The emergence of antibiotic-resistant Gram-positive bacterial infections, notably with *Staphylococcus aureus* and *Streptococcus pneumoniae*, has prompted development of new chemotherapeutic agents that selectively attack new bacterial targets.1) Quinolones represent an extremely successful family of antibacterial drugs that are active against a wide range of multiresistant pathogens since their mode of action is against different molecular targets than other antimicrobial classes.2) Quinolone antibacterial agents have been known to inhibit DNA gyrase and topoisomerase IV, bacterial topoisomerase II enzymes.3) DNA topoisomerase II enzyme is an essential cellular enzyme that catalyzes the double strand breakage of DNA to allow strand passage and thereby control the topology and conformation of DNA.4)

Following the discovery of the therapeutic effects of nalidixic acid in the 1970s, medicinal chemists began to probe every position within the quinolone nucleus in an attempt to improve potency, broaden the spectrum of antibacterial activity, and reduce recognized side effects. To date, several thousand related compounds have been synthesized. In the 1980s, the addition both of a fluorine atom at the 6-position and a piperazine substitution at the 7-position of the basic quinolone structure was found to enhance quinolone antibacterial activity, gaining effectiveness against such organisms as *Pseudomonas aeruginosa* and Gram-positive cocci, and to increase the extent of oral drug absorption and tissue distribution.5,6) Norfloxacin 1 (patented in 1978) was the first compound to combine a piperazinyl side chain in position 7 and a fluoro group in position 6. Although the addition of a piperazinyl ring at the 7-position resulted in increased activity against bacteria such as *P. aeruginosa* and Gram-positive cocci, norfloxacin continued to suffer from poor bioavailability.7) The addition of a cyclopropyl ring at the N-1 position led to the development of ciprofloxacin 2. While the spectra and antibacterial activities of quinolones such as norfloxacin 1 and ciprofloxacin 2 have been improved to include most Gram-negative bacteria, their activities against Gram-positive bacteria remained limited.8) The medicinal chemists have synthesized a large number of norfloxacin and ciprofloxacin analogues. With these compounds, there seems to be an inverse relationship between a compound’s Gram-positive and Gram-negative activity such that enhanced activity against one often accompanies reduced activity against the other. Another drawback to increased activity is the potential for increased host toxicity. For example, several broad-spectrum quinolones approved for use in human medicine, such as temafloxacin and grepafloxacin, have been voluntarily withdrawn from clinical use as a result of emerging safety concerns.8)
It is known that at least two factors determine the potency of quinolones against bacteria: the transport of the drug into the cells and the inhibition of the target enzyme, DNA gyrase or topoisomerase IV. A substituent on the 7-position would play a key role in both events.9) Recently, we have synthesized N-substituted piperazinyl quinolones 4 differing from norfloxacin 1, ciprofloxacin 2 or enoxacin 3 (Fig. 1) solely by the linkage of various 2-(thiophen-2-yl)ethyl groups to the piperazinyl residue at C-7 of the parent drug and explored the linkage of various 2-(thiophen-2-yl)ethyl groups to the norfloxacin, ciprofloxacin or enoxacin 8a to give exclusively (E)-5d and (E)-5f. However, in the reaction of norfloxacin 1 with α-bromooxime derivatives 8a, (E)- and (Z)-isomers of 5e were isolated in approximately a 65/35 ratio based upon comparison of the NMR integration of corresponding peaks from an aliquot of the principal product mixture. Among oxime ether derivatives 5g–l, O-benzyl-oximes 5j and 5l were isolated as pure (Z)-isomer while all O-methyl oximes 5g–i and O-benzylxime analog of norfloxacin 5k were obtained as a mixture of (E)- and (Z)-isomers, with (Z)-isomers predominating. Physicochemical data of these compounds are shown in Table 1. The stereochemistry of the oxime derivatives 5d–l was elucidated by 1H-NMR spectroscopy. The 1H-NMR chemical shifts of the methylene and thiophene ring protons for the synl—synl— isomers (Table 2). Experiences in oximes and oxime ethers suggest that proximity to the oxygen of the oxime in the α-syn configuration will deshield the proton and cause a downfield shift in the signals of related protons.13—15) The protons of thiophene ring which is syn to the oxime moiety shifted downfield in (E)-isomers. However, this anisotropic deshielding effect is largely dependent of the dihedral angle between the oxime oxygen and the thiophene ring, and proximity of oxime oxygen to the H3, H4 and H5 protons of thiophene. In contrast, the protons of methylene which is syn to the oxime moiety shifted downfield in (Z)-isomers (Table 2).

Antibacterial Activity Compounds 5a—l were evaluated for their antibacterial activity by determination of MIC (minimum inhibitory concentration) values using conventional agar-dilution method.16) The results of antibacterial

Table 1. Structures and Physicochemical Data of N-[2-(Thiophen-3-yl)ethyl] Piperazinyl Quinolone Derivatives 5a—l

<table>
<thead>
<tr>
<th>Compd.</th>
<th>X</th>
<th>Y</th>
<th>R</th>
<th>mp (°C)</th>
<th>Yield (%)</th>
<th>Reaction time (d)</th>
</tr>
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<tr>
<td>5a</td>
<td>N</td>
<td>O</td>
<td>Et</td>
<td>170—172</td>
<td>58</td>
<td>2</td>
</tr>
<tr>
<td>5b</td>
<td>CH</td>
<td>O</td>
<td>Et</td>
<td>224—226</td>
<td>52</td>
<td>4</td>
</tr>
<tr>
<td>5c</td>
<td>CH</td>
<td>O</td>
<td>c-Pr</td>
<td>192—194</td>
<td>41</td>
<td>2</td>
</tr>
<tr>
<td>5d (E)</td>
<td>N</td>
<td>NOH</td>
<td>Et</td>
<td>207—210</td>
<td>51</td>
<td>3</td>
</tr>
<tr>
<td>5e (E/Z=65:35)</td>
<td>CH</td>
<td>NOH</td>
<td>Et</td>
<td>217—220</td>
<td>60</td>
<td>2</td>
</tr>
<tr>
<td>5f (E)</td>
<td>CH</td>
<td>NOH</td>
<td>c-Pr</td>
<td>256—258</td>
<td>31</td>
<td>3</td>
</tr>
<tr>
<td>5g (E/Z=17:83)</td>
<td>N</td>
<td>NOCH3</td>
<td>Et</td>
<td>177—178</td>
<td>50</td>
<td>7</td>
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<tr>
<td>5h (E/Z=21:79)</td>
<td>CH</td>
<td>NOCH3</td>
<td>Et</td>
<td>205—207</td>
<td>35</td>
<td>2</td>
</tr>
<tr>
<td>5i (E/Z=9:91)</td>
<td>CH</td>
<td>NOCH3</td>
<td>c-Pr</td>
<td>200—203</td>
<td>36</td>
<td>7</td>
</tr>
<tr>
<td>5j (Z)</td>
<td>N</td>
<td>NOBn</td>
<td>Et</td>
<td>191—193</td>
<td>48</td>
<td>7</td>
</tr>
<tr>
<td>5k (E/Z=20:80)</td>
<td>CH</td>
<td>NOBn</td>
<td>Et</td>
<td>160—162</td>
<td>46</td>
<td>5</td>
</tr>
<tr>
<td>5l (Z)</td>
<td>CH</td>
<td>NOBn</td>
<td>c-Pr</td>
<td>170—171</td>
<td>58</td>
<td>4</td>
</tr>
</tbody>
</table>

a) Recrystallized from EtOH–CHCl3.  b) Yield after recrystallization.
testing of \( N-[2-(thiophen-3-yl)-2-oxoethyl] \) piperazinyl quinolones 5a—c and their oxime derivatives 5d—I against a panel of selected Gram-positive [ \( S. aureus \) ATCC 6538p, \( S. epidermidis \) ATCC 12228, \( B. subtilis \) ATCC 6633], and Gram-negative (Escherichia coli ATCC 8739, Klebsiella pneumoniae ATCC 10031 and Pseudomonas aeruginosa ATCC 9027) bacteria, are reported in Table 3, in comparison with those of the reference drugs norfloxacin 1 and ciprofloxacin 2 against Selected Strains (MICs in \( \mu g/mL \)).

In general, the MIC values of test derivatives indicate that the compounds 5a—I and SI showed significant antibacterial activity, whereas the compounds 5j and 5k exhibited moderate to poor activity against Gram-negative and Gram-positive bacteria.

The novel \( N-[2-(thiophen-3-yl)ethyl] \) piperazinyl quinolones 5a—I demonstrated a high inhibition of all the tested Gram-positive microorganisms and a lot of compounds have MIC values in the range of 0.098—3.13 \( \mu g/mL \). Most of the compounds demonstrated an excellent antimicrobial activity against \( S. aureus \) (MICs 0.098—3.13 \( \mu g/mL \)), \( S. epidermidis \) (MICs 0.098—1.56 \( \mu g/mL \)) and \( B. subtilis \) (MICs 0.049—1.56 \( \mu g/mL \)). It is worth noting that compounds 5a—I are potent also towards clinical isolates of methicillin-resistant \( S. aureus \) (MRSA I and MRSA II) that are inhibited by many compounds at concentrations of 0.19—1.56 \( \mu g/mL \). Furthermore, the data obtained indicate that the antibacterial activity against methicillin-resistant \( S. aureus \) is comparable to that exhibited against the methicillin-susceptible one. Table 3 reveals that compound 5f followed by 5i are superior in inhibiting the growth of staphylococci (MICs 0.098—0.19 \( \mu g/mL \)), while the remaining compounds 5b—e
and 5g–h are equivalent in antibacterial activity against these microorganisms with respectable activity (MICs 0.78—
1.56 µg/ml). More potent compounds 5f and 5i possessed comparable or better activity with respect to the reference
drugs norfloxacin and ciprofloxacin against staphylococci.

Compound 5a–i showed moderate or poor activity, ex-
pressed as minimal inhibitory concentrations (MIC), against
Pseudomonas aeruginosa (MIC>3.13–100 µg/ml). But, whereas they exhibited a strong effectiveness towards other Gram-
negative bacteria (MIC<3.13 µg/ml). More significant inhib-
itory properties were detected for ketone derivative 5c
against Escherichia coli (MIC=0.049 µg/ml) and Klebsiella
pneumoniae (MIC=0.025 µg/ml) as well as towards Pseu-
domonas aeruginosa at the concentration of 3.13 µg/ml. It is
also interesting to note that Gram-positive microorganism
Bacillus subtilis was more susceptible to 5c than to 5f (the
more potent compound against staphylococci).

Considering the varied structure–activity relationships of
different series of compounds, it can be inferred that the an-
bacterial properties of compounds is determined by the com-
bination influence of N-1 substituent (ethyl or cyclopropyl)
and functionality on ethyl linker of thiophene and piperazine.
In accordance to previous antibacterial studies, among ke-
tones, oxime, O-methyl oxime and O-benzyl oxime deriva-
tives of 2-(oxygeniminoethyl) piperazinyl quinolones, lower sus-
ceptibilities (higher MICs) were observed with
trifluoracetylation of ketone to oxime group caused a significant in-

General Procedure for the Synthesis of Compounds 5a–i
A mixture of 2-bromo-1-(thiophen-3-yl)ethanone 7 or 2-bromo-1-(thiophen-3-yl)-
ethanone oxime derivatives 8a–c (0.55 mmol), quinolone 1—3 (0.5 mmol) and NaHCO3 (0.5 mmol) in DMF (5 ml), was stirred at room temperature for 2—7d. After consumption of quinolone 1—3, water (20 ml) was added and the precipitate was filtered, washed with water and crystallized from EtOH—CHCl3 to give compounds 5a–i.

1-Ethyl-6-fluoro-1,4-dihydro-7-[4-(2-thiophen-3-yl)-2-oxoethyl]piper-

alkyl]piperazin-1-yl]-4-oxo-1,8-naphthyridine-3-carboxylic Acid (5a): 1H-NMR
(MDMSO-d6) δ: 1.40 (t, 3H, CH3—, J=7.0 Hz), 2.68—2.72 (m, 4H, piperazine), 3.85 (s, 2H, COCH2), 3.86—3.90 (m, 4H, piperazine), 4.49 (q, 2H, CH2—Me), 7.54 (dd, 1H, H2-thiophene, J=5.0, 1.1 Hz), 7.63 (dd, 1H, H1-thiophene, J=5.0, 2.8 Hz), 8.11 (d, 1H, H1-quinolone, J=13.5 Hz), 8.60 (dd, 1H, H1-thiophene, J=2.8, 1.1 Hz), 8.99 (s, 1H, H2-quinolone), 15.33 (s, 1H, COOH). IR (KBr) cm−1: 1635, 1678, 1713 (C=O-

Kofler hot stage apparatus (C. Reichert, Vienna, Austria) and are uncor-
rected. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (potassium bromide disks; Shimadzu, Tokyo, Japan). 1H-NMR spectra were measured using a Bruker 500 spectrometer (Bruker, Rheinstetten, Ger-
many), and chemical shifts are expressed as δ (ppm) with tetramethylsilane as
internal standard. Elemental analyses were carried out on a CHN rapid ele-
mental analyzer (GmbH-Germany) for C, H and N, and the results are within
±0.4% of the theoretical values.

Experimental

Chemicals and all solvents used in this study were purchased from Merck
AG and Aldrich Chemical. The 2-bromo-1-(thiophen-3-yl)ethanone 7 and 2-
bromo-1-(thiophen-3-yl)ethanone oxime derivatives 8 were prepared accord-

Metabolizing enzymes of (2-oxyiminoethyl) piperazinyl quinolones, lower sus-
ceptibilities (higher MICs) were observed with

Experimental

Chemicals and all solvents used in this study were purchased from Merck
AG and Aldrich Chemical. The 2-bromo-1-(thiophen-3-yl)ethanone 7 and 2-
bromo-1-(thiophen-3-yl)ethanone oxime derivatives 8 were prepared accord-
ing to the literature methods.10—12 Melting points were determined on a
tute of (E)- and (Z)-isomers (E/Z = 17:83). 1H-NMR (DMSO-d6) δ: 1.53 (t, 3H, CH3, J = 7.1 Hz), 2.68—2.75 (m, 4H, piperazine), 3.48 (s, 2H, N=CCH3—, E-isomer), 3.70 (s, 2H, N=CCH3—, Z-isomer), 3.87—3.93 (m, 4H, piperazine), 4.00 (s, 3H, OCH3—, Z-isomer), 4.05 (s, 3H, OCH3—, E-isomer), 4.43 (q, 2H, CH2–Me, J = 7.0 Hz), 7.31—7.35 (m, 1H, H-thiophene, E and Z isomers), 7.56 (d, 1H, H-thiophene, Z-isomer, J = 5.0 Hz), 7.74 (d, 1H, H-thiophene, E-isomer, J = 5.0 Hz), 7.88 (d, 1H, H-thiophene, Z-isomer, J = 2.1 Hz), 8.14 (d, 1H, H-thiophene, J = 13.5 Hz), 8.37 (d, 1H, H-thiophene, E-isomer, J = 2.0 Hz), 8.73 (s, 1H, H-quinoline), 15.09 (s, 1H, COOH). IR (KBr) cm⁻¹: 1629, 1721 (C=O).

1-Ethyl-6-fluoro-1,4-dihydro-7-[(2-thiophen-3-yl)-2-methoxyimino-ethyl]piperazin-1-yl]-4-oxo-3-quinolone Carboxylic Acid (5H): Mixture of (E)- and (Z)-isomers (E/Z = 21:79). 1H-NMR (DMSO-d6) δ: 1.40 (t, 3H, CH3, J = 6.8 Hz), 2.61—2.68 (m, 4H, piperazine), 3.22—3.33 (m, 4H, piperazine), 3.44 (2H, N=CCH3—, E-isomer), 3.66 (s, 2H, N=CCH3—, Z-isomer), 3.90 (s, 3H, OCH3—, E-isomer), 4.59 (t, 2H, CH2–Me, J = 6.6 Hz), 7.17 (d, 1H, H-quinoline, J = 6.6 Hz), 7.44 (d, 1H, H-thiophene, Z-isomer, J = 4.4 Hz), 7.52—7.58 (m, 1H, H-thiophene, E and Z isomers), 7.66 (d, 1H, H-thiophene, E-isomer, J = 4.5 Hz), 7.92 (d, 1H, H-quinoline, J = 13.2 Hz), 8.02 (d, 1H, H-thiophene, Z-isomer, J = 1.9 Hz), 8.36 (d, 1H, H-thiophene, E-isomer, J = 2.0 Hz), 8.95 (s, 1H, H-quinoline), 15.34 (s, 1H, COOH). IR (KBr) cm⁻¹: 1618, 1718 (C=O).

1-Ethyl-6-fluoro-1,4-dihydro-7-[(2-thiophen-3-yl)-2-methoxyimino-ethyl]piperazin-1-yl]-4-oxo-3-quinolone Carboxylic Acid (5I): Mixture of (E)- and (Z)-isomers (E/Z = 9:91). 1H-NMR (DMSO-d6) δ: 1.13—1.20 (m, 2H, cyclopropyl), 1.28—1.33 (m, 2H, cyclopropyl), 2.63—2.71 (m, 4H, piperazine), 3.27—3.33 (m, 4H, piperazine), 3.45 (s, 2H, N=CCH3—, E-isomer), 3.67 (s, 2H, N=CCH3—, Z-isomer), 3.74—3.84 (m, 1H, cyclopro- pylon), 3.91 (s, 3H, OCH3—, Z-isomer), 3.92 (s, 3H, OCH3—, E-isomer), 7.45 (d, 1H, H-thiophene, Z-isomer, J = 5.0 Hz), 7.52—7.55 (m, 1H, H-thiophene, E-isomer, J = 1.27—1.31 (m, 2H, cyclopropyl), 2.61—2.67 (m, 4H, piperazine), 3.26—3.29 (m, 4H, piperazine), 3.71 (s, 2H, N=CCH3—), 3.78—3.83 (m, 1H, cyclopro- pylon), 5.20 (s, 2H, OCH3—), 7.32—7.45 (m, 1H, H-thiophene and 5H, phenyl), 7.53—7.57 (m, 1H, H-thiophene and 1H, H-quinoline), 9.07 (d, 1H, H-quinoline, J = 13.2 Hz), 8.02 (dd, 1H, H-thiophene, J = 2.8, 1.0 Hz), 8.66 (s, 1H, H-quinoline), 15.21 (s, 1H, COOH). IR (KBr) cm⁻¹: 1627, 1733 (C=O).

Antibacterial Activity

Compounds 5a—l were evaluated for their anti- bacterial activity using conventional agar-dilution method. 19 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 μg/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 × 10⁸ CFU/ml). The suspensions were then diluted in 0.85% saline to give 10⁸ CFU/ml. Petri dishes were spot-inoculated with 1 μl of each prepared bacterial suspension (10⁸ 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.

Acknowledgments

This work was supported by a grant from the Research Council of Ministry of Health and Medical Education of Iran.

References