Synthesis, and Antimycobacterial and Cytotoxic Evaluation of Certain Fluoroquinolone Derivatives

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Certain 1-ethyl- and 1-aryl-6-fluoro-1,4-dihydroquinol-4-one derivatives were synthesized and evaluated for antimycobacterial and cytotoxic activities. Preliminary results indicated that, for 1-aryl-6-fluoroquinolones, both 7-(piperazin-1-yl)- and 7-(4-methylpiperazin-1-yl) derivatives, **9b** and **11a**, are able to completely inhibit the growth of *M. tuberculosis* at a concentration of 6.25 μg/ml, while the 7-[4-(2-oxo-2-phenylethyl)piperazin-1-yl] derivative **13** exhibits only 31% growth inhibition at the same concentration. For 1-ethyl-6-fluoroquinolones, both 7-[4-(2-oxopropyl)piperazin-1-yl]- and 7-[4-(2-oxo-2-phenylethyl)piperazin-1-yl]-derivatives, **2a** and **2b**, respectively, show complete inhibition, while their 2-iminoethyl and substituted phenyl counterparts **3a** and **2c** are less active. In addition, the 6,8-difluoro derivative was a more-favorable inhibitor than its 6-fluoro counterpart (**2b** *vs.* **2d**). These results deserve full attention especially because **2a**, **2b**, **9b**, and **11a** are non-cytotoxic at a concentration of 100 μM. Furthermore, compound **9b** proved to be a potent anti-TB agent with selective index (SI) > 40 and an EC_{90} value of 5.75 μg/ml.

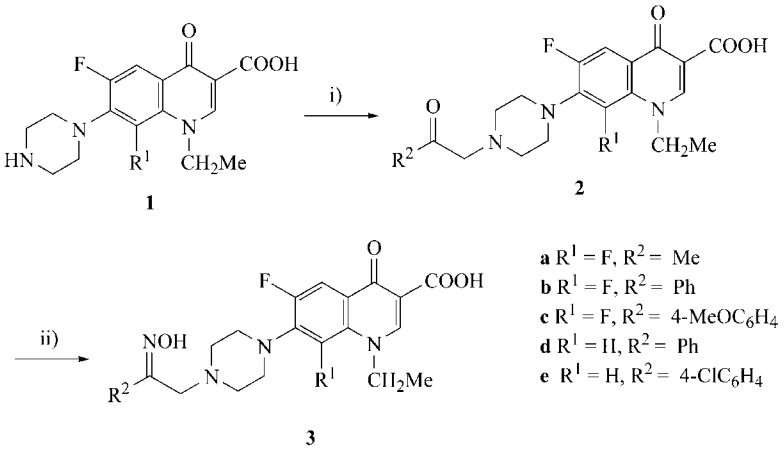
Introduction. – The drugs currently used for the treatment of tuberculosis (TB) infection are streptomycin, isoniazid, ethambutol, pyrazinamide, and rifampicin [1]. However, the current TB treatment regimens, although highly effective, are far from ideal. With the optimal combination of available drugs, the duration of treatment required for curing patients cannot be reduced to less than six months. As a result, noncompliance, *i.e.*, patients stopping therapy as soon as an improvement in the symptoms is felt, allows the remaining, more-resistant bacteria to survive and spread. Recently, the incidence of TB infection has been further complicated by an increase in cases that are resistant to conventional drug therapy, especially the emergence of multi-drug resistant tuberculosis (MDRTB) [2][3]. Furthermore, the association of TB and HIV infection is so dramatic that, in some cases, nearly two-thirds of the patients diagnosed with TB are also HIV-1 seropositive [4]. Numerous studies showing that TB may be a cofactor in the progression of HIV has caused an urgent need to search for alternative chemotherapeutics for *Mycobacterium 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 easy absorbtion after oral administration and ready penetration into mammalian cells [9–14]. These properties are important for the treatment of intracellular pathogens, such as TB [15]. Synthesis of new fluoroquinolones and the evaluation of these agents for antimycobacterial activity has continued,

and a series of SAR studies has been performed with *M. avium*, *M. tuberculosis*, and other mycobacteria [16–20]. 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 [21–24]. Since the antibacterial mechanism of these fluoroquinolones is unique and absolutely different from that of the currently used anti-TB drugs, they will become very important drug candidates if proved to be active against the growth of *M. tuberculosis.*

Results and Discussion. – The preparation of (*E*)-1-ethyl-6,8-difluoro-1,4-dihydro-7-{4-[2-(hydroxyimino)propyl]piperazin-1-yl}-4-oxoquinoline-3-carboxylic acid (**3a**) is outlined in *Scheme 1*. 8-Fluoronorfloxacin (**1a**) was treated with $NaHCO_3$ and chloroacetone to give its *N*-(2-oxopropyl) intermediate **2a**, which, upon treatment with NH_2OH in EtOH, afforded **3a** in 79% overall yield. Synthesis and antibacterial activities of **3b**–**3e** have been reported previously [22][23].

Scheme 1

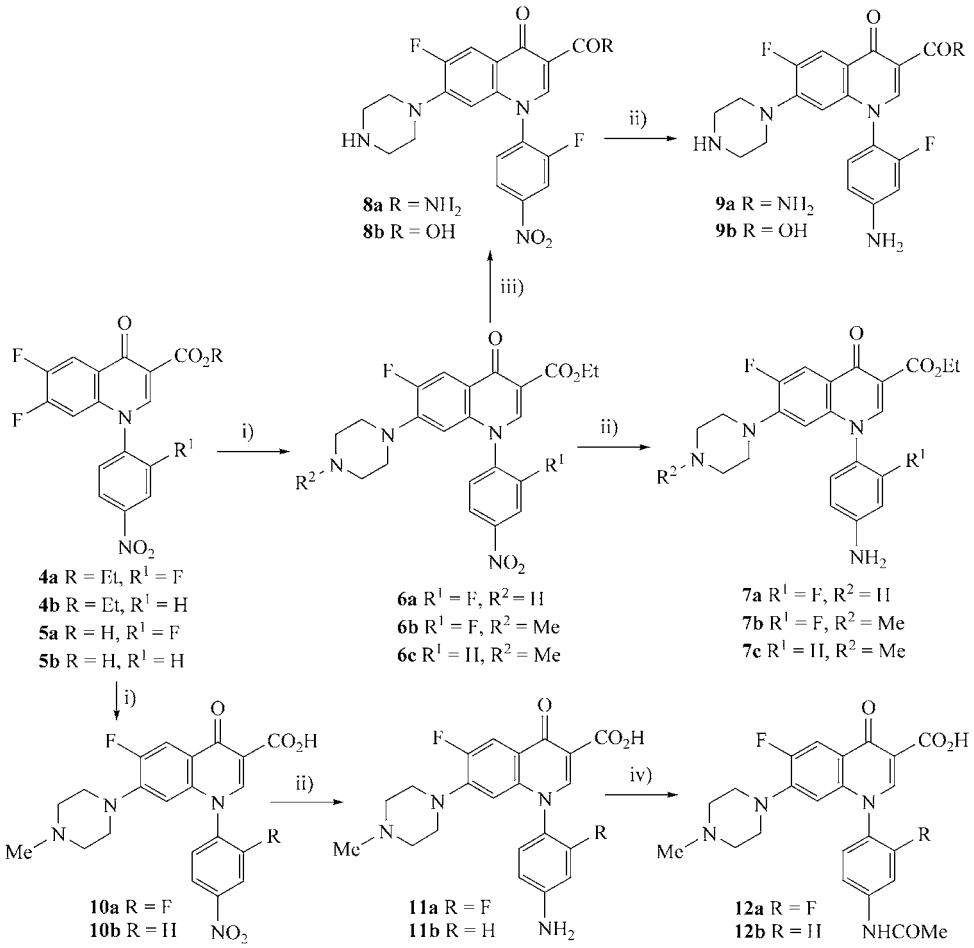


i) $NaHCO_3$, R_2COCH_2Cl in DMF. ii) NH_2OH in EtOH.

The synthetic pathways for preparation of 1-aryl-6-fluoro-1,4-dihydro-7-(piperazin-1-yl)-4-oxoquinoline-3-carboxylic acids **6**–**12** are described in *Scheme 2*. Reaction of ethyl 6,7-difluoro-1-(2-fluoro-4-nitrophenyl)-1,4-dihydro-4-oxoquinoline-3-carboxylate (**4a**) with piperazine or 1-methylpiperazine in MeCN afforded ethyl 6-fluoro-1-(2-fluoro-4-nitrophenyl)-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylate (**6a**) and its 7-(4-methylpiperazin-1-yl) derivative **6b**, respectively. Accordingly, ethyl 6-fluoro-1,4-dihydro-7-(4-methylpiperazin-1-yl)-1-(4-nitrophenyl)-4-oxoquinoline-3-carboxylate (**6c**) was obtained by the treatment of **4b** with 1-methylpiperazine. Compounds **6a**–**6c** were reduced by catalytic hydrogenation on Pd/C to their corresponding 1-(4-aminophenyl) counterparts **7a**–**7c**. Reaction of **6a** with 28% NH_4OH in a sealed bomb afforded 6-fluoro-1-(2-fluoro-4-nitrophenyl)-1,4-dihydro-4-

oxo-7-(piperazin-1-yl)quinoline-3-carboxamide (**8a**), which was reduced by catalytic hydrogenation to give 1-(4-amino-2-fluorophenyl)-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxamide (**9a**) in 59% overall yield. Compounds **8b** and **9b** were synthesized according to our previous report [23].

Scheme 2



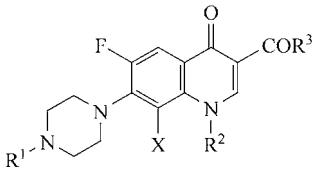
i) Piperazine or 1-methylpiperazine in MeCN. ii) H_2, Pd/C in CH_2Cl_2. iii) NH_4OH in a sealed bomb (for **7a** from **5a**); HCl/AcOH 1:4 (for **7b** from **5a**). iv) Ac_2O, pyridine.

1-(4-Amino-2-fluorophenyl)-6-fluoro-1,4-dihydro-7-(4-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic acid (**11a**) was obtained by catalytic hydrogenation of its nitro precursor **10a**, which, in turn, was prepared by the reaction of 6,7-difluoro-1-(2-fluoro-4-nitrophenyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**5a**) and 1-methylpiperazine. Acetylation of **11a** with Ac_2O afforded 1-[4-(acetylamino)-2-fluorophenyl]-6-fluoro-1,4-dihydro-7-(4-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic acid (**12a**)

in 93% yield. Accordingly, compound **12b** was synthesized from **5b**. Compound **13** was prepared from **5a** according to our previous report [23].

The anti-TB activity of fluoroquinolones is summarized in *Table 1*. Compound **2a** was found to be a very potent inhibitor, being able to inhibit 99% growth of *M. tuberculosis* at a concentration of 6.25 μg/ml. Of the 6-fluoro derivatives, compound **2d** exhibited 53% inhibition of the growth of *M. tuberculosis* and was more active than its 4-Cl and the oxime congeners (**2d** *vs.* **2e** and **2d** *vs.* **3d**). The same order has been observed for 6,8-difluoro derivatives, in which **2a** is a more-potent anti-TB agent than its oxime counterpart **3a**, and **2b** was more potent than its 4-MeO derivative **2c**. Compound **2b** was able to completely inhibit the growth of *M. tuberculosis*, while its 6-F counterpart **2d** showed only marginal inhibitory activity, indicating that the 6,8-difluoro derivative was a more-favorable inhibitor than its 6-F counterpart. The enhancement of anti-TB activity by 8-F group could be attributed to the higher lipophilicity, which facilitated its penetration into mammalian cells.

Table 1. *Inhibitory Activity of Fluoroquinolones against* M. tuberculosis *at a Concentration of 6.25 μg/ml*



Compound	R^1	R^2	R^3	X	Inhibition [%]
2a	$MeCOCH_2$	$MeCH_2$	OH	F	99
2b	$PhCOCH_2$	$MeCH_2$	OH	F	100
2c	4-$MeOC_6H_4COCH_2$	$MeCH_2$	OH	F	29
2d	$PhCOCH_2$	$MeCH_2$	OH	H	53
2e	4-ClC_6H_4CO	$MeCH_2$	OH	H	24
3a	$MeC(=NOH)CH_2$	$MeCH_2$	OH	F	34
3d	$PhC(=NOH)CH_2$	$MeCH_2$	OH	H	28
7a	H	4-NH_2,2F-C_6H_3	EtO	H	13
7b	Me	4-NH_2,2F-C_6H_3	EtO	H	0
7c	Me	4-$NH_2C_6H_4$	EtO	H	0
8a	H	2-F,4-NO_2-C_6H_3	NH_2	H	0
8b	H	2-F,4-NO_2-C_6H_3	OH	H	37
9a	H	4-NH_2,2-F-C_6H_3	NH_2	H	1
9b	H	4-NH_2,2-F-C_6H_3	OH	H	100
11a	Me	4-NH_2,2-F-C_6H_3	OH	H	100
11b	Me	4-$NH_2C_6H_4$	OH	H	84
12a	Me	4-(NHAC),2-F-C_6H_3	OH	H	18
12b	Me	4-(NHAC)-C_6H_4	OH	H	15
13	4-$MeOC_6H_4COCH_2$	4-NH_2,2-F-C_6H_3	OH	H	31

The esters **7a**–**7c** and amides **8a** and **9a** were devoid of anti-TB activity. Compound **11b** exhibited good inhibitory activity (84% inhibition), while its 4-amino-2-fluorophenyl derivative **11a** further improved the potency. Both **9b** and **11a** were able to completely inhibit the growth of *M. tuberculosis*, indicating that the 7-(piperazin-1-yl) can be substituted with a Me group. However, a [(4-methoxyphenyl)carbonyl]methyl

substituent (instead of Me; *i.e.*, **13**) drastically decreased potency. Acetylation of amino group led to decreased anti-TB activity (**11a** *vs.* **12a** and **11b** *vs.* **12b**).

Compounds **2a**, **2b**, and **9b**, which demonstrated at least 90% inhibition in the primary screen, were retested at lower concentrations against *M. tuberculosis* $H_{37}Rv$ to determine the actual minimum inhibitory concentration (*MIC*) and the cytotoxicity (IC_{50}) [25] (*Table 2*). Among them, **9b**, which possessed the selective index (*SI*, IC_{50}/MIC) > 40, was then tested for killing of *M. tuberculosis* Erdman (ATCC 35801) in monolayers of mouse bone marrow macrophages [26] at four-fold concentrations equivalent to 0.25, 1, 4, and 16 × the *MIC*. The results indicated **9b** to be a potent anti-TB fluoroquinolone with an EC_{90} value of 5.75 μg/ml ($EC_{90} > 16 \times MIC$ are considered inactive).

Table 2. *Anti-TB Activity of* **2a**, **2b**, *and* **9b**

Compound	*MIC* [μg/ml][a])	IC_{50} [μg/ml][b])	*SI*[c])	EC_{90} [μg/ml][d])
2a	3.13	> 10	> 3.19	n.d.[e])
2b	3.13	> 10	> 3.19	n.d.
9b	1.56	> 62.5	> 40.06	5.75

[a]) Minimium inhibitory concentration (*MIC*) against *M. tuberculosis* $H_{37}Rv$. [b]) Cytotoxicity (IC_{50}) in VERO cells at concentrations of 62.5 μg/ml or 10 × the *MIC* for *M. tuberculosis* $H_{37}Rv$. [c]) Selective index (*SI*, IC_{50}/MIC). [d]) Lowest concentration effecting a 90% reduction in colony forming units at 7 days compared to drug-free controls. [e]) Not determined.

All compounds were evaluated *in vitro* against a three-cell-line panel consisting of MCF 7 (Breast), NCI-H460 (Lung), and SF-268 (CNS). In this protocol, each cell line is inoculated and preincubated on a microtiter plate. Test agents are then added at a single concentration (100 μM), and the culture was incubated for 48 h. End-point determinations are made with sulforhodamine B, a protein-binding dye. Results for each test agent are reported as the percent of growth of the treated cells when compared to the untreated control cells. Compounds, which reduced the growth of any one of the cell lines to 32% or less (negative numbers indicate cell kill), are passed on for evaluation in the full panel of 60 cell lines over a 5-log dose range [27]. Results from *Table 3* indicated that all of them, with the exception of compounds **2c**–**2e**, **3d**, and **13**, whose mean GI_{50} value ranged from 22 to 83 μM, are inactive.

Conclusions. – A number of fluoroquinolone derivatives were synthesized, and evaluated for antimycobacterial and cytotoxic activities. Preliminary results are *1*) for 1-ethyl 6,8-difluoroquinolones, 7-[4-(2-oxopropyl)piperazin-1-yl] and 7-[4-(2-oxo-2-phenylethyl)piperazin-1-yl] derivatives, **2a** and **2b**, respectively, are active anti-TB agents, while their 2-iminoethyl and substituted phenyl counterparts are inactive. *2*) For 1-aryl-6-fluoroquinolones, both 7-(piperazin-1-yl) and 7-(4-methylpiperazin-1-yl) derivatives, **9b** and **11a**, respectively, are active against the growth of *M. tuberculosis*, while the 7-[4-(2-oxo-2-phenylethyl)piperazin-1-yl] counterpart, **13**, becomes inactive. These results deserve full attention, especially because **2a**, **2b**, **9b**, and **11a** are non-cytotoxic. Furthermore, compound **9b** proved to be a potent anti-TB agent with the selective index (*SI*) > 40 and an EC_{90} value of 5.75 μg/ml.

Table 3. In Vitro *Anticancer Assay of Fluoroquinolone Derivatives*

Compound	Growth Percentages			Mean GI_{50} [μM][a])
	NCI-H460 (Lung)	MCF 7 (Breast)	SF-268 (CNS)	
2a	81	66	72	Inactive
2b	50	37	23	> 100
2c	84	69	− 2	83.0
2d	93	91	6	69.9
2e	94	89	22	72.0
3d	9	25	3	29.4
6a	92	74	76	Inactive
6b	101	114	120	Inactive
6c	104	116	115	Inactive
7a	96	64	109	Inactive
7b	111	94	78	Inactive
9a	96	105	89	Inactive
9b	116	89	86	Inactive
11a	95	96	106	Inactive
11b	100	109	118	Inactive
12a	85	90	84	Inactive
12b	96	108	121	Inactive
13	4	11	29	22.1

[a]) Mean values over all 60 cancer cell lines tested [27].

Experimental Part

General. M.p.: *Electrothermal IA9100* digital melting-point apparatus; uncorrected. TLC: silica gel *60 F-254* plates from *EM Laboratories, Inc.*; detection by UV light (254 nm). NMR (^{1}H and ^{13}C) spectra; *Varian Unity-400* spectrometer or *Varian Gemini-200* spectrometer, chemical shifts δ in ppm with Me_4Si as an internal standard (= 0 ppm), coupling constants J in Hz. Elemental analyses were carried out on a *Heraeus CHN-O-Rapid* elemental analyzer, and results were within ± 0.4% of calc. values.

1-Ethyl-6,8-difluoro-1,4-dihydro-4-oxo-7-[4-(2-oxopropyl)piperazin-1-yl]quinoline-3-carboxylic acid (**2a**). A mixture of 8-fluoronorfloxacin (0.5 g, 1.48 mmol), $KHCO_3$ (0.15 g, 1.48 mmol), KI (0.08 g, 0.5 mmol), and chloroacetone (0.19 g, 2 mmol) in DMF (40 ml) was stirred at r.t. for 24 h, poured into ice-water (50 ml), and filtered to give a solid, which was washed with H_2O, crystallized from EtOH/CH_2Cl_2 10:1 to give **2a** (0.49 g, 85%). M.p. 198° (dec.). ^{1}H-NMR (400 MHz, DMSO): 1.44 (*t*, *J* = 6.0, Me); 2.11 (*s*, MeCO); 2.58, 3.28 (2 br. *s*, 8 H-(piperazine)); 3.15 (*s*, NCH_2CO); 4.59 (*m*, N(1)CH_2); 7.90 (*d*, *J* = 11.6, H−C(5)); 8.92 (*s*, H−C(2)); 14.90 (br. *s*, COOH). Anal. calc. for $C_{19}H_{21}F_2N_3O_4 \cdot 0.2\ H_2O$: C 57.34, H 5.42, N 10.56; found: C 57.48, H 5.37, N 10.52.

*1-Ethyl-6,8-difluoro-1,4-dihydro-7-[(*E*)-4-[2-(hydroxyimino)propyl]piperazin-1yl]-4-oxoquinoline-3-carboxylic Acid* (**3a**). To a suspension of **2a** (0.39 g, 1 mmol) in MeOH (20 ml) was added a soln. of $NH_2OH \cdot HCl$ (0.14 g, 2 mmol) and $NaHCO_3$ (0.17 g, 2 mmol) in H_2O (2 ml). The mixture was stirred for at r.t. 4 h, H_2O (20 ml) and CH_2Cl_2 (50 ml) were then added. The org. phase was washed successively with H_2O and brine, dried (Na_2SO_4), and evaporated to give a residual solid, which was purified by flash column chromatography (FC) (silica gel; CH_2Cl_2/MeOH 10:1) and crystallized from CH_2Cl_2/MeOH 10:1 to give **3a** (0.38 g, 93%). M.p. 231° (dec.). ^{1}H-NMR (400 MHz, DMSO): 1.44 (*t*, *J* = 6.6, Me); 1.81 (*s*, MeC=N); 2.52, 3.36 (2 br. *s*, 8 H (piperazine)); 3.02 (*s*, NCH_2C=N); 4.58 (*m*, N(1)CH_2); 7.84 (*d*, *J* = 13.2, H−C(5)); 8.91 (*s*, H−C(2)); 10.61 (*s*, NOH); 15.30 (br. *s*, COOH). Anal. calc. for $C_{19}H_{22}F_2N_4O_4$: C 55.88, H 5.43, N 13.72; found: C 55.69, H 5.23, N 13.57.

Ethyl 6-Fluoro-1-(2-fluoro-4-nitrophenyl)-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylate (**6a**). A mixture of **4a** (2.68 g, 6.83 mmol) and piperazine (1.72 g, 20 mmol) in MeCN (100 ml) was refluxed under N_2 for 5 h (TLC monitoring). The solvent was evaporated to give a residual solid, which was purified by FC (CH_2Cl_2/MeOH 10:1) and crystallized from EtOH to afford **6a** (2.66 g, 85%). M.p. 288° (dec.). ^{1}H-NMR (400 MHz, DMSO): 1.24 (*t*, *J* = 7.2, Me); 2.77, 2.91 (2 br. *s*, 8 H (piperazine)); 4.19 (*q*, *J* = 7.2, CH_2O); 6.24 (*d*, *J* = 6.0, H−C(8)); 7.80 (*d*, *J* = 13.2, H−C(5)); 8.18 (*dd*, *J* = 8.8, 8.0, H−C(6′)); 8.35 (*ddd*, *J* = 8.8, 2.8, 2.0,

H–C(5′)); 8.51 (*dd*, *J* = 9.6, 2.8, H–C(3′)); 8.54 (*s*, H–C(2)). Anal. calc. for $C_{22}H_{20}F_2N_4O_5 \cdot 0.2\ H_2O$: C 57.02, H 4.45, N 12.13; found: C 57.09, H 4.46, N 11.96.

Ethyl 6-Fluoro-1-(2-fluoro-4-nitrophenyl)-1,4-dihydro-7-(4-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxylate (**6b**). Compound **6b** was obtained from **4a** and 1-methylpiperazine as described for **6a**. FC (CH_2Cl_2/MeOH 20:1): 63% yield. M.p. 184–185°. ^{1}H-NMR (200 MHz, DMSO): 1.26 (*t*, *J* = 7.2, Me); 2.21 (*s*, MeN); 2.44, 3.03 (2 br. *s*, 8 H (piperazine)); 4.21 (*q*, *J* = 7.2, CH_2O); 6.28 (*d*, *J* = 7.0, H–C(8)); 7.83 (*d*, *J* = 13.4, H–C(5)); 8.18 (*dd*, *J* = 8.4, 8.0, H–C(6′)); 8.36 (*dd*, *J* = 8.8, 2.0, H–C(5′)); 8.53 (*m*, H–C(3′, H–C(2)). Anal. calc. for $C_{23}H_{22}F_2N_4O_5 \cdot 4.0\ H_2O$: C 50.73, H 5.55, N 10.29; found: C 50.63, H 5.29, N 10.54.

Ethyl 6-Fluoro-1,4-dihydro-7-(4-methylpiperazin-1-yl)-1-(4-nitrophenyl)-4-oxoquinoline-3-carboxylate (**6c**). Compound **6c** was obtained from **4b** and 1-methylpiperazine as described for **6a**. FC (CH_2Cl_2/MeOH 20:1): 90% yield. M.p. 231–233°. ^{1}H-NMR (200 MHz, DMSO): 1.24 (*t*, *J* = 7.2, Me); 2.17 (*s*, MeN); 2.38, 2.96 (2 br. *s*, 8 H (piperazine)); 4.20 (*q*, *J* = 7.2, CH_2O); 6.33 (*d*, *J* = 7.2, H–C(8)); 7.82 (*d*, *J* = 13.6, H–C(5)); 7.97 (*m*, H–C(2′), H–C(6′)); 8.48 (*m*, H–C(3′), H–C(5′), H–C(2)). Anal. calc. for $C_{23}H_{23}FN_4O_5 \cdot 0.5\ H_2O$: C 59.61, H 5.22, N 12.09; found: C 59.25, H 5.23, N 11.91.

Ethyl 1-(4-Amino-2-fluorophenyl)-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylate (**7a**). A mixture of **6a** (3.40 g, 7.42 mmol) and 5% Pd/C (0.50 g) in CH_2Cl_2/EtOH 3:1 (100 ml) was stirred under H_2 for 24 h (TLC monitoring). The resulting mixture was filtered, and the soln. was evaporated under reduced pressure. The residual solid was purified by FC (CH_2Cl_2/MeOH 30:1) and crystallized from EtOH to give **7a** (2.45 g, 77%). M.p. 260–261°. ^{1}H-NMR (400 MHz, DMSO): 1.26 (*t*, *J* = 7.2, Me); 3.40 (br. *s*, 8 H (piperazine)); 4.20 (*q*, *J* = 7.2, CH_2O); 6.04 (br. *s*, NH_2); 6.36 (*d*, *J* = 7.2, H–C(8)); 6.59 (*m*, H–C(3′), H–C(5′)); 7.34 (*dd*, *J* = 9.2, 8.4, H–C(6′)); 7.85 (*d*, *J* = 13.2, H–C(5)); 8.37 (*s*, H–C(2)). Anal. calc. for $C_{22}H_{22}F_2N_4O_3 \cdot 2.5\ H_2O$: C 55.80, H 5.75, N 11.83; found: C 55.57, H 5.74, N 11.60.

Ethyl 1-(4-Amino-2-fluorophenyl)-6-fluoro-1,4-dihydro-7-(4-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxylate (**7b**). Compound **7b** was obtained from **6b** as described for **7a**. FC (CH_2Cl_2/MeOH 20:1): 47% yield. M.p. 132–133°. ^{1}H-NMR (200 MHz, DMSO): 1.25 (*t*, *J* = 7.2, Me); 2.18 (*s*, MeN); 2.42, 2.99 (2 br. *s*, 8 H (piperazine)); 4.18 (*q*, *J* = 7.2, CH_2O); 5.99 (br. *s*, NH_2); 6.33 (*d*, *J* = 7.2, H–C(8)); 6.57 (*m*, H–C(3′), H–C(5′)); 7.32 (*dd*, *J* = 9.2, 8.4, H–C(6′)); 7.79 (*d*, *J* = 13.6, H–C(5)); 8.32 (*s*, H–C(2)). Anal. calc. for $C_{23}H_{24}F_2N_4O_3 \cdot 0.8\ H_2O$: C 60.46, H 5.65, N 12.26; found: C 60.68, H 5.68, N 11.98.

Ethyl 1-(4-Aminophenyl)-6-fluoro-1,4-dihydro-7-(4-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxylate (**7c**). Compound **7c** was obtained from **6c** as described for **7a**. FC (CH_2Cl_2/MeOH 30:1): 60% yield. M.p. 227–229°. ^{1}H-NMR (400 MHz, DMSO): 1.25 (*t*, *J* = 7.2, Me); 2.38 (*s*, MeN); 2.71, 3.06 (2 br. *s*, 8 H (piperazine)); 4.19 (*q*, *J* = 7.2, CH_2O); 5.65 (br. *s*, NH_2); 6.43 (*d*, *J* = 7.6, H–C(8)); 6.74 (*m*, 2 H–C(2′, 6′)); 7.23 (*m*, 2 H–C(3′, 5′); 7.79 (*d*, *J* = 13.6, H–C(5)); 8.30 (*s*, H–C(2)). Anal. calc. for $C_{23}H_{25}FN_4O_3 \cdot 2.0\ H_2O$: C 59.99, H 6.35, N 12.17; found: C 60.23, H 6.05, N 12.03.

6-Fluoro-1-(2-fluoro-4-nitrophenyl)-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxamide (**8a**). A mixture of the **6a** (2.18 g, 4.75 mmol) and 28% NH_4OH (40 ml) in MeOH (10 ml) was heated in a steel bomb at 125° for 5 h. The mixture was evaporated to give a residual solid, which was purified by FC (CH_2Cl_2/MeOH 20:1) and crystallized from EtOH to afford **8a** (2.66 g, 76%). M.p. 259–260° (dec.). ^{1}H-NMR (200 MHz, DMSO): 2.78, 2.94 (2 br. *s*, 8 H (piperazine)); 6.31 (*dd*, *J* = 7.0, 1.4, H–C(8)); 7.64 (*d*, *J* = 4.4, CONH); 7.92 (*d*, *J* = 13.6, H–C(5)); 8.17 (*dd*, *J* = 8.8, 7.6, H–C(6′)); 8.37 (*ddd*, *J* = 8.8, 2.4, 1.0, H–C(5′)); 8.55 (*dd*, *J* = 9.8, 2.4, H–C(3′)); 8.65 (*s*, H–C(2)); 9.13 (*d*, *J* = 4.4, CONH). Anal. calc. for $C_{20}H_{17}F_2N_5O_4 \cdot 0.8\ H_2O$: C 54.13, H 4.22, N 15.78; found: C 54.44, H 4.08, N 15.41.

1-(4-Amino-2-fluorophenyl)-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxamide (**9a**). Compound **9a** was obtained from **8a** as described for **7a**: 78% yield. M.p. 276–278°. ^{1}H-NMR (400 MHz, DMSO): 2.80, 2.92 (2 br. *s*, 8 H (piperazine)); 6.01 (br. *s*, NH_2); 6.38 (*d*, *J* = 6.8, H–C(8)); 6.59 (*m*, H–C(3′), H–C(5′)); 7.34 (*dd*, *J* = 9.2, 8.4, H–C(6′)); 7.57 (*d*, *J* = 4.4, CONH); 7.88 (*d*, *J* = 13.2, H–C(5)); 8.44 (*s*, H–C(2)); 9.20 (*d*, *J* = 4.4, CONH). Anal. calc. for $C_{21}H_{21}F_2N_5O_2 \cdot 1.0\ H_2O$: C 58.46, H 5.37, N 16.23; found: C 58.38, H 5.11, N 16.05.

6-Fluoro-1-(2-fluoro-4-nitrophenyl)-1,4-dihydro-7-(4-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic Acid (**10a**). Compound **10a** was obtained from **5a** and 1-methylpiperazine as described for **6a**. FC (CH_2Cl_2/MeOH 3:1): 91% yield. M.p. 288–289°. ^{1}H-NMR (400 MHz, DMSO): 2.18 (*s*, MeN); 2.40, 3.09 (2 br. *s*, 8 H (piperazine)); 6.37 (*d*, *J* = 7.2, H–C(8)); 8.00 (*d*, *J* = 13.2, H–C(5)); 8.18 (*dd*, *J* = 8.8, 8.0, H–C(6′)); 8.39 (*d*, *J* = 8.0, H–C(5′)); 8.57 (*dd*, *J* = 8.8, 2.4, H–C(3′)); 8.92 (*s*, H–C(2)); 14.80 (br. *s*, COOH). Anal. calc. for $C_{21}H_{18}F_2N_4O_5$: C 56.76, H 4.08, N 12.61; found: C 56.74, H 4.08, N 12.61.

6-Fluoro-1,4-dihydro-7-(4-methylpiperazin-1-yl)-1-(4-nitrophenyl)-4-oxoquinoline-3-carboxylic Acid (**10b**). Compound **10b** was obtained from **5b** and 1-methylpiperazine as described for **6a**. FC (CH_2Cl_2/MeOH

2:1): 72% yield. M.p. 297–298°. ^{1}H-NMR (400 MHz, DMSO/CF_3COOH): 2.88 (*s*, MeN); 3.14, 3.59 (2 br. *s*, 8 H (piperazine)); 6.53 (*d*, *J* = 7.2, H–C(8)); 8.06 (*m*, H–C(5), H–C(2′), H–C(6′)); 8.54 (*m*, H–C(3′), H–(5′)); 8.77 (*s*, H–C(2)). Anal. calc. for $C_{23}H_{22}F_2N_4O_4 \cdot 0.3\ H_2O$: C 58.41, H 4.57, N 12.98; found: C 58.46, H 4.55, N 13.12.

1-(4-Amino-2-fluorophenyl)-6-fluoro-1,4-dihydro-7-(4-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic Acid (**11a**). Compound **11a** was obtained from **10a** as described for **7a**. FC (CH_2Cl_2/MeOH 10:1): 77% yield. M.p. 278–280°. ^{1}H-NMR (400 MHz, DMSO): 2.18 (*s*, MeN); 2.42, 3.07 (2 br. *s*, 8 H (piperazine)); 6.03 (br. *s*, NH_2); 6.43 (*d*, *J* = 7.2, H–C(8)); 6.58 (*m*, H–C(3′), H–C(5′)); 7.34 (*dd*, *J* = 8.8, 8.4, H–C(6′)); 7.94 (*d*, *J* = 13.2, H–C(5)); 8.59 (*s*, H–C(2)). Anal. calc. for $C_{21}H_{20}F_2N_4O_3$: C 60.86, H 4.86, N 13.52; found: C 60.83, H 5.07, N 13.27.

1-(4-Aminophenyl)-6-fluoro-1,4-dihydro-7-(4-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic Acid (**11b**). Compound **11b** was obtained from **10b** as described for **7a**. FC (CH_2Cl_2/MeOH 20:1): 51% yield. M.p. 268–269°. ^{1}H-NMR (200 MHz, DMSO): 2.19 (*s*, MeN); 2.44, 3.06 (2 br. *s*, 8 H (piperazine)); 5.69 (br. *s*, NH_2); 6.55 (*d*, *J* = 7.0, H–C(8)); 6.76 (*m*, H–C(3′), H–C(5′)), 7.27 (*m*, H–C(2′), H–C(6′)); 8.50 (*s*, H–C(2)). Anal. calc. for $C_{21}H_{21}FN_4O_3$: C 63.63, H 5.34, N 14.13; found: C 63.41, H 5.40, N 13.94.

1-[4-(Acetylamino)-2-fluorophenyl]-6-fluoro-1,4-dihydro-7-(4-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic Acid (**12a**). A mixture of **11a** (0.23 g, 0.55 mmol), Ac_2O (30 ml), and pyridine (30 ml) was stirred at r.t. for 24 h. The resulting mixture was concentrated under reduced pressure, and then CH_2Cl_2 (100 ml) was added, and the mixture was washed with H_2O (3 × 80 ml), dried (Na_2SO_4), and evaporated. The residual solid was purified by FC (CH_2Cl_2/MeOH 50:1) to give **12a** (0.23 g, 93%). M.p. 246–247°. ^{1}H-NMR (200 MHz, DMSO): 2.14 (*s*, MeCO); 2.18 (*s*, MeN); 2.42, 3.08 (2 br. *s*, 8 H (piperazine)); 6.38 (*d*, *J* = 7.0, H–C(8)); 7.53 (*dd*, *J* = 8.8, 1.4, H–C(5′)); 7.74 (*dd*, *J* = 8.8, 8.4, H–C(6′)); 7.95 (*m*, H–C(3′), H–C(5)); 8.75 (*s*, H–C(2)); 10.54 (br. *s*, CONH). Anal. calc. for $C_{23}H_{22}F_2N_4O_4 \cdot 0.5\ H_2O$: C 57.14, H 5.18, N 10.66; found: C 56.82, H 5.33, N 10.85.

1-[4-(Acetylamino)phenyl]-6-fluoro-1,4-dihydro-7-(4-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic Acid (**12b**). Compound **12b** was obtained from **11b** as described for **12a**. FC (CH_2Cl_2/MeOH 50:1): 71% yield. M.p. 287–288°. ^{1}H-NMR (200 MHz, DMSO): 2.12 (*s*, MeCO); 2.19 (*s*, MeN); 2.43, 3.06 (2 br. *s*, 8 H (piperazine)); 6.46 (*d*, *J* = 7.4, H–C(8)); 7.61 (*m*, H–C(2′), H–C(6′)); 7.86 (*m*, H–C(3′), H–C(5′)); 7.96 (*d*, *J* = 13.4, H–C(5)); 8.58 (*s*, H–C(2)); 10.35 (br. *s*, CONH). Anal. calc. for $C_{23}H_{22}FN_4O_4 \cdot AcOH \cdot 0.2\ H_2O$: C 59.81, H 5.51, N 11.16; found: C 59.73, H 5.59, N 11.09.

Antimycobacterium Activity. Primary screening is conducted at 6.25 μg/ml against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium with a broth microdilution assay, the Microplate Alamar Blue Assay (MABA) [25]. Compounds exhibiting fluorescence are tested in the BACTEC 460 radiometric system [25]. Compounds demonstrating at least 90% inhibition in the primary screen are retested at lower concentrations against *M. tuberculosis* $H_{37}Rv$ to determine the actual minimum inhibitory concentration (*MIC*) with MABA. The *MIC* is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls. Concurrent with the determination of *MIC*s, compounds are tested for cytotoxicity (IC_{50}) in VERO cells at concentrations of 62.5 μg/ml or 10 × the MIC for *M. tuberculosis* $H_{37}Rv$. After 72-h exposure, viability is assessed on the basis of cellular conversion of MTT to a formazan product with the *Promega Cell Titer 96 Nonradioactive Cell Proliferation Assay.* Compounds for which the selective index (*SI*, IC_{50}/MIC) > 10 will have *in vitro* activity confirmed in the BACTEC 460 at 6.25 μg/ml. Compounds are then tested for killing of *M. tuberculosis* Erdman (ATCC 35801) in monolayers of mouse bone marrow macrophages [26] (EC_{90}; lowest concentration effecting a 90% reduction in colony forming units at seven days compared to drug-free controls) at four-fold concentrations equivalent to 0.25, 1, 4, and 16 × the MIC. Compounds with EC_{90} > 16 × MIC are considered inactive in the model.

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