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Synthesis and antibacterial activity of *N*-[2-[5-(methylthio)thiophen-2-yl]-2-oxoethyl] and *N*-[2-[5-(methylthio)thiophen-2-yl]-2-(oxyimino)ethyl]piperazinylquinolone derivatives

Alireza Foroumadi,[a,d] Mehdi Oboudiat,[b] Saeed Emami,[c] Alireza Karimollah,[b] Lotfollah Saghaee,[b] Mohammad Hassan Moshafi[d] and Abbas Shafiee[a,*]

[a]*Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran 14174, Iran*
[b]*Department of Medicinal Chemistry, Faculty of Pharmacy, Esfahan University of Medical Sciences, Esfahan, Iran*
[c]*Department of Medicinal Chemistry, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran*
[d]*Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran*

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Abstract—A number of *N*-substituted piperazinylquinolone derivatives were synthesized and evaluated for antibacterial activity against Gram-positive and Gram-negative bacteria. Preliminary results indicated that most compounds tested in this study demonstrated comparable or better activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* than their parent piperazinylquinolones as reference drugs. Among these derivatives, ciprofloxacin derivative **5a**, containing *N*-[2-[5-(methylthio)thiophen-2-yl]-2-oxoethyl] residue, showed significant improvement of potency against staphylococci, maintaining Gram-negative coverage.

1. Introduction

The earliest antibacterial agents to be used clinically in the 1920s were the synthetic sulfonamides. Since then, quinolones were the only synthetic agents that play a major role in the treatment of bacterial community or hospital acquired diseases. The excellent pharmacokinetic properties, high antimicrobial activity, and few side effects that most quinolones demonstrate explain their widespread use in clinical practice.[1] The compounds have had good success against Gram-negative bacteria, but resistance of Gram-positive pathogens, such as *Staphylococcus aureus*, has become a problem.[2–8] In addition, certain adverse events (e.g., CNS side effects, phototoxicity, and arthropathy) became apparent, although the more serious events are rare.[9,10] Thus, despite many advances in the fluoroquinolone field, there exists a continuous need for novel quinolones to overcome the limitations of existing drugs.

Quinolones inhibit DNA synthesis by interacting with two essential bacterial type II topoisomerases, DNA gyrase and topoisomerase IV.[11–13] They inhibit enzyme function by binding to the catalytic intermediate enzyme–DNA complex. The stabilization of the resulting quinolone–enzyme–DNA complex leads to the generation of double-strand DNA breaks that trigger a cascade of events leading to cell death.[12–15] The molecular organization of the complex is presently unknown although several models have been suggested.[16–18] According to these models, differences in enzyme inhibitory potency are mainly determined by the binding strength of the drug to a DNA receptor site on the enzyme–substrate complex, while the interaction of the C-7 substituent with the enzyme plays a supporting role.

The 1,4-dihydro-4-oxopyridine-3-carboxylic acid associated with a 5,6-fused aromatic ring is the common chemical feature of bactericidal quinolones (Fig. 1). In the resulting bicyclic ring, the 1-, 5-, 6-, 7-, and 8-positions are the major targets of chemical variation but,

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* Corresponding author. Tel.: +98 21 66406757; fax: +98 21 66461178; e-mail: ashafiee@ams.ac.ir

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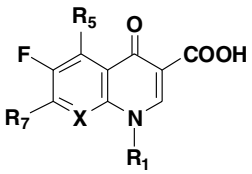


Figure 1. Common pharmacophore of quinolones and structure of some piperazinylquinolones. **1**, Ciprofloxacin; R_1 = cyclopropyl, R_5 = H, R_7 = piperazin-1-yl, X = CH. **2**, Norfloxacin; R_1 = ethyl, R_5 = H, R_7 = piperazin-1-yl, X = CH. **3**, Enoxacin; R_1 = ethyl, R_5 = H, R_7 = piperazin-1-yl, X = N.

synthetic efforts for improved potency have been the main focus on 7-position.[19–21] Both activity spectrum and kinetic profile can be controlled at C-7. The most common substituents are cyclic amino groups, for example, piperazine or pyrrolidine rings; other groups have been less successful. Piperazine rings are particularly common (e.g., ciprofloxacin **1**, norfloxacin **2** or enoxacin **3**) and confer potency against Gram-negative bacteria.[19–21] The first generation of 7-piperazinyl quinolones, exemplified by ciprofloxacin, norfloxacin, and enoxacin, had high activity against Gram-negative species and atypicals, but moderate activity against Gram-positive bacteria.[2]

To explore the potential of tethered 7-piperazinylquinolones as anti-Gram-positive agents, we have reported a number of *N*-substituted piperazinylquinolones with high activity against staphylococci.[22–24] In addition, a number of quinolones **4** with a 2-oxoethyl or a 2-oximinoethyl moiety attached to the piperazine ring at C-7 position were synthesized and evaluated for antibacterial activity by us.[25–28] The results demonstrated that the introduction of thiophen-2-yl or 5-bromothiophen-2-yl group instead of phenyl, substituted phenyl, and furan-2-yl groups at 2 position of 2-oxoethyl or 2-oximinoethyl moiety attached to the piperazine ring in 7-piperazinyl quinolones improved the overall antibacterial activity against Gram-positive bacteria.[25–28]

In continuation of our research program to establish the structure–activity relationships of *N*-[2-(thiophen-2-yl)-2-oxoethyl] and *N*-[2-(thiophen-2-yl)-2-(oxyimino)ethyl]piperazinyl quinolones and their structural features for anti-Gram-positive activity, we focused mainly on 5-(methylthio)thiophene as the side-chain aryl residue that, in itself, shows some antimicrobial activity,[29] and investigation of its combination with other substitutions. Thus, we herein report the synthesis and antibacterial activity of *N*-[2-[5-(methylthio)thiophen-2-yl]-2-oxoethyl]piperazinylquinolones **5a–c** and *N*-[2-[5-(methylthio)thiophen-2-yl]-2-(oxyimino)ethyl]piperazinylquinolone derivatives **6–8** (**a–c**) (Fig. 2).

2. Chemistry

Our synthetic pathway to intermediates **11** and **12a–c**, and target compounds **5a–c** and **6–8** (**a–c**) is presented in Schemes 1 and 2. Compound 1-[5-(methylthio)thiophen-2-yl]ethanone **10** was obtained from 2-bromothiophene **9** according to the method reported in the literature.[30,31] Ketone **10** was brominated with copper (II) bromide in refluxing $CHCl_3$–EtOAc to give corresponding α-bromoketone **11**.[32] Compound **11** was converted to oxime derivative **12a** by stirring with excess $HONH_2{\cdot}HCl$ in methanol at room temperature. Similarly, the *O*-methyloxime ethers **12b** and *O*-benzyloxime ethers **12c** were synthesized by reaction of compound **11** with *O*-methylhydroxylamine and *O*-benzylhydroxylamine hydrochloride, respectively.[27,28] Reaction of quinolones **1**, **2** or **3** with α-bromoketone **11** or α-bromooxime derivatives **12a–c** in DMF, in the presence of $NaHCO_3$ at room temperature, afforded corresponding

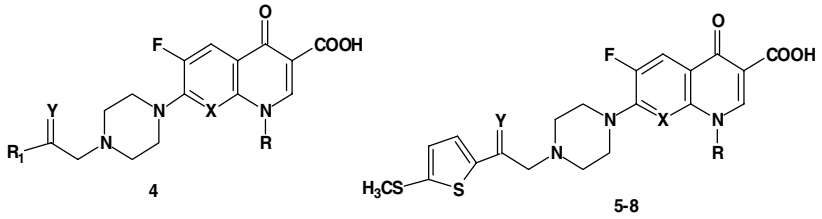
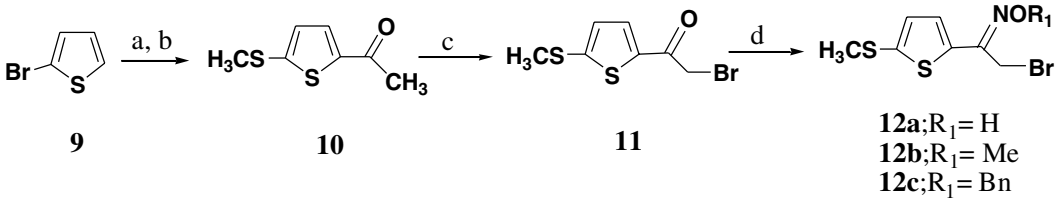
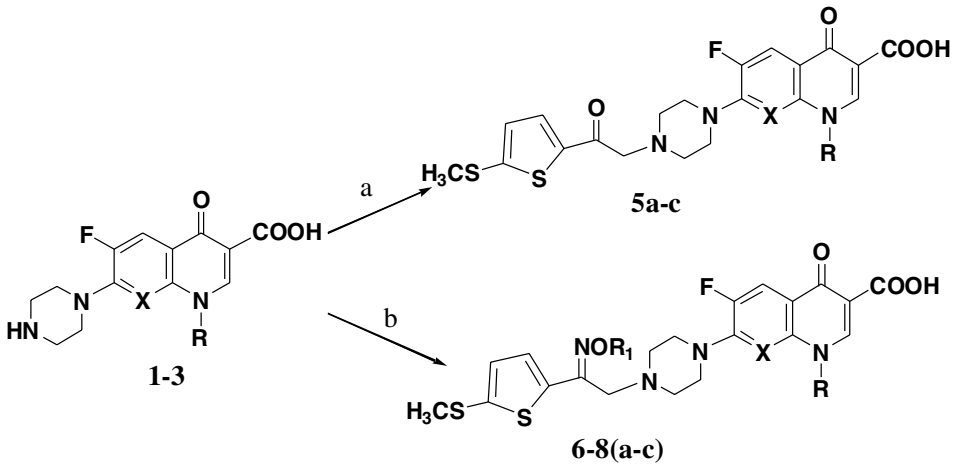


Figure 2. X = CH, N. Y = O, NOH, NOMe, $NOCH_2Ar$. R = cyclopropyl, ethyl. R_1 = Ph, substituted-Ph, furan-2-yl thiophen-2-yl, 5-bromothiophen-2-yl.



Scheme 1. Synthesis of α-bromoketone **11** and α-bromooxime derivatives**12a–c**. Reagents and conditions: (a) Mg, S_8, CH_3I, Et_2O; (b) Ac_2O, H_3PO_4; (c) $CuBr_2$, $CHCl_3$–AcOEt, reflux; (d) hydroxylamine hydrochloride or *O*-methyl hydroxylamine hydrochloride or *O*-benzyl hydroxylamine hydrochloride, MeOH, rt.



Scheme 2. Synthesis of compounds **5a–c** and **6–8** (**a–c**). Reagents and conditions: (a) α-bromoketone **11**, $NaHCO_3$, DMF, rt; (b) α-bromooxime derivatives **12a–c**, $NaHCO_3$, DMF, rt.

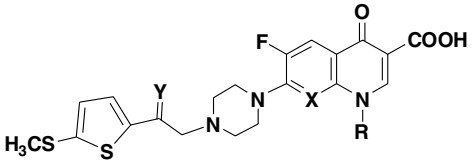
ketones **5a–c** and oxime derivatives **6–8** (**a–c**), respectively.[27,28]

3. Results and discussion

Compounds **5a–c** and **6–8** (**a–c**) were evaluated for their antibacterial activity against Gram-positive (*S. aureus* ATCC 6538p, *Staphylococcus epidermidis* ATCC 12228, and *Bacillus subtilis* PTCC 1023) and Gram-negative (*Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 10031, and*Pseudomonas aeruginosa* ATCC 9027) bacteria using conventional agar-dilution method.[33] The MIC (minimum inhibitory concentration) values were determined by comparison to ciprofloxacin **1**, norfloxacin **2**, and enoxacin **3** as reference drugs. The individual minimum inhibitory concentrations (MICs, μg/mL) obtained for compounds **5a–c** and **6–8** (**a–c**) are presented in Table 1.

The MIC values of the test derivatives against *Staphylococcus* strains indicate that most compounds possessed a comparable or better activity (MIC = 0.03–1 μg/mL) with respect to the reference drugs (MIC = 0.25–1 μg/mL). Compounds **7a**, **5a**, and **6a** followed by **7b** and **5b** are superior in inhibiting the growth of *S. aureus* (MIC = 0.03–0.5 μg/mL). Compound **7a** was the most active compound against *S. aureus*, its activity found to be 16–32 times better than reference drugs. Derivatives **5a**, **6a**, and **7a** were the most active against *S. epidermidis*, showing MIC values of 0.03 μg/mL, their activities 8- to 16-fold more than reference drugs.

Table 1. Structures and in vitro antibacterial activities of compounds **5a–c** and **6–8** (**a–c**) against selected strains (MICs in μg/mL)



Compound	X	Y	R	*S. aureus*	*S. epidermidis*	*B. subtilis*	*K. pneumoniae*	*E. coli*	*P. aeruginosa*
5a	CH	O	Cyclopropyl	0.06	0.03	0.125	0.125	0.125	1
5b	CH	O	Ethyl	0.5	4	8	8	8	16
5c	N	O	Ethyl	0.5	0.5	1	1	1	4
6a	CH	NOH	Cyclopropyl	0.06	0.03	2	2	1	32
6b	CH	NOH	Ethyl	1	2	64	>64	>64	>64
6c	N	NOH	Ethyl	>64	64	>64	>64	>64	>64
7a	CH	NOMe	Cyclopropyl	0.03	0.03	0.5	2	1	16
7b	CH	NOMe	Ethyl	0.25	0.25	8	8	8	32
7c	N	NOMe	Ethyl	1	1	8	8	8	>64
8a	CH	NOBn	Cyclopropyl	16	32	64	64	64	>64
8b	CH	NOBn	Ethyl	32	8	>64	>64	>64	>64
8c	N	NOBn	Ethyl	>64	64	64	64	64	>64
1	(Ciprofloxacin)			0.5	0.25	0.015	0.03	0.125	1
2	(Norfloxacin)			1	0.5	0.06	0.125	0.25	4
3	(Enoxacin)			1	0.5	0.125	0.25	0.25	4

Antibacterial screening of compounds **5a–c** and **6–8** (**a–c**) against *B. subtilis* reveals that compounds **5a** and **7a** possessed a significant activity. Derivative **5a** was the most potent against *B. subtilis* (MIC = 0.125 μg/mL), being equipotent to enoxacin.

Generally, most compounds showed poor or no activity (MIC > 16 μg/mL) against Gram-negative bacteria. However, compound **5a** was the most potent against all Gram-negative bacteria, with a MIC value of 0.125–1 μg/mL. Its activity was found to be comparable to those of reference drugs.

The first information obtained in this study is that most compounds exhibit high activity against Gram-positive bacteria and less activity against Gram-negative bacteria. Thus, introduction of 2-[5-(methylthio)thiophen-2-yl]ethyl residue carrying 2-oxo- or 2-oxyimino groups at the N-4 position of piperazine ring changes the antibacterial profile of piperazinylquinolones. In general, *N*-[2-[5-(methylthio)thiophen-2-yl]-2-oxoethyl]- and *N*-[2-[5-(methylthio)thiophen-2-yl]-2-oximinooethyl] groups are well tolerated in terms of Gram-positive activity. In many cases, the compounds had activity superior to the their parent quinolones. However, the improved activity against Gram-positive bacteria can often be at the expense of activity against Gram-negative bacteria. In addition, ketones **5**, oximes **6**, and *O*-methyl oximes **7** showed more potent antibacterial activity than *O*-benzyl oximes **8** against both Gram-positive and Gram-negative bacteria. Comparison between MIC values of ketones **5**, oximes **6**, and *O*-methyl oximes **7** revealed that oximation of ketones seemed to have different influence on the antibacterial activity against various bacteria strains. For example, ketone **5a**, oxime **6a**, and *O*-methyl oxime **7a** in ciprofloxacin series showed equipotent activity against staphylococci, while ketones **5** exhibited more potent activity than oxime derivatives **6** against Gram-negative bacteria.

The results of MIC tests against both Gram-positive and Gram-negative bacteria revealed that ciprofloxacin derivatives (R = cyclopropyl and X = CH) were more active than norfloxacine and enoxacine derivatives (R = ethyl and X = CH or N). These data confirm that the effect of changes in the side-chain of the 7-piperazinyl ring mainly depends on the substituent at N-1 position.

First approach in the series of new 2-[5-(methylthio)thiophen-2-yl]ethyl derivatives of piperazinyl quinolones bearing different structural features on the quinolone ring and piperazine moiety points out that the compound **5a** exerts significant in vitro antibacterial activity against staphylococci and it was more potent than the reference drugs against *S. aureus* and *S. epidermidis*, maintaining its Gram-negative coverage. Indeed, the overall activity of compound **5a** against Gram-positive (especially staphylococci) and Gram-negative bacteria was the best among thiophene derivatives and other aryl side chains in *N*-(2-oxoethyl) and *N*-[(2-(oxyimino)ethyl]piperazinyl quinolone series **4**.[25–28] Staphylococci have been recognized as important pathogens. These organisms can be resistant to multiple antimicrobial agents, which severely limits therapeutic options in selected instances. The susceptibility of an organism to a particular quinolone is determined by both the ability of the agent to penetrate the cell envelope and the inhibitory activity of the agent against its target.[34] It has been reported that the activity of some quinolones against staphylococci is dependent on the bulkiness of the substituents at C-7 position, demonstrating that molecular mass is not a limiting factor for activity against staphylococci.[35] In addition, the C-7 substituent affects the interaction with the target, and both activity spectrum and kinetic profile can be controlled at C-7.[2,16–18] Therefore, our methodology to achieve a better antimicrobial profile against staphylococci, by focusing on the functionality at C-7 position and introducing a certain residue on piperazine ring, would be useful.

In conclusion, we have described the synthesis and antibacterial evaluation of a new series of *N*-substituted piperazinylquinolones characterized by having *N*-[2-[5-(methylthio)thiophen-2-yl]ethyl residue as a bulky side-chain. Preliminary results indicated that most compounds demonstrated comparable or better activity against *S. aureus* and *S. epidermidis* than their parent piperazinylquinolones as reference drugs. Among these derivatives, ciprofloxacin derivative **5a**, containing *N*-[2-[5-(methylthio)thiophen-2-yl]-2-oxoethyl] residue, showed significant improvement of potency against staphylococci, maintaining Gram-negative coverage.

4. Experimental

Chemicals and all solvents used in this study were purchased from Merck AG and Aldrich Chemical. The 1-[5-(methylthio)thiophen-2-yl]ethanone **10** was prepared according to the literature.[30,31] 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 FT-80 spectrometer, and chemical shifts are expressed as δ (ppm) with tetramethylsilane as internal standard. The mass spectra were run on a Finnigan TSQ-70 spectrometer (Finnigan, USA) at 70 eV. Elemental analyses were carried out on a CHN-O 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; column chromatography was performed on Merck silica gel (70–230 mesh). Yields are of purified product and were not optimized.

4.1. 2-Bromo-1-[5-(methylthio)thiophen-2-yl]ethanone (11)

A vigorously stirred solution of compound **10** (5.0 g, 29 mmol) in $CHCl_3$–EtOAc (1:1, 50 mL) was refluxed and then, pulverized copper (II) bromide (12.2 g, 55.0 mmol) was added portionwise during 4 h. The resulting reaction mixture was refluxed with vigorous

stirring for additional 2 h to ensure complete exposure of the copper (II) bromide to the reaction medium until the reaction was completed as judged by a color change of the solution from green to amber, disappearance of all black solid, and cessation of HBr evolution. After removal of the copper (I) bromide (white solid) by filtration, the solvents were evaporated from the filtrate under reduced pressure. The residue was purified by column chromatography, eluting with hexane–chloroform and crystallized from *n*-heptane to give compound **11** (4.3 g). Yield 62%; mp 58–59 °C; ν_{max} (KBr) 1660 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.60 (s, 3H, S-CH_3), 4.30 (s, 2H, CH_2Br), 6.95 (d, 1H, J = 4.4 Hz, thiophene), 7.65 (d, 1H, J = 4.4 Hz, thiophene).

4.2. 2-Bromo-1-[5-(methylthio)thiophen-2-yl]ethanone oxime (12a).

A solution of **11** (300 mg, 1.2 mmol), hydroxylamine hydrochloride (4.8 mmol), and concentrated HCl (0.7 mL) in CH_3OH (20 mL) was stirred at room temperature for 7 days. Then, H_2O (25 mL) was added and the precipitate was filtered, washed with water, and crystallized from *n*-hexane to give compound **12a** (229 mg). Yield 72%; mp 150–151 °C; ν_{max} (KBr) 3200 (OH), 1610 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.55 (s, 3H, S-CH_3), 4.50 (s, 2H, CH_2Br), 7.00 (d, 1H, J = 4.5 Hz, thiophene), 7.45 (d, 1H, J = 4.5 Hz, thiophene). MS (*m*/*z*) 265 (M^+), 267 (M^++2).

4.3. 2-Bromo-1-[5-(methylthio)thiophen-2-yl]ethanone *O*-methyloxime (12b)

A solution of **11** (400 mg, 1.6 mmol) and *O*-methylhydroxylamine hydrochloride (266 mg, 3.2 mmol) in CH_3OH (15 mL) was stirred at room temperature for 7 days. Water (40 mL) was added and extracted with $CHCl_3$. The organic layer was washed (H_2O), dried (Na_2SO_4), and evaporated in vacuo to give **12b** as a viscous oil. Yield 85%; ^1H NMR ($CDCl_3$) δ 2.55 (s, 3H, S-CH_3), 4.10 (s, 3H, O-CH_3), 4.50 (s, 2H, CH_2Br), 6.95 (d, 1H, J = 4.5 Hz, thiophene), 7.20 (d, 1H, J = 4.5 Hz, thiophene).

4.4. 2-Bromo-1-[5-(methylthio)thiophen-2-yl]ethanone *O*-benzyloxime (12c)

A solution of **11** (251 mg, 1.0 mmol) and *O*-benzylhydroxylamine hydrochloride (240 mg, 1.5 mmol) in CH_3OH (20 mL) was stirred at room temperature for 48 h. Water (30 mL) was added and extracted with $CHCl_3$. The organic layer was washed (H_2O), dried (Na_2SO_4), and evaporated in vacuo to give **12c** as a viscous oil. Yield 92%; ^1H NMR ($CDCl_3$) δ 2.50 (s, 3H, S-CH_3), 4.50 (s, 2H, CH_2Br), 5.25 (s, 2H, O-CH_2), 6.95 (d, 1H, J = 4.4 Hz, thiophene), 7.15 (d, 1H, J = 4.4 Hz, thiophene), 7.20–7.50 (m, 5H, phenyl).

4.5. General procedure for the synthesis of *N*-[2-[5-(methylthio)thiophen-2-yl]-2-oxoethyl]piperazinylquinolones 5a–c

A mixture of compound **11** (83 mg, 0.33 mmol), piperazinylquinolone **1**–**3** (0.3 mmol), and $NaHCO_3$ (26 mg, 0.3 mmol) in DMF (10 mL) was stirred at room temperature for 8–10 h. After consumption of piperazinylquinolone (monitored by TLC), H_2O (20 mL) was added and the precipitate was filtered, washed with water, and crystallized from EtOH–$CHCl_3$ to give compounds **5a**–**c**.

4.5.1. 1-Cyclopropyl-6-fluoro-1,4-dihydro-7-[4-[2-[5-(methylthio)thiophen-2-yl]-2-oxoethyl]piperazin-1-yl]-4-oxo-3-quinolone carboxylic acid (5a). Yield 29%; mp 174–175 °C (dec); ν_{max} (KBr) 1660, 1710 and 1725 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) 1.10–1.40 (m, 4H, cyclopropyl), 2.55 (s, 3H, S-CH_3), 2.70 (br s, 4H, piperazine), 3.45 (br s, 4H, piperazine), 3.35–3.64 (m, 1H, cyclopropyl), 3.69 (s, 2H, $COCH_2N$), 6.90 (d, 1H, J = 4.4 Hz, thiophene), 7.30 (d,1H, H_8, $J_{H,F}$ = 7.0 Hz), 7.81 (d, 1H, J = 4.4 Hz, thiophene), 7.90 (d, 1H, H_5-quinoline, $J_{H,F}$ = 12.5 Hz), 8.70 (s, 1H, H_2-quinoline).

4.5.2. 1-Ethyl-6-fluoro-1,4-dihydro-7-[4-[2-[5-(methylthio)thiophen-2-yl]-2-oxoethyl]piperazin-1-yl]-4-oxo-3-quinolone carboxylic acid (5b). Yield 65%; mp 197–198 °C (dec); ν_{max} (KBr) 1660, 1715 and 1735 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) 1.60 (t, 3H, J = 7.2 Hz, -CH_3 ethyl), 2.60 (s, 3H, S-CH_3), 2.90 (br s, 4H, piperazine), 3.41 (br s, 4H, piperazine), 3.70 (s, 2H, $COCH_2N$), 4.45 (q, 2H, J = 7.2 Hz, -CH_2- ethyl), 6.95 (d, 1H, J = 4.4 Hz, thiophene), 7.00 (d,1H, H_8, $J_{H,F}$ = 7.0 Hz), 7.85 (d, 1H, J = 4.4 Hz, thiophene), 7.99 (d, 1H, H_5-quinoline, $J_{H,F}$ = 12.5 Hz), 8.81 (s, 1H, H_2-quinoline).

4.5.3. 1-Ethyl-6-fluoro-1,4-dihydro-7-[4-[2-[5-(methylthio)-thiophen-2-yl]-2-oxoethyl] piperazin-1-yl]-4-oxo-1,8-naphthyridine-3-carboxylic acid (5c). Yield 71%; mp 155–156 °C (dec.); ν_{max} (KBr) 1660, 1710 and 1730 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) 1.45 (t, 3H, J = 7.2 Hz, -CH_3 ethyl), 2.62 (s, 3H, S-CH_3), 2.75 (br s, 4H, piperazine), 3.70 (s, 2H, $COCH_2N$), 3.95 (br s, 4H, piperazine), 4.35 (q, 2H, J = 7.2 Hz, -CH_2-ethyl), 6.91 (d, 1H, J = 4.4 Hz, thiophene), 7.75 (d, 1H, J = 4.4 Hz, thiophene), 8.01 (d, 1H, H_5-quinoline, $J_{H,F}$ = 12.6 Hz), 8.65 (s, 1H, H_2-quinoline).

4.6. General procedure for the synthesis of *N*-[2-[5-(methylthio)thiophen-2-yl]-2- hydroxyiminoethyl]piperazinylquinolones 6a–c

A mixture of compound **12a** (88 mg, 0.33 mmol), piperazinylquinolone **1**–**3** (0.3 mmol), and $NaHCO_3$ (26 mg, 0.3 mmol) in DMF (10 mL) was stirred at room temperature for 6 h. After consumption of piperazinylquinolone (monitored by TLC), water (20 mL) was added and the precipitate was filtered, washed with water, and crystallized from EtOH–DMF to give compounds **6a**–**c**.

4.6.1. 1-Cyclopropyl-6-fluoro-1,4-dihydro-7-[4-[2-[5-(methylthio)thiophen-2-yl]-2-hydroxyiminoethyl]piperazin-1-yl]-4-oxo-3- quinolone carboxylic acid (6a). Yield 69%; mp 249–250 °C (dec); ν_{max} (KBr) 1710 and 1725 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) 1.15–1.40 (m, 4H, cyclopropyl), 2.49 (s, 3H, S-CH_3), 2.61 (br s, 4H, piperazine), 3.36 (br s, 4H, piperazine), 3.50 (s, 2H, CH_2N), 3.61–3.92 (m, 1H, cyclopropyl), 7.10 (d, 1H,

J = 4.4 Hz, thiophene), 7.60 (d,1H, H_8, $J_{H,F}$ = 7.0 Hz), 7.75 (d, 1H, J = 4.4 Hz, thiophene), 7.90 (d, 1H, H_5-quinoline, $J_{H,F}$ = 12.6 Hz), 8.65 (s, 1H, H_2-quinoline), 12.00 (s, 1H, OH).

4.6.2. 1-Ethyl-6-fluoro-1,4-dihydro-7-[4-[2-[5-(methylthio)thiophen-2-yl]-2- hydroxyiminoethyl]piperazin-1-yl]-4-oxo-3-quinolone carboxylic acid (6b). Yield 81%; mp 240–241 °C (dec); ν_{max} (KBr) 1715 and 1725 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) 1.40 (t, 3H, J = 7.0 Hz, -CH_3 ethyl), 2.56 (s, 3H, S-CH_3), 2.65 (br s, 4H, piperazine), 3.30 (br s, 4H, piperazine), 3.55 (s, 2H, CH_2N), 4.59 (q, 2H, J = 7.0 Hz, -CH_2- ethyl), 7.05 (d, 1H, J = 4.4 Hz, thiophene), 7.20 (d,1H, H_8, $J_{H,F}$ = 7.0 Hz), 7.75 (d, 1H, J = 4.4 Hz, thiophene), 7.90 (d, 1H, H_5-quinoline, $J_{H,F}$ = 12.6 Hz), 8.89 (s, 1H, H_2-quinoline), 11.91 (s, 1H, OH).

4.6.3. 1-Ethyl-6-fluoro-1,4-dihydro-7-[4-[2-[5-(methylthio)thiophen-2-yl]-2- hydroxyiminoethyl]piperazin-1-yl]-4-oxo-1,8-naphthyridine-3-carboxylic acid (6c). Yield 73%; mp 248–249 °C (dec); ν_{max} (KBr) 1710 and 1725 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) 1.40 (t, 3H, J = 7.0 Hz, -CH_3 ethyl), 2.49 (s, 3H, S-CH_3), 2.62 (br s, 4H, piperazine), 3.50 (s, 2H, CH_2N), 3.85 (br s, 4H, piperazine), 4.48 (q, 2H, J = 7.0 Hz, -CH_2- ethyl), 7.11 (d, 1H, J = 4.4 Hz, thiophene), 7.70 (d, 1H, J = 4.4 Hz, thiophene), 8.10 (d, 1H, H_5-quinoline, $J_{H,F}$ = 12.6 Hz), 8.95 (s, 1H, H_2-quinoline), 11.95 (s, 1H, OH).

4.7. General procedure for the synthesis of *N* -[2-[5-(methylthio)thiophen-2-yl]-2-methoxyiminoethyl] piperazinylquinolones 7a–c

A mixture of compound **12b** (92 mg, 0.33 mmol), piperazinylquinolone **1**–**3** (0.3 mmol), and $NaHCO_3$ (26 mg, 0.3 mmol) in DMF (10 mL) was stirred at room temperature for 6–10 h. After consumption of piperazinylquinolone (monitored by TLC), H_2O (25 mL) was added and extracted with $CHCl_3$. The organic layer was washed (H_2O), dried (Na_2SO_4), and evaporated in vacuo. The residue was crystallized from EtOH–DMF to give compounds **7a**–**c**.

4.7.1. 1-Cyclopropyl-6-fluoro-1,4-dihydro-7-[4-[2-[5-(methylthio)thiophen-2-yl]-2-methoxyiminoethyl]piperazin-1-yl]-4-oxo-3- quinolone carboxylic acid (7a). Yield 54%; mp 175–176 °C (dec.); ν_{max} (KBr) 1710 and 1720 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) 1.22–1.46 (m, 4H, cyclopropyl), 2.61 (s, 3H, S-CH_3), 2.75 (br s, 4H, piperazine), 3.36 (br s, 4H, piperazine), 3.55 (s, 2H, CH_2N), 3.40–3.70 (m, 1H, cyclopropyl), 4.11 (s, 3H, OCH_3), 6.95 (d, 1H, J = 4.4 Hz, thiophene), 7.35 (d, 1H, H_8, $J_{H,F}$ = 7.0 Hz), 7.75 (d, 1H, J = 4.4 Hz, thiophene), 8.05 (d, 1H, H_5-quinoline, $J_{H,F}$ = 12.6 Hz), 8.75 (s, 1H, H_2-quinoline).

4.7.2. 1-Ethyl-6-fluoro-1,4-dihydro-7-[4-[2-[5-(methylthio)thiophen-2-yl]-2-methoxyiminoethyl]piperazin-1-yl]-4-oxo-3-quinolone carboxylic acid (7b). Yield 72%; mp 180–181 °C; ν_{max} (KBr) 1710 and 1725 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) 1.50 (t, 3H, J = 7.0 Hz, -CH_3 ethyl), 2.45 (s, 3H, S-CH_3), 2.70 (br s, 4H, piperazine), 3.25 (br s, 4H, piperazine), 3.55 (s, 2H, CH_2N), 3.90 (s, 3H, OCH_3), 4.25 (q, 2H, J = 7.0 Hz, -CH_2- ethyl), 6.85 (d,1H, H_8, $J_{H,F}$ = 7.0 Hz), 6.90 (d, 1H, J = 4.4 Hz, thiophene), 7.30 (d, 1H, J = 4.4 Hz, thiophene), 8.00 (d, 1H, H_5-quinoline, $J_{H,F}$ = 12.6 Hz), 8.60 (s, 1 H, H_2-quinoline).

4.7.3. 1-Ethyl-6-fluoro-1,4-dihydro-7-[4-[2-[5-(methylthio)thiophen-2-yl]-2-methoxyiminoethyl]piperazin-1-yl]-4-oxo-1,8-naphthyridine-3-carboxylic acid (7c). Yield 64%; mp 177–178 °C (dec); ν_{max} (KBr) 1710 and 1725 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) 1.50 (t, 3H, J = 7.0 Hz, -CH_3 ethyl), 2.49 (s, 3H, S-CH_3), 2.64 (br s, 4H, piperazine), 3.55 (s, 2H, CH_2N), 3.89 (br s, 4H, piperazine), 3.90 (s, 3H, OCH_3), 4.30 (q, 2H, J = 7.0 Hz, -CH_2- ethyl), 6.90 (d, 1H, J = 4.3 Hz, thiophene), 7.30 (d, 1H, J = 4.3 Hz, thiophene), 8.05 (d, 1H, H_5-quinoline, $J_{H,F}$ = 12.8 Hz), 8.70 (s, 1H, H_2-quinoline).

4.8. General procedure for the synthesis of *N*-[2-[5-(methylthio)thiophen-2-yl]-2-(benzyloxyimino)ethyl]piperazinylquinolones 8a–c

A mixture of compound **12c** (100 mg, 0.28 mmol), piperazinylquinolone **1**–**3** (0.25 mmol), and $NaHCO_3$ (21 mg, 0.25 mmol) in DMF (8 mL) was stirred at room temperature for 12 h. After consumption of piperazinylquinolone (monitored by TLC), H_2O (25 mL) was added and extracted with $CHCl_3$. The organic layer was washed (H_2O), dried (Na_2SO_4), and evaporated in vacuo. The residue was crystallized from ethanol to give compounds **8a**–**c**.

4.8.1. 1-Cyclopropyl-6-fluoro-1,4-dihydro-7-[4-[2-[5-(methylthio)thiophen-2-yl]-2-(benzyloxyimino)ethyl]piperazin-1-yl]-4-oxo-3-quinolone carboxylic acid (8a). Yield 50%; mp 164–165 °C; ν_{max} (KBr) 1710 and 1725 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) 1.21–1.50 (m, 4H, cyclopropyl), 2.55 (s, 3H, S-CH_3), 2.75 (br s, 4H, piperazine), 3.40 (br s, 4H, piperazine), 3.50 (m, 1H, cyclopropyl), 3.70 (s, 2H, CH_2N), 5.22 (s, 2H, OCH_2), 6.95 (d, 1H, J = 4.4 Hz, thiophene), 7.00 (d, 1H, H_8, $J_{H,F}$ = 7.0 Hz), 7.25–7.50 (m, 5H, phenyl), 7.70 (d, 1H, J = 4.4 Hz, thiophene), 8.00 (d, 1H, H_5-quinoline, $J_{H,F}$ = 12.6 Hz), 8.75 (s, 1H, H_2-quinoline).

4.8.2. 1-Ethyl-6-fluoro-1,4-dihydro-7-[4-[2-[5-(methylthio)thiophen-2-yl]-2-(benzyloxyimino)ethyl]piperazin-1-yl]-4-oxo- 3-quinolone carboxylic acid (8b). Yield 62%; mp 166–167 °C; ν_{max} (KBr) 1710 and 1720 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) 1.55 (t, 3H, J = 7.0 Hz, -CH_3 ethyl), 2.49 (s, 3H, S-CH_3), 2.65 (br s, 4H, piperazine), 3.35 (br s, 4H, piperazine), 3.71 (s, 2H, CH_2N), 4.30 (q, 2H, J = 7.0 Hz, -CH_2- ethyl), 5.21 (s, 2H, OCH_2), 6.85 (d,1H, H_8, $J_{H,F}$ = 7.0 Hz), 6.95 (d, 1H, J = 4.2 Hz, thiophene), 7.20–7.50 (m, 5H, phenyl),7.70 (d, 1H, J = 4.2 Hz, thiophene), 8.05 (d, 1H, H_5-quinoline, $J_{H,F}$ = 12.6 Hz), 8.66 (s, 1H, H_2-quinoline).

4.8.3. 1-Ethyl-6-fluoro-1,4-dihydro-7-[4-[2-[5-(methylthio)thiophen-2-yl]-2-(benzyloxyimino)ethyl]piperazin-1-yl]-4-oxo-1,8-naphthyridine-3- carboxylic acid (8c). Yield 60%; mp 116–117 °C; ν_{max} (KBr) 1710 and 1725

(C=O) cm^{-1}; ^1H NMR ($CDCl_3$) 1.50 (t, 3H, $J = 7.0$ Hz, -CH_3 ethyl), 2.45 (s, 3H, S-CH_3), 2.50 (br s, 4H, piperazine), 3.65 (s, 2H, CH_2N), 3.70 (br s, 4H, piperazine), 4.35 (q, 2H, $J = 7.0$ Hz, -CH_2- ethyl), 5.15 (s, 2H, OCH_2), 6.90 (d, 1H, $J = 4.4$ Hz, thiophene), 7.16–7.44 (m, 5H, phenyl), 7.70 (d, 1H, $J = 4.4$ Hz, thiophene), 8.00 (d, 1H, H_5-quinoline, $J_{H,F} = 12.6$ Hz), 8.60 (s, 1H, H_2-quinoline).

4.9. Antibacterial activity

Compounds **5**–**8** were evaluated for their antibacterial activity using conventional agar-dilution method.[33] 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 was diluted with water (9 mL). Further progressive double dilution with melted Muller–Hinton agar was performed to obtain the required concentrations of 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.06, 0.03, and 0.015 μg/mL. Petri dishes were incubated with 1–5×10^4 colony-forming units (cfu) and incubated at 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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