

# Antibacterial Activity of Lipophilic Fluoroquinolone Derivatives

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**Abstract:** We report in this work the antibacterial evaluation of 12 lipophilic fluoroquinolone derivatives containing diaminoalkyl side chains at C-7 position. The compounds were investigated against 15 bacterial strains including Gram-negative and Gram-positive species of clinical and microbiological importance. Three compounds (5, 10 and 11) were as active as or more efficient than gatifloxacin against Gram-positive bacteria *M. lentus*. When compared with gatifloxacin compound 10 was 16 times more active. Two compounds (11 and 12) were twice more active than the reference compound against *S. aureus*.

**Key Words:** Fluoroquinolones, lipophilicity, antibacterial activity, gatifloxacin, moxifloxacin, diamines, alkyl chains.

## INTRODUCTION

The discovery of the fluoroquinolones (FQs) during the 1980s improved the treatment of infectious diseases, due to their fewer toxic side effects when compared with the existing drugs [1-4]. Overall these compounds have enhanced pharmacokinetics properties and extensive and potent activity against various parasites, bacteria, and mycobacteria, including bacterial strains resistant to other antimicrobial agents [4, 5]. According to the medical literature, FQs are to be considered the first-line therapy for complicated urinary tract infection and diarrhea, considering the bacterial aetiology. They are also alternative agents for the treatment of many sexually transmitted diseases, as well as osteomyelitis, wound infection and respiratory infections [1-4].

After the discovery of the fluoroquinolone norfloxacin, structure-activity relationships (SAR) analysis of the fluoroquinolonic nucleus led to the development of new derivatives with better solubility, higher antimicrobial profile, prolonged serum half-life, fewer adverse side effects, and allowing for both oral and parenteral routes of administration [1, 3, 4].

It is assumed that the bactericidal activity generated by FQs is caused by the inhibition of two bacterial enzymes: DNA gyrase (topoisomerase II) and topoisomerase IV enzymes. Gatifloxacin and moxifloxacin are 8-methoxy-fluoroquinolones with enhanced anti-Gram-positive activity *in vitro* compared with other FQs such as ciprofloxacin and ofloxacin [5-8].

Considering the antimicrobial resistance phenomenon as one of the greatest challenges of 21<sup>st</sup> century facing the mod-

ern medicine, it is assumed that the discovery of new substances with potential effect against pathogenic bacteria is needed. In this regard, prospective studies on synthesis or modification of drugs may help to improve the antimicrobial chemotherapy. Though quinolones are still presently active against many bacterial strains, prospective studies aimed in the characterization of new derivatives are needed to help overcome the antimicrobial resistance phenomenon.

We recently described the synthesis of lipophilic FQ derivatives containing diaminoalkyl side chains at C-7 position with very good activities against *Mycobacterium tuberculosis* [9]. In this context, this work describes the antibacterial evaluation of these 12 lipophilic 8-methoxy-fluoroquinolones, analogues of gatifloxacin and moxifloxacin, against 11 Gram-negative and 4 Gram-positive bacteria species of clinical and microbiological relevance.

## MATERIALS AND METHODS

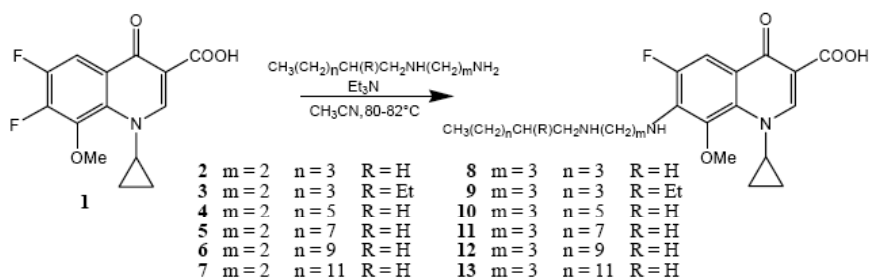
### Chemistry

The synthesis of different lipophilic FQ derivatives was achieved by a simple synthetic method previously described [9]. The reaction between fluoroquinolone 1 and the respective *N*-alkyl-diamines afforded the desired fluoroquinolone derivatives 2-13 in 61-68% yields (Scheme 1).

### Antibacterial Activity

Using the Broth Dilution Method [10] the antibacterial activity of the synthesized compounds was investigated *in vitro* against 11 Gram-negative American Type Culture Collection (ATCC) (*Shigella sonnei* ATCC 25931, *Enterobacter cloacae* ATCC 13047, *E. aerogenes* CDC 1680, *Citrobacter freundii* ATCC 8090, *Klebsiella pneumonia* ATCC 13866, *Salmonella typhi* ATCC 19430, *Escherichia coli* ATCC 11229, *Proteus mirabilis* CDC 305, *Serratia marscescens* ATCC 4133, *Pseudomonas aeruginosa* ATCC 27853 and

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Scheme 1. Synthesis of different FQ derivatives

*Providencia stuartii* ATCC 29914) and 4 Gram-positive ATCC (*Staphylococcus epidermidis* ATCC 12228, *S. aureus* ATCC 25923, *Micrococcus lentus* ATCC 10240, and *Enterococcus faecalis* ATCC 51299) bacteria species. The tests were performed in duplicate. The inoculum for each tested strain, previously aerobically grown in Tryptic Soy Agar (Difco) at 35.5 °C for 24h, was adjusted to the turbidity of 0.5 McFarland ( $1.5 \times 10^8$  CFU/ml) with sterile saline solution (0.85% NaCl). Tested compounds 2-13 (5 mg) were dissolved in ethanol (10 ml) and the solutions were diluted with Mueller Hinton Broth (Difco) to obtain the concentrations of 0.0625 to 16.0 µg/ml, in a final volume of 3 ml. The tubes were inoculated with 100 µl of the bacterial culture and incubated aerobically at 35.5 °C for 24h.

The MIC was defined as the lowest concentration of tested compound resulting in no bacterial growth (100% inhibition rate). Control experiments were performed using only sterile saline solution inoculated in the Mueller Hinton medium and using gatifloxacin as standard antimicrobial agent for comparison purposes.

## RESULTS AND DISCUSSION

The *in vitro* antibacterial activities of FQ derivatives 2-13 was summarized in Table 1. Overall, the MIC ranged between 0.06 and 16 µg/ml. Compounds 10 and 11 were more effective against the Gram-positive bacteria *M. lentus* (MIC = 0.062 and 0.125 µg/ml, respectively) than the reference compound gatifloxacin (MIC = 1.0 µg/ml), while compound

Table 1. *In Vitro* Antibacterial Activity of Lipophilic FQ Derivatives 2-13

		Minimal Inhibitory Concentrations - MICs (µg/ml)												
Bacteria		Compounds												
		2	3	4	5	6	7	8	9	10	11	12	13	Gat
Gram (+)	<i>S. epidermidis</i>	nd	16	16	16	nd	nd	4	8	1	4	8	8	0.062
	<i>M. lentus</i>	4	8	2	1	16	>16	4	4	0.062	0.125	8	8	1
	<i>S. aureus</i>	16	8	4	8	16	16	16	2	2	0.5	0.5	4	1
	<i>E. faecalis</i>	nd	8	8	16	nd	nd	16	16	8	>16	0.2	4	0.25
Gram (-)	<i>S. sonei</i>	>16	8	2	2	>16	>16	nd	>16	2	8	nd	nd	0.25
	<i>E. cloacae</i>	8	4	2	2	16	16	nd	>16	>16	>16	nd	nd	0.25
	<i>C. freundii</i>	nd	>16	8	16	nd	nd	nd	4	8	8	nd	nd	0.5
	<i>K. pneumoniae</i>	nd	16	>16	16	nd	nd	nd	>16	>16	>16	nd	nd	0.5
	<i>E. aerogenes</i>	8	>16	2	1	8	16	nd	16	>16	>16	nd	nd	0.062
	<i>S. tiphy</i>	nd	16	4	>16	nd	nd	nd	16	4	8	nd	nd	0.125
	<i>P. mirabilis</i>	nd	>16	4	8	nd	nd	nd	>16	16	16	nd	nd	0.062
	<i>S. marscenceses</i>	nd	>16	>16	>16	nd	nd	nd	>16	>16	>16	nd	nd	0.062
	<i>E. coli</i>	nd	16	16	>16	nd	nd	4	8	2	0.256	16	>16	0.062
	<i>P. stuartii</i>	>16	16	2	>16	>16	>16	nd	>16	>16	16	nd	nd	0.125
	<i>P. aeruginosa</i>	nd	16	>16	>16	nd	nd	nd	16	16	>16	nd	nd	0.125

Gat, Gatifloxacin; n.d, not determined based in the high MIC values obtained for other bacteria.

5 showed the same activity as the control FQ. Compounds 11 and 12 were also more active than the control drug against Gram positive bacteria *S. aureus* (MIC = 0.5, 0.5 and 1.0 µg/ml, respectively). All the tested compounds were less effective against Gram-negative bacteria when compared to gatifloxacin, and the most active was compound 11 (MIC = 0.256 µg/ml against *E. coli*).

The size of the carbon spacer (m=2 or 3) seems to be important for the activity against Gram-positive bacteria, and 1,3-propanediamine derivatives 8-13 were generally much more active than their 1,2-ethanediamine analogues. Concerning the size of the lipophilic alkyl chain, the best activities were observed for substances with 8 carbons (compounds 4 and 10), 10 carbons (compounds 5 and 11) or 12 carbons (compound 12). Ramified compounds 3 and 9 showed a lower activity than their linear analogues 4 and 10, may be due to their greater volume. As observed in the results, antibacterial activities of the different FQ derivatives vary with the lipophilic moiety length and the best results were obtained with 8-10 carbons. Considering the commercial FQ derivatives, the most common substituent at C-6 is a piperazinyl group having a two-carbon spacer chain between the amino groups [4]. From the results obtained in the present work a three-carbon spacer could perhaps enhance the antibacterial activity, and overcome the bacterial resistance against quinolones.

## CONCLUSION

Among the tested synthetic FQ derivatives, three compounds (5, 10 and 11) were as active as or more efficient than gatifloxacin against Gram-positive bacteria *M. lentus*. When compared with gatifloxacin compound 10 was 16 times more active. Two compounds (11 and 12) were twice more active than the reference compound against *S. aureus*. To enhance the antibacterial activity the ideal carbon chain should have about 10 carbon atoms, and the spacer 3 carbon atoms. The biological activities of these compounds make them potentially interesting new lead structures for the development of more active antibacterial agents useful in both human and veterinary medicine.

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