Full Paper

Synthesis and *In-Vitro* Antimycobacterial Activity of Fluoroquinolone Derivatives Containing a Coumarin Moiety

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A series of gatifloxacin, ciprofloxacin, and 8-OCH_3 ciprofloxacin coumarin derivatives with remarkable improvement in lipophilicity as compared to the parent fluoroquinolones was designed, synthesized, and characterized by ^{1}H-NMR, MS, and HRMS. These derivatives were evaluated for their *in-vitro* activity against *Mycobacterium smegmatis* CMCC 93202 and MTB H37Rv ATCC 27294. All of the synthesized compounds were less active than the parent compounds against *M. smegmatis* CMCC 93202, but the activity of compound **6** was found to be 2–8-fold more potent than ciprofloxacin, 8-OCH_3 ciprofloxacin, moxifloxacin, and rifampin, and comparable to gatifloxacin against MTB H37Rv ATCC 27294. These results indicated that the lipophilicity of the tested compounds is not the sole parameter affecting antimycobacterial activity.

Keywords: 8-OCH_3 ciprofloxacin / Antimycobacterial activity / Ciprofloxacin / Coumarin / Gatifloxacin

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Introduction

Tuberculosis (TB) is a common deadly contagious disease caused by various mycobacteria, mainly by *Mycobacterium tuberculosis* (MTB) in human [1]. The global epidemic of TB is assuming alarming proportions. The World Health Organization (WHO) has estimated that approximately 2 billion people have been infected with MTB, and about 2 million people are killed by this bacterial pathogen annually [2]. The increasing emergence of drug-resistant TB (DR-TB), especially multidrug-resistant TB (MDR-TB) is causing particular concern; Besides the emergence of DR-TB and MDR-TB, another contributing factor underlying the resurgence of TB is HIV co-infection with TB, in which TB is the leading cause of death among HIV-positive patients [1, 3]. Accordingly, there is an urgent need to develop novel and highly effective anti-TB drugs.

Fluoroquinolone (FQ) antibacterial agents act by inhibiting bacterial type II DNA topoisomerases, DNA gyrase, and topoisomerase IV which are both required for cell growth and division [4]. DNA gyrase seems to be the sole topoisomerase drug target of FQs in MTB [5]. Resistance to FQs remains relatively low in clinical isolates of MTB currently, and there are no reports of cross-resistance or antagonism with other classes of antimycobacterial agents [6, 7]. Some early FQs, including ciprofloxacin (CPFX, Fig. 1), ofloxacin, and sparfloxacin were recommended as second-line agents for the treatment of TB mainly in cases involving resistance or intolerance to first-line anti-TB therapy by WHO in 1996 [8]. It was noted that newer FQs as moxifloxacin (MXFX, Fig. 1) and gatifloxacin (GTFX, Fig. 1), having a particularly strong *in-vitro* and *in-vivo* activity against MTB, could be promising agents for the treatment of TB [9].

Structure–activity relationship studies of FQs have been extensively investigated and the substituent at the C-7 position has great influence on their potency, spectrum, and

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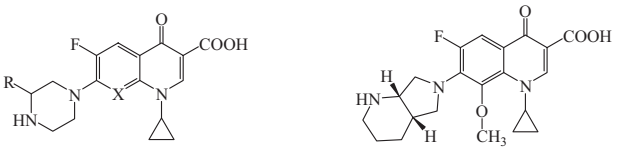


Figure 1. Chemical structures of some fluoroquinolones.

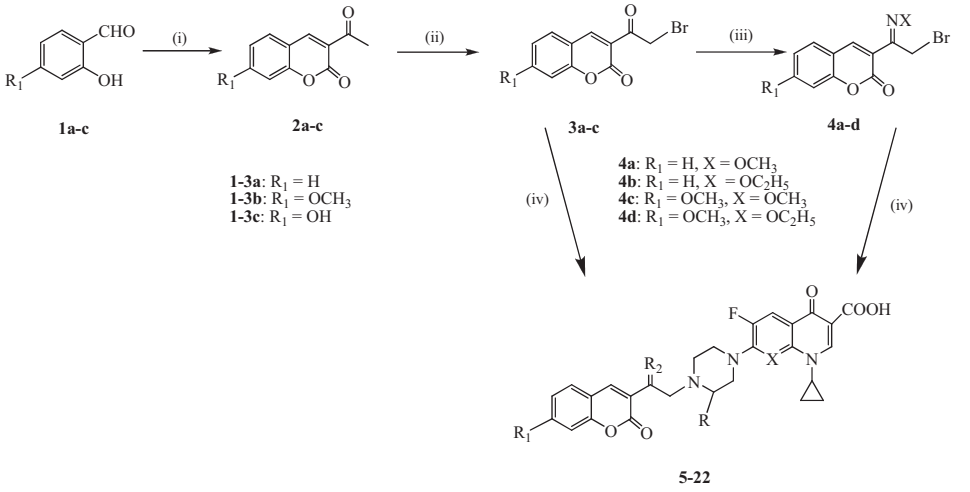
safety [10–12]. The lipophilicity of FQs was suggested to play an important role in the penetration of them into bacterial cells, and simply increasing the lipophilic character at C-7 position could also increase the anti-TB activity [7, 13]. For example, some of FQs (CPFX, GTFX, and balofloxacin) derivatives containing a lipophilic isatin moiety were found to be far more active than the respective parent drugs [1, 7, 13].

Coumarin is an endogenous compound identified in many organisms [14], and its derivatives have variety of biological activities like antibacterial [15], anti-inflammatory [16], antitumor [17], and anti-TB activity [18]. Emami et al. synthesized a series of norfloxacin-, enoxacin-, and CPFX-coumarin conjugates and found that one of them showed comparable or better antibacterial activity than the parent FQ [19]. It was a pity that antimycobacterial activity of these derivatives with remarkable improvement in the lipophilicity was not assayed. It was reported that 8-OCH_3 FQs with N1-cyclopropyl substitution are much more active against resistant MTB than C-8 hydrogen analogs [20]. Accordingly, in this report, three classes of GTFX, a 8-OCH_3 FQ with strong anti-TB activity, CPFX, one of anti-TB agents, and its 8-OCH_3 analog (8-OCH_3 CPFX, Fig. 1) derivatives containing an (un)substituted coumarin moiety were designed and synthesized to explore the effect of lipophilic character at 7-position of these FQs on activity against mycobacteria.

Results and discussion

Chemistry

Detailed synthetic pathways to GTFX, CPFX and 8-OCH_3 CPFX coumarin derivatives **5–22** are depicted in Scheme 1. Aldol condensation and cyclization of 2-hydroxybenzaldehydes **1a–c** with ethyl acetoacetate in the presence of diethylamine gave 3-acetyl coumarins **2a–c** (40.6–72.8%). The compounds **2a–c** were converted to 3-(bromoacetyl)coumarins **3a–c** (54.6–80.6%) by refluxing with Br_2 in $CHCl_3$ or *tetra-n*-butylammonium tribromide ($TBABr_3$) in CH_2Cl_2. The oxime functional groups were introduced into the α-bromoketones **3a–c** by coupling with methoxyamine hydrochloride or ethoxyamine hydrochloride in the presence of NaOAc to afford

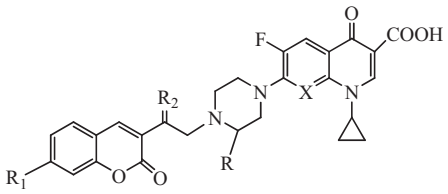


5: R_1 = H, R_2 = O, X = C-OCH_3, R = H
6: R_1 = OCH_3, R_2 = O, X = C-OCH_3, R = H
7: R_1 = OH, R_2 = O, X = C-OCH_3, R = H
8: R_1 = H, R_2 = $NOCH_3$, X = C-OCH_3, R = H
9: R_1 = H, R_2 = NOC_2H_5, X = C-OCH_3, R = H
10: R_1 = OCH_3, R_2 = $NOCH_3$, X = C-OCH_3, R = H
11: R_1 = OCH_3, R_2 = NOC_2H_5, X = C-OCH_3, R = H
12: R_1 = OCH_3, R_2 = O, X = C-OCH_3, R = CH_3
13: R_1 = H, R_2 = $NOCH_3$, X = C-OCH_3, R = CH_3
14: R_1 = H, R_2 = NOC_2H_5, X = C-OCH_3, R = CH_3
15: R_1 = OCH_3, R_2 = $NOCH_3$, X = C-OCH_3, R = CH_3
16: R_1 = OCH_3, R_2 = NOC_2H_5, X = C-OCH_3, R = CH_3
17: R_1 = OCH_3, R_2 = O, X = CH, R = H
18: R_1 = OH, R_2 = O, X = CH, R = H
19: R_1 = H, R_2 = $NOCH_3$, X = CH, R = H
20: R_1 = H, R_2 = NOC_2H_5, X = CH, R = H
21: R_1 = OCH_3, R_2 = $NOCH_3$, X = CH, R = H
22: R_1 = OCH_3, R_2 = NOC_2H_5, X = CH, R = H

(i) $CH_3COCH_2COOCH_2CH_3$, Et_2NH, EtOH; (ii) Br_2, $CHCl_3$ (for **2a**) or $TBABr_3$, CH_2Cl_2 (for **2b,c**);
(iii) CH_3ONH_2.HCl or $C_2H_5ONH_2$.HCl, NaOAc, MeOH; (iv) GTFX, CPFX or 8-OCH_3 CPFX, $NaHCO_3$, DMF

Scheme 1. Synthetic route of fluoroquinolone derivatives **5–22**.

Table 1. Structures, lipophilicity, and antimycobacterial activity of compounds **5–22**



5-22

Compd.	R_1	R_2	R	X	log*P* [a]	MIC (μg/mL)	
						M.s. [b]	MTB [c]
5	H	O	H	C–OCH_3	2.24	6.25	1.0
6	OCH_3	O	H	C–OCH_3	2.11	6.25	0.25
7	OH	O	H	C–OCH_3	1.85	6.25	1.0
8	H	$NOCH_3$	H	C–OCH_3	2.89	6.25	1.0
9	H	NOC_2H_5	H	C–OCH_3	3.23	6.25	>128
10	OCH_3	$NOCH_3$	H	C–OCH_3	2.76	6.25	>128
11	OCH_3	NOC_2H_5	H	C–OCH_3	3.10	6.25	>128
12	OCH_3	O	CH_3	C–OCH_3	2.43	>12.5	1.0
13	H	$NOCH_3$	CH_3	C–OCH_3	3.21	>12.5	1.0
14	H	NOC_2H_5	CH_3	C–OCH_3	3.55	>12.5	>128
15	OCH_3	$NOCH_3$	CH_3	C–OCH_3	3.08	6.25	1.0
16	OCH_3	NOC_2H_5	CH_3	C–OCH_3	3.42	6.25	1.0
17	OCH_3	O	H	CH	2.24	12.5	>128
18	OH	O	H	CH	1.98	3.12	0.5
19	H	$NOCH_3$	H	CH	3.02	12.5	1.0
20	H	NOC_2H_5	H	CH	3.36	>12.5	2.0
21	OCH_3	$NOCH_3$	H	CH	2.89	12.5	4.0
22	OCH_3	NOC_2H_5	H	CH	3.23	12.5	4.0
GTFX					1.51	0.10	0.25
CPFX					1.32	0.39	1.0
8-OCH_3CPFX					1.20	0.1	0.5
MXFX					1.68	0.1	0.5
RIF						3.12	2.0
INH						0.78	0.125

[a]The log*P* is calculated by ChemOffice 2009 software; [b]M.s.: *M. smegmatis* CMCC 93202; [c]MTB: MTB H37Rv ATCC 27294.

α-bromooxime derivatives **4a–d** (68.2-93.8%) [14, 19, 21, 22]. Nucleophilic substitution reactions of FQs (GTFX, CPFX, and 8-OCH_3 CPFX) with α-bromoketones **3a–c** or α-bromooximes **4a–d** in DMF, in the presence of $NaHCO_3$, provide FQs derivatives **5–22** (35.4-68.6%).

Lipophilicity

Lipophilicity of the synthesized GTFX, CPFX, and 8-OCH_3 CPFX coumarin derivatives **5–22**, the parent compounds GTFX, CPFX, and 8-OCH_3 CPFX are expressed in the terms of their log*P* values which were calculated with ChemOffice 2009 software. As shown in Table 1, a remarkable improvement was seen in the lipophilicity of the derivatives **5–22** (1.85–3.55) as evidenced by log*P* values which were higher than compared with the respective parent GTFX, CPFX, and 8-OCH_3 CPFX (1.20–1.51) (statistically significant at $p < 0.0001$ using *t*-test). This may be rendering them more capable of penetrating various biomembranes, consequently improving their penetrability toward mycobacterial cell membrane. In other words, the improvement of the lipophilic character of the target compounds **5–22** probably enhances their antimycobacterial activity.

Pharmacology

The target compounds **5–22** were initially evaluated for their *in-vitro* antimycobacterial activity against *M. smegmatis* CMCC 93202 using serial double dilution technique in duplicate, and then against MTB H37Rv ATCC 27294 using rapid direct susceptibility test technique [1]. The minimum inhibitory concentration (MIC) is defined as the concentration of the compound required to give complete inhibition of mycobacterial growth, and MICs of the synthesized compounds along

with GTFX, CPFX, 8-OCH_3 CPFX, MXFX, and rifampin (RIF) for comparison are presented in Table 1. These data suggested that all of the target compounds had considerable activity against *M. smegmatis* CMCC 93202 (MIC: 3.12–>12.5 μg/mL), although less active than the respective parent GTFX, CPFX, and 8-OCH_3 CPFX (MIC: 0.1–0.39 μg/mL, respectively).

Compounds **5–8**, **12**, **13**, **15**, **16**, **18**, and **19** (MIC: 0.25–1.0 μg/mL) showed good potency in inhibiting the growth of MTB H37Rv ATCC 27294. Among them, the activity of compound **18** (MIC: 0.5 μg/mL) was 2-fold more potent than its parent CPFX (1.0 μg/mL) and comparable to MXFX. The most active compound **6** (MIC: 0.25 μg/mL) was found to be 2–8-fold more potent than 8-OCH_3 CPFX, CPFX, MXFX, and RIF and comparable to GTFX against this strain.

Conclusion

In summary, a series of GTFX, CPFX, and 8-OCH_3 CPFX coumarin derivatives was designed, synthesized, and characterized by ^{1}H-NMR, MS, and HRMS. These derivatives were initially evaluated for their *in-vitro* antimycobacterial activity against *M. smegmatis* CMCC 93202, and then against MTB H37Rv ATCC 27294. The data showed that all of the target compounds with improved lipophilicity were less active than the respective parent GTFX, CPFX, and 8-OCH_3 CPFX against *M. smegmatis* CMCC 93202. Some of them showed good activity against MTB H37Rv ATCC 27294 and the activity of 8-OCH_3 CPFX derivative **6** (MIC: 0.25 μg/mL) was found to be 2–8-fold more potent than 8-OCH_3 CPFX, CPFX, MXFX, and RIF and comparable to GTFX against this strain.

The relative contribution of GTFX, CPFX, and 8-OCH_3 CPFX moieties to activity against MTB H37Rv ATCC 27294 is as follows: (1) for 7-OCH_3 coumarin, 8-OCH_3 CPFX (MIC: 0.25 μg/mL) > GTFX (MIC: 0.5 μg/mL) > CPFX (MIC: 1.0 μg/mL), when R = O; GTFX (MIC: 1.0 μg/mL) > CPFX (MIC: 4.0 μg/mL) > 8-OCH_3 CPFX (MIC: >128 μg/mL), when R = $NOCH_3$ or NOC_2H_5; (2) for 7-OH coumarin, CPFX (MIC: 0.5 μg/mL) > 8-OCH_3 CPFX (MIC: 1.0 μg/mL), when R = O; (3) for 7-H coumarin, GTFX (MIC: 1.0 μg/mL) ≈ CPFX (MIC: 1.0 μg/mL) ≈ 8-OCH_3 CPFX (MIC: 1.0 μg/mL), when R = $NOCH_3$; CPFX (MIC: 2.0 μg/mL) >> GTFX (MIC: >128 μg/mL) ≈ 8-OCH_3 CPFX (MIC: >128 μg/mL), when R = NOC_2H_5. Our results indicated that the lipophilicity of the tested compounds was not the sole parameter affecting antimycobacterial activity.

Experimental section

Chemistry

Melting points were determined in open capillaries and are uncorrected. ^{1}H-NMR spectra were determined on a Varian Mercury-400 spectrometer in DMSO-d_6 or $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. Electrospray ionization (ESI) mass spectra and high resolution mass spectra (HRMS) were obtained on a MDSSCIEX Q-Tap mass spectrometer and AccuTOF CS JMS-T100CS (JEOL) mass spectrometer, respectively. Unless otherwise noted, the reagents were obtained from commercial supplier and used without further purification. TLC was performed on silica gel plates (Merck, ART5554 60F_{254}).

General procedure for the synthesis of 2a–c

A solution of 2-hydroxybenzaldehydes **1a–c** (0.38 mol), ethyl acetoacetate (50 mL, 0.39 mol), and diethylamine (1 mL, 0.02 mol) in ethanol (100 mL) was stirred at room temperature for 10 h. The yellow solid obtained was filtered, washed with ether and recrystallized from alcohol to afford the title compounds **2a–c**.

*3-Acetylcoumarin **2a***

Yield: 72.8%. mp: 115–118°C ([23], mp: 118°C). ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 2.57 (3H, s, $COCH_3$), 7.39–7.46 (2H, m, Ph-H), 7.71–7.75 (1H, m, Ph-H), 7.93–7.95 (1H, m, Ph-H), 8.64 (1H, s, Ph-H). ESI-MS: *m/z* 189 $(M+H)^+$.

*3-Acetyl-7-methoxycoumarin **2b***

Yield: 65.5%. mp: 171–173°C ([23], mp: 170°C). ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 2.54 (3H, s, $COCH_3$), 3.88 (3H, s, OCH_3), 6.99–7.05 (2H, m, Ph-H), 7.85–7.87 (1H, m, Ph-H), 8.62 (1H, s, Ph-H). ESI-MS: *m/z* 219 $(M+H)^+$.

*3-Acetyl-7-hydroxycoumarin **2c***

Yield: 40.6%. mp: 244–245°C ([24], mp: 240°C). ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 2.54 (3H, s, $COCH_3$), 6.74–6.85 (2H, m, Ph-H), 7.76–7.79 (1H, m, Ph-H), 8.58 (1H, s, Ph-H). ESI-MS: *m/z* 204 $(M+H)^+$.

Synthesis of 3a–c

*3-Bromoacetylcoumarin **3a***

To a solution of **2a** (10.0 g, 53.0 mmol) in $CHCl_3$ (100 mL) was added dropwise a solution of Br_2 (3.1 mL, 57.7 mmol) in $CHCl_3$ (20 mL) over a period of 0.5 h. The reaction mixture was stirred for 15 min at 60°C and concentrated under reduced pressure. The residue was treated with acetic acid (100 mL), filtered, and dried to provide the title compound **3a** (11.4 g, 80.6%) as a white solid. Mp: 163–164°C ([14], mp: 163–165°C). ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 4.88 (2H, s, $BrCH_2CO$), 7.42–7.50 (2H, m, Ph-H), 7.75–7.80 (1H, m, Ph-H), 7.97–7.99 (1H, m, Ph-H), 8.82 (1H, s, Ph-H). ESI-MS: *m/z* 267 $(M+H)^+$, 269 $(M+2+H)^+$.

General procedure for the synthesis of 3b,c

A solution of **2b,c** (9.0 mmol) and $TBABr_3$ (8.80 g, 18.0 mmol) in CH_2Cl_2 (100 mL) was stirred at room temperature for 12 h. The off-white solid obtained was collected to give the title compounds **3b,c**.

3-Bromoacetyl-7-methoxycoumarin ***3b***

Yield: 63.5%. mp: 206–207°C ([25], mp: 210–211°C). ^{1}H-NMR (400 MHz, $CDCl_3$) δ_{ppm}: 3.93 (3H, s, OCH_3), 4.75 (2H, s, $BrCH_2CO$), 6.85–6.94 (2H, m, Ph-H), 7.57–7.59 (1H, m, Ph-H), 8.61 (1H, s, Ph-H). ESI-MS: *m/z* 297 $(M+H)^+$, 299 $(M+2+H)^+$.

3-Bromoacetyl-7-hydroxycoumarin ***3c***

Yield: 54.6%. mp: 214–216°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 4.82 (2H, s, $BrCH_2CO$), 6.74–6.88 (2H, m, Ph-H), 7.76–7.89 (1H, m, Ph-H), 8.58–8.85 (1H, m, Ph-H). ESI-MS: *m/z* 283 $(M+H)^+$, 285 $(M+2+H)^+$.

General procedure for the synthesis of 4a–d

A mixture of **3a–c** (1.8 mmol), methoxyamine hydrochloride or ethoxyamine hydrochloride (3.7 mmol), and anhydrous sodium acetate (0.20 g, 2.7 mmol) in methanol (30 mL) was stirred at 40°C for 10–12 h and concentrated under reduced pressure. The residue was treated with water (20 mL) and then filtered. The solid obtained was recrystallized from alcohol to afford the title compounds **4a–d**.

3-[2-Bromo-1-(methoxyimino)ethyl]coumarin ***4a***

Yield: 93.8%. mp: 147–149°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 4.01 (3H, s, OCH_3), 4.47 (2H, s, $BrCH_2CN$), 7.37–7.46 (2H, m, Ph-H), 7.65–7.87 (2H, m, Ph-H), 8.28 (1H, s, Ph-H). ESI-MS: *m/z* 296 $(M+H)^+$, 298 $(M+2+H)^+$.

3-[2-Bromo-1-(ethoxyimino)ethyl]coumarin ***4b***

Yield: 90.5%. mp: 126–127°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 1.28 (3H, t, $J = 7.2$ Hz, CH_2CH_3), 4.27 (2H, q, $J = 7.2$ Hz, CH_2CH_3), 4.49 (2H, s, $BrCH_2CN$), 7.38–7.47 (2H, m, Ph-H), 7.66–7.70 (1H, m, Ph-H), 7.86–7.88 (1H, m, Ph-H), 8.30 (1H, s, Ph-H). ESI-MS: *m/z* 310 $(M+H)^+$, 312 $(M+2+H)^+$.

3-[2-Bromo-1-(methoxyimino)ethyl]-7-methoxycoumarin ***4c***

Yield: 74.6%. mp: 120–121°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 3.87 (3H, s, OCH_3), 4.00 (3H, s, OCH_3), 4.48 (2H, s, $BrCH_2CN$), 6.99–7.06 (2H, m, Ph-H), 7.77–7.79 (1H, m, Ph-H), 8.23 (1H, s, Ph-H). MS (ESI, *m/z*): 326 $(M+H)^+$, 328 $(M+2+H)^+$.

3-[2-Bromo-1-(ethoxyimino)ethyl]-7-methoxycoumarin ***4d***

Yield: 68.2%. mp: 106–108°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 1.28 (3H, t, $J = 7.2$ Hz, CH_2CH_3), 3.87 (3H, s, OCH_3), 4.26 (2H, q, $J = 7.2$ Hz, CH_2CH_3), 4.48 (2H, s, $BrCH_2CN$), 6.99–7.07 (2H, m, Ph-H), 7.77–7.80 (1H, m, Ph-H), 8.22 (1H, s, Ph-H). ESI-MS: *m/z* 340 $(M+H)^+$, 342 $(M+2+H)^+$.

General procedure for the synthesis of 5–22

A mixture of GTFX, CPFX or 8-OCH_3 CPFX (2.5 mmol), **4a–d** (2.7 mmol), and sodium bicarbonate (0.42 g, 5.0 mmol) in DMF (30 mL) was stirred at 0–10°C for 10–15 h. Water (20 mL) was added and the precipitate was filtered. The white solid obtained was recrystallized from alcohol to give the title compounds **5–22.**

1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(coumarin-3-yl)-2-oxo]ethylpiperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid ***5***

Yield: 40.6%. mp: 219–221°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ_{ppm}: 1.01–1.26 (4H, m, 2 cyclopropyl CH_2), 3.36–3.66 (4H, m, piperazine), 3.84 (3H, s, OCH_3), 3.95–4.02 (4H, m, piperazine), 4.42 (2H, s, NCH_2CO), 4.70 (1H, s, cyclopropyl CH), 7.42–7.45 (2H, m, Ph-H), 7.75–7.79 (2H, m, Ph-H), 7.91–7.94 (1H, m, Ph-H), 8.82–8.84 (2H, m, Ph-H). ESI-MS: *m/z* 548 $(M+H)^+$. HRMS-ESI: *m/z* calcd. for $C_{29}H_{27}FN_3O_7$ $(M+H)^+$: 548.18330; Found: 548.18225.

1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(7-methoxycoumarin-3-yl)-2-oxo]ethylpiperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid ***6***

Yield: 42.6%. mp: 216–218°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 1.01–1.11 (4H, m, 2 cyclopropyl CH_2), 2.65–2.72 (4H, m, piperazine), 3.30 (3H, s, OCH_3), 3.34–3.35 (4H, m, piperazine), 3.75 (2H, s, NCH_2CO), 3.90 (3H, s, OCH_3), 4.15 (1H, s, cyclopropyl CH), 7.02–7.08 (2H, m, Ph-H), 7.72–7.89 (2H, m, Ph-H), 8.66–8.68 (2H, m, Ph-H). ESI-MS: *m/z* 578 $(M+H)^+$. HRMS-ESI: *m/z* calcd. for $C_{30}H_{29}FN_3O_8$ $(M+H)^+$: 578.19387; Found: 578.19461.

1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(7-hydroxycoumarin-3-yl)-2-oxo]ethylpiperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid ***7***

Yield: 36.5%. mp: 210–212°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 0.99–1.13 (4H, m, 2 cyclopropyl CH_2), 2.48–2.49 (4H, m, piperazine), 3.34 (4H, s, piperazine), 3.75 (3H, s, OCH_3), 3.87 (2H, s, NCH_2CO), 4.13–4.17 (1H, m, cyclopropyl CH), 6.61–6.75 (2H, m, Ph-H), 7.69–7.74 (2H, m, Ph-H), 8.54 (1H, s, Ph-H), 8.68 (1H, s, C_2-H). ESI-MS: *m/z* 564 $(M+H)^+$. HRMS-ESI: *m/z* calcd. for $C_{29}H_{27}FN_3O_8$ $(M+H)^+$: 564.17822; Found: 564.17646.

1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(coumarin-3-yl)-2-(methoxyimino)]ethylpiperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid ***8***

Yield: 66.2%. mp: 207–209°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 0.98–1.10 (4H, m, 2 cyclopropyl CH_2), 2.49–2.61 (4H, m, piperazine), 3.10–3.27 (4H, m, piperazine), 3.61 (3H, s, OCH_3), 3.70 (3H, s, OCH_3), 3.74–3.92 (2H, m, NCH_2CN), 4.08–4.13 (1H, m, cyclopropyl CH), 7.38–7.46 (2H, m, Ph-H), 7.62–7.81 (3H, m, Ph-H), 8.15–8.19 (1H, m, Ph-H), 8.65–8.67 (1H, m, C_2-H). ESI-MS: *m/z* 577 $(M+H)^+$. HRMS-ESI: *m/z* calcd. for $C_{30}H_{30}FN_4O_7$ $(M+H)^+$: 577.20985; Found: 577.21041.

1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(coumarin-3-yl)-2-(ethoxyimino)]ethylpiperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid ***9***

Yield: 64.5%. mp: 226–228°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 0.97–1.07 (4H, m, 2 cyclopropyl CH_2), 1.26 (3H, t, $J = 6.8$ Hz, CH_2CH_3), 2.49–2.51 (4H, m, piperazine), 3.14 (4H, s, piperazine), 3.64 (3H, s, OCH_3), 3.71 (2H, s, NCH_2CN), 4.09–4.11 (1H, m, cyclopropyl CH), 4.18 (2H, q, $J = 6.8$ Hz, CH_2CH_3), 7.36–7.46 (2H, m, Ph-H), 7.62–7.81 (3H, m, Ph-H), 8.19 (1H, s, Ph-H), 8.65 (1H, s, C_2-H). ESI-MS: m/z 591 $(M+H)^+$. HRMS-ESI: m/z calcd. for $C_{31}H_{32}FN_4O_7$ $(M+H)^+$: 591.22550; Found: 591.22327.

1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(7-methoxycoumarin-3-yl)-2-(methoxyimino)]ethylpiperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid ***10***

Yield: 58.6%. mp: 235–237°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 0.93–1.06 (4H, m, 2 cyclopropyl CH_2), 2.49 (4H, s, piperazine), 3.13 (4H, s, piperazine), 3.66 (3H, s, OCH_3), 3.69 (1H, s, cyclopropyl CH), 3.86 (3H, s, OCH_3), 3.90 (2H, s, NCH_2CN), 6.96–7.06 (2H, m, Ph-H), 7.64–7.72 (2H, m, Ph-H), 8.11 (1H, s, Ph-H), 8.60 (1H, s, C_2-H). ESI-MS: m/z 607 $(M+H)^+$. HRMS-ESI: m/z calcd. for $C_{31}H_{32}FN_4O_8$ $(M+H)^+$: 607.22042; Found: 607.22036.

1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(ethoxyimino)-2-(7-methoxycoumarin-3-yl)]ethylpiperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid ***11***

Yield: 65.3%. mp: 211–212°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 0.97–1.08 (4H, m, 2 cyclopropyl CH_2), 1.25 (3H, t, $J = 7.2$ Hz, CH_2CH_3), 2.49 (4H, s, piperazine), 3.15 (4H, s, piperazine), 3.66 (3H, s, OCH_3), 3.70 (2H, s, NCH_2CN), 3.86 (3H, s, OCH_3), 4.13–4.19 (3H, m, CH_2CH_3 and cyclopropyl CH), 6.69–7.05 (2H, m, Ph-H), 7.67–7.72 (2H, m, Ph-H), 8.11 (1H, s, Ph-H), 8.65 (1H, s, C_2-H). ESI-MS: m/z 621 $(M+H)^+$. HRMS-ESI: m/z calcd. for $C_{32}H_{34}FN_4O_8$ $(M+H)^+$: 621.23607; Found: 621.23835.

1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(7-methoxycoumarin-3-yl)-2-oxo]ethyl-3-methylpiperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid ***12***

Yield: 62.6%. mp: 231–233°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 1.01–1.09 (7H, m, 2 cyclopropyl CH_2 and CH_3), 2.31–2.43 (1H, m, piperazine), 2.65–3.25 (6H, m, piperazine), 3.30 (3H, s, OCH_3), 3.74 (2H, s, NCH_2CO), 3.89 (3H, s, OCH_3), 4.09–4.13 (1H, m, cyclopropyl CH), 7.03–7.09 (2H, m, Ph-H), 7.72–7.94 (2H, m, Ph-H), 8.61 (1H, s, Ph-H), 8.68 (1H, s, C_2-H). ESI-MS: m/z 592 $(M+H)^+$. HRMS-ESI: m/z calcd. for $C_{31}H_{31}FN_3O_8$ $(M+H)^+$: 592.20952; Found: 592.20786.

1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(coumarin-3-yl)-2-(methoxyimino)]ethyl-3-methylpiperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3- carboxylic acid ***13***

Yield: 54.5%. mp: 247–250°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 0.96–1.07 (7H, m, 2 cyclopropyl CH_2 and CH_3), 2.33–2.38 (1H, m, piperazine), 2.55–3.23 (6H, m, piperazine), 3.60 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 3.94–3.98 (2H, m, NCH_2CN), 4.07–4.11 (1H, m, cyclopropyl CH), 7.36–7.42 (2H, m, Ph-H), 7.62–7.69 (2H, m, Ph-H), 7.80–7.82 (1H, m, Ph-H), 8.18 (1H, s, Ph-H), 8.65 (1H, s, C_2-H). ESI-MS: m/z 591 $(M+H)^+$. HRMS-ESI: m/z calcd. for $C_{31}H_{32}FN_4O_7$ $(M+H)^+$: 591.22550; Found: 591.22784.

1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(coumarin-3-yl)-2-(ethoxyimino)]ethyl-3-methylpiperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid ***14***

Yield: 68.6%. mp: 229–231°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 0.97–1.07 (7H, m, 2 cyclopropyl CH_2 and CH_3), 1.26 (3H, t, $J = 6.8$ Hz, CH_2CH_3), 2.34–2.38 (1H, m, piperazine), 2.57–3.23 (6H, m, piperazine), 3.58 (1H, s, cyclopropyl CH), 3.60 (3H, s, OCH_3), 3.95–4.09 (2H, m, NCH_2CN), 4.18 (2H, q, $J = 6.8$ Hz, CH_2CH_3), 7.36–7.47 (2H, m, Ph-H), 7.62–7.70 (2H, m, Ph-H), 7.81–7.83 (1H, m, Ph-H), 8.17 (1H, s, Ph-H), 8.65 (1H, s, C_2-H). ESI-MS: m/z 605 $(M+H)^+$. HRMS-ESI: m/z calcd. for $C_{32}H_{34}FN_4O_7$ $(M+H)^+$: 605.24115; Found: 605.24459.

1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(7-methoxycoumarin-3-yl)-2-(methoxyimino)]ethyl-3-methylpiperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid ***15***

Yield: 57.0%. mp: 235–237°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 0.89–1.07 (7H, m, 2 cyclopropyl CH_2 and CH_3), 2.32–2.37 (1H, m, piperazine), 2.49–3.20 (6H, m, piperazine), 3.28 (1H, s, cyclopropyl CH), 3.62 (3H, s, OCH_3), 3.86 (2H, s, NCH_2CN), 3.90 (3H, s, OCH_3), 6.96–7.06 (2H, m, Ph-H), 7.62–7.73 (2H, m, Ph-H), 8.09 (1H, s, Ph-H), 8.57 (1H, s, C_2-H). ESI-MS: m/z 621 $(M+H)^+$. HRMS-ESI: m/z calcd. for $C_{32}H_{34}FN_4O_8$ $(M+H)^+$: 621.23607; Found: 621.23851.

1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(ethoxyimino)-2-(7-methoxycoumarin-3-yl)]ethyl-3-methylpiperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid ***16***

Yield: 64.3%. mp: 231–233°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 0.84–1.03 (7H, m, 2 cyclopropyl CH_2 and CH_3), 1.26 (3H, t, $J = 7.2$ Hz, CH_2CH_3), 2.32–2.37 (1H, m, piperazine), 2.53–3.28 (6H, m, piperazine), 3.56–3.60 (1H, m, cyclopropyl CH), 3.62 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.88-3.93 (2H, m, NCH_2CN), 4.16 (2H, q, $J = 7.2$ Hz, CH_2CH_3), 6.96–7.06 (2H, m, Ph-H), 7.58–7.74 (2H, m, Ph-H), 8.09 (1H, s, Ph-H), 8.50 (1H, s, C_2-H). ESI-MS: m/z 635 $(M+H)^+$. HRMS-ESI: m/z calcd. for $C_{33}H_{36}FN_4O_8$ $(M+H)^+$: 635.25172; Found: 635.25391.

1-Cyclopropyl-6-fluoro-7-{4-[2-(7-methoxycoumarin-3-yl)-2-oxo]ethylpiperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid ***17***

Yield: 35.4%. mp: 222–224°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 1.16–1.29 (4H, m, 2 cyclopropyl CH_2), 2.49–2.88

(4H, m, piperazine), 3.22–3.28 (5H, m, piperazine and cyclopropyl CH), 3.89 (3H, s, OCH_3), 3.81 (2H, s, NCH_2CO), 7.01–7.08 (2H, m, Ph-H), 7.55–7.57 (1H, m, Ph-H), 7.88–7.90 (2H, m, Ph-H), 8.66 (1H, s, C_2-H). ESI-MS: *m/z* 548 $(M+H)^+$. HRMS-ESI: *m/z* calcd. for $C_{29}H_{27}FN_3O_7$ $(M+H)^+$: 548.18330; Found: 548.18487.

1-Cyclopropyl-6-fluoro-7-{4-[2-(7-hydroxycoumarin-3-yl)-2-oxo]ethylpiperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid ***18***

Yield: 45.7%. mp: >300°C. ^{1}H-NMR (400 MHz, DMSO-d_6 + D_2O) δ_{ppm}: 1.09–1.28 (4H, m, 2 cyclopropyl CH_2), 2.71 (4H, s, piperazine), 3.29 (4H, s, piperazine), 3.71 (1H, s, cyclopropyl CH), 3.78 (2H, s, NCH_2CO), 6.14–6.17 (1H, m, Ph-H), 7.23–7.26 (1H, m, Ph-H), 7.48–7.50 (1H, m, Ph-H), 7.81–7.85 (1H, m, Ph-H), 8.18 (1H, s, Ph-H), 8.59 (1H, s, C_2-H). ESI-MS: *m/z* 534 $(M+H)^+$. HRMS-ESI: *m/z* calcd. for $C_{28}H_{25}FN_3O_7$ $(M+H)^+$: 534.16765; Found: 534.16671.

1-Cyclopropyl-6-fluoro-7-{4-[2-(coumarin-3-yl)-2-(methoxyimino)]ethylpiperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid ***19***

Yield: 43.5%. mp: 142–144°C ([19], mp: 138–140°C). ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 1.10–1.24 (4H, m, 2 cyclopropyl CH_2), 2.53–2.57 (4H, m, piperazine), 3.13–3.16 (4H, m, piperazine), 3.72 (2H, s, NCH_2CN), 3.73–3.74 (1H, m, cyclopropyl CH), 3.92 (3H, s, OCH_3), 7.35–7.86 (6H, m, Ph-H), 8.20 (1H, s, Ph-H), 8.60 (1H, s, C_2-H). ESI-MS: *m/z* 547 $(M+H)^+$. HRMS-ESI: *m/z* calcd. for $C_{29}H_{28}FN_4O_6$ $(M+H)^+$: 547.19929; Found: 547.20188.

1-Cyclopropyl-6-fluoro-7-{4-[2-(ethoxyimino)-2-(coumarin-3-yl)]ethylpiperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid ***20***

Yield: 38.2%. mp: 218–220°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 1.11–1.28 (7H, m, 2 cyclopropyl CH_2 and CH_2CH_3), 2.56–2.58 (4H, m, piperazine), 3.13–3.14 (4H, m, piperazin), 3.73–3.75 (3H, m, NCH_2CN and cyclopropyl CH), 4.16–4.21 (2H, m, CH_2CH_3), 7.35–7.47 (3H, m, Ph-H), 7.60–7.86 (3H, m, Ph-H), 8.20 (1H, s, Ph-H), 8.61 (1H, s, C_2-H). ESI-MS: *m/z* 561 $(M+H)^+$. HRMS-ESI: *m/z* calcd. for $C_{30}H_{30}FN_4O_6$ $(M+H)^+$: 561.21494; Found: 561. 21820.

1-Cyclopropyl-6-fluoro-7-{4-[2-(7-methoxycoumarin-3-yl)-2-(methoxyimino)]ethylpiperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid ***21***

Yield: 56.6%. mp: 249–250°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 1.11–1.24 (4H, m, 2 cyclopropyl CH_2), 2.49 (4H, s, piperazine), 3.14 (4H, s, piperazine), 3.70 (2H, s, NCH_2CN), 3.75 (1H, s, cyclopropyl CH), 3.85 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 6.95–7.03 (2H, m, Ph-H), 7.45–7.47 (1H, m, Ph-H), 7.70–7.87 (2H, m, Ph-H), 8.12 (1H, s, Ph-H), 8.62 (1H, s, C_2-H). ESI-MS: *m/z* 577 $(M+H)^+$. HRMS-ESI: *m/z* calcd. for $C_{30}H_{30}FN_4O_7$ $(M+H)^+$: 577.20985; Found: 577.21007.

1-Cyclopropyl-6-fluoro-7-{4-[2-(ethoxyimino)-2-(7-methoxycoumarin-3-yl)]ethylpiperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid ***22***

Yield: 54.3%. mp: 193–195°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_{ppm}: 1.09–1.27 (7H, m, 2 cyclopropyl CH_2 and CH_2CH_3), 2.56 (4H, s, piperazine), 3.13 (4H, s, piperazine), 3.71 (3H, s, OCH_3), 3.84–3.87 (3H, m, CH_2CH_3 and cyclopropyl CH), 4.14–4.19 (2H, m, NCH_2CN), 6.95–7.03 (2H, m, Ph-H), 7.43-7.45 (1H, m, Ph-H), 7.70–7.85 (2H, m, Ph-H), 8.12 (1H, s, Ph-H), 8.59 (1H, s, C_2-H). ESI-MS: *m/z* 591 $(M+H)^+$. HRMS-ESI: *m/z* calcd. for $C_{31}H_{32}FN_4O_7$ $(M+H)^+$: 591.22550; Found: 591.22910.

MIC determination

The target compounds **5–22** were initially evaluated for their *in-vitro* activity against *M. smegmatis* CMCC 93202. The compounds were dissolved in DMSO and two-fold diluted at concentrations from 12.5 to 0.05 μg/ml. The tested strains were prepared in 54 medium in a volume of 150 μL in 96-well microplates. The plates were incubated at 37°C for 72 h.

The entire compounds **5–22** were further evaluated for their *in-vitro* activity against MTB H37Rv ATCC 27294 using rapid direct susceptibility test technique. The tested compounds were dissolved in DMSO and two-fold diluted at concentrations from 128 to 0.125 μg/ml. The strain was obtained from Jiangsu Province Hospital, Nanjing, China.

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