F}$= 20.25 Hz, C-5), 109.26 (C-7), 112.94 (C-5''), 121.02 (C-3''), 123.48(C-9a), 124.46 (C-5a), 128.43 (C-4''), 128.88 (C-3a), 130.76 (C-9b), 132.89 (C-6''), 139.26 (C-1''), 147.25 (d, $^1J_{C-F}$ = 251.23 Hz, C-4), 149.55 (C-8), 154.35 (C-2''), 165.98 (COOH), 176.42 (C-6).

HRMS ((+ve)-ESI): *m/z* calculated for $C_{21}H_{20}FN_4O_4$ [M+H]$^+$: 411.14686, found: 411.14631

Compound 5b: Synthesis of 9-butyl-4-fluoro-3-(3-methoxy phenyl)-6-oxo-6,9-dihydro-3H-[1,2,3] triazolo[4,5-h]quinolone-7-carboxylic acid (5b)

Trizolo **5b** was synthesized following similar general procedure starting from compound **4b.** The reduced **4b** (0.50g, 1.21mmol) in RBF, then 20 mL aqueous HCl was added and the mixture was stirred in ice bath (0-5°C) for 30 minutes, Sodium nitrite ($NaNO_2$, 0.5g, 1.75mmol) dissolved in 10 mL H_2O was added. The reaction mixture was left for 24hrs. Thereafter, upon cooling the reaction mixture was poured onto crushed ice (250g) and the resulting brown precipitate was filtered, washed with cold water (2 x 15 mL) and left to dry. Yield= 0.3g (≈ 66 %). mp = 205-208°C; *Rf* value in system 1= 0.33

^{1}H NMR (500 MHz, DMSO-d_6): δ 0.90 (t, *J*=8.8 Hz, 3H, CH_3-4'), 1.42 (m, 2H, CH_2-3'), 1.87 (m, 2H, CH_2-2'), 3.82 (s, 3H, OCH_3), 5.20 (brs, 2H, N-CH_2-1'), 7.05-7.77 (m, 3H, Ar.H, 2'', 4'', 6''), 7.96-8.32 (m, 2H, H-5'', H-5), 9.18 (s, 1H, H-8), 11.99 (br s, 1H, COOH).

^{13}C NMR (125MHz, DMSO-d_6): 14.03 (CH_3-4'), 19.45 (CH_2-3'), 32.14 (CH_2-2'), 56.19(OCH_3), 57.61 (N-CH_2-1'), 110.01 (C-7), 110.48 (C-2''), 113.32 (d, *J*=23.1Hz, C-5), 117.94 (d, *J*=19.2Hz, C-3a), 118.25 (C-6''), 118.90 (C-4''), 123.57 (C-9a), 127.93 (C-5a), 130.75 (C-5''), 139.76 (C-9b), 145.08 (C-1''), 150.25 (C-8), 155.22 (*d, $^1J_{C-F}$ =248.6Hz,C-4),* 160.60 (C-3''), 165.86 (COOH), 176.25 (C-6).

HRMS (ESI, -ve): *m/z* calculated for $C_{20}H_{18}FN_4O_2$ [M-1, -COOH]$^+$ 365.14138, Found 365.25601.

Compound 5c: Synthesis of 9-butyl-4-fluoro-3-(4-methoxy-phenyl)-6-oxo-6,9-dihydro-3H-[1,2,3] triazolo[4,5-h]quinolone-7-carboxylic acid (5c)

Compound **5c** was synthesized following similar general procedure starting from compound **4b** (0.75g, 1.82mmol) in RBF, then adding 20 mL aqueous HCl and the mixture left stirring in ice bath (0-5°C) for 30 minutes. Sodium nitrite ($NaNO_2$, 0.5g, 1.75mmol)

Dissolved in 10 mL H_2O was added and the reaction mixture was left for 24hrs. The reaction was monitored by TLC and completed within 24 hrs. Thereafter, upon cooling the reaction mixture was poured onto crushed ice (150g) and the resulting brown precipitate was filtered, washed with cold water (2 x 15mL) and left to dry. Yield= 0.3g (≈ 40 %). mp = 180.5-183°C; *Rf* value in system 1= 0.31

^{1}H NMR (500 MHz, DMSO-d_6): δ 0.91 (m, 3H, CH_3-4'), 1.42 (m, 2H, CH_2-3'), 1.88 (m, 2H, CH_2-2'), 3.88 (s, 3H, OCH_3), 5.22 (brt, 2H, N-CH_2-1'), 7.19 (m, 2H, Ar.H, 2'', 6''), 7.41 (m, 2H, H-3'' & H-5''), 8.10 (d, $^1J_{H-F}$ =13.2Hz, H-5), 9.15 (s, 1H, H-2), 11.19 (br s, 1H, COOH).

^{13}C NMR (75 MHz, DMSO-d_6): 14.02 (CH_3-4'), 19.45 (CH_2-3'), 32.16 (CH_2-2'), 56.78(OCH_3), 57.60 (N-CH_2-1'), 109.27 (d, C-5), 110.06 (C-7), 114.67 (C-2'' & C-6''), 114.82 (d, *J*=24Hz, C-3a), 123.56 (C-9a), 128.31 (C-9b), 130.97 (C-3'' & C-5''), 132.21 (C-5a), 139.29 (C-1''), 149.92 (C-8), 153.23 (d, $^1J_{C-F}$=252Hz, C-4),162.49 (C-4''), 165.96 (COOH), 176.51 (C-6).

HRMS (ESI, -ve): *m/z* calculated for $C_{20}H_{18}FN_4O_2$ [M-1, -COOH]$^+$ 365.14138, Found 365.1567.

Compound 5d: 9-butyl-4-fluoro-3-(2,4-dimethoxy-phenyl)-6-oxo-6,9-dihydro-3H-[1,2,3] triazolo[4,5-h]quinolone-7-carboxylic acid (5d).

Compound 5e: 9-butyl-4-fluoro-3-(3,4,5-t trimethoxy-phenyl)-6-oxo-6,9-dihydro-3H-[1,2,3] triazolo[4,5-h]quinolone-7-carboxylic acid (5e).

Preparation of **5d, 5e** was impossible due to low yields of their starting **4d, 4e.** Low yields and difficult separation was the major limitation to prepare both compounds **4d**, **4e** suffered severe oxidation /degradation upon any attempts of separation. To other more, they suffer similar problem upon heating on analysis or work up.

Attempts synthesis of hydroxyl derivatives 6 and 7 (b, d, e) (Scheme 2)

Compound 6b: Synthesis of 1-Butyl-7-(3-hydroxyphenylamino) 6-fluoro--8-nitro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (6b)

The ester **2b** (1.5g, 13.74mmol) in 10mL conc. HBr (48%) was refluxed at 110 °C. Phenolic ether hydrolysis of methoxy groups was followed by TLC and completed within 3-4 days. Then, the reaction mixture was cooled, poured onto crushed ice and the resulting brown precipitate was collected, washed with cold water (2 x 30 mL) and left to dry. Yield ≈ 8% (0.12g). Mp = 150-151.5 °C; *Rf* value in system 2 = 0.12.

HRMS (ESI, +ve): *m/z* calculated for $C_{20}H_{18}FN_3NaO_6$ $[M+Na1]^+$ 438.10773, Found 438.19983.

Compound 6d: Synthesis of 1-Butyl-7-(2,4-dihydroxyphenylamino) 6-fluoro--8-nitro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (6d)

A combination of the ester **2d**,(1g, 7.98mmol) in 10mL conc. HBr (48%) was refluxed at 110 °C. Phenolic ether hydrolysis of methoxy groups was followed by TLC and completed within 3-4days. Then, the reaction mixture was cooled, poured onto crushed ice and the resulting brown orange precipitate was collected, washed with cold water (2 x 30 mL) and left to dry. Yield ≈ 30% (0.3g). Mp = 140-142.5 °C; *Rf* value in system 2 = 0.15. TLC of this product showed many colored spots of which 3 were major. Crystallization of the product gave more pure solid with 3spots of which 2 are major spots: the product (major) and starting dimethoxy acid (major) and starting dimethoxy ester (minor). Our dihydroxy target 6d was major in the mixture (50%).

^{1}H-NMR (500MHz, DMSO-d_6, major):*δ* 0.75 (t, *J*=7.45Hz, 3H, CH_3-4'.19 (m, 2H, CH_2-3'), 1.56 (m, 2H, CH_2-2'), 4.26 (t, *J*=7.8 Hz, 2H, N-CH_2-1'), 6.72 (brs, 1H, OH), 6.94 (d, J=7.8Hz, 1H, H-5''), 7.34 (brs, 1H, OH), 7.39 (d, *J*=2.4Hz, 1H, H-3''), 7.46 (d, *J*=8.1Hz, 1H, H-6''), 7.86 (d,$^3J_{H\text{-}F=}$12.10Hz,1H, H-5), 8.62 (brs, 1H, NH), 8.84 (s, 1H, H-2), 15.88 (brs, 1H, COOH)

HRMS (ESI, +ve): *m/z* calculated for $C_{20}H_{18}FN_3NaO_7$ $[M+Na]^+$ 454.10265, Found 454.16942; LRMS (ESI, +ve): *m/z* calculated for $C_{20}H_{18}FN_3NaO_7$: 454.16 (21%, M+Na), 413.26 (40%, M+-F), 381.29 (20%).

Other peaks for dimethoxy starting: 2d (487.47), 3d acid 459.42

Compound 6e: Synthesis of 1-Butyl-7-(3, 4, 5-trihydroxyphenylamino) 6-fluoro--8-nitro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (6e)

A combination of the ester **2e**,(1g, 7.08mmol) in 10mL concentrated HBr (48%) was refluxed at 110 °C. Phenolic ether hydrolysis of methoxy groups was followed by TLC and completed within 3-4days. Then, the reaction mixture was cooled, poured onto crushed ice and the resulting brown orange precipitate was collected, washed with cold water (2 x 30 mL) and left to dry. Yield ≈ 30% (0.3g). Mp = 137-138.2C; *Rf* value in system 2 = 0.13.

HRMS (ESI, -ve): *m/z* calculated for $C_{20}H_{19}FN_3O_8$ $[M-F]^+$ 429.11722, Found 429.23797; LRMS (ESI, -ve): *m/z* calculated for $C_{20}H_{20}FN_4NaO_8$ $[M+Na+NH3]^+$ 486.29(95%),: 339.20 (47%), 284.27 (11%), 381.29 (20%).

Other peaks for trimethoxy starting: 3d (540.53, M+Na ester), 5%., 3e (M+Na) acid 512.49 (6%).

Compound 7b: 8-Amino-1-butyl-6-fluoro-7-(3-hydroxy phenylamino)-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (7b)

Compound 7d: 8-Amino-1-butyl-6-fluoro-7-(2,4-dihydroxy phenylamino)-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (7d)

Compound 7e: 8-Amino-1-butyl-6-fluoro-7-(3,4,5-trihydroxy phenylamino)-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (7e)

Due to low yields, many derivatives obtained, difficult separation, these derivatives were difficult to prepare at this time limited project.