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Lipophilic quinolone derivatives: Synthesis and *in vitro* antibacterial evaluation

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ABSTRACT

This paper reports on the design of a series of 10 novel lipophilic piperazinyl derivatives of the 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, their synthesis, their characterisation by ¹H, ¹³C and ¹⁹F NMR, IR spectroscopy and HRMS, as well as their biological activity against bacteria of medical interest. Among these derivatives, 2 were as potent as the parent quinolone against *Neisseria gonorrhoeae* whereas all the compounds displayed lower activity than the parent quinolone against other bacteria of medical interest. Our results showing that the increased lipophilicity was deleterious for antibacterial activity may help to design new quinolone derivatives in the future, especially lipophilic quinolones which have been poorly investigated previously.

Since the discovery of norfloxacin, the first fluoroquinolone (FQ),¹ which is structurally characterised by a R⁶ fluorine atom in the quinolone ring that results in improved potency and spectrum of activity, FQ have become a significant class of clinically useful antibacterial agents. The development of new FQ gave rise to several FDA-approved drugs, such as ciprofloxacin (CPX) and moxifloxacin (MXF), in which the 1-substituted-1,4-dihydro-6-fluoro-4-oxo-7-piperazinyl (or 7-octahydro-1H-pyrrolo^{3,4-b}-pyridinyl for MXF)-3-carboxylic acid moiety is the basic

scaffold (Fig. 1 for FQ numbering system).²

FQ are broad-spectrum antibacterial agents that are used for the treatment of various bacterial infections such as urinary tract infections, sexually transmitted diseases, respiratory tract infections etc.³⁻⁴ They are also recommended as second-line antituberculosis agents by the World Health Organization (WHO).⁵

However, excessive use of FQ has led to the emergence of FQ-resistant (FQ-R) bacteria. The prevalence and spread of FQ-R bacteria

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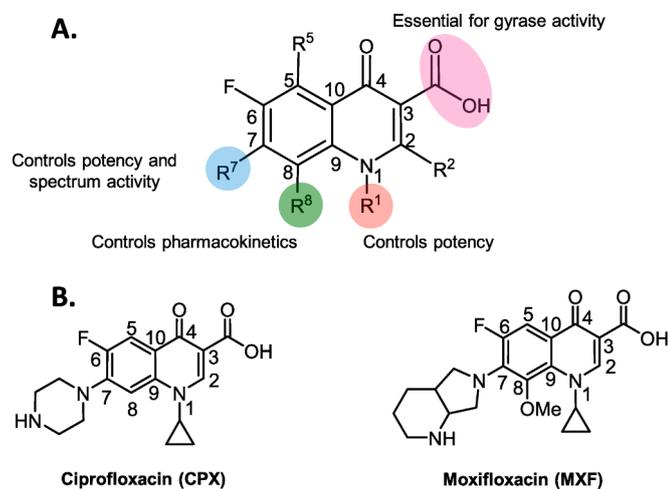


Fig. 1. Structure activity relationship of 1-substituted-1,4-dihydro-6-fluoro-4-oxo-3-carboxylic acid key scaffold, adapted from² (A) and structures of CPX and MXF (B).

have been reported among various important human pathogens including the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) as well as *Escherichia coli*, *Streptococcus pneumoniae* and *Neisseria gonorrhoeae*, and they have become a major public health concern over the past years.^{6–9}

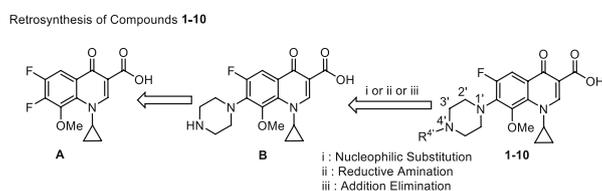
FQ are bactericidal by interfering with type II topoisomerases, especially DNA gyrase in gram-negative bacteria and topoisomerase IV (Topo IV) in gram-positive bacteria. These two topoisomerases, which are heterotetrameric A₂B₂ complexes comprised of two GyrA/GyrB and ParC/ParE subunits for DNA gyrase and Topo IV, respectively, regulate DNA topology during replication.¹⁰ Resistance to FQ mainly involves one or more amino acid substitutions in the quinolone resistance-determining region (QRDR) of the *gyrA* and/or *parC* genes, and more rarely of the *gyrB* and/or *parE* genes.¹¹

In addition, infectious diseases caused by multidrug-resistant pathogens have been associated with a higher mortality rate and longer hospital stay because of the lack of therapeutically effective drugs.^{12,13} In this context, the development of new agents active against emerging resistant bacteria is strongly desired.

In the literature, there are many examples of attempts to optimize the scaffold of FQ to improve their oral and parenteral dosing, to increase their spectrum of activity, including FQ-resistant strains, and to reduce their side effects.¹⁴

Lipophilicity of FQ is a key factor for their penetration into mammalian cells and the central nervous system. For example, introduction of a lipophilic fluorine atom at R⁶ position was a triggering event for quinolone use, but the increased lipophilicity of FQ with the fluorine at R⁸ was gained only at the expense of higher toxicity. FQ prodrugs were designed to increase their lipophilicity and thereby their biological activity¹⁵ but, apart from the incomplete work of Grohe *et al.* in 1986 on some FQ bearing alkylated piperazines, the impact of the lipophilicity of quinolones with a long alkyl chain has not been investigated extensively.¹⁶

Examples of the synthesis and evaluation of FQ derivatives have been reported, with the main modifications made at R⁷ position where aminopyrrolidines and piperazines are the most effective substituents for improvement of the antimicrobial activity (Fig. 1 for FQ numbering).¹⁷ The introduction of alkyl groups to these R⁷ substituents improved FQ activities also against gram-positive bacteria.¹⁸ In addition, adding a methyl group to the amino group of aminopyrrolidine at R⁷ position could be effective for avoiding the inhibitory effect of cytochrome P450 3A4.¹⁹ Moreover, Jordi *et al.* demonstrated that introduction of a methyl or ethyl group to the R⁷ aminoazetidine improved the pharmacokinetic



Compound	R ⁴	Experimental Conditions	clogP	clogD
CPX	-	-	-0.73	-5.24
MXF	-	-	-0.08	-4.59
1	n-C ₇ H ₁₅	i	2.96	-1.55
2	n-C ₈ H ₁₇	i	3.49	-1.02
3	n-C ₉ H ₁₉	i	4.02	-0.49
4	n-C ₁₀ H ₂₁	i	4.58	0.07
5	n-C ₁₁ H ₂₃	i	5.08	0.57
6	n-C ₁₂ H ₂₅	i	5.61	1.10
7	n-C ₁₄ H ₂₉	i	6.67	2.16
8	(CH ₂ CH ₂ O) ₂ CH ₂ CH ₃	ii	0.19	-4.32
9	2-ethylhexyl	ii	3.36	-1.15
10	(CO) ₇ H ₁₅	iii	4.25	-0.26

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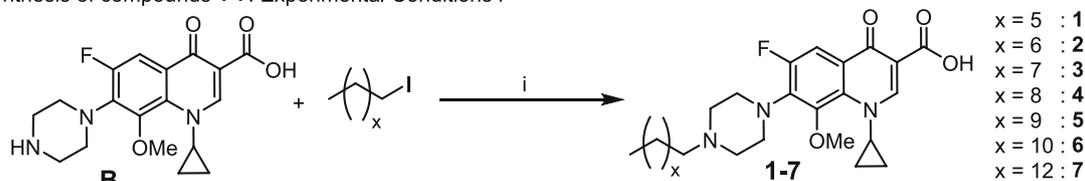
Fig. 2. Retrosynthesis of compounds 1–10 and their clogP and clogD values. The piperazine nucleophilic aromatic substitution was added at the R⁷ position of the synthon A to obtain synthon B. Synthon B was used to synthesized compounds 1–7 by nucleophilic substitution (i), compounds 8–9 by reductive amination (ii) and compound 10 by addition elimination (iii).

properties but reduced the antibacterial activity against gram-negative bacteria.²⁰

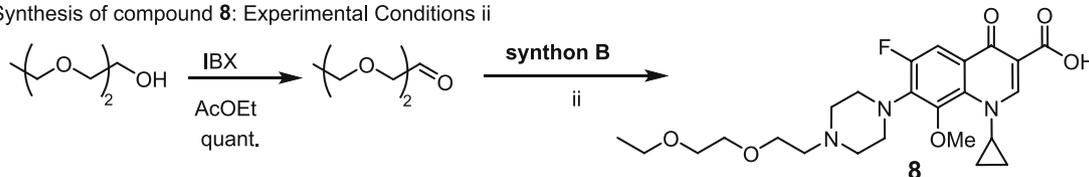
Concerning piperazine or piperazine-like quinolones, extensive research on the substitution at R^{3'} positions of the piperazine ring (Fig. 2 for piperazine numbering) have been carried out, and numerous aromatic derivatives at R^{4'} position of the piperazine ring have been reported.^{2,21} But despite the statement by Haemers *et al.* that “derivatives with higher alkyl substitutions should be investigated in detail”,²² very few examples of quinolones with long alkyl chains at the R^{4'} position of the piperazine ring have been described. Grohe *et al.* synthesised ciprofloxacin-like molecules alkylated with short and long carbon chains (CH₃, C₂H₅, n-C₃H₇, i-C₃H₇, n-C₄H₉, i-C₄H₉, n-C₅H₁₁, i-C₅H₁₁, or n-C₁₂H₂₅) but did not provide information regarding the antibacterial activities for most of them (n-C₄H₉, i-C₄H₉, n-C₅H₁₁, or n-C₁₂H₂₅).¹⁶ Haemers *et al.* evaluated the antimycobacterial activity of several ciprofloxacin-like quinolones with short alkyl chains (CH₃, C₂H₅, n-C₃H₇, i-C₃H₇) at position R^{4'} and showed that the derivatives with C₂H₅ and i-C₃H₇ were the most active.²² More recently, De Almeida *et al.* described quinolones with long aminoalkyl chains (–NH–[CH₂]_m–NH–[CH₂]_n–CH₃) with m = 2 or 3 and 5 < n < 13), instead of a piperazine ring at R⁷.²³ Among these two series, the highest activity was displayed by the two compounds with an alkyl chain length of 10 carbon atoms, whereas the compounds with the shortest alkyl chains were least active. Some authors have shown that triazole rings (1,2,4-triazole or 1,3-thiazolidinone) at the R^{4'} position of the piperazine group of norfloxacin²⁴ or between positions R⁷ and R⁸,^{25,26} may potentiate the antimicrobial activity against both gram-positive and gram-negative bacteria.

Taking into account these data, our main goal was to develop new potent antibacterial FQ. Since the impact of a substitution at the R^{4'} position of the piperazine group (Fig. 2) has been poorly studied, we designed and synthesised 10 new FQ-derivatives based on the CPX skeleton on which a methoxy group (R⁸ = OMe) has been added since replacing a R⁸–H atom by a R⁸–OMe group increased bactericidal effect of quinolones, especially against gram positive bacteria such as *S. aureus*.²⁷ The originality of our work lies in the addition of a long linear alkyl chain (compounds 1–7: 7 to 14 carbon atoms) to modulate the lipophilicity of those CPX “OMe” skeleton (clogD between –1.55 and +2.16) (Fig. 2). Moreover, we investigated slight modifications of the alkyl chain on the piperazine ring: one with a long glycol chain and a low lipophilicity (compound 8, clogD = –4.32), another one with an alkyl chain substituted with an ethyl to assess the impact of substitution (compound 9, clogD = 1.15), and the last one with a carboxy octyl chain

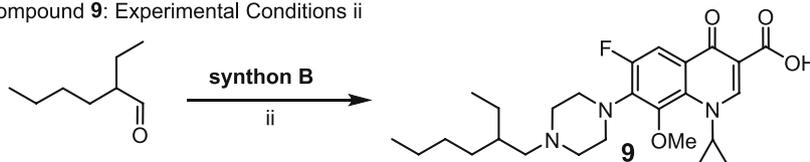
Synthesis of compounds 1-7: Experimental Conditions i



Synthesis of compound 8: Experimental Conditions ii



Synthesis of compound 9: Experimental Conditions ii



Synthesis of compound 10: Experimental Conditions iii

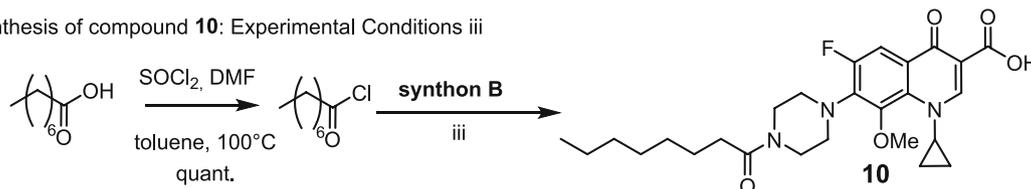


Fig. 3. Synthesis of compounds 1–10, reagents and conditions: i) synthon **B**, anhydrous DMF, NaHCO₃, 40 °C, 40 h then LiOH, EtOH/H₂O, RT, overnight, 25–35% over 2 steps. ii) synthon **B**, DCM, NaBH(OAc)₃, CH₃COOH, RT, overnight, 20–40%; iii) synthon **B**, triethylamine, DCM, RT, 1 h, 40%.

on the piperazine ring (compound **10**), to evaluate the impact of an amide bond rather than a simple N–C bond.

Herein, we described the synthesis of these 10 FQ-derivatives having the piperazine group substituted and their *in vitro* antibacterial activities against various bacterial species of medical interest, including FQ-R isolates.

As numerous FQ, those compounds bear a cyclopropyl group in R¹, a piperazinyl group in R⁷ and a methoxy group in R⁸. Therefore, we used 1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (synthon **A**) as starting material, so those compounds synthesis can be achieved in three steps only. The synthesis of compounds 1–10 described in this study is outlined in Fig. 3. Following the procedures described by Guruswamy and Arul,²⁸ 1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (synthon **A**) was refluxed for 6 days in anhydrous acetonitrile in the presence of triethylamine, the piperazine nucleophilic aromatic substitution proceeded regioselectively on the R⁷ position of the difluoroquinolone nucleus and therefore synthon **B** was obtained with 50% yield. Interestingly, no activation of the R⁷ position was required for this step, as it sometimes was.²⁹ Then, in order to synthesize compounds 1–7, synthon **B** was reacted under basic conditions with the corresponding iodoalkane. But, for each reaction, as the alkylation of the carboxylic acid function was observed during the process, a mixture of the ester and the desired products was obtained. Therefore, a basic hydrolysis was necessary as a last step to obtain compounds 1–7 with moderate yields (25–35%). For compounds 8–9, we proceeded via a direct reductive amination. Firstly, oxidation of 2-(2-ethoxyethoxy)ethan-1-ol gave 2-(2-ethoxyethoxy) acetaldehyde using 2-iodoxybenzoic acid (IBX) with a quantitative yield. Then, the coupling between the corresponding

aldehydes (2-(2-ethoxyethoxy) acetaldehyde for **8**, and 2-ethylhexanal, which is commercially available, for **9**, with synthon **B** in the presence of sodium triacetoxyborohydride, a mild reducing agent, enabled to achieve the direct reductive amination with a poor to moderate yield (20–40%) (Fig. 3). Finally, for compound **10**, the carboxylic acid function of the octanoic acid was activated through an acyl chloride, then a peptide coupling between this acyl chloride and synthon **B** gave compound **10** with a moderate yield (40%).

In order to explore the importance of substituents at the R⁴ position on the piperazine ring, antimicrobial activity of compounds 1–10 against gram-positive and gram-negative bacteria was compared to CPX and MXF (Table 1).

Overall, high MICs (≥0.5 mg/L) were observed for bacteria of medical interest, except *N. gonorrhoeae*, whatever the activity profiles of other drugs, and neither compound was more active than CPX or MXF among all species (Table 1). This latter point may be due to lower membrane penetration.³¹

Among the derivatives enabling to explore the impact of the size of the alkyl chain (compounds 1–7), the derivative with the smallest alkyl chain (compound **1**, C₇) displayed lower MICs than the other compounds, against gram negative strains (*A. baumannii*, *E. coli*, *P. aeruginosa*) and *S. aureus*. Surprisingly, the increase of MICs values against *S. aureus* strains was lower for compound **1** than for CPX or MXF, possibly because the presence of the R⁸ methoxy group as described by Lu *et al.*³⁰

Regarding compounds bearing a long glycol chain (compound **8**), a 2-ethylhexyl chain (compound **9**) and a carboxy octyl chain (compound **10**) on the piperazine ring, compound **9** exhibited the highest MIC whatever the bacterial species (Table 1). Regarding *E. coli*, MIC of compound **8** was similar to this of compound **1**. For *N. gonorrhoeae*, none

Table 1
Minimum inhibitory concentrations (MICs) of ciprofloxacin, moxifloxacin, and the compounds 1 to 10 for several clinical and reference bacteria of medical interest carrying various susceptibility profile to beta-lactams and fluoroquinolones.

Strains		Resistance profile to beta-lactams and fluoroquinolones	MICs (mg/L)											
			CPX	MXF	1	2	3	4	5	6	7	8	9	10
<i>A. baumannii</i>	ATCC 19606	No resistance	0.5	[0.25–0.5]	8	16	64	64	64	64	64	nd	nd	nd
<i>A. baumannii</i>	139	Resistant to beta-lactams and FQ	>32	2	[4–8]	[16–32]	64	≥64	≥64	≥64	≥64	nd	nd	nd
<i>A. baumannii</i>	140	Resistant to beta-lactams and FQ ^a	>32	[4–8]	64	64	64	64	64	64	≥64	nd	nd	nd
<i>E. coli</i>	ATCC 35218	No resistance	0.008	0.06	1	≥64	8	[32–>64]	[16–32]	64	[32–64]	[0.5–1]	≥64	≥64
<i>E. coli</i>	202	Resistant to beta-lactams ^b	0.06	[0.5–1]	[2–4]	[2–4]	[8–16]	≥64	≥64	[32–64]	64	nd	nd	nd
<i>E. coli</i>	203	Resistant to beta-lactams and FQ ^c	>32	8	>64	>64	>64	>64	>64	>64	>64	nd	nd	nd
<i>N. gonorrhoeae</i>	ATCC 19424	No resistance	0.004	0.06	[0.25–0.5]	0.25	0.25	[0.12–0.25]	[0.5–1]	[0.12–0.5]	[0.12–0.5]	2	>32	1
<i>P. aeruginosa</i>	ATCC 27853	No resistance	[0.25–0.5]	1	64	>64	>64	>64	>64	>64	>64	nd	nd	nd
<i>P. aeruginosa</i>	142	Resistant to beta-lactams and FQ ^d	>32	[8–16]	>64	>64	≥64	>64	>64	>64	>64	nd	nd	nd
<i>P. aeruginosa</i>	143	Resistant to beta-lactams and FQ	>32	[16–32]	>64	>64	≥64	>64	>64	>64	>64	nd	nd	nd
<i>S. pneumoniae</i>	ATCC 49169	No resistance	[0.5–1]	[0.06–0.12]	[4–8]	32	2	>64	8	[4–8]	[8–16]	1	2	0.5
<i>S. aureus</i>	ATCC 29213	No resistance	0.25	[0.06–0.125]	[1–2]	[4–8]	8	32	[32–64]	[32–64]	[32–64]	0.5	[32–64]	[1–2]
<i>S. aureus</i>	196	MRSA	>32	0.5	[2–4]	[4–8]	≥64	≥64	64	≥64	>64	nd	nd	nd
<i>S. aureus</i>	197	MRSA	>32	[1–2]	[8–16]	[8–16]	>64	>64	≥64	≥64	>64	nd	nd	nd

Abbreviations: CPX, ciprofloxacin; MXF, moxifloxacin; MRSA, methicillin-resistant *S. aureus*; ESBL, extended-spectrum beta-lactamase.

^a OXA-23-producing.

^b ESBL- and OXA-48-producing.

^c ESBL-producing.

^d Producing metallo-beta-lactamase VIM.

Table 2

Minimum inhibitory concentrations (MICs) of ciprofloxacin, moxifloxacin, compounds 2 and 4 for several clinical and reference *N. gonorrhoeae* carrying various susceptibility profile to FQ, macrolides and tetracyclines.

<i>N. gonorrhoeae</i> strains	Resistance profile to beta-lactams, fluoroquinolones, macrolides and tetracyclines	MICs (mg/L)			
		CPX	MXF	2	4
ATCC 19424	No resistance	0.004	0.06	0.25	[0.12–0.25]
WHO A	No resistance	[0.002–0.004]	[0.004–0.008]	[0.03–0.06]	0.5
WHO B	Resistant to tetracyclines	0.008	[0.008–0.016]	[0.12–0.25]	1
WHO C	Resistant to tetracyclines	0.004	0.016	[0.5–1]	1
WHO E	Resistant to tetracyclines	[0.002–0.004]	[0.004–0.008]	[0.03–0.12]	[0.25–0.5]
WHO M	Resistant to tetracyclines and FQ ^a	2	2	[16–32]	≥64
WHO O	Resistant to tetracyclines	[0.004–0.008]	[0.016–0.03]	[0.03–0.5]	[1–2]
WHO Q	Resistant to tetracyclines	[0.008–0.016]	[0.016–0.03]	[0.5–1]	2
Barla194	Resistant to tetracyclines, FQ and macrolides ^b	32	16	128	256
WHO G	Resistant to tetracyclines and FQ ^c	[0.125–0.25]	[0.03–0.06]	[0.25–2]	8
WHO K	Resistant to beta-lactams, tetracyclines and FQ ^d	≥32	[2–16]	256	256
WHO Z	Resistant to beta-lactams, tetracyclines and FQ ^d	>32	2	>32	≥64

Abbreviations: CPX, ciprofloxacin; MXF, moxifloxacin.

^a S91F and D95G substitutions in GyrA.

^b E91G substitution in ParC, and S91F and D95G substitution in GyrA.

^c S91F substitution in GyrA.

^d S87R and S88P substitutions in ParC, and S91F and D95G substitutions in GyrA.

of the compounds displayed MIC lower than compounds 1–7. For *S. pneumoniae*, MICs of these compounds were similar or lower than MICs of compounds 1–7, and for *S. aureus* the sole compound exhibiting lower MIC than compounds 1–7 was the compound 8. Against gram negative bacteria none of these compounds displayed lower MICs than CPX or MXF, whereas against gram positive bacteria, compound 8 or 10 displayed similar MICs than CPX and higher than MXF. It can be hypothesized that, for *E. coli* and *S. aureus*, low lipophilicity leads to lower MIC in case of equivalent steric hindrance.

Besides, remarkably, compounds 1–7 bearing a long linear alkyl chain on the piperazine ring, showed significantly lower MICs against the *N. gonorrhoeae* ATCC 19,424 reference strain than compounds 8–10. However, the MICs values of compounds 1–7 were higher than MICs of CPX (16 to 128-fold) and similar to- or higher than MICs of MXF (2 to 16-fold). In order to evaluate the impact of the size of the alkyl chain, we determined MICs of compound 2 and compound 4 (displaying the lowest MIC with the shortest and longest side alkyl chain, respectively) against 11 *N. gonorrhoeae* strains harbouring different antibiotic susceptibility profiles (Table 2).

Compounds 2 and 4 displayed interesting antimicrobial activities against *N. gonorrhoeae* strains with MICs values ranging from 0.03 to 256 mg/L for compound 2, and from 0.25 to 256 mg/L for compound 4. The MICs of compound 2 were similar to, or lower than, the MICs of compound 4 for all strains. Interestingly, strains for which the MICs of compounds 2 and 4 were the lowest (WHO A, WHO B, WHO C, WHO E, WHO O, WHO Q), were those that were susceptible to FQ and whose *gyrA* and *parC* genes were wild-type (MICs < 2 mg/L), whereas the strains resistant to FQ and harbouring mutations in the *gyrA* and/or *parC* genes (WHO M, WHO G, WHO K, WHO Z and barla194) displayed the highest MICs (≥2 mg/L). Unfortunately, the MICs increase for bacteria with mutations in *gyrA* and *parC* is known to confer resistance to FQ. These results strongly suggest cross resistance between the two compounds and the FQ in clinical use, whereas, as expected, no cross resistance was observed with antibiotics of other classes (*i.e.* beta-lactams, macrolides and tetracyclines) (Tables 1 and 2).

In conclusion, this work describes the synthesis, characterization and evaluation of ten FQ-derivatives. Antibacterial activity of the designed compounds against gram-positive and gram-negative was evaluated with microdilution assays. Considering all biological results, neither of these FQ-derivatives was as active as CPX and MXF (Tables 1 and 2). These results suggest that the introduction of a long alkyl chain or a 2-ethylhexyl chain or a carboxy octyl chain on the FQ piperazine ring at the R⁴ position reduces the antibacterial activity. More SAR needs to be conducted, but compound 8, which has the lowest clogD value (-4.32)

among the 10 FQ-derivatives (-1.55 for 1 to 2.16 for 7) displayed the lowest MICs against *E. coli* and *S. aureus*, which suggests that increasing lipophilicity is deleterious for antibacterial activity. Moreover, we concluded that a common mechanism led to cross resistance with FQ in clinical use in *N. gonorrhoeae*.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bmcl.2021.128450>.

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