Synthesis and Antibacterial Activity of New *N*-[2-(Thiophen-3-yl)ethyl] Piperazinyl Quinolones

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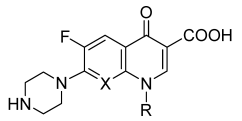
As a part of continuing search for potential antibacterial agents in the quinolones field, we have synthesized novel quinolone agents bearing *N*-[2-(thiophen-3-yl)ethyl] piperazinyl moiety in the 7-position of the quinolone ring. *In vitro* antibacterial evaluation of the target compounds showed that *N*-[2-(thiophen-3-yl)ethyl] group attached to piperazine ring served as promising C-7 substituent for piperazinyl quinolone antibacterials. Among these derivatives, ciprofloxacin analogues, containing *N*-[2-(thiophen-3-yl)-2-hydroxyiminoethyl] or *N*-[2-(thiophen-3-yl)-2-methoxyiminoethyl] residue provided a high inhibition against all the tested Gram-positive organisms including methicillin-resistant *Staphylococcus aureus* comparable or superior with respect to the reference drugs norfloxacin and ciprofloxacin.

Key words synthesis; quinolone; thiophene; oxime; antibacterial activity

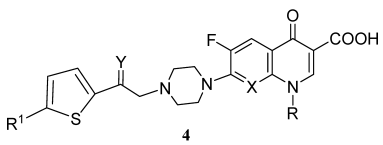
The emergence of antibiotic-resistant Gram-positive bacterial infections, notably with *Staphylococcus aureus* and *Streptococcus pneumoniae*, has prompted development of new chemotherapeutic agents that selectively attack new bacterial targets.[1)]

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)]

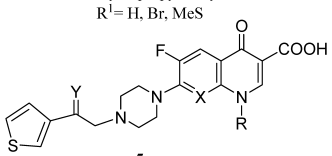
Following the discovery of the therapeutic effects of nalidixic acid in the 1970s, medicinal chemists began to probe every position within the quinolone nucleus in an attempt to improve potency, broaden the spectrum of antibacterial activity, and reduce recognized side effects. To date, several thousand 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.[5)] 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)]



1, Norfloxacin; R= ethyl, X= CH
2, Ciprofloxacin; R= cyclopropyl, X= CH
3, Enoxacin; R= ethyl, X= N



4
X= CH, N
Y= O, NOH, $NOCH_3$, NOBn
R= cyclopropyl, ethyl
R^1= H, Br, MeS



5
X= CH, N
Y= O, NOH, NOMe, NOBn
R= cyclopropyl, ethyl

Fig. 1. Chemical Structures of Piperazinyl Quinolones

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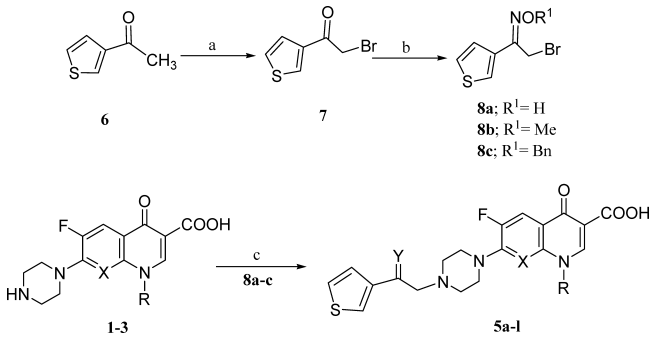
It is known that at least two factors determine the potency of quinolones against bacteria: the transport of the drug into the cells and the inhibition of the target enzyme, DNA gyrase or topoisomerase IV. A substituent on the 7-position would play a key role in both events.[9] Recently, we have synthesized *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 their antibacterial activities.[10—12] By considering the considerable activity of *N*-[2-(thiophen-2-yl)ethyl] piperazinyl quinolone derivatives **4** against Gram-negative and Gram-positive bacteria, a new series of *N*-[2-(thiophen-3-yl)ethyl] derivatives of piperazinyl quinolone (**5**, Fig. 1) were also synthesized and evaluated for antibacterial activity as positional isomers of **4**.

Results and Discussion

Chemistry The *N*-[2-(thiophen-3-yl)ethyl] piperazinyl quinolone analogues **5** were prepared by the synthetic route diagrammed in Fig. 2. The commercially available ketone **6** was brominated with $CuBr_2$ in refluxing $CHCl_3$–AcOEt to give corresponding α-bromoketone **7**.[12] Compound **7** was converted to oxime derivative **8a** by stirring with excess of hydroxylamine hydrochloride in methanol at room temperature. Similarly, the *O*-methyloxime ethers **8b** and *O*-benzyloxime ethers **8c** were synthesized by reaction of compound **7** with methoxyamine hydrochloride and *O*-benzylhydroxylamine hydrochloride, respectively.[10—12] Reaction of 7-piperazinylquinolones (**1**, **2** or **3**) with 3-(bromoacetyl)thiophene **7** or α-bromooxime derivatives **8a**—**c** in DMF, in the presence of $NaHCO_3$ at room temperature afforded corresponding ketones **5a**—**c** and oxime derivatives **5d**—**l**, respectively.[10—12] Accordingly, enoxacin **3** and ciprofloxacin **2** reacted with α-bromooxime **8a** to give exclusively (*E*)-**5d** and (*E*)-**5f**. However, in the reaction of norfloxacin **1** with α-bromooxime **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*-benzyloximes **5j** and **5l** were isolated as pure (*Z*)-isomer while all *O*-methyl oximes **5g**—**i** and *O*-benzyloxime 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 ^{1}H-NMR spectroscopy. The ^{1}H-NMR chemical shifts of the methylene and thiophene ring protons for the *E* and *Z* isomers of oxime derivatives **5d**—**l** have notable deviations (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 H_2, H_4 and H_5 protons of thiophene. In contrast, the protons of methylene which is *syn* to the oxime moiety shifted downfield in (*Z*)-isomers (Table 2).

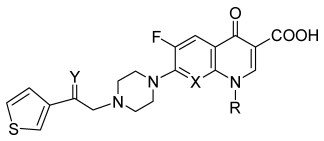


Reagents and conditions: (a) $CuBr_2$, $CHCl_3$–EtOAc, reflux; (b) $HONH_2 \cdot HCl$ or $MeONH_2 \cdot HCl$ or $BnONH_2 \cdot HCl$, MeOH, r.t.; (c) DMF, $NaHCO_3$, r.t.

Fig. 2. Synthesis of *N*-[2-(Thiophen-3-yl)ethyl] Piperazinyl Quinolones **5a**—**l**

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

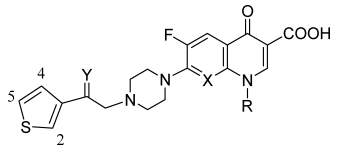
Table 1. Structures and Physicochemical Data of *N*-[2-(Thiophen-3-yl)ethyl] Piperazinyl Quinolone Derivatives **5a**—**l**



Compd.	X	Y	R	mp (°C)[a]	Yield (%)[b]	Reaction time (d)
5a	N	O	Et	170—172	58	2
5b	CH	O	Et	224—226	52	4
5c	CH	O	*c*-Pr	192—194	41	2
5d (*E*)	N	NOH	Et	207—210	51	3
5e (*E*/*Z*=65 : 35)	CH	NOH	Et	217—220	60	2
5f (*E*)	CH	NOH	*c*-Pr	256—258	31	3
5g (*E*/*Z*=17 : 83)	N	$NOCH_3$	Et	177—178	50	7
5h (*E*/*Z*=21 : 79)	CH	$NOCH_3$	Et	205—207	35	2
5i (*E*/*Z*=9 : 91)	CH	$NOCH_3$	*c*-Pr	200—203	36	7
5j (*Z*)	N	NOBn	Et	191—193	48	7
5k (*E*/*Z*=20 : 80)	CH	NOBn	Et	160—162	46	5
5l (*Z*)	CH	NOBn	*c*-Pr	170—171	58	4

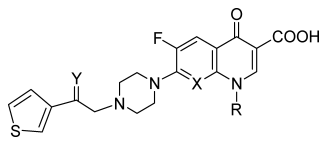
a) Recrystallized from EtOH–$CHCl_3$. *b*) Yield after recrystallization.

Table 2. Selected ^{1}H-NMR Chemical Shifts of Oximes **5d**—**f** and *O*-Substituted Oximes **5g**—**l**



Compd.	X	Y	R	δ CH_2		δ H-2 thiophene		δ H-4 thiophene	
				E	*Z*	*E*	*Z*	*E*	*Z*
5d (*E*)	N	NOH	Et	3.41	—	8.41	—	7.73	—
5e (*E*/*Z*)	CH	NOH	Et	3.45	3.68	8.40—8.44	7.93—7.96	7.73	7.45
5f (*E*)	CH	NOH	*c*-Pr	3.45	—	8.42	—	7.73	—
5g (*E*/*Z*)	N	$NOCH_3$	Et	3.48	3.70	8.37	7.88	7.74	7.56
5h (*E*/*Z*)	CH	$NOCH_3$	Et	3.44	3.66	8.36	8.02	7.66	7.44
5i (*E*/*Z*)	CH	$NOCH_3$	*c*-Pr	3.45	3.67	8.37	8.02	7.67	7.45
5j (*Z*)	N	NOBn	Et	—	3.67	—	8.02	—	7.30—7.45
5k (*E*/*Z*)	CH	NOBn	Et	3.45	3.70	8.39	8.01	7.69	7.31—7.45
5l (*Z*)	CH	NOBn	*c*-Pr	—	3.71	—	8.02	—	7.32—7.45

Table 3. *In Vitro* Antibacterial Activities of Compounds **5a**—**l** in Comparison with Reference Drugs Norfloxacin **1** and Ciprofloxacin **2** against Selected Strains (MICs in μg/ml)



Compd.	X	Y	R	*S. a.*[a)]	MRSA I	MRSA II	*S. e.*	*B. s.*	*E. c.*	*K. p*	*P. a.*
5a	N	O	Et	3.13	1.56	1.56	1.56	0.39	0.39	0.19	25
5b	CH	O	Et	1.56	1.56	1.56	1.56	0.098	0.19	0.098	6.25
5c	CH	O	*c*-Pr	0.78	0.78	0.78	0.39	0.049	0.049	0.025	3.13
5d	N	NOH	Et	0.78	0.78	0.78	0.78	0.098	0.098	0.098	6.25
5e	CH	NOH	Et	0.39	0.78	0.78	0.39	0.78	0.78	0.049	50
5f	CH	NOH	*c*-Pr	0.098	0.19	0.19	0.098	0.78	0.39	0.049	>100
5g	N	$NOCH_3$	Et	1.56	1.56	1.56	1.56	25	12.5	3.13	>100
5h	CH	$NOCH_3$	Et	0.39	0.78	0.78	0.78	1.56	0.78	0.39	50
5i	CH	$NOCH_3$	*c*-Pr	0.19	0.19	0.19	0.19	0.39	0.39	0.19	25
5j	N	NOBn	Et	50	>100	>100	50	50	50	25	>100
5k	CH	NOBn	Et	25	50	50	25	6.25	6.25	3.13	>100
5l	CH	NOBn	*c*-Pr	0.78	3.13	3.13	0.78	0.78	0.39	0.19	50
1, norfloxacin				0.39	0.78	0.78	0.39	0.098	0.049	0.025	3.13
2, ciprofloxacin				0.19	0.39	0.39	0.19	0.025	0.013	0.013	0.39

a) *S. a.*: *Staphylococcus aureus* ATCC 6538p, MRSA I and II: methicillin-resistant *Staphylococcus aureus* (clinical isolates I and II), *S. e.*: *Staphylococcus epidermidis* ATCC 12228, *B. s.*: *Bacillus subtilis* ATCC 6633, *E. c.*: *Escherichia coli* ATCC 8739, *K. p.*: *Klebsiella pneumoniae* ATCC 10031, *P. a.*: *Pseudomonas aeruginosa* ATCC 9027.

testing of *N*-[2-(thiophen-3-yl)-2-oxoethyl] piperazinyl quinolones **5a**—**c** and their oxime derivatives **5d**—**l** against a panel of selected Gram-positive [*Staphylococcus aureus* ATCC 6538p, methicillin-resistant *Staphylococcus aureus* (MRSA I and MRSA II, clinical isolates), *Staphylococcus epidermidis* ATCC 12228, *Bacillus 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 and ciprofloxacin.

In general, the MIC values of test derivatives indicate that the compounds **5a**—**i** and **5l** 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 μg/ml. Most of the compounds demonstrated an excellent antimicrobial activity against *Staphylococcus aureus* (MICs 0.098—3.13 μg/ml), *Staphylococcus epidermidis* (MICs 0.098—1.56 μg/ml) and *Bacillus subtilis* (MICs 0.049—1.56 μg/ml). It is worth noting that compounds **5a**—**i** are potent also towards clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA I and MRSA II) that are inhibited by many compounds at concentrations of 0.19—1.56 μg/ml. Furthermore, the data obtained indicate that the antibacterial activity against methicillin-resistant *Staphylococcus 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 μg/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, expressed as minimal inhibitory concentrations (MIC), against *Pseudomonas aeruginosa* (MIC>3.13—100 μg/ml), whereas they exhibited a strong effectiveness towards other Gram-negative bacteria (MIC<3.13 μg/ml). More significant inhibitory 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 *Pseudomonas 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 antibacterial properties of compounds is determined by the combination influence of N-1 substituent (ethyl or cyclopropyl) and functionality on ethyl linker of thiophene and piperazine. In accordance to previous antibacterial studies, among ketones, oxime, *O*-methyl oxime and *O*-benzyl oxime derivatives of (2-oxyiminoethyl) piperazinyl quinolones, lower susceptibilities (higher MICs) were observed with *O*-benzyl oxime derivatives. Thus, the *O*-benzyl oxime moiety diminished the activity against both Gram-positive and Gram-negative bacteria. As we can see, the most potent compound against staphylococci (compound **5f**) belongs to the oxime series, whereas the most potent compound against Gram-negativies (compound **5c**) belongs to the ketone series. Thus, the type of functionality on ethyl spacer has a different influence on antibacterial profile against Gram-positives and Gram-negatives. Comparison between MIC values of ketone analogs **5a**—**c** and their oxime counterpart revealed that alteration of ketone to oxime group caused a significant increase in antibacterial activity against staphylococci only in ciprofloxacin series (**5f** *vs.* **5c**). In addition, *O*-methylation or *O*-benzylation of oxime derivatives could not improved the antibacterial activity. However, in some cases, the presence or introduction of various functional groups in a compound does not allow to accurately explain the kind and intensity of its antibacterial activity.

In conclusion, we have synthesized novel quinolone agents bearing *N*-[2-(thiophen-3-yl)ethyl] piperazinyl moiety in the 7-position of the quinolone ring. *In vitro* antibacterial evaluation of target compounds showed that *N*-[2-(thiophen-3-yl)ethyl] group attached to piperazine ring served as promising C-7 substituents for piperazinyl quinolones. Among these derivatives, ciprofloxacin derivatives, containing *N*-[2-(thiophen-3-yl)-2-hydroxyimino ethyl] or *N*-[2-(thiophen-3-yl)-2-methoxyimino ethyl] residue provided a high inhibition of all the tested Gram-positive organisms including MRSA comparable or superior with respect to the reference drugs norfloxacin and ciprofloxacin.

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 according to the literature methods.[10—12] Melting points were determined on a Kofler hot stage apparatus (C. Reichert, Vienna, Austria) and are uncorrected. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (potassium bromide disks; Shimadzu, Tokyo, Japan). ^{1}H-NMR spectra were measured using a Bruker 500 spectrometer (Bruker, Rheinstetten, Germany), 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 $\pm$0.4% of the theoretical values.

General Procedure for the Synthesis of Compounds 5a—l 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 $NaHCO_3$ (0.5 mmol) in DMF (5 ml), was stirred at room temperature for 2—7 d. 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 **5a**—**l**.

1-Ethyl-6-fluoro-1,4-dihydro-7-[4-[2-(thiophen-3-yl)-2-oxoethyl]piperazin-1-yl]-4-oxo-1,8-naphthyridine-3-carboxylic Acid (**5a**): ^{1}H-NMR (DMSO-d_6) δ: 1.40 (t, 3H, CH_3, J=7.0 Hz), 2.68—2.76 (m, 4H, piperazine), 3.85 (s, 2H, $COCH_2$), 3.86—3.90 (m, 4H, piperazine), 4.49 (q, 2H, CH_2-Me), 7.54 (dd, 1H, H_4-thiophene, J=5.0, 1.1 Hz), 7.63 (dd, 1H, H_5-thiophene, J=5.0, 2.8 Hz), 8.11 (d, 1H, H_5-quinolone, J=13.5 Hz), 8.60 (dd, 1H, H_2-thiophene, J=2.8, 1.1 Hz), 8.99 (s, 1H, H_2-quinolone), 15.33 (s, 1H, COOH). IR (KBr) cm^{-1}: 1635, 1678, 1713 (C=O).

1-Ethyl-6-fluoro-1,4-dihydro-7-[4-[2-(thiophen-3-yl)-2-oxoethyl]piperazin-1-yl]-4-oxo-3-quinolone Carboxylic Acid (**5b**): ^{1}H-NMR (DMSO-d_6) δ: 1.42 (t, 3H, CH_3, J=7.0 Hz), 2.72—2.80 (m, 4H, piperazine), 3.32—3.40 (m, 4H, piperazine), 3.85 (s, 2H, $COCH_2$), 4.60 (q, 2H, CH_2-Me, J=7.0 Hz), 7.20 (d, 1H, H_8-quinolone, J=7.2 Hz), 7.56 (dd, 1H, H_4-thiophene, J=5.0, 1.0 Hz), 7.64 (dd, 1H, H_5-thiophene, J=5.0, 2.8 Hz), 7.92 (d, 1H, H_5-quinolone, J=13.3 Hz), 8.62 (dd, 1H, H_2-thiophene, J=2.7, 1.0 Hz), 8.96 (s, 1H, H_2-quinolone), 15.36 (s, 1H, COOH). IR (KBr) cm^{-1}: 1624, 1682, 1718 (C=O).

1-Cyclopropyl-6-fluoro-1,4-dihydro-7-[4-[2-(thiophen-3-yl)-2-oxoethyl]-piperazin-1-yl]-4-oxo-3-quinolone Carboxylic Acid (**5c**): ^{1}H-NMR (DMSO-d_6) δ: 1.14—1.24 (m, 2H, cyclopropyl), 1.28—1.40 (m, 2H, cyclopropyl), 2.74—2.82 (m, 4H, piperazine), 3.35—3.40 (m, 4H, piperazine), 3.80 (m, 1H, cyclopropyl), 3.86 (s, 2H, $COCH_2$), 7.56 (d, 1H, H_4-thiophene, J=4.9 Hz), 7.59 (d, 1H, H_8-quinolone, J=5.6 Hz), 7.62—7.67 (m, 1H, H_5-thiophene), 7.91 (d, 1H, H_5-quinolone, J=13.2 Hz), 8.62 (m, 1H, H_2-thiophene), 8.67 (s, 1H, H_2-quinolone), 15.21 (s, 1H, COOH). IR (KBr) cm^{-1}: 1618, 1679, 1731 (C=O).

1-Ethyl-6-fluoro-1,4-dihydro-7-[4-[2-(thiophen-3-yl)-2-hydroxyiminoethyl]piperazin-1-yl]-4-oxo-1,8-naphthyridine-3-carboxylic Acid (**5d**): (*E*)-isomer. ^{1}H-NMR (DMSO-d_6) δ: 1.38 (t, 3H, CH_3, J=6.8 Hz), 2.58—2.63 (m, 4H, piperazine), 3.41 (s, 2H, –N=CCH_2–), 3.77—3.84 (m, 4H, piperazine), 4.49 (q, 2H, CH_2-Me, J=7.0 Hz), 7.54 (dd, 1H, H_5-thiophene, J=5.0, 3.0 Hz), 7.73 (dd, 1H, H_4-thiophene, J=5.0, 1.0 Hz), 8.09 (d, 1H, H_5-quinolone, J=13.5 Hz), 8.41 (dd, 1H, H_2-thiophene, J=2.9, 1.0 Hz), 8.98 (s, 1H, H_2-quinolone), 11.42 (s, 1H, NOH), 15.32 (s, 1H, COOH). IR (KBr) cm^{-1}: 1634, 1720 (C=O).

1-Ethyl-6-fluoro-1,4-dihydro-7-[4-[2-(thiophen-3-yl)-2-hydroxyiminoethyl]piperazin-1-yl]-4-oxo-3-quinolone Carboxylic Acid (**5e**): Mixture of (*E*)- and (*Z*)-isomers (*E*/*Z*=65 : 35). ^{1}H-NMR (DMSO-d_6) δ: 1.40 (t, 3H, CH_3, J=6.9 Hz), 2.59—2.71 (m, 4H, piperazine), 3.25—3.33 (m, 4H, piperazine), 3.45 (s, 2H, –N=CCH_2–, *E*-isomer), 3.68 (s, 2H, –N=CCH_2–, *Z*-isomer), 4.58 (q, 2H, CH_2-Me, J=7.0 Hz), 7.19 (d, 1H, H_8-quinolone, J=6.8 Hz), 7.45 (d, 1H, H_4-thiophene, *Z*-isomer, J=5.0 Hz), 7.50—7.55 (m, 1H, H_5-thiophene, *Z* and *E* isomers), 7.73 (d, 1H, H_4-thiophene, *E*-isomer, J=5.1 Hz), 7.92 (d, 1H, H_5-quinolone, J=13.6 Hz), 7.93—7.96 (m, 1H, H_2-thiophene, *Z*-isomer), 8.40—8.44 (m, 1H, H_2-thiophene, *E*-isomer), 8.95 (s, 1H, H_2-quinolone), 11.27 (s, 1H, NOH, *Z*-isomer), 11.40 (s, 1H, NOH, *E*-isomer), 15.35 (s, 1H, COOH). IR (KBr) cm^{-1}: 1625, 1714 (C=O).

1-Cyclopropyl-6-fluoro-1,4-dihydro-7-[4-[2-(thiophen-3-yl)-2-hydroxyiminoethyl]piperazin-1-yl]-4-oxo-3-quinolone Carboxylic Acid (**5f**): (*E*)-isomer. ^{1}H-NMR (DMSO-d_6) δ: 1.15—1.19 (m, 2H, cyclopropyl), 1.27—1.32 (m, 2H, cyclopropyl), 2.62—2.70 (m, 4H, piperazine), 3.25—3.33 (m, 2H, piperazine), 3.45 (s, 2H, –N=CCH_2–), 3.78—3.83 (m, 1H, cyclopropyl), 7.53 (dd, 1H, H_5-thiophene, J=5.0, 3.0 Hz), 7.57 (d, 1H, H_8-quinolone, J=7.36 Hz), 7.73 (d, 1H, H_4-thiophene, J=5.0 Hz), 7.91 (d, 1H, H_5-quinolone, J=13.2 Hz), 8.42 (d ,1H, H_2-thiophene, J=2.9 Hz), 8.66 (s, 1H, H_2-quinolone), 11.43 (s, 1H, NOH), 15.23 (s, 1H, COOH). IR (KBr) cm^{-1}: 1618, 1731 (C=O).

1-Ethyl-6-fluoro-1,4-dihydro-7-[4-[2-(thiophen-3-yl)-2-methoxyiminoethyl]piperazin-1-yl]-4-oxo-1,8-naphthyridine-3-carboxylic Acid (**5g**): Mix-

ture of (*E*)- and (*Z*)-isomers (*E*/*Z*=17 : 83). ^{1}H-NMR (DMSO-d_6) δ: 1.53 (t, 3H, CH_3, *J*=7.1 Hz), 2.68—2.75 (m, 4H, piperazine), 3.48 (s, 2H, N=CCH_2–, *E*-isomer), 3.70 (s, 2H, N=CCH_2–, *Z*-isomer), 3.87—3.93 (m, 4H, piperazine), 4.00 (s, 3H, OCH_3, *Z*-isomer), 4.05 (s, 3H, OCH_3, *E*-isomer), 4.43 (q, 2H, CH_2-Me, *J*=7.0 Hz), 7.31—7.35 (m, 1H, H_5-thiophene, *E* and *Z* isomers), 7.56 (d, 1H, H_4-thiophene, *Z*-isomer, *J*=5.0 Hz), 7.74 (d, 1H, H_4-thiophene, *E*-isomer, *J*=5.0 Hz), 7.88 (d, 1H, H_2-thiophene, *Z*-isomer, *J*=2.1 Hz), 8.14 (d, 1H, H_5-quinolone, *J*=13.5 Hz), 8.37 (d, 1H, H_2-thiophene, *E*-isomer, *J*=2.0 Hz), 8.73 (s, 1H, H_2-quinolone), 15.09 (s, 1H, COOH). IR (KBr) cm^{-1}: 1629, 1721 (C=O).

1-Ethyl-6-fluoro-1,4-dihydro-7-[4-[2-(thiophen-3-yl)-2-methoxyiminoethyl]piperazin-1-yl]-4-oxo-3-quinolone Carboxylic Acid (**5h**): Mixture of (*E*)- and (*Z*)-isomers (*E*/*Z*=21 : 79). ^{1}H-NMR (DMSO-d_6) δ: 1.40 (t, 3H, CH_3, *J*=6.8 Hz), 2.61—2.68 (m, 4H, piperazine), 3.22—3.33 (m, 4H, piperazine), 3.44 (s, 2H, –N=CCH_2–, *E*-isomer), 3.66 (s, 2H, –N=CCH_2–, *Z*-isomer), 3.90 (s, 3H, OCH_3, *Z*-isomer), 3.91 (s, 3H, OCH_3, *E*-isomer), 4.58 (q, 2H, CH_2-Me, *J*=6.6 Hz), 7.17 (d, 1H, H_8-quinolone, *J*=6.6 Hz), 7.44 (d, 1H, H_4-thiophene, *Z*-isomer, *J*=4.4 Hz), 7.52—7.58 (m, 1H, H_5-thiophene, *E* and *Z* isomers), 7.66 (d, 1H, H_4-thiophene, *E*-isomer, *J*=4.5 Hz), 7.92 (d, 1H, H_5-quinolone, *J*=13.2 Hz), 8.02 (d, 1H, H_2-thiophene, *Z*-isomer, *J*=1.9 Hz), 8.36 (d, 1H, H_2-thiophene, *E*-isomer, *J*=2.0 Hz), 8.95 (s, 1H, H_2-quinolone), 15.34 (s, 1H, COOH). IR (KBr) cm^{-1}: 1618, 1718 (C=O).

1-Cyclopropyl-6-fluoro-1,4-dihydro-7-[4-[2-(thiophen-3-yl)-2-methoxyiminoethyl]piperazin-1-yl]-4-oxo-3-quinolone Carboxylic Acid (**5i**): Mixture of (*E*)- and (*Z*)-isomers (*E*/*Z*=9 : 91). ^{1}H-NMR (DMSO-d_6) 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=CCH_2–, *E*-isomer), 3.67 (s, 2H, –N=CCH_2–, *Z*-isomer), 3.74—3.84 (m, 1H, cyclopropyl), 3.91 (s, 3H, OCH_3, *Z*-isomer), 3.92 (s, 3H, OCH_3, *E*-isomer), 7.45 (d, 1H, H_4-thiophene, *Z*-isomer, *J*=5.0 Hz), 7.52—7.55 (m, 1H, H_5-thiophene), 7.59 (d, 1H, H_8-quinolone, *J*=7.7 Hz), 7.67 (d, 1H, H_4-thiophene, *E*-isomer, *J*=5.0 Hz), 7.90 (d, 1H, H_5-quinolone, *J*=13.2 Hz), 8.02 (d, 1H, H_2-thiophene, *Z*-isomer, *J*=2.1 Hz), 8.37 (d, 1H, H_2-thiophene, *E*-isomer, *J*=2.0 Hz), 8.66 (s, 1H, H_2-quinolone), 15.21 (s, 1H, COOH). IR (KBr) cm^{-1}: 1629, 1731 (C=O).

1-Ethyl-6-fluoro-1,4-dihydro-7-[4-[2-(thiophen-3-yl)-2-(phenylmethoxyimino)ethyl]piperazin-1-yl]-4-oxo-1,8-naphthyridine-3-carboxylic Acid (**5j**): (*Z*)-isomer. ^{1}H-NMR (DMSO-d_6) δ: 1.38 (m, 3H, CH_3, *J*=6.9 Hz), 2.57—2.60 (m, 4H, piperazine), 3.67 (s, 2H, N=CCH_2–), 3.73—3.80 (m, 4H, piperazine), 4.49 (q, 2H, CH_2-Me, *J*=6.6 Hz), 5.18 (s, 2H, CH_2-ph), 7.30—7.45 (m, 1H, H_4-thiophene and 5H, phenyl), 7.54 (dd, 1H, H_5-thiophene, *J*=4.9, 2.9 Hz), 8.02 (d, 1H, H_2-thiophene, *J*=2.7 Hz), 8.10 (d, 1H, H_5-quinolone, *J*=13.5 Hz), 8.98 (s, 1H, H_2-quinolone), 15.33 (s, 1H, COOH). IR (KBr) cm^{-1}: 1627, 1721 (C=O).

1-Ethyl-6-fluoro-1,4-dihydro-7-[4-[2-(thiophen-3-yl)-2-(phenylmethoxyimino)ethyl]piperazin-1-yl]-4-oxo-3-quinolone Carboxylic Acid (**5k**): Mixture of (*E*)- and (*Z*)-isomers (*E*/*Z*=20 : 80). ^{1}H-NMR (DMSO-d_6) δ: 1.40 (t, 3H, CH_3, *J*=6.6 Hz), 2.58—2.67 (m, 4H, piperazine), 3.20—3.30 (m, 4H, piperazine), 3.45 (s, 2H, –N=CCH_2–, *E*-isomer), 3.70 (s, 2H, –N=CCH_2–, *Z*-isomer), 4.57 (q, 2H, CH_2-Me, *J*=5.8 Hz), 5.19 (s, 2H, OCH_2-ph, *Z*-isomer), 5.21 (s, 2H, OCH_2-ph, *E*-isomer), 7.16 (d, 1H, H_8-quinolone, *J*=7.1 Hz), 7.31—7.45 (m, 1H, H_4-thiophene, *Z*-isomer and 5H, phenyl), 7.53 (dd, 1H, H_5-thiophene, *Z*-isomer, *J*=5.0, 2.9 Hz), 7.57 (dd, 1H, H_5-thiophene, *E*-isomer, *J*=5.0, 2.8 Hz), 7.69 (dd, 1H, H_4-thiophene, *E*-isomer, *J*=4.6, 1.1 Hz), 7.91 (d, 2H, H_5-quinolone, *J*=12.99 Hz), 8.01 (dd, 1H, H_2-thiophene, *Z*-isomer, *J*=2.8, 1.0 Hz), 8.39 (dd, 1H, H_2-thiophene, *E*-isomer, *J*=1.0, 2.9 Hz), 8.94 (s, 1H, H_2-quinolone), 15.34 (s, 1H, COOH). IR (KBr) cm^{-1}: 1613, 1731 (C=O).

1-Cyclopropyl-6-fluoro-1,4-dihydro-7-[4-[2-(thiophen-3-yl)-2-(phenylmethoxyimino)ethyl]piperazin-1-yl]-4-oxo-3-quinolone Carboxylic Acid (**5l**): (*Z*)-isomer. ^{1}H-NMR (DMSO-d_6) δ: 1.15—1.20 (m, 2H, cyclopropyl), 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=CCH_2–), 3.78—3.83 (m, 1H, cyclopropyl), 5.20 (s, 2H, OCH_2), 7.32—7.45 (m, 1H, H_4-thiophene and 5H, phenyl), 7.53—7.57 (m, 1H, H_5-thiophene and 1H, H_8-quinolone), 7.90 (d, 1H, H_5-quinolone, *J*=13.2 Hz), 8.02 (dd, 1H, H_2-thiophene, *J*=2.8, 1.0 Hz), 8.66 (s, 1H, H_2-quinolone), 15.21 (s, 1H, COOH). IR (KBr) cm^{-1}: 1627, 1733 (C=O).

Antibacterial Activity Compounds **5a**—**l** were evaluated for their antibacterial activity using conventional agar-dilution method.[16] 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 (1.5×10^8 CFU/ml). The suspensions were then diluted in 0.85% saline to give 10^7 CFU/ml. Petri dishes were spot-inoculated with 1 μl 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.

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