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Lipophilic quinolone derivatives: Synthesis and in vitro antibacterial evaluation

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Aim of the study: to synthesise and characterise a class of lipophilic quinolone derivatives, and to evaluate their in vitro antibacterial activity against Neisseria gonorrhoeae.

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 15F 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), which is structurally characterised by a R6 fluorine atom in the quinoline 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-1,4-)-pyridinyl for MXF)-3-carboxylic acid moiety is the basic scaffold (Fig. 1 for FQ numbering system).

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. They are also recommended as second-line antituberculosis agents by the World Health Organization (WHO).

However, excessive use of FQ has led to the emergence of FQ-resistant (FQ-R) bacteria. The prevalence and spread of FQ-R bacteria...
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. 1–3

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 A$_2$B$_2$ 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. 1,5,13 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$^5$ position was a triggering event for quinolone use, but the increased lipophilicity of FQ with the fluorine at R$^5$ 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. 15

Examples of the synthesis and evaluation of FQ derivatives have been reported, with the main modifications made at R$^7$ position where amidopyrrolidines and piperazines are the most effective substruments 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 amidopyrrolidine 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$^7$ aminoaazetidine 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$^7$ positions of the piperazine ring (Fig.2 for piperazine numbering) have been carried out, and numerous aromatic derivatives at R$^7$ 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$^7$ 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$_2$H$_5$, n-C$_3$H$_7$, i-C$_3$H$_7$, n-C$_4$H$_9$, i-C$_4$H$_9$, n-C$_5$H$_{11}$, i-C$_5$H$_{11}$, or n-C$_6$H$_{13}$) but did not provide information regarding the antibacterial activities for most of them (n-C$_3$H$_7$, i-C$_3$H$_7$, n-C$_5$H$_{11}$, or n-C$_6$H$_{13}$). 10 Haemers et al. evaluated the antimycobacterial activity of several ciprofloxacin-like quinolones with short alkyl chains (CH$_3$, C$_2$H$_5$, n-C$_3$H$_7$, i-C$_3$H$_7$, n-C$_4$H$_9$, i-C$_4$H$_9$, n-C$_5$H$_{11}$, i-C$_5$H$_{11}$) at position R$^7$ and showed that the derivatives with C$_2$H$_5$ and i-C$_3$H$_7$ were the most active. 23 More recently, De Almeida et al. described quinolones with long aminoalkyl chains (–NH–[CH$_2$]$_m$–NH–[CH$_2$]$_n$) with m = 2 or 3 and 5 < n < 13), instead of a piperazine ring at R$^7$. 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$^7$ position of the piperazine group of norfloxacin 24 or between positions R$^5$ and R$^7$ 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$^7$ 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$^7$–OMe) has been added since replacing a R$^7$–H atom by a R$^7$–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 skeleton on which a methoxy group (R$^7$–OMe) has been poorly studied, we investigated slight modifications of the alkyl chain on the piperazine ring: one with a long glycol chain and a low lipophilicity (compound 1, 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
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 R² and a methoxy group in R³. 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 R² position of the difluorquinolone nucleus and therefore synthon B was obtained with 50% yield. Interestingly, no activation of the R² 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 iodoaldehyde. 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 R⁴ 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 | nd |
| *A. baumannii* 139     | Resistant to beta-lactams and FQ                         | ≥32         | 2   | [4–8] | [16–32] | 64 | ≥64 | ≥64 | ≥64 | ≥64 | nd | nd | nd | nd |
| *A. baumannii* 140     | Resistant to beta-lactams and FQ                         | ≥32         | [4–8] | 64 | 64 | 64 | 64 | 64 | 64 | 64 | nd | 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 | ≥64 |
| *E. coli* 202          | Resistant to beta-lactams                                 | 0.06        | [0.5–1] | [2–4] | [2–4] | [8–16] | ≥64 | ≥64 | [32–64] | 64 | nd | nd | nd | nd |
| *E. coli* 203          | Resistant to beta-lactams and FQ                         | ≥32         | 8   | >64 | >64 | >64 | >64 | >64 | >64 | >64 | nd | 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 | nd |
| *P. aeruginosa* 142    | Resistant to beta-lactams and FQ                         | ≥32         | [8–16] | >64 | >64 | >64 | >64 | >64 | >64 | >64 | nd | 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 | nd |
| *S. pneumoniae* ATCC 49169 | No resistance                                           | [0.5–1] | [0.06–0.12] | [4–8] | 32  | 2   | >64 | 8  | [4–8] | 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 | nd |
| *S. aureus* 197        | MRSA                                                     | ≥32         | [1–2] | [8–16] | [8–16] | >64 | >64 | >64 | >64 | >64 | nd | 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.
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 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 compounds 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′ 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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