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► To cite this version:

Elodie Sadowski, Beatrice Bercot, Aurélie Chauffour, Catherine Gomez, Emmanuelle Varon, et al.. Lipophilic quinolone derivatives: Synthesis and in vitro antibacterial evaluation. *Bioorganic and Medicinal Chemistry Letters*, 2022, 55, pp.128450. 10.1016/j.bmcl.2021.128450 . hal-03448562v1

HAL Id: hal-03448562

<https://hal.sorbonne-universite.fr/hal-03448562v1>

Submitted on 25 Nov 2021 (v1), last revised 25 Nov 2021 (v2)

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1 **Lipophilic quinolone derivatives: synthesis and *in vitro* antibacterial evaluation**

2

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51 **ABSTRACT**

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

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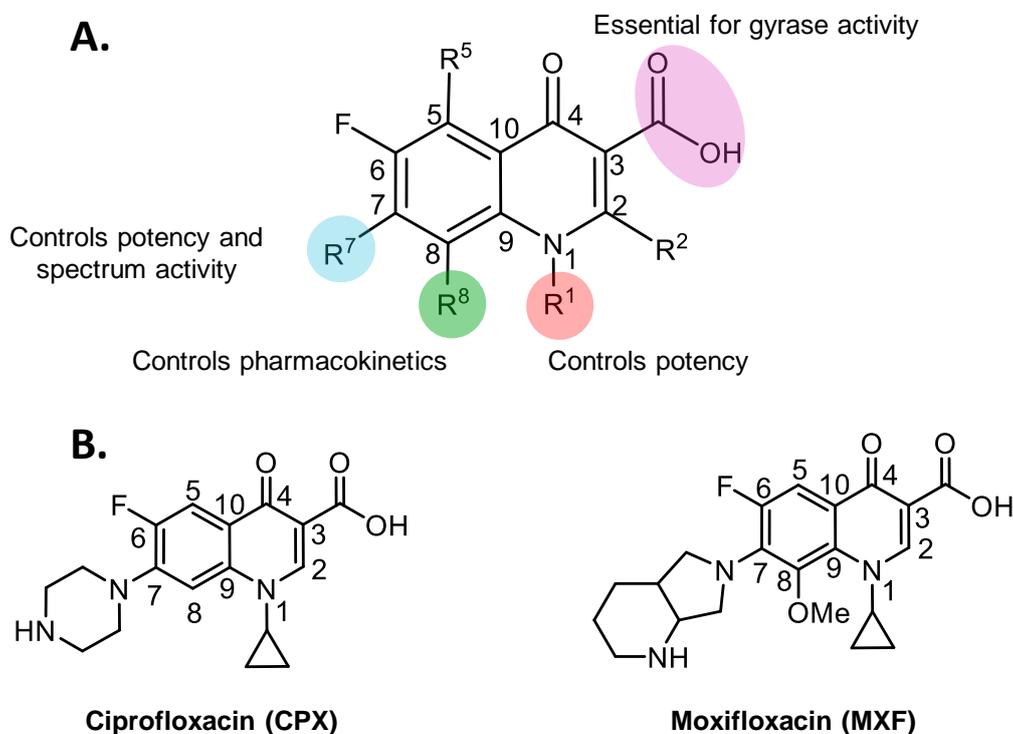
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64 **Keywords:** antibacterial, fluoroquinolones, synthesis, *Neisseria gonorrhoeae*,

65 ESKAPE

66

67 Since the discovery of norfloxacin, the first fluoroquinolone (FQ),¹ which is structurally
 68 characterised by a R⁶ fluorine atom in the quinolone ring that results in improved
 69 potency and spectrum of activity, FQ have become a significant class of clinically useful
 70 antibacterial agents. The development of new FQ gave rise to several FDA-approved
 71 drugs, such as ciprofloxacin (CPX) and moxifloxacin (MXF), in which the 1-substituted-
 72 1,4-dihydro-6-fluoro-4-oxo-7-piperazinyl (or 7-octahydro-1H-pyrrolo[3,4-b]-
 73 pyridinyl for MXF)-3-carboxylic acid moiety is the basic scaffold (Fig. 1 for FQ
 74 numbering system).²



75

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

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

82 However, excessive use of FQ has led to the emergence of FQ-resistant (FQ-R)
83 bacteria. The prevalence and spread of FQ-R bacteria have been reported among
84 various important human pathogens including the ESKAPE pathogens (*Enterococcus*
85 *faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*,
86 *Pseudomonas aeruginosa*, and *Enterobacter* species) as well as *Escherichia coli*,
87 *Streptococcus pneumoniae* and *Neisseria gonorrhoeae*, and they have become a
88 major public health concern over the past years.⁶⁻⁹

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

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

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

103 Lipophilicity of FQ is a key factor for their penetration into mammalian cells and the
104 central nervous system. For example, introduction of a lipophilic fluorine atom at R⁶
105 position was a triggering event for quinolone use, but the increased lipophilicity of FQ

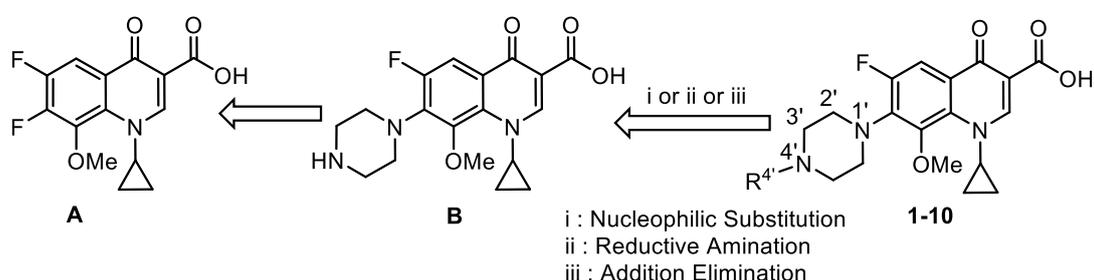
106 with the fluorine at R⁸ was gained only at the expense of higher toxicity. FQ prodrugs
107 were designed to increase their lipophilicity and thereby their biological activity¹⁵ but,
108 apart from the incomplete work of Grohe *et al.* in 1986 on some FQ bearing alkylated
109 piperazines, the impact of the lipophilicity of quinolones with a long alkyl chain has not
110 been investigated extensively.¹⁶

111 Examples of the synthesis and evaluation of FQ derivatives have been reported, with
112 the main modifications made at R⁷ position where aminopyrrolidines and piperazines
113 are the most effective substituents for improvement of the antimicrobial activity (Fig.1
114 for FQ numbering).¹⁷ The introduction of alkyl groups to these R⁷ substituents improved
115 FQ activities also against gram-positive bacteria.¹⁸ In addition, adding a methyl group
116 to the amino group of aminopyrrolidine at R⁷ position could be effective for avoiding
117 the inhibitory effect of cytochrome P450 3A4.¹⁹ Moreover, Jordi *et al.* demonstrated
118 that introduction of a methyl or ethyl group to the R⁷ aminoazetidine improved the
119 pharmacokinetic properties but reduced the antibacterial activity against gram-
120 negative bacteria.²⁰

121 Concerning piperazine or piperazine-like quinolones, extensive research on the
122 substitution at R³ positions of the piperazine ring (Fig.2 for piperazine numbering) have
123 been carried out, and numerous aromatic derivatives at R⁴ position of the piperazine
124 ring have been reported.^{2,21} But despite the statement by Haemers *et al.* that
125 “derivatives with higher alkyl substitutions should be investigated in detail”,²² very few
126 examples of quinolones with long alkyl chains at the R⁴ position of the piperazine ring
127 have been described. Grohe *et al.* synthesised ciprofloxacin-like molecules alkylated
128 with short and long carbon chains (CH₃, C₂H₅, n-C₃H₇, i-C₃H₇, n-C₄H₉, i-C₄H₉, n-C₅H₁₁,
129 i-C₅H₁₁, or n-C₁₂H₂₅) but did not provide information regarding the antibacterial
130 activities for most of them (n-C₄H₉, i-C₄H₉, n-C₅H₁₁, or n-C₁₂H₂₅).¹⁶ Haemers *et al.*

131 evaluated the antimycobacterial activity of several ciprofloxacin-like quinolones with
 132 short alkyl chains (CH₃, C₂H₅, n-C₃H₇, i-C₃H₇) at position R^{4'} and showed that the
 133 derivatives with C₂H₅ and i-C₃H₇ were the most active.²² More recently, De Almeida *et*
 134 *al.* described quinolones with long aminoalkyl chains (-NH-[CH₂]_m-NH-[CH₂]_n-CH₃)
 135 with m = 2 or 3 and 5 < n < 13), instead of a piperazine ring at R⁷.²³ Among these two
 136 series, the highest activity was displayed by the two compounds with an alkyl chain
 137 length of 10 carbon atoms, whereas the compounds with the shortest alkyl chains were
 138 least active. Some authors have shown that triazole rings (1,2,4-triazole or 1,3-
 139 thiazolidinone) at the R^{4'} position of the piperazine group of norfloxacin²⁴ or between
 140 positions R⁷ and R⁸,^{25,26} may potentiate the antimicrobial activity against both gram-
 141 positive and gram-negative bacteria.

Retrosynthesis of Compounds 1-10



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

142

143 Fig. 2. Retrosynthesis of compounds **1-10** and their clogP and clogD values. The
144 piperazine nucleophilic aromatic substitution was added at the R⁷ position of the
145 synthon **A** to obtain synthon **B**. Synthon **B** was used to synthesize compounds **1-7** by
146 nucleophilic substitution (i), compounds **8-9** by reductive amination (ii) and compound
147 **10** by addition elimination (iii).

148

149 Taking into account these data, our main goal was to develop new potent antibacterial
150 FQ. Since the impact of a substitution at the R^{4'} position of the piperazine group (Fig.
151 2) has been poorly studied, we designed and synthesised 10 new FQ-derivatives
152 based on the CPX skeleton on which a methoxy group (R⁸=OMe) has been added
153 since replacing a R⁸-H atom by a R⁸-OMe group increased bactericidal effect of
154 quinolones, especially against gram positive bacteria such as *S. aureus*²⁷. The
155 originality of our work lies in the addition of a long linear alkyl chain (compounds **1-7**:
156 7 to 14 carbon atoms) to modulate the lipophilicity of those CPX "OMe" skeleton (clogD
157 between -1.55 and +2.16) (Fig. 2). Moreover, we investigated slight modifications of
158 the alkyl chain on the piperazine ring: one with a long glycol chain and a low lipophilicity
159 (compound **8**, clogD = -4.32), another one with an alkyl chain substituted with an ethyl
160 to assess the impact of substitution (compound **9**, clogD = 1.15), and the last one with
161 a carboxy octyl chain on the piperazine ring (compound **10**), to evaluate the impact of
162 an amide bond rather than a simple N-C bond.

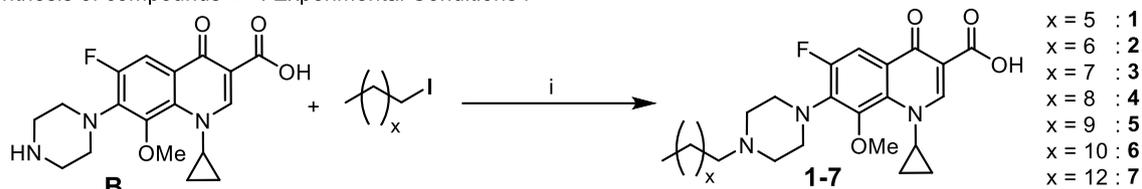
163 Herein, we described the synthesis of these 10 FQ-derivatives having the piperazine
164 group substituted and their *in vitro* antibacterial activities against various bacterial
165 species of medical interest, including FQ-R isolates.

166 As numerous FQ, those compounds bear a cyclopropyl group in R¹, a piperazinyl group
167 in R⁷ and a methoxy group in R⁸. Therefore, we used 1-cyclopropyl-6,7-difluoro-8-
168 methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (synthon **A**) as starting material,
169 so those compounds synthesis can be achieved in three steps only. The synthesis of
170 compounds **1-10** described in this study is outlined in Fig. 3. Following the procedures
171 described by Guruswamy and Arul,²⁸ 1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-
172 dihydroquinoline-3-carboxylic acid (synthon **A**) was refluxed for 6 days in anhydrous
173 acetonitrile in the presence of triethylamine, the piperazine nucleophilic aromatic
174 substitution proceeded regiospecifically on the R⁷ position of the difluoroquinolone
175 nucleus and therefore synthon **B** was obtained with 50% yield. Interestingly, no
176 activation of the R⁷ position was required for this step, as it sometimes was.²⁹ Then, in
177 order to synthesise compounds **1-7**, synthon **B** was reacted under basic conditions
178 with the corresponding iodoalkane. But, for each reaction, as the alkylation of the
179 carboxylic acid function was observed during the process, a mixture of the ester and
180 the desired products was obtained. Therefore, a basic hydrolysis was necessary as a
181 last step to obtain compounds **1-7** with moderate yields (25-35%). For compounds **8-**
182 **9**, we proceeded via a direct reductive amination. Firstly, oxidation of 2-(2-
183 ethoxyethoxy)ethan-1-ol gave 2-(2-ethoxyethoxy) acetaldehyde using 2-
184 iodoxybenzoic acid (IBX) with a quantitative yield. Then, the coupling between the
185 corresponding aldehydes (2-(2-ethoxyethoxy) acetaldehyde for **8**, and 2-ethylhexanal,
186 which is commercially available, for **9**, with synthon **B** in the presence of sodium
187 triacetoxyborohydride, a mild reducing agent, enabled to achieve the direct reductive
188 amination with a poor to moderate yield (20-40%) (Fig. 3). Finally, for compound **10**,
189 the carboxylic acid function of the octanoic acid was activated through an acyl chloride,

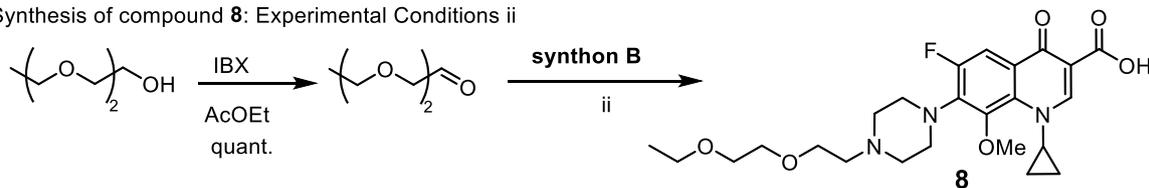
190 then a peptide coupling between this acyl chloride and synthon **B** gave compound **10**
191 with a moderate yield (40%).

192

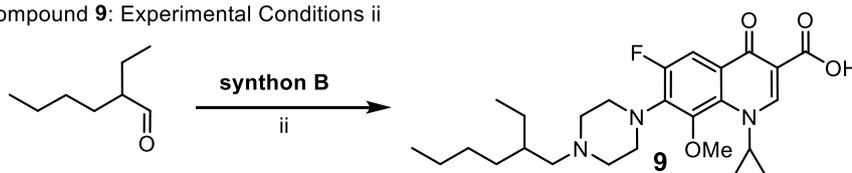
Synthesis of compounds **1-7**: Experimental Conditions i



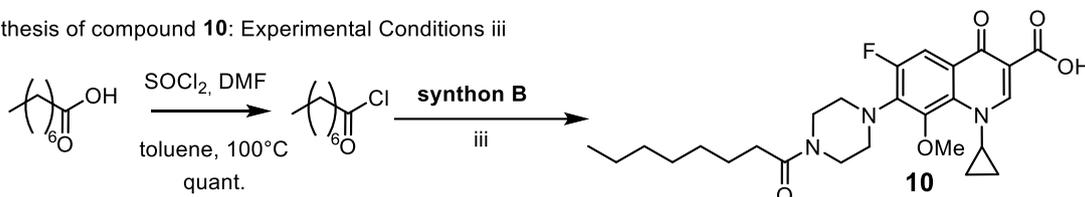
Synthesis of compound **8**: Experimental Conditions ii



Synthesis of compound **9**: Experimental Conditions ii



Synthesis of compound **10**: Experimental Conditions iii



193

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

198

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

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

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

212 Regarding compounds bearing a long glycol chain (compound **8**), a 2-ethylhexyl chain
213 (compound **9**) and a carboxy octyl chain (compound **10**) on the piperazine ring,
214 compound **9** exhibited the highest MIC whatever the bacterial species (Table 1).
215 Regarding *E. coli*, MIC of compound **8** was similar to this of compound **1**. For
216 *N. gonorrhoeae*, none of the compounds displayed MIC lower than compounds **1-7**.
217 For *S. pneumoniae*, MICs of these compounds were similar or lower than MICs of
218 compounds **1-7**, and for *S. aureus* the sole compound exhibiting lower MIC than
219 compounds **1-7** was the compound **8**. Against gram negative bacteria none of these
220 compounds displayed lower MICs than CPX or MXF, whereas against gram positive
221 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).

246 In conclusion, this work describes the synthesis, characterization and evaluation of ten
247 FQ-derivatives. Antibacterial activity of the designed compounds against gram-positive
248 and gram-negative was evaluated with microdilution assays. Considering all biological
249 results, neither of these FQ-derivatives was as active as CPX and MXF (Tables 1 and
250 2). These results suggest that the introduction of a long alkyl chain or a 2-ethylhexyl
251 chain or a carboxy octyl chain on the FQ piperazine ring at the R^{4'} position reduces the
252 antibacterial activity. More SAR needs to be conducted, but compound **8**, which has
253 the lowest clogD value (-4.32) among the 10 FQ-derivatives (-1.55 for **1** to 2.16 for **7**)
254 displayed the lowest MICs against *E. coli* and *S. aureus*, which suggests that
255 increasing lipophilicity is deleterious for antibacterial activity. Moreover, we concluded
256 that a common mechanism led to cross resistance with FQ in clinical use in
257 *N. gonorrhoeae*.

258

259

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

261 Table 1: Minimum inhibitory concentrations (MICs) of ciprofloxacin, moxifloxacin, and the compounds **1** to **10** for several clinical and reference bacteria of

262 medical interest carrying various susceptibility profile to beta-lactams and fluoroquinolones.

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

264 ^aOXA-23-producing.

265 ^bESBL- and OXA-48-producing.

266 ^cESBL-producing.

267 ^dProducing metallo-beta-lactamase VIM.

268

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

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 ^aS91F and D95G substitutions in GyrA

274 ^bE91G substitution in ParC, and S91F and D95G substitution in GyrA

275 ^cS91F substitution in GyrA

276 ^dS87R and S88P substitutions in ParC, and S91F and D95G substitutions in GyrA

277 **Declaration of competing interests.**

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

284

285 **Funding**

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

289

290 **Acknowledgments**

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

293 **Appendix A. Experimental section is described in Supporting Information**

294 **References**

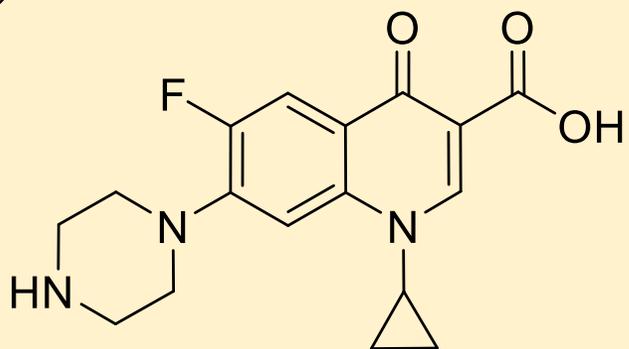
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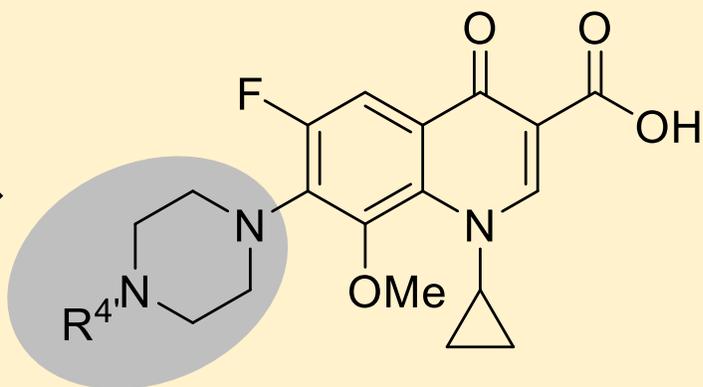
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404



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: $R^{4'} = 2\text{-ethylhexyl}$

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)

⇒ **increased lipophilicity is deleterious against antibacterial activity**

1 **Supporting Information**

2 **Chemistry**

3 Thin-layer chromatography was performed on TLC plastic sheets of silica gel 60F254
4 (layer thickness 0.2 mm) from Merck. Column chromatographic purification was carried
5 out on silica gel 60 (70-230 mesh ASTM, Merck). IR, ¹H, ¹⁹F and ¹³C NMR spectra
6 were used to characterize the structures of all compounds. IR spectra were recorded
7 on a Perkin Elmer Spectrum 100 FT-IR spectrometer and NMR spectra were recorded,
8 using CDCl₃, CD₂Cl₂, CD₃CN, D₂O or DMSO-d₆ as solvent, on a Bruker AC 300 or 400
9 spectrometer at 300 or 400 MHz for ¹H, 75 or 100 MHz for ¹³C and 282 or 377 MHz for
10 ¹⁹F spectra. Chemical shifts (δ) were expressed in parts per million relative to the signal
11 indirectly (i) to CHCl₃ (δ 7.27) for ¹H and (ii) to CDCl₃ (δ 77.2) for ¹³C, and directly (iii)
12 to CFCl₃ (internal standard) (δ 0) for ¹⁹F. Chemical shifts are given in ppm and peak
13 multiplicities are designated as follows: s, singlet; br s, broad singlet; d, doublet; dd,
14 doublet of doublet; t, triplet; q, quadruplet; quint, quintuplet; m, multiplet. HRMS were
15 obtained from the "Service Central d'analyse de Solaize" (Centre National de la
16 Recherche Scientifique) and were recorded on a Waters spectrometer using
17 electrospray ionization-Time-of-Flight (ESI-TOF). The purity of the final compounds
18 (> 99%) were assessed by HPLC analyses (flow of 1 mL/min) using a Waters
19 photodiode array detector apparatus (PDA, UV detector from 210 to 295 nm) using a
20 X-Bridge (5 μm)-packed C18 column (150 mm*4.6) or a Sunfire (3.5 μm)-packed C8
21 column (150 mm*4.6) and three solvent systems, *i.e.* solvent A (H₂O + 0,02%
22 HCOOH), solvent C (NH₄COOH 5 mM pH 8) and solvent D (CH₃CN). Elemental
23 analysis was performed by the "Service de Microanalyse" de l'Institut de Chimie des
24 Substances Naturelles (Gif-sur-Yvette, France). The clogP and clogD theoretical values

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

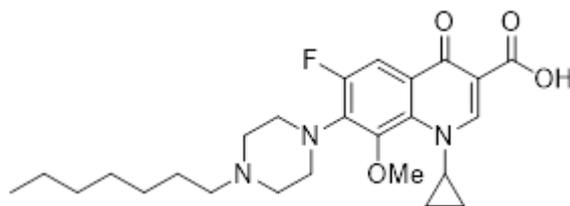
28 **Experimental conditions i (compounds 1-7) (Fig. 2 and 3)**

29 To a suspension of 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(piperazin-1-yl)-1,4-
30 dihydroquinoline-3-carboxylic acid (synthon **B**, 75 mg, 0.20 mmol), in dry
31 dimethylformamide (DMF) (18 mL), were added 1-iodoalkyl (2.75 eq.) and NaHCO₃ (6
32 eq.). The reaction mixture was stirred at 40°C for 40h and then concentrated under
33 reduced pressure. The residue was taken up in dichloromethane (DCM) (40 mL) and
34 the organic layer was washed with water (30 mL) and dried over anhydrous MgSO₄ to
35 give a mixture of 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-alkylpiperazin-1-yl)-1,4-
36 dihydroquinoline-3-carboxylic acid and 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-
37 alkylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic alkyl ester.

38 The mixture was dissolved in an EtOH/H₂O (5/2) mixture (20 mL), and LiOH (8 eq.)
39 was added. The reaction mixture was stirred overnight at room temperature and then
40 acidified with HCl_{aq} 1 N to pH 3. Ethanol was removed under reduced pressure and
41 the aqueous layer was extracted with DCM (40 mL). The organic layer was washed
42 with saturated aqueous NaHCO₃ (20 mL), dried over anhydrous MgSO₄ and
43 concentrated in vacuo. The crude solid obtained was washed with Et₂O (3x5 mL) to
44 yield 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-alkylpiperazin-1-yl)-1,4-
45 dihydroquinoline-3-carboxylic acid (25-35%) as a white-yellow powder.

46

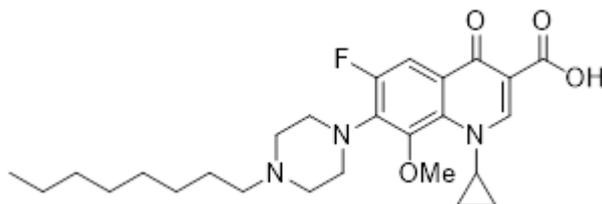
47 **Preparation of 1-cyclopropyl-6-fluoro-8-methoxy-7-(4-heptylpiperazin-1-yl)-4-**
48 **oxo-1,4-dihydroquinoline-3-carboxylic acid (compound 1).**



50 Likewise, the experimental conditions i when applied to 1-iodoheptane (125 mg,
51 0.55 mmol) afforded a mixture of 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-
52 heptylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid and 1-cyclopropyl-6-
53 fluoro-8-methoxy-4-oxo-7-(4-heptylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic
54 heptyl ester. After the basic hydrolysis, the crude solid obtained was washed with Et₂O
55 (3x5 mL) to yield 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-heptylpiperazin-1-yl)-
56 1,4-dihydroquinoline-3-carboxylic acid (28 mg, 30% over 2 steps) as a white powder.

57 **¹H NMR (300 MHz, CDCl₃, δ):** 8.80 (s, 1H, H₂), 7.85 (d, 1H, ³J_{H-F} = 12.0 Hz, H₅), 4.01
58 (m, 1H, CH(cPr)), 3.76 (s, 3H, OCH₃), 3.48 (br s, 4H, H_{2'} and H_{3'}), 2.64 (br s, 4H, H_{1'}
59 and H_{4'}), 2.45 (m, 2H, NCH₂CH₂CH₂), 1.56 (m, 2H, NCH₂CH₂CH₂), 1.32-1.29 (m, 10H,
60 CH₂), 1.02-0.95 (m, 2H, CH₂(cPr)), 0.87 (t, ³J_{H-H} = 6.8 Hz, 3H, CH₃). **¹³C NMR (75 MHz,**
61 **CDCl₃, δ):** 177.1 (d, J = 3.1 Hz, C₄), 166.9 (s, CO₂H), 156.2 (d, J = 250.7 Hz, C₆), 150.0
62 (s, C₂), 145.4 (d, J = 5.8 Hz, C₈), 139.5 (s, C₇), 134.1 (s, C₉), 121.4 (s, C₁₀), 108.1 (s,
63 C₃), 108.0 (d, J = 16.3 Hz, C₅), 62.7 (s, OCH₃), 59.1 (s, NCH₂CH₂CH₂), 53.9 (s, C_{1'} and
64 C_{4'}), 50.5 (d, C_{2'} and C_{3'}), 40.7 (s, CH(cPr)), 31.9 (s, CH₂), 29.3 (s, CH₂), 29.0 (s, CH₂),
65 27.6 (s, CH₂), 26.7 (s, CH₂), 22.7 (s, CH₂), 14.2 (s, CH₃), 9.7 (s, 2CH₂(cPr)). **Elemental**
66 **Analysis:** C = 64.62%, H = 7.52%, N = 8.83%, calcd C = 65.34%, H = 7.46%,
67 N = 9.14%. **Melting Point** = 157.2°C. **HPLC:** R_t = 11.1 min (solvent C/D: 5/95).

68 **Preparation of 1-cyclopropyl-6-fluoro-8-methoxy-7-(4-octylpiperazin-1-yl)-4-oxo-**
69 **1,4-dihydroquinoline-3-carboxylic acid (compound 2).**



70
71 Likewise, the experimental conditions i when applied to 1-iodooctane (133 mg,
72 0.55 mmol) afforded a mixture of 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-
73 octylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid and 1-cyclopropyl-6-fluoro-
74 8-methoxy-4-oxo-7-(4-octylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic octyl
75 ester. After the basic hydrolysis, the crude solid obtained was washed with Et₂O
76 (3x5 mL) to yield 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-octylpiperazin-1-yl)-
77 1,4-dihydroquinoline-3-carboxylic acid (30 mg, 33% over 2 steps) as a yellow powder.

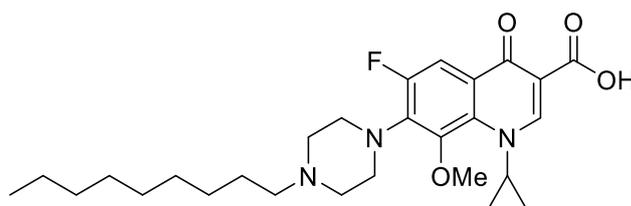
78 **¹H NMR (400 MHz, CD₂Cl₂, δ):** 14.77 (broad s, 1H, CO₂H), 8.77 (s, 1H, H₂), 7.80 (d,
79 1H, ³J_{H-F} = 12.4 Hz, H₅), 4.04 (m, 1H, CH(cPr)), 3.77 (s, 3H, OCH₃), 3.43 (br s, 4H, H₂
80 and H₃'), 2.58 (br s, 4H, H₁' and H₄'), 2.39 (m, 2H, NCH₂CH₂CH₂), 1.50 (m, 2H,
81 NCH₂CH₂CH₂), 1.42-1.24 (m, 10H, 5 CH₂), 1.20 (q, 2H, J = 6.9 Hz, CH₂(cPr)), 1.02-
82 0.95 (m, 2H, CH₂(cPr)), 0.89 (t, 3H, ³J_{H-H} = 6.8 Hz, CH₃). **¹⁹F NMR (376 MHz, CD₂Cl₂,**
83 **δ):** -120.1 (s, F₆). **¹³C NMR (100 MHz, CD₂Cl₂, δ):** 177.5 (d, J = 3.1 Hz, C₄), 166.8 (s,
84 CO₂H), 156.7 (d, J = 250.8 Hz, C₆), 150.3 (s, C₂), 145.9 (d, J = 5.8 Hz, C₈), 140.1 (d,
85 J = 11.7 Hz, C₇), 134.6 (s, C₉), 121.9 (d, J = 9.2 Hz, C₁₀), 108.0 (s, C₃), 107.9 (d,
86 J = 23.3 Hz, C₅), 62.8 (s, OCH₃), 59.3 (s, NCH₂CH₂CH₂), 54.3 (s, C₁' and C₄'), 51.2 (d,
87 J = 4.6 Hz, C₂' and C₃'), 41.0 (s, CH(cPr)), 32.3 (s, CH₂), 30.0 (s, CH₂), 29.7 (s, CH₂),
88 27.9 (s, CH₂), 27.2 (s, CH₂), 23.1 (s, CH₂), 14.3 (s, CH₃), 9.8 (s, 2CH₂(cPr)). **IR (neat):**
89 **ν** = 3084, 2926, 2853, 2809, 2770, 1728, 1617, 1601, 1554, 1539, 1505, 1436, 1383,

90 1376, 1312, 1280, 1238, 1204, 1187, 1144, 1128, 1115, 1091, 1055, 1040, 1008, 993,
91 957, 934, 887, 831, 821, 805, 730, 710 cm^{-1} ; **HRMS (ESI+)** m/z $[\text{M}+\text{H}]^+$ calcd for
92 $\text{C}_{26}\text{H}_{36}\text{FN}_3\text{O}_4$: 474.2769, found: 474.2765. **Elemental Analysis:** C = 66.11%,
93 H = 7.78%, N = 8.82%, calcd C = 65.94%, H = 7.66%, N = 8.87%. **Melting**
94 **Point** = 146.3°C. **HPLC:** R_t = 12.0 min (solvent C/D: 5/95).

95

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

98



99 Likewise, the experimental conditions i when applied to 1-iodononane (144 mg,
100 0.57 mmol) afforded a mixture of 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-
101 nonylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid and 1-cyclopropyl-6-
102 fluoro-8-methoxy-4-oxo-7-(4-nonylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic
103 nonyl ester. After the basic hydrolysis, the crude solid obtained was washed with Et_2O
104 (3x5 mL) to yield 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-nonylpiperazin-1-yl)-
105 1,4-dihydroquinoline-3-carboxylic acid (33 mg, 34% over 2 steps) as a white powder.

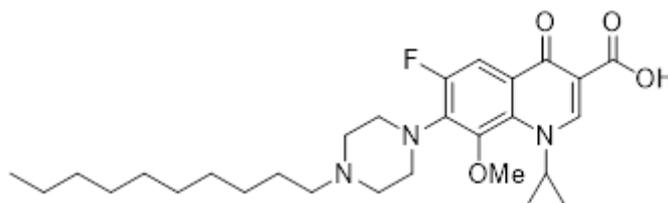
106 **$^1\text{H NMR}$ (300 MHz, CDCl_3 , δ):** 8.95 (s, 1H, H_2), 7.90 (d, 1H, $^3J_{\text{H-F}} = 12.0$ Hz, H_5), 4.07
107 (m, 1H, $\text{CH}(\text{cPr})$), 3.80 (s, 3H, OCH_3), 3.50 (br s, 4H, H_2' and H_3'), 2.65 (br s, 4H, H_1'
108 and H_4'), 2.46 (m, 2H, $\text{NCH}_2\text{CH}_2\text{CH}_2$), 1.58 (m, 2H, $\text{NCH}_2\text{CH}_2\text{CH}_2$), 1.40-1.24 (m, 14H,
109 $6\text{CH}_2 + \text{CH}_2(\text{cPr})$), 1.05-1.02 (m, 2H, $\text{CH}_2(\text{cPr})$), 0.92 (t, 3H, $^3J_{\text{H-H}} = 6.8$ Hz, CH_3); **^{13}C**

110 **NMR (75 MHz, CDCl₃, δ):** 177.1 (d, *J* = 3.1 Hz, C₄), 166.9 (s, CO₂H), 156.2 (d,
111 *J* = 250.0 Hz, C₆), 154.5 (s, C₂), 145.3 (d, *J* = 5.7 Hz, C₈), 139.7 (d, *J* = 12.0 Hz, C₇),
112 134.0 (d, *J* = 1.6 Hz, C₉), 121.6 (d, *J* = 9.0 Hz, C₁₀), 108.4 (s, C₃), 108.0 (d, *J* = 18.8
113 Hz, C₅), 62.6 (s, OCH₃), 59.1 (s, NCH₂CH₂CH₂), 54.0 (s, C_{1'} and C_{4'}), 50.8 (d, *J* = 4.6
114 Hz, C_{2'} and C_{3'}), 40.6 (s, CH(cPr)), 32.0 (s, CH₂), 29.7 (s, CH₂), 29.4 (s, CH₂), 27.7 (s,
115 CH₂), 26.9 (s, CH₂), 22.8 (s, CH₂), 14.2 (s, CH₃), 9.6 (s, 2CH₂(cPr)). **Elemental**
116 **Analysis:** C = 66.36%, H = 7.86%, N = 8.50%, calcd C = 66.51%, H = 7.85%,
117 N = 8.62%. **Melting Point** = 144.4°C. **HPLC:** *R*_t = 12.9 min (solvent C/D: 5/95).

118

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

121



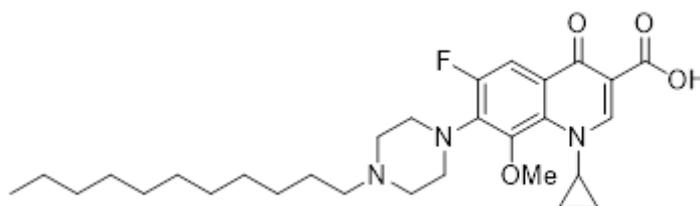
122

123 Likewise, the experimental conditions i when applied to 1-iododecane (163 mg, 0.61
124 mmol) afforded a mixture of 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-
125 decylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid and 1-cyclopropyl-6-fluoro-
126 8-methoxy-4-oxo-7-(4-decylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic decyl
127 ester. After the basic hydrolysis, the crude solid obtained was washed with Et₂O
128 (3×5 mL) to yield 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-decylpiperazin-1-yl)-
129 1,4-dihydroquinoline-3-carboxylic acid (30 mg, 29% over 2 steps) as a white powder.

130 **¹H NMR (400 MHz, CD₂Cl₂, δ):** 14.78 (br s, 1H, CO₂H), 8.78 (s, 1H, H₂), 7.82 (d, ³J_{H-F}
131 = 12.4 Hz, 1H, H₅), 4.04 (m, 1H, CH(cPr)), 3.76 (s, 3H, OCH₃), 3.43 (br s, 4H, H_{2'} and
132 H_{3'}), 2.57 (br s, 4H, H_{1'} and H_{4'}), 2.39 (m, 2H, NCH₂CH₂CH₂), 1.51 (m, 2H,
133 NCH₂CH₂CH₂), 1.37-1.24 (m, 14H, CH₂), 1.20 (m, 2H, CH₂(cPr)), 1.02-0.95 (m, 2H,
134 CH₂(cPr)), 0.89 (t, ³J_{H-H} = 6.7 Hz, 3H, CH₃). **¹⁹F NMR (376 MHz, CD₂Cl₂, δ):** -120.1 (s,
135 F₆). **¹³C NMR (100 MHz, CD₂Cl₂, δ):** 177.5 (d, J = 3.1 Hz, C₄), 166.9 (s, CO₂H), 156.7
136 (d, J = 250.9 Hz, C₆), 150.3 (s, C₂), 145.9 (d, J = 5.8 Hz, C₈), 140.1 (d, J = 11.8 Hz,
137 C₇), 134.6 (s, C₉), 121.9 (d, J = 9.1 Hz, C₁₀), 108.0 (s, C₃), 107.9 (d, J = 23.2 Hz, C₅),
138 62.8 (s, OCH₃), 59.3 (s, NCH₂CH₂CH₂), 54.3 (s, C_{1'} and C_{4'}), 51.2 (d, J = 4.7 Hz, C_{2'}
139 and C_{3'}), 41.0 (s, CH(cPr)), 32.3 (s, CH₂), 30.1 (s, CH₂), 30.0 (s, 2CH₂), 29.8 (s, CH₂),
140 27.9 (s, CH₂), 27.3 (s, CH₂), 23.1 (s, CH₂), 14.3 (s, CH₃), 9.8 (s, 2CH₂(cPr)). **IR (neat):**
141 ν = 3071; 2924, 2852, 2770, 1729, 1618, 1601, 1536, 1506, 1441, 1394, 1384, 1313,
142 1281, 1239, 1206, 1188, 1148, 1129, 1116, 1091, 1055, 1042, 1003, 959, 937, 888,
143 879, 831, 821, 805, 730, 710 cm⁻¹; **HRMS (ESI+) m/z:** [M+H]⁺ calcd for C₂₈H₄₀FN₃O₄:
144 502.3082, found, 502.3077. **Elemental Analysis:** C = 67.28%, H = 8.21%, N = 8.31%,
145 calcd C = 67.04%, H = 8.04%, N = 8.38%. **Melting Point** = 136.1°C. **HPLC:**
146 R_t = 13.9 min (solvent C/D: 5/95).

147

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

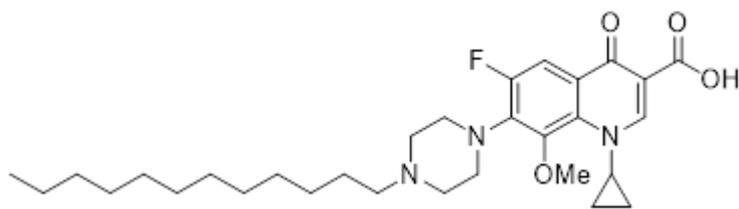


150

151 Likewise, the experimental conditions i when applied to 1-iodoundecane (160 mg,
152 0.54 mmol) afforded a mixture of 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-
153 undecylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid and 1-cyclopropyl-6-
154 fluoro-8-methoxy-4-oxo-7-(4-undecylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic
155 undecyl ester. After the basic hydrolysis, the crude solid obtained was washed with
156 Et₂O (3x5 mL) to yield 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-undecylpiperazin-
157 1-yl)-1,4-dihydroquinoline-3-carboxylic acid (36 mg, 35% over 2 steps) as a white
158 powder.

159 **¹H NMR (300 MHz, CDCl₃, δ):** 8.84 (s, 1H, H₂), 7.89 (d, 1H, ³J_{H-F} = 12.0 Hz, H₅), 4.07
160 (m, 1H, CH(cPr)), 3.80 (s, 3H, OCH₃), 3.50 (br s, 4H, H_{2'} and H_{3'}), 2.66 (br s, 4H, H_{1'}
161 and H_{4'}), 2.46 (m, 2H, NCH₂CH₂CH₂), 1.58 (m, 2H, NCH₂CH₂CH₂), 1.36-1.23 (m, 18H,
162 6CH₂ + CH₂(cPr)), 1.05-1.03 (m, 2H, CH₂(cPr)), 0.92 (t, 3H, ³J_{H-H} = 6.8 Hz, CH₃); **¹³C**
163 **NMR (75 MHz, CDCl₃, δ):** 177.1 (d, J = 3.1 Hz, C₄), 166.9 (s, CO₂H), 156.2 (d,
164 J = 250.5 Hz, C₆), 154.6 (s, C₂), 145.4 (d, J = 5.6 Hz, C₈), 139.6 (s, C₇), 134.1 (s, C₉),
165 121.7 (d, J = 9.0 Hz, C₁₀), 108.5 (s, C₃), 108.0 (d, J = 18.2 Hz, C₅), 62.6 (s, OCH₃),
166 59.2 (s, NCH₂CH₂CH₂), 54.0 (s, C_{1'} and C_{4'}), 50.8 (s, C_{2'} and C_{3'}), 40.7 (s, CH(cPr)),
167 32.1 (s, CH₂), 29.8 (s, CH₂), 29.7 (s, CH₂), 29.5 (s, CH₂), 27.7 (s, CH₂), 26.9 (s, CH₂),
168 22.8 (s, CH₂), 14.3 (s, CH₃), 9.7 (s, 2CH₂(cPr)). **Elemental Analysis:** C = 67.27%,
169 H = 8.14%, N = 8.02%, calcd C = 67.55%, H = 8.21%, N = 8.15%. **Melting**
170 **Point** = 136.3°C. **HPLC:** R_t = 14.9 min (solvent C/D: 5/95).

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

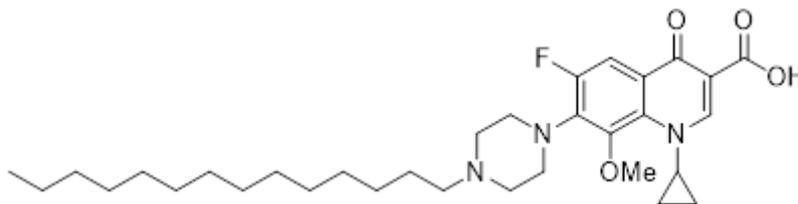


173

174 Likewise, the experimental conditions i when applied to 1-iodododecane (163 mg,
 175 0.61 mmol) afforded a mixture of 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-
 176 dodecylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid and 1-cyclopropyl-6-
 177 fluoro-8-methoxy-4-oxo-7-(4-dodecylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic
 178 dodecyl ester. After the basic hydrolysis, the crude solid obtained was washed with
 179 Et₂O (3x5 mL) to yield 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-dodecylpiperazin-
 180 1-yl)-1,4-dihydroquinoline-3-carboxylic acid (30 mg, 28% over 2 steps) as a white
 181 powder.

182 **¹H NMR (300 MHz, CDCl₃, δ):** 8.84 (s, 1H, H₂), 7.90 (d, 1H, ³J_{H-F} = 12.0 Hz, H₅), 4.06
 183 (m, 1H, CH(cPr)), 3.80 (s, 3H, OCH₃), 3.50 (br s, 4H, H_{2'} and H_{3'}), 2.66 (br s, 4H, H_{1'}
 184 and H_{4'}), 2.46 (m, 2H, NCH₂CH₂CH₂), 1.58 (m, 2H, NCH₂CH₂CH₂), 1.36-1.24 (m, 20H,
 185 6CH₂ + CH₂(cPr)), 1.04-1.02 (m, 2H, CH₂(cPr)), 0.92 (t, 3H, ³J_{H-H} = 6.8 Hz, CH₃); **¹³C**
 186 **NMR (75 MHz, CDCl₃, δ):** 177.0 (d, J = 3.1 Hz, C₄), 166.9 (s, CO₂H), 156.2 (d,
 187 J = 250.4 Hz, C₆), 154.6 (s, C₂), 145.3 (d, J = 5.6 Hz, C₈), 139.6 (d, J = 1.6 Hz, C₇),
 188 134.0 (s, C₉), 121.7 (d, J = 9.2 Hz, C₁₀), 108.4 (s, C₃), 108.1 (d, J = 18.2 Hz, C₅), 62.6
 189 (s, OCH₃), 59.1 (s, NCH₂CH₂CH₂), 53.9 (s, C_{1'} and C_{4'}), 50.7 (s, C_{2'} and C_{3'}), 40.6 (s,
 190 CH(cPr)), 32.0 (s, CH₂), 29.7 (2s, 3CH₂), 29.6 (2s, 2CH₂), 29.4 (s, CH₂), 27.6 (s, CH₂),
 191 26.8 (s, CH₂), 22.8 (s, CH₂), 14.2 (s, CH₃), 9.6 (s, 2CH₂(cPr)). **Elemental Analysis:**
 192 C = 67.89%, H = 8.49%, N = 7.82%, calcd C = 68.03%, H = 8.37%, N = 7.93%. **Melting**
 193 **Point** = 134.6°C. **HPLC:** R_t = 10.4 min (solvent A/solvent D: 65/35).

194 **Preparation of 1-cyclopropyl-7-(4-tetradecylpiperazin-1-yl)-6-fluoro-8-methoxy-**
195 **4-oxo-1,4-dihydroquinoline-3-carboxylic acid (compound 7).**



197 Likewise, the experimental conditions i when applied to 1-iodotetradecane (163 mg,
198 0.61 mmol) afforded a mixture of 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-
199 tetradecylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid and 1-cyclopropyl-6-
200 fluoro-8-methoxy-4-oxo-7-(4-tetradecylpiperazin-1-yl)-1,4-dihydroquinoline-3-
201 carboxylic tetradecyl ester. After the basic hydrolysis, the crude solid obtained was
202 washed with Et₂O (3x5 mL) to yield 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-
203 tetradecylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (28 mg, 25% over 2
204 steps) as a yellow powder.

205 **¹H NMR (300 MHz, CDCl₃, δ):** 8.85 (s, 1H, H₂), 7.90 (d, 1H, ³J_{H-F} = 12.0 Hz, H₅), 4.06
206 (m, 1H, CH(cPr)), 3.80 (s, 3H, OCH₃), 3.49 (br s, 4H, H_{2'} and H_{3'}), 2.65 (br s, 4H, H_{1'}
207 and H_{4'}), 2.45 (m, 2H, NCH₂CH₂CH₂), 1.58 (m, 2H, NCH₂CH₂CH₂), 1.34-1.24 (m, 24H,
208 6CH₂ + CH₂(cPr)), 1.04-1.02 (m, 2H, CH₂(cPr)), 0.92 (t, 3H, ³J_{H-H} = 6.8 Hz, CH₃); **¹³C**
209 **NMR (75 MHz, CDCl₃, δ):** 177.1 (d, J = 3.1 Hz, C₄), 166.9 (s, CO₂H), 156.2 (d,
210 J = 250.6 Hz, C₆), 154.5 (s, C₂), 145.3 (d, J = 5.6 Hz, C₈), 139.6 (d, J = 11.6Hz, C₇),
211 134.0 (s, C₉), 121.7 (d, J = 8.9 Hz, C₁₀), 108.4 (s, C₃), 108.1 (d, J = 19.0 Hz, C₅), 62.5
212 (s, OCH₃), 59.1 (s, NCH₂CH₂CH₂), 54.0 (s, C_{1'} and C_{4'}), 50.7 (s, C_{2'} and C_{3'}), 40.6 (s,
213 CH(cPr)), 32.0 (s, CH₂), 29.7 (3s, 3CH₂), 29.6 (2s, 2CH₂), 29.4 (s, CH₂), 27.6 (s, CH₂),
214 26.9 (s, CH₂), 22.7 (s, CH₂), 14.2 (s, CH₃), 9.6 (s, 2CH₂(cPr)). **Elemental Analysis:**

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

217

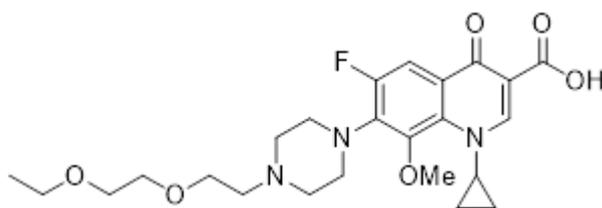
218 **Experimental conditions ii (compounds 8 and 9) (Fig. 2 and 3)**

219 To a suspension of 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(piperazin-1-yl)-1,4-
220 dihydroquinoline-3-carboxylic acid (1eq), in dry DCM, were added the aldehyde
221 (1.2 eq) and acetic acid (6 eq.). $\text{NaBH}(\text{OAc})_3$ (1.3 eq.) was added portion wise and the
222 mixture was stirred at room temperature overnight.

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

229 **Preparation of 1-cyclopropyl-7-(4-(2-(2-ethoxyethoxy)ethyl)piperazin-1-yl)-6-** 230 **fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (compound 8).**

231



232

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

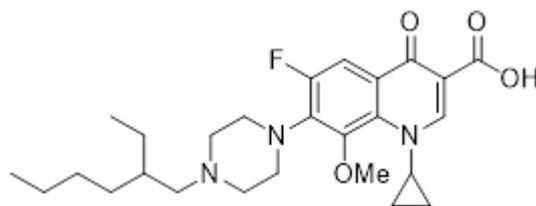
236 column chromatography, 1-cyclopropyl-7-(4-(2-(2-ethoxyethoxy)ethyl)piperazin-1-yl)-
237 6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid as a yellow solid
238 (1.05 g, 20%).

239 **¹H NMR (300 MHz, CDCl₃, δ):** 8.84 (s, 1H, H₂), 7.90 (d, 1H, ³J_{H-F} = 12.0 Hz, H₅), 4.05
240 (m, 1H, CH(cPr)), 3.77 (s, 3H, OCH₃), 3.70-3.52 (m, 12H, 4CH₂O and 2CH₂N), 2.77
241 (br s, 4H, H_{1'} and H_{4'}), 1.26 (t, 3H, ³J_{H-H} = 6.8 Hz, CH₃), 1.21-1.16 (m, 2H, CH₂(cPr)),
242 1.04-1.02 (m, 2H, CH₂(cPr)); **¹³C NMR (75 MHz, CDCl₃, δ):** 177.4 (d, J = 3.1 Hz, C₄),
243 167.2 (s, CO₂H), 156.5 (d, J = 250.3 Hz, C₆), 150.2 (s, C₂), 145.7 (d, J = 5.6 Hz, C₈),
244 139.9 (d, J = 11.8 Hz, C₇), 134.3 (d, J = 1.6 Hz, C₉), 121.9 (d, J = 9.1 Hz, C₁₀), 108.6
245 (s, C₃), 108.1 (d, J = 20.1 Hz, C₅), 70.8 (s, C_{1'} and C_{4'}), 70.2 (s, C_{2'} and C_{3'}), 69.0 (s,
246 CH(cPr)), 67.0 (s, CH₂), 62.9 (s, OCH₃), 58.4 (s, 2CH₂), 54.5 (s, C_{1'} and C_{4'}), 50.8 (s,
247 CH₂), 50.7 (s, C_{2'} and C_{3'}), 41.0 (s, CH(cPr)), 15.5 (s, CH₃), 9.9 (s, 2CH₂(cPr)).
248 **Elemental Analysis:** C = 59.16%, H = 6.71%, N = 8.28%, calcd C = 59.76%,
249 H = 6.75%, N = 8.80%. **HPLC:** R_f = 8.7 min (solvent C/D: 5/95).

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

252

253



254

255 Likewise, the experimental conditions ii when applied to 2-ethylhexanal (1.32 g,
256 10.3 mmol), synthon **B** (2.48 g, 6.85 mmol), acetic acid (2.47 g, 41.1 mmol) and
257 NaBH(OAc)₃ (1.89 g, 8.90 mmol) afforded, after column chromatography, 1-

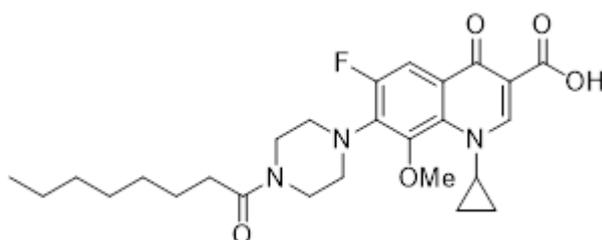
258 cyclopropyl-7-(2-(ethyl)hexylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-
259 dihydroquinoline-3-carboxylic acid as a white solid (1.30 g, 40%).
260
261 **¹H NMR (300 MHz, CDCl₃, δ):** 8.85 (s, 1H, H₂), 7.90 (d, 1H, ³J_{H-F} = 12.0 Hz, H₅), 4.10-
262 4.02 (m, 1H, CH(cPr)), 3.82 (s, 3H, OCH₃), 3.58-3.48 (br s, 4H, H_{2'} and H_{3'}), 2.70 (br
263 s, 4H, H_{1'} and H_{4'}), 2.28 (m, 2H, NCH₂CH), 1.70 (m, 1H, NCH₂CH), 1.34-1.21 (m, 10H,
264 4CH₂ + CH₂(cPr)), 1.06-1.00 (m, 2H, CH₂(cPr)), 0.97-0.87 (m, 6H, 2CH₃); **¹³C NMR (75**
265 **MHz, CDCl₃, δ):** 177.4 (d, J = 3.1 Hz, C₄), 167.3 (s, CO₂H), 156.6 (d, J = 250.5 Hz,
266 C₆), 150.2 (s, C₂), 145.6 (d, J = 5.8 Hz, C₈), 140.0 (d, J = 11.6 Hz, C₇), 134.4 (d,
267 J = 1.5 Hz, C₉), 121.8 (d, J = 9.1 Hz, C₁₀), 108.7 (s, C₃), 108.2 (d, J = 24.8 Hz, C₅),
268 62.7 (s, OCH₃), 59.1 (s, NCH₂CH₂CH₂), 54.7 (s, C_{1'} and C_{4'}), 51.2 (s, C_{2'} and C_{3'}), 41.0
269 (s, CH(cPr)), 36.5 (s, CH) 31.9 (s, CH₂), 29.4 (s, CH₂), 25.0 (s, CH₂), 23.6 (s, CH₂),
270 14.5 (s, CH₃), 11.2 (s, CH₃), 10.0 (s, 2CH₂(cPr)). **Elemental Analysis:** C = 65.66%,
271 H = 7.49%, N = 8.57%, calcd C = 65.94%, H = 7.66%, N = 8.87%. **HPLC:** R_t = 18.6 min
272 (solvent C/D: 5/95).

273 **Experimental conditions iii (Fig. 2 and 3)**

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

276

277



278

279 To a suspension of 1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(piperazin-1-yl)-1,4-
280 dihydroquinoline-3-carboxylic acid (2.28 g, 6.30 mmol, 1 eq) in dichloromethane, at 0°C
281 were added triethylamine (0.83 g, 8.19 mmol, 1.3 eq.) and acyl chloride (1.537 g,
282 9.45 mmol, 1.5 eq.). The mixture was stirred at room temperature for 1 hour. Hexane
283 was added and the mixture was filtered. The filtrate was evaporated and the resulted
284 solid was purified by column chromatography (silicagel, gradient DCM/Methanol 95/5)
285 resulting in the expected product white solid (1.23 g, 2.52 mmol, 40%).

286 **¹H NMR (300 MHz, CDCl₃, δ):** 8.87 (s, 1H, H₂), 7.95 (d, 1H, ³J_{H-F} = 12.0 Hz, H₅), 4.06
287 (m, 1H, CH(cPr)), 3.86-3.71 (m, 7H, OCH₃, H_{2'} and H_{3'}), 3.44 (br s, 4H, H_{1'} and H_{4'}),
288 2.42 (t, 2H, NCOCH₂), 1.43-1.23 (m, 12H, 6 CH₂), 1.20 (q, J = 7.0 Hz, 2H), 1.07-1.02
289 (m, 2H, CH₂(cPr)), 0.89 (m, 3H, CH₃). **¹³C NMR (75 MHz, CDCl₃, δ):** 177.4 (d, J = 3.1
290 Hz, C₄), 167.3 (s, CO₂H), 156.5 (d, J = 250.2 Hz, C₆), 150.2 (s, C₂), 145.6 (d, J = 5.4
291 Hz, C₈), 139.4 (d, J = 12.0 Hz, C₇), 134.4 (s, C₉), 122.9 (d, J = 8.9 Hz, C₁₀), 108.7 (s,
292 C₃), 108.5 (d, J = 27.6 Hz, C₅), 62.7 (s, OCH₃), 59.1 (s, NCH₂CH₂CH₂), 54.7 (s, C_{1'} and
293 C_{4'}), 51.2 (s, C_{2'} and C_{3'}), 41.0 (s, CH(cPr)), 36.5 (s, CH) 31.9 (s, CH₂), 29.4 (s, CH₂),
294 25.0 (s, CH₂), 23.6 (s, CH₂), 14.5 (s, CH₃), 11.2 (s, CH₃), 10.0 (s, 2CH₂(cPr)).
295 **Elemental Analysis:** C = 63.31%, H = 7.10%, N = 8.08%, calcd C = 64.05%, H =
296 7.03%, N = 8.62%. **HPLC:** R_t = 13.3 min (solvent A/solvent D: 65/35).

297

298 **Antibacterial activity evaluation**

299 *Antibiotics*

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

302

303 *Bacterial strains*

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

315

316 *Antibacterial susceptibility testing*

317 The minimum inhibitory concentrations (MICs) of the compounds **1-10**, MXF and CPX
318 were determined by broth microdilution, according to the Clinical and Laboratory
319 Standards Institute (CLSI) guidelines,⁴ for *A. baumannii*, *E. coli*, *P. aeruginosa*, *S.*
320 *aureus* and *S. pneumoniae* strains, and by the agar dilution method for *N. gonorrhoeae*
321 *strains*. Briefly, suspensions of *N. gonorrhoeae* strains (10^5 CFU/mL) were spotted, in
322 duplicate, on GC agar base with 2% v/v Vitox and 1% haemoglobin and containing
323 various concentrations of each compound tested. Antibiotic-containing agar plates
324 were incubated for 24-48h at 37°C in a 5% CO₂ atmosphere.

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

328

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