

Synthesis and antimycobacterial evaluation of certain fluoroquinolone derivatives

Yue-Ling Zhao,^{a,b} Yeh-Long Chen,^a Jia-Yuh Sheu,^c I-Li Chen,^c
Tai-Chi Wang^c and Chheng-Chyi Tzeng^{a,*}

^aFaculty of Medicinal and Applied Chemistry, College of Life Science, Kaohsiung Medical University, Kaohsiung City 807, Taiwan

^bGraduate Institute of Pharmaceutical Sciences, College of Pharmacy, Kaohsiung Medical University, Kaohsiung City 807, Taiwan

^cDepartment of Pharmacy, Department of Environmental Engineering and Health, Tajen Institute of Technology, Pingtung 907, Taiwan

Received 19 March 2005; revised 6 April 2005; accepted 6 April 2005

Available online 28 April 2005

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 ofloxacin 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**).

© 2005 Elsevier Ltd. All rights reserved.

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 pharmacoki-

netic 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 fluoroquinolones 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.

*Corresponding author. Tel.: +886 7 3121101x6985; fax: +886 7 3125339; e-mail: tzengch@kmu.edu.tw

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 CH₃CN 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

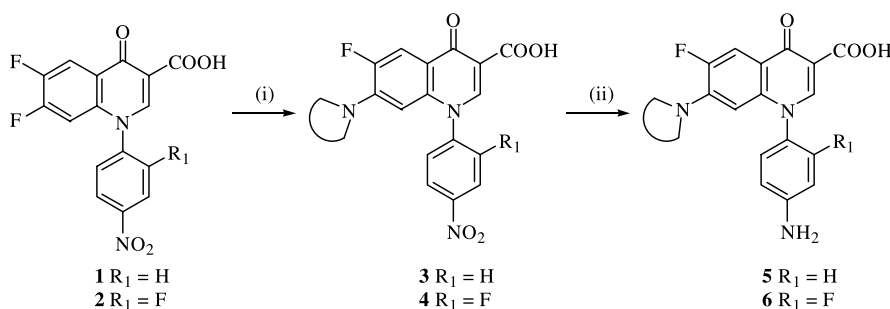
The preparation of bifunctional fluoroquinolone-hydroxyquinoline derivatives **9a–e** is described in Scheme 2. Treatment of **7**²² with fluoroquinolone

derivatives **8a–e** afforded desired products **9a–e** in 58–63% yield.

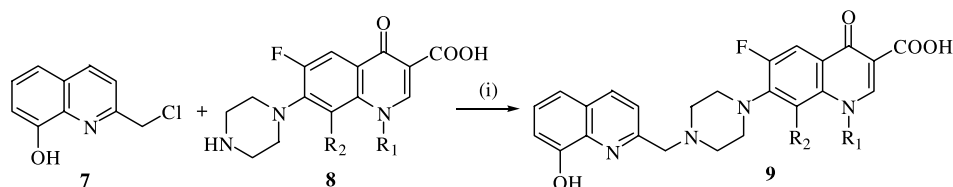
3. Pharmacological results and discussion

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.

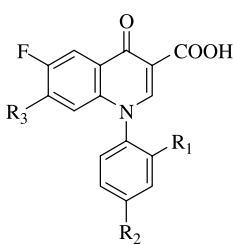
The anti-TB activity of bifunctional fluoroquinolone-hydroxyquinoline complexes is depicted in Table 2. Ciprofloxacin and ofloxacin derivatives, which exhibited the same anti-TB activity (98% inhibition), are more potent than norfloxacin counterpart, which in turn is more potent than 1-aryl congeners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

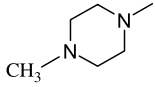
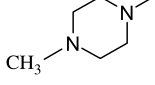
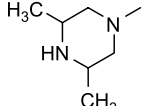
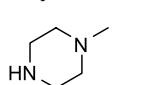


Scheme 1. Reagents and conditions: (i) cyclic amine, Et₃N, CH₃CN; (ii) Pd/C, H₂, DMF.

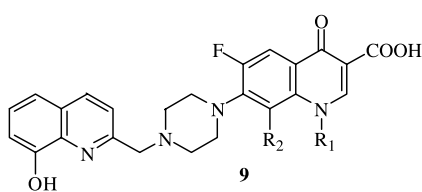


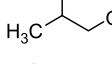
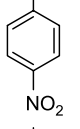
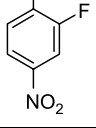
Scheme 2. Reagents and conditions: (i) K₂CO₃, DMF.

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


Compound	R ₁	R ₂	Inhibition (%)
9a	—CH ₂ CH ₃	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 ofloxacin 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 F₂₅₄ 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. ¹H NMR spectra: Varian-Unity-400 spectrometer at 400 MHz or Varian-Gemini-200 spectrometer at 200 MHz, chemical shifts δ in ppm with SiMe₄ 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 $\pm 0.4\%$ 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). ^1H NMR (200 MHz, TFA): δ 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 $\text{C}_{21}\text{H}_{18}\text{FN}_3\text{O}_5 \cdot 0.1\text{H}_2\text{O}$: 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. ^1H NMR (400 MHz, TFA): δ 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 $\text{C}_{20}\text{H}_{16}\text{FN}_3\text{O}_6 \cdot \text{H}_2\text{O}$: 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. ^1H NMR (400 MHz, TFA): δ 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 $\text{C}_{21}\text{H}_{19}\text{FN}_4\text{O}_5 \cdot 0.33\text{H}_2\text{O}$: 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. ^1H NMR (200 MHz, DMSO + TFA): δ 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 $\text{C}_{21}\text{H}_{17}\text{F}_2\text{N}_3\text{O}_5 \cdot 0.5\text{H}_2\text{O}$: 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. ^1H 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 $\text{C}_{20}\text{H}_{15}\text{F}_2\text{N}_3\text{O}_6$: 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. ^1H 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 $\text{C}_{21}\text{H}_{18}\text{F}_2\text{N}_4\text{O}_5$: 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. ^1H NMR (400 MHz, DMSO + TFA): 1.26 (d, 6H, J = 6.4 Hz, $2 \times \text{CH}_3$), 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 $\text{C}_{22}\text{H}_{20}\text{F}_2\text{N}_4\text{O}_5 \cdot 1.0\text{H}_2\text{O}$: 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-nitrophenylfluororoquinolone **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. ^1H 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. 1H 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. 1H NMR (200 MHz, DMSO + TFA): 1.56 (m, 6H, 7-piperidiny-H), 3.05 (m, 4H, 7-piperidiny-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. 1H 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. 1H 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_2N), 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. 1H 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_2N), 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-6H-1-oxa-3a-azaphenalene-5-carboxylic acid (9c). Yield: 63%. Mp: 204–205 °C. 1H NMR (TFA): 1.82 (d, 3H, $J = 6.6$ Hz, 3- CH_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_2N), 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. 1H NMR (TFA): 3.75–4.12 (m, 8H, 7-piperazinyl-H), 5.38 (s, 2H, 2'- CH_2N), 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. 1H NMR ($CDCl_3$): 2.72, 3.18 (2m, 8H, 7-piperazinyl-H), 5.91 (s, 2H, 2'- CH_2N), 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 $\mu g/mL$ against *M. tuberculosis* H₃₇Rv (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.

Acknowledgements

Financial support of this work by the National Science Council of the Republic of China is gratefully acknowledged. Antimycobacterial data were provided by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) through a research and development contract with the US National Institute of Allergy and Infectious diseases. We also thank US National Cancer Institute (NCI) for the anticancer

screenings and the National Center for High-Performance Computing of the Republic of China for providing computer resources and chemical database services.

References and notes

- Houston, S.; Fanning, A. *Drugs* **1994**, *48*, 689.
- Jacobs, R. F. *Clin. Infect. Dis.* **1994**, *19*, 1.
- Weltman, A. C.; Rose, D. N. *Arch. Intern. Med.* **1994**, *154*, 2161.
- Alland, D.; Kalkut, G. E.; Moss, A. R.; McAdam, R. A.; Hahn, J. A.; Bosworth, W.; Drucker, E.; Bloom, B. R. *N. Engl. J. Med.* **1994**, *330*, 1710.
- Wallis, R. S.; Vjecha, M.; Amir-Tahmassebi, M.; Okwera, A.; Byekwaso, F.; Nyole, S.; Kabengeru, S.; Mugerwa, R. D.; Elinor, J. J. *J. Infect. Dis.* **1993**, *167*, 43.
- Bakkestuen, A. K.; Gundersen, L. L.; Langli, G.; Liu, F.; Nolsoe, J. M. *J. Bioorg. Med. Chem. Lett.* **2000**, *10*, 1207.
- Lin, Y. M.; Flavin, M. T.; Cassidy, C. S.; Mar, A.; Chen, F. C. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2101.
- Lin, Y. M.; Zhou, Y.; Flavin, M. T.; Zhou, L. M.; Nie, W.; Chen, F. C. *Bioorg. Med. Chem.* **2002**, *10*, 2795.
- Leysen, D. C.; Haemers, A.; Pattyn, S. R. *Antimicrob. Agents Chemother.* **1989**, *33*, 1.
- Haemers, A.; Leysen, D. C.; Bollaert, W.; Zhang, M.; Pattyn, S. R. *Antimicrob. Agents Chemother.* **1990**, *34*, 496.
- Garcia-Rodriguez, J. A.; Gomez Garcia, A. C. *J. Antimicrob. Chemother.* **1993**, *32*, 797.
- Renau, T. E.; Sanchez, J. P.; Shapiro, M. A.; Dever, J. A.; Gracheck, S. J.; Domagala, J. M. *J. Med. Chem.* **1995**, *38*, 2974.
- Renau, T. E.; Sanchez, J. P.; Gage, J. W.; Dever, J. A.; Shapiro, M. A.; Gracheck, S. J.; Domagala, J. M. *J. Med. Chem.* **1996**, *39*, 729.
- Berning, S. E. *Drugs* **2001**, *61*, 9.
- Tzeng, C. C.; Chen, Y. L. *Chin. Pharm. J.* **2002**, *54*, 229.
- Sheu, J. Y.; Chen, Y. L.; Fang, K. C.; Wang, T. C.; Peng, C. F.; Tzeng, C. C. *J. Heterocycl. Chem.* **1998**, *35*, 955.
- Fang, K. C.; Chen, Y. L.; Sheu, J. Y.; Wang, T. C.; Tzeng, C. C. *J. Med. Chem.* **2000**, *43*, 3809.
- Chen, Y. L.; Fang, K. C.; Sheu, J. Y.; Hsu, S. L.; Tzeng, C. C. *J. Med. Chem.* **2001**, *44*, 2374.
- Hsu, S. L.; Chen, Y. L.; Tzeng, C. C.; Fang, K. C.; Sheu, J. Y. *Helv. Chim. Acta* **2001**, *84*, 874.
- Sheu, J. Y.; Chen, Y. L.; Tzeng, C. C.; Hsu, S. L.; Fang, K. C.; Wang, T. C. *Helv. Chim. Acta* **2003**, *86*, 2481.
- Murugasu-Oei, B.; Dick, T. *Int. J. Antimicrob. Agents* **2001**, *18*, 579.
- Yoneda, A.; Newkome, G. R.; Theriot, K. J. *J. Organomet. Chem.* **1991**, *401*, 217.
- Collins, L.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004.