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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 ^{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.

Keywords: antibacterial, fluoroquinolones, synthesis, *Neisseria gonorrhoeae*, ESKAPE

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]

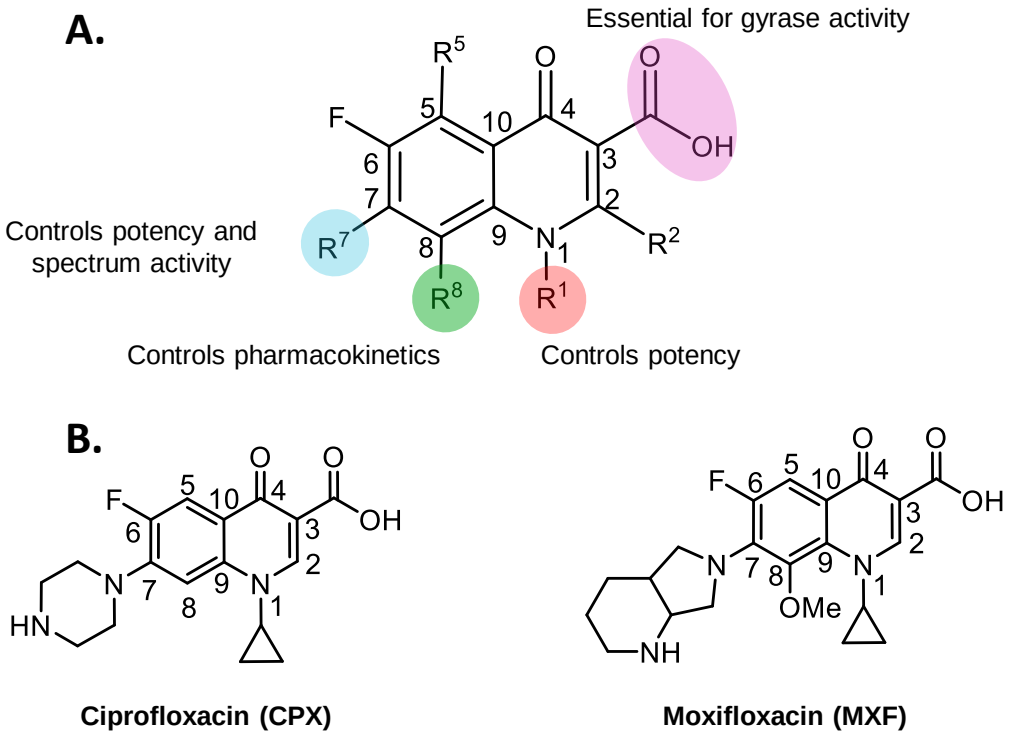


Fig. 1. Structure activity relationship of 1-substituted-1,4-dihydro-6-fluoro-4-oxo-3-carboxylic acid key scaffold, adapted from[2] (A) and structures of CPX and MXF (B).

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 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

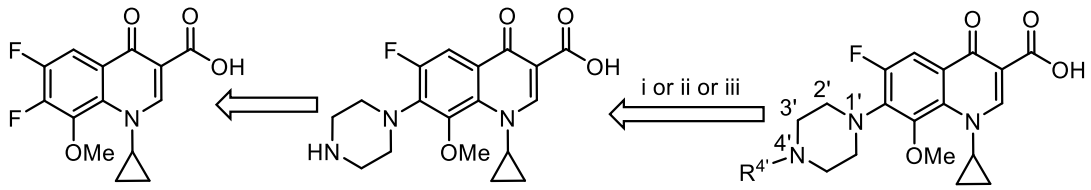
with the fluorine at R^8 was gained only at the expense of higher toxicity. FQ prodrugs were designed to increase their lipophilicity and thereby their biological activity[15] but, apart from the incomplete work of Grohe *et al*. in 1986 on some FQ bearing alkylated piperazines, the impact of the lipophilicity 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.

Retrosynthesis of Compounds **1-10**



i : Nucleophilic Substitution
ii : Reductive Amination
iii : Addition Elimination

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^7 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).

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 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%).

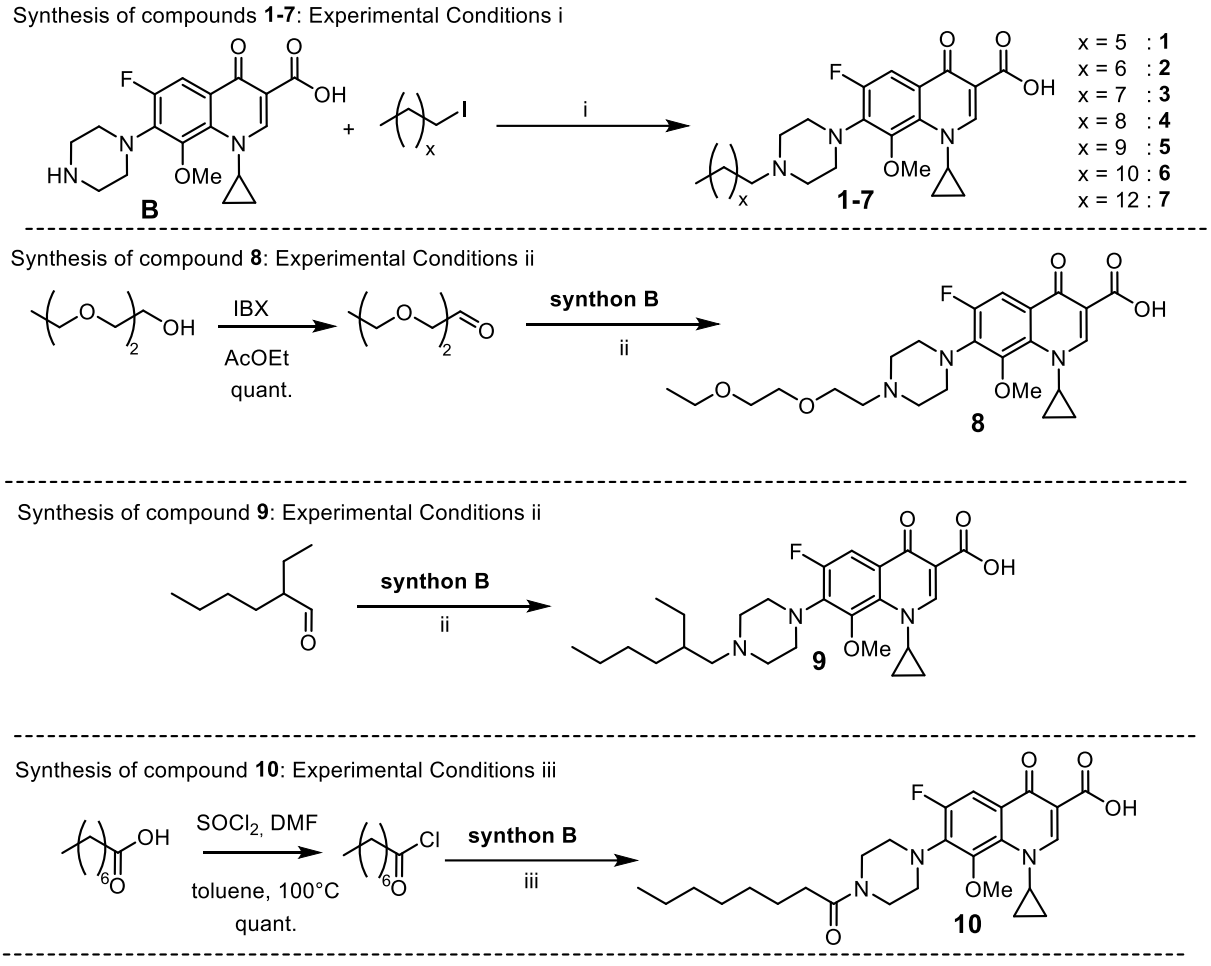


Fig 3. Synthesis of compounds **1-10**, reagents and conditions: i) synthon **B,** anhydrous DMF, $NaHCO_3$, 40°C, 40h then LiOH, EtOH/H_2O, RT, overnight, 25-35% over 2 steps. ii) synthon **B,** DCM, $NaBH(OAc)_3$, CH_3COOH, RT, overnight, 20-40%; iii) synthon **B,** triethylamine, DCM, RT, 1h, 40%.

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 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

222 can be hypothesized that, for *E. coli* and *S. aureus*, low lipophilicity leads to lower MIC
223 in case of equivalent steric hindrance.

224 Besides, remarkably, compounds **1-7** bearing a long linear alkyl chain on the
225 piperazine ring, showed significantly lower MICs against the *N. gonorrhoeae* ATCC
226 19424 reference strain than compounds **8-10**. However, the MICs values of
227 compounds **1-7** were higher than MICs of CPX (16 to 128-fold) and similar to- or higher
228 than MICs of MXF (2 to 16-fold). In order to evaluate the impact of the size of the alkyl
229 chain, we determined MICs of compound **2** and compound **4** (displaying the lowest
230 MIC with the shortest and longest side alkyl chain, respectively) against 11
231 *N. gonorrhoeae* strains harbouring different antibiotic susceptibility profiles (Table 2).

232 Compounds **2** and **4** displayed interesting antimicrobial activities against
233 *N. gonorrhoeae* strains with MICs values ranging from 0.03 to 256 mg/L for compound
234 **2**, and from 0. 25 to 256 mg/L for compound **4**. The MICs of compound **2** were similar
235 to, or lower than, the MICs of compound **4** for all strains. Interestingly, strains for which
236 the MICs of compounds **2** and **4** were the lowest (WHO A, WHO B, WHO C, WHO E,
237 WHO O, WHO Q), were those that were susceptible to FQ and whose *gyrA* and *parC*
238 genes were wild-type (MICs < 2 mg/L), whereas the strains resistant to FQ and
239 harbouring mutations in the *gyrA* and/or *parC* genes (WHO M, WHO G, WHO K,
240 WHO Z and barla194) displayed the highest MICs (≥ 2 mg/L). Unfortunately, the MICs
241 increase for bacteria with mutations in *gyrA* and *parC* is known to confer resistance to
242 FQ. These results strongly suggest cross resistance between the two compounds and
243 the FQ in clinical use, whereas, as expected, no cross resistance was observed with
244 antibiotics of other classes (*i.e.* beta-lactams, macrolides and tetracyclines) (Tables 1
245 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.*

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

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.

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.

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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

270 Table *2*: Minimum inhibitory concentrations (MICs) of ciprofloxacin, moxifloxacin, compounds 2 and 4 for several clinical and reference *N. gonorrhoeae*
271 carrying various susceptibility profile to FQ, macrolides and tetracyclines.
272 Abbreviations: CPX, ciprofloxacin; MXF, moxifloxacin
273 [a]S91F and D95G substitutions in GyrA
274 [b]E91G substitution in ParC, and S91F and D95G substitution in GyrA
275 [c]S91F substitution in GyrA
276 [d]S87R and S88P substitutions in ParC, and S91F and D95G substitutions in GyrA

Declaration of competing interests.

Declarations of interest: none, except for Alexandra Aubry. She declares the following financial interests/personal relationships which may be considered to be potential competing interests: she received support for travel and congress fees (MSD, BD, Eumedica); her research unit receives recurrent financial support from J&J (no personal remuneration) and she is a member of two IMI consortia aiming to develop new antimycobacterial drugs (RespiriTB and RespiriNTM).

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Appendix A. Experimental section is described in Supporting Information

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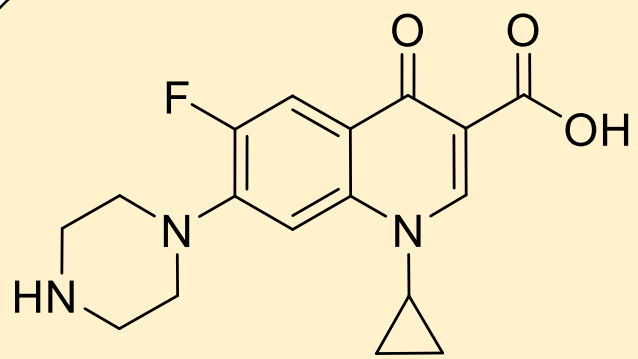
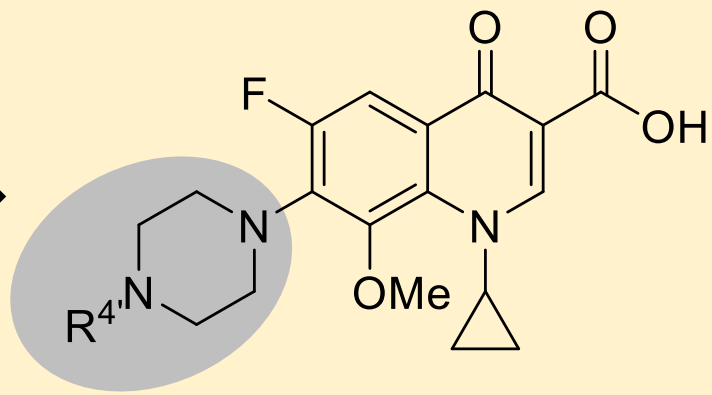


Ciprofloxacin (CPX)



Ciprofloxacin: $R^{4'}$ = H et R^8 = H

1 to **10**: R^8 = OMe

1: $R^{4'}$ = n-C_7H_{15}
2: $R^{4'}$ = n-C_8H_{17}
3: $R^{4'}$ = n-C_9H_9
4: $R^{4'}$ = n-$C_{10}H_{21}$
5: $R^{4'}$ = n-$C_{11}H_{23}$
6: $R^{4'}$ = n-$C_{12}H_{25}$
7: $R^{4'}$ = n-$C_{14}H_{29}$
8: $R^{4'}$ = $(CH_2CH_2O)_2CH_2CH_3$
9: $R^{4'}$ = 2-ethylhexyl
10: $R^{4'}$ = $(CO)C_7H_{15}$

Compounds **2** and **4** 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 (ESKAPE)

$\Rightarrow$ **increased lipophilicity is deleterious against antibacterial activity**

Supporting Information

Chemistry

Thin-layer chromatography was performed on TLC plastic sheets of silica gel 60F254 (layer thickness 0.2 mm) from Merck. Column chromatographic purification was carried out on silica gel 60 (70-230 mesh ASTM, Merck). IR, ^{1}H, ^{19}F and ^{13}C NMR spectra were used to characterize 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_2Cl_2, CD_3CN, D_2O or DMSO-d_6 as solvent, on a Bruker AC 300 or 400 spectrometer at 300 or 400 MHz for ^{1}H, 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 ^{1}H and (ii) to $CDCl_3$ (δ 77.2) for ^{13}C, and directly (iii) to $CFCl_3$ (internal standard) (δ 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. HRMS were obtained from the “Service Central d’analyse de Solaize” (Centre National de la Recherche Scientifique) and were recorded on a Waters spectrometer using electrospray ionization-Time-of-Flight (ESI-TOF). The purity of the final compounds (> 99%) were assessed by HPLC analyses (flow of 1 mL/min) using a Waters photodiode array detector apparatus (PDA, UV detector from 210 to 295 nm) using a X-Bridge (5 µm)-packed C18 column (150 mm*4.6) or a Sunfire (3.5 µm)-packed C8 column (150 mm*4.6) and three solvent systems, *i.e.* solvent A (H_2O + 0,02% HCOOH), solvent C (NH_4COOH 5 mM pH 8) and solvent D (CH_3CN). Elemental analysis was performed by the “Service de Microanalyse” de l’Institut de Chimie des Substances Naturelles (Gif-sur-Yvette, France). The clogP and clogD theoritical values

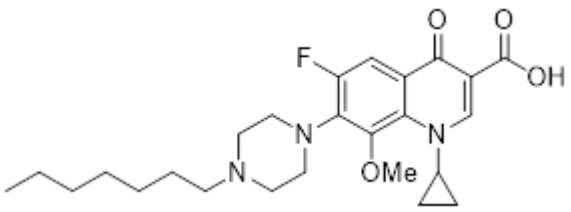
were assessed using ChemDraw 2019. Synthon **B** was prepared from synthon **A** (Sigma-Aldrich) as described in literature.[1] Compounds **8-10** were designed by Guillaume Anquetin and synthesized by Diverchim, France.

Experimental conditions i (compounds 1-7) (Fig. 2 and 3)

To a suspension of 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (synthon **B**, 75 mg, 0.20 mmol), in dry dimethylformamide (DMF) (18 mL), were added 1-iodoalkyl (2.75 eq.) and $NaHCO_3$ (6 eq.). The reaction mixture was stirred at 40°C for 40h and then concentrated under reduced pressure. The residue was taken up in dichloromethane (DCM) (40 mL) and the organic layer was washed with water (30 mL) and dried over anhydrous $MgSO_4$ to give a mixture of 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-alkylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid and 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-alkylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic alkyl ester.

The mixture was dissolved in an EtOH/H_2O (5/2) mixture (20 mL), and LiOH (8 eq.) was added. The reaction mixture was stirred overnight at room temperature and then acidified with HCl_{aq} 1 N to pH 3. Ethanol was removed under reduced pressure and the aqueous layer was extracted with DCM (40 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ (20 mL), dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crude solid obtained was washed with Et_2O (3×5 mL) to yield 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-alkylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (25-35%) as a white-yellow powder.

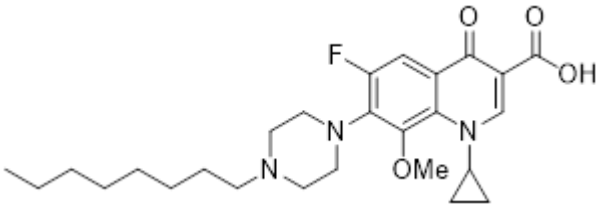
Preparation of 1-cyclopropyl-6-fluoro-8-methoxy-7-(4-heptylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (compound 1).



Likewise, the experimental conditions i when applied to 1-iodoheptane (125 mg, 0.55 mmol) afforded a mixture of 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-heptylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid and 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-heptylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic heptyl ester. After the basic hydrolysis, the crude solid obtained was washed with Et_2O (3×5 mL) to yield 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-heptylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (28 mg, 30% over 2 steps) as a white powder.

^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.80 (s, 1H, H_2), 7.85 (d, 1H, $^3J_{H\text{-}F}$ = 12.0 Hz, H_5), 4.01 (m, 1H, C*H*(cPr)), 3.76 (s, 3H, OC*H*$_3$), 3.48 (br s, 4H, $H_{2'}$ and $H_{3'}$), 2.64 (br s, 4H, $H_{1'}$ and $H_{4'}$), 2.45 (m, 2H, NC*H*$_2$$CH_2CH_2$), 1.56 (m, 2H, NCH_2C*H*$_2$$CH_2$), 1.32-1.29 (m, 10H, CH_2), 1.02-0.95 (m, 2H, C*H*$_2$(cPr)), 0.87 (t, $^3J_{H\text{-}H}$ = 6.8 Hz, 3H, C*H*$_3$). **^{13}C NMR (75 MHz, $CDCl_3$, δ):** 177.1 (d, *J* = 3.1 Hz, C_4), 166.9 (s, *C*O_2H), 156.2 (d, *J* = 250.7 Hz, C_6), 150.0 (s, C_2), 145.4 (d, *J* = 5.8 Hz, C_8), 139.5 (s, C_7), 134.1 (s, C_9), 121.4 (s, C_{10}), 108.1 (s, C_3), 108.0 (d, *J* = 16.3 Hz, C_5), 62.7 (s, O*C*H_3), 59.1 (s, N*C*$H_2CH_2CH_2$), 53.9 (s, $C_{1'}$ and $C_{4'}$), 50.5 (d, $C_{2'}$ and $C_{3'}$), 40.7 (s, *C*H(cPr)), 31.9 (s, CH_2), 29.3 (s, CH_2), 29.0 (s, CH_2), 27.6 (s, CH_2), 26.7 (s, CH_2), 22.7 (s, CH_2), 14.2 (s, CH_3), 9.7 (s, 2CH_2(cPr)). **Elemental Analysis:** C = 64.62%, H = 7.52%, N = 8.83%, calcd C = 65.34%, H = 7.46%, N = 9.14%. **Melting Point** = 157.2°C. **HPLC**: R_t = 11.1 min (solvent C/D: 5/95).

Preparation of 1-cyclopropyl-6-fluoro-8-methoxy-7-(4-octylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (compound 2).

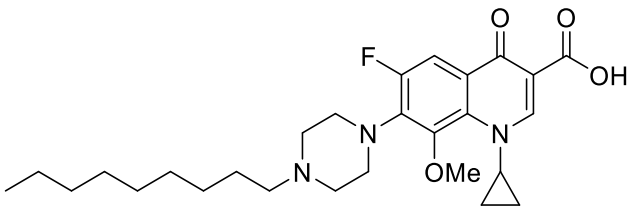


Likewise, the experimental conditions i when applied to 1-iodooctane (133 mg, 0.55 mmol) afforded a mixture of 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-octylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid and 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-octylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic octyl ester. After the basic hydrolysis, the crude solid obtained was washed with Et_2O (3×5 mL) to yield 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-octylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (30 mg, 33% over 2 steps) as a yellow powder.

^{1}H NMR (400 MHz, CD_2Cl_2, δ): 14.77 (broad s, 1H, CO_2H), 8.77 (s, 1H, H_2), 7.80 (d, 1H, $^3J_{H\text{-}F}$ = 12.4 Hz, H_5), 4.04 (m, 1H, C*H*(cPr)), 3.77 (s, 3H, OCH_3), 3.43 (br s, 4H, $H_{2'}$ and $H_{3'}$), 2.58 (br s, 4H, $H_{1'}$ and $H_{4'}$), 2.39 (m, 2H, NC$H_2$$CH_2CH_2$), 1.50 (m, 2H, NCH_2C$H_2$$CH_2$), 1.42-1.24 (m, 10H, 5 CH_2), 1.20 (q, 2H, *J* = 6.9 Hz, CH_2(cPr)), 1.02-0.95 (m, 2H, CH_2(cPr)), 0.89 (t, 3H, $^3J_{H\text{-}H}$ = 6.8 Hz, CH_3). **^{19}F NMR (376 MHz, CD_2Cl_2, δ):** -120.1 (s, F_6). **^{13}C NMR (100 MHz, CD_2Cl_2, δ):** 177.5 (d, *J* = 3.1 Hz, C_4), 166.8 (s, *C*O_2H), 156.7 (d, *J* = 250.8 Hz, C_6), 150.3 (s, C_2), 145.9 (d, *J* = 5.8 Hz, C_8), 140.1 (d, *J* = 11.7 Hz, C_7), 134.6 (s, C_9), 121.9 (d, *J* = 9.2 Hz, C_{10}), 108.0 (s, C_3), 107.9 (d, *J* = 23.3 Hz, C_5), 62.8 (s, OCH_3), 59.3 (s, $NCH_2CH_2CH_2$), 54.3 (s, $C_{1'}$ and $C_{4'}$), 51.2 (d, *J* = 4.6 Hz, $C_{2'}$ and $C_{3'}$), 41.0 (s, *C*H(cPr)), 32.3 (s, CH_2), 30.0 (s, CH_2), 29.7 (s, CH_2), 27.9 (s, CH_2), 27.2 (s, CH_2), 23.1 (s, CH_2), 14.3 (s, CH_3), 9.8 (s, 2CH_2(cPr)). **IR (neat):** v = 3084, 2926, 2853, 2809, 2770, 1728, 1617, 1601, 1554, 1539, 1505, 1436, 1383,

1376, 1312, 1280, 1238, 1204, 1187, 1144, 1128, 1115, 1091, 1055, 1040, 1008, 993, 957, 934, 887, 831, 821, 805, 730, 710 cm^{-1}; **HRMS (ESI+)** *m/z*: $[M+H]^+$ calcd for $C_{26}H_{36}FN_3O_4$: 474.2769, found: 474.2765. **Elemental Analysis:** C = 66.11%, H = 7.78%, N = 8.82%, calcd C = 65.94%, H = 7.66%, N = 8.87%. **Melting Point** = 146.3°C. **HPLC**: R_t = 12.0 min (solvent C/D: 5/95).

Preparation of 1-cyclopropyl-7-(4-nonylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (compound 3).

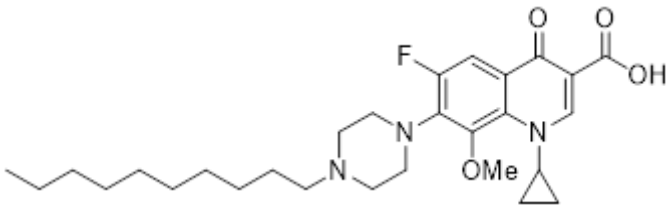


Likewise, the experimental conditions i when applied to 1-iodononane (144 mg, 0.57 mmol) afforded a mixture of 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-nonylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid and 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-nonylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic nonyl ester. After the basic hydrolysis, the crude solid obtained was washed with Et_2O (3×5 mL) to yield 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-nonylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (33 mg, 34% over 2 steps) as a white powder.

^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.95 (s, 1H, H_2), 7.90 (d, 1H, $^3J_{H\text{-}F}$ = 12.0 Hz, H_5), 4.07 (m, 1H, C*H*(cPr)), 3.80 (s, 3H, OCH_3), 3.50 (br s, 4H, $H_{2'}$ and $H_{3'}$), 2.65 (br s, 4H, $H_{1'}$ and $H_{4'}$), 2.46 (m, 2H, NC$H_2$$CH_2CH_2$), 1.58 (m, 2H, NCH_2CH_2CH_2), 1.40-1.24 (m, 14H, $6CH_2$ + CH_2(cPr)), 1.05-1.02 (m, 2H, CH_2(cPr)), 0.92 (t, 3H, $^3J_{H\text{-}H}$ = 6.8 Hz, CH_3) ; **^{13}C**

NMR (75 MHz, $CDCl_3$, δ): 177.1 (d, *J* = 3.1 Hz, C_4), 166.9 (s, *C*O_2H), 156.2 (d, *J* = 250.0 Hz, C_6), 154.5 (s, C_2), 145.3 (d, *J* = 5.7 Hz, C_8), 139.7 (d, *J* = 12.0 Hz, C_7), 134.0 (d, *J* = 1.6 Hz, C_9), 121.6 (d, *J* = 9.0 Hz, C_{10}), 108.4 (s, C_3), 108.0 (d, *J* = 18.8 Hz, C_5), 62.6 (s, O*C*H_3), 59.1 (s, N*C*$H_2$$CH_2$$CH_2$), 54.0 (s, $C_{1'}$ and $C_{4'}$), 50.8 (d, *J* = 4.6 Hz, $C_{2'}$ and $C_{3'}$), 40.6 (s, *C*H(cPr)), 32.0 (s, CH_2), 29.7 (s, CH_2), 29.4 (s, CH_2), 27.7 (s, CH_2), 26.9 (s, CH_2), 22.8 (s, CH_2), 14.2 (s, CH_3), 9.6 (s, 2CH_2(cPr)). **Elemental Analysis:** C = 66.36%, H = 7.86%, N = 8.50%, calcd C = 66.51%, H = 7.85%, N = 8.62%. **Melting Point** = 144.4°C. **HPLC**: R_t = 12.9 min (solvent C/D: 5/95).

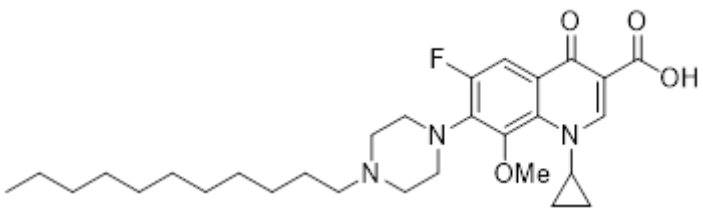
Preparation of 1-cyclopropyl-7-(4-decylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (compound 4).



Likewise, the experimental conditions i when applied to 1-iododecane (163 mg, 0.61 mmol) afforded a mixture of 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-decylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid and 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-decylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic decyl ester. After the basic hydrolysis, the crude solid obtained was washed with Et_2O (3×5 mL) to yield 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-decylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (30 mg, 29% over 2 steps) as a white powder.

^{1}H NMR (400 MHz, CD_2Cl_2, δ): 14.78 (br s, 1H, CO_2H), 8.78 (s, 1H, H_2), 7.82 (d, $^{3}J_{H\text{-}F}$ = 12.4 Hz, 1H, H_5), 4.04 (m, 1H, C*H*(cPr)), 3.76 (s, 3H, OCH_3), 3.43 (br s, 4H, $H_{2'}$ and $H_{3'}$), 2.57 (br s, 4H, $H_{1'}$ and $H_{4'}$), 2.39 (m, 2H, NC$H_2$$CH_2CH_2$), 1.51 (m, 2H, NCH_2C$H_2$$CH_2$), 1.37-1.24 (m, 14H, CH_2), 1.20 (m, 2H, CH_2(cPr)), 1.02-0.95 (m, 2H, CH_2(cPr)), 0.89 (t, $^{3}J_{H\text{-}H}$ = 6.7 Hz, 3H, CH_3). **^{19}F NMR (376 MHz, CD_2Cl_2, δ):** -120.1 (s, F_6). **^{13}C NMR (100 MHz, CD_2Cl_2, δ):** 177.5 (d, *J* = 3.1 Hz, C_4), 166.9 (s, *C*O_2H), 156.7 (d, *J* = 250.9 Hz, C_6), 150.3 (s, C_2), 145.9 (d, *J* = 5.8 Hz, C_8), 140.1 (d, *J* = 11.8 Hz, C_7), 134.6 (s, C_9), 121.9 (d, *J* = 9.1 Hz, C_{10}), 108.0 (s, C_3), 107.9 (d, *J* = 23.2 Hz, C_5), 62.8 (s, O*C*H_3), 59.3 (s, N*C*$H_2CH_2CH_2$), 54.3 (s, $C_{1'}$ and $C_{4'}$), 51.2 (d, *J* = 4.7 Hz, $C_{2'}$ and $C_{3'}$), 41.0 (s, *C*H(cPr)), 32.3 (s, CH_2), 30.1 (s, CH_2), 30.0 (s, 2CH_2), 29.8 (s, CH_2), 27.9 (s, CH_2), 27.3 (s, CH_2), 23.1 (s, CH_2), 14.3 (s, CH_3), 9.8 (s, 2CH_2(cPr)). **IR (neat):** v = 3071; 2924, 2852, 2770, 1729, 1618, 1601, 1536, 1506, 1441, 1394, 1384, 1313, 1281, 1239, 1206, 1188, 1148, 1129, 1116, 1091, 1055, 1042, 1003, 959, 937, 888, 879, 831, 821, 805, 730, 710 cm^{-1}; **HRMS (ESI+)** *m/z*: $[M+H]^+$ calcd for $C_{28}H_{40}FN_3O_4$: 502.3082, found, 502.3077. **Elemental Analysis:** C = 67.28%, H = 8.21%, N = 8.31%, calcd C = 67.04%, H = 8.04%, N = 8.38%. **Melting Point** = 136.1°C. **HPLC**: R_t = 13.9 min (solvent C/D: 5/95).

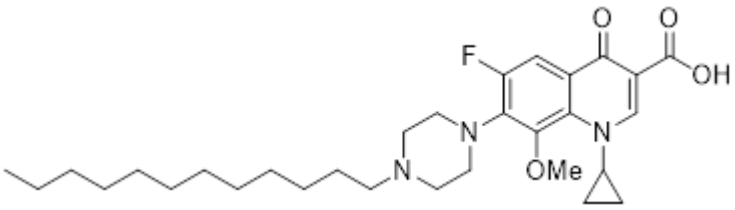
Preparation of 1-cyclopropyl-7-(4-undecylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (compound 5).



Likewise, the experimental conditions i when applied to 1-iodoundecane (160 mg, 0.54 mmol) afforded a mixture of 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-undecylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid and 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-undecylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic undecyl ester. After the basic hydrolysis, the crude solid obtained was washed with Et_2O (3×5 mL) to yield 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-undecylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (36 mg, 35% over 2 steps) as a white powder.

^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.84 (s, 1H, H_2), 7.89 (d, 1H, $^3J_{H\text{-}F}$ = 12.0 Hz, H_5), 4.07 (m, 1H, C*H*(cPr)), 3.80 (s, 3H, OCH_3), 3.50 (br s, 4H, $H_{2'}$ and $H_{3'}$), 2.66 (br s, 4H, $H_{1'}$ and $H_{4'}$), 2.46 (m, 2H, NCH_2CH_2CH_2), 1.58 (m, 2H, $NCH_2CH_2CH_2$), 1.36-1.23 (m, 18H, $6CH_2$ + CH_2(cPr)), 1.05-1.03 (m, 2H, CH_2(cPr)), 0.92 (t, 3H, $^3J_{H\text{-}H}$ = 6.8 Hz, CH_3) ; **^{13}C NMR (75 MHz, $CDCl_3$, δ):** 177.1 (d, *J* = 3.1 Hz, C_4), 166.9 (s, *C*O_2H), 156.2 (d, *J* = 250.5 Hz, C_6), 154.6 (s, C_2), 145.4 (d, *J* = 5.6 Hz, C_8), 139.6 (s, C_7), 134.1 (s, C_9), 121.7 (d, *J* = 9.0 Hz, C_{10}), 108.5 (s, C_3), 108.0 (d, *J* = 18.2 Hz, C_5), 62.6 (s, O*C*H_3), 59.2 (s, N$CH_2CH_2CH_2$), 54.0 (s, $C_{1'}$ and $C_{4'}$), 50.8 (s, $C_{2'}$ and $C_{3'}$), 40.7 (s, *C*H(cPr)), 32.1 (s, CH_2), 29.8 (s, CH_2), 29.7 (s, CH_2), 29.5 (s, CH_2), 27.7 (s, CH_2), 26.9 (s, CH_2), 22.8 (s, CH_2), 14.3 (s, CH_3), 9.7 (s, $2CH_2$(cPr)). **Elemental Analysis:** C = 67.27%, H = 8.14%, N = 8.02%, calcd C = 67.55%, H = 8.21%, N = 8.15%. **Melting Point** = 136.3°C. **HPLC**: R_t = 14.9 min (solvent C/D: 5/95).

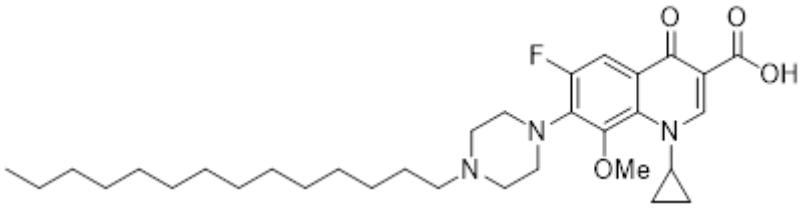
Preparation of 1-cyclopropyl-7-(4-dodecylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (compound 6).



Likewise, the experimental conditions i when applied to 1-iodododecane (163 mg, 0.61 mmol) afforded a mixture of 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-dodecylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid and 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-dodecylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic dodecyl ester. After the basic hydrolysis, the crude solid obtained was washed with Et_2O (3×5 mL) to yield 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-dodecylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (30 mg, 28% over 2 steps) as a white powder.

^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.84 (s, 1H, H_2), 7.90 (d, 1H, $^3J_{H\text{-}F}$ = 12.0 Hz, H_5), 4.06 (m, 1H, C*H*(cPr)), 3.80 (s, 3H, OCH_3), 3.50 (br s, 4H, $H_{2'}$ and $H_{3'}$), 2.66 (br s, 4H, $H_{1'}$ and $H_{4'}$), 2.46 (m, 2H, NC$H_2$$CH_2CH_2$), 1.58 (m, 2H, NCH_2C$H_2$$CH_2$), 1.36-1.24 (m, 20H, 6$CH_2$ + CH_2(cPr)), 1.04-1.02 (m, 2H, CH_2(cPr)), 0.92 (t, 3H, $^3J_{H\text{-}H}$ = 6.8 Hz, CH_3) ; **^{13}C NMR (75 MHz, $CDCl_3$, δ):** 177.0 (d, *J* = 3.1 Hz, C_4), 166.9 (s, *C*O_2H), 156.2 (d, *J* = 250.4 Hz, C_6), 154.6 (s, C_2), 145.3 (d, *J* = 5.6 Hz, C_8), 139.6 (d, *J* = 1.6 Hz, C_7), 134.0 (s, C_9), 121.7 (d, *J* = 9.2 Hz, C_{10}), 108.4 (s, C_3), 108.1 (d, *J* = 18,2 Hz, C_5), 62.6 (s, O*C*H_3), 59.1 (s, N*C*$H_2CH_2CH_2$), 53.9 (s, $C_{1'}$ and $C_{4'}$), 50.7 (s, $C_{2'}$ and $C_{3'}$), 40.6 (s, *C*H(cPr)), 32.0 (s, CH_2), 29.7 (2s, 3CH_2), 29.6 (2s, 2CH_2), 29.4 (s, CH_2), 27.6 (s, CH_2), 26.8 (s, CH_2), 22.8 (s, CH_2), 14.2 (s, CH_3), 9.6 (s, 2CH_2(cPr)). **Elemental Analysis:** C = 67.89%, H = 8.49%, N = 7.82%, calcd C = 68.03%, H = 8.37%, N = 7.93%. **Melting Point** = 134.6°C. **HPLC**: R_t = 10.4 min (solvent A/solvent D: 65/35).

Preparation of 1-cyclopropyl-7-(4-tetradecylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (compound 7).



Likewise, the experimental conditions i when applied to 1-iodotetradecane (163 mg, 0.61 mmol) afforded a mixture of 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-tetradecylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid and 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-tetradecylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic tetradecyl ester. After the basic hydrolysis, the crude solid obtained was washed with Et_2O (3×5 mL) to yield 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-tetradecylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (28 mg, 25% over 2 steps) as a yellow powder.

^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.85 (s, 1H, H_2), 7.90 (d, 1H, $^3J_{H\text{-}F}$ = 12.0 Hz, H_5), 4.06 (m, 1H, C*H*(cPr)), 3.80 (s, 3H, OC*H*$_3$), 3.49 (br s, 4H, $H_{2'}$ and $H_{3'}$), 2.65 (br s, 4H, $H_{1'}$ and $H_{4'}$), 2.45 (m, 2H, NC*H*$_2$$CH_2CH_2$), 1.58 (m, 2H, NCH_2C*H*$_2$$CH_2$), 1.34-1.24 (m, 24H, $6CH_2$ + C*H*$_2$(cPr)), 1.04-1.02 (m, 2H, C*H*$_2$(cPr)), 0.92 (t, 3H, $^3J_{H\text{-}H}$ = 6.8 Hz, C*H*$_3$) ; **^{13}C NMR (75 MHz, $CDCl_3$, δ):** 177.1 (d, *J* = 3.1 Hz, C_4), 166.9 (s, *C*O_2H), 156.2 (d, *J* = 250.6 Hz, C_6), 154.5 (s, C_2), 145.3 (d, *J* = 5.6 Hz, C_8), 139.6 (d, *J* = 11.6Hz, C_7), 134.0 (s, C_9), 121.7 (d, *J* = 8.9 Hz, C_{10}), 108.4 (s, C_3), 108.1 (d, *J* = 19.0 Hz, C_5), 62.5 (s, O*C*H_3), 59.1 (s, N*C*$H_2CH_2CH_2$), 54.0 (s, $C_{1'}$ and $C_{4'}$), 50.7 (s, $C_{2'}$ and $C_{3'}$), 40.6 (s, *C*H(cPr)), 32.0 (s, CH_2), 29.7 (3s, $3CH_2$), 29.6 (2s, $2CH_2$), 29.4 (s, CH_2), 27.6 (s, CH_2), 26.9 (s, CH_2), 22.7 (s, CH_2), 14.2 (s, CH_3), 9.6 (s, $2CH_2$(cPr)). **Elemental Analysis:**

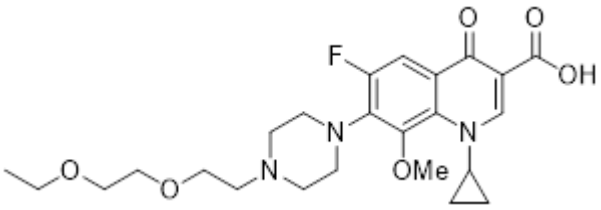
C = 69.10%, H = 8.85%, N = 7.44%, calcd C = 68.91%, H = 8.67%, N = 7.53%. **Melting Point** = 131.1°C. **HPLC**: R_t = 10.9 min (solvent A/solvent D: 65/35).

Experimental conditions ii (compounds 8 and 9) (Fig. 2 and 3)

To a suspension of 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (1eq), in dry DCM, were added the aldehyde (1.2 eq) and acetic acid (6 eq.). $NaBH(OAc)_3$ (1.3 eq.) was added portion wise and the mixture was stirred at room temperature overnight.

Water (450 mL) was added and the mixture was filtered on celite. The cake was washed with dichloromethane and the phases were separated. The organic phase was washed with water, dried over magnesium sulfate, filtered and concentrated to dryness. The product was purified two times by column chromatography (silicagel, gradient DCM/Methanol 90/10, 0.5% AcOH) resulting in the expected product (yellow solid, isolated yields between 20 and 40%).

Preparation of 1-cyclopropyl-7-(4-(2-(2-ethoxyethoxy)ethyl)piperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (compound 8).

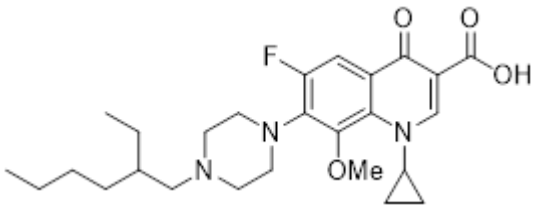


Likewise, the experimental conditions ii when applied to 2-(2-ethoxyethoxy)acetaldehyde (2.18 g, 16.5 mmol), synthon **B** (3.98 g, 11.0 mmol), acetic acid (3.96 g, 66 mmol) and $NaBH(OAc)_3$ (3.03 g, 14.3 mmol) afforded, after

column chromatography, 1-cyclopropyl-7-(4-(2-(2-ethoxyethoxy)ethyl)piperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid as a yellow solid (1.05 g, 20%).

^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.84 (s, 1H, H_2), 7.90 (d, 1H, $^3J_{H\text{-}F}$ = 12.0 Hz, H_5), 4.05 (m, 1H, C*H*(cPr)), 3.77 (s, 3H, OC*H*$_3$), 3.70-3.52 (m, 12H, 4CH_2O and 2CH_2N), 2.77 (br s, 4H, $H_{1'}$ and $H_{4'}$), 1.26 (t, 3H, $^3J_{H\text{-}H}$ = 6.8 Hz, C*H*$_3$), 1.21-1.16 (m, 2H, C*H*$_2$(cPr)), 1.04-1.02 (m, 2H, C*H*$_2$(cPr)); **^{13}C NMR (75 MHz, $CDCl_3$, δ):** 177.4 (d, *J* = 3.1 Hz, C_4), 167.2 (s, *C*O_2H), 156.5 (d, *J* = 250.3 Hz, C_6), 150.2 (s, C_2), 145.7 (d, *J* = 5.6 Hz, C_8), 139.9 (d, *J* = 11.8 Hz, C_7), 134.3 (d, *J* = 1.6 Hz, C_9), 121.9 (d, *J* = 9.1 Hz, C_{10}), 108.6 (s, C_3), 108.1 (d, *J* = 20.1 Hz, C_5), 70.8 (s, $C_{1'}$ and $C_{4'}$), 70.2 (s, $C_{2'}$ and $C_{3'}$), 69.0 (s, *C*H(cPr)), 67.0 (s, CH_2), 62.9 (s, OCH_3), 58.4 (s, 2CH_2), 54.5 (s, $C_{1'}$ and $C_{4'}$), 50.8 (s, CH_2), 50.7 (s, $C_{2'}$ and $C_{3'}$), 41.0 (s, *C*H(cPr)), 15.5 (s, CH_3), 9.9 (s, 2CH_2(cPr)). **Elemental Analysis:** C = 59.16%, H = 6.71%, N = 8.28%, calcd C = 59.76%, H = 6.75%, N = 8.80%. **HPLC**: R_t = 8.7 min (solvent C/D: 5/95).

Preparation of 1-cyclopropyl-7-(2-(ethyl)hexylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (compound 9).



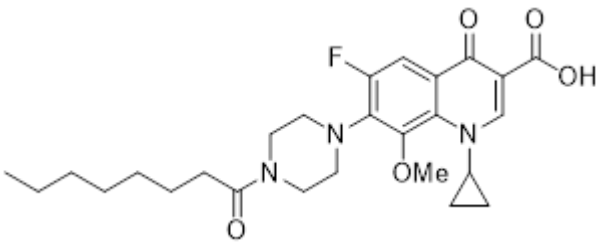
Likewise, the experimental conditions ii when applied to 2-ethylhexanal (1.32 g, 10.3 mmol), synthon **B** (2.48 g, 6.85 mmol), acetic acid (2.47 g, 41.1 mmol) and $NaBH(OAc)_3$ (1.89 g, 8.90 mmol) afforded, after column chromatography, 1-

cyclopropyl-7-(2-(ethyl)hexylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid as a white solid (1.30 g, 40%).

^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.85 (s, 1H, H_2), 7.90 (d, 1H, $^{3}J_{H\text{-}F}$ = 12.0 Hz, H_5), 4.10-4.02 (m, 1H, C*H*(cPr)), 3.82 (s, 3H, OCH_3), 3.58-3.48 (br s, 4H, $H_{2'}$ and $H_{3'}$), 2.70 (br s, 4H, $H_{1'}$ and $H_{4'}$), 2.28 (m, 2H, NCH_2CH), 1.70 (m, 1H, NCH_2CH), 1.34-1.21 (m, 10H, $4CH_2$ + CH_2(cPr)), 1.06-1.00 (m, 2H, CH_2(cPr)), 0.97-0.87 (m, 6H, 2CH_3); **^{13}C NMR (75 MHz, $CDCl_3$, δ):** 177.4 (d, *J* = 3.1 Hz, C_4), 167.3 (s, CO_2H), 156.6 (d, *J* = 250.5 Hz, C_6), 150.2 (s, C_2), 145.6 (d, *J* = 5.8 Hz, C_8), 140.0 (d, *J* = 11.6 Hz, C_7), 134.4 (d, *J* = 1.5 Hz, C_9), 121.8 (d, *J* = 9.1 Hz, C_{10}), 108.7 (s, C_3), 108.2 (d, *J* = 24.8 Hz, C_5), 62.7 (s, OCH_3), 59.1 (s, $NCH_2CH_2CH_2$), 54.7 (s, $C_{1'}$ and $C_{4'}$), 51.2 (s, $C_{2'}$ and $C_{3'}$), 41.0 (s, *C*H(cPr)), 36.5 (s, CH) 31.9 (s, CH_2), 29.4 (s, CH_2), 25.0 (s, CH_2), 23.6 (s, CH_2), 14.5 (s, CH_3), 11.2 (s, CH_3), 10.0 (s, $2CH_2$(cPr)). **Elemental Analysis:** C = 65.66%, H = 7.49%, N = 8.57%, calcd C = 65.94%, H = 7.66%, N = 8.87%. **HPLC**: R_t = 18.6 min (solvent C/D: 5/95).

Experimental conditions iii (Fig. 2 and 3)

Preparation of 1-cyclopropyl-6-fluoro-8-methoxy-7-(4-octanoylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (compound 10).



To a suspension of 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (2.28 g, 6.30 mmol, 1eq) in dichloromethane, at 0°C were added triethylamine (0.83 g, 8.19 mmol, 1.3 eq.) and acyl chloride (1.537 g, 9.45 mmol, 1.5 eq.). The mixture was stirred at room temperature for 1 hour. Hexane was added and the mixture was filtered. The filtrate was evaporated and the resulted solid was purified by column chromatography (silicagel, gradient DCM/Methanol 95/5) resulting in the expected product white solid (1.23 g, 2.52 mmol, 40%).

^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.87 (s, 1H, H_2), 7.95 (d, 1H, $^{3}J_{H\text{-}F}$ = 12.0 Hz, H_5), 4.06 (m, 1H, C*H*(cPr)), 3.86-3.71 (m, 7H, OC*H*$_3$, $H_{2'}$ and $H_{3'}$), 3.44 (br s, 4H, $H_{1'}$ and $H_{4'}$), 2.42 (t, 2H, NCOC*H*$_2$), 1.43-1.23 (m, 12H, 6 CH_2), 1.20 (q, *J* = 7.0 Hz, 2H), 1.07-1.02 (m, 2H, C*H*$_2$(cPr)), 0.89 (m, 3H, C*H*$_3$). **^{13}C NMR (75 MHz, $CDCl_3$, δ):** 177.4 (d, *J* = 3.1 Hz, C_4), 167.3 (s, *C*O_2H), 156.5 (d, *J* = 250.2 Hz, C_6), 150.2 (s, C_2), 145.6 (d, *J* = 5.4 Hz, C_8), 139.4 (d, *J* = 12.0 Hz, C_7), 134.4 (s, C_9), 122.9 (d, *J* = 8.9 Hz, C_{10}), 108.7 (s, C_3), 108.5 (d, *J* = 27.6 Hz, C_5), 62.7 (s, O*C*H_3), 59.1 (s, N*C*H_2*C*H_2*C*H_2), 54.7 (s, $C_{1'}$ and $C_{4'}$), 51.2 (s, $C_{2'}$ and $C_{3'}$), 41.0 (s, *C*H(cPr)), 36.5 (s, CH) 31.9 (s, CH_2), 29.4 (s, CH_2), 25.0 (s, CH_2), 23.6 (s, CH_2), 14.5 (s, CH_3), 11.2 (s, CH_3), 10.0 (s, 2CH_2(cPr)). **Elemental Analysis:** C = 63.31%, H = 7.10%, N = 8.08%, calcd C = 64.05%, H = 7.03%, N = 8.62%. **HPLC**: R_t = 13.3 min (solvent A/solvent D: 65/35).

Antibacterial activity evaluation

Antibiotics

Moxifloxacin (MXF) and ciprofloxacin (CPX) were obtained from Sigma-Aldrich (France) and used without further purification.

Bacterial strains

Bacterial strains were cultured on two media depending on the species. Several bacteria were studied and cultured on Mueller-Hinton agar (Bio-Rad) (3 *Acinetobacter baumannii,* 3 *Escherichia coli,* , 3 *Pseudomonas aeruginosa*, 3 *Staphylococcus aureus* and 1 *Streptococcus pneumoniae*), whereas, both reference and clinical *Neisseria gonorrhoeae* strains were studied and cultured on GC agar (CM0367, Oxoid) plates supplemented with 2% v/v Vitox (SR0090A, Oxoid) and 1% haemoglobin (LP0053, Oxoid) (a reference strain ATCC 19424, a collection of 10 WHO international reference strains (WHO A, WHO B, WHO C, WHO E, WHO G, WHO K, WHO M, WHO O, WHO Q, WHOZ)[2,3] and one clinical strain highly resistant to macrolides (named Barla 194) available at the French National Reference Centre for bacterial sexually transmitted infections) (Tables 1 and 2).

Antibacterial susceptibility testing

The minimum inhibitory concentrations (MICs) of the compounds **1-10**, MXF and CPX were determined by broth microdilution, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines,[4] for *A. baumannii*, *E. coli*, *P. aeruginosa*, *S. aureus and S. pneumoniae* strains, and by the agar dilution method for *N. gonorrhoeae strains.* Briefly, suspensions of *N. gonorrhoeae* strains (10^5 CFU/mL) were spotted, in duplicate, on GC agar base with 2% v/v Vitox and 1% haemoglobin and containing various concentrations of each compound tested. Antibiotic-containing agar plates were incubated for 24-48h at 37°C in a 5% CO_2 atmosphere.

The MIC was defined as the lowest concentration of compound that inhibited bacterial growth and the MIC values were determined at least three times in independent experiments.

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