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Synthesis and antimicrobial evaluation of novel alkylated piperazine-based fluoroquinolone carboxylate derivatives

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In the present study, to synthesize quinolone-piperazine alkylated analogues, initially 3,4-difluoro nitro benzene is reduced to yield 3,4-difluorobenzenamine which is treated with diethyl ethoxymethyl enemalonate to yield diethyl 2-((3,4-difluorophenylamino) methylene) malonate. This has been further cyclized to produce ethyl 6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate which is treated with iodoethane to produce ethyl 1-ethyl-6,7-diifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate. This is hydrolyzed and subsequent nucleophilic substitution with piperazine at 7th position gives quinolone-piperazine derivative. This is alkylated to yield 1-ethyl-6-fluoro-7-(4-alkylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid which is treated with ethanol to afford the desired ethyl 1-ethyl-6-fluoro-7-(4-hexylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate derivatives. Based on the antimicrobial activity studies, hexyl analog **8a** exhibits potent antimicrobial activity against *Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 121, *Micrococcus luteus* MTCC 2470 (MIC value 1.9µg/mL), with respect to other synthesized compounds and reference drug ciprofloxacin. **8b** (heptyl analogue) and **8c** (nonyl analogue) show significant activity with MIC value 3.9µg/mL. In the case of antifungal screening, **8a**, **8b** and **8c** show significant antifungal activity against *Candida albicans* MTCC 3017with MIC value ranged from 3.9 to 7.8µg/mL. Compounds **8a** (butyl) shows potent minimum bactericidal concentration activity with MIC value 3.9µg/mL against the tested strains.

Keywords: Piperazine, fluoroquinolone, quinolone, antimicrobial, antifungal

Diseases caused by microbial pathogens posing a big challenge to health of public due to the extensive occurrence of drug resistance. Therefore, of late, researchers focused on addressing the issue of drug resistant microbes generated from the misuse and unlimited use of antimicrobial agents. To deal with this problem researcher need to investigate for new powerful antimicrobial agents by modifying the structure of well-known antimicrobial agents.

Quinolones are the most important common frameworks present in a large variety of molecules with various pharmacological activities. Especially, quinolone antibacterial agents represent a fast-growing group of antibiotics. Fluoroquinolones (FQs) are the important heterocyclic compounds belong to the class of quinolones showing a broad spectrum of biological activities against various bacteria, mycobacteria, and parasites[1-9]. Fluoroquinolones emerged as an important class of antibiotics for treating various bacterial infections namely, urinary tract, respiratory tract, *gastrointestinal*, abdominal, skin, soft tissue, bone and joints and further, it can be used to treat sexually transmitted diseases[10].

Various studies revealed that fluoroquinolone derivatives also showed the antiproliferative activities in some tumor cells such as breast cancer cells[11], bladder transitional cell carcinoma[12-14], non small cell lung carcinoma[15], prostate carcinoma[16, 17] and colorectal carcinoma[18]. The well known Fluoroquinolone derivative ciprofloxacin exhibited antiproliferative and apoptosis inducing activities both on prostate and bladder cancer cells[11, 19, 16, 17]. The suppression of DNA gyrase and cell permeability of fluoroquinolones is effectively influenced by substitution of a big functional group at the C-7 position and greatly effects their antibacterial efficacy, spectrum and safety[20, 21]. In recent years a number of quinolone compounds with substitution of piperazine ring at C-7 position were synthesized and evaluated for antibacterial activities[22-25]. Generally, the action of fluoroquinolones increases with an increase in lipophilicity[26]. In addition, oils and fats are the most significant renewable raw materials for the

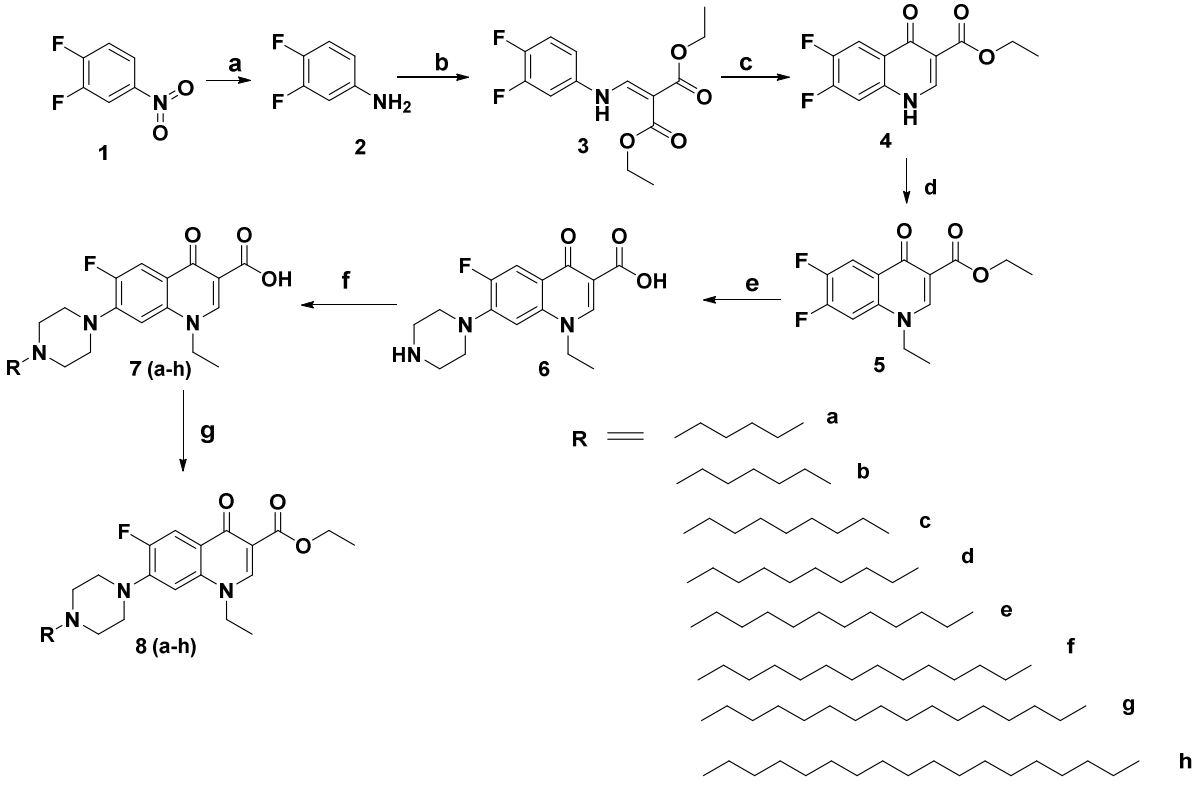
chemical industry. Fatty acids are aliphatic carboxylic acids produced from the cleavage of fats and oils regarded as non toxic, bio-compatible and bio-degradable. These fatty acid derivatives are found to be associated with diverse types of biological activities, such as antimicrobial[26, 27], antifungal[28], anticancer[29-31] and pesticidal[32] activities. Hybrid molecules, in general designed based on taking two or more pharmacophores into a single molecule. The hybrid molecules with novelty are expected to exhibit optimal activities.

Motivated by the above-mentioned findings and in continuation of our studies towards synthesis of carboxylated quinolones having alkyl chain moiety, herein we plan to synthesize a new class of carboxylated fluoroquinolones with the alkyl chain moiety on the piperazinyl ring by alkylation to generate hybrid molecules with potential biological activities. In the present study, a series of ethyl 1-ethyl-6-fluoro-7-(4-alkylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate derivatives were synthesized and evaluated for their biological activities.

Results and Discussion

The synthesis of targeted fluoroquinolones, ethyl 7-(4-alkylpiperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylate is outlined in Scheme I.

The starting material, 3,4-difluoro nitro benzene was reduced to yield 3,4-difluorobenzenamine by using iron and ammonium chloride in methanol and water. Resultant reduced 3,4-difluorobenzenamine was treated with diethyl ethoxymethyl enemalonate to yield diethyl 2-((3,4-difluorophenylamino) methylene)malonate. The intermediate ethyl 6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate was achieved by the cyclization of diethyl 2-((3,4-difluorophenylamino)methylene) malonate in presence of diphenyl ether. Ethyl 6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate was treated with iodoethane in presence of K_2CO_3 and dry DMF to produce ethyl 1-ethyl-6,7-diifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate. This was hydrolyzed with 2N HCl and acetic acid followed by the nucleophilic substitution with piperazine at 7th position in the presence of triethyl amine to give respective quinolone-piperazine derivative.



Scheme I — Reagents and conditions: (a) Fe, NH_4Cl, H_2O, MeOH, 90°C, 12 h; (b) Diethyl ethoxymethyl enemalonate, 120°C, 4 h; (c) Diphenyl ether, 255°C, 6 h; (d) K_2CO_3, C_2H_5-I, DMF, 85°C, 16 h; (e) (1) MeOH, 10% HCl, 80°C, 12 h; (2) Piperazine, Et_3N, reflux for 8 h; (f) Alkyl bromide, Et_3N, DMF, 80-90°C, 2 h; (g) $SOCl_2$, MeOH, reflux for 12 h

Further the quinolone piperazine derivative was alkylated by using various alkyl bromides in the presence of triethyl amine and DMF to yield 1-ethyl-6-fluoro-7-(4-alkylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline -3-carboxylic acid. Then this compound was reacted with thionyl chloride in presence of ethanol to yield the required ethyl 1-ethyl-6-fluoro-7-(4-hexylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate derivatives. The synthesized compounds were characterized by spectral data (^{1}H NMR, ^{13}C NMR, IR, and ESI-MS).

Antimicrobial Activity

All the newly synthesized compounds **8a–h** were directly screened for *in vitro* antimicrobial, minimum bactericidal activity and biofilm inhibition activity against different gram-positive bacterial strains such as *Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 121, *S. aureus* MLS16 MTCC 2940, *Micrococcus luteus* MTCC 2470, and gram-negative bacterial strains such as *Klebsiella planticola* MTCC 530, *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 2453 and fungi such as *Candida albicans* MTCC 3017.

The antimicrobial activity results showed (Table I) that majority of the compounds exhibited either promising or significant activities. Compound **8a** exhibited potent antimicrobial activity against *Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 121, *Micrococcus luteus* MTCC 2470 (MIC value 1.9µg/mL) and compounds **8a** (against *S. aureus* MLS16 MTCC 2940 and *Klebsiella planticola* MTCC 530 strains)**, 8b** (against *Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 121, *Micrococcus luteus* MTCC 2470 strains), **8c** (against *Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 121 and *Pseudomonas aeruginosa* MTCC 2453 strains) showed significant activity with MIC value 3.9µg/mL and remaining compounds showed good activity against various strains with MIC value ranged from 7.8 to 15.6 µg/mL. In the case of antifungal screening, **8a**, **8b**, and **8c** showed significant antifungal activity against *Candida albicans* MTCC 3017with MIC value ranged from 3.9 to7.8 µg/mL. The activity results in this regard are furnished in Table I. Compound **8a** (hexyl) showed potent minimum bactericidal concentration activity against *Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 121 and *Micrococcus luteus* MTCC 2470 (MIC value 3.9µg/mL). Compounds **8b** (heptyl) against *Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 121, *Micrococcus luteus* MTCC 2470 and *Candida albicans* MTCC 3017 (MIC value 7.8µg/mL) and **8c** (nonyl) against *Staphylococcus aureus* MTCC96, *Bacillus subtilis* MTCC 121 and *Pseudomonas aeruginosa* MTCC 2453 (MIC value 7.8µg/mL) displayed promising activities. The activity results in this regard are furnished in Table II.

It is noteworthy that compounds with short and medium chain fatty alkyl substituents on the piperazinyl ring exhibited potent activity. This is

Table I — Antimicrobial activity of synthesized compounds

Test compd	Minimum inhibitory concentration (µg/mL)							
	Staphylococcus aureus MTCC 96	*Bacillus subtilis* MTCC 121	*S. aureus* MLS16 MTCC 2940	*Micrococcus luteus* MTCC 2470	*Klebsiella planticola* MTCC 530	*Escherichia coli* MTCC 739	*Pseudomonas aeruginosa* MTCC 2453	*Candida albicans* MTCC 3017
8a	1.9	1.9	3.9	1.9	3.9	7.8	7.8	3.9
8b	3.9	3.9	7.8	3.9	7.8	7.8	7.8	3.9
8c	3.9	3.9	7.8	7.8	7.8	15.6	3.9	7.8
8d	15.6	7.8	>125	15.6	>125	>125	7.8	>125
8e	>125	7.8	>125	15.6	>125	>125	15.6	>125
8f	>125	7.8	>125	15.6	>125	>125	15.6	>125
8g	>125	>125	>125	>125	>125	>125	15.6	>125
8h	>125	>125	>125	>125	>125	>125	15.6	>125
Miconazole (Standard control)	–	–	–	–	–	–	–	7.8
Ciprofloxacin (Standard control)	0.9	0.9	0.9	0.9	0.9	0.9	0.9	–

evident from compound **8a** (hexyl analogue), **8b** (heptyl analogue) and **8c** (nonyl analogue) which are either short chain or medium chain analogues. The long chain compounds **8e** (12 carbon chain), **8f** (14 carbon chain), **8g** (16 carbon chain) and **8h** (18 carbon chain) were found to exhibit no significant activity. Based on the antimicrobial activities of the synthesized derivatives, all the compounds were screened for antibiofilm activity against *Staphylococcus aureus* MLS-16 MTCC 2940, *Micrococcus luteus* MTCC 2470, *Klebsiella planticola* MTCC 530, *Pseudomonas aeruginosa* MTCC 2453 and *Candida albicans* MTCC 3017which are important nosocomial pathogens encountered in medical establishments and devices and have the ability to form biofilms. The results to this regard are summarized in Table III, which clearly reveal that these compounds exhibited moderate to low activities against all the tested strains.

Experimental Section

All the chemicals used in this study were of analytical grade and they were procured from various commercial sources and used without any further purification. Progress of reactions was monitored on micro TLC plates (coated with TLC grade silica gel, procured from Merck). Column chromatography was carried out by using silica gel (100-200 mesh) obtained from Qualigens (India) using freshly distilled solvents. ^{1}H-NMR and ^{13}C-NMR spectra were recorded using a Bruker Avance (for ^{1}H-NMR at 300 MHz, 400 MHz, 500 MHz and for ^{13}C-NMR at 75 MHz, 100 MHz, 125 MHz) spectrometer, using TMS for proton NMR (δ = 0 ppm) and δ 77.00 ppm

Table II — Minimum bactericidal concentration (MBC) of synthesized compounds

Test compd	Minimum bactericidal concentration (μg/mL)							
	Staphylococcus aureus MTCC 96	*Bacillus subtilis* MTCC 121	*S. aureus* MLS16 MTCC 2940	*Micrococcus luteus* MTCC 2470	*Klebsiella planticola* MTCC 530	*Escherichia coli* MTCC 739	*Pseudomonas aeruginosa* MTCC 2453	*Candida albicans* MTCC 3017
8a	3.9	3.9	7.8	3.9	37.8	15.6	15.6	7.8
8b	7.8	7.8	15.6	7.8	15.6	15.6	15.6	7.8
8c	7.8	7.8	15.6	15.6	15.6	31.2	7.8	15.6
8d	31.2	15.6	>125	31.2	15.6	>125	15.6	>125
8e	>125	15.6	>125	31.2	15.6	>125	31.2	>125
8f	>125	15.6	>125	31.2	15.6	>125	31.2	>125
8g	>125	>125	>125	>125	>125	>125	31.2	>125
8h	>125	>125	>125	>125	>125	>125	31.2	>125
Miconazole (Standard control)	–	–	–	–	–	–	–	7.8
Ciprofloxacin (Standard control)	0.9	0.9	0.9	0.9	0.9	0.9	0.9	–

Table III — Biofilm activity of synthesized compounds

Test compd	IC_{50} values (μM)				
	S. aureus MLS16 MTCC 2940	*Micrococcus luteus* MTCC 2470	*Klebsiella planticola* MTCC 530	*Pseudomonas aeruginosa* MTCC 2453	*Candida albicans* MTCC 3017
8a	NA	31.4 ± 0.06	27.21 ± 0.12	48.7 ± 0.13	30.54 ± 0.12
8b	NA	44.94 ± 0.08	NA	NA	112.35 ± 0.14
8c	NA	32.15 ± 0.07	NA	42.6 ± 0.09	41.8 ± 0.07
8d	NA	27.10 ± 0.09	NA	NA	NA
8e	58.96 ± 0.08	29.12 ± 0.08	NA	NA	38.83 ± 0.09
8f	NA	31.30 ± 0.08	NA	NA	32.40 ± 0.016
8g	NA	NA	NA	NA	35.02 ± 0.09
Ciprofloxacin	0.7 ± 0.09	0.8 ± 0.11	0.8 ± 0.09	0.5 ± 0.12	–
Miconazole	–	–	–	–	2.5 ± 0.09

NA = No activity.

for ^{13}C-NMR as internal standard for chemical shifts (δ) in $CDCl_3$ at 25°C. The chemical shift values are showed in ppm (parts per million) units. Infrared spectral analysis was carried out in chloroform solvent on a Perkin-Elmer FT-IR spectrum BX. Using High Resolution Mass Spectrometry (HRMS) mass spectral data was recorded.

3,4-Difluoroaniline, 2: 1,2-difluoro-4-nitrobenzene (4 g, 1 mmol) was taken in methanol and water (25 mL: 5 mL) at rt and stirred, to this stirred solution iron powder (2.5 g) and ammonium chloride (2 g) were added. This stirred solution was then refluxed for 12 h and the progress of reaction was monitored by TLC. After 12 h reaction period the reaction mixture was filtered with the aid of celite pad. Then the solvent was removed under reduced pressure, water was added and the product was extracted using ethyl acetate solvent and dried over anhydrous sodium sulphate. Silica gel column chromatography was employed to purify crude product and the required product was eluted in a solvent mixture (Hexane: EtOAc, 30: 70, v/v) as a brown liquid with 77% yield. ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) = 6.88-6.94 (m, 1H, Ar-H), 6.43-6.47 (m, 1H, Ar-H), 6.31-6.34 (m, 1H, Ar-H), 3.60 (s, 2H, NH_2); ^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) = 150.8, 147.6, 143.3, 140.0, 115.8, 108.5, 101.8; IR ($CHCl_3$ ν_{max} cm^{-1}): 3383, 2360, 1617, 1521, 1266, 1213; MS (ESI, *m/z*) $[M+H]^+$ 130.

Diethyl 2-((3,4-difluorophenylamino) methylene) malonate, 3: A mixture of 3,4-difluoroaniline (2.5 g, 1 mmol), diethyl [(ethyloxy) methylidene] propanedioate (2.5 g, 1 mmol) was heated at 120°C for 4 h. The reaction mixture was cooled to room temperature and reduced to dryness in vacuum to obtain the desired diethyl 2-((3,4-difluorophenylamino) methylene) malonate. The product so obtained was used for the next step without further purification. IR ($CHCl_3$ ν_{max} cm^{-1}): 3442, 2361, 1635, 1225, 1080, 800; HRMS (ESI) *m/z*$[M+H^+]$- calc for $C_{14}H_{16}F_2NO_4$ is 300.10419 found 300.10425 ($C_{14}H_{16}F_2NO_4$).

Ethyl 6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate, 4: Small portions of 2-((3,4-difluorophenylamino) methylene) malonate (4 g, 1 mmol) was added to boiling diphenyl ether (30 mL) over a period of 10 min at about 255°C and the contents were heated for 6 h. Then the reaction mixture was cooled to 50°C and diethyl ether was added to dilute it and cooled further to obtain a precipitate. Then filtration was carried out to collect the solid residue and this was washed with diethyl ether and dried in vacuum to get ethyl 6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate. The attained title compound (1.8 g, 53% yield, white solid) was used in the next reaction step without further purification. IR ($CHCl_3$ ν_{max} cm^{-1}):3441, 2360, 1678, 759; MS (ESI, *m/z*) $[M+H]^+$ 254.

Ethyl1-ethyl-6,7-difluoro-4-oxo-1,4-dihydroquinoline -3-carboxylate, 5: DMF (20 mL) was used to dissolve Ethyl-6, 7-difluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylate (2 g, 1 mmol). Potassium carbonate (3.5 g, 1.5 mmol) and iodoethane (2 mL, 1 mmol) were added consecutively to this solution and the contents were magnetically stirred at 85 °C for 16 h. Micro TLC with UV detection was used to monitor the progress of reaction. At the end of reaction, water was added and ethyl acetate was used to extract the title compound, dried using anhydrous sodium sulphate. Silica gel column chromatography was employed to purify the crude product and ethyl acetate was used to elute the required product as a light yellow solid with 63% yield. ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) = 8.49 (s, 1H, Ar-H), 8.28 –8.32 (m, 1H, Ar-H), 7.26 - 7.29 (m, 1H, Ar-H), 4.37 – 4.42 (m, 2H, CH_2), 4.19 - 4.23 (m, 2H, CH_2), 1.54 – 1.57 (t, J = 7.2 Hz, 3H, CH_3), 1.40 – 1.42 (t, J = 6.9 Hz, 3H, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm) =171.0, 163.2, 153.4, 150.0, 145.1, 134.3, 124.8, 113.6, 109.1, 104.0, 59.1, 47.8, 13.0; IR ($CHCl_3$ ν_{max} cm^{-1}): 3684, 3020, 2400, 2130, 1616, 1473, 1215, 1025, 762, 669; HRMS (ESI) *m/z* $[M+Na^+]$: calc for $C_{14}H_{13}F_2NO_3Na$ is 304.07557 found 304.07538 ($C_{14}H_{13}F_2NO_3Na$).

1-Ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid, 6: 10% HCl (2 mL) was added to the stirred solution of Ethyl-1-ethyl-6, 7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (55mg, 0.020 mmol) in methanol (2mL) and the contents were heated at 80°C for 12 h. After 12 h period, the mixture was cooled and water (2 mL) was added, the solid precipitate was filtered and dried to obtain the carboxylic acid (49 mg) compound. This acid was suspended in water (2 mL), to this solution triethyl amine (0.075 mL, 0.5 mmol), piperazine (47 mg, 0.5 mmol) were added consecutively the mixture was refluxed for 8 h period. Precipitation was

obtained after the reaction mixture was cooled and treated with acetic acid. Subsequently, filtered to get the precipitate and this was water washed and dried to obtain norfloxacin (55mg, 84% yield). MS (ESI, *m/z*) $[M+H]^+$ 320.

General Procedure for the Synthesis of Target Compounds, 7a-h: The contents 1-Ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (2 g, 1 mmol), triethylamine (1.5 g, 0.015 mol) and alkyl bromide (0.012-0.02 mol) in DMF solvent (40 mL) were heated at 80-90°C temperature for 2 h period under magnetic stirring. The progression in reaction was supervised by TLC under UV detection. After conformation with TLC, solvent present in the reaction mixture was evaporated to dryness. The suspension was made using residue and water and the solid obtained was filtered and dried to get the unpurified 1-ethyl-6-fluoro-7-(4-alkylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid products. Silica gel-based column chromatography was employed to purify the crude compound and the required product was eluted as a white solid in chloroform, methanol, (99: 1, v/v) solvent mixture.

1-Ethyl-6-fluoro-7-(4-hexylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, 7a: The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Chloroform: Methanol, 99: 1, v/v) as a white solid with 79% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 15.14 (s, 1H, COOH), 8.67 (s, 1H, Ar-H), 8.05 (d, *J* = 13.12 Hz, 1H, Ar-H), 6.84 (d, *J* = 6.86 Hz, 1H, Ar-H), 4.36-4.32 (m, 2H, N-CH_2), 3.35 (broad-s, 4H, $_2$HC-N-CH_2), 2.67 (broad-s, 4H, $_2$HC-N-CH_2), 2.44-2.41 (t, *J* = 7.47Hz, 2H, CH_2), 1.60-1.53 (m, 5H, CH_2, CH_3), 1.39-1.29 (m, 6H, $(CH_2)_{3,}$), 0.90-0.88 (t, *J* = 6.56Hz, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 176, 167, 154, 146, 145, 137, 120, 112, 107, 103, 58, 57, 53, 52, 49, 49, 31, 28, 27, 26, 22, 22, 14, 13; IR ($CHCl_3$ ν_{max} cm^{-1}): 3424, 2929, 2856, 1718, 1626, 1584, 1490, 1382, 1220, 771.

1-Ethyl-6-fluoro-7-(4-heptylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, 7b: The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Chloroform: Methanol, 99: 1, v/v) as a white solid with 73% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 15.10 (s, 1H, COOH), 8.67 (s, 1H, Ar-H), 8.06 (d, *J* = 13.12 Hz, 1H, Ar-H), 6.83 (d, *J* = 6.71 Hz, 1H, Ar-H), 4.34-4.30 (m, 2H, N-CH_2), 3.34 (broad-s, 4H, $_2$HC-N-CH_2), 2.67 (broad-s, 4H, $_2$HC-N-CH_2), 2.43-2.40 (t, *J* = 7.47Hz, 2H, CH_2), 1.62-1.50 (m, 5H, CH_2, CH_3), 1.31-1.27 (m, 8H, $(CH_2)_{4,}$), 0.89-0.87 (t, *J* = 6.86Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 176.9, 167.2, 154.7, 147.0, 146.2, 137.0, 120.4, 112.8, 108.3, 103.6, 58.6, 52.8, 49.8, 49.7, 31.8, 29.5, 29.2, 27.4, 26.8, 22.6, 14.3, 14.0; IR ($CHCl_3$ ν_{max} cm^{-1}): 3424, 2929, 2856, 1717, 1626, 1546, 1490, 1382, 1220, 1089, 771.

1-Ethyl-6-fluoro-7-(4-nonylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, 7c: The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Chloroform: Methanol, 99: 1, v/v) as a white solid with 85% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 15.12 (s, 1H, COOH), 8.66 (s, 1H, Ar-H), 8.03 (d, *J* = 13.08 Hz, 1H, Ar-H), 6.84 (d, *J* = 6.96 Hz, 1H, Ar-H), 4.36-4.31 (m, 2H, N-CH_2), 3.35 (broad-s, 4H, $_2$HC-N-CH_2), 2.67 (broad-s, 4H, $_2$HC-N-CH_2), 2.44-2.40 (t, *J* = 7.58Hz, 2H, CH_2), 1.60-1.51 (m, 5H, CH_2, CH_3), 1.41-1.28 (m, 12H, $(CH_2)_{6,}$), 0.90-0.87 (t, *J* = 6.72Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 176.7, 167.1, 154.6, 146.9, 146.1, 137.0, 120.1, 112.4, 108.0, 103.6, 58.5, 53.4, 52.7, 49.7, 31.7, 29.4, 29.1, 27.4, 26.6, 22.5, 14.3, 13.9; IR ($CHCl_3$ ν_{max} cm^{-1}): 3424, 2929, 2856, 1719, 1626, 1584, 1490, 1220, 771.

7-(4-Decylpiperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, 7d: The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Chloroform: Methanol, 99: 1, v/v) as a white solid with 80% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 15.11(s, 1H, COOH), 8.67 (s, 1H, Ar-H), 8.06 (d, *J* = 13.08 Hz, 1H, Ar-H), 6.83 (d, *J* = 6.96 Hz, 1H, Ar-H), 4.35-4.29 (m, 2H, N-CH_2), 3.34 (t, *J* = 4.76 Hz, 4H, $_2$HC-N-CH_2), 2.67 (t, *J* = 4.89 Hz, 4H, $_2$HC-N-CH_2), 2.44-2.40 (t, *J* = 7.58Hz, 2H, CH_2), 1.60-1.49 (m, 5H, CH_2, CH_3), 1.39-1.31 (m, 14H, $(CH_2)_{7,}$), 0.91-0.88 (t, *J* = 6.48Hz, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 176.9, 167.1, 154.7, 147.0, 146.1, 137., 120.3, 112.7, 108.2, 103.6, 58.5, 52.7, 49.8, 49.7, 31.7, 27.1, 26.7, 22.5, 14.3, 14.0; IR ($CHCl_3$ ν_{max} cm^{-1}): 3424, 2929, 2856, 1719, 1626, 1584, 1490, 1220, 771.

7-(4-Dodecylpiperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, 7e: The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Chloroform: Methanol, 99: 1, v/v) as a white solid with 74% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 15.10(s, 1H, COOH), 8.67 (s, 1H, Ar-H), 8.06 (d, *J* = 13.12 Hz, 1H, Ar-H), 6.83 (d, *J* = 6.86 Hz, 1H, Ar-H), 4.34-4.29 (m, 2H, N-CH_2), 3.35-3.33 (t, *J* = 4.57 Hz, 4H, $_2$HC-N-CH_2), 2.67-2.66 (t, *J* = 4.88 Hz, 4H, $_2$HC-N-CH_2), 2.43-2.40 (t, *J* = 7.62Hz, 2H, CH_2), 1.61-1.50 (m, 5H, CH_2, CH_3), 1.32-1.26 (m, 18H, $(CH_2)_{9,}$), 0.89-0.86 (t, *J* = 6.86Hz, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 176.8, 167.1, 154.7, 146.9, 146.1, 137.0, 120.3, 112.7, 108.2, 103.6, 58.6, 52.8, 49.8, 31.8, 29.6, 29.3, 27.4, 26.8, 22.6, 14.3, 14.0; IR ($CHCl_3$ ν_{max} cm^{-1}): 3431, 2919, 2850, 1721, 1620, 1504, 1462, 1382, 1219, 1089, 768.

1-Ethyl-6-fluoro-4-oxo-7-(4-tetradecylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid, 7f: The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Chloroform: Methanol, 99: 1, v/v) as a white solid with 79% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 15.11 (s, 1H, COOH), 8.67 (s, 1H, Ar-H), 8.06 (d, *J* = 13.08 Hz, 1H, Ar-H), 6.83 (d, *J* = 6.96 Hz, 1H, Ar-H), 4.35-4.30 (m, 2H, N-CH_2), 3.36-3.34 (t, *J* = 4.76 Hz, 4H, $_2$HC-N-CH_2), 2.69-2.66 (t, *J* = 4.89 Hz, 4H, $_2$HC-N-CH_2), 2.45-2.41 (t, *J* = 7.58Hz, 2H, CH_2), 1.61-1.50 (m, 5H, CH_2, CH_3), 1.32-1.27 (m, 22H, $(CH_2)_{11,}$), 0.90-0.87 (t, *J* = 6.72Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 176.8, 167.1, 154.7, 147.0, 146.1, 137.0, 120.3, 112.75, 108.2, 103.6, 58.5, 52.8, 49.8, 49.7, 31.8, 29.6, 29.3, 27.4, 26.7, 22.6, 14.3, 14.0; IR ($CHCl_3$ ν_{max} cm^{-1}): 3430, 2918, 2850, 1721, 1620, 1582, 1492, 1382, 1220, 1089, 771.

1-Ethyl-6-fluoro-7-(4-hexadecylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, 7g: The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Chloroform: Methanol, 99: 1, v/v) as a white solid with 78% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 15.11 (s, 1H, COOH), 8.67 (s, 1H, Ar-H), 8.05 (d, *J* = 13.20 Hz, 1H, Ar-H), 6.83 (d, *J* = 6.05 Hz, 1H, Ar-H), 4.38-4.24 (m, 2H, N-CH_2), 3.36-3.34 (t, *J* = 4.76 Hz, 4H, $_2$HC-N-CH_2), 2.69-2.66 (t, *J* = 4.89 Hz, 4H, $_2$HC-N-CH_2), 2.44-2.39(t, *J* = 7.42Hz, 2H, CH_2), 1.70-1.53 (m, 5H, CH_2, CH_3), 1.39-1.25 (m, 26H, $(CH_2)_{13,}$), 0.90-0.85 (t, *J*= 6.05Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 176.9, 167.1, 154.7, 146.9, 146.1, 137.0, 120.3, 112.7, 108.2, 103.6, 58.6, 52.8, 49.8, 49.8, 49.7, 31.8, 29.5, 29.3, 27.4, 26.8, 22.6, 14.3, 14.0; IR ($CHCl_3$ ν_{max} cm^{-1}): 3428, 2917, 2849, 1721, 1620, 1504, 1462, 1383, 1221, 1089, 771.

1-Ethyl-6-fluoro-7-(4-octadecylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, 7h: The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (Chloroform: Methanol, 99: 1, v/v) as a white solid with 83% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 15.11 (s, 1H, COOH), 8.67 (s, 1H, Ar-H), 8.06 (d, *J* = 13.08 Hz, 1H, Ar-H), 6.83 (d, *J* = 6.96 Hz, 1H, Ar-H), 4.35-4.30 (m, 2H, N-CH_2), 3.36-3.34 (t, *J* = 4.76 Hz, 4H, $_2$HC-N-CH_2), 2.69-2.66 (t, *J* = 4.89 Hz, 4H, $_2$HC-N-CH_2), 2.45-2.41 (t, *J* = 7.58Hz, 2H, CH_2), 1.61-1.50 (m, 5H, CH_2, CH_3), 1.32-1.27 (m, 30H, $(CH_2)_{15}$), 0.90-0.87 (t, *J* = 6.67Hz, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 176.9, 167.2, 154.5, 147.0, 146.0, 137.0, 120.4, 112.7, 108.2, 103.6, 58.5, 52.7, 49.7, 36.4, 31.8, 31.4, 29.6, 29.3, 27.4, 26.7, 22.6, 14.3, 14.0; IR ($CHCl_3$ ν_{max} cm^{-1}): 3428, 2917, 2849, 1721, 1620, 1504, 1462, 1383, 1221, 1089, 771.

General Procedure for the Synthesis of Target Compounds, 8a-h: 1-Ethyl-6-fluoro-7-(4-alkylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid(2 g, 1 mmol) was taken in methanol (20 mL) and slowly $SOCl_2$ (3.5 g, 1.5 mmol) was added and the contents were refluxed under stirring for 12 h period. The reaction progression was monitored by micro TLC with UV detection. The solvent was removed under rotary evaporation after the 12 h reaction period. The title compound was extracted with ethyl acetate and water washings given and dried over anhydrous sodium sulphate. This crude compound was purified by silica gel column chromatography and the required product was eluted as an off white solid in hexane, ethyl acetate (20: 80, v/v) solvent mixture.

Ethyl 1-ethyl-6-fluoro-7-(4-hexylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate, 8a: The crude compound was purify employing silica gel-

based column chromatography and the desired product was eluted in hexane, ethyl acetate mixture (20: 80, v/v) as an off white solid in 63% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 8.41(s, 1H, Ar-H), 8.10(d, *J* = 13.27 Hz, 1H, Ar-H), 6.74 (d, *J* = 6.40 Hz, 1H, Ar-H), 4.37-4.41 (m, 2H, N-CH_2), 4.19-4.20 (m, 2H, OCH_2), 3.27 (broad-s, 4H, $_2$HC-N-CH_2), 2.66 (broad-s, 4H, $_2$HC-N-CH_2), 2.41-2.44 (t, *J* = 7.47Hz, 2H, CH_2), 1.52-1.54 (m, 5H, CH_2, CH_3), 1.39-1.42 (t, *J* = 7.17Hz, 3H, CH_3), 1.31-1.34 (m, 6H, $(CH_2)_3$), 0.88-0.1 (t, *J* = 6.56Hz, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 173.0, 165.8, 154.1, 147.9, 144.7, 136.0, 123.7, 113.6, 110.4, 103.6, 60.7, 58.6, 52.8, 49.9, 48.9, 31.7, 27.1, 26.6, 22.5, 14.3, 14.3, 14.00; IR ($CHCl_3$ ν_{max} cm^{-1}): 3692, 3423, 2925, 2857, 1742, 1626, 1479, 1444, 1360, 1256, 753; HRMS (ESI) *m/z* [M+H$^+$]: calc for $C_{24}H_{35}O_3N_3F$ is 432.26570 found 432.26466 ($C_{24}H_{35}O_3N_3F$).

Ethyl 1-ethyl-6-fluoro-7-(4-heptylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate, 8b: The crude compound was purified by silica gel-based column chromatography and the desired product was eluted in a solvent mixture of hexane and ethyl acetate (20: 80, v/v) as an off white solid with 65% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 8.42 (s, 1H, Ar-H), 8.10 (d, *J* = 13.42 Hz, 1H, Ar-H), 6.75 (d, *J* = 6.71 Hz, 1H, Ar-H), 4.37-4.41 (m, 2H, N-CH_2), 4.17-4.22 (m, 2H, OCH_2), 3.32 (broad-s, 4H, $_2$HC-N-CH_2), 2.73 (broad-s, 4H, $_2$HC-N-CH_2), 2.47-2.49 (t, *J* = 6.56Hz, 2H, CH_2), 1.52-1.60 (m, 5H, CH_2, CH_3), 1.39-1.42 (t, *J* = 7.17Hz, 3H, CH_3), 1.25-1.34 (m, 8H, $(CH_2)_4$), 0.87-0.90 (t, *J* = 6.86Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 172.9, 165.4, 154.1, 147.7, 144.4, 135.8, 123.4, 113.3, 110.0, 103.7, 60.5, 58.4, 52.6, 49.5, 48.9, 31.6, 29.0, 27.2, 26.3, 22.4, 14.3, 14.2, 13.9; IR ($CHCl_3$ ν_{max} cm^{-1}): 3423, 2925, 2857, 1742, 1615, 1479, 1444, 1360, 1256, 753; HRMS (ESI) *m/z* [M+H$^+$]: calc for $C_{23}H_{38}O_3N_3FNa$ is 446.27894 found 446.28041 ($C_{23}H_{38}O_3N_3FNa$).

Ethyl 1-ethyl-6-fluoro-7-(4-nonylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3- carboxylate, 8c: The crude compound was purified by silica gel-based column chromatography and the required product (off white solid) was eluted from the column using a solvent mixture (Hexane: EtOAc, 20: 80, v/v) with 68% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 8.43 (s, 1H, Ar-H), 8.03 (d, *J* = 12.66 Hz, 1H, Ar-H), 6.83 (d, *J* = 6.56 Hz, 1H, Ar-H), 4.36-4.40 (m, 2H, N-CH_2), 4.19-4.23(m, 2H, OCH_2), 3.66 (broad-s, 4H, $_2$HC-N-CH_2), 3.33 (broad-s, 4H, $_2$HC-N-CH_2), 2.99-3.02(t, *J* = 7.01Hz, 2H, CH_2), 1.89-1.92 (m, 5H, CH_2, CH_3), 1.53-1.56 (t, *J* = 7.17Hz, 3H, CH_3), 1.26-1.42 (m, 12H, $(CH_2)_6$), 0.87-0.90 (t, *J* = 6.71Hz, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 172.8, 164.9, 153.6, 151.6, 147.9, 142.9, 135.8, 124.3, 113.2, 110.1, 105.0, 60.6, 57.4, 51.5, 49.0, 46.8, 31.6, 29.2, 29.0, 28.9, 26.7, 23.7, 22.5, 14.4, 14.3, 13.9; IR ($CHCl_3$ ν_{max} cm^{-1}): 3424, 2925, 2857, 1742, 1615, 1479, 1444, 1360, 1215, 754; HRMS (ESI) *m/z* [M+H$^+$]: calc for $C_{27}H_{41}O_3N_3F$ is 474.31265 found 474.31150 ($C_{27}H_{41}O_3N_3F$).

Ethyl 7-(4-decylpiperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate, 8d: The crude compound was subjected to silica gel column chromatography and the targeted product (off white solid) was eluted in a hexane and ethyl acetate (20: 80, v/v) solvent mixture with 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.41 (s, 1H, Ar-H), 8.10 (d, *J* = 13.32 Hz, 1H, Ar-H), 6.74 (d, *J* = 5.25Hz, 1H, Ar-H), 4.41-4.36 (m, 2H, N-CH_2), 4.19-4.20 (m, 2H, OCH_2), 3.28 (broad-s, 4H, $_2$HC-N-CH_2), 2.68 (broad-s, 4H, $_2$HC-N-CH_2), 2.41-2.45 (t, *J* = 7.33Hz, 2H, CH_2), 1.51-1.53 (m, 5H, CH_2, CH_3), 1.39-1.42(t, *J* = 7.09Hz, 3H, CH_3), 1.27-1.32 (m, 14H, $(CH_2)_7$), 0.86-0.90 (t, *J* = 6.48Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 172.9, 165.7, 154.3, 147.8, 144.5, 135.9, 123.6, 113.5, 110.3, 103.7, 60.7, 58.5, 52.8, 49.7, 48.9, 31.8, 29.4, 29.2, 27.4, 26.6, 22.6, 14.3, 14.2, 14.0; IR ($CHCl_3$ ν_{max} cm^{-1}): 3423, 2924, 2857, 1742, 1615, 1479, 1444, 1360, 1255, 753; HRMS (ESI) *m/z* [M+H$^+$]: 488.32718.

Ethyl 7-(4-dodecylpiperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate, 8e: The crude compound purified using silica gel column chromatography and the targeted product was eluted in a solvent mixture (Hexane: EtOAc, 20: 80, v/v) as an off white solid with 70% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.41 (s, 1H, Ar-H), 8.10 (d, *J* = 13.32 Hz, 1H, Ar-H), 6.74 (d, *J* = 6.84Hz, 1H, Ar-H), 4.36-4.41 (m, 2H, N-CH_2), 4.17-4.22 (m, 2H, OCH_2), 3.28 (broad-s, 4H, $_2$HC-N-CH_2), 2.67 (broad-s, 4H, $_2$HC-N-CH_2), 2.40-2.44 (t, *J* = 7.45Hz, 2H, CH_2), 1.51-1.60 (m, 5H, CH_2, CH_3), 1.39-1.42 (t, *J* = 7.09Hz, 3H, CH_3), 1.26-1.32 (m, 18H, $(CH_2)_9$), 0.86-0.89 (t, *J* = 6.60Hz, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 172.9, 165.7, 154.0, 147.8,

144.7, 135.9, 123.6, 113.5, 110.3, 103.6, 60.7, 58.6, 52.8, 52.7, 49.8, 48.9, 31.8, 29.5, 29.2, 27.4, 26.6, 22.6, 14.3, 14.2, 14.0; IR ($CHCl_3$ ν_{max} cm^{-1}): 3019, 2914, 2851, 1743, 1614, 1475, 1444, 1361, 1214, 757; HRMS (ESI) *m/z* [M+H$^+$]: calc for $C_{30}H_{47}O_3N_3F$ is 516.35960 found 516.35866 ($C_{27}H_{41}O_3N_3F$).

Ethyl 1-ethyl-6-fluoro-4-oxo-7-(4-tetradecylpiperazin -1-yl)-1,4-dihydroquinoline-3 carboxylate, 8f: The crude compound was subjected to silica gel column chromatography and the required off white (solid) coloured product was eluted in a solvent mixture of hexane and ethyl acetate (20: 80, v/v) with 78% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.43(s, 1H, Ar-H), 8.04 (d, *J* = 12.83 Hz, 1H, Ar-H), 6.82 (d, *J* = 6.72Hz, 1H, Ar-H), 4.35-4.41 (m, 2H, N-CH_2), 4.18-4.23 (m, 2H, OCH_2), 3.66 (broad-s, 4H, $_2$HC-N-CH_2), 3.31 (broad-s, 4H, $_2$HC-N-CH_2), 2.97-3.01 (t, *J* = 6.96Hz, 2H, CH_2), 1.88-1.91 (m, 5H, CH_2, CH_3), 1.52-1.56 (t, *J* = 7.21Hz, 3H, CH_3), 1.26-1.42 (m, 22H, $(CH_2)_{11}$), 0.86-0.90 (t, *J* = 6.72Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 172.8, 165.0, 153.8, 147.9, 143.0, 135.8, 124.3, 113.4, 110.2, 104.9, 60.6, 57.5, 51.5, 49.0, 46.9, 31.8, 29.5, 29.2, 28.9, 26.7, 23.7, 22.5, 14.4, 14.3, 14.0; IR ($CHCl_3$ ν_{max} cm^{-1}): 3019, 2914, 2851, 1744, 1614, 1479, 1444, 1361, 1288, 771; HRMS (ESI) *m/z* [M+H$^+$]: calc for $C_{32}H_{51}O_3N_3F$ is 544.39090 found 544.39042 ($C_{32}H_{51}O_3N_3F$).

Ethyl 1-ethyl-6-fluoro-7-(4-hexadecylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate, 8g: The crude compound was subjected to silica gel column chromatography and the chosen product (off white solid) was obtained in a hexane, ethyl acetate (20: 80, v/v) solvent mixture in 73% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.41 (s, 1H, Ar-H), 8.10 (d, *J* = 13.32 Hz, 1H, Ar-H), 6.74 (d, *J* = 6.84Hz, 1H, Ar-H), 4.36-4.41 (m, 2H, N-CH_2), 4.16-4.21 (m, 2H, OCH_2), 3.27 (broad-s, 4H, $_2$HC-N-CH_2), 2.66 (broad-s, 4H, $_2$HC-N-CH_2), 2.40-2.44 (t, *J* = 7.45Hz, 2H, CH_2), 1.51-1.59 (m, 5H, CH_2, CH_3), 1.38-1.42 (t, *J* = 7.09Hz, 3H, CH_3), 1.26-1.31 (m, 26H, $(CH_2)_{13}$), 0.85-0.89 (t, *J* = 6.60Hz, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 172.9, 165.3, 153.9, 147.8, 144.1, 135.9, 123.7, 113.4, 110.1, 104.1, 60.6, 58.2, 52.4, 48.9, 31.8, 29.5, 29.4, 29.3, 29.2, 27.2, 25.6, 22.5, 14.3, 14.3, 14.0; IR ($CHCl_3$ ν_{max} cm^{-1}): 3424, 2913, 2850, 1743, 1614, 1479, 1443, 1380, 1219, 772; HRMS (ESI) *m/z* [M+H$^+$]: calc for $C_{34}H_{55}O_3N_3F$ is 572.42220 found 572.42177 ($C_{34}H_{55}O_3N_3F$).

Ethyl 1-ethyl-6-fluoro-7-(4-octadecylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate, 8h: Silica gel column chromatography of crude compound was performed and the aimed product was eluted in a hexane and ethyl acetate solvent mixture (20: 80, v/v) as an off white solid (60% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.42 (s, 1H, Ar-H), 8.10 (d, *J* = 13.32 Hz, 1H, Ar-H), 6.75 (d, *J* = 6.72 Hz, 1H, Ar-H), 4.36-4.41 (m, 2H, N-CH_2), 4.17-4.22 (m, 2H, OCH_2), 3.33 (broad-s, 4H, $_2$HC-N-CH_2), 2.74 (broad-s, 4H, $_2$HC-N-CH_2), 2.48-2.51 (t, *J* = 6.72Hz, 2H, CH_2), 1.51-1.60 (m, 5H, CH_2, CH_3), 1.39-1.42 (t, *J* = 7.09Hz, 3H, CH_3), 1.25-1.32 (m, 30H, $(CH_2)_{15}$), 0.86-0.89 (t, *J* = 6.60Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 173.0, 165.6, 154.3, 147.8, 144.5, 135.9, 123.7, 113.5, 110.2, 103.8, 60.7, 58.4, 52.7, 49.5, 48.9, 31.8, 29.6, 29.2, 27.3, 26.3, 22.60, 14.3, 14.3, 14.0; IR ($CHCl_3$ ν_{max} cm^{-1}): 3442, 2913, 2850, 1744, 1614, 1479, 1443, 1380, 1219, 772; HRMS (ESI) *m/z* [M+H$^+$]: calc for $C_{36}H_{59}O_3N_3F$ is 600.45350 found 600.45363 ($C_{36}H_{59}O_3N_3F$).

Antibacterial and antifungal assays

The antibacterial and antifungal activities of the synthesized compounds were determined using well diffusion method[32] against different pathogenic bacterial strains such as *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS-16 MTCC 2940, *Bacillus subtilis* MTCC 121, *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 2453 and *Klebsiella planticola* MTCC 530 along with different *Candida* strain such as *Candida albicans* MTCC 3017. All these bacterial and fungal strains were procured from the Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India. The pathogenic reference strains were seeded on the surface of the media Petri plates, containing Muller-Hinton agar with 0.1 mL of previously prepared microbial suspensions individually containing 1.5×10^8 cfu ml^{-1} (equal to 0.5 McFarland standard). Wells of 6.0 mm diameter were prepared in the media plates using a cork borer and the synthesized compounds dissolved in 10% DMSO at a dose range of 125 - 0.97 µg were added in each well under sterile conditions in a laminar air flow chamber. Standard antibiotic solutions of ciprofloxacin (bacterial strains) and miconazole (*Candida* strains) at a dose range of 125 - 0.97 µg $well^{-1}$, served as positive controls, while the

well containing DMSO served as negative control. The plates were incubated for 24 h at 30°C and the well containing the least concentration showing the inhibition zone is considered as the minimum inhibitory concentration. All experiments were carried out in duplicates and mean values are represented.

Minimum bactericidal concentration

Minimum bactericidal concentration assay (NCCLS, 2000) were performed in sterile 2.0 mL microfuge tubes against *Bacillus subtilis* MTCC 121 which were procured from the Microbial Type Culture Collection (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India. The pathogenic *Bacillus subtilis* strain was cultured overnight in Mueller Hinton broth. Serial dilutions of synthesized compounds were prepared in Mueller Hinton broth with different concentrations ranging from 0 to150 µg/mL. To the serially diluted the synthesized derivatives, 100 µL of overnight cultured bacterial suspension was added to reach a final concentration of 1.5×10^8 cfu ml^{-1} (equal to 0.5 McFarland) and incubated at 37ºC for 24 h. After 24 h of incubation, the minimum bactericidal concentration (MBC) was determined by sampling 10 µL of suspension from the tubes onto Mueller Hinton agar plates and were incubated for 24 h at 37ºC to observe the growth of test organisms. MBC is the lowest concentration of synthesized derivatives required to kill a particular bacterium. All the experiments were carried out in duplicates.

Biofilm inhibition assay

The test compounds were screened in sterile 96 well polystyrene microtiter plates using the modified biofilm inhibition assay[34], against a panel of pathogenic bacterial strains including *Staphylococcus aureus* MTCC 196, *Staphylococcus aureus* MLS-16 MTCC 2940, *Micrococcus luteus* MTCC 2470 and *Klebsiella planticola* MTCC 530, which were cultured overnight in tryptone soy broth (supplemented with 0.5% glucose). The test compounds of predetermined concentrations ranging from 0 to 250 µg/mL were mixed with the bacterial suspensions having an initial inoculum concentration of 5×10^5 CFU/mL. Aliquots of 100 µL were distributed in each well and then incubated at 37ºC for 24 h under static conditions. The medium was then discarded and washed with phosphate buffered saline to remove the non-adherent bacteria. Each well of the microtiter plate was stained with 100 µL of 0.1% crystal violet solution followed by 30 min incubation at room temperature. Later the crystal violet solution from the plates was discarded, thoroughly washed with distilled water for 3 to 4 times and air dried at room temperature. The crystal violet stained biofilm was solubilised in 95% ethanol (100 µL) and the absorbance was recorded at 540 nm using TRIAD multimode reader (Dynex Technologies, Inc, Chantilly, VA, USA). Blank wells were employed as background check. The inhibition data were interpreted from the dose-response curves, where IC_{50} value is defined as the concentration of inhibitor required to inhibit 50% of biofilm formation under the above assay conditions. All the experiments were carried out in triplicates and the values are indicated as mean ± S.D.

Conclusions

In conclusion, in the present study a series of ethyl 1-ethyl-6-fluoro-7-(4-alkylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate derivatives were synthesized. All the newly prepared compounds were evaluated for their antimicrobial activities. Most of the compounds showed significant antimicrobial activities. Compound **8a** having hexyl alkyl chain not only exhibited potent antimicrobial activity against the selective strains but also it was proved to be potent for antifungal activity. Compounds **8b** (heptyl chain derivative) and **8c** (nonyl chain derivative) also showed promising antimicrobial activities. These kinds of compounds would represent a promising class of antimicrobial agents that deserves further investigation and derivatization.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

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