Synthesis and evaluation of 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(2-(4-substitutedpiperazin -1-yl)acetyl)piperazin-1-yl) quinoline-3-carboxylic acid derivatives as anti-tu...

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Synthesis and evaluation of 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(2-(4-substitutedpiperazin-1-yl)acetyl)piperazin-1-yl) quinoline-3-carboxylic acid derivatives as anti-tubercular and antibacterial agents

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ABSTRACT

A series of twenty two novel 1-cyclopropyl-6-fluoro-4-oxo-7-(4-substitutedpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid analogues were synthesized, characterized (^{1}H NMR, ^{13}C NMR and LCMS) and screened for their *in vitro* anti-tubercular and antibacterial activity. Many of these compounds exhibited MIC values in the range 7.32–136.10 μM against *Mycobacterium tuberculosis* H_{37}Rv. Eight compounds were further subjected to cytotoxic studies. Furthermore, the title compounds were screened for antibacterial activity against *Staphylococcus aureus* ATCC 29213 (gram positive) and *Escherichia coli* ATCC 25922 (gram negative) bacteria. Many of these compounds exhibited MIC values in the range 0.44–34.02 μM. Compound **3f** was found to be the most active with an MIC of 0.44 and 0.8 μM respectively against both the strains. In general, the antibacterial activity of title compounds was more prominent.

1. Introduction

Tuberculosis (TB) is the foremost airborne contagious disease caused by *Mycobacterium tuberculosis* (MTB). TB is the second leading cause of death from an infectious disease worldwide, after the human immunodeficiency virus (HIV). TB is known to be one of the major causes of death in HIV patients. According to the World Health Organization (WHO), 8.7 million people were infected with TB in 2011 resulting in the death of 1.4 million people. Furthermore, about 0.43 million deaths due to TB were reported amongst people affected HIV. In 2011 almost 60% of Multidrug Resistant-TB (MDR-TB) cases were reported from India, China and Russian federation only. The MTB commonly attacks the lungs, kidney, spine, and brain. Hence, if TB is not treated properly, it can be serious and fatal [1].

Presently, TB treatment using DOTS (directly observed therapy short-course) requires a combination of three to four drugs, which includes isoniazid, rifampin, pyrazinamide, and ethambutol over a period of 6–12 months. The long treatment schedule for TB is due to the presence of a non-replicating persistent MTB phenotype [2] and hence novel drugs are urgently required for shortening the treatment period and to target MDR-TB.

Quinolones possess exceptional antibacterial activity against MTB due to their excellent oral bioavailability and an ability to penetrate the macrophages [3–6]. Among the quinolone core antibacterial agents, fluoroquinolones (FQ) are an imperative class of compounds which possess remarkable biological activities such as antibacterial, antitubercular, antitumour, nosocomial infections of the respiratory, gastrointestinal infections, urinary tract infections, skin and soft tissue infections, chronic osteomyelitis, and sexually transmitted diseases [3,7–11].

FQs have divulged victory against gram-negative bacteria, but nevertheless they are resistant to gram-positive pathogens, such as *Staphylococcus aureus* [12]. However, some associated side effects such as CNS, phototoxicity, and arthropathy are still inevitable [13]. Rest of the molecule tailored to piperazine nucleus at C-7 position

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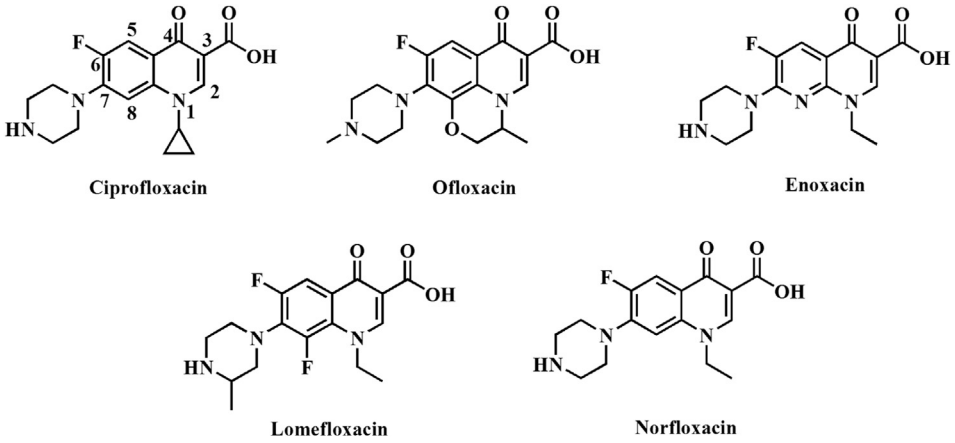


Fig. 1. Structures of second line anti-tubercular fluoroquinolone agents.

of FQs is the domain to enhance the drug–enzyme interaction [14–16]. Hence, there is a need for the development of novel molecules to treat efficiently the resistant forms of this bacterial pathogen.

FQs like ciprofloxacin (CP), ofloxacin, enoxacin, lomefloxacin, and norfloxacin are used as second line drugs to treat TB (Fig. 1). FQs are known to be active against isoniazid and rifampin resistant MTB [3]. These FQs are highly concentrated in the host cells and enhance their antimycobacterial action [17]. FQs target and inhibit the action of bacterial type II topoisomerase, deoxyribonucleic acid (DNA) gyrase, and topoisomerase IV. These enzymes are found to be vital for DNA supercoiling [18,19].

According to structure activity relationship (SAR) studies of FQ antibacterial agents, substituents at the C-7 position are crucial and attribute to the physicochemical properties of FQs exhibiting antibacterial activity, bioavailability and safety [20–22]. Carboxylic acid group at C-3 position and keto group at C-4 position are essential for hydrogen bonding interactions with DNA bases [23]. Mycobacteria have lipid rich cell walls, and lipophilicity is an imperative consideration in the design and activity of novel molecules [24]. In FQs substitution at C-7 affects the pharmacokinetic profile and the spectrum of activity [25]. Hence assimilation of the right substituent on the 4th position of piperazine at C-7 position of FQ creates interest in exploring molecular diversity to synthesize therapeutically important framework thereby anticipating greater lipophilicity. Based on this concept, several FQ derivatives have been reported and evaluated for their anti-tubercular and antimicrobial [26–30]. This avenue encouraged us further to explore the substituted piperazine CP derivatives anticipating good activity against this infectious bacterial disease.

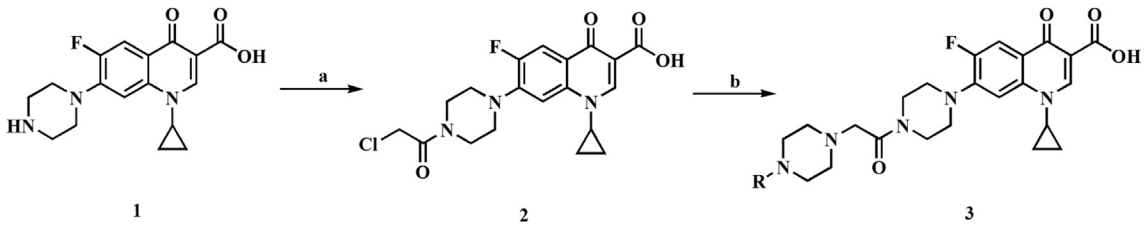
2. Chemistry

The synthetic strategy adopted to obtain the title compounds is depicted in Scheme 1. Acylation of CP (**1**) with chloroacetyl chloride was carried out as reported earlier [31]. A series of 1-cyclopropyl-6-fluoro-4-oxo-7-(4-substitutedpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid derivatives **3a**–**v** of CP derivatives were prepared by coupling commercially available substituted piperazines with **2** in *N*,*N*-dimethylformamide. In general ^{1}H NMR of all the title compounds displayed two triplets in the range 1.10–1.37 ppm and a multiplet in the range 3.75–3.90 ppm corresponding to the protons of cyclopropyl ring. Two multiplets of piperazine protons resonated in the range 3.30–3.70 ppm. Two sharp doublets resonated in the range 7.20–7.90 ppm due to C-5 and C-8 protons of the FQ moiety. The C-2 protons of FQ showed a sharp singlet in the range 8.63–8.71 ppm. A broad peak due to the proton of carboxylic acid functional group resonated in the range 15.15–15.25 ppm. The acetyl link protons showed multiplet in the range 3.29–3.39 and second piperazine protons resonated in the range of 2.20–2.60. Further, the structure of the title compounds was substantiated from ^{13}C NMR and ESI-MS respectively. All the compounds were evaluated for their antimycobacterial and antimicrobial activity and the results are summarized in Table 1.

3. Results and discussion

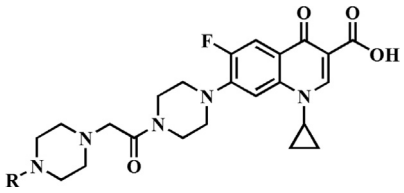
3.1. Antimycobacterial activity

The compounds, **3a**–**v**, were tested for anti-tubercular activity against MTB H_{37}Rv strain. The active compounds exhibited MIC in the range 7.32–136.10 μM. Compounds **3c**, **3d** and **3f** were the most active compounds with MIC 16.05, 7.32 and 28.25 μM respectively (Table 1). The SAR study revealed that when 'R' is phenyl (**3e**) the compound was found to be inactive. Introducing electron withdrawing and releasing groups at various positions on phenyl ring exhibited moderate anti-tubercular activity. Eventually, introduction of nitrogen atom in the phenyl ring (**3o** and **3p**) didn't alter the activity spectrum. Replacing 'R' by aliphatic chain with/without



Scheme 1. Synthesis of 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(2-(4-substitutedpiperazin-1-yl)acetyl)piperazin-1-yl)quinoline-3-carboxylic acid derivatives (**3a**–**v**). Reagents and conditions: (a) Et_3N, $ClCH_2COCl$, CH_2Cl_2, 0 °C–RT; (b) Et_3N, KI, substituted piperazines, 125 °C.

Table 1
In vitro antimycobacterial and antibacterial activity of CP derivatives **3a**–**v**.



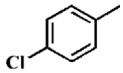
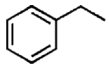
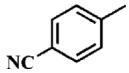
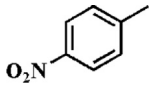
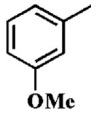
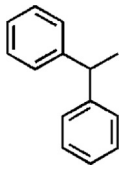
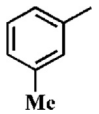
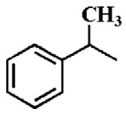
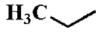
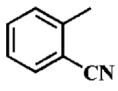
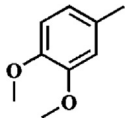
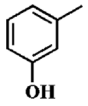
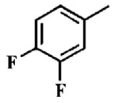
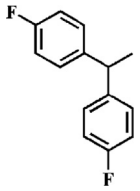
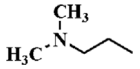
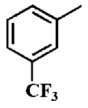
Entry	R	Yield (%)	*clog P*[a]	MIC (μM)		
				M. tuberculosis $H_{37}Rv$	*S. aureus* ATCC 29213	*E. coli* ATCC 25922
3a		57	−1.20	136.10	34.02	34.02
3b		68	2.54	113.03	1.76	7.06
3c		88	−0.81	16.05	4.01	16.05
3d		89	1.95	7.32	1.83	1.83
3e		88	1.66	>120.24	0.93 (7.51)[b]	1.87 (7.51)[b]
3f		95	2.54	28.25	0.44 (0.88)[b]	0.88 (7.06)[b]
3g		68	1.49	>114.85	7.17	7.17
3h		92	1.77	>110.87	6.92	13.85
3i		87	1.61	>113.82	0.88	3.55
3j		82	3.30	25.71	0.80	12.85
3k		65	2.09	29.29	1.83	7.32
3l		94	2.19	28.55	1.78	3.56
3m		94	−0.67	33.04	16.52	16.52
3n		63	1.49	>114.85	0.89	3.58

Table 1 (*continued*)

Entry	R	Yield (%)	*c*log *P*[a]	MIC (μM)		
				M. tuberculosis $H_{37}Rv$	*S. aureus* ATCC 29213	*E. coli* ATCC 25922
3o		81	0.71	>120.02	1.87	3.75
3p		91	−0.05	59.89	1.87	3.74
3q		69	1.27	>108.06	1.68	6.75
3r		79	0.02	>116.73	1.82	3.64
3s		72	2.10	>112.63	>225.26	>225.26
3t		66	3.58	>97.22	1.51	>24.30
3u		75	−0.82	121.37	7.58	7.58
3v		56	2.84	>106.62	0.83	6.66
Ciprofloxacin			−0.72	ND	0.37	0.021
Rifampicin			ND	0.14	ND	ND

ND: not determined.
[a] *c*log *P* was calculated by software (Chem Draw Ultra 10.0).
[b] MBC minimum bactericidal concentration was determined for selective compounds.

nitrogen atom (**3a**, **3m**, and **3u**) was found to be critical. Interestingly, immediate branching at α-position of the aliphatic chain (**3c**, MIC 16.05 μM) enhanced the activity by two fold as compared to **3m**. The enhanced activity of **3c** might be attributed to the presence of carbonyl functional group. Also, immediate branching at α-position of aromatic ring (**3j**, **3l**, and **3t**) showed moderate activity except **3d**, which exhibited excellent activity amongst the series with MIC 7.32 μM. These encouraging results further pave the way to explore different substituents on the benzyl group.

3.2. Antibacterial activity

The title compounds exhibited good antibacterial activity against *S. aureus* ATCC 29213 (gram positive) and *E. coli* ATCC 25922 (gram negative) bacteria. The MICs for these compounds were observed in the range 0.44–34.02 μM. When 'R' is methyl group (**3a**, MIC = 34.02 μM) the compound showed moderate activity against gram positive and gram negative bacteria. Lengthening by one methylene group resulted in a two fold enhancement in the activity (**3m**, MIC = 16.52 μM). Inclusion of tertiary nitrogen atom further enhanced the activity by four fold (**3u**, MIC = 7.58 μM). Replacing 'R' with an acetyl group significantly enhanced the activity against gram positive bacteria (**3c**, MIC = 4.01 μM). 'R' being branched diaryl and aryl–alkyl methylene group or mono aryl methylene group displayed good activity (**3j**, **3l**, and **3t**, MIC = 0.80–1.78 μM). The presence of various electron withdrawing and releasing substituents on the phenyl ring, unfolds good activity. In particular, **3n** and **3v** bearing cyano and trifluoromethyl groups at the ortho and meta position, inhibited 99% growth of gram positive bacteria at 0.83–0.89 μM, whereas, **3i** and **3k** comprising methoxy and methyl group at meta position inhibited 99% growth of gram positive bacteria at 0.88–1.83 μM. Compound **3f** ('R' is 2-chlorophenyl) was found to be the most active with MIC 0.44 and 0.88 μM respectively towards *S. aureus*

ATCC 29213 and *E. coli* ATCC 25922 bacteria. Further, **3e** and **3f** were also bactericidal in nature (Table 1). However, in general the title compounds displayed good activity against *S. aureus* ATCC 29213 as compared to *E. coli* ATCC 25922 strain. All these derivatives were less active compared to the standard ciprofloxacin.

3.3. Cytotoxicity assay

Compounds with MIC $\leq$ 59.89 μM were further examined for their toxicity in mouse macrophage cell lines (RAW 264.7) at 50 μM concentration. The approximate IC_{50} values [32] and selectivity index (SI) are tabulated in Table 2. Among the eight compounds tested, two compounds **3c** and **3d** were found to have SI values 9.59 and 22 respectively. This indicates their effectiveness towards drug development for TB. These encouraging results unravelled the interest to consider lipophilicity as a vital platform to fetch CP derivatives as potential therapeutic agent.

3.4. DNA gyrase supercoiling assay

DNA gyrase is a bacterial type II DNA topoisomerase which controls the topological state of DNA [33]. The relaxed form of DNA is crucial for its replication and transcription. In order to catalyze the negative supercoiling of double-stranded circular DNA, the free energy of ATP hydrolysis is utilized [34]. Therefore, suppressing the role of DNA gyrase hampers the relaxation process of super coiled DNA, which ultimately causes bacterial cell death. The supercoiling activity studies were carried out at 20, 10, 7, and 5 μM concentrations of compound **3d** using inspiralis kit and the results are illustrated in Fig. 2. The activity results indicated the IC_{50} of compound **3d** to be 7 μM.

4. Conclusion

In conclusion, this work has revealed the synthesis, and *in vitro* antimycobacterial as well as antibacterial activity of the new CP derivatives. Amongst, the synthesized compounds, **3p** exhibited 99% inhibition of MTB $H_{37}Rv$ strain with MIC 59.89 μM. Compounds **3f**, **3j**–**m** were significantly active against MTB with MIC 25.71–33.04 μM. Compound **3c** and **3d** exhibited good activity with MIC 16.05 and 7.32 μM respectively. In general, antibacterial activity was found to be more prominent than anti-tubercular activity. Particularly, compound **3e** inhibit 99% of *S. aureus* ATCC 29213 with MIC 0.93 μM and minimum bactericidal concentration (MBC) 7.51 μM. Further, *E. coli* ATCC 25922 was inhibited at MIC 1.87 μM and MBC 7.51 μM respectively. However, the most active compound **3f** was found to inhibit *S. aureus* ATCC 29213 with MIC 0.44 μM and MBC 0.88 μM respectively. For *E. coli* ATCC 25922 it was found to inhibit with MIC 0.88 μM and MBC 7.06 μM. The anti-tubercular SAR profile suggests that tailoring benzyl and acetyl group by means of appropriate substituents or functional groups might provide an insight to obtain the lead compound. From the antibacterial SAR summary, it is evident that introducing suitable halogens, heteroatom or in combination at the ortho position of phenyl ring would provide rewarding information for further lead molecule development towards inhibiting existing drug resistant forms of bacterial pathogens.

Table 2
IC_{50} (μM) and selectivity index (SI) values of active compounds (**3c**,**d**, **3f**, **3j**–**m** and **3p**) against mouse macrophage cell lines (RAW 264.7).

Entry	Compound	MIC (μM) in MTB $H_{37}Rv$[a]	% Cell inhibition at 50 μM	IC_{50} (μM) approximation[b]	SI value[c]
1	**3c**	16.05	16.24	153.94	9.59
2	**3d**	7.32	15.51	161.18	22.01
3	**3f**	28.25	14.94	167.33	5.92
4	**3j**	25.71	25.88	96.59	3.75
5	**3k**	29.29	16.22	154.13	5.26
6	**3l**	28.55	16.92	147.75	5.17
7	**3m**	33.04	13.31	187.82	5.68
8	**3p**	59.89	11.00	227.27	3.79

[a] Minimum inhibitory concentration against $H_{37}Rv$ strain of *M. tuberculosis* (μM).
[b] Measurement of cytotoxicity in RAW 264.7 cells: 50% inhibitory concentrations (μM).
[c] Selectivity index (*in vitro*): IC_{50} in RAW 264.7 cells/MIC against *M. tuberculosis*.

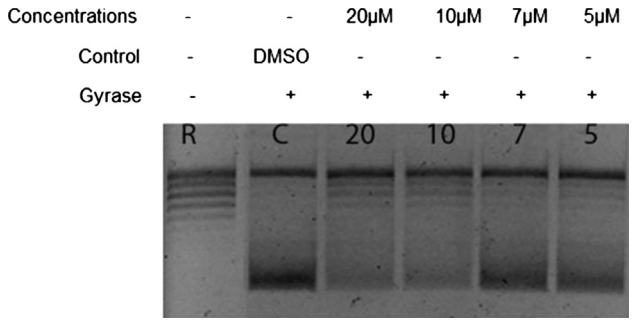


Fig. 2. Illustration of the supercoiling activity of compound **3d**. Lane 1: relaxed circular DNA (R), lane 2: supercoiling reaction (control-C) in presence of 4% DMSO, lane 3–6: reaction in the presence of 20, 10, 7, and 5 μM of compound **3d** respectively.

5. Experimental protocols

5.1. General

All reagents were purchased from commercial available sources and used without further purification. CP was purchased from Sigma Aldrich (>98%). All reactions were monitored by analytical Thin Layer Chromatography (TLC) preformed on E-Merck 0.25 mm pre-coated silica gel aluminium plates (60 F254) using mixture of dichloromethane and methanol. Visualization of the spots on TLC plates was achieved either by exposure to UV light (254 nm). Column chromatography was performed using silica gel (Acme, 100–200 mesh). Solvents were dried according to standard procedures prior to use. Melting points were obtained using Stuart SMP30 system and are uncorrected. Infrared (IR) spectra were recorded in KBr pellets on Shimadzu IR Prestige-21 FT-IR spectrophotometer (cm^{-1}). ^1H and ^{13}C NMR spectra were recorded on Avance 300 (300.132 MHz for ^1H, 75 MHz for ^{13}C), in $CDCl_3$ or DMSO-d_6. Chemical shifts have been expressed in parts per million (δ) relative to tetramethylsilane ($\delta = 0.0$) as an internal standard and coupling constants (J) in Hertz. Low-resolution mass spectra (ESI-MS) were recorded using LC/MS-2020 Shimadzu instrument.

5.2. Synthesis of 7-[4-(2-chloroacetyl) piperazin-1-yl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**2**)

The compound **2** was synthesized according to the literature protocol [31]. Mp: 262–263 °C (lit. mp: >260 °C [31]).

5.3. General procedure for the preparation of 1-cyclopropyl-6-fluoro-4-oxo-7-(4-substituted piperazin-1-yl)-1,4-dihydro quinoline-3-carboxylic acid derivatives of CP (**3a**–**v**)

To a solution of substituted piperazines (0.9819 mmol) in dry DMF (4 mL), triethylamine (0.27 mL, 1.9638 mmol) and potassium iodide (16.29 mg, 0.0981 mmol) were added at RT under N_2

atmosphere. Compound **2** (0.4 g, 0.9819 mmol) was added to the above reaction mixture and resultant mixture was heated at 125 °C. After the reaction was complete, as indicated by TLC, DMF was evaporated in vacuo. The obtained residue was diluted with 20 mL of water. The compound was extracted with CH_2Cl_2 (3 × 5 mL). The organic layers were collected, washed with saturated brine solution, dried over anhydrous $MgSO_4$ and concentrated in vacuo. The resultant crude was purified by column chromatography [CH_2Cl_2/MeOH (1–10%)] to get the title compounds.

*5.3.1. 1-Cyclopropyl-6-fluoro-7-[4-(2-{4-methylpiperazin-1-yl} acetyl)piperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3a**)*

M.p. 248–250 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.18 (t, 2H, J = 7.2 Hz), 1.29 (t, 2H, J = 6.9 Hz), 2.67 (m, 4H), 2.89 (m, 4H), 3.02 (s, 3H), 3.16 (m, 4H), 3.28 (m, 2H), 3.76 (m, 4H), 3.81 (tt, 1H, J = 7.2 Hz, J = 6.9 Hz), 7.52 (d, 1H, J_{H-F} = 7.5 Hz), 7.89 (d, 1H, J_{H-F} = 13.2 Hz), 8.63 (s, 1H), 15.18 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 53.67, 51.12, 50.89, 49.16, 45.91, 41.39, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{24}H_{30}FN_5O_4$ 471.23, found 472.39 $[M + H]^+$.

*5.3.2. 7-[4-(2-{4-(4-Chlorophenyl)piperazin-1-yl}acetyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3b**)*

M.p. 249–250 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (t, 2H, J = 7.2 Hz), 1.29 (t, 2H, J = 6.9 Hz), 2.67 (m, 4H), 2.98 (m, 4H), 3.26 (m, 4H), 3.32 (m, 2H), 3.67 (m, 4H), 3.79 (tt, 1H, J = 7.2 Hz, J = 6.9 Hz), 6.52 (d, 2H, J = 8.5 Hz), 7.10 (d, 2H, J = 5.8 Hz), 7.52 (d, 1H, J_{H-F} = 7.5 Hz), 7.89 (d, 1H, J_{H-F} = 13.2 Hz), 8.63 (s, 1H), 15.18 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.84, 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 132.57, 125.51, 119.91 (d, J_{C-F} = 8.1 Hz), 116.38, 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 53.89, 51.12, 50.46, 49.12, 45.84, 35.82, 8.43. ESI-MS (m/z): calcd. for $C_{29}H_{31}ClFN_5O_4$ 567.20, found 568.29 $[M + H]^+$.

*5.3.3. 7-[4-(2-{4-Acetylpiperazin-1-yl}acetyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3c**)*

M.p. 230–231 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.18 (t, 2H, J = 7.2 Hz), 1.29 (t, 2H, J = 6.9 Hz), 2.15 (s, 3H), 2.52 (m, 4H), 2.86 (m, 4H), 3.21 (m, 4H), 3.31 (m, 2H), 3.76 (m, 4H), 3.82 (tt, 1H, J = 7.2 Hz, J = 6.9 Hz), 7.52 (d, 1H, J_{H-F} = 7.5 Hz), 7.89 (d, 1H, J_{H-F} = 13.2 Hz), 8.63 (s, 1H), 15.18 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 168.39, 167.56, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 62.45, 52.83, 51.15, 48.86, 35.81, 25.14, 8.17. ESI-MS (m/z): calcd. for $C_{25}H_{30}FN_5O_5$ 499.22, found 500.42$[M + H]^+$.

*5.3.4. 7-[4-(2-{4-Benzylpiperazin-1-yl}acetyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3d**)*

M.p. 104–106 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.18 (t, 2H, J = 7.2 Hz), 1.29 (t, 2H, J = 6.9 Hz), 2.72 (m, 4H), 2.93 (m, 4H), 3.15 (s, 2H), 3.32 (m, 4H), 3.46 (m, 2H), 3.62 (m, 4H), 3.71 (tt, 1H, J = 7.2 Hz, J = 6.9 Hz), 7.30 (m, 5H), 7.52 (d, 1H, J_{H-F} = 7.5 Hz), 7.89 (d, 1H, J_{H-F} = 13.2 Hz), 8.63 (s, 1H), 15.18 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.02 (d, J_{C-F} = 2.2 Hz), 168.33, 153.60 (d, J_{C-F} = 249.3 Hz), 146.21, 145.49 (d, J_{C-F} = 10.3 Hz), 139.01, 127.12, 126.75, 126.13, 120.15 (d, J_{C-F} = 8.1 Hz), 112.51 (d, J_{C-F} = 24.14 Hz), 108.16, 105.08 (d, J_{C-F} = 3.7 Hz), 61.41, 54.31, 51.17, 51.48, 49.11, 45.07, 35.12, 8.65. ESI-MS (m/z): calcd. for $C_{30}H_{34}FN_5O_4$ 547.26, found 548.62 $[M + H]^+$.

*5.3.5. 1-Cyclopropyl-6-fluoro-4-oxo-7-[4-(2-{4-phenylpiperazin-1-yl}acetyl)piperazin-1-yl]-1,4-dihydroquinoline-3-carboxylic acid (**3e**)*

M.p. 212–214 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.18 (t, 2H, J = 7.2 Hz), 1.29 (t, 2H, J = 6.9 Hz), 2.62 (m, 4H), 2.91 (m, 4H), 3.28 (m, 4H), 3.44 (m, 2H), 3.68 (m, 4H), 3.81 (tt, 1H, J = 7.2 Hz, J = 6.9 Hz), 7.23 (m, 5H), 7.52 (d, 1H, J_{H-F} = 7.5 Hz), 7.89 (d, 1H, J_{H-F} = 13.2 Hz), 8.63 (s, 1H), 15.18 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.02 (d, J_{C-F} = 2.2 Hz), 168.33, 166.82, 153.60 (d, J_{C-F} = 249.3 Hz), 147.53, 145.49 (d, J_{C-F} = 10.3 Hz), 144.21, 139.01, 130.09, 128.41, 127.74, 120.15 (d, J_{C-F} = 8.1 Hz), 112.51 (d, J_{C-F} = 24.14 Hz), 108.16, 105.08 (d, J_{C-F} = 3.7 Hz), 54.65, 52.42, 51.17, 49.33, 45.40, 34.86, 8.18. ESI-MS (m/z): calcd. for $C_{29}H_{32}FN_5O_4$ 533.24, found 534.31 $[M + H]^+$.

*5.3.6. 7-[4-(2-{4-(2-Chlorophenyl)piperazin-1-yl}acetyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3f**)*

M.p. 138–139 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.18 (t, 2H, J = 7.2 Hz), 1.29 (t, 2H, J = 6.9 Hz), 2.56 (m, 4H), 2.82 (m, 4H), 3.18 (m, 4H), 3.37 (m, 2H), 3.72 (m, 4H), 3.76 (tt, 1H, J = 7.2 Hz, J = 6.9 Hz), 7.05 (t, 1H), 7.13 (d, 1H, J = 7.9 Hz), 7.55 (m, 2H), 7.52 (d, 1H, J_{H-F} = 7.5 Hz), 7.89 (d, 1H, J_{H-F} = 13.2 Hz), 8.63 (s, 1H), 15.18 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.02 (d, J_{C-F} = 2.2 Hz), 168.33, 166.82, 153.60 (d, J_{C-F} = 249.3 Hz), 147.53, 145.49 (d, J_{C-F} = 10.3 Hz), 143.84, 139.01, 130.95, 129.13, 128.64, 124.20, 122.26, 120.15 (d, J_{C-F} = 8.1 Hz), 112.51 (d, J_{C-F} = 24.14 Hz), 108.16, 105.08 (d, J_{C-F} = 3.7 Hz), 53.11, 51.75, 50.83, 48.96, 44.72, 35.42, 7.87. ESI-MS (m/z): calcd. for $C_{29}H_{31}ClFN_5O_4$ 567.20, found 568.35 $[M + H]^+$.

*5.3.7. 7-[4-(2-{4-(4-Cyanophenyl)piperazin-1-yl}acetyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3g**)*

M.p. 278–279 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.18 (t, 2H, J = 7.2 Hz), 1.29 (t, 2H, J = 6.9 Hz), 2.45 (m, 4H), 2.79 (m, 4H), 3.21 (m, 4H), 3.32 (m, 2H), 3.67 (m, 4H), 3.71 (tt, 1H, J = 7.2 Hz, J = 6.9 Hz), 6.65 (d, 2H, J = 8.5 Hz), 7.30 (d, 2H, J = 5.8 Hz), 7.52 (d, 1H, J_{H-F} = 7.5 Hz), 7.89 (d, 1H, J_{H-F} = 13.2 Hz), 8.63 (s, 1H), 15.18 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.84, 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 131.86, 126.93, 119.91 (d, J_{C-F} = 8.1 Hz), 117.12, 116.91, 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 53.43, 51.75, 50.22, 49.72, 45.89, 35.23, 7.13. ESI-MS (m/z): calcd. for $C_{30}H_{31}FN_6O_4$ 558.24, found 559.38 $[M + H]^+$.

*5.3.8. 1-Cyclopropyl-6-fluoro-7-[4-(2-{4-(4-nitrophenyl)piperazin-1-yl}acetyl)piperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3h**)*

M.p. 242–244 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.18 (t, 2H, J = 7.2 Hz), 1.29 (t, 2H, J = 6.9 Hz), 2.54 (m, 4H), 2.83 (m, 4H), 3.23 (m, 4H), 3.38 (m, 2H), 3.69 (m, 4H), 3.79 (tt, 1H, J = 7.2 Hz, J = 6.9 Hz), 6.70 (d, 2H, J = 8.5 Hz), 7.90 (d, 2H, J = 5.8 Hz), 7.52 (d, 1H, J_{H-F} = 7.5 Hz), 7.89 (d, 1H, J_{H-F} = 13.2 Hz), 8.63 (s, 1H), 15.18 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 148.42, 146.93, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 132.45, 128.41, 119.91 (d, J_{C-F} = 8.1 Hz), 117.12, 116.91, 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 53.39, 51.52, 50.79, 49.23, 35.85, 8.34. ESI-MS (m/z): calcd. for $C_{29}H_{31}FN_6O_6$ 578.23, found 579.32 $[M + H]^+$.

*5.3.9. 1-Cyclopropyl-6-fluoro-7-[4-(2-{4-(3-methoxyphenyl) piperazin-1-yl}acetyl)piperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3i**)*

M.p. 144–145 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.18 (t, 2H, J = 7.2 Hz), 1.29 (t, 2H, J = 6.9 Hz), 2.41 (m, 4H), 2.72 (m, 4H), 3.14 (m, 4H), 3.46 (m, 2H), 3.69 (m, 4H), 3.76 (s, 3H), 3.79 (tt, 1H, J = 7.2 Hz, J = 6.9 Hz), 6.42 (d, 1H, J = 8.2 Hz), 6.50 (s, 1H), 6.57 (d, 1H, J = 7.5 Hz), 7.15 (t, 1H), 7.52 (d, 1H, J_{H-F} = 7.5 Hz), 7.89 (d, 1H, J_{H-F} = 13.2 Hz), 8.63 (s, 1H), 15.18 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.02 (d, J_{C-F} = 2.2 Hz), 168.33, 166.82, 153.6 (d, J_{C-F} = 249.3 Hz), 146.27, 145.49 (d, J_{C-F} = 10.3 Hz), 144.56, 139.01, 131.29, 129.72, 129.13, 123.18, 120.15 (d, J_{C-F} = 8.1 Hz), 112.51 (d, J_{C-F} = 24.14 Hz), 110.3, 108.16, 105.08 (d, J_{C-F} = 3.7 Hz), 53.85, 52.11, 51.73, 48.17, 46.14, 44.72, 35.42, 8.18. ESI-MS (m/z): calcd. for $C_{30}H_{34}FN_5O_5$ 563.25, found 564.42 $[M + H]^+$.

*5.3.10. 7-[4-(2-{4-Benzhydrylpiperazin-1-yl}acetyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3j**)*

M.p. 199–200 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (t, 2H, J = 7.2 Hz), 1.32 (t, 2H, J = 6.9 Hz), 2.43 (m, 4H), 2.68 (m, 4H), 2.96 (s, 3H), 3.18 (m, 4H), 3.48 (m, 2H), 3.72 (m, 4H), 3.83 (tt, 1H, J = 7.2 Hz, J = 6.9 Hz), 4.12 (s, 1H), 7.25 (m, 10H), 7.52 (d, 1H, J_{H-F} = 7.5 Hz), 7.89 (d, 1H, J_{H-F} = 13.2 Hz), 8.63 (s, 1H), 15.18 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.02 (d, J_{C-F} = 2.2 Hz), 168.33, 166.82, 153.6 (d, J_{C-F} = 249.3 Hz), 147.53, 145.49 (d, J_{C-F} = 10.3 Hz), 142.60, 139.01, 128.52, 127.86, 127.00, 120.15 (d, J_{C-F} = 8.1 Hz), 112.51 (d, J_{C-F} = 24.14 Hz), 108.16, 105.08 (d, J_{C-F} = 3.7 Hz), 76.27, 53.35, 51.92, 50.65, 49.45, 45.48, 35.33, 8.26. ESI-MS (m/z): calcd. for $C_{36}H_{38}FN_5O_4$ 623.29, found 624.41 $[M + H]^+$.

*5.3.11. 1-Cyclopropyl-6-fluoro-4-oxo-7-[4-(2-{4-m-tolylpiperazin-1-yl}acetyl)piperazin-1-yl]-1,4-dihydroquinoline-3-carboxylic acid (**3k**)*

M.p. 158–160 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (t, 2H, J = 7.2 Hz), 1.29 (t, 2H, J = 6.9 Hz), 2.19 (s, 3H), 2.38 (m, 4H), 2.66 (m, 4H), 3.38 (m, 4H), 3.41 (m, 2H), 3.72 (m, 4H), 3.76 (tt, 1H, J = 7.2 Hz, J = 6.9 Hz), 6.55 (d, 1H, J = 7.9 Hz), 6.68 (d, 1H, J = 7.2 Hz), 6.71 (s, 1H), 7.04 (t, 1H), 7.42 (d, 1H, J_{H-F} = 7.5 Hz), 7.99 (d, 1H, J_{H-F} = 13.2 Hz), 8.73 (s, 1H), 15.18 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.02 (d, J_{C-F} = 2.2 Hz), 168.33, 166.82, 153.6 (d, J_{C-F} = 249.3 Hz), 146.03, 145.49 (d, J_{C-F} = 10.3 Hz), 141.48, 139.01, 129.50, 127.59, 127.11, 126.58, 122.34, 120.15 (d, J_{C-F} = 8.1 Hz), 112.51 (d, J_{C-F} = 24.14 Hz), 108.16, 105.08 (d, J_{C-F} = 3.7 Hz), 53.87, 51.12, 50.86, 49.43, 45.12, 35.39, 22.29, 8.18. ESI-MS (m/z): calcd. for $C_{30}H_{34}FN_5O_4$ 547.26, found 548.39 $[M + H]^+$.

*5.3.12. 1-Cyclopropyl-6-fluoro-4-oxo-7-[4-(2-{4-(1-phenylethyl) piperazin-1-yl}acetyl)piperazin-1-yl]-1,4-dihydroquinoline-3-carboxylic acid (**3l**)*

M.p. 212–214 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.18 (t, 2H, J = 7.2 Hz), 1.20 (d, 3H), 1.29 (t, 2H, J = 6.9 Hz), 2.39 (m, 4H), 2.52 (m, 4H), 3.06 (q, 1H), 3.31 (m, 4H), 3.38 (m, 2H), 3.64 (m, 4H), 3.78 (tt, 1H, J = 7.2 Hz, J = 6.9 Hz), 7.30 (m, 5H), 7.52 (d, 1H, J_{H-F} = 7.5 Hz), 7.89 (d, 1H, J_{H-F} = 13.2 Hz), 8.63 (s, 1H), 15.18 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.02 (d, J_{C-F} = 2.2 Hz), 168.33, 166.82, 153.60 (d, J_{C-F} = 249.3 Hz), 147.53, 145.49 (d, J_{C-F} = 10.3 Hz), 142.60, 139.01, 128.52, 127.86, 127.00, 120.15 (d, J_{C-F} = 8.1 Hz), 112.51 (d, J_{C--F} = 24.14 Hz), 108.16, 105.08 (d, J_{C-F} = 3.7 Hz), 72.21, 54.11, 52.19, 51.07, 48.69, 46.18, 34.04, 22.67, 8.13. ESI-MS (m/z): calcd. for $C_{31}H_{36}FN_5O_4$ 561.28, found 562.41 $[M + H]^+$.

*5.3.13. 1-Cyclopropyl-7-[4-(2-{4-ethylpiperazin-1-yl}acetyl) piperazin-1-yl]-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3m**)*

M.p. 239–240 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.08 (t, 3H), 1.18 (t, 2H, J = 7.2 Hz), 1.29 (t, 2H, J = 6.9 Hz), 2.90 (q, 2H), 2.36 (m, 4H), 2.48 (m, 4H), 3.32 (m, 4H), 3.52 (m, 2H), 3.67 (m, 4H), 3.79 (tt, 1H, J = 7.2 Hz, J = 6.9 Hz), 7.55 (d, 1H, J_{H-F} = 7.5 Hz), 7.89 (d, 1H, J_{H-F} = 13.2 Hz), 8.63 (s, 1H), 15.18 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 54.56, 52.23, 51.67, 49.71, 48.87, 44.76, 34.74, 14.87, 8.45. ESI-MS (m/z): calcd. for $C_{25}H_{32}FN_5O_4$ 485.24, found 486.37 $[M + H]^+$.

*5.3.14. 7-[4-(2-{4-(2-Cyanophenyl)piperazin-1-yl}acetyl) piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3n**)*

M.p. 222–223 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.18 (t, 2H, J = 7.2 Hz), 1.29 (t, 2H, J = 6.9 Hz), 2.62 (m, 4H), 3.14 (m, 4H), 3.34 (m, 4H), 3.66 (m, 2H), 3.77 (m, 4H), 3.82 (tt, 1H, J = 7.2 Hz, J = 6.9 Hz), 7.05 (t, 1H), 7.13 (d, 1H J = 8.5 Hz), 7.55 (m, 2H), 7.66 (d, 1H, J_{H-F} = 7.5 Hz), 7.92 (d, 1H, J_{H-F} = 13.2 Hz), 8.63 (s, 1H), 15.18 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 142.43, 138.95, 128.20, 127.43, 126.32, 122.78, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 108.23, 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 81.42, 53.59, 51.12, 50.58, 49.33, 45.06, 35.99, 8.54. ESI-MS (m/z): calcd. for $C_{30}H_{31}FN_6O_4$ 558.24, found 559.36 $[M + H]^+$.

*5.3.15. 1-Cyclopropyl-6-fluoro-4-oxo-7-[4-(2-{4-(pyridine-2-yl) piperazin-1-yl}acetyl) piperazin-1-yl]-1,4-dihydroquinoline-3-carboxylic acid (**3o**)*

M.p. 204–205 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.18 (t, 2H, J = 7.2 Hz), 1.29 (t, 2H, J = 6.9 Hz), 2.57 (m, 4H), 3.23 (m, 4H), 3.31 (m, 4H), 3.56 (m, 2H), 3.61 (m, 4H), 3.77 (tt, 1H, J = 7.2 Hz, J = 6.9 Hz), 7.16 (t, 1H), 7.21 (d, 1H, J = 8.2 Hz), 7.62 (m, 2H), 7.66 (d, 1H, J_{H-F} = 7.5 Hz), 7.92 (d, 1H, J_{H-F} = 13.2 Hz), 8.63 (s, 1H), 15.18 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 154.34, 153.21 (d, J_{C-F} = 249.3 Hz), 150.78, 148.43, 147.23, 146.07, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 121.56, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 53.78, 53.12, 50.96, 48.76, 45.78, 37.23, 8.45. ESI-MS (m/z): calcd. for $C_{28}H_{31}FN_6O_4$ 534.24, found 535.36 $[M + H]^+$.

*5.3.16. 1-Cyclopropyl-6-fluoro-4-oxo-7-[4-(2-{4-(pyrimidine-2-yl) piperazin-1-l}acetyl)piperazin-1-yl]-1,4-dihydroquinoline-3-carboxylic acid (**3p**)*

M.p. 189–190 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.18 (t, 2H, J = 7.2 Hz), 1.29 (t, 2H, J = 6.9 Hz), 2.46 (m, 4H), 2.58 (m, 4H), 3.05 (m, 4H), 3.34 (m, 2H), 3.67 (m, 4H), 3.77 (tt, 1H, J = 7.2 Hz, J = 6.9 Hz), 7.01(t, 1H), 7.55 (d, 1H, J_{H-F} = 7.5 Hz), 7.89 (d, 1H, J_{H-F} = 13.2 Hz), 8.31 (d, 2H, J = 4.6 Hz), 8.63 (s, 1H), 15.18 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 160.67, 154.89, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 122.45, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 54.12, 52.23, 51.08, 49.67, 44.97, 36.24, 8.14. ESI-MS (m/z): calcd. for $C_{27}H_{30}FN_7O_4$ 535.23, found 536.39 $[M + H]^+$.

*5.3.17. 1-Cyclopropyl-7-[4-(2-{4-(3,4-dimethoxyphenyl)piperazin-1-yl}acetyl)piperazin-1-yl]-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3q**)*

M.p. 189–190 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (t, 2H, J = 7.2 Hz), 1.29 (t, 2H, J = 6.9 Hz), 2.35 (m, 4H), 2.42 (m, 4H), 3.25

(m, 4H), 3.38 (m, 2H), 3.71 (m, 4H), 3.73 (s, 6H), 3.79 (tt, 1H, $J = 7.2$ Hz, $J = 6.9$ Hz), 6.25 (s, 1H), 6.56 (d, 1H, $J = 7.5$ Hz), 6.63 (d, 1H, $J = 7.9$ Hz), 7.55 (d, 1H, $J_{H-F} = 7.5$ Hz), 7.89 (d, 1H, $J_{H-F} = 13.2$ Hz), 8.63 (s, 1H), 15.18 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, $J_{C-F} = 2.2$ Hz), 167.56, 166.43, 153.21 (d, $J_{C-F} = 249.3$ Hz), 147.23, 145.12 (d, $J_{C-F} = 10.3$ Hz), 143.24, 140.67, 138.08, 138.95, 121.78, 116.02, 119.91 (d, $J_{C-F} = 8.1$ Hz), 117.32, 111.97 (d, $J_{C-F} = 24.14$ Hz), 107.12, 104.89 (d, $J_{C-F} = 3.7$ Hz), 61.45, 60.95, 59.34, 53.35, 51.92, 50.65, 49.45, 35.33, 8.26. ESI-MS (m/z): calcd. for $C_{31}H_{36}FN_5O_6$ 593.26, found 594.39 $[M + H]^+$.

5.3.18. 1-Cyclopropyl-6-fluoro-7-[4-(2-{4-(3-hydroxyphenyl) piperazin-1-yl}acetylpiperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3r**)

M.p. 220–222 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.18 (t, 2H, $J = 7.2$ Hz), 1.29 (t, 2H, $J = 6.9$ Hz), 2.32 (m, 4H), 2.43 (m, 4H), 3.34 (m, 4H), 3.46 (m, 2H), 3.67 (m, 4H), 3.81 (tt, 1H, $J = 7.2$ Hz, $J = 6.9$ Hz), 5.23 (s, 1H), 6.39 (d, 1H, $J = 8.5$ Hz), 6.45 (s, 1H), 6.52 (d, 1H, $J = 7.5$ Hz), 7.05 (t, 1H), 7.52 (d, 1H, $J_{H-F} = 7.5$ Hz), 7.89 (d, 1H, $J_{H-F} = 13.2$ Hz), 8.63 (s, 1H), 15.18 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, $J_{C-F} = 2.2$ Hz), 167.56, 166.43, 153.21 (d, $J_{C-F} = 249.3$ Hz), 147.23, 146.58, 145.12 (d, $J_{C-F} = 10.3$ Hz), 142.03, 138.95, 134.61, 119.91 (d, $J_{C-F} = 8.1$ Hz), 111.97 (d, $J_{C-F} = 24.14$ Hz), 107.28, 107.12, 106.07, 104.89 (d, $J_{C-F} = 3.7$ Hz), 101.72, 54.15, 50.91, 51.27, 49.81, 44.73, 35.99, 8.12. ESI-MS (m/z): calcd. for $C_{29}H_{32}FN_5O_5$ 549.24, found 550.45 $[M + H]^+$.

5.3.19. 1-Cyclopropyl-7-[4-(2-{4-(3,4-difluorophenyl)piperazin-1-yl}acetyl)piperazin-1-yl]-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3s**)

M.p. 198–200 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.24 (t, 2H, $J = 7.2$ Hz), 1.37 (t, 2H, $J = 6.9$ Hz), 2.32 (m, 4H), 2.56 (m, 4H), 3.35 (m, 4H), 3.39 (m, 2H), 3.79 (m, 4H), 3.85 (tt, 1H, $J = 7.2$ Hz, $J = 6.9$ Hz), 6.98 (dd, 1H, $J_{H-F} = 7.1$ Hz), 7.15 (dt, 1H, $J_{H-F} = 13.2$ Hz, 6.8 Hz), 7.25 (dd, 1H, $J_{H-F} = 12.11$ Hz, 7.01 Hz), 7.63 (d, 1H, $J_{H-F} = 7.5$ Hz), 7.97 (d, 1H, $J_{H-F} = 13.2$ Hz), 8.71 (s, 1H), 15.22 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, $J_{C-F} = 2.2$ Hz), 167.56, 166.43, 162.58 (dd, $J_{C-F} = 252.67$ Hz, 22.68 Hz), 153.21 (d, $J_{C-F} = 249.3$ Hz), 151.52 (dd, $J_{C-F} = 247.28$ Hz, 21.39 Hz), 147.23, 145.12 (d, $J_{C-F} = 10.3$ Hz), 141.49 (d, $J_{C-F} = 2.8$ Hz), 138.95, 125.23 (d, $J_{C-F} = 3.07$ Hz), 119.91 (d, $J_{C-F} = 8.1$ Hz), 111.97 (d, $J_{C-F} = 24.14$ Hz), 110.87 (dd, $J_{C-F} = 23.81$ Hz, 3.7 Hz), 109.42 (dd, $J_{C-F} = 20.65$ Hz, 2.95 Hz), 107.12, 104.89 (d, $J_{C-F} = 3.7$ Hz), 55.32, 51.49, 51.11, 49.72, 45.29, 35.37, 8.42. ESI-MS (m/z): calcd. for $C_{29}H_{30}F_3N_5O_4$ 569.22, found 570.49 $[M + H]^+$.

5.3.20. 7-[4-(2-{4-(Bis(4-fluoroophenyl)methyl)piperazin-1-yl} acetyl)piperazin-1-yl]-1-cyclo propyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3t**)

M.p. 195–196 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19 (t, 2H, $J = 7.2$ Hz), 1.32 (t, 2H, $J = 6.9$ Hz), 2.39 (m, 4H), 2.56 (m, 4H), 3.25 (m, 4H), 3.35 (m, 2H), 3.74 (m, 4H), 3.86 (tt, 1H, $J = 7.2$ Hz, $J = 6.9$ Hz), 4.20 (s, 1H), 7.13–7.21 (dd, 4H, $J_{H-F} = 8.5$ Hz), 7.42–7.50 (dd, 4H, $J_{H-F} = 5.8$ Hz, $J_{H-F} = 7.5$ Hz) 7.59–7.61 (d, 1H, $J_{H-F} = 7.5$ Hz), 7.94–7.99 (d, 1H, $J_{H-F} = 13.2$ Hz), 8.71 (s, 1H), 15.22 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.97 (d, $J_{C-F} = 2.2$ Hz), 168.23, 166.76, 161.83 (d, $J_{C-F} = 246.49$ Hz), 153.57 (d, $J_{C-F} = 251.52$ Hz), 147.50, 145.45 (d, = 10.3 Hz), 139.01, 138.07 (d, $J_{C-F} = 2.9$ Hz), 129.21 (d, $J_{C-F} = 7.3$ Hz), 120.08 (d, d, $J_{C-F} = 8.1$ Hz), 115.43 (d, $J_{C-F} = 21.27$ Hz), 112.46 (d, $J_{C-F} = 23.48$ Hz), 108.11, 105.05 (d, $J_{C-F} = 3.7$ Hz), 74.43, 53.31, 51.70, 50.61, 49.39, 45.45, 35.32, 8.25. ESI-MS (m/z): calcd. for $C_{36}H_{36}F_3N_5O_4$ 659.27, found 660.62 $[M + H]^+$.

5.3.21. 1-Cyclopropyl-7-[4-(2-{4-(2-(dimethylamino)ethyl) piperazin-1-yl}acetyl)piperazin-1-yl]-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3u**)

M.p. 138–140 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.24 (t, 2H, $J = 7.2$ Hz), 1.37 (t, 2H, $J = 6.9$ Hz), 2.42 (m, 4H), 2.59 (m, 4H), 2.70 (t, 4H), 2.83 (s, 6H), 3.35 (m, 4H), 3.39 (m, 2H), 3.76 (m, 4H), 3.89 (tt, 1H, $J = 7.2$ Hz, $J = 6.9$ Hz), 7.63 (d, 1H, $J_{H-F} = 7.5$ Hz), 7.97 (d, 1H, $J_{H-F} = 13.2$ Hz), 8.71 (s, 1H), 15.22 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, $J_{C-F} = 2.2$ Hz), 167.56, 166.43, 153.21 (d, $J_{C-F} = 249.3$ Hz), 147.23, 145.12 (d, $J_{C-F} = 10.3$ Hz), 138.95, 119.91 (d, $J_{C-F} = 8.1$ Hz), 111.97 (d, $J_{C-F} = 24.14$ Hz), 107.12, 104.89 (d, $J_{C-F} = 3.7$ Hz), 60.95, 53.53, 51.67, 50.22, 49.20, 48.63, 47.97, 41.11, 35.12, 8.09. ESI-MS (m/z): calcd. for $C_{27}H_{37}FN_6O_4$ 528.29, found 530.41 $[M + H]^+$.

5.3.22. 1-Cyclopropyl-6-fluoro-4-oxo-7-[4-(2-{4-(3-(trifluoromethyl)phenyl)piperazin-1-yl} acetyl)piperazin-1-yl]-1,4-dihydroquinoline-3-carboxylic acid (**3v**)

M.p. 190–192 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24 (t, 2H, $J = 7.2$ Hz), 1.37 (t, 2H, $J = 6.9$ Hz), 2.42 (m, 4H), 2.59 (m, 4H), 3.35 (m, 4H), 3.39 (m, 2H), 3.76 (m, 4H), 3.89 (tt, 1H, $J = 7.2$ Hz, $J = 6.9$ Hz), 7.14 (d, 1H, $J = 7.7$ Hz), 7.26 (s, 1H), 7.30 (d, 1H, $J = 8.3$ Hz), 7.48 (t, 1H, 8.03 Hz), 7.63 (d, 1H, $J_{H-F} = 7.5$ Hz), 7.97 (d, 1H, $J_{H-F} = 13.2$ Hz), 8.71 (s, 1H), 15.22 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, $J_{C-F} = 2.2$ Hz), 167.56, 166.43, 153.21 (d, $J_{C-F} = 249.3$ Hz), 148.38 (d, $J_{C-F} = 249.3$ Hz), 147.23, 145.12 (d, $J_{C-F} = 10.3$ Hz), 138.95, 132.94, 126.39, 124.62, 119.91 (d, $J_{C-F} = 8.1$ Hz), 119.19, 116.47, 112.72, 111.97 (d, $J_{C-F} = 24.14$ Hz), 107.12, 104.89 (d, $J_{C-F} = 3.7$ Hz), 54.11, 50.89, 50.16, 49.92, 45.14, 35.49, 8.08. ESI-MS (m/z): calcd. for $C_{30}H_{31}F_4N_5O_4$ 601.23, found 602.56 $[M + H]^+$.

5.4. Antimycobacterial assay

MIC was determined by broth dilution method against *M. tuberculosis* $H_{37}Rv$ (ATCC 27294; American Type Culture Collection, Manassas, VA, USA) using micro-broth dilution method [35,36]. The two fold serial dilutions of compounds were prepared in Middlebrook 7H9 broth (Difco Laboratories, Detroit, Mich.) supplemented with 0.5% (v/v) glycerol, 0.25% (v/v) Tween 80 (Himedia, Mumbai India), and 10% ADC (albumin dextrose catalase, Becton Dickinson, Sparks, MD) in 96-well U bottom microtitre plates (Tarson, Mumbai, India). The bacterial suspension was prepared from an actively growing bacterial culture and adjusted to 1 McFarland standard equivalent to 1.0×10^7 CFU/mL. The suspension was further diluted in the ratio 1:50 in the Middlebrook 7H9 media. Hundred microlitres of this diluted inoculum was added to each well of the plate resulting in the final inoculum of 1.0×10^6 CFU/mL in the well and the final concentrations of compounds ranged between 0.03 and 64 μg/mL. The plates were incubated at 37 °C for 3-weeks in 5% CO_2. The plates were read visually and the minimum concentration of the compound showing no turbidity was recorded as MIC.

5.5. Anti-bacterial assay

The antibacterial activity of the compounds was performed using microdilution method [37] against *S. aureus* ATCC 29213 (gram positive) and *E. coli* ATCC 25922 (gram negative). Bacterial suspensions were prepared in sterile normal saline from 24-h grown culture. The MIC was performed in Muller Hinton Broth (MHB; BD Biosciences, USA). Two-fold serial dilutions of the compounds were prepared in MHB in 100 μl volume in a 96 well U bottom microtitre plates (Tarson, Mumbai, India). The final concentrations of the compounds ranged between 0.06 and 128 μg/mL. The turbidity of bacterial suspensions was adjusted to 0.5 McFarland ($\sim 1.5 \times 10^8$ CFU/mL), which was further diluted in MHB and, a

100 μL volume of this diluted inoculum was added to each well of the plate, resulting in a final inoculum of 5×10^6 CFU/mL. The plates were incubated at 37 °C for 24 h and were read visually. The minimum concentration of the sample showing no turbidity was recorded as MIC. The MBC was also determined from the same microtitre plates after 24 h incubation. 20 μl of the suspension from the well showing MIC value and wells containing 2×, 4×, 8× and 16× concentration of MIC value was spotted onto the Muller Hinton Agar plate. The spotted plate was incubated for 24 h and the CFU count was taken simultaneously. The minimum concentration of the compound showing 3 log reduction in the inoculum size as compared to the original inoculum size was considered as the MBC.

5.6. Cytotoxicity assay

The most active compounds (**3c**,**d**, **3f**, **3j**–**m** and **3p**) were further examined for cytotoxicity in mouse macrophage cell lines (RAW 264.7) at 50 μM concentration. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 non-radioactive cell proliferation assay [38].

5.7. DNA gyrase supercoiling assay

Supercoiling assay was performed using inspiralis kit (Inspiralis Limited Norwich Bioincubator Norwich Research ParkColney Lane Norwich). The assay was done in 30 μL reaction volume for 30 min at 37 °C in an assay buffer containing 50 mM HEPES. KOH (pH 7.9), 6 mM magnesium acetate, 4 mM DTT, 1 mM ATP, 100 mM potassium glutamate, 2 mM spermidine and 0.05 mg/ml of albumin. During the assay 1 unit of DNA gyrase was incubated with 0.5 μg of relaxed pBR322 in the assay buffer for 30 min. DNA was prepared later for electrophoresis by addition of an equal volume of chloroform:isoamylalcohol (24:1), followed by brief vortexing, centrifugation and addition of 30 mL STEB (40% sucrose, 100 mM Tris–HCl (pH 8.0), 100 mM EDTA and 0.5 mg/ml bromophenol blue) to quench the reaction. The products were analysed by electrophoresis on 1% agarose gels and stained with ethidium bromide. Using Image lab software (Biorad) the intensity of bands were measured and analyzed to know the enzyme activity inhibition.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.10.055.

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