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# Synthesis and antimycobacterial evaluation of certain fluoroquinolone derivatives

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**Abstract**—A number of fluoroquinolone derivatives were synthesized and evaluated for antimycobacterial activity. Preliminary results are (1) for 1-aryl fluoroquinolones, 1-(4-nitrophenyl) derivatives were inactive while their 1-(2-fluoro-4-nitrophenyl) counterparts were active anti-TB agents (**3a** vs **4a**; **3b** vs **4b**) indicated the fluoro substituent at C-2 position is important. For the 1-(2-fluoro-4-nitrophenyl)quinolones, 7-piperidinyl derivative **4a** and 7-(3,5-dimethylpiperazinyl) derivative **4e**, which exhibited 97% and 98% inhibition, respectively, were more active than their 7-morpholinyl, 7-(4-methylpiperazinyl) and 7-piperazinyl congeners, **4b**,**4c** and **4d**, respectively. In addition, 7-[4-(8-hydroxyquinolin-2-ylmethyl)piperazin-1-yl] derivative **9d** exhibited 44% inhibition on the growth of *Mycobacterium tuberculosis* while its 7-(4-methylpiperazin-1-yl) counterpart **3c** was inactive implied the metal-chelating 8-hydroxyquinoline moiety was capable of enhancing the anti-TB activity, (2) for the bifunctional fluoroquinolone–hydroxyquinoline complexes, ciprofloxacin and ofloxacine derivatives, which exhibited the same anti-TB activity (98% inhibition), are more potent than norfloxacin counterpart, which in turn is more potent than 1-aryl congeners (**9b**, **9c** > **9a** > **9d**, **9e**).


## 1. Introduction

The first-line drugs currently used for the treatment of tuberculosis (TB), an infection of *Mycobacterium tuberculosis*, are streptomycin, isoniazid (INH), ethambutol, pyrazinamide and rifampicin.[1] However, TB is still a challenging worldwide health problem, especially the emergence of multi-drug resistant (MDR) strains of *M. tuberculosis*, which are insensitive to one or more of the first-line anti-TB drugs.[2–4] Furthermore, the association of TB and HIV infections has caused an urgent need in search of alternative chemotherapeutics for *M. tuberculosis* infection.[5–8] During the past decade, several of the fluoroquinolone antibacterial drugs have been examined as potential chemotherapeutics for *M. tuberculosis* infection because of their favorable pharmacokinetic profiles such as easily absorbed after oral administration and readily penetrated into mammalian cells.[9–14] However, the structural modification of the fluoroquinolones with respect to their optimum anti-TB activity has not been thoroughly explored.

Over the past few years, we were particularly interested in the synthesis and evaluation of fluoroquinolons for their antibacterial and anticancer activities.[15–20] Recently, we described the synthesis and antimycobacterial activity of certain fluoroquinolones with substituents at N-1 and C-7. Several compounds, such as 1-(4-amino-2-fluorophenyl)-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid **6d** is able to completely inhibit the growth of *M. tuberculosis* at a concentration of 6.25 μg/mL.[20] In the continuation of our search for more potent anti-TB agents, we report herein preparation and evaluation on certain isosteric isomers of **6d**. In addition, we have also prepared a new class of bifunctional fluoroquinolone–hydroxyquinoline derivatives in which the metal-chelating 8-hydroxyquinoline is linked to the 7-(piperazin-4-yl) moiety of fluoroquinolones

*Keywords*: Fluoroquinolone; Antimycobacterial activity; Metal-chelating agents; Fluoroquinolone–hydroxyquinoline complexes.
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through a methylene bridge. 8-Hydroxyquinoline was reported to possess a strong antimycobacterium activity, more potent than the antibiotic nitroxoline (5-nitro-8-hydroxyquinoline) against the growth of *Mycobacterium bovis* BCG.[21]

## 2. Chemistry

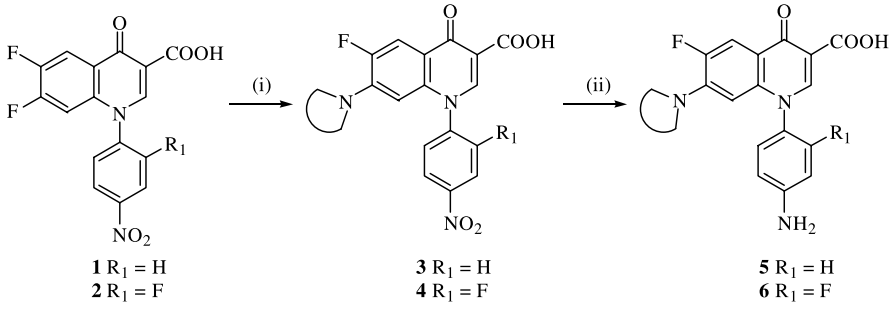
The preparation of 7-substituted 1-(4-aminophenyl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **5** and its 2′-fluoro derivative **6** is outlined in Scheme 1. Treatment of 6,7-difluoro-1-(4-nitrophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**1**) and its 2′-fluoro derivative **2** with piperidine in $CH_3CN$ afforded 6-fluoro-1-(4-nitrophenyl)-4-oxo-7-(piperidin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**3a**) and its 2′-fluoro derivative **4a**, respectively, which were reduced by catalytic hydrogenation on Pd/C to give their respective 1-(4-aminophenyl) counterpart **5a** and 1-(2-fluoro-4-aminophenyl) counterpart **6a**. Accordingly, 1-(4-aminophenyl)-6-fluoro-7-(morpholin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**5b**) and its 2′-fluoro derivative **6b** were obtained by catalytic hydrogenation of their respective 1-(4-nitrophenyl) precursor **3b** and 1-(2-fluoro-4-nitrophenyl) precursor **4b**, which in turn were obtained by the treatment of **1** and **2**, respectively, with morpholine. Reaction of **2** with 1-methylpiperazine and 2,6-dimethylpiperazine gave 6-fluoro-1-(2-fluoro-4-nitrophenyl)-7-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4c**) and its 7-(3,5-dimethylpiperazin-1-yl) congener **4e**, respectively. Synthesis of **4d** was described in our previous report.[20]

The preparation of bifunctional fluoroquinolone–hydroxyquinoline derivatives **9a–e** is described in Scheme 2. Treatment of **7**[22] with fluoroquinolone derivatives **8a–e** afforded desired products **9a–e** in 58–63% yield.

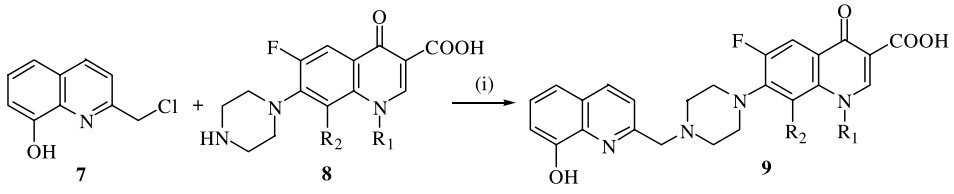
## 3. Pharmacological results and discussion

The anti-TB activity of 1-aryl fluoroquinolones is summarized in Table 1. With an exception of **4c**, 1-(4-nitrophenyl) derivatives were inactive while their 1-(2-fluoro-4-nitrophenyl) counterparts were active anti-TB agents (**3a** vs **4a**; **3b** vs **4b**) indicated the fluoro substituent at C-2 position is important. The same SAR has been observed for 1-(4-aminophenyl) derivatives and their 1-(4-amino-2-fluorophenyl) counterparts (**5a** vs **6a**; **5b** vs **6b**) in which **6a** was found to be a very potent inhibitor, being able to inhibit 95% growth of *M. tuberculosis* at a concentration of 6.25 μg/mL while **5a** was inactive. For the 1-(2-fluoro-4-nitrophenyl)quinolones, 7-piperidinyl derivative **4a** and 7-(3,5-dimethylpiperazinyl) derivative **4e**, which exhibited 97% and 98% inhibition, respectively, were more active than their 7-morpholinyl, 7-(4-methylpiperazinyl) and 7-piperazinyl congeners, **4b**,**4c** and **4d**, respectively. The same SAR has been observed for 1-(4-amino-2-fluorophenyl)quinolones in which 7-piperidinyl derivative **6a** exhibited 95% inhibition on the growth of *M. tuberculosis* while its 7-morpholinyl counterpart **6b** showed only marginal inhibitory activity.

The anti-TB activity of bifunctional fluoroquinolone–hydroxyquinoline complexes is depicted in Table 2. Ciprofloxacin and ofloxacine derivatives, which exhibited the same anti-TB activity (98% inhibition), are more potent than norfloxacin counterpart, which in turn is more potent than 1-aryl congeners (**9b**, **9c** > **9a** > **9d**, **9e**). For 1-aryl fluoroquinolones, 7-[4-(8-hydroxyquinolin-2-ylmethyl)piperazin-1-yl] derivative **9d** exhibited

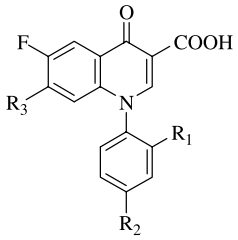


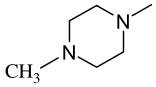
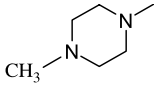
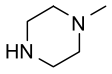
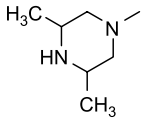
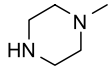

**Scheme 1.** Reagents and conditions: (i) cyclic amine, $Et_3N$, $CH_3CN$; (ii) Pd/C, $H_2$, DMF.




**Scheme 2.** Reagents and conditions: (i) $K_2CO_3$, DMF.

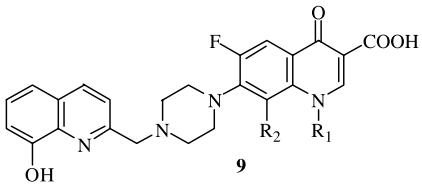
**Table 1.** Anti-TB activity of 1-aryl fluoroquinolone derivatives at 6.25 μg/mL



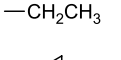
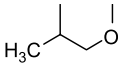
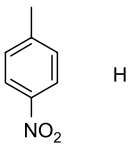
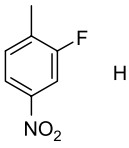
| Compound | $R_1$ | $R_2$ | $R_3$ | Inhibition (%) |
|---|---|---|---|---|
| **3a** | H | $NO_2$ |  | 6 |
| **3b** | H | $NO_2$ |  | 0 |
| **3c** | H | $NO_2$ |  | 0 |
| **4a** | F | $NO_2$ |  | 97 |
| **4b** | F | $NO_2$ |  | 49 |
| **4c** | F | $NO_2$ |  | 0 |
| **4d** | F | $NO_2$ |  | 37 |
| **4e** | F | $NO_2$ |  | 98 |
| **5a** | H | $NH_2$ |  | 0 |
| **5b** | H | $NH_2$ |  | 0 |
| **6a** | F | $NH_2$ |  | 95 |
| **6b** | F | $NH_2$ |  | 48 |
| **6d** | F | $NH_2$ |  | 100 |

44% inhibition on the growth of *M. tuberculosis* while its 7-(4-methylpiperazin-1-yl) counterpart **3c** was inactive implied the metal-chelating 8-hydroxyquinoline moiety was capable of enhancing the anti-TB activity. The same SAR was observed in which **9e** exhibited 47% inhibition on the growth of *M. tuberculosis* while **4c** was inactive.

**Table 2.** Anti-TB activity of bifunctional fluoroquinolone–hydroxyquinoline derivatives at 6.25 μg/mL[a]



**9**

| Compound | $R_1$ | $R_2$ | Inhibition (%) |
|---|---|---|---|
| **9a** |  | H | 86 |
| **9b** |  | H | 98 |
| **9c** |  | H | 98 |
| **9d** |  | H | 44 |
| **9e** |  | H | 47 |

[a] For ofloxacin, a range of MICs of 0.5–3.1 μg/mL was reported.[11]

## 4. Conclusion

For 1-aryl fluoroquinolones, most 1-(4-nitrophenyl) derivatives were inactive while their 1-(2-fluoro-4-nitrophenyl) counterparts were active anti-TB agents indicated the fluoro substituent at C-2 position is important. Among the 1-(2-fluoro-4-nitrophenyl)quinolones, 7-piperidinyl derivative **4a** and 7-(3,5-dimethylpiperazinyl) derivative **4e**, which exhibited 97% and 98% inhibition, respectively, are two of the best. For the bifunctional fluoroquinolone–hydroxyquinoline complexes, ciprofloxacin and ofloxacine derivatives are two of the most potent anti-TB agents, which exhibited 98% inhibition on the growth of *M. tuberculosis*.

## 5. Experimental

### 5.1. General

TLC: precoated (0.2 mm) silica gel 60 $F_{254}$ plates from EM Laboratories, Inc.; detection by UV light (254 nm). All chromatographic separations were performed using silica gel (Merck 60 230–400 mesh). Mp: Electrothermal IA9100 digital melting-point apparatus; uncorrected. $^1$H NMR spectra: Varian-Unity-400 spectrometer at 400 MHz or Varian-Gemini-200 spectrometer at 200 MHz, chemical shifts $\delta$ in ppm with $SiMe_4$ as an internal standard (= 0 ppm), coupling constants $J$ in hertz. Mass spectra (HRMS) were recorded on Finnigan/Thermo Quest MAT 95XL. Elemental analyses were carried out on a Heraeus CHN–O-Rapid elemental

analyzer and results were within ±0.4% of calculated values.

### 5.2. General procedure for coupling of 6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acids with appropriate cyclic amines

A mixture of 6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **1** or **2** (4 mmol), appropriate cyclic amine (20 mmol), $Et_3N$ (9 mL) and acetonitrile (100 mL) were refluxed for 6–12 h (monitored until the reactant disappeared by TLC). After cooling, the resulting precipitate was collected by filtration and washed with EtOH to give a crude residue, which was purified by flash column chromatography (FC, silica gel) and recrystallized.

**5.2.1. 6-Fluoro-1-(4-nitrophenyl)-4-oxo-7-(piperidin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (3a).** This compound was obtained from **1** and piperidine, which was purified by FC ($CH_2Cl_2$–MeOH = 10:1) and recrystallized from EtOH in 88% yield. Mp 320 °C (dec). $^1$H NMR (200 MHz, TFA): $\delta$ 1.88 (m, 2H, 7-piperidinyl-H), 2.13 (m, 4H, 7-piperidinyl-H), 3.95 (m, 4H, 7-piperidinyl-H), 7.97 (m, 3H, 8-, 2′-, 6′-H), 8.66 (m, 3H, 5-, 3′-, 5′-H), 9.36 (s, 1H, 2-H). Anal. Calcd for $C_{21}H_{18}FN_3O_5 \cdot 0.1H_2O$: C, 61.03; H, 4.49; N, 10.17. Found: C, 60.64; H, 4.61; N, 10.12.

**5.2.2. 6-Fluoro-7-(morpholin-4-yl)-1-(4-nitrophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3b).** This compound was obtained from **1** and morpholine, which was purified by FC ($CH_2Cl_2$–MeOH = 10:1) and recrystallized from EtOH in 82% yield. Mp: 319–320 °C. $^1$H NMR (400 MHz, TFA): $\delta$ 3.56 (m, 4H, 7-morpholinyl-H), 4.13 (m, 4H, 7-morpholinyl-H), 6.71 (d, 1H, $J$ = 6.0 Hz, 8-H), 7.95 (m, 2H, 2′-, 6′-H), 8.33 (d, 1H, $J$ = 12.8 Hz, 5-H), 8.71 (m, 2H, 3′-, 5′-H), 9.21 (s, 1H, 2-H). Anal. Calcd for $C_{20}H_{16}FN_3O_6 \cdot H_2O$: C, 55.68; H, 4.21; N, 9.74. Found: C, 56.05; H, 3.99; N, 9.34.

**5.2.3. 6-Fluoro-7-(4-methylpiperazin-1-yl)-1-(4-nitrophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3c).** This compound was obtained from **1** and 4-methylpiperazine, which was purified by FC ($CH_2Cl_2$–MeOH = 2:1) and recrystallized from EtOH in 72% yield. Mp: 297–298 °C. $^1$H NMR (400 MHz, TFA): $\delta$ 2.88 (s, 3H, N–Me), 3.03–3.26 (m, 4H, 7-piperazinyl-H), 3.50–3.68 (m, 4H, 7-piperazinyl-H), 6.54 (d, 1H, $J$ = 7.2 Hz, 8-H), 8.05–8.10 (m, 3H, 2′-, 5′-, 6′-H), 8.55 (m, 2H, 3′-, 5′-H), 8.77 (s, 1H, 2-H). Anal. Calcd for $C_{21}H_{19}FN_4O_5 \cdot 0.33H_2O$: C, 58.33; H, 4.58; N, 12.96. Found: C, 58.46; H, 4.55; N, 13.12.

**5.2.4. 6-Fluoro-1-(2-fluoro-4-nitrophenyl)-4-oxo-7-(piperidin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (4a).** This compound was obtained from **2** and piperidine, which was purified by FC ($CH_2Cl_2$–MeOH = 100:1) and recrystallized from EtOH in 88% yield. Mp: 292–293 °C. $^1$H NMR (200 MHz, DMSO + TFA): $\delta$ 1.55 (m, 6H, 7-piperidinyl-H), 3.07 (m, 4H, 7-piperidinyl-H), 6.35 (d, 1H, $J$ = 7.0 Hz, 8-H), 7.95 (d, 1H, $J$ = 13.2 Hz, 5-H), 8.18 (m, 1H, 6′-H), 8.40 (d, 1H, $J$ = 8.4 Hz, 5′-H), 8.56 (dd, 1H, $J$ = 9.6, 1.8 Hz, 3′-H), 8.90 (s, 1H, 2-H). Anal. Calcd for $C_{21}H_{17}F_2N_3O_5 \cdot 0.5H_2O$: C, 57.53; H, 4.15; N, 9.59. Found: C, 57.50; H, 4.07; N, 9.69.

**5.2.5. 6-Fluoro-1-(2-fluoro-4-nitrophenyl)-7-(morpholin-4-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (4b).** This compound was obtained from **2** and morpholine, which was purified by FC ($CH_2Cl_2$–MeOH = 50:1) and recrystallized from EtOH in 93% yield. Mp: 284–285 °C. $^1$H NMR (200 MHz, DMSO + TFA): 3.09 (m, 4H, 7-morpholinyl-H), 3.70 (m, 4H, 7-morpholinyl-H), 6.39 (d, 1H, $J$ = 6.6 Hz, 8-H), 7.98 (d, 1H, $J$ = 13.6 Hz, 5-H), 8.17 (m, 1H, 6′-H), 8.40 (d, 1H, $J$ = 8.4 Hz, 5′-H), 8.56 (dd, 1H, $J$ = 10.0, 2.2 Hz, 3′-H), 8.92 (s, 1H, 2-H). Anal. Calcd for $C_{20}H_{15}F_2N_3O_6$: C, 55.69; H, 3.51; N, 9.74. Found: C, 55.65; H, 3.40; N, 9.59.

**5.2.6. 6-Fluoro-1-(2-fluoro-4-nitrophenyl)-7-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (4c).** This compound was obtained from **2** and 4-methylpiperazine, which was purified by FC ($CH_2Cl_2$–MeOH = 3:1) and recrystallized from EtOH in 91% yield. Mp: 288–289 °C. $^1$H NMR (400 MHz, TFA): 2.18 (s, 3H, N–Me), 2.42 (m, 4H, 7-piperazinyl-H), 3.09 (m, 4H, 7-piperazinyl-H), 6.38 (d, 1H, $J$ = 7.2 Hz, 8-H), 8.00 (d, 1H, $J$ = 13.2 Hz, 5-H), 8.18 (dd, 1H, $J$ = 8.0, 8.4 Hz, 6′-H), 8.40 (m, 1H, 5′-H), 8.58 (dd, 1H, $J$ = 9.6, 2.4 Hz, 3′-H), 8.92 (s, 1H, 2-H). Anal. Calcd for $C_{21}H_{18}F_2N_4O_5$: C, 56.76; H, 4.08; N, 12.61. Found: C, 56.74; H, 4.26; N, 12.32.

**5.2.7. 6-Fluoro-1-(2-fluoro-4-nitrophenyl)-7-(3,5-dimethylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (4e).** This compound was obtained from **2** and *cis*-2,6-dimethylpiperazine, which was purified by FC ($CH_2Cl_2$–MeOH = 20:1) and recrystallized from EtOH in 93% yield. Mp: 265–267 °C. $^1$H NMR (400 MHz, DMSO + TFA): 1.26 (d, 6H, $J$ = 6.4 Hz, $2 \times CH_3$), 2.85 (m, 2H, 7-piperazinyl-H), 3.48 (m, 2H, 7-piperazinyl-H), 3.62 (m, 2H, 7-piperazinyl-H), 6.55 (d, 1H, $J$ = 6.4 Hz, 8-H), 8.10 (m, 2H, 5-, 6′-H), 8.40 (m, 2H, 3′-, 5′-H), 8.84 (s, 1H, 2-H). Anal. Calcd for $C_{22}H_{20}F_2N_4O_5 \cdot 1.0H_2O$: C, 55.45; H, 4.66; N, 11.76. Found: C, 55.54; H, 4.40; N, 11.39.

### 5.3. General procedure for reduction of 1-nitrophenyl-fluororoquinolones by catalytic hydrogenation

A mixture of 1-nitrophenylfluororquinolone **3a**,**b** or **4a**,**b** (2 mmol) and 5% Pd/C (80 mg) in DMF (80 mL) was stirred under $H_2$ for 12–24 h (monitored until the reactant disappeared by TLC). The resulting mixture was filtered and the solvent was evaporated under reduced pressure. The residue was purified by FC ($CH_2Cl_2$–MeOH = 20:1) and recrystallized from EtOH.

**5.3.1. 1-(4-Aminophenyl)-6-fluoro-4-oxo-7-(piperidin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (5a).** Yield: 75%. Mp: 286–287 °C. $^1$H NMR (200 MHz, DMSO + TFA): 1.57 (m, 6H, 7-piperidinyl-H), 3.03 (m, 4H, 7-piperidinyl-H), 5.69 (br s, 2H, $NH_2$), 6.53 (d, 1H, $J$ = 7.6 Hz, 8-H), 6.75 (m, 2H, Ar–H), 7.26 (m, 2H, Ar–H),

7.87 (d, 1H, $J$ = 13.6 Hz, 5-H), 8.48 (s, 1H, 2-H). Anal. Calcd for $C_{21}H_{20}FN_3O_3$: C, 66.12; H, 5.30; N, 11.02. Found: C, 65.88; H, 5.35; N, 10.90.

**5.3.2. 1-(4-Aminophenyl)-6-fluoro-7-(morpholin-4-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5b).** Yield: 95%. Mp: 275–277 °C. $^1$H NMR (200 MHz, DMSO + TFA): 3.04 (m, 4H, 7-morpholinyl-H), 3.72 (m, 4H, 7-morpholinyl-H), 5.70 (br s, 2H, $NH_2$), 6.56 (d, 1H, $J$ = 7.8 Hz, 8-H), 6.73 (m, 2H, Ar–H), 7.26 (m, 2H, Ar–H), 7.93 (d, 1H, $J$ = 13.4 Hz, 5-H), 8.50 (s, 1H, 2-H). Anal. Calcd for $C_{20}H_{18}FN_3O_4{\cdot}0.3H_2O$: C, 61.78, H, 4.83, N, 10.81. Found: C, 61.86; H, 4.76; N, 10.83.

**5.3.3. 1-(4-Amino-2-fluorophenyl)-6-fluoro-4-oxo-7-(piperidin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (6a).** Yield: 68%. Mp: 271–272 °C. $^1$H NMR (200 MHz, DMSO + TFA): 1.56 (m, 6H, 7-piperidinyl-H), 3.05 (m, 4H, 7-piperidinyl-H), 6.04 (br s, 2H, $NH_2$), 6.43 (d, 1H, $J$ = 7.4 Hz, 8-H), 6.62 (m, 2H, 3′-, 5′-H), 7.35 (m, 1H, 6′-H), 7.89 (d, 1H, $J$ = 13.6 Hz, 5-H), 8.59 (s, 1H, 2-H). Anal. Calcd for $C_{21}H_{19}F_2N_3O_3$: C, 63.14; H, 4.80; N, 10.52. Found: C, 62.95; H, 4.88; N, 10.48.

**5.3.4. 1-(4-Amino-2-fluorophenyl)-6-fluoro-7-(morpholin-4-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6b).** Yield: 59%. Mp: 293–294 °C. $^1$H NMR (200 MHz, DMSO + TFA): 3.07 (m, 4H, 7-morpholinyl-H), 3.72 (m, 4H, 7-morpholinyl-H), 6.06 (br s, 2H, $NH_2$), 6.47 (d, 1H, $J$ = 7.4 Hz, 8-H), 6.59 (m, 2H, 3′-, 5′-H), 7.36 (m, 1H, 6′-H), 7.96 (d, 1H, $J$ = 13.6 Hz, 5-H), 8.62 (s, 1H, 2-H). Anal. Calcd for $C_{20}H_{17}F_2N_3O_4$: C, 59.84; H, 4.28; N, 10.47. Found: C, 59.65; H, 4.34; N, 10.37.

### 5.4. General procedure for the coupling of 6-fluoro-7-(piperazin-1-yl)-4-quinolone-3-carboxylic acids with 2-(chloromethyl)-8-hydroxyquinoline (7)

A mixture of **7**[22] (1 mmol), $K_2CO_3$ (0.14 g, 1 mmol), KI (50 mg) and corresponding 6-fluoro-7-(piperazin-1-yl)-4-quinolone-3-carboxylic acids **8a**–**e** (1 mmol) in DMF (50 mL) was stirred at room temperature (monitored until the reactant disappeared by TLC). Evaporation of the solvent gave a residue, which was poured into ice water (100 mL). The resulting solid was collected and crystallized from EtOH.

**5.4.1. 1-Ethyl-6-fluoro-7-[4-(8-hydroxyquinolin-2-ylmethyl)-piperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (9a).** Yield: 63%. Mp: 121–122 °C. $^1$H NMR (TFA): 1.78 (t, 3H, $J$ = 7.0 Hz, N(1)$CH_2CH_3$), 3.72–4.30 (m, 8H, 7-piperazinyl-H), 4.88 (d, 2H, $J$ = 7.0 Hz, N(1)-$CH_2CH_3$), 5.44 (s, 2H, 2′-$CH_2$N), 7.54 (d, 1H, $J$ = 6.6 Hz, 8-H), 7.77–8.07 (m, 3H, 6′-, 7′-, 8′-H), 8.39 (m, 2H, 3′-, 5-H), 9.28 (d, 1H, $J$ = 8.4 Hz, 4′-H), 9.34 (s, 1H, 2-H). Anal. Calcd for $C_{26}H_{25}FN_4O_4{\cdot}2.5H_2O$: C, 59.88, H, 5.79, N, 10.74. Found: C, 60.01; H, 5.70; N, 10.56.

**5.4.2. 1-Cyclopropyl-6-fluoro-7-[4-(8-hydroxyquinolin-2-ylmethyl)piperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (9b).** Yield: 61%. Mp 140–141 °C. $^1$H NMR (TFA): 1.44 (m, 2H, N(1)-c-Pro-H), 1.67 (m, 2H, N(1)-c-Pro-H), 3.77–4.31 (m, 9H, 7-piperazinyl-H, N(1)-c-Pro-H), 5.44 (s, 2H, 2′-$CH_2$N), 7.78 (d, 1H, $J$ = 7.2 Hz, 8-H), 7.98 (m, 3H, 6′-, 7′-, 8′-H), 8.33 (d, 1H, $J$ = 12.4 Hz, 5-H), 8.42 (d, 1H, $J$ = 8.4 Hz, 3′-H), 9.29 (d, 1H, $J$ = 8.4 Hz, 4′-H), 9.35 (s, 1H, 2-H). Anal. Calcd for $C_{27}H_{25}FN_4O_4{\cdot}0.7H_2O$: C, 64.70; H, 5.32; N, 11.18. Found: C, 64.81; H, 5.30; N, 10.95.

**5.4.3. 8-Fluoro-9-[4-(8-hydroxyquinolin-2-ylmethyl)piperazin-1-yl]-3-methyl-6-oxo-2,3-dihydro-6*H*-1-oxa-3a-azaphenalene-5-carboxylic acid (9c).** Yield: 63%. Mp: 204–205 °C. $^1$H NMR (TFA): 1.82 (d, 3H, $J$ = 6.6 Hz, 3-C$H_3$), 3.84–4.10 (m, 8H, 9-piperazinyl-H), 4.71 (m, 2H, 2-H), 5.16 (q, 1H, $J$ = 6.6 Hz, 3-H), 5.39 (s, 2H, 2′-$CH_2$N), 7.78 (dd, 1H, $J$ = 8.4, 1.2 Hz, 7′-H), 7.92–8.09 (m, 3H, 6′-, 8′-, 7-H), 8.41 (d, 1H, $J$ = 8.4 Hz, 3′-H), 9.29 (m, 2H, 4′-, 4-H). Anal. Calcd for $C_{27}H_{25}FN_4O_5{\cdot}0.8H_2O$: C, 62.49; H, 5.25; N, 10.79. Found: C, 62.63; H, 5.02; N, 10.53. HRFABMS calcd for $C_{27}H_{24}FN_4O_5$: 503.1730. Found $[M-H]^-$: 503.1729.

**5.4.4. 6-Fluoro-7-[4-(8-hydroxyquinolin-2-ylmethyl)piperazin-1-yl]-1-(4-nitrophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (9d).** Yield: 58%. Mp: 201–202 °C. $^1$H NMR (TFA): 3.75–4.12 (m, 8H, 7-piperazinyl-H), 5.38 (s, 2H, 2′-$CH_2$N), 6.80 (d, 1H, $J$ = 6.8 Hz, 8-H), 7.75–8.01 (m, 5H, 6′-, 7′-, 8′-H, Ar–H), 8.38 (m, 2H, 3′-, 5-H), 8.68 (m, 2H, Ar–H), 9.26 (m, 2H, 4′-, 2-H). HRFABMS calcd for $C_{30}H_{24}FN_5O_6$: 569.1711. Found $[M]^-$: 569.1722.

**5.4.5. 6-Fluoro-1-(2-fluoro-4-nitrophenyl)-7-[4-(8-hydroxyquinolin-2-ylmethyl)-piperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (9e).** Yield: 58%. Mp 201–202 °C. $^1$H NMR ($CDCl_3$): 2.72, 3.18 (2m, 8H, 7-piperazinyl-H), 5.91 (s, 2H, 2′-$CH_2$N), 6.17 (dd, 1H, $J$ = 6.8, 1.8 Hz, 8-H), 7.16 (dd, 1H, $J$ = 7.4, 1.4 Hz, 7′-H), 7.30 (dd, 1H, $J$ = 8.2, 1.4 Hz, 5′-H), 7.44 (dd, 1H, $J$ = 8.2, 7.4 Hz, 6′-H), 7.56 (d, 1H, $J$ = 8.6 Hz, 3′-H), 7.77 (m, 1H, Ar–H), 8.09 (d, 1H, $J$ = 13.0 Hz, 5-H), 8.12 (d, 1H, $J$ = 8.6 Hz, 4′-H), 8.33 (m, 2H, Ar–H), 8.57 (s, 1H, 2-H). HRFABMS calcd for $C_{30}H_{23}F_2N_5O_6$: 587.1617. Found $[M]^-$: 587.1692.

### 5.5. Antimycobacterium activity

Primary screening is conducted at 6.25 μg/mL against *M. tuberculosis* $H_{37}$Rv (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA).[23] The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls.

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