Lipophilic quinolone derivatives: Synthesis and in vitro antibacterial evaluation

Elodie Sadowski, Beatrice Bercot, Aurélie Chauffour, Catherine Gomez, Emmanuelle Varon, Mary Mainardis, Wladimir Sougakoff, Claudine Mayer, Emmanuelle Sachon, Guillaume Anquetin, et al.

Bioorg. Med. Chem. Lett. 55 (2022) 128450

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

Elodie Sadowski [a,b], Beatrice Bercot [c,d,e], Aurélie Chauffour [a], Catherine Gomez [f], Emmanuelle Varon [g,h], Mary Mainardis [c,d,e], Wladimir Sougakoff [a,i], Claudine Mayer [j,k,l], Emmanuelle Sachon [b,m], Guillaume Anquetin [n], Alexandra Aubry [a,l,*]

[a] *Sorbonne Université, INSERM, Centre d'Immunologie et des Maladies Infectieuses, U1135, AP-HP, Hôpital Pitié-Salpètrière, Centre National de Référence des Mycobactéries et de la Résistance des Mycobactéries aux Antituberculeux, F-75013 Paris, France*

[b] *Sorbonne Université, École normale supérieure, PSL University, CNRS, Laboratoire des Biomolécules, LBM, 4 place Jussieu, 75252 Cedex 05 Paris, France*

[c] *Paris University, INSERM UMR1137, Infection, Antimicrobials, Modelling, Evolution, IAME, 16 rue Henri Huchard, 75870 Paris Cedex 18, France*

[d] *French National Reference Centre for Bacterial Sexually Transmitted Infections, Associated Laboratory for Gonococci, Assistance Publique - Hôpitaux de Paris (APHP), 1 Avenue Claude Vellefaux, 75010 Paris, France*

[e] *Infectious Agents Department, Bacteriology Unit, Saint Louis Hospital, Assistance Publique - Hôpitaux de Paris (APHP), 1 Avenue Claude Vellefaux, 75010 Paris, France*

[f] *Laboratoire de Génomique, Bioinformatique et Chimie Moléculaire (EA7528), Equipe Chimie Moléculaire, Conservatoire National des Arts et Métiers (CNAM), HESAM Université, 2 rue Conté, 75003 Paris, France*

[g] *Laboratory of Medical Biology, Centre Hospitalier Intercommunal de Créteil, 40 avenue de Verdun, 94010 Créteil, France*

[h] *National Reference Center for Pneumococci, Centre Hospitalier Intercommunal de Créteil, 40 avenue de Verdun, 94010 Créteil, France*

[i] *AP-HP. Sorbonne-Université, Centre National de Référence des Mycobactéries et de la Résistance des Mycobactéries aux Antituberculeux, Laboratoire de Bactériologie-Hygiène, Groupe Hospitalier Pitié-Salpètrière, 47-83 Boulevard de l'Hôpital, 75651 Paris Cedex 13, France*

[j] *Department of Computer Science, ICube UMR 7357, CNRS, University of Strasbourg, 300 bd Sébastien Brant, 67400 Illkirch, France*

[k] *Unité de Microbiologie Structurale, Institut Pasteur, CNRS UMR 3528, 25 rue du Docteur Roux, 75724 Paris Cedex 15, France*

[l] *Université de Paris, 5 rue Thomas-Mann, 75013 Paris, France*

[m] *Sorbonne Université, MS^{3}U Platform, Mass Spectrometry Sciences Sorbonne Université, 4 place Jussieu, 75252 Cedex 05 Paris, France*

[n] *Université de Paris, ITODYS (Interfaces Traitements Organisation et DYnamique des Systèmes), CNRS, F-75006 Paris, France*

ARTICLE INFO

Keywords:
Antibacterial
Fluoroquinolones
Synthesis
Neisseria gonorrhoeae
ESKAPE

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 ^{1}H, ^{13}C and ^{19}F 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 R^6 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

* Corresponding author at: Faculté de médecine Sorbonne-Université, Cimi-Paris-U1135, 91, Boulevard de l'Hôpital, 75013 Paris, France.
E-mail address: alexandra.aubry@sorbonne-universite.fr (A. Aubry).

https://doi.org/10.1016/j.bmcl.2021.128450
Received 16 August 2021; Received in revised form 25 October 2021; Accepted 7 November 2021
Available online 11 November 2021

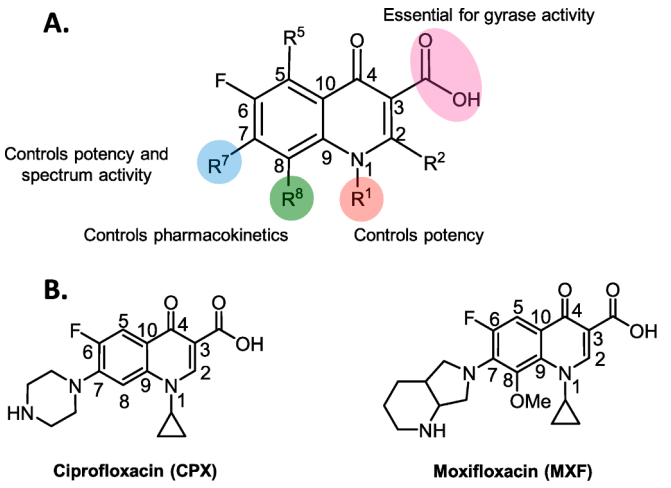
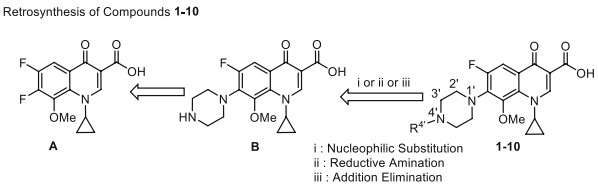


Fig. 1. Structure activity relationship of 1-substituted-1,4-dihydro-6-fluoro-4-oxo-3-carboxylic acid key scaffold, adapted from[2] (A) and structures of CPX and MXF (B).



Compound	$R^{4'}$	Experimental Conditions	clogP	clogD
CPX	-	-	-0.73	-5.24
MXF	-	-	-0.08	-4.59
1	n-C_7H_{15}	i	2.96	-1,55
2	n-C_8H_{17}	i	3.49	-1.02
3	n-C_9H_{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	$(CH_2CH_2O)_2CH_2CH_3$	ii	0.19	-4.32
9	2-ethylhexyl	ii	3.36	-1.15
10	$(CO)C_7H_{15}$	iii	4.25	-0.26

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

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 heterotetrametric A_2B_2 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 R^6 position was a triggering event for quinolone use, but the increased lipophilicity of FQ with the fluorine at R^8 was gained only at the expense of higher toxicity. FQ prodrugs were designed to increase their lipophilicity and thereby their biological activity[15] 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 R^7 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 R^7 substituents improved FQ activities also against gram-positive bacteria. [18] In addition, adding a methyl group to the amino group of aminopyrrolidine at R^7 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 R^7 aminoazetidine improved the pharmacokinetic 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 (CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9, i-C_4H_9, n-C_5H_{11}, i-C_5H_{11}, or n-$C_{12}H_{25}$) but did not provide information regarding the antibacterial activities for most of them (n-C_4H_9, i-C_4H_9, n-C_5H_{11}, or n-$C_{12}H_{25}$).[16] Haemers *et al.* evaluated the antimycobacterial activity of several ciprofloxacin-like quinolones with short alkyl chains (CH_3, C_2H_5, n-C_3H_7, i-C_3H_7) at position $R^{4'}$ and showed that the derivatives with C_2H_5 and i-C_3H_7 were the most active.[22] More recently, De Almeida *et al.* described quinolones with long aminoalkyl chains (–NH-[CH_2]m-NH-[CH_2]n-CH_3) with $m = 2$ or 3 and $5 < n < 13$), instead of a piperazine ring at R^7. [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 norfloxacin [24] or between positions R^7 and R^8,[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 (R^8 = OMe) has been added since replacing a R^8-H atom by a R^8-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

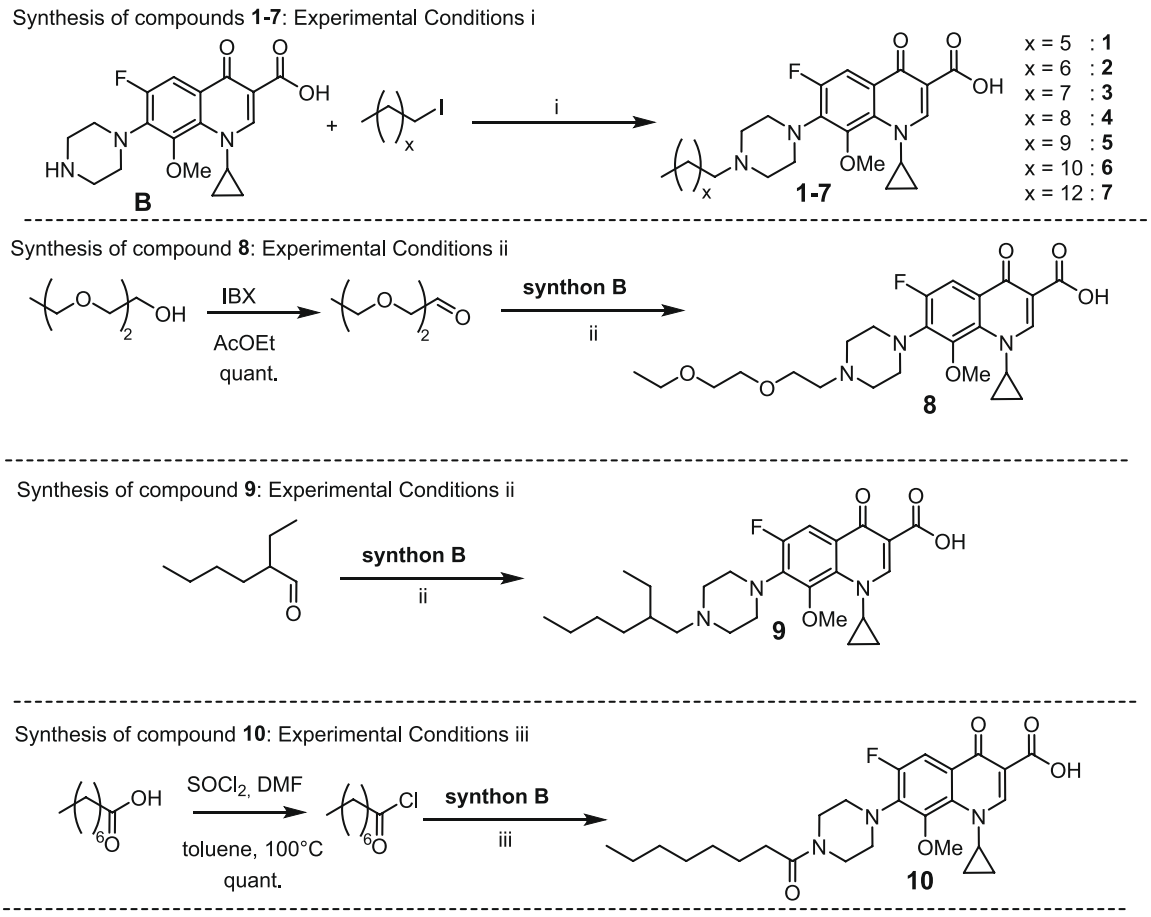


Fig. 3. Synthesis of compounds **1**–**10**, reagents and conditions: i) synthon **B,** anhydrous DMF, $NaHCO_3$, 40 °C, 40 h then LiOH, $EtOH/H_2O$, RT, overnight, 25–35% over 2 steps. ii) synthon **B,** DCM, $NaBH(OAc)_3$, CH_3COOH, RT, overnight, 20–40%; iii) synthon **B,** triethylamine, DCM, RT, 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 R^1, a piperazinyl group in R^7 and a methoxy group in R^8. 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 regiospecifically on the R^7 position of the difluoroquinolone nucleus and therefore synthon **B** was obtained with 50% yield. Interestingly, no activation of the R^7 position was required for this step, as it sometimes was.[29] Then, in order to synthesise 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 $R^{4'}$ 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**, C_7) 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 R^8 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
A. baumannii	ATCC 19606	No resistance	0.5	[0.25–0.5]	8	16	64	64	64	64	64	nd	nd	nd
A. baumannii	139	Resistant to beta-lactams and FQ	>32	2	[4–8]	[16–32]	64	≥64	≥64	≥64	≥64	nd	nd	nd
A. baumannii	140	Resistant to beta-lactams and FQ[a]	>32	[4–8]	64	64	64	64	64	64	≥64	nd	nd	nd
E. coli	ATCC 35218	No resistance	0.008	0.06	1	≥64	8	[32–>64]	[16–32]	64	[32–64]	[0.5–1]	≥64	≥64
E. coli	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
E. coli	203	Resistant to beta-lactams and FQ[c]	>32	8	>64	>64	>64	>64	>64	>64	>64	nd	nd	nd
N. gonorrhoeae	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
P. aeruginosa	ATCC 27853	No resistance	[0.25–0.5]	1	64	>64	>64	>64	>64	>64	>64	nd	nd	nd
P. aeruginosa	142	Resistant to beta-lactams and FQ[d]	>32	[8–16]	>64	>64	≥64	>64	>64	>64	>64	nd	nd	nd
P. aeruginosa	143	Resistant to beta-lactams and FQ	>32	[16–32]	>64	>64	≥64	>64	>64	>64	>64	nd	nd	nd
S. pneumoniae	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
S. aureus	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]
S. aureus	196	MRSA	>32	0.5	[2–4]	[4–8]	≥64	≥64	64	≥64	>64	nd	nd	nd
S. aureus	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.

N. gonorrhoeae 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 $R^{4'}$ 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.*

Funding

The work was supported by grants from the order of Malta Grants for Leprosy Research, QUIET Lutech MA077/SL00091 – ref UPMCX13042 from the SATT LUTECH and ANR DETONATOR.

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.

Acknowledgments

We acknowledge the ITODYS's (Interfaces Traitements Organisation et DYnamique des Systèmes) NMR facility and Ekkehard Collatz for English editing.

Appendix A. Supplementary data

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

References

[1] Koga H, Itoh A, Murayama S, Suzue S, Irikura T. Structure-activity relationships of antibacterial 6,7- and 7,8-disubstituted 1-alkyl-1,4-dihydro-4-oxoquinoline-3-carboxylic acids. *J Med Chem.* 1980;23(12):1358–1363. https://doi.org/10.1021/jm00186a014.
[2] Suaifan GARY, Mohammed AAM. Fluoroquinolones structural and medicinal developments (2013–2018): Where are we now? *Bioorg Med Chem.* 2019;27(14):3005–3060. https://doi.org/10.1016/j.bmc.2019.05.038.
[3] Dalhoff A. Global fluoroquinolone resistance epidemiology and implictions for clinical use. *Interdisciplinary Perspectives on Infectious Diseases.* 2012;2012:1–37. https://doi.org/10.1155/2012/976273.
[4] Wolfson JS, Hooper DC. Fluoroquinolone antimicrobial agents. *Clin Microbiol Rev.* 1989;2(4):378–424.
[5] World Health Organization. *WHO Consolidated Guidelines on Drug-Resistant Tuberculosis Treatment.*; 2019. Accessed September 1, 2020. http://www.ncbi.nlm.nih.gov/books/NBK539517/.
[6] Lockhart SR, Abramson MA, Beekmann SE, et al. Antimicrobial Resistance among Gram-Negative Bacilli Causing Infections in Intensive Care Unit Patients in the

United States between 1993 and 2004. *J Clin Microbiol*. 2007;45(10):3352–3359. https://doi.org/10.1128/JCM.01284-07.

[7] Zhanel GG, DeCorby M, Nichol KA, et al. Antimicrobial susceptibility of 3931 organisms isolated from intensive care units in Canada: Canadian National Intensive Care Unit Study, 2005/2006. *Diagn Microbiol Infect Dis*. 2008;62(1): 67–80. https://doi.org/10.1016/j.diagmicrobio.2008.04.012.

[8] Donegan EA, Wirawan DN, Muliawan P, et al. Fluoroquinolone-Resistant Neisseria gonorrhoeae in Bali, Indonesia: 2004. *Sexually Transmitted Diseases*. 2006;33(10): 625-629. doi:10.1097/01.olq.0000216012.83990.bd.

[9] Wi T, Lahra MM, Ndowa F, et al. Antimicrobial resistance in Neisseria gonorrhoeae: Global surveillance and a call for international collaborative action. *PLoS Med*. 2017;14(7):e1002344. https://doi.org/10.1371/journal.pmed.1002344.

[10] Ashley RE, Dittmore A, McPherson SA, Turnbough CL, Neuman KC, Osheroff N. Activities of gyrase and topoisomerase IV on positively supercoiled DNA. *Nucleic Acids Res*. 2017;45(16):9611–9624. https://doi.org/10.1093/nar/gkx649.

[11] Hooper DC, Jacoby GA. Topoisomerase Inhibitors: Fluoroquinolone Mechanisms of Action and Resistance. *Cold Spring Harb Perspect Med*. 2016;6(9):a025320. https://doi.org/10.1101/cshperspect.a025320.

[12] Dent LL, Marshall DR, Pratap S, Hulette RB. Multidrug resistant Acinetobacter baumannii: a descriptive study in a city hospital. *BMC Infect Dis*. 2010;10(1):196. https://doi.org/10.1186/1471-2334-10-196.

[13] Morata L, Cobos-Trigueros N, Martínez JA, et al. Influence of multidrug resistance and appropriate empirical therapy on the 30-day mortality rate of pseudomonas aeruginosa bacteremia. *Antimicrob Agents Chemother*. 2012;56(9):4833–4837. https://doi.org/10.1128/AAC.00750-12.

[14] Ezelarab HAA, Abbas SH, Hassan HA, Abuo-Rahma G-D. Recent updates of fluoroquinolones as antibacterial agents. *Arch Pharm*. 2018;351(9):1800141. https://doi.org/10.1002/ardp.v351.910.1002/ardp.201800141.

[15] Fan Y-L, Wu J-B, Cheng X-W, Zhang F-Z, Feng L-S. Fluoroquinolone derivatives and their anti-tubercular activities. *Eur J Med Chem*. 2018;146:554–563. https://doi.org/10.1016/j.ejmech.2018.01.080.

[16] Grohe K, Petersen U, Kuck K-H. MICROBICIDAL AGENTS BASED ON QUINOLONECARBOXYLIC ACID. Published online 1986. Accessed June 15, 2020. https://patentimages.storage.googleapis.com/01/64/be/a2e9540afd7cca/US4563459.pdf.

[17] Domagala JM. Structure-activity and structure-side-effect relationships for the quinolone antibacterials. *J Antimicrob Chemother*. 1994;33(4):685–706. https://doi.org/10.1093/jac/33.4.685.

[18] Domagala JM, Hagen SE, Joannides T, et al. Quinolone antibacterials containing the new 7-[3-(1-aminoethyl)-1-pyrrolidinyl] side chain: the effects of the 1-aminoethyl moiety and its stereochemical configurations on potency and in vivo efficacy. *J Med Chem*. 1993;36(7):871–882. https://doi.org/10.1021/jm00059a012.

[19] Odagiri T, Inagaki H, Sugimoto Y, et al. Design, Synthesis, and Biological Evaluations of Novel 7-[7-Amino-7-methyl-5-azaspiro[2.4]heptan-5-yl]-8-methoxyquinolines with Potent Antibacterial Activity against Respiratory Pathogens. *J Med Chem*. 2013;56(5):1974–1983. https://doi.org/10.1021/jm301650g.

[20] Frigola J, Vano D, Torrens A, Gomez-Gomar A, Ortega E, Garcia-Granda S. 7-Azetidinylquinolones as Antibacterial Agents. 3. Synthesis, Properties and Structure-Activity Relationships of the Stereoisomers Containing a 7-(3-Amino-2-methyl-1-azetidinyl) Moiety. *J Med Chem*. 1995;38(7):1203–1215. https://doi.org/10.1021/jm00007a017.

[21] El-wahab HAAA, Accietto M, Marino LB, et al. Design, synthesis and evaluation against Mycobacterium tuberculosis of azole piperazine derivatives as dicyclotyrosine (cYY) mimics. *Bioorg Med Chem*. 2018;26(1):161–176. https://doi.org/10.1016/j.bmc.2017.11.030.

[22] Haemers A, Leysen DC, Bollaert W, Zhang MQ, Pattyn SR. Influence of N substitution on antimycobacterial activity of ciprofloxacin. *Antimicrob Agents Chemother*. 1990;34(3):496–497. https://doi.org/10.1128/AAC.34.3.496.

[23] de Almeida C, Diniz C, Silva V, Saraiva M, Le Hyaric M, de Almeida M. Antibacterial Activity of Lipophilic Fluoroquinolone Derivatives. *MC*. 2009;5(5): 419–421. https://doi.org/10.2174/157340609789117859.

[24] Mentese MY, Bayrak H, Uygun Y, et al. Microwave assisted synthesis of some hybrid molecules derived from norfloxacin and investigation of their biological activities. *Eur J Med Chem*. 2013;67:230–242. https://doi.org/10.1016/j.ejmech.2013.06.045.

[25] Abu-Sini M, Mayyas A, Al-Karablieh N, et al. Synthesis of 1,2,3-Triazolo[4,5-h] quinolone Derivatives with Novel Anti-Microbial Properties against Metronidazole Resistant Helicobacter pylori. *Molecules*. 2017;22(5):841. https://doi.org/10.3390/molecules22050841.

[26] Gao L-Z, Xie Y-S, Li T, Huang W-L, Hu G-Q. Synthesis and antibacterial activity of novel [1,2,4]triazolo[3,4-h][1,8]naphthyridine-7-carboxylic acid derivatives. *Chin Chem Lett*. 2015;26(1):149–151. https://doi.org/10.1016/j.cclet.2014.09.017.

[27] Zhao X, Xu C, Domagala J, Drlica K. DNA topoisomerase targets of the fluoroquinolones: A strategy for avoiding bacterial resistance. *Proc Natl Acad Sci U S A*. 1997;94(25):13991–13996.

[28] Guruswamy B, Arul R. Synthesis, Characterization, and Antimicrobial Activities of Novel N-substituted β-Hydroxy Amines and β-Hydroxy Ethers that Contained 8-Methoxy Fluoroquinolones. *J Heterocycl Chem*. 2016;53(1):284–293. https://doi.org/10.1002/jhet.v53.110.1002/jhet.1927.

[29] Anquetin G, Greiner J, Mahmoudi N, et al. Design, synthesis and activity against Toxoplasma gondii, Plasmodium spp., and Mycobacterium tuberculosis of new 6-fluoroquinolones. *Eur J Med Chem*. 2006;41(12):1478–1493. https://doi.org/10.1016/j.ejmech.2006.07.003.

[30] Lu T, Zhao X, Li X, et al. Enhancement of Fluoroquinolone Activity by C-8 Halogen and Methoxy Moieties: Action against a Gyrase Resistance Mutant of Mycobacterium smegmatis and a Gyrase-Topoisomerase IV Double Mutant of Staphylococcus aureus. *Antimicrob Agents Chemother*. 2001;45(10):2703–2709. https://doi.org/10.1128/AAC.45.10.2703-2709.2001.

[31] Redgrave LS, Sutton SB, Webber MA, Piddock LJV. Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success. *Trends Microbiol*. 2014;22(8):438–445. https://doi.org/10.1016/j.tim.2014.04.007.