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Synthesis of gatifloxacin derivatives and their biological activities against *Mycobacterium leprae* and *Mycobacterium tuberculosis*

Catherine Gomez [a], Prishila Ponien [a], Nawal Serradji [a], Aazdine Lamouri [a], Alix Pantel [b,c], Estelle Capton [b], Vincent Jarlier [b,c,d], Guillaume Anquetin [a,*], Alexandra Aubry [b,c,d,*]

[a] Université Paris Diderot, Sorbonne Paris Cité, ITODYS, UMR CNRS 7086, 15 rue J-A de Baïf, 75205 Paris Cedex 13, France
[b] UPMC Université Paris 06, ER5 (EA 1541), laboratoire de Bactériologie-Hygiène, F-75005 Paris, France
[c] AP-HP, Hôpital Pitié-Salpêtrière, laboratoire de Bactériologie-Hygiène, F-75013 Paris, France
[d] Centre National de Référence des Mycobactéries et de la Résistance des Mycobactéries aux Antituberculeux, F-75013 Paris, France

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ABSTRACT

Novel 3′-piperazinyl derivatives of the 8-hydrogeno and 8-methoxy-6-fluoro-1-cyclopropyl-4-quinolone-3-carboxylic acid scaffolds were designed, synthesized and characterized by ^{1}H, ^{13}C and ^{19}F NMR, and HRMS. The activity of these derivatives against pathogenic mycobacteria (*M. leprae* and *M. tuberculosis*), wild-type (WT) strains or strains harboring mutations implicated in quinolone resistance, were determined by measuring drug concentrations inhibiting cell growth (MIC) and/or DNA supercoiling by DNA gyrase (IC_{50}), or inducing 25% DNA cleavage by DNA gyrase (CC_{25}). Compound **4** (with a methoxy in R_8 and a secondary carbamate in R_3') and compound **5** (with a hydrogen in R_8 and an ethyl ester in R_3') displayed biological activities close to those of ofloxacin but inferior to those of gatifloxacin and moxifloxacin against *M. tuberculosis* and *M. leprae* WT DNA gyrases, whereas all of the compounds were less active in inhibiting *M. tuberculosis* growth and *M. leprae* mutant DNA gyrases. Since R_3' substitutions have been poorly investigated previously, our results may help to design new quinolone derivatives in the future.

1. Introduction

Controlling leprosy and tuberculosis (TB), two communicable diseases caused by *Mycobacterium leprae* and *Mycobacterium tuberculosis*, respectively, is often challenging due to the emergence of multidrug-resistant strains.[1] The World Health Assembly decided, in 1991, to 'eliminate leprosy as a public health problem' by the year 2000. After an important decrease in the number of new cases of leprosy detected each year worldwide, from 763,000 in 2000 to 299,000 in 2005, the decrease was gradual since 2005, with 228,000 new cases at the end of 2010.[1] In conjunction with this still high number of new cases, leprosy resistant to dapsone and rifampin therapy emerged. Similarly, TB affecting millions of people worldwide remains a public health challenge, partly due to the emergence of multidrug-resistant tuberculosis (MDR-TB), that is, TB resistant to both isoniazid and rifampin, which currently represents a serious obstacle to TB control. As a consequence of the threat of drug-resistant TB and leprosy, the WHO Stop TB Strategy[2] and the Final Push strategy to eliminate leprosy[3] emphasize the need to increase surveillance, control, and treatment efforts. The development of new antimycobacterial drugs should have two objectives: shorten treatment to improve compliance and maintain activity against drug-resistant strains. Quinolones are good candidates for the development of more powerful agents against leprosy and tuberculosis.[4] They play a critical role in the treatment of MDR-TB and drug-resistant leprosy. Moxifloxacin and gatifloxacin (Fig. 1) are the most active quinolones against *M. tuberculosis* and *M. leprae*, and are under evaluation to shorten the treatment of TB and leprosy caused by susceptible mycobacteria.[4] Unfortunately, MDR-TB strains resistant also to quinolones have emerged, leading to virtually untreatable 'eXtensively Drug Resistant' (XDR) tuberculosis. Cases of leprosy resistant to dapsone, rifampin and quinolones have been reported also.[5] In the future, quinolone resistance will likely increase in TB and leprosy due to (i) poor MDR-TB management, (ii) increasing use of quinolones in susceptible TB, and (iii) wide use of quinolones for empirical treatment of a larger range of non-mycobacterial infections such as urinary and respiratory tract infections, diarrhea and typhoid fever, which are common infections in the areas of high prevalence of tuberculosis and leprosy.[6,7]

Quinolones act by forming a reversible ternary complex with their bacterial targets (DNA gyrase and topoisomerase IV) and DNA, blocking bacterial growth and chromosome fragmentation,

* Corresponding authors. Tel.: +33 1 57 27 72 57; fax: +33 1 57 27 72 63 (G.A.). Present address: Laboratoire de Bactériologie, Faculté de Médecine Pierre et Marie Curie, 91 Boulevard de l'hôpital, 75634 Paris Cedex 13, France. Tel.: +33 1 40 77 97 46; fax: +33 1 45 82 75 77 (A.A.).
E-mail addresses: guillaume.anquetin@univ-paris-diderot.fr (G. Anquetin), alexandra.aubry@upmc.fr (A. Aubry).

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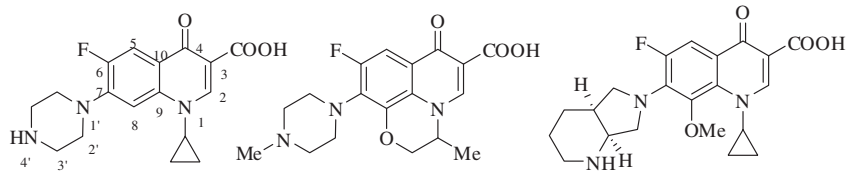
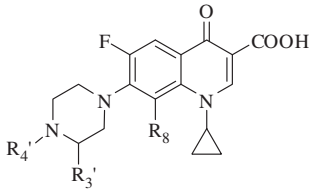


Ciprofloxacin **Ofloxacin** **Moxifloxacin**



General structure of **gatifloxacin** and **compounds 1-10**

Compound	R_3'	R_4'	R_8
Gatifloxacin	CH_3	H	OMe
1	$CH_2OC(O)NEt_2$	H	H
2		H	OMe
3	$CH_2OC(O)NHBu$	H	H
4		H	OMe
5	COOEt	H	H
6		H	OMe
7	COOH	H	H
8		H	OMe
9		Et	H
10		Et	OMe

Figure 1. General structure of ciprofloxacin, ofloxacin, moxifloxacin, gatifloxacin and compounds **1–10.**

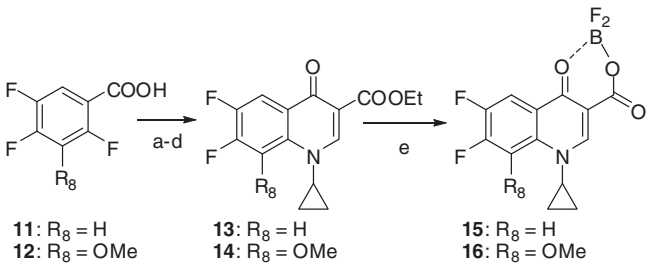
which leads to irreversible lethal damage.[8–10] Mutations in the sole target of *M. tuberculosis* and *M. leprae*, that is, DNA gyrase ($GyrA_2$-$GyrB_2$), are the prominent established mechanisms of acquired resistance to quinolones. Recently we established structure activity relationships (SAR) for quinolones with respect to *M. leprae* and *M. tuberculosis*.[11–13] These data underline the importance of (i) a cyclopropyl substituent at the N_1 position, (ii) a fluorine atom at the R_6 position (except for garenoxacin), (iii) a substituted carbon atom at the C_8 position (except for ciprofloxacin), and (iv) a cycle or a bicycle containing a nitrogen atom at the R_7 position (Fig. 1). Despite the fact that modifications at the R_7 position have been extensively studied, the few modifications of the piperazine core that have been investigated were restricted to R_3' = Me, diMe, Et, CH_2OH, CH_2F, CH_2CN, $CH_2N(CH_3)_2$ and C_6H_5.[14–17] The R_7 position is fundamental for biological activity as illustrated by two recent crystal structures of topoisomerase IV with DNA and moxifloxacin.[18,19] The *M. tuberculosis* DNA gyrase reaction core resembles closely the core of these structures.[20] The bulky substituent in R_7, which occupies a large and solvent-accessible pocket of DNA gyrase, interacts with the beginning of the α2 helix (residues 498–502 of GyrB) of one of the three regions of the TOPRIM domain which are part of the quinolone-binding pocket. These observations prompted us to select gatifloxacin, that contains a 3′-methyl piperazine in R_7 (Fig. 1), as the starting compound for the search of more potent quinolones against *M. leprae* and *M. tuberculosis* in order to take advantage of the interaction between R_7 and the large quinolone-binding pocket of DNA gyrase. Hence, the methyl group of gatifloxacin was replaced by different functional groups (with R_3' being an ethyl ester, carboxylic acid, secondary or tertiary carbamates) in order to assess the impact of the functionalization of this R_3'-position on the antimycobacterial activity of those novel gatifloxacin derivatives (Fig. 1).

2. Results and discussion

2.1. Chemistry

Compounds **1–6** and **7–10** were prepared as shown in Scheme 1 and Table 1, and Table 2, respectively. The synthetic investigations began with 3-hydrogeno (**11**) and 3-methoxy (**12**) 2,4,5-trifluorobenzoic acids. The first four steps of the synthesis were adapted from earlier work.[21,22] After conversion of the carboxylic acid to the corresponding acyl chloride, the key step was the reaction between ethyl 3-(diethylamino) acrylate and the corresponding trifluorobenzoyl chloride in the presence of triethyl amine (Scheme 1). After transaminolysis with cyclopropylamine, the quinolone ring formation proceeded smoothly with potassium carbonate in hot DMF to afford **13** and **14** with good yields (62% and 65%, respectively, after 4 steps).

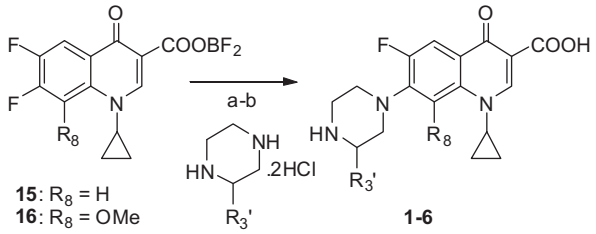
Due to the poor reactivity of the C_7–F bond towards nucleophilic aromatic substitution, the C_7 atom of **13** and **14** was rendered more electrophile by the coordination of a Lewis acid, $-BF_2$, onto both carbonyl functions (Scheme 1). In a recently described procedure, 1.2 equiv of K_2CO_3 and 1.4 equiv of $BF_3{\cdot}Et_2O$[23] instead of 10 equiv of $BF_3{\cdot}Et_2O$ were used.[21] Using this improved non-chromatographic process, the difluoro–boron complexes **15** and **16**, required for the nucleophilic aromatic substitutions, were obtained with good yield (89% and 69%, respectively). Finally, compounds **1–6** were obtained by coupling **15** and **16** with various piperazines[24] in refluxing acetonitrile for 1–7 days with poor to moderate yield (Table 1) in agreement with published data.[17,22] In our case, the poor yield observed for compounds **2–4** (approximately 20%) can be correlated to the steric hindrance between the large substituent on the piperazine



11: R_8 = H **12**: R_8 = OMe **13**: R_8 = H **14**: R_8 = OMe **15**: R_8 = H **16**: R_8 = OMe

Scheme 1. Synthetic pathway to difluoro–boron complexes **15** and **16.** Reagents and conditions: (a) oxalyl chloride, DMF, DCM, 24 h; (b) Et_2N–CH=CH–COOEt, Et_3N, toluene, 90 °C, 5h (75–79%); (c) Et_2O/EtOH:2:1, cyclopropylamine, 25 °C, 3h; (d) DMF, K_2CO_3, 90 °C, 5h (82–83%); (e) $BF_3{\cdot}Et_2O$, THF, K_2CO_3 (69–89%).

Table 1
Synthesis of compounds **1–6**



Compounds	R_8	R_3'	Yield (%)
1	H	$CH_2OC(O)NEt_2$	55
2	OMe	$CH_2OC(O)NEt_2$	23
3	H	$CH_2OC(O)NHBu$	23
4	OMe	$CH_2OC(O)NHBu$	18
5	H	COOEt	47
6	OMe	COOEt	51

Reagents and conditions: (a) CH_3CN, NEt_3, reflux, 1–7 days, Only for **5**: (b) MeOH, NEt_3, reflux, 24 h.

ring (carbamate function) and the quinolone core (especially for R_8 = OMe)**.** But, due to the steric hindrance, this nucleophilic aromatic substitution appeared to be regiospecific and prevented us from time-consuming and unnecessary selective protection and deprotection of the N_4' secondary amine of the piperazine ring. To confirm this assessment, we used a selectively N_1'-protected piperazine, **17**, bearing the smallest functional group in R_3' (i.e., COOEt), and the less hindered difluoro–boron complex, that is, **15** (Scheme 2)**.** After 70 h at reflux in acetonitrile, no reaction occurred between **15** and **17**. This absence of reactivity confirmed the observed regiospecificity of the nucleophilic substitution of N_1', due to the steric hindrance caused by bulky substituent's in R_3'. Moreover, the use of microwaves to shorten reaction time and improve yield,[17,25] in order to obtain compounds **1–6**, did not prove to be efficient. Concerning the synthesis of compounds **7–10** (Table 2), **7–8** were directly obtained by basic hydrolysis of **5** and **6**, respectively. Recently, quinolones bearing an ethyl group at the N_4' position were shown to reduce the emergence of resistant mutants.[26] Hence, we synthesized N_4'–Et quinolones (**9–10**) in order to assess the impact of N_4'-ethylation on their biological activity against quinolone-resistant strains. Thus, to begin with, compounds **5Et** and **6Et** were obtained after alkylation of both secondary amine and carboxylic acid (Table 2). The last step was the basic hydrolysis of both ethyl ester groups to obtain the corresponding dicarboxylic acids **9–10** with moderate to good yields**.**

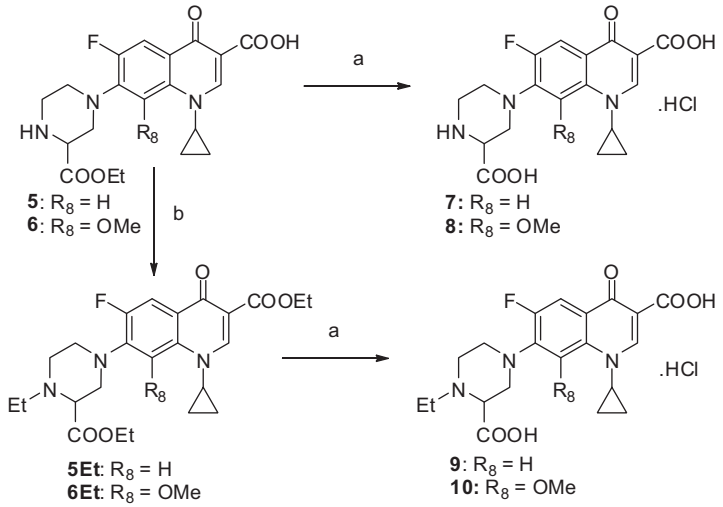
2.2. Antimicrobial activity

In order to explore the importance of substituents in the R_3' position on the piperazine rings, compounds **1–10** were compared to well known quinolones (ofloxacin, ciprofloxacin, moxifloxacin and gatifloxacin). The ten compounds were screened for their capacity to inhibit (i) the growth of *M. tuberculosis* (MIC) and (ii) the supercoiling activity of wild-type (WT) *M. tuberculosis* and *M. leprae* DNA gyrases (IC_{50}); then (iii) they were screened for their capacity to induce 25% cleavage (CC_{25}) of DNA from *M. leprae* WT and mutant (G89C and A91 V) strains. Residues 89 and 91 correspond to amino acids 88 and 90, and 81 and 83, in the *M. tuberculosis* and *Escherichia coli* numbering systems, respectively. The results are shown in Table 3.

2.2.1. MIC determination

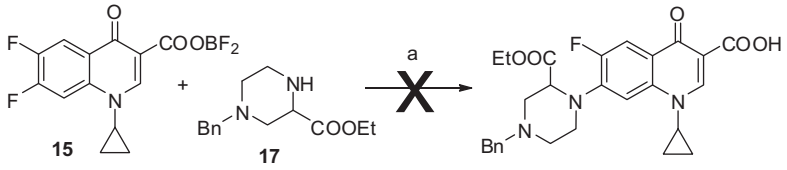
The compounds were screened for their in vitro activity against *M. tuberculosis* H37Rv comparatively to the in vitro activity of well known quinolones. Compound **5**, with a MIC of 4 μg/mL, was the most potent inhibitor in the series. Indeed,

Table 2
Synthesis of compounds **7–10**



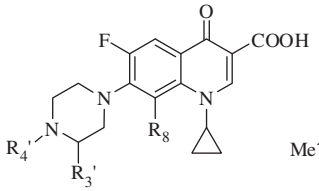
Compounds	R_8	R_3'	R_4'	Yield (%)
5Et	H	COOEt	Et	39
6Et	OMe	COOEt	Et	44
7	H	COOH	H	56
8	OMe	COOH	H	78
9	H	COOH	Et	75
10	OMe	COOH	Et	41

Reagents and conditions: (a) CH_3OH/H_2O:4:1, LiOH, rt, 1–5 days; (b) DMF, Cs_2CO_3 or $NaHCO_3$, EtI, 60 °C, 1–3 days.

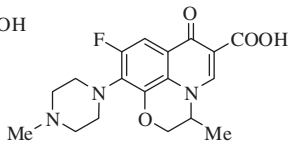


Scheme 2. Synthetic pathway explored to selectively obtain novel 2′-substituted piperazinyl quinolones. Reagents and conditions: (a) CH_3CN, NEt_3, reflux, 70 h.

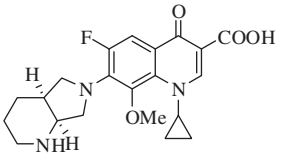
Table 3
Activities of compounds **1**–**10** inhibiting *M. leprae* DNA cleavable complex formation (CC_{25}) with wild-type and modified DNA gyrase, *M. tuberculosis* H37Rv growth (MIC), and DNA supercoiling of DNA gyrase (IC_{50})



General structure for **ciprofloxacin, gatifloxacin** and **compounds 1-10**



Ofloxacin



Moxifloxacin

Compound	R_3'	R_4'	R_8	*c*Log*P*[a]	*M. tuberculosis*		*M. leprae*			
					MIC (μg/mL)	IC_{50} (μg/mL)	IC_{50} (μg/mL)	CC_{25} (μg/mL)		
							WT	WT	A91 V	G89C
Gatifloxacin	CH_3	H	OMe	−0.27	0.125	3	3	1	15	No
Moxifloxacin	—	—	OMe	−1.50	0.5	2	2	2	12	No
Ciprofloxacin	H	H	H	−0.73	0.5	6	2	2	30	No
Ofloxacin	H	Me	Bridge C1–C8	−0.51	1	6	8	10	>240	No
1	$CH_2OC(O)NEt_2$	H	H	−0.22	128	265	118	>240	No	No
2	$CH_2OC(O)NEt_2$	H	OMe	0.86	32	50	20	60	>240	No
3	$CH_2OC(O)NHBu$	H	H	−0.33	16	30	22	30	no	No
4	$CH_2OC(O)NHBu$	H	OMe	0.76	8	17	10	10	>240	No
5	COOEt	H	H	−1.35	4	27	20	20	>240	No
6	COOEt	H	OMe	−0.27	>32	21	17	80	>240	No
7	COOH	H	H	−1.42	32	20	30	30	>240	No
8	COOH	H	OMe	−1.48	16	16	20	27	>240	No
9	COOH	Et	H	−0.28	32	48	35	>240	No	No
10	COOH	Et	OMe	−0.34	32	87	55	>240	>240	No

No: not observable.
>240: observable but 25% of cut was not reached.
[a] *c*Log*P* values were calculated using ChemDrawUltra10.0.

compared to that of ofloxacin, the MICs of compounds **5** and **4** were four to eightfold higher, whereas the MICs of compounds **1**–**3** and **6**–**10** were at least 16-fold higher. With R_8 = H, and considering the functional group in R_3', the rank of order for antimycobacterial activity decreased from COOEt (as in **5**), $CH_2OC(O)NHBu$ (as in **3**), COOH (as in **7** and **9**), to $CH_2OC(O)NEt_2$ (as in **1**). For R_8 = OMe, the rank of order was similar except that the compound with COOEt (as in **6**) in R_3' was the least active (Table 3). Considering the nature of the substitution in R_8, compounds harboring OMe were more potent than their hydrogeno counterparts, except for compound **6**.

2.2.2. Inhibition of *M. tuberculosis* and *M. leprae* wild-type DNA gyrases

Among the compounds tested against *M. tuberculosis* and *M. leprae* WT DNA gyrases, compounds **3**–**8** proved to be the most efficient inhibitors, with an $IC_{50} \leqslant 30$ μg/mL. IC_{50}s varied depending on the nature of the 3′ substituent on the piperazine ring. For R_8 = H, IC_{50}s decreased in the order $CH_2OC(O)NHBu$ (as in **3**), COOEt (as in **5**), and COOH (as in **7**), to $CH_2OC(O)NEt_2$ (as in **1**) and COOH (with R_4' = Et as in **9**) while for R_8 = OMe, IC_{50}s decreased in the order $CH_2OC(O)NHBu$ (as in **4**), COOEt (as in **6**), and COOH (as in **8**), to $CH_2OC(O)NEt_2$ (as in **2**) and COOH (with R_4' = Et as in **10**). As observed for MICs, considering the nature of the substitution in R_8, compounds harboring a OMe were more potent than their hydrogeno counterparts, except for compound **10** where it resulted in a small decrease of the inhibitory activity.

2.2.3. Drug-inducible DNA cleavage by *M. leprae* gyrase

With *M. leprae* WT DNA gyrase, compounds **3**–**5** and **7**–**8** showed the best induction of DNA cleavage at CC_{25}s below 30 μg/mL. Compound **4** was as active as ofloxacin with a CC_{25} of 10 μg/mL (Table 3), whereas compounds **1**, **6, 9** and **10** had CC_{25}s ranging from 8-fold higher (**6**) to undeterminable (**1** and **9**–**10**). CC_{25} values varied depending on the nature of the 3′ substituent on the piperazine ring. For R_8 = H, CC_{25} values decreased in the order: $CH_2OC(O)NHBu$ (as in **3**), COOEt (as in **5**), and COOH (as in **7**), to $CH_2OC(O)NEt_2$ (as in **1**) and COOH (with R_4' = Et as in **9**) while for R_8 = OMe they decreased in the order: $CH_2OC(O)NHBu$ (as in **4**) and COOH (as in **8**), to $CH_2OC(O)NEt_2$ (as in **2**) and COOEt (as in **6**), and to COOH (with R_4' = Et as in **10**). In contrast to what was observed for MICs and IC_{50}s, no clear conclusion concerning cleavage activity can be drawn from the nature of the substitution in R_8. Indeed, with carbamates in R_3' (i.e., **1**–**4**), compounds bearing a OMe in R_8 were equally or more active than their hydrogeno counterparts, whereas with a carboxylic acid function in R_3' (i.e.,

7–**10**), the presence or absence of a OMe did not make any difference, and compound **5** with an ethyl ester function in R_3' was four-times more active than its OMe derivative **6**.

Regarding mutated *M. leprae* DNA gyrases, all compounds were equally poorly active against the two mutated strains (Table 3).

2.3. General discussion of structure–activity relationships

Considering all biological results, the most successful substitutions at R_3' were secondary carbamates (**4**) and ethyl ester (**5**) functions, whereas a tertiary carbamate function in R_3' (**1**–**2**) and ethylation in N_4' (**9**–**10**) abolished activity. Concerning the impact of the number of hydrogen bond donors/acceptors or of lipophilicity (Table 3) on the biological results, a few trends can be observed. Concerning carbamates, compounds with secondary carbamates were more potent than tertiary carbamates in all assays. Indeed, as compounds **1** and **3**, and compounds **2** and **4,** had the same lipophilicity, the enhanced activities of compounds **3** and **4** were linked to secondary carbamates, which was due to their hydrogen atom that might play the role of a hydrogen bond donor. But the lack of a hydrogen bond donor in R_3' did not abolish all activity, as compound **5**, substituted with an ethyl ester function at the R_3' position, proved to be very active. Concerning carboxylic acid function, compounds not ethylated at N_4' (**7**–**8**) were more active than compounds that were (**9**–**10**), and they were as active as secondary carbamates (**3**–**4**), except with respect to the MIC for *M. tuberculosis*. Finally, ethylation at N_4' did not enhance biological activities against *M. leprae* with gyrase mutations implicated in quinolone resistance.

2.4. Correlation between MICs, IC_{50}s and CC_{25}s

The concentrations of compounds that inhibited 50% of the DNA supercoiling activity of the *M. tuberculosis* DNA gyrase were well correlated with the concentrations required to inhibit 50% of the corresponding activity of *M. leprae* (Fig. 2, $R^2 = 0.95$).

On the other hand, MICs and IC_{50}s for *M. tuberculosis* were not proportional (data not shown)**.** This absence of nonproportionality has been noted previsouly,[13,17,27] presumably reflecting differences in the cell-permeating properties and accumulation of the different quinolones.[28] Penetration of the *M. tuberculosis* cell wall by quinolones has not been evaluated yet, because the study of the mycobacterial cell wall is still a difficult and uncertain task.[28] Nevertheless, penetration of the *M. tuberculosis* cell wall seems to be at least 100-fold less efficient than that of *E. coli*.[29] Thus, the MICs of compounds **7**–**10**, which differed only by N_4'-ethylation and the substitution in R_8, were similar but the IC_{50} values varied: the IC_{50}s of compounds **9** and **10,** ethylated in N_4', were at least twice as high as those of **7** and **8,** which were not ethylated in N_4'**.** Ethyl substitution decreased bacterial target affinity, which could be compensated for by a better cell-wall penetration, as suggested by their higher *c*log*P* values.

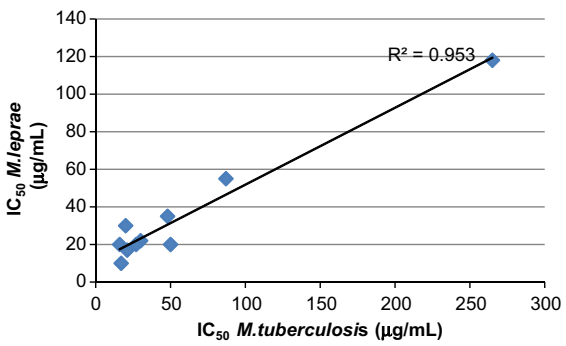


Figure 2. Correlation between IC_{50} against *M. leprae* and IC_{50} against *M. tuberculosis*.

It was noticed that the concentration of quinolones required to inhibit DNA supercoiling by gyrase is substantially higher than that required to inhibit growth, as observed previously,[13,30] due to the poisoning effect of quinolones interacting with the topoisomerases.

Finally, the DNA cleavage assay was thought to be more relevant than the supercoiling inhibition assay for measurement of the activity of a gyrase inhibitor.[27,31] However, it seems that this conclusion does not apply to *M. leprae*, as the effective quinolone concentrations measured by the DNA cleavage assay were slightly different from those measured by the supercoiling assay and were less well correlated with the concentrations inhibiting *M. tuberculosis* growth. The DNA gyrase supercoiling inhibition assay and DNA gyrase cleavable-complex assay are distinct in that the former measures catalytic inhibition, whereas the latter probes an established equilibrium between the ternary DNA–enzyme–drug complexes in which the DNA is either broken or intact.[31]

2.5. Conclusions

In conclusion, this work describes the synthesis, characterization, and evaluation, using *M. tuberculosis* and *M. leprae*, of ten gatifloxacin derivatives obtained by reaction of 8-hydrogeno (**15**) and 8-methoxy (**16**) 1-cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylato-difluoro–boron with several substituted piperazines. Taking into account all biological results, none of our gatifloxacin derivatives were more active than existing quinolones (Table 3). With biological activities close to those of ofloxacin, the two most active compounds were **4** (R_8 = OMe and R_3' = $CH_2OC(O)NEt_2$) and **5** (R_8 = H and R_3' = COOEt). It is evident then that a OMe substituent in R_8 is not mandatory in the search for potent antimycobacterial quinolones. Finally, since R_3' substitutions have been poorly investigated to date and never with the substituents explored here, our results will help the future design of new quinolones. Indeed, our results prove that piperazines can be functionalized in R_3' in various ways (e.g., by secondary carbamate or ester functions) without a deleterious effect on the biological activity. Moreover, as the N_4'–H bond has a crucial importance in the quinolone backbone, this R_3' substitution offers a supplementary possibility for the synthesis of quinolone–drug conjugates, quinolone hybrids or quinolone prodrugs.

3. Materials and methods

3.1. Reagents

The following four quinolones were provided by their corresponding manufacturers: gatifloxacin (Grünenthal, Levallois-Perret, France); ciprofloxacin and moxifloxacin (Bayer Pharma, Puteaux, France); and ofloxacin (Sigma–Aldrich Chimie, Saint Quentin Fallavier, France).

3.2. In vitro antimicrobial activity

M. tuberculosis H37Rv was grown on Löwenstein-Jensen medium. MICs were determined by the proportion method as described previously.[32] Briefly, 10^3 and 10^5 CFU were spread onto 7H11 agar supplemented with 10% oleic acid–albumin–dextrose-catalase and containing serial twofold dilutions of the compound[2]. Colonies were enumerated after 21–30 days of incubation at 37 °C. The MIC was defined as the drug concentration at which the bacterial growth was reduced to 1% or less of that of the drug-free control culture.[33]

3.3. DNA supercoiling assay

Recently, we developed an in vitro assay that replaces the time-consuming mouse footpad system for antimicrobial evaluation in *M. leprae*.[11] *M. tuberculosis* and *M. leprae* DNA gyrase was purified as described previously.[11,13] The reaction mixture (total volume, 30 μl) contained DNA gyrase assay buffer (40 mM Tris–HCl [pH 7.5], 25 mM KCl, 6 mM magnesium acetate, 2 mM spermidine, 4 mM dithiothreitol, bovine serum albumin [0.36 μg/mL], 10 mM potassium glutamate, 1 mM ATP [pH 8.0]) and relaxed pBR322 DNA (0.4 μg) as the substrate. Gyrase proteins (300 ng of GyrA and 250 ng of GyrB) were mixed in the presence of increasing concentrations of quinolones for 1 h at 37 °C for *M. tuberculosis* and 2 h at 30 °C for *M. leprae*. Reactions were terminated by the addition of 50% glycerol containing 0.25% bromophenol blue, and the total reaction mixture was subjected to electrophoresis in a 1% agarose gel in 0.5× TBE (Tris–borate–EDTA, pH 8.3) buffer. After electrophoresis for 5.5 h at 50 V, the gel was stained with ethidium bromide (0.7 μg/mL). The inhibitory effect of quinolones on DNA gyrase was assessed by determining the concentration of drug required to inhibit the supercoiling activity of the enzyme by 50% (IC_{50}). Supercoiling activity was assessed by tracing the brightness of the bands corresponding to the supercoiled pBR322 DNA with Molecular Analyst software (Bio-Rad).

3.4. DNA cleavage assay

M. leprae DNA gyrases were purified as described previously.[11,12] The reaction mixture (total volume, 20 μL) contained the DNA gyrase assay buffer (40 mM Tris–HCl [pH 7.5], 25 mM KCl, 20 mM magnesium acetate, 2 mM spermidine, 4 mM dithiothreitol, 0.1 μg/mL of yeast tRNA, bovine serum albumin [0.36 μg/mL], 3 mM ATP [pH 8.0]) and supercoiled pBR322 DNA (0.4 μg) as the substrate. Three hundred nanograms of *M. leprae* GyrA and 250 ng of *M. leprae* GyrB were mixed in the presence of increasing concentrations of quinolones for 1 h at 30 °C. Three microliters of 2% SDS were added to separate the free DNA from the cleaved DNA covalently linked to DNA gyrase, and 3 μL of a 1 μg/mL solution of proteinase K was added to remove the covalently bound GyrA protein. Incubation was continued for 30 min at 37 °C. The reactions were stopped as described above for supercoiling. The DNA products were examined by agarose gel electrophoresis, and the drug concentration that resulted in 25% DNA cleavage (CC_{25}) was determined.

3.5. Correlation between MICs, IC_{50}s and CC_{25}s

The relationships between the MICs and IC_{50}s or CC_{50}s were assessed by estimating a linear regression between two components. The strength of this relationship was quantified by the R^2 coefficient and displayed graphically by the regression line.

3.6. Synthesis

3.6.1. General chemistry methods

All materials were obtained from commercial suppliers and used without further purification. Thin-layer chromatography was performed on TLC plastic sheets of silica gel 60F254 (layer thickness 0.2 mm) from Merck. Column chromatography purification was carried out on silica gel 60 (70–230 mesh ASTM, Merck). Melting points were determined either on a digital melting point apparatus (Electrothermal IA 8103) and are uncorrected or on a Kofler bench type WME (Wagner & Munz). IR, ^1H, ^{19}F and ^{13}C NMR spectra were used to confirm the structures of all compounds. IR spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrometer and NMR spectra were recorded, using $CDCl_3$, CD_3CN, D_2O or DMSO-d_6 as solvent, on a BRUKER AC 300 or 400 spectrometer at 300 or 400 MHz for ^1H, 75 or 100 MHz for ^{13}C and 282 or 377 MHz for ^{19}F spectra. Chemical shifts (δ) were expressed in parts per million relative to the signal indirectly (i) to $CHCl_3$ (δ 7.27 for ^1H and (ii) to $CDCl_3$ (δ 77.2) for ^{13}C and directly (iii) to $CFCl_3$ (internal standard) (δ 0.0) for ^{19}F. Chemical shifts are given in ppm and peak multiplicities are designated as follows: s, singlet; br s, broad singlet; d, doublet; dd, doublet of doublet; t, triplet; q, quadruplet; quint, quintuplet; m, multiplet. High resolution mass spectra (HRMS) of the ten final compounds (**1–10**) were obtained from the ′Service Central d′analyse de Solaize′ (Centre Nationale de la Recherche Scientifique) and were recorded on a Waters spectrometer using electrospray ionization-TOF (ESI-TOF).

The synthesis of compounds **13–16** has been previously described.[21,23,34] Compounds **1–10** were synthesized according to procedures described below and were dissolved in DMSO or NaOH for biological tests.

3.6.2. Ethyl 1-cyclopropyl-7-(3′-(ethoxycarbonyl)-4′-ethylpiperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate 5Et

A solution of **5** (0.107 g, 0.26 mmol) in 10 mL of DMF, $NaHCO_3$ (61.2 mg, 0.73 mmol) and iodoethane (0.074 mL, 0.925 mmol) was vigorously stirred at 90 °C during 60 h. DCM and water were added, after extraction with DCM (3 × 30 mL), the organic layers were collected, washed with water (3 × 30 mL), dried over $MgSO_4$, filtered and evaporated. The oily residue was purified by flash chromatography on silica gel (DCM/MeOH: 96:4) affording the product $\mathbf{5_{Et}}$ as a colorless oil (44 mg, 0.102 mmol, 39%).

Note: $\mathbf{5_{Et}}$ was obtained as a mixture with the corresponding carboxylic acid in C_3, which was not a problem as the next step consisted in the hydrolysis of both ester functions.

^1H NMR ($CDCl_3$): δ 1.07–1.11 (m, 5H, CH_2(cPr) and NCH_2CH_3), 1.24–1.28 (m, 5H, CH_2(cPr) and OCH_2CH_3), 1.36 (t, 3H, OCH_2CH_3, J_{H-H} = 7.14Hz), 2.57 (m, 2H, H_2' and NC*H*), 2.79 (m, 1H, NC*H*), 3.29–3.47 (m, 7H, H_2', H_3', H_5' and H_6'), 4.17 (q, 2H, $COO(CH_2)CH_3$, J_{H-H} = 7.1Hz), 4.20 (q, 2H, $COOCH_2$, J_{H-H} = 7.1Hz), 4.36 (q, 2H, $COOCH_2$, J_{H-H} = 7.1Hz), 7.23 (d, 1H, H_8, J_{H-F} = 5.9Hz), 8.00 (d, 1H, H_5, J_{H-F} = 13.1Hz), 8.45 (s, 1H, H_2).

^{19}F NMR ($CDCl_3$) δ −123.9 s, 1F, F_6).

3.6.3. Ethyl 1-cyclopropyl-7-(3′-(ethoxycarbonyl)-4′-ethylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate 6Et

A solution of **6** (152 mg, 0.35 mmol) in 10 mL of DMF, Cs_2CO_3 (392 mg, 1.20 mmol) and iodoethane (0.20 mL, 0.315 mmol) was vigorously stirred at 60 °C during 19 h. DCM and water were added, after extraction with DCM (3 × 30 mL), the organic layers were collected, washed with water (3 × 30 mL), dried over $MgSO_4$, filtered and evaporated. The oily residue was purified by flash chromatography on silica gel (DCM/MeOH: 96:4) affording the product $\mathbf{6_{Et}}$ as a brown oil (75 mg, 0.153 mmol, 44%). ^1H NMR ($CDCl_3$): δ 0.89 (m, 2H, CH_2(cPr)), 1.07–1.12 (m, 5H, NCH_2CH_3 and CH_2(cPr)), 1.21 (t, 2H, OCH_2, J_{H-H} = 7.0Hz), 1.34 (t, 2H, OCH_2, J_{H-H} = 7.0Hz), 2.54 (m, 2H, H_2' and NCH_2), 2.75–2.80 (m, 1H, NCH_2), 3.25–3.30 (m, 1H, H_2') 3.30–3.35 (m, 3H, H_3' and $2H_6'$), 3.53–3.56 (m, 1H, H_5'), 3.69 (s, 3H, OCH_3), 3.83 (quint., 1H, CH(cPr)), 4.15 (q, 2H, OCH_2, J_{H-H} = 7.1Hz), 4.32 (q, 2H, OCH_2, J_{H-H} = 7.1Hz), 7.81 (d, 1H, H_5, J_{H-F} = 12.4Hz), 8.51 (s, 1H, H_2). ^{19}F NMR ($CDCl_3$) δ −121.6 s, 1F, F_6). ^{13}C NMR ($CDCl_3$): 9.3 and 9.4 ($2CH_2$(cPr)), 14.2 and 14.4 (2 OCH_2CH_3), 39.4 (CH(cPr)), 49.0 ($C_{2'}$), 49.6 (NCH_2), 50.7 ($C_{5'}$), 53.3 ($C_{6'}$), 60.7 and 60.8 (2 OCH_2), 62.6 (OCH_3), 63.5 ($C_{3'}$), 108.9 (d, C_5, $^3J_{C-F}$ = 23.3 Hz), 109.9 (C_3), 125.6 (d, C_{10}, $^3J_{C-F}$ = 8.0 Hz), 132.8 (s, C_9), 137.5 (d, C_8, $^3J_{C-F}$ = 12.0 Hz), 145.7 (s, C_7), 150.6 (s, C_2), 155.8 (d, C_6, $^1J_{C-F}$ = 257.1 Hz), 165.6 (COOH), 166.2 and 171.4 (2 COOEt), 172.7 (d, C_4, $^4J_{C-F}$ = 2.2 Hz).

3.6.4. General procedure for the synthesis of compounds (1–6)

A mixture of the suitable difluoro–boron quinolone **15–16** (1.0 equiv) and the suitable piperazine (2.3 equiv) and dry NEt_3 (5.0 equiv) was stirred at refluxed of dry CH_3CN (3 mL) between one day and one week. After evaporation, the crude residue was taken up in a 1:1 $CHCl_3/H_2O$ mixture. After extraction, the organic layer was washed with water (2 × 5 mL) and dried over $MgSO_4$. The oily residue was purified by flash chromatography ($CHCl_3$/MeOH: 10:0 to 9:1) and then by recrystallization (H_2O/CH_3CN: 9:1) and lyophilization to afford the desired target compounds (**1–6**).

3.6.4.1. 1-Cyclopropyl-7-(3′-((diethylcarbamoyloxy)methyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (1).

Compound **1** was synthesized (yellow powder, 114 mg, 0.25 mmol, **55%**) according to the general procedure described above, starting from **15** (140 mg, 0.45 mmol) and piperazin-2-ylmethyl diethyl carbamate (297 mg, 1.03 mmol). ^{1}H NMR (CD_3CN) δ 1.10–1.13 (m, 8H, CH_2(cPr) and 2CH_3), 1.27–1.34 (m, 2H, CH_2(cPr)), 2.76 (app t, 1H, $H_{2'}$, $^3J_{H-H}$ = 10.7 Hz), 2.93–3.02 (m, 2H, $H_{6'}$ and $H_{5'}$), 3.09–3.20 (m, 2H, $H_{3'}$ and $H_{5'}$), 3.27 (q, 4H, NCH_2, $^3J_{H-H}$ = 7.0 Hz), 3.58–3.71 (m, 3H, $H_{2'}$,$H_{6'}$ and CH(cPr)), 4.04 (d, 2H, OCH_2, $^3J_{H-H}$ = 5.8 Hz), 7.54 (d, 1H, H_8, $^4J_{H-F}$ = 7.4 Hz), 7.94 (d, 1H, H_5, $^3J_{H-F}$ = 13.4 Hz), 8.70 (s, 1H, H_2), 15.18 (br s, 1H, OH). ^{19}F NMR (CD_3CN) δ −123.0 s, 1F, F_6). ^{13}C NMR (CD_3CN) δ 9.2 (s, CH_2(cPr)), 14.3 and 15.1 (s, 2CH_3), 37.3 (s, CH(cPr)), 42.8 and 43.1 (s, 2CH_2), 46.3 (s, $C_{5'}$), 52.2 (d, $C_{6'}$, $^4J_{C-F}$ = 4.6 Hz), 54.6 (d, $C_{2'}$, $^4J_{C-F}$ = 4.4 Hz), 55.6 (s, $C_{3'}$), 67.7 (s, OCH_2), 107.9 (d, C_8, $^3J_{C-F}$ = 3.4 Hz), 109.0 (s, C_3), 112.8 (d, C_5, $^2J_{C-F}$ = 23.3 Hz), 120.9 (d, C_{10}, $^3J_{C-F}$ = 7.8 Hz), 141.2 (s, C_9), 147.6 (d, C_7, $^2J_{C-F}$ = 10.0 Hz), 149.6 (s, C_2), 155.2 (d, C_6, $^1J_{C-F}$ = 247.7 Hz), 157.0 (s, NC(O)O), 168.2 (s, COOH), 178.8 (d, C_4, $^4J_{C-F}$ = 2.4 Hz). HR-ESMS: *m/z*: calculated for $C_{23}H_{30}FN_4O_5$: 461.2200; found 461.2174 $[M+H]^+$ IR (ATR): 2988 (OH acide), 1696 (ester), 1625 (amide), 1483 (C=C), 1456 (C=C), 1385 (C–N, C–C, C–O), 1268 (C–N, C–C, C–O), 1171 (C–N, C–C, C–O), 1072 (C–F). Mp = 124 °C.

3.6.4.2. 1-Cyclopropyl-7-(3′-((diethylcarbamoyloxy)methyl)piperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (2).

Compound **2** was synthesized (yellow powder, 50 mg, 0.10 mmol, 23%) according to the general procedure described above, starting from **16** (150 mg, 0.44 mmol) and piperazin-2-ylmethyl diethyl carbamate (292 mg, 1.02 mmol). ^{1}H NMR (CD_3CN) δ 0.85–0.89 (m, 2H, CH_2(cPr)), 0.95–1.06 (m, 8H, 2CH_3 and CH_2(cPr)), 2.87–2.98 (m, 3H, $H_{2'}$ and 2$H_{5'}$), 3.05–3.20 (m, 2H, $H_{3'}$ and $H_{6'}$), 3.18 (q, 4H, 2CH_2, $^3J_{H-H}$ = 7.0 Hz), 3.32 (d, 1H, $H_{6'}$, $^3J_{H-H}$ = 10.8 Hz), 3.43 (d, 1H, $H_{2'}$, $^3J_{H-H}$ = 11.5 Hz), 3.68 (s, 3H, OCH_3), 3.91–3.96 (m, 2H, OCH_2), 4.00–4.07 (m, 1H, CH(cPr)), 7.71 (d, 1H, H_5, $^3J_{H-F}$ = 12.4 Hz), 8.66 (s, 1H, H_2), 14.85 (br s, 1H, OH). ^{19}F NMR (CD_3CN) δ −121.3 s, 1F, F_6). ^{13}C NMR (CD_3CN) δ 9.9 (s, 2CH_2(cPr)), 13.7 and 14.5 (s, 2CH_3), 41.7 (s, CH(cPr)), 42.1 and 42.6 (s, CH_2), 46.6 (s, $C_{5'}$), 52.1 (d, $C_{6'}$, $^4J_{C-F}$ = 17.2 Hz), 54.7 (d, $C_{2'}$, $^4J_{C-F}$ = 14.6 Hz), 55.6 (s, $C_{3'}$), 63.5 (s, OCH_3), 66.9 (s, OCH_2), 107.9 (d, C_5, $^2J_{C-F}$ = 23.3 Hz), 108.2 (s, C_3), 122.6 (d, C_{10}, $^3J_{C-F}$ = 8.9 Hz), 135.6 (s, C_9), 140.7 (d, C_7, $^2J_{C-F}$ = 11.6 Hz), 147.2 (s, C_8), 151.6 (s, C_2), 156.5 (s, NC(O)O), 157.2 (d, C_6, $^1J_{C-F}$ = 249.0 Hz), 167.3 (s, C(O)O), 178.1 (s, C_4). HR-ESMS: *m/z*: calculated for $C_{24}H_{32}FN_4O_6$: 491.2306; found 491.2279 $[M+H]^+$ IR (ATR): 2975 (OH acide), 1728 (ester), 1689 (cetone), 1617 (amide), 1438 (C=C), 1315 (C–N, C–C, C–O), 1272 (C–N, C–C, C–O), 1172 (C–N, C–C, C–O), 1058 (C–F). Mp = 100 °C.

3.6.4.3. 7-(3′-((Butylcarbamoyloxy)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3).

Compound **3** was synthesized (beige powder, 51 mg, 0.11 mmol, 23%) according to the general procedure described above, starting from **15** (149 mg, 0.48mmol) and piperazin-2-ylmethyl butyl carbamate (356 mg, 1.24 mmol). ^{1}H NMR (CD_3CN) δ 0.84–0.88 (m, 2H, CH_2(cPr)), 0.86 (appt, 3H, CH_3, $^3J_{H-H}$ = 7.2 Hz), 1.22–1.47 (m, 6H, CH_2(cPr) and CH_2), 2.86–2.95 (m, 1H, $H_{2'}$), 2.99–3.14 (m, 4H, $H_{6'}$, $H_{5'}$, NCH_2), 3.16–3.24 (m, 2H, $H_{2'}$, $H_{5'}$), 3.26–3.33 (m, 1H, $H_{3'}$), 3.56–3.71 (m, 3H, $H_{2'}$, $H_{6'}$, CH(cPr)), 4.05–4.15 (m, 2H, OCH_2), 7.54 (d, 1H, H_8, $^4J_{H-F}$ = 6.9 Hz), 7.90 (d, 1H, H_5, $^3J_{H-F}$ = 13.4 Hz), 8.73 (s, 1H, H_2), 14.85 (br s, 1H, OH). ^{19}F NMR (CD_3CN) δ −121.2 s, 1F, F_6). ^{13}C NMR (CD_3CN) δ 8.6 (s, CH_2(cPr)), 14.1 (s, CH_3), 20.6 and 32.5 (s, CH_2), 36.9 (s, CH(cPr)), 41.3 (s, NCH_2), 44.7 (s, $C_{5'}$), 49.9 (s, $C_{6'}$), 52.1 (s, $C_{2'}$), 54.6 (s, $C_{3'}$), 65.1 (s, OCH_2), 107.7 (d, C_8, $^3J_{C-F}$ = 4.0 Hz), 108.1 (s, C_3), 112.4 (d, C_5, $^2J_{C-F}$ = 23.2 Hz), 120.9 (d, C_{10}, $^3J_{C-F}$ = 8.0 Hz), 135.5 (s, C_9), 140.5 (s, C_7), 149.5 (s, C_2), 156.2 (d, C_6, $^1J_{C-F}$ = 247.7 Hz), 157.8 (s, NC(O)O), 174.9 (s, COOH), 178.1 (s, C_4). HR-ESMS: *m/z*: calculated for $C_{23}H_{30}FN_4O_5$: 461.2200; found 461.2178 $[M+H]^+$ IR (ATR): 2960 (OH acide), 1718 (ester), 1624 (amide), 1488 (C=C), 1303 (C–N, C–C, C–O), 1266 (C–N, C–C, C–O), 1102 (C–F). Mp = 225 °C.

3.6.4.4. 7-(3′-((Butylcarbamoyloxy)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (4).

Compound **4** was synthesized (yellow powder, 35 mg, 0.072 mmol, 18%) according to the general procedure described above, starting from **16** (138 mg, 0.40 mmol) and piperazin-2-ylmethyl butyl carbamate (300 mg, 1.05 mmol). ^{1}H NMR (CD_3CN) δ 0.90 (app. t, 3H, CH_3, $^3J_{H-H}$ = 7.3 Hz), 0.94–0.99 (m, 2H, CH_2(cPr)), 1.11–1.48 (m, 6H, CH_2(cPr) and 2 CH_2), 2.94–3.15 (m, 6H, $H_{5'}$, $H_{6'}$ and NCH_2), 3.19–3.29 (m, 1H, $H_{3'}$), 3.36–3.49 (m, 2H, $H_{2'}$), 3.95–4.01 (m, 1H, CH(cPr)), 4.08–4.16 (m, 2H, OCH_2), 7.80 (d, 1H, H_5, $^3J_{H-F}$ = 12.5 Hz), 8.75 (s, 1H, H_2), 14.90 (br s, 1H, OH). ^{19}F NMR (CD_2Cl_2) δ −120.1 s, 1F, F_6). ^{13}C NMR (CD_2Cl_2) δ 9.8 (s, 2CH_2(cPr)), 13.9 (s, CH_3), 20.3 and 32.4 (s, 2CH_2), 40.9 (s, CH(cPr)), 41.2 (s, NCH_2), 46.1 (s, $C_{5'}$), 51.6 (s, $C_{6'}$), 51.7 (s, $C_{2'}$), 55.1 (s, $C_{3'}$), 62.9 (s, OCH_3) 66.1 (s, OCH_2), 107.6 (d, C_5, $^2J_{C-F}$ = 24.5 Hz), 108.1 (s, C_3), 122.3 (d, C_{10}, $^3J_{C-F}$ = 8.8 Hz), 134.5 (s, C_9), 140.0 (d, C_7, $^2J_{C-F}$ = 11.4 Hz), 146.1 (d, C_8, $^3J_{C-F}$ = 5.1 Hz) 150.4 (s, C_2), 156.6 (d, C_6, $^1J_{C-F}$ = 251.0 Hz), 156.5 (s, NC(O)O), 166.9 (s, COOH), 177.5 (d, C_4, $^3J_{C-F}$ = 3.0 Hz). HR-ESMS: *m/z*: calculated for $C_{24}H_{32}FN_4O_6$: 491.2306; found 491.2298 $[M+H]^+$ IR (ATR): 3320 (OH acide), 1714 (ester), 1616 (cetone), 1442 (C=C), 1316 (C–N, C–C, C–O), 1270 (C–N, C–C, C–O), 1246 (C–N, C–C, C–O), 1057 (C–F). Mp = 104 °C.

3.6.4.5. 1-Cyclopropyl-7-(3′-ethoxycarbonylpiperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-carboxylic acid (5).

Compound **5** was synthesized (yellow powder, 103 mg, 0.24 mmol, 47%) according to the general procedure described above, starting from **15** (158 mg, 0.51 mmol) and ethyl-piperazinyl-2-carboxylate (304 mg, 1.32 mmol). ^{1}H NMR (CD_3CN) δ 1.02–1.10 (m, 2H, CH_2(cPr)), 1.18 (t, 3H, CH_3, $^3J_{H-H}$ = 7.0 Hz), 1.26–1.28 (m, 2H, CH_2(cPr)), 3.02–3.15 (m, 1H, $H_{5'}$), 3.19–3.33 (m, 2H, $H_{6'}$ and $H_{5'}$), 3.39–3.50 (m, 2H, $H_{2'}$ and $H_{6'}$), 3.53–3.63 (m, 1H, CH(cPr)), 3.72 (d, 1H, $H_{2'}$, $^3J_{H-H}$ = 12.4 Hz), 3.94 (d, 1H, $H_{3'}$, $^3J_{H-H}$ = 4.8 Hz), 4.15 (q, 2H, CH_2, $^3J_{H-H}$ = 7.0 Hz), 7.49 (d, 1H, H_8, $^4J_{H-F}$ = 7.3 Hz), 7.84 (d, 1H, H_5, $^3J_{H-F}$ = 13.0 Hz), 8.60 (s, 1H, H_2). ^{19}F NMR (CD_3CN) δ −123.3 s, 1F, F_6), −151,6 s, 0.44F, BF_2). ^{13}C NMR (CD_3CN) δ 8.7 (s, CH_2(cPr)), 14.5 (s, CH_3), 36.8 (s, CH(cPr)), 44.2 (s, $C_{5'}$), 49,7 (d, $C_{6'}$, $^4J_{C-F}$ = 3.1 Hz), 51.9 (d, $C_{2'}$, $^4J_{C-F}$ = 5.2 Hz), 57.3 (s, $C_{3'}$), 62.8 (s, CH_2), 107.9 (d, C_8, $^3J_{C-F}$ = 3.0 Hz), 108.4 (s, C_3), 112.4 (d, C_5, $^2J_{C-F}$ = 23.2 Hz), 120.9 (s, C_{10}, $^3J_{C-F}$ = 7.9 Hz), 140.4 (s, C_9), 146.0 (d, C_7, $^2J_{C-F}$ = 10.5 Hz), 149.2 (s, C_2), 154.6 (d, C_6, $^1J_{C-F}$ = 247.9 Hz), 167.6 (s, COOH), 170.6 (s, COOEt), 178.0 (s, C_4). HR-ESMS: *m/z*: calculated for $C_{20}H_{23}FN_3O_5$: 404.1621; found 404.1610 $[M+H]^+$ IR (ATR): 3002 (OH acide), 1730 (ester), 1628 (cetone), 1492 (C=C), 1458 (C=C), 1381 (C–N, C–C, C–O), 1337 (C–N, C–C, C–O), 1268 (C–N, C–C, C–O), 1055 (C–F). Mp >300 °C.

3.6.4.6. 1-Cyclopropyl-7-(3′-ethylcarbonylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-carboxylic acid (6). Compound **6** was synthesized (yellow powder, 97 mg, 0.22 mmol, 51%) according to the general procedure described above, starting from **16** (152 mg, 0.44 mmol) and ethyl-piperazinyl-2-carboxylate (266 mg, 1.15 mmol). ^{1}H NMR (CD_3CN) δ 0.91–1.01 (m, 2H, CH_2(cPr)), 1.08–1.12 (m, 2H, CH_2(cPr)), 1.16 (t, 3H, CH_3, $^3J_{H-H}$ = 7.1 Hz), 2.99–3.05 (m, 1H, $H_{4'}$), 3.18–3.22 (m, 1H, $H_{4'}$), 3.31–3.36 (m, 2H, $H_{3'}$), 3.45–3.53 (m, 1H, $H_{2'}$), 3.66–3.74 (m, 5H, OCH_3, $H_{1'}$ and $H_{2'}$), 4.00 (quint., 1H, CH(cPr)), 4.15 (q, 2H, CH_2, $^3J_{H-H}$ = 7.1 Hz), 7.85 (d, 1H, H_5, $^3J_{H-F}$ = 11.2 Hz), 8.79 (s, 1H, H_2). ^{19}F NMR (CD_3CN) δ −120.8 s, 1F, F_6). ^{13}C NMR (CD_3CN) δ 9.6 and 9.9 (s, 2CH_2(cPr)), 14.4 (s, CH_3), 40.7 (s, CH(cPr)), 44.9 (s, $C_{5'}$), 51.3 (d, $C_{6'}$, $^4J_{C-F}$ = 3.6 Hz), 52.9 (d, $C_{2'}$, $^4J_{C-F}$ = 4.6 Hz), 57.6 (s, $C_{3'}$), 61.7 (s, CH_2), 62.2 (s, OCH_3), 107.7 (s, C_3), 108.4 (d, C_5, $^2J_{C-F}$ = 23.4 Hz), 122.7 (d, C_{10}, $^3J_{C-F}$ = 11.4 Hz), 134.0 (s, C_9), 139.5 (d, C_7, $^2J_{C-F}$ = 10.4 Hz), 146.0 (d, C_8, $^3J_{C-F}$ = 5.6 Hz), 150.2 (s, C_2), 156.2 (d, C_6, $^1J_{C-F}$ = 248.1 Hz), 166.9 (s, COOH), 171.2 (s, C$OOEt$), 177.2 (s, C_4). HR-ESMS: m/z: calculated for $C_{21}H_{25}FN_3O_6$: 434.1727; found 434.1717 $[M+H]^+$ IR (ATR): 3348 (COO–H), 1742 (ester), 1617 (ketone), 1579 (N–H), 1462 (C=C), 1446 (C=C), 1290 (C–N, C–C, C–O), 1090 (C–N, C–C, C–O), 1058 (C–F). Mp >300 °C.

3.6.5. General procedure for the synthesis of compounds (7–10)

LiOH (10 equiv) was added to a solution of the suitable quinolone (1.0 equiv) in MeOH/H_2O: 8:2, and the resulting mixture was stirred during 3 days at room temperature. After addition of HCl (1M) until pH = 1, the precipitate was filtered and dried affording the desired targeted compounds (**7–10**).

3.6.5.1. 7-(3′-Carboxypiperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (7). Compound **7** was synthesized (white powder, 65 mg, 0.149 mmol, 56%) according to the general procedure described above, starting from **5** (107 mg, 0.265 mmol). ^{1}H NMR (D_2O/CD_3CN: 9:1): δ 1.11 (m, 2H, CH_2(cPr)), 1.36 (m, 2H, CH_2(cPr)), 3.35 (m, 2H, $H_{5'}$ and $H_{2'}$), 3.53 (m, 2H, CH(cPr) and $H_{6'}$), 3.72 (m, 2H, $H_{6'}$ and $H_{5'}$), 3.96 (m, 1H, $H_{2'}$), 4.06 (m, 1H, $H_{3'}$), 7.63 (d, 1H, H_8, J_{H-F} = 7.3Hz), 7.92 (d, 1H, H_5, J_{H-F} = 13.1Hz), 8.80 (s, 1H, H_2). ^{19}F NMR (D_2O/CD_3CN: 9:1) δ −121.0 s, 1F, F_6). ^{13}C NMR (D_2O/CD_3CN: 9:1): 6.7 (s, 2CH_2(cPr)), 33.5 (s, CH(cPr)), 37.1 (s, $C_{5'}$), 43.1 (s, $C_{6'}$), 47.0 (s, $C_{2'}$), 57.0 (s, $C_{3'}$), 107.6 (s, C_3), 108.5 (d, C_8, $^3J_{C-F}$ = 2.23 Hz), 112.5 (d, C_5, $^2J_{C-F}$ = 23.3 Hz), 121.1 (d, C_{10}, $^3J_{C-F}$ = 8.0 Hz), 140.3 (s, C_9), 145.2 (d, C_7, $^2J_{C-F}$ = 10.0 Hz), 149.7 (s, C_2), 154.5 (d, C_6, $^1J_{C-F}$ = 250.0 Hz), 169.1 (COOH), 170.0 (COOH), 178.0 (d, C_4, $^4J_{C-F}$ = 2.0 Hz). HR-ESMS: m/z: calculated for $C_{18}H_{19}FN_3O_5$: 376.1308; found 376.1303 $[M+H]^+$. Mp = 219–222 °C.

3.6.5.2. 1-Cyclopropyl-7-(3′-carboxypiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline carboxylic acid, (8). Compound **8** was synthesized (yellow powder, 120 mg, 0.272 mmol, 78%) according to the general procedure described above, starting from **6** (151 mg, 0.348 mmol). ^{1}H RMN (D_2O/CD_3CN: 9:1): δ 0.95 (m, 2H, CH_2(cPr)), 1.14 (m, 2H, CH_2(cPr)), 3.28 (m, 1H, $H_{5'}$), 3.53 (m, 2H, $H_{5'}$ and $H_{6'}$), 3.56 (m, 1H, $H_{6'}$), 3.65 (dd, 1H, $H_{3'}$ J_{H-H} = 13.7Hz, J_{H-H} = 8.0Hz), 3.74 (s, 3H, OCH_3), 3.85 (dd, 1H, H_2', J_{H-H} = 14Hz, J_{H-H} = 3.7Hz), 4.38 (dd, 1H, $H_{2'}$, J_{H-H} = 8.9Hz, J_{H-H} = 3.7Hz), 7.76 (d, 1H, H_5, J_{H-F} = 12.0Hz), 8.84 (s, 1H, H_2). ^{19}F NMR (D_2O/CD_3CN: 9:1) δ −119.5 s, 1F, F_6). ^{13}C RMN (D_2O/CD_3CN: 9:1): 8.5 and 8.8 (CH_2(cPr)), 40.9 (CH(cPr)), 42.8, 46.7 and 49.5 (CH_2), 56.4 ($C_{3'}$), 62.6 (OCH_3), 107.0 (d, C_5, $^2J_{C-F}$ = 23.8 Hz), 151.2 (C_2), 168.4 (COOH). *Other peaks were not visible due to poor solubility*. HR-ESMS: m/z: calculated for $C_{19}H_{21}FN_3O_6$: 406.1414; found 406.1409 $[M+H]^+$ Mp = 215–218 °C.

3.6.5.3. 1-Cyclopropyl-7-(3′-carboxy-4′-ethylpiperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline carboxylic acid (9). Compound **9** was synthesized (white powder, 20.6 mg, 0.047 mmol, **75**%) according to the general procedure described above, starting from **5Et** (29 mg, 0.063 mmol). ^{1}H RMN (D_2O/CD_3CN: 9:1): δ 1.46 (m, 2H, CH_2(cPr)), 1.66 (m, 5H, NCH_2(CH_3)), and CH_2(cPr)), 3.60–3.70 (m, 2H, NCH and NCH(CH_3)), 3.83 (m, 3H, NCH_2 and NCH(CH_3)), 4.02 (m, 1H, CH(cPr)), 4.18 (m, 2H), 4.33 (m, 1H), 4.48 (m, 1H), 7.87 (d, 1H, H_8, J_{H-H} = 5.6Hz), 8.05 (d, 1H, H_5, J_{H-H} = 12.8Hz), 9.02 (s, 1H, H_2). ^{19}F NMR (D_2O/CD_3CN: 9:1) δ −121.2 s, 1F, F_6). ^{13}C RMN (D_2O/CD_3CN: 9:1): 7.2 (CH_2(cPr)), 8.5 (NCH_2(CH_3)), 35.9 (CH(cPr)), 45.4, 48.7, 48.9, 50.7 (NCH_2(CH_3)), 67.1 (NCH(COOH) 105.9 (C_3), 106.8 (C_8), 111.1 (d, C_5, $^2J_{C-F}$ = 23.4 Hz), 119.3 (C_{10}), 139.1 (C_9), 143.4 (C_7), 148.3 (C_2), 153.1 (d, C_6, $^1J_{C-F}$ = 253.89 Hz), 168.4 (COOH), 176.4 (C_4). HR-ESMS: m/z: calculated for $C_{20}H_{23}FN_3O_5$: 404.1621; found 404.1611 $[M+H]^+$ Mp = 208–209 °C.

3.6.5.4. 1-Cyclopropyl-7-(3′-carboxy-4′-ethylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline carboxylic acid (10). Compound **10** was synthesized (white powder, 55 mg, 0.117 mmol, 41%) according to the general procedure described above, starting from **6Et** (75 mg, 0.286 mmol). ^{1}H RMN (D_2O/CD_3CN: 9:1): 1.26 (m, 2H, CH_2(cPr)), 1.47 (m, 2H, CH_2(cPr)), 1.62 (t, 3H, NCH_2(CH_3)), J_{H-H} = 7.1Hz), 3.60–3.70 (m, 3H, NCH and NCH_2(CH_3)), 3.80–3.90 (m, 3H), 4.02 (s, 3H, OCH_3), 4.10–4.20 (m, 2H), 4.36 (m, 1H CH(cPr)), 7.81 (d, 1H, H_5, J_{H-H} = 12.8Hz), 9.06 (s, 1H, H_2). ^{19}F NMR (D_2O/CD_3CN: 9:1) δ −119.6 s, 1F, F_6). ^{13}C RMN (D_2O/CD_3CN: 9:1): 8.4 (CH_2(cPr)), 8.7 (NCH_2(CH_3)), 40.8 (CH(cPr)), 46.4, 49.8, 50.9 (NCH_2), 62.5 (s, OCH_3), 105.7 (C_3), 106.6 (d, C_5, $^2J_{C-F}$ = 23.8 Hz), 122.0 (C_{10}), 133.8 (C_9), 137.5 (C_8), 143.4 (C_7), 150.9 (C_2), 155.7 (d, C_6, $^1J_{C-F}$ = 250.7 Hz), 168.2 (COOH), 176.5 (C_4, $^4J_{C-F}$ = 3.1Hz). HR-ESMS: m/z: calculated for $C_{21}H_{25}FN_3O_6$: 434.1727; found 434.1714 $[M+H]^+$ Mp = 201–202 °C.

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