

Novel Levofloxacin Derivatives as Potent Antibacterial Agents

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Abstract

In this report, a new series of fluoroquinolone agents which was derived from levofloxacin was synthesized and evaluated against Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Micrococcus luteus*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Serratia marcescens*) organisms. The results showed that some of the synthesized compounds are strong antibacterial agents. All the synthesized compounds showed remarkable activities against *Staphylococcus epidermidis* and *Staphylococcus aureus* (MIC = 0.03-1 µg/mL and 0.06-1 µg/mL respectively).

Keywords: Antibacterial evaluation; Fluoroquinolone; Levofloxacin.

Introduction

Infections, caused by bacteria, parasites, viruses and fungi threaten the global public health by causing mild to severe disease. To reduce illness and death from infectious disease, antibiotics and similar drugs, together called antimicrobial agents have been widely used. Antibacterial agents work by killing or arresting the growth of bacterium [1]. Misuse and overuse of them led to the resistance of a bacteria to the antibacterial drugs which have become a public issue worldwide [2-5]. This drug resistance led to the increased risk of death due to the unsuccessful responses to the standard treatments. Therefore, an ever-increasing efforts which was encouraged by FDA, have

been devoted to the development of novel antimicrobial agents with no or little bacterial resistance.

Among various antibacterial agents and their different mechanism of action in fighting against bacteria, fluoroquinolones (FQ) have been extensively used to combat bacterial infections [6-9]. This class of antibiotics exhibited the significant ability to combat Gram-positive, Gram-negative and anaerobic bacterial infections. In addition, the target of these agents is two type II bacterial topoisomerase enzymes, DNA gyrase and/or topoisomerase IV [10]. The ability of this category in the treatment of tuberculosis (TB) [11], respiratory and urinary tract disease, typhoid fever, bone-joint infections, acute bronchitis and sinusitis is among the therapeutic applications of these agents.

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Levofloxacin (LVFX), ciprofloxacin (CPFX), gemifloxacin (GMFX), moxifloxacin, norfloxacin and ofloxacin belong to this group [Fig. 1] [12, 13]. The presence of 4-quinolone/naphthyridone-3-carboxylic acid as the main core and a secondary amino group at the C-7 position of the heterocyclic core are the required common feature of this group, however, recently novel cores have been found effective. The presence of five or six-membered nitrogen-containing heterocycles at the C-7 position of the basic quinolone strongly impressed the antibacterial potency of FQs [14], due to its role in the drug-enzyme interaction domain [15] and helping in the easily transportation of the drug into the cells [16].

Dealing with the drug resistance challenge, new solutions have been introduced, among them, producing novel analogs of existing drugs by chemical modification led to positive outcomes [17-21]. To achieve this, several factors should be considered involving excellent bioavailability, tissue penetrability and low toxic effects. Levofloxacin [22], a chiral analogue of ofloxacin, was approved for the treatment of a number of bacterial infections by targeting topoisomerase II (DNA-gyrase) in Gram-negative bacteria and topoisomerase IV in Gram-positive bacteria [23]. With respect to excellent activity of levofloxacin, we decided to study the effect of structural modification on the antibacterial activity of new LVFX'S derivatives.

Materials and Methods

Determination of antibacterial activity

Compounds **5(a-c)**, **6(a-c)**, **7(a-c)** were evaluated for their antibacterial activity using conventional agar-dilution method. Two-fold serial dilutions of the compounds and reference drugs were prepared in Mueller–Hinton agar. Drugs (10.0 mg) were dissolved in DMSO (1 mL) and the solution was diluted with water (9 mL). Further progressive double dilution with melted Mueller–Hinton agar was performed to obtain the required concentrations of 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, 0.19, 0.098, 0.049, 0.025, 0.013, 0.006 and 0.003 mg/mL. The bacteria inocula were prepared by suspending overnight colonies from Mueller–Hinton agar media in 0.85% saline. The inocula were adjusted photometrically at 600 nm to a cell density equivalent to approximately 0.5 McFarland standard (1.5×10^8 CFU/mL). The suspensions were then diluted in 0.85% saline to give 10^7 CFU/ml. Petri dishes were spot-inoculated with 1 mL of each prepared bacterial suspension (10^4 CFU/spot) and incubated at 35-37 °C for 18 h. The minimum inhibitory concentration (MIC) was the lowest concentration of the test compound, which resulted in no visible growth on the plate. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment.

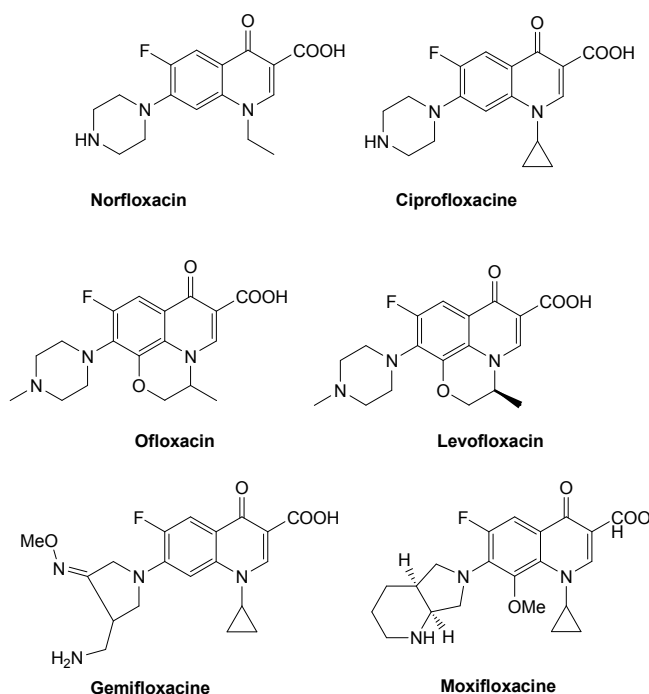


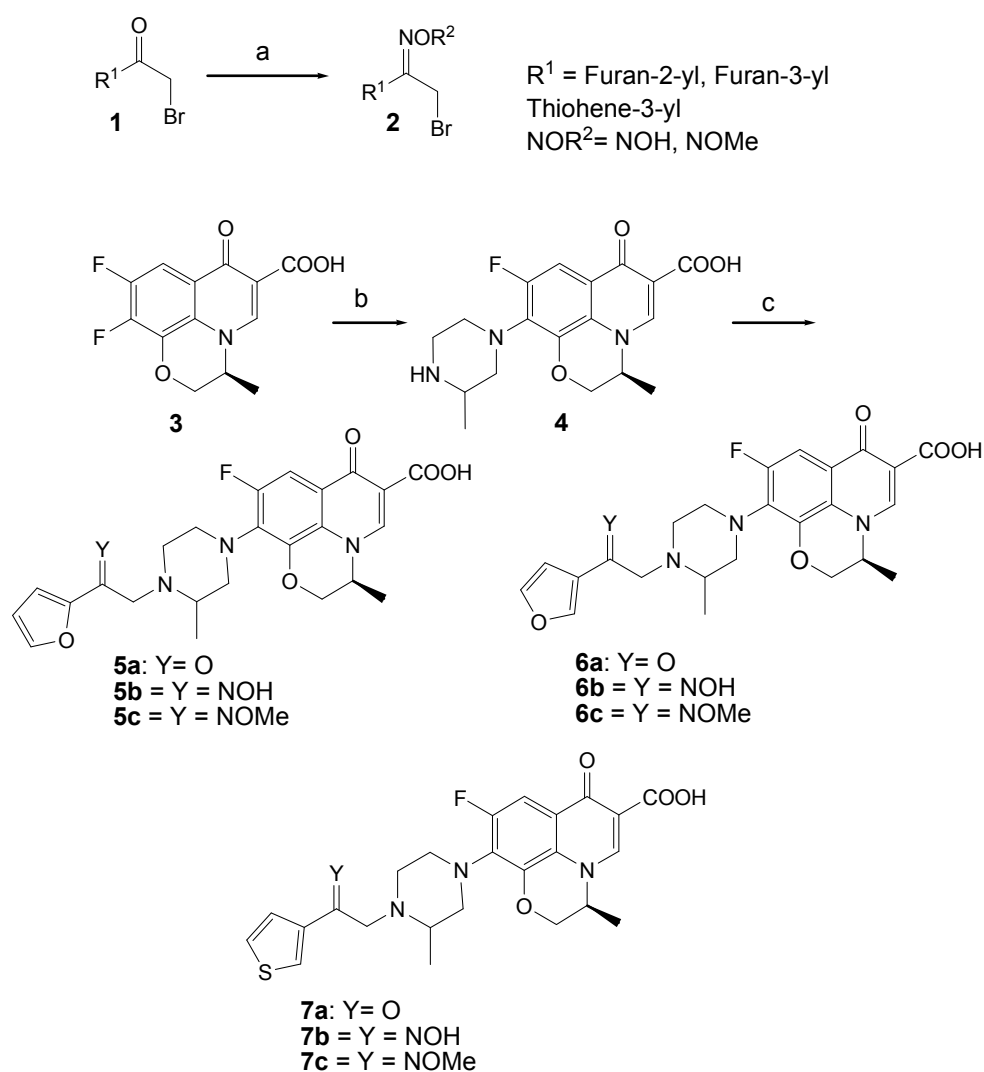
Figure 1. The structures of known fluoroquinolone antibacterial agents.

Results and Discussion

Following our recent program in developing potent bioactive compounds [24, 25] especially fluoroquinolone derivatives by making structural modifications, which normally resulted in excellent activities against many Gram-positive and Gram-negative microorganisms [26, 27], herein, we synthesized and evaluated novel levofloxacin-based derivatives. Four Gram-positive and four Gram-negative bacteria were chosen and the potency of the synthesized compounds was determined. Due to the importance of levofloxacin against Gram-positive bacteria series, better results were obtained in the case of these organisms. However, some of the synthesized compounds exhibited good results against Gram-negative strains.

Chemistry. At first, the reaction of ketones **1** with hydroxylamine hydrochloride or *O*-methylhydroxylamine hydrochloride gave **2** [28, 29]. The substitution of 2-methyl piperazine at C-7 position was carried out according the literature [30]. The target compounds were prepared in good yields in the presence of sodium hydrogen carbonate at room temperature.

Antibacterial activity. The minimum inhibitory concentration (MIC) of compounds was determined by agar-dilution method against four Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Serratia marcescens*) and four Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Micrococcus luteus*) bacteria panel along with levofloxacin for comparison. The MIC values are presented in Table 1. In general, all



Scheme 1. Synthesis of target compounds: Reagents and conditions: (a) $\text{NH}_2\text{OH}\cdot\text{HCl}$ or MeONH_2HCl , MeOH, r.t.; (b) 2-methyl piperazine; (c) **1** or **2**, DMF, NaHCO_3 , r.t.

Table 1. Antibacterial activities (MICs in $\mu\text{g}/\text{mL}$) of compounds.

Compd.	Gram-positive			Gram-negative				
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>M. luteus</i>	<i>S. epidermidis</i>	<i>K. pneumoniae</i>	<i>E. Coli</i>	<i>P. aeruginosa</i>	<i>S. marcescens</i>
5a	0.06	0.06	8	0.12	0.25	0.25	8	2
5b	0.06	0.03	>64	0.03	0.12	2	8	32
5c	0.25	0.03	32	0.25	0.25	1	16	>64
6a	0.12	0.06	32	0.03	0.12	0.5	32	16
6b	0.12	0.06	64	0.03	0.12	0.5	32	32
6c	0.12	0.12	32	0.06	0.06	1	16	32
7a	0.5	0.25	>64	1	2	8	64	64
7b	1	0.25	64	0.5	2	16	>64	64
7c	0.12	0.5	>64	0.12	32	32	>64	64
Levofloxacin	1	0.12	2	0.5	0.03	0.03	1	0.5

the synthesized compounds showed remarkable activities against *Staphylococcus epidermidis* and *Staphylococcus aureus* (MIC = 0.03-1 $\mu\text{g}/\text{mL}$ and 0.06-1 $\mu\text{g}/\text{mL}$ respectively). The potency of compounds against Gram-positive bacteria was better or the same with levofloxacin's amount, except for *Micrococcus lutes*. Compound **5a** showed the best activity against Gram-positive and Gram-negative microorganisms and its most potent activity was observed against *Staphylococcus aureus* (MIC = 0.06 $\mu\text{g}/\text{mL}$), 16-times more active than levofloxacin. In some other cases, superior results compared to reference drug was obtained. In *Staphylococcus epidermidis*, *Bacillus subtilis* and *Klebsiella pneumonia*, the presence of oximes (**5b**, **6b**, **7b**) led to the better or same activity compared to ketone counterparts (**5a**, **6a**, **7a**). In the case of methyl oxime derivatives, compound **7c** exhibited potent activity against *Staphylococcus epidermidis* and *Staphylococcus aureus*, than that of reference compound (MIC = 0.12, 0.12 $\mu\text{g}/\text{mL}$ respectively). Compounds **5c** and **6c** also had superior activity against *Bacillus subtilis* and *Klebsiella pneumonia* than other derivatives (MIC = 0.03, 0.06 $\mu\text{g}/\text{mL}$ respectively). Thiophene containing derivatives **7a-c** resulted in the weakest activity.

Conclusion

In conclusion, *in vitro* antibacterial activity of levofloxacin derivatives against Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Micrococcus Luteu*) and Gram-negative (*E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Serratiamarcescens*) was evaluated. By using agar dilution technique, compounds **5a**, **6a**, **7b** were found to be more potent against *Staphylococcus aureus* and *Staphylococcus epidermidis* respectively, which are stronger than levofloxacin.

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