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In-vitro Antibacterial Evaluation of Some Fluoroquinolone Derivatives Against Food Borne Bacteria

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Abstract

Food poisoning has been emerged as a worldwide health issue and related illness in both developed and developing countries confirms its significance as an important public health priority. *In vitro* antibacterial evaluation of nine fluoroquinolone derivatives against food borne bacteria including Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Salmonella enterica*, *Escherichia coli*, *Yersinia enterocolitica*, *Vibrio cholerae*) organisms was developed using agar dilution technique and compared with ciprofloxacin and norfloxacin as reference drugs. Most compounds showed good activity against Gram-positive and the Gram-negative bacteria. All compounds were active against *Staphylococcus aureus* (MIC=3.12-6.12µg/mL) and compounds 1a, 1c-e, 2a, and 3c (MIC=3.12µg/mL) exhibited excellent antibacterial activity in comparison to ciprofloxacin and norfloxacin (MIC=6.25µg/mL). Fluoroquinolone derivatives exhibited good activity against food borne bacteria and are appropriate candidates for food poisoning prevention.

Keywords: Antibacterial evaluation; Fluoroquinolone; Food borne bacteria.

Introduction

Recently, food poisoning has been emerged as a worldwide health issue [1, 2] and estimates of foodborne illness in different countries demonstrate the increasing demand for food safety as a significant public health priority [3-5]. Foodborne illness include a wide range of diseases caused by bacteria, parasites, viruses, toxins, and metals which need more concentrated attention [6, 7].

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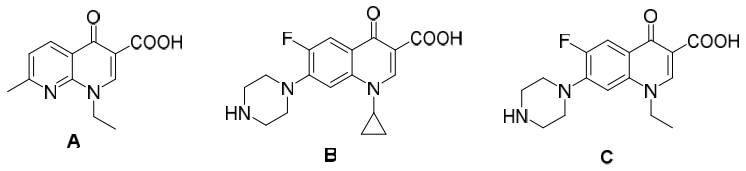


Figure 1. Nalidixic acid A, ciprofloxacin B, and norfloxacin C.

Since food-poisoning bacteria are often present in many foods, knowing the characteristics of food borne bacteria such as *Staphylococcus aureus*, *Salmonella enterica*, *Escherichia coli*, *Yersinia enterocolitica*, *Vibrio cholerae*, etc. is crucial to conduct an actual control program. At this juncture, numerous antibacterial agents have been introduced for treating infectious diseases causing from the penetration of food borne bacteria into the human population [8-12]. Although they have significantly improved the health of humans as well as the health of animals, there is an expressive increasing in resistance of bacteria to antibacterial agents [13-16].

In recent studies, a lot of effort has been devoted to recognize the efficient naturally occurring [17, 18] or synthetic antibacterial agents [19, 20]. Among a wide spectrum of antibacterial compounds, quinolones have shown brilliant activity and have been frequently utilized for the treatment of bacterial infections [21-23].

By the advent of nalidixic acid (A, Fig. 1) which exhibited good activity against gram-negative bacilli, such as *Escherichia coli*, *Klebsiella* and *Proteus* species, new horizons were opened to synthetic antibiotics [24]. The appearance of various limitations such as need to high doses of drug and unsatisfactory activity against gram-positive bacteria led to design and synthesis of novel quinolone-based compounds particularly fluoroquinolones such as ciprofloxacin (B, Fig. 1) and norfloxacin (C, Fig. 1) in 1980s [25, 26]. In spite of lots of improvements obtained by these drugs, various modifications have been made to increase their efficacy. In this manner, various antibiotics such as enoxacine, clinafloxacin, gatifloxacin, grepafloxacin, levofloxacin, moxifloxacin, sparfloxacin, and trovafloxacin were synthesized and used for various infections [27].

Due to the importance of heterocycles in bioactive compounds [28, 29] and in continuation of our research program to find a novel antimicrobial agent [30-31], in this research we would like to report the antibacterial activities of some novel *N*-substituted piperazinyl fluoroquinolone derivatives 1a-1e, 2a, and 3a-3c.

Materials and Methods

All compounds were prepared according to our previous reports [32-35].

Determination of Minimum Inhibitory Concentration (MIC) of the synthesized compounds on the test organisms

The minimum inhibitory concentration was measured using agar dilution technique. Briefly, the synthesized compounds were dissolved in dimethyl sulfoxide (DMSO, Merck) and serially diluted in molten Mueller Hinton broth (MHA, Sigma) in petri dishes (100 mm×15 mm) to obtain final concentrations: 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.195, 0.098, 0.049, 0.025, 0.012, 0.006 and 0.003 μg mL^{-1}.The solvent did not exceed 1% concentration and did not affect the growth of the organisms. All bacterial strains were grown in Mueller Hinton broth (MHB, Sigma) for 4 h at 37 °C. Bacterial suspensions with 0.5 McFarland standard turbidity, which is equivalent to 108 cfu/mL, were prepared by dilution with Mueller Hinton broth. The diluted inoculum was added to a Steer's replicator calibrated and incubated for 24 h at 37 °C. After incubation, all dishes were observed for microbial inhibition.

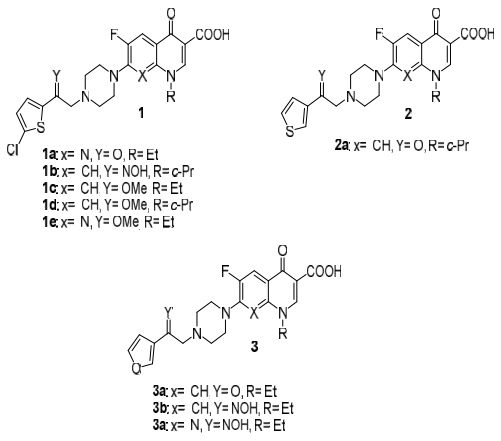


Figure 2. Structure of quinolinone derivatives.

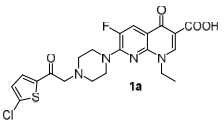
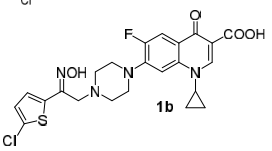
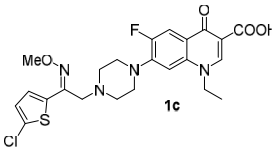
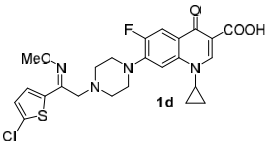
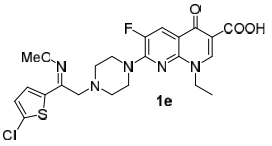
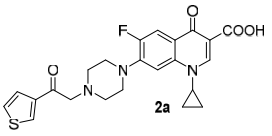
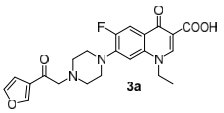
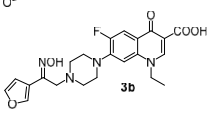
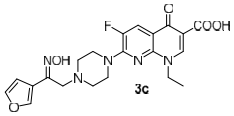
Results and Discussion

As synthesis and evaluation of antibacterial agents particularly fluoroquinolones is an important part of our research program (30-33); herein, we have evaluated antibacterial activity of some of previously prepared fluoroquinolone derivatives containing chlorothiophen 1, thiophen 2, and furan moieties 3 in their structure (30-33) (Fig. 2) against food contaminating bacteria including such as *Staphylococcus aureus*, *Salmonella enterica*, *Escherichia coli*, *Yersinia enterocolitica*, *Vibrio cholerae*. The results are summarized in Table 1.

Antibacterial activity

All fluoroquinolone derivatives 1a-1e, 2a, and 3a-3c

Table 1. Antibacterial activity of fluoroquinolone derivatives

Compound	Gram-positive	Gram-negative			
	S. aureus	***S. enterica***	***E. Coli***	***Y. enterocolitica***	***V. cholerae***
 1a	3.12	3.12	3.12	3.12	6.25
 1b	6.25	3.12	12.50	3.12	0.78
 1c	3.12	3.12	3.12	3.12	12.50
 1d	3.12	3.12	3.12	3.12	12.50
 1e	3.12	3.12	6.25	3.12	6.25
 2a	3.12	3.12	3.12	3.12	6.25
 3a	6.25	1.65	3.12	6.25	6.25
 3b	6.25	3.12	3.12	3.12	0.78
 3c	3.12	3.12	3.12	3.12	6.25
Ciprofloxacin (B)	6.25	3.12	6.25	3.12	0.78
Norfloxacin (C)	6.25	0.39	6.25	0.39	6.25

were evaluated for their antibacterial activity against a panel of microorganisms including *Staphylococcus aureus* ATCC 25913, *Salmonella enterica* PTCC 1709, *Escherichia coli* ATCC 8739, *Yersinia enterocolitica* PTCC 1477, and *Vibrio cholerae* PTCC 1611 using agar dilution technique (34). The MIC (minimum inhibitory concentration) values were determined and compared with ciprofloxacin B and norfloxacin C as reference drugs (Table 1).

As can be seen in Table 1; most compounds showed good activity against Gram-positive and the Gram-negative bacteria. Our results revealed that all compounds were active against *Staphylococcus aureus* (MIC = 3.12-6.12 µg/mL) and compounds 1a, 1c-e, 2a, and 3c (MIC = 3.12 µg/mL) exhibited excellent antibacterial activity in comparison to ciprofloxacin B and norfloxacin C (MIC = 6.25 µg/mL).

The MIC values of evaluated compounds indicated that most derivatives have noticeable activity against Gram-negative microorganisms. *Salmonella enterica* was inhibited by all derivatives at concentration of 1.65-3.12 µg/mL. Although they acted as well as ciprofloxacin B (MIC = 3.12 µg/mL) they showed poor activity in comparison to norfloxacin C (MIC = 0.39 µg/mL). Our results related to *Escherichia coli* inhibition depicted that all compounds (MIC = 3.12 µg/mL) except 1b and 1e (MIC = 12.50 and 6.25 µg/mL, respectively) were much stronger than ciprofloxacin B and norfloxacin C (MIC = 6.25 µg/mL). Also it was found that all compounds except 3a, showed good activity against *Yersinia enterocolitica* (MIC = 3.12 µg/mL) in comparison to ciprofloxacin B (MIC = 3.12 µg/mL); however, they showed poor activity compared with norfloxacin (MIC = 0.39 µg/mL). Finally, our data revealed that all compounds except 1c and 1d (MIC = 12.50 µg/mL) inhibited the growth of *Vibrio cholerae* efficiently. It is worth mentioning that 1b and 3b (MIC = 0.78 µg/mL) acted as well as ciprofloxacin B (MIC = 0.78 µg/mL) and showed the best activity among the fluoroquinolone derivatives. Nevertheless, all compounds except **1c** and **1d** showed good activity compared with norfloxacin **B** (MIC = 6.25 µg/mL).

Conclusion

In conclusion, *in vitro* antibacterial activity of some fluoroquinolone derivatives against food borne bacteria including Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Salmonella enterica*, *Escherichia coli*, *Yersinia enterocolitica*, *Vibrio cholerae*) organisms was evaluated using agar dilution technique and compared with ciprofloxacin and norfloxacin as reference drugs. Most compounds showed good activity and in some cases superior activity was obtained in comparison to reference drugs. Our results revealed that fluoroquinolones are appropriate candidates for food poisoning prevention.

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