# Synthesis and *in-vitro* antibacterial activity of *N*-piperazinyl quinolone derivatives with 5-chloro-2-thienyl group

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**ABSTRACT**

Background and the purpose of the study: Fluoroquinolones are an important group of antimicrobial agents that are used widely in the treatment of various infectious diseases. The purpose of the present study was to synthesize new *N*-piperazinyl quinolone derivatives with 5-chloro-2-theinyl group having possible antimicrobial activity. Methods: Reaction of ciprofloxacin (**1**), norfloxacin (**2**) and enoxacin (**3**) with α-bromoketone **10** or α-bromooxime derivatives **11a-c** in DMF, in the presence of $NaHCO_3$ at room temperature, afforded corresponding ketones **4a-c** or oxime derivatives **5-7(a-c)**, respectively. Results and major conclusion: The synthesized compounds were tested against a series of Gram-positive and Gram-negative bacteria. The results of MIC tests against both Gram-positive and Gram-negative bacteria revealed that ciprofloxacin derivatives (compounds **4a**, **5a**, **6a** and **7a**) were more active than norfloxacin and enoxacin analogues. Compound **5a**, containing *N*-[2-(5-chlorothiophen-2-yl)-2-hydroxyiminoethyl] residue provided a high *in vitro* antibacterial activity against Gram-positive bacteria, with MIC of 0.06, 0.125, 0.5 and 0.125 μg/mL against *S. aureus*, *S. epidermidis*, *E. feacalis* and *B. subtilis*, respectively. Its activity was found to be 4 to 8 times better than reference drug (ciprofloxacin) against all Gram-positive bacteria with the exception of *E. feacalis*.

**Keywords:** Quinolones, *N*-substituted piperazinyl quinolones, In vitro antibacterial activity, Thiophene derivatives, Oximes

## INTRODUCTION

Fluoroquinolones are an important group of antimicrobial agents that are used widely in the treatment of various infectious diseases as a result of activity, significant tissue penetration and convenient routes of administration. However, growing use of fluoroquinolones has impacted the Gram-negative bacilli (e.g., *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella*), causing their resistant rates to approach the critical points (1).

The problem of drug-resistant bacteria has been the driving force for the development of newer quinolones. The extensive research efforts have enabled a better definition of the structural moieties or elements around the basic pharmacophore of quinolones that offer the best combination of clinical efficacy and reduced resistance selection in Gram-positive and Gram-negative bacteria. Most of the quinolones currently on the market have only moderate activity against many Gram-positive cocci, including staphylococci and streptococci. Therefore, recent efforts have been directed toward the synthesis of new quinolone antibacterial that can provide improved Gram-positive antibacterial activity, while retaining good Gram-negative activity (2, 3).

Quinolone inhibit DNA synthesis by interacting with two essential bacterial type II topoisomerases, DNA gyrase and topoisomerase IV (4). They inhibit enzyme function by binding to the catalytic intermediate enzyme-DNA complex. The stabilization of the resulting enzyme-DNA complex leads to the generation of double-strand DNA breaks that trigger a cascade of events leading to cell death (5). According to the inhibition mechanisms of the quinolones, proposed by Shen *et al*, the site near the C-7 substituent is regarded as drug-enzyme

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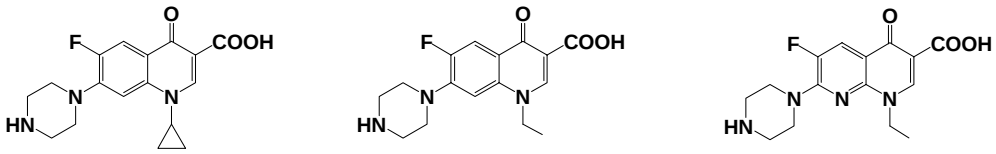


**1** **2** **3**

**Figure 1.** Structures of ciprofloxacin (**1**), norfloxacin (**2**) and enoxacin (**3**).

interaction domain (6). In addition, Klopman *et al*, also concluded that the cell permeability is dominantly controlled by C-7 substituent (7).

Both activity spectrum and kinetic profile can be controlled at C-7. The most common substituents are cyclic amino group, for example, piperazine and pyrrolidine ring. Piperazine ring is particularly common (e.g., ciprofloxacin **1**, norfloxacin **2** and enoxacin **3**) (Fig. 1) and confer potency against Gram-negative bacteria.

To explore the potential of 7-piperazinylquinolone derivatives as anti-Gram-positive agents, we have reported a number of *N*-substituted piperazinyl quinolones with high activity against staphylococci (8, 9). In addition, a number of quinolones with a 2-oxoethyl or a 2-oxyiminoethyl moiety attached to the piperazine ring were synthesized and evaluated for their antibacterial activity by us (10, 11) and others (12). The results demonstrated that the introduction of thiophen-2-yl or 5-bromo thiophen-2-yl group instead of phenyl at 2 position of 2-oxoethyl or 2-oxyimino ethyl moiety attached to the piperazine ring in 7-piperazinyl quinolones improved the overall antibacterial activity against Gram-positive bacteria (13).

In continuation of our research program to establish the structure-activity relationships of *N*-substituted piperazinyl quinolone derivatives, herein we report the synthesis and antibacterial activity of *N*-[2-(5-cholorothiophen-2-yl)-2-oxoethyl]piperazinyl quinolone derivatives **4a-c** and *N*-[2-(5-cholorothiophen-2-yl)-2-(oxyimino)ethyl]piperazinyl quinolones **5-7(a-c)**.

## MATERIAL AND METHODS

*Chemistry*

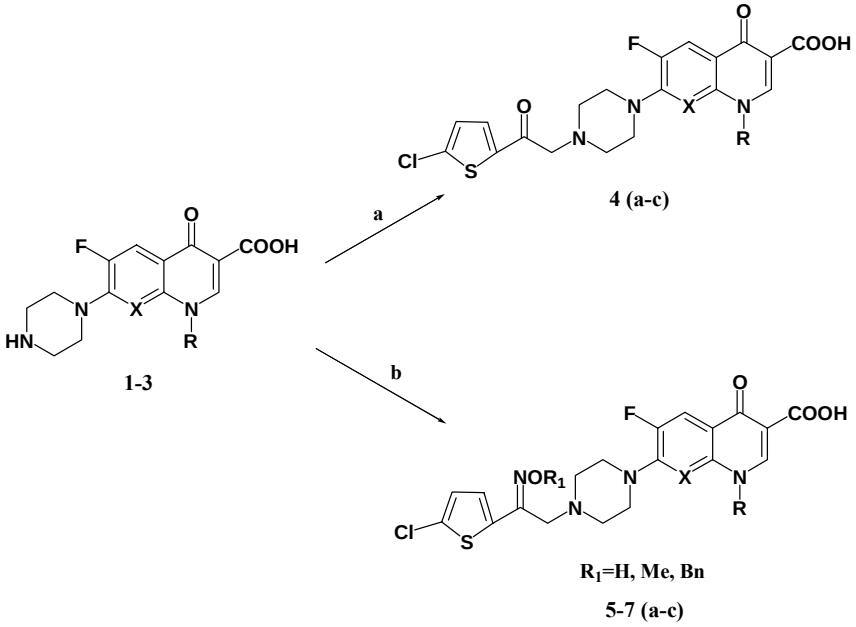
Chemicals and all solvents used in this study were purchased from Merck AG and Aldrich Chemical. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (potassium bromide disks). $^1$H-NMR spectra were measured using a Bruker 500 spectrometer, and chemical shifts are expressed as δ (ppm) with tetramethylsilane as internal standard. Elemental analyses were carried out on a CHN rapid elemental analyzer (GmbH-Germany) for C, H and N, and the results are within ± 0.4% of the theoretical values. Merck silica gel 60 F254 plates were used for analytical TLC. Yields are of purified product and were not optimized.

*General procedure for the preparation of **4-7(a-c)**.*

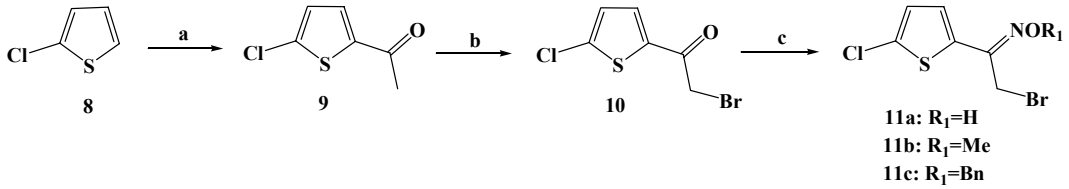
A mixture of 2-bromo-1-(5-chlorothiophen-2-yl)ethanone **10** or 2-bromo-1-(5-chlorothiophen-2-yl)ethanone oxime derivatives **11a-c** (0.55 mmol), quinolone **1-3** (0.5 mmol) and $NaHCO_3$ (0.5 mmol) in DMF (5 mL), was stirred at room temperature for 2-7 days. After consumption of quinolone **1-3**, water (20 mL) was added and the precipitate was filtered, washed with water and crystallized from EtOH-$CHCl_3$ to give compounds **4-7(a-c)**.

*Antimicrobial activity*

Compounds **4-7(a-c)** were screened for their antibacterial activity against Gram-positive (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 14940, *Bacillus subtilis* ATCC 6051) and Gram-negative (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 10031, *Salmonella typhi* ATCC 19430, *Shigella flexneri* NCTC 8516, *Serratia marcescens* PTCC 1111, *Pseudomonas aeruginosa* ATCC 27853) bacteria by the conventional agar dilution method (14). Twofold serial dilutions of the compounds and reference drugs were prepared in Muller Hinton agar. Drugs (6.4 mg) were dissolved in DMSO (1 mL) and the solution diluted with water (9 mL). Further progressive double dilution with melted Muller-Hinton agar was performed to obtain the required concentrations of 4, 2, 1, 0.5, 0.25, 0.125, 0.06, 0.03 and 0.015 µg/mL. Petri dishes were inoculated with 1-5×$10^4$ colony-forming units (cfu) and incubated at 37°C for 18 h. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the test compound, which resulted in no visible growth on the plate. To insure 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.




**Scheme 1.** Synthesis of compounds **4-7**. Reagents and conditions: (a) α-bromoketone **10**, $NaHCO_3$, DMF, rt; (b) α-bromooxime **11a-c**, $NaHCO_3$, DMF, rt.



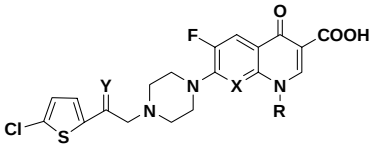

**Scheme 2.** Synthesis of intermediates α-Bromoketone **10** and α-Bromooxime **11**. Reagents and conditions: (a) acetyl chloride, $AlCl_3$, $CS_2$, rt; (b) $CuBr_2$, $CHCl_3$-EtOAc, reflux; (c) hydroxylamine hydrochloride or *O*-methyl hydroxylamine hydrochloride or *O*-benzyl hydroxylamine hydrochloride, MeOH, rt.

## RESULTS AND DISCUSSIN

Our synthetic pathways to intermediates **10** and **11a-c,** and target compounds **4a-c** and **5-7(a-c)** are presented in Schemes 1 and 2. Compound 1-(5-chlorothiophen-2-yl)ethanone **9** was obtained from 2-cholorothiophen **8** according to the reported method (15). Ketone **9** was brominated with copper (II) bromide in refluxing $CHCl_3$-EtOAc to give corresponding α-bromoketone **10** (16). Compound **10** was converted to oxime derivative **11a** by stirring with excess $HONH_2$.HCl in methanol at room temperature. Similarly, the *O*-methyloxime ether **11b** and *O*-benzyloxime ether **11c** were synthesized by reaction of compound **10** with *O*-methylhydroxylamine or *O*-benzylhydroxylamine hydrocholoride, respectively (13). Reaction of quinolones **1**, **2** or **3** with α-bromoketone **10** or α-bromooxime derivatives **11a-c** in DMF, in the presence of $NaHCO_3$ at room temperature, afforded corresponding ketones **4a-c** or oxime derivatives **5-7(a-c)**, respectively (17). Physicochemical and spectroscopic data of these compounds are shown in Tables 1 and 2, respectively.

Compounds **4-7(a-c)** were tested against a panel of microorganisms including Gram-positive (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 14940, *Enterococcus feacalis* NCTC 6013 and *Bacillus subtilis* ATCC 6051) and Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 10031, *Salmonella typhi* ATCC 19430, *Shigella flexneri* NCTC 8516, *Serratia marcescens* PTCC 1111 and *Pseudomonas aeruginosa* ATCC 27853) using conventional agar-dilution method. The MIC (minimum inhibitory concentration) values were determined by comparison to ciprofloxacin as reference drug (Table 3).

**Table 1.** Structures and physicochemical data of compounds **4-7**.




| Compd | X | R | Y | Mp (°C) | Yield (%) | Formula | Elemental analysis Found (Calcd) % | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | C | H | N |
| **4a** | CH | c-Pr | O | 199-200 | 56 | $C_{23}H_{21}ClFN_3O_4S$ | 56.06 (56.38) | 4.21 (4.32) | 8.68 (8.58) |
| **4b** | CH | Et | O | 187-188 | 61 | $C_{22}H_{21}ClFN_3O_4S$ | 55.16 (55.29) | 4.28 (4.43) | 8.60 (8.79) |
| **4c** | N | Et | O | 180-182 | 70 | $C_{21}H_{20}ClFN_4O_4S$ | 52.55 (52.66) | 4.21 (4.21) | 11.78 (11.70) |
| **5a** | CH | c-Pr | NOH | 275-276 | 67 | $C_{23}H_{22}ClFN_4O_4S$ | 57.99 (58.29) | 4.37 (4.47) | 8.60 (8.87) |
| **5b** | CH | Et | NOH | 259-261 | 58 | $C_{22}H_{22}ClFN_4O_4S$ | 56.96 (57.20) | 4.45 (4.58) | 8.98 (9.10) |
| **5c** | N | Et | NOH | 251-253 | 73 | $C_{21}H_{21}ClFN_5O_4S$ | 54.56 (54.49) | 4.60 (4.35) | 12.18 (12.10) |
| **6a** | CH | c-Pr | NOMe | 226-228 | 50 | $C_{24}H_{24}ClFN_4O_4S$ | 55.30 (55.54) | 4.29 (4.66) | 10.98 (10.80) |
| **6b** | CH | Et | NOMe | 199-200 | 71 | $C_{23}H_{24}ClFN_4O_4S$ | 54.16 (54.49) | 4.44 (4.77) | 11.05 (11.05) |
| **6c** | N | Et | NOMe | 171-172 | 62 | $C_{22}H_{23}ClFN_5O_4S$ | 51.90 (52.02) | 4.66 (4.56) | 14.00 (13.79) |
| **7a** | CH | c-Pr | NOBn | 196-198 | 52 | $C_{30}H_{28}ClFN_4O_4S$ | 60.56 (60.55) | 4.71 (4.74) | 9.68 (9.41) |
| **7b** | CH | Et | NOBn | 200-201 | 58 | $C_{29}H_{28}ClFN_4O_4S$ | 57.01 (59.74) | 4.64 (4.84) | 9.66 (9.61) |
| **7c** | N | Et | NOBn | 142-143 | 60 | $C_{28}H_{27}ClFN_5O_4S$ | 57.66 (57.58) | 4.48 (4.66) | 11.75 (11.99) |

Generally, compounds **4-5(a-c)** and **6a** showed significant activity against Gram-positive bacteria, but remaining tested compounds **6b, 6c** and **7(a-c)** exhibited poor or no activity at concentrations <4 μg/mL. Compound **5a** was found to exhibit the most potent *in vitro* antibacterial activity against Gram-positive bacteria, with MIC of 0.06, 0.125, 0.5 and 0.125 μg/mL against *S. aureus*, *S. epidermidis*, *E. feacalis* and *B. subtilis*, respectively. Its activity was found to be 4 to 8 times better than reference drug against all Gram-positives with the exception of *E. feacalis*. Its activity against *E. feacalis* was comparable to reference drug ciprofloxacin (MIC =0.5 μg /mL). In addition, compounds **4a**, **5b, 5c** and **6a** showed good activity against *S. aureus*, *S. epidermidis*, and *B. subtilis* comparable to ciprofloxacin.

As noted in Table 3, the MIC values of the tested compounds indicated that compounds **4(a-c)** and **5a** showed moderate to significant activity (MIC =0.125-4 μg /mL) against Gram-negative bacteria, with the exception of *P. aeruginosa*. Indeed, all compounds showed no activity against *P. aeruginosa* at concentrations <4 μg/mL; the MIC of ciprofloxacin was 2 μg/mL against this microorganism. Generally, most compounds are less active than reference drugs against Gram-negative bacteria. However, MIC data reveals that compound **4a** followed by **4b** and **5a**, which are superior in inhibiting the growth of Gram-negative bacteria, show comparable activity in respect to ciprofloxacin against some Gram-negative strains.

The results of MIC tests against both Gram-positive and Gram-negative bacteria revealed that ciprofloxacin derivatives (compound **4a**, **5a**, **6a** and **7a**) were more active than norfloxacin and enoxacin derivatives. Comparison between MIC values of ketones **4(a-c)** and oximes **5(a-c)** revealed that oximation of ketones seemed to have positive effect for improvement of activity against Gram-positives but this improvement can often be at the expense of activity against Gram-negative bacteria. However, as it is evident from the data for oxime **5a**, higher susceptibilities (lower MICs) were observed with this compound against Gram-positives while the activity against Gram-negatives was retained. Furthermore, *O*-substitution of oxime moiety by methyl or benzyl

**Table 2.** Spectral data of compounds **4-7**.

| Compd | $^1$H NMR (500 MHz, $CDCl_3$); δ ppm | IR (KBr, $cm^{-1}$) |
|---|---|---|
| **4a** | 1.17-1.22 (m, 4H, cyclopropyl), 2.76-2.78 (m, 4H, piperazine), 3.36-3.41 (m, 4H, piperazine), 3.74 (s, 2H, $COCH_2$), 3.84-3.85 (m, 1H, cyclopropyl), 7.30 (d, 1H, thiophene, *J*= 4.1 Hz ), 7.61(d, 1H, $H_8$, *J*= 7.4 Hz), 7.89 (d, 1H, thiophene, *J*= 4.2 Hz), 7.95 (d, 1H, $H_5$, *J*= 10.3 Hz), 8.67 (s, 1H, $H_2$), 15.20 (s, 1H, COOH). | 1624 and 1731 (C=O) |
| **4b** | 1.42 (t, 3H, $CH_3$, *J*= 6.6 Hz), 2.72-2.79 (m, 4H, piperazine), 3.39-3.42 (m, 4H, piperazine), 3.73 (s, 2H, $COCH_2$), 4.60 (q, 2H, $CH_2$-$CH_3$, *J*= 6 Hz), 7.23 (d, 1H, $H_8$, *J*= 6.1 Hz), 7.30 (d, 1H, thiophene, *J*= 3.9 Hz), 7.93 (d, 1H, $H_5$, *J*= 6.5 Hz), 7.94 (d, 1H, thiophene, *J*= 3.9 Hz), 8.96 (s, 1H, $H_2$), 15.32 (s, 1H, COOH). | 1619, 1680 and 1730 (C=O) |
| **4c** | 1.39 (t, 3H, $CH_3$, *J*= 6.9 Hz), 2.72-2.73 (m, 4H, piperazine), 3.73 (s, 2H, $COCH_2$), 3.88-3.90 (m, 4H, piperazine), 4.50 (q, 2H, $CH_2$-$CH_3$, *J*= 6.7 Hz), 7.30 (d, 1H, thiophene, *J*= 4.1 Hz), 7.94 (d, 1H, thiophene, *J*= 4.1 Hz), 8.10 (d, 1H, $H_5$, *J*= 13.4 Hz), 9.00 (s, 1H, $H_2$), 15.23 (s, 1H, COOH). | 1629 and 1716 (C=O) |
| **5a** | 1.17-1.32 (m, 4H, cyclopropyl), 2.66-2.74 (m, 4H, piperazine), 2.86-3.12 (m, 4H, piperazine), 3.54 (s, 2H, N=$CCH_2$), 3.75-3.85 (m, 1H, cyclopropyl), 7.20 (d, 1H, thiophene, *J*=3.8 Hz), 7.56 (d, 1H, $H_8$, *J*= 7.4 Hz), 7.71 (d, 1H, thiophene, *J*= 3.8 Hz), 7.90 (d, 1H, $H_5$, *J*= 13.1 Hz), 8.65 (s, 1H, $H_2$), 12.24 (s, 1H, NOH), 15.17 (s, 1H, COOH). | 1632, 1724 (C=O), 3218 (NOH) |
| **5b** | 1.40 (t, 3H, $CH_3$, *J*= 6.9 Hz), 2.62-2.88 (m, 4H, piperazine), 3.27-3.30 (m, 4H, piperazine), 3.54 (s, 2H, N=$CCH_2$), 4.58 (q, 2H, $CH_2$-$CH_3$, *J*= 6.7 Hz), 7.18 (s, 1H, $H_8$), 7.20 (d, 1H, thiophene, *J*= 4.2 Hz), 7.71 (d, 1H, thiophene, *J*= 4.1 Hz), 7.92 (d, 1H, $H_5$, *J*= 13.1 Hz), 8.95 (s, 1H, $H_2$), 12.25 (s, 1H, NOH), 15.34 (s, 1H, COOH). | 1634, 1725 (C=O), 3235 (NOH) |
| **5c** | 1.38 (t, 3H, $CH_3$, *J*= 6.7 Hz), 2.57-2.65 (m, 4H, piperazine), 3.51 (s, 2H, N=$CCH_2$), 3.75-3.86 (m, 4H, piperazine), 4.47-4.52 (m, 2H, $CH_2$-$CH_3$), 7.20 (d, 1H, thiophene, *J*= 3.8 Hz), 7.70 (d, 1H, thiophene, *J*= 3.7 Hz), 8.08 (d, 1H, $H_5$, *J*= 13.4 Hz), 8.97 (s, 1H, $H_2$), 12.25 (s, 1H, NOH), 15.32 (s, 1H, COOH). | 1630, 1717 (C=O), 3197 (NOH) |
| **6a** | Mixture of *E* and *Z* isomer *E*/*Z* : 34/67, 1.16-1.31 (m, 4H, cyclopropyl), 2.61-2.72 (m, 4H, piperazine), 3.54 and 3.67 (2s, 2H, $CH_2$- *E* isomer and $CH_2$- *Z* isomer respectively), 3.90-4.01 (m, 4H, piperazine), 4.02 (s, 3H, $NOCH_3$), 4.04 (m, 1H, cyclopropyl), 7.12 (d, thiophene-*Z* isomer, *J*= 2.5 Hz), 7.23 (d, thiophen-*E* isomer, *J*= 2.5 Hz), 7.47 (d, 1H, $H_8$, *J*= 7.4 Hz), 7.56 (s, thiophene–*Z* isomer), 7.78 (s, thiophene-*E* isomer), 7.90 (d, 1H, $H_5$, *J*= 12.7 Hz), 8.66 (s, 1H, $H_2$), 15.18 (s, 1H, COOH). | 1626, 1733 (C=O) |
| **6b** | Mixture of *E* and *Z* isomer *E*/*Z* : 75/25; 1.41 (t, 3H, $CH_3$, *J*= 7.0 Hz), 2.64-2.71 (m, 4H, piperazine), 3.30-3.31 (m, 4H, piperazine), 3.54 and 3.67 (2s, 2H, $CH_2$- *E* isomer and $CH_2$-*Z* isomer respectively), 3.90 and 4.01 (2s, 3H, $NOCH_3$-*Z* isomer and $NOCH_3$-*E* isomer respectively), 4.58 (q, 2H, $CH_2$-$CH_3$, *J*= 7.0 Hz), 7.11 (d, thiophene- Z isomer, *J*= 3.9 Hz), 7.19 (d, 1H, $H_8$, *J*= 7.21 Hz), 7.23 (d, thiophene- *E* isomer, *J*= 4.1 Hz), 7.47 (d, thiophene-*Z* isomer, *J*= 4.0 Hz), 7.77 (d, thiophene-*E* isomer, *J*= 4.2 Hz), 7.92 (d, 1H, $H_5$, *J*= 13.1 Hz), 8.94 (s, 1H, $H_2$), 15.31 (s, 1H, COOH). | 1629, 1746 (C=O) |
| **6c** | Mixture of *E* and *Z* isomer *E*/*Z*: 37/63; 1.38 (t, 3H, $CH_3$, *J*= 6.9 Hz), 2.58-2.65 (m, 4H, piperazine), 3.51 and 3.67 (2s, 2H, $CH_2$-*E* isomer and $CH_2$-*Z* isomer respectively), 3.78-3.81 (m, 4H, piperazine), 3.88 and 3.99 (2s, 3H, $NOCH_3$-*Z* isomer and $NOCH_3$-*E* isomer respectively), 4.49 (q, 2H, $CH_2$-$CH_3$, *J*= 6.9 Hz), 7.12 (d, thiophene–*Z* isomer, *J*= 3.9 Hz), 7.24 (d, thiophene–*E* isomer, *J*= 4.2 Hz), 7.48 (d, 1H, thiophene–*Z* isomer, *J*= 4.0 Hz), 7.77 (d, thiophene–*E* isomer, *J*= 4.2 Hz), 8.08 (d, 1H, $H_5$, *J*= 13.4 Hz), 8.98 (s, 1H, $H_2$), 15.29 (s, 1H, COOH). | 1635, 1714 (C=O) |
| **7a** | Mixture of *E* and *Z* isomer *E*/*Z*: 29/71; 1.13-1.30 (m, 4H, cyclopropyl), 2.62-2.64 (m, 4H, piperazine), 3.21-3.39 (m, 4H, piperazine), 3.53 and 3.70 (2s, 2H, $CH_2$-*E* isomer and $CH_2$-*Z* isomer respectively), 3.75 (m, 1H, cyclopropyl), 5.18 and 5.31 (2s, 2H, $NOCH_2$ –*Z* isomer and NO-$CH_2$-*E* isomer respectively), 7.11 (d, 1H, thiophene-*Z* isomer, *J*= 4.0 Hz), 7.24 (d, 1H, thiophene-*E* isomer, *J*= 4.1 Hz), 7.33-7.43 (m, 5H, phenyl), 7.46 (d, thiophene-*Z* isomer, *J*= 4.0 Hz), 7.52 (d, 1H, $H_8$, *J*= 6.8 Hz), 7.77 (d, thiophene-*E* isomer, *J*= 4.2 Hz), 7.87 (d, 1H, $H_5$, *J*= 13.1 Hz), 8.62 (s, 1H, $H_2$), 15.21 (s, 1H, COOH). | 1621, 1712 (C=O) |
| **7b** | Mixture of *E* and *Z* isomer *E*/*Z*: 30/70; 1.40 (t, 3H, $CH_3$, *J*= 6.7 Hz), 2.62-2.63 (m, 4H, piperazine), 3.17-3.31 (m, 4H, piperazine), 3.53 and 3.70 (2s, 2H, $CH_2$-*E* isomer and $CH_2$-*Z* isomer respectively), 4.57 (q, 2H, $CH_2$-$CH_3$, *J*= 4.5 Hz), 5.18 and 5.30 (2s, 2H, $NOCH_2$ –*Z* isomer and $NOCH_2$ –*E* isomer respectively), 7.11 (d, thiophene-*Z* isomer, *J*= 4.0 Hz), 7.17 (d, 1H, $H_8$, *J*= 7.17 Hz), 7.24 (d, thiophene-*E* isomer, *J*= 4.2 Hz), 7.32-7.43 (m, 5H, phenyl), 7.46 (d, thiophene-*Z* isomer, *J*= 4.0 Hz), 7.76 (d, thiophene-*E* isomer, *J*= 4.2 Hz), 7.92 (d, 1H, $H_5$, *J*= 12.8 Hz), 8.95 (s, 1H, $H_2$), 15.34 (s, 1H, COOH). | 1624, 1721 (C=O) |
| **7c** | Mixture of *E* and *Z* isomer *E*/*Z*: 78/22; 1.38 (t, 3H, $CH_3$, *J*= 2.7 Hz), 2.54-2.59 (m, 4H, piperazine), 3.50 and 3.66 (2s, 2H, $CH_2$-*E* isomer and $CH_2$-*Z* isomer respectively), 3.77-3.79 (m, 4H, piperazine), 4.49 (q, 2H, $CH_2$-$CH_3$, *J*= 6.9 Hz), 5.16 and 5.29 (2s, 2H, $NOCH_2$-*Z* isomer and $NOCH_2$-*E* isomer respectively), 7.11 (d, thiophene-*Z* isomer, *J*= 4.0 Hz), 7.23 (d, thiophene-*E* isomer, *J*= 4.2 Hz), 7.32-7.41 (m, 5H, phenyl), 7.46 (d, thiophene-*Z* isomer, *J*= 4.2 Hz), 7.76 (d, thiophene-*E* isomer, *J*= 4.2 Hz), 8.09 (d, 1H, $H_8$, *J*= 13.5 Hz), 8.98 (s, 1H, $H_2$), 15.32 (s, 1H, COOH). | 1641, 1722 (C=O) |

**Table 3.** *In vitro* antibacterial activities of compounds **4-7** against selected strains[a] (MICs in μg /mL)

| Compd | *S. a.* | *S. e.* | *E. f.* | *B. s.* | *E. c.* | *K. p.* | *S. t.* | *S. f.* | *S. m.* | *P. a.* |
|---|---|---|---|---|---|---|---|---|---|---|
| **4a** | 0.5 | 1 | 2 | 0.125 | 0.125 | 0.25 | 0.125 | 0.125 | 0.5 | >4 |
| **4b** | 2 | 2 | 4 | 0.5 | 0.25 | 1 | 0.25 | 0.25 | 1 | >4 |
| **4c** | 2 | >4 | >4 | 1 | 1 | 2 | 0.5 | 0.5 | 2 | >4 |
| **5a** | 0.06 | 0.125 | 0.5 | 0.125 | 0.25 | 2 | 0.25 | 0.25 | >4 | >4 |
| **5b** | 0.125 | 1 | 4 | 0.25 | >4 | >4 | 2 | 2 | >4 | >4 |
| **5c** | 0.5 | 1 | >4 | 0.25 | >4 | >4 | >4 | >4 | >4 | >4 |
| **6a** | 0.5 | 1 | 4 | 1 | 2 | >4 | 1 | 2 | >4 | >4 |
| **6b** | >4 | >4 | >4 | 1 | >4 | >4 | >4 | >4 | >4 | >4 |
| **6c** | >4 | >4 | >4 | 1 | >4 | >4 | >4 | >4 | >4 | >4 |
| **7a** | >4 | >4 | >4 | >4 | 1 | >4 | 1 | 1 | >4 | >4 |
| **7b** | >4 | >4 | >4 | >4 | >4 | >4 | >4 | >4 | >4 | >4 |
| **7c** | >4 | >4 | >4 | >4 | >4 | >4 | >4 | >4 | >4 | >4 |
| **Cip**[b] | 0.5 | 0.5 | 0.5 | 0.5 | 0.025 | 0.06 | 0.125 | 0.125 | 0.25 | 2 |

[a] *S. a.*: *Staphylococcus aureus* ATCC 25923, *S. e.*: *Staphylococcus epidermidis* ATCC 14940, *E. f.*: *Enterococcus feacalis* NCTC 6013, *B. s.*: *Bacillus subtilis* ATCC 6051, *E. c.*: *Escherichia coli* ATCC 25922, *K. p.*: *Klebsiella pneumoniae* ATCC 10031, *S. t.*: *Salmonella typhi* ATCC 19430, *S. f.*: *Shigella flexneri* NCTC 8516, *S. m.*: *Serratia marcescens* PTCC 1111, *P. a.*: *Pseudomonas aeruginosa* ATCC 27853.

[b] Cip: ciprofloxacin

did not improve activity against both Gram-positive and Gram-negative bacteria. Notably, *O*-methyl oxime derivative **6a** (ciprofloxacin analog) showed some activities at concentrations ≤ 4 μg/mL. These data suggested that in *N*-[2-(5-chlorothiophen-2-yl)ethyl]piperazinyl quinolone series, differences in the moiety present at N-1 position of quinolone ring or at ethyl residue attached to piperazine ring markedly influence microbiological properties and the effect of changes in the side chain of the 7-piperazinyl ring mainly depend on substituent at N-1 position.

In conclusion, some of the new *N*-[2-(5-chlorothiophen-2-yl)ethyl]piperazinyl quinolones showed respectable antibacterial activity and modification of N-1 substituent on quinolone ring and side chain of the 7-piperazinyl ring produced remarkable changes in activity.

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## REFERENCES

1. Saubolle MA. Antimicrobial resistance: current status and future direction. Am. J. Rhinol. 2006; 20(6): 667-671.
2. Hooper DC. Fluoroquinolone resistance among Gram-positive cocci. Lancet Infect. *Dis.* 2002; 2: 530-538.
3. Emami S, Shafiee A, Foroumadi A. Structural features of new Quinolones and relationship to antibacterial activity against Gram-positive bacteria. Mini-Rev. Med. Chem. 2006; 6: 375-386.
4. Anderson, VE, Osherhoff N. Type II topoisomerases as targets for quinolone antibacterials: turning Dr. Jekyll into Mr. Hyde. Curr. Pharm. Des. 2001; 7: 337-353.
5. Hooper DC. Mechanisms of action of antimicrobial: focus on fluoroquinolones. Clin. Infect. Dis. 2001; 32(suppl 1): S9-S15.
6. Shen LL, Baranowski J, Pernet AG. Mechanism of inhibition of DNA gyrase by quinolone antibacterials: specificity and cooperativity of drug binding to DNA. Biochemistry, 1989; 28: 3879-3885.
7. Klopman G, Macina OT, Levinson ME, Rosenkranz HS. Computer automated structure evaluation of quinolone antibacterial agents. Antimicrob. Agents Chemother. 1987; 31: 1831-1840.

8. Foroumadi A, Firoozpour L, Emami S, Mansouri S, Ebrahimabadi AH, Asadipour A, Amini M, Saeid-Adeli N, Shafiee A. Synthesis and antibacterial activity of *N*-[5-(chlorobenzylthio)-1,3,4-thiadiazol-2-yl] piperazinyl quinolone derivatives., Arch. Pharm. Res. 2007; 30 (2): 138-145.
9. Foroumadi A, Mansouri S, Emami S, Mirzaei J, Sorkhi M, Saeid-Adeli N, Shafiee A. Synthesis and Antibacterial Activity of Nitroaryl Thiadiazole-Levofloxacin Hybrids., Arch. Pharm. Chem. Life Sci. 2006; 339: 621–624.
10. Foroumadi A, Mohammadhosseini N, Emami S, Letafat B, Faramarzi MA, Samadi N, Shafiee A. Synthesis and antibacterial activity of new 7-piperazinyl-quinolones containing a functionalized 2-(furan-3-yl)ethyl. Arch. Pharm. Chem. Life Sci. 2007; 340: 47-52.
11. Foroumadi A, Emami S, Mansouri S, Javidnia A, Saeid-Adeli N, Shirazi FH, Shafiee A. Synthesis and antibacterial activity of levofloxacin derivatives with certain bulky residues on piperazine rings. Eur. J. Med. Chem. 2007; 42: 985-992.
12. Fang KC, Chen YL, Sheu JY, Wang TC, Tzeng CC. Synthesis, antibacterial, and cytotoxic evaluation of certain 7-substituted norfloxacin derivatives, J. Med. Chem. 2000; 43 (20): 3809-3812.
13. Foroumadi A, Emami S, Mehni M, Moshafi M, Shafiee A. Synthesis and antibacterial activity of ***N***-[2-(5-bromothiophen-2-yl)-2-oxoethyl] and *N*-[(2-(5-bromo thiophen-2-yl)-2-Oximinoethyl] derivatives of piperazinyl quinolones. Bioorg. Med. Chem. Lett. 2005; 15: 4536-4539.
14. Baron EJ, Finegold SM. Bailey Scott's Diagnostic Microbiology, 11th edition, The C. V. Mosby Company: St. Louis. 2002; 235-236.
15. Kreuz KL, Fishkill NY, Hurd CD**.** Nitrothiophenyl ketones, 1953; US patent 2642440.
16. Buu-Hoi NH. Thiophen series III. Indoles, naphthindoles, pyrrocolines, and pyrimidazoles derived from the thiophene nucleus, Rec. Trav. Chim. 1949; 68: 441-472.
17. Foroumadi A, Oboudiat M, Emami S, Karimollah A, Saghaee L, Moshafi MH, Shafiee A. Synthesis and antibacterial activity of *N*-[2-[5-(methylthio)thiophen-2-yl]-2-oxoethyl] and *N*-[2-[5-(methylthio)thio phen-2-yl]-2-(oxyimino)ethyl] piperazinylquinolone derivatives. Bioorg. Med. Chem. 2006; 14: 3421-3427.