NOTE

Side chain length is more important than stereochemistry in the antibacterial activity of enantiomerically pure 4-aminoalcohol quinoline derivatives

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Mefloquine (MQ) is a quinoline methanol derivative used in the preventive and curative treatment of malaria. MQ was also previously found to be effective on Gram-positive bacteria such as *Staphylococcus aureus*, *Streptococcus pneumoniae* or, to a lesser extent, *Enterococcus faecalis*¹ as well as on *Mycobacterium avium* complex.² In addition, it has been reported as a potential inhibitor of efflux pumps in Gram-negative bacteria.³ However, the use of MQ as an antibiotic has been limited by relatively high minimum inhibitory concentrations (MICs) as well as adverse effects, such as neurotoxicity,⁴ highlighted through its use as an antimalarial drug. A way to enhance the activity and reduce the neurotoxicity of MQ derivatives could be the synthesis of enantiomerically pure 4-aminoalcohol quinolines, as enantiomerism could have a part in the *in vitro* activity and toxicity of these molecules. MQ possesses two asymmetric carbon atoms and is commercially available as a racemic mixture of its *erythro*-enantiomers (Figure 1). Inhibitory concentrations 50 (IC₅₀) values for the (+)-enantiomer of MQ were found to be lower than those of the (–)-enantiomer by a factor of 1.6–1.8 on some strains (D6 and W2),⁵ and we recently showed that (S)-4-aminoalcohol quinolines derivatives were 2- to 15-fold more active than their (R) counterparts on W2 and 3D7 *Plasmodium falciparum* strains.⁶ The antibacterial activity of MQ has also recently been proposed to be dependent on enantiomerism, as (+)-(11S, 12R) MQ was found to be more efficient in clearing *M. avium* from tissues of infected mice.⁷

Furthermore, the (–)-enantiomer was found to block central nervous system adenosine receptors, whereas the (+)-enantiomer did not. This blockage of central adenosine receptors by the (–)-enantiomer is believed to result in neuropsychiatric symptoms associated with MQ.⁸ With these differences in mind, a new enantioselective pathway to synthesize pure enantiomers of MQ amino-analogs was recently proposed.⁹ The structures of these derivatives are displayed in Figure 1. As they only bear one

asymmetric carbon at the position 11, the importance of the stereochemistry of this asymmetric carbon and of the length of the lateral chain on the antibacterial activity have been evaluated in this work.

MQ and MQ derivatives were tested on *Escherichia coli* DSM 1103, *Pseudomonas aeruginosa* DSM 1117, *S. aureus* CIP 103.429 and *E. faecalis* CIP 103.214. In addition, *P. aeruginosa* AM85, a strain formerly identified as resistant to ciprofloxacin through an efflux-mediated mechanism,¹⁰ as well as ten recent clinical strains (isolated in 2010) of *S. aureus* resistant to methicillin, ciprofloxacin and levofloxacin were tested for their susceptibility to MQ derivatives. For MIC determinations, the broth microdilution technique was carried out as advised by the Clinical and Laboratory Standards Institute using drug concentrations ranging from 0.0625 to 128 µg ml^{–1}.¹¹ In the series of MQ derivatives with a linear side chain, with the exception of (S)-6, compounds were found to be at least as effective as MQ on *S. aureus* and *E. faecalis*. For these species, the lower MICs were witnessed for compounds 3, 4, 5 and 7 with a 4- to 16-fold gain in activity compared with MQ and MICs ranging from 1 to 4 µg ml^{–1} (Table 1). In addition, *in vitro* susceptibility evaluation showed that (R)- and (S)-enantiomers displayed similar MICs against the Gram-positive bacteria tested. The only noticeable exception was compound (R)-6 that was found to be more effective than (S)-6 (Table 1). For *E. coli*, MQ derivatives with shorter side chains (compounds 1, 2 and (S)-3) were the most effective as well as compound 8, bearing an hydroxyphenyl substituent (Table 1). A two-fold difference in favor of the (R)-enantiomers was found for 1, 2 and 8. Interestingly, for methicillin-resistant *S. aureus* strains, MIC₅₀ and MIC₉₀ (the MIC capable of inhibiting the growth of at least 50 and 90% of the strains in the panel tested, respectively) for MQ and MQ derivatives only differed by a two-fold factor, whereas this factor was of eightfold for ciprofloxacin. The overall antibacterial activity of

R	Compound number
Butyl	1
Pentyl	2
Hexyl	3
Heptyl	4
Octyl	5
Nonyl	6
Phenyl	7
Para-hydroxyphenyl	8

Figure 1 Structures of mefloquine (MQ) and 4-aminoalcohol quinoline derivative enantiomers.

4-aminoalcohol quinoline derivatives on Gram-positive bacteria did not seem to be dependent on their stereochemistry but rather on the length of the side chain, as compounds bearing 6, 7 and 8 carbons on this side chain were the more efficient on both methicillin-sensitive and -resistant *S. aureus* strains as well as on *E. faecalis*. This finding is somewhat at odds with what was previously described for the antibacterial activity of MQ, as stereochemistry was recently reported to be of importance in MQ activity in a mouse model of *M. avium* infection, with the (+)-(11*S*,12*R*)-enantiomer of MQ being the more efficient.⁷ In addition, the antimalarial activity of the same MQ derivatives, as those tested here, was shown to be significantly better for (*S*)-enantiomers on two *P. falciparum* strains, one resistant and one sensitive to chloroquine.⁶ The mechanism underlying the differential activity of MQ and MQ derivative enantiomers against *P. falciparum* has not clearly been elucidated but a possible explanation might reside in the stereospecific recognition by transporters located on the digestive vacuole membrane such as *Plasmodium falciparum* Chloroquine Resistance Transporter (PfCRT) or *Plasmodium falciparum* MultiDrug Resistance 1 (PfMDR1), which enable quinoline derivatives to reach and/or remain at their site of action.¹² A hypothesis to explain the lack of stereospecificity on *S. aureus* and *E. faecalis* might, therefore, be that MQ derivatives do not depend on a transport system to enter Gram-positive bacteria, whereas such a system, possibly allowing the preferential entry of

one of MQ or MQ derivatives enantiomers, could be necessary to enable their activity on *M. avium* and/or Gram-negative bacteria. Indeed, whereas the MQ derivatives tested in this work were inactive against *P. aeruginosa*, possibly through lack of penetration, an activity dependant on the stereochemistry at the position 11 was witnessed for *E. coli*, with (*R*)-enantiomers of MQ derivatives having shorter side chains being the more efficient (Table 1).

clog*P* values calculated for MQ derivatives ranged from 3.51 for **8** to 5.54 for **6** (Pallas 3.5 software, Compudrug International, Inc., Sedona, AZ, USA) and fell within the range of values usually found for drugs (that is, 2–5).¹³ Moreover, MICs witnessed for the more active compounds were of 1–2 µg ml⁻¹ for Gram-positive bacteria and 8–16 µg ml⁻¹ for *E. coli*. If a parallel is drawn with MQ pharmacokinetics, the active concentrations against Gram-positive bacteria, but not those against *E. coli*, should be achievable in clinical situations. Indeed, previously published papers have shown that MQ concentrations can reach up to 3 µg ml⁻¹ after a 25-mg kg⁻¹ oral dose of MQ.¹⁴ Interestingly, the screening of **3**, **4** and **5** against recent clinical strains of *S. aureus* resistant to both methicillin and fluoroquinolones showed that MQ derivatives retained their activity against those strains, hence offering an alternative to treat such resistant strains and pointing out that a different mechanism of action from that of β-lactams and fluoroquinolones could be involved.

Table 1 Antimicrobial activities (MIC $\mu\text{g ml}^{-1}$) of 4-aminoalcohol quinoline derivatives

Compound	S. aureus		E. faecalis		E. coli	
	CIP	MRSA	CIP	DSM	P. aeruginosa	P. aeruginosa
	103.429 (10 strains)		103.214	1103	DSM 1117	AM 85
Ciprofloxacin	0.25	16/128 ^a	0.25	0.0625	0.125	16
Mefloquine	16	16/32	16	64	>128	>128
(R)-1	16	ND	16	32	>128	>128
(S)-1	16	ND	16	64	>128	>128
(R)-2	16	8/16	16	16	>128	>128
(S)-2	16	8/16	16	32	>128	>128
(R)-3	4	4/8	4	>128	>128	>128
(S)-3	4	4/4	4	16	>128	>128
(R)-4	1	2/4	1	>128	>128	>128
(S)-4	2	2/4	2	>128	>128	>128
(R)-5	1	2/2	1	>128	>128	>128
(S)-5	1	2/4	1	>128	>128	>128
(R)-6	16	ND	16	>128	>128	>128
(S)-6	>128	ND	>128	>128	>128	>128
(R)-7	4	4/8	4	>128	>128	>128
(S)-7	4	4/8	4	>128	>128	>128
(R)-8	8	ND	8	8	>128	>128
(S)-8	8	ND	8	16	>128	>128

Abbreviations: MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *S. aureus*; ND, not determined.

^aMIC₅₀/MIC₉₀.

After this first screening of *in vitro* antibacterial activity, only (R)-enantiomers from **3**, **4** and **5** were retained for further investigation, as they displayed the better MICs on Gram-positive bacteria of all MQ derivatives tested. Time-kill studies were carried out for *S. aureus* CIP 103.429 and *S. aureus* strain SAAM 27 (resistant to methicillin, ciprofloxacin and levofloxacin). Compound (R)-5 was the most efficient, with a 99.9% decrease of the inoculum as early as 2 h after inoculation when the concentration was superior or equal to the MIC. Contrary to what was observed with compound (R)-4, no regrowth at 24 h was witnessed for (R)-5 at concentrations equal or superior to the MIC (for detailed results see Supplementary Figure S1 in Supplementary Information). Previously, time-kill assays showed that MQ was rapidly lytic for *S. aureus*.¹ The same result was found here with MQ derivatives. However, the actual antibacterial mechanism of action of MQ remains poorly understood but DNA gyrase inhibition, a mechanism of action described for fluoroquinolone, was not implied.¹ Along with the retained activity of our compounds on fluoroquinolone-resistant *S. aureus* strains, this further reinforces the hypothesis of a different mechanism of action for MQ derivatives.

As the tested MQ derivatives were not active against *P. aeruginosa*, we wanted to investigate whether this lack of activity could be linked to an efflux of the molecules from the bacterial cytoplasm through pumps such as MexAB-OprM.¹⁰ PA β N, a known inhibitor of *P. aeruginosa* efflux pumps, was then added for MIC determinations on *P. aeruginosa* DSM 1117 and AM 85 (fluoroquinolone-resistant strain presenting a characterized efflux). All MICs remained above 128 $\mu\text{g ml}^{-1}$ for MQ as well as all of the other compounds tested. These results rule out efflux as a mechanism explaining the lack of sensitivity of *P. aeruginosa* to 4-aminoalcohol quinolines. As MQ has also been described as a possible inhibitor of efflux pumps in *E. coli*

and *P. aeruginosa*,³ we also used *P. aeruginosa* AM 85 strain to assess whether MQ derivatives would bear the same inhibitory activity when used in conjunction with ciprofloxacin, a known substrate for *P. aeruginosa* efflux pumps.¹⁰ Although PA β N induced a ready decrease in the MIC of ciprofloxacin (from 16 to 1 $\mu\text{g ml}^{-1}$), neither MQ nor MQ derivatives at concentrations ranging from 25 to 100 $\mu\text{g ml}^{-1}$ were able to trigger a similar decrease (for details see Supplementary Tables S1 and S2 in Supplementary Information). The discrepancy witnessed for MQ with previously published results in the efflux inhibition³ might be due to a methodological difference, as we only used phenotypical screening, or to a possible importance of the side chain in the inhibition process.

Finally, as MQ is mainly metabolized by the liver, the cytotoxicity of MQ and MQ derivatives was evaluated on hepatocellular carcinoma HepG2 cells with an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. IC₅₀s, concentrations killing 50% of the cells, were calculated after a 24-h incubation with MQ or MQ derivatives. Compounds (R)-3, (R)-4 and (R)-5 IC₅₀ values were found to be of 19.4, 16.6 and 13.4 $\mu\text{g ml}^{-1}$, respectively. MQ was also tested on this cell line and gave an IC₅₀ value of 11 $\mu\text{g ml}^{-1}$, below its MIC values. The safety index of MQ derivatives therefore ranged between 3- and 19-fold for (R)-3 and (R)-5, respectively, better on this model than the one from their parent molecule, which is currently used as an antimalarial.

In summary, the MQ derivatives tested in this paper were found to be mainly active on Gram-positive bacteria with a gain of 4- to 16-fold compared with MQ. This activity did not appear to depend on the stereochemistry of the asymmetric carbon at the position 11 of the molecule but rather on the length of the side chain, with compound (R)-5 being the most effective and less toxic, making it the best candidate for further modifications and/or development. Gram-negative bacteria were less sensitive to MQ derivatives as these were found to be inactive against *P. aeruginosa* but still enabling a gain in activity of up to eightfold as compared with MQ for *E. coli*. Against the latter species, compounds with the shorter side chain length were the most effective, contrary to what was observed with Gram-positive bacteria, and (R)-enantiomers tended to have slightly lower MICs than their (S) counterparts. This observation vouched for a potential implication of stereochemistry in the target of MQ derivative compounds in Gram-negative bacteria and/or in the transport of these molecules in the bacterial cytoplasm. Further investigations are needed to thoroughly elucidate the underlying mechanism of action of these compounds as well as that of MQ. Additional modifications on the side chain are also planned, which could lead to compounds with lower MICs and gain information on the implication of the second asymmetric carbon at the position 12 of the molecules in their antibacterial activity.

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