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ORIGINAL RESEARCH

7-Piperazinylquinolones with methylene-bridged nitrofuran scaffold as new antibacterial agents

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Abstract Quinolone class of antibacterial agents has considerable attention to find new useful antibacterial agents. Therefore, a series of *N*-substituted piperazinylquinolones bearing (5-nitrofuran-2-yl)methyl moiety were synthesized and evaluated against a variety of bacteria. The methylene-bridged nitrofuran functionality has been recently used in oxazolidinone class of antibacterial agents containing piperazinyl moiety by introducing ranbezolid as a 5-nitrofuran analog of eperzolid. The results of antibacterial evaluation revealed that the influence of (5-nitrofuran-2-yl) attachment to the 7-piperazinylquinolones against different bacterial species depends on the type of substituents at the N-1 and C-8 positions. Better results were obtained with ethyl at N-1 and CF at C-8 in the term of activity against *Bacillus subtilis* and *E. coli*. While, the optimum activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* was entailed by a molecule possessing cyclopropyl at N-1 and CH at C-8.

Keywords Antibacterial activity · Quinolones · 5-Nitrofuran · Structure–activity relationship

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Introduction

Quinolones are an important class of chemotherapeutic agents, which are widely used for the treatment of various bacterial infections in both community and hospital settings due to their broad spectrum and excellent oral bioavailability (Mugnaini *et al.*, 2009; Emami *et al.*, 2005). Quinolones exert their antibacterial action by the inhibition of type II bacterial topoisomerases such as DNA gyrase and topoisomerase IV (Ball, 2000). DNA gyrase is an essential bacterial enzyme responsible for the maintenance of DNA topology by introducing negative supercoils in DNA within transcription and replication processes. Topoisomerase IV provides a potent ATP-dependent chromosome decatenation and relaxation activity (Cheng *et al.*, 2007). Therefore, the quinolones by inhibiting these enzymes block the transcription and the DNA replication, which results in cell death (Willmott *et al.*, 1994). The selective action of quinolones as antibacterial agents lies behind the differences between prokaryotic and eukaryotic topoisomerases (Gootz *et al.*, 1990).

Although, the new generation of quinolones achieved significant improvements in terms of potency, spectrum, and pharmacokinetics, but these agents faced a growing incidence of resistance especially to Gram-positive bacteria (e.g., *Staphylococcus aureus*, *Streptococcus pneumonia*, and *Enterococci*) (Canton *et al.*, 2003; Emami *et al.*, 2006).

Furthermore, some of the adverse effects, such as phototoxicity, cardiotoxicity, and hypoglycemia are unacceptable for quinolones. For example, grepafloxacin and trovafloxacin were withdrawn from market due to the increased cases of cardiotoxicity and liver toxicity, respectively (Graul *et al.*, 1999). Similarly, gatifloxacin which was associated with potentially severe dysglycemia, was removed from the US market in 2006 (Mehlhorn and Brown, 2007). Therefore, there is an utmost need to develop newer quinolone antibacterials with better profile of activity and desired property.

Historically, since the discovery of norfloxacin and ciprofloxacin in the early 1980s, most of the attentions have been focused on the basic group at the C-7 position of quinolones which greatly influences their spectrum, potency, and safety (Koga *et al.*, 1980). Structure–activity relationship (SAR) studies of quinolones have indicated that the 5- or 6-membered cyclic amines such as pyrrolidine or piperazine have been proven to be the optimal substituents for chemical modification. Piperazine substituent at the C-7 position of quinolone core structure has resulted in a large number of marketed antibacterial agents including norfloxacin, ciprofloxacin, enoxacin, pefloxacin, ofloxacin, levofloxacin, fleroxacin, lomefloxacin, sparfloxacin, and gatifloxacin (Emami *et al.*, 2005, 2006). The previous studies have revealed that the piperazine ring of 7-piperazinylquinolones is the most adaptable site for chemical manipulation and possesses enough structural flexibility to allow lead optimization (Emami *et al.*, 2006). Moreover, some studies have indicated that increased bulkiness of substituents at this site enhances protection from efflux exporter proteins, and decreases bacterial drug-resistance (Beyer *et al.*, 2000; Davies *et al.*, 2000; Pestova *et al.*, 2000; Costa *et al.*, 2011). Also, the bulkiness of substituent at C-7 position leads to reduced side effects, improved potency and in vivo efficacy of quinolones against Gram-positive bacteria (Minovski *et al.*, 2011; Emami *et al.*, 2006).

Previously, as part of an ongoing program to find new antibacterial agents that display strong antiGram-positive activities, we have focused on introducing appropriate functional groups to the piperazine ring of 7-piperazinylquinolones (Mirzaei and Foroumadi, 2000; Foroumadi *et al.*, 2003, 2005, 2006a, b, c; Jazayeri *et al.*, 2009). In the present communication, for introducing new functionality through N-atom of 7-piperazinylquinolones, we selected methylene-bridged nitrofuran scaffold (Fig. 1). Since the antibacterial activity of 5-nitrofurans was well-documented, thus, this type of pharmacophore combination could be improved the antibacterial potential of quinolones. This strategy was previously employed for oxazolidinone

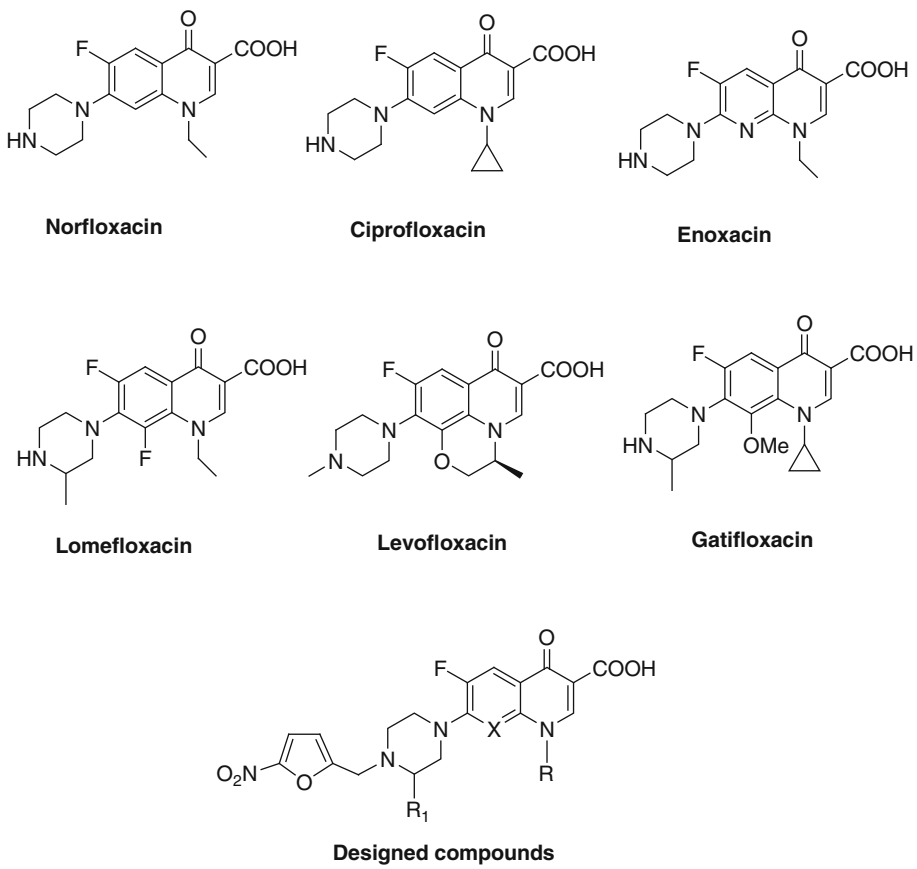


Fig. 1 Structures of some marketed 7-piperazinylquinolone antibacterials norfloxacin, ciprofloxacin, enoxacin, lomefloxacin, levofloxacin, and gatifloxacin, and designed 7-piperazinylquinolones with methylene-bridged nitrofuran scaffold

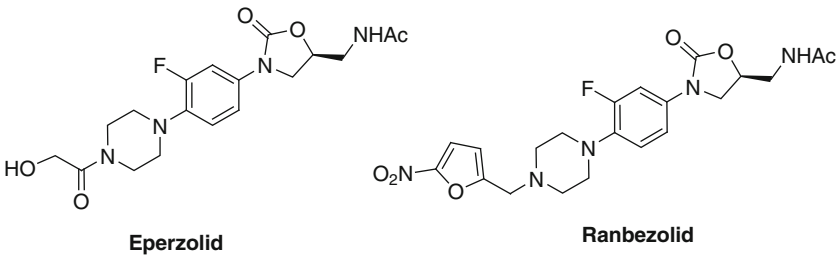
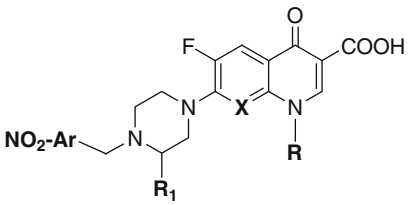
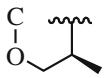


Fig. 2 Oxazolidinone antibacterial agents containing piperazinyl moiety. Ranbezolid was introduced as a 5-nitrofuran analog of eperzolid

Table 1 Physiochemical properties of the compounds **4a–g**



Compounds	NO_2–Ar	X	R	R_1	Formula	MW	Analysis (calcd/found)		
							C %	H %	N %
4a	5-NO_2-Furan-2-yl-	CH	Et	H	$C_{21}H_{21}FN_4O_6$	444.4	56.75	4.76	12.61
							56.88	4.69	12.66
4b	5-NO_2-Furan-2-yl-	CH	*c*-Pr	H	$C_{22}H_{21}FN_4O_6$	456.4	57.89	4.64	12.28
							58.01	4.48	12.11
4c	5-NO_2-Furan-2-yl-	COMe	*c*-Pr	Me	$C_{24}H_{25}FN_4O_7$	500.5	57.60	5.03	11.19
							57.63	4.89	10.99
4d	5-NO_2-Furan-2-yl-	CF	Et	Me	$C_{22}H_{22}F_2N_4O_6$	476.4	55.46	4.65	11.76
							55.50	4.58	11.77
4e	5-NO_2-Furan-2-yl-	N	Et	H	$C_{20}H_{20}FN_5O_6$	445.4	53.93	4.53	15.72
							54.04	4.70	15.61
4f	5-NO_2-Furan-2-yl-			H	$C_{22}H_{21}FN_4O_7$	472.4	55.93	4.48	11.86
							55.84	4.56	11.79
4g	4-NO_2-Phenyl-	CH	*c*-Pr	H	$C_{24}H_{23}FN_4O_5$	466.5	61.80	4.97	12.01
							61.86	5.03	12.00

class of antibacterial agents by introducing ranbezolid as a 5-nitrofuran analog of eperzolid (Fig. 2) (Das *et al.*, 2005). Hence, we report herein, the synthesis and antibacterial activity of some *N*-substituted piperazinylquinolones bearing (5-nitrofuran-2-yl)methyl moiety.

Materials and methods

Chemistry

N-Desmethyllevofloxacin was prepared by the reaction of piperazine with (−)-9,10-difluoro-2,3-dihydro-3-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid (Atarashi *et al.*, 1987). Other parent 7-piperazinylquinolones were purchased from Sigma-Aldrich. The structures and physicochemical data of synthesized *N*-substituted piperazinylquinolones **4a–g** are presented in Table 1. All reagents and solvents were purchased from Merck AG and used without further purification.

The progress of reactions and the purity of compounds were checked by thin-layer chromatography (TLC) using Merck silica gel 60 F254 plates, and visualization was achieved with UV light (254 nm). Yields are based on purified material and were not optimized. Melting points were determined in open glass capillaries using Bibby Stuart Scientific SMP3 apparatus (Stuart Scientific, Stone, UK) and are uncorrected. The ^{1}H NMR spectra were recorded using a Bruker 400 or 500 spectrometers, and chemical shifts are expressed as δ (ppm) with tetramethylsilane as internal standard. The spin multiplicities are reported as s (singlet), br s (broad singlet), d (doublet),

t (triplet), q (quartet), and m (multiplet). Coupling constants (*J*) are reported in Hertz (Hz). The IR spectra were obtained on a PerkinElmer FT-IR spectrophotometer (potassium bromide disks). 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.

Synthesis of (5-nitrofuran-2-yl)methanol ***(2)***

5-Nitrofurfural (282 mg, 2 mmol) was dissolved in methanol (6 mL) and the solution was cooled to 0 °C. Then, sodium borohydride (83 mg, 2.2 mmol) was added portion-wise and the mixture was stirred for a further 0.5 h. After completion of the reaction, the solvent was evaporated under reduced pressure and the residue was dissolved in small amount of water. The solution was extracted with diethyl ether (3 × 10 mL). The organic phase was washed with water, dried with Na_2SO_4, and evaporated to afford compound **2** as a pale yellow oil. Yield 30 %; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.5 (br s, 1H, OH), 4.73 (s, 2H, CH_2), 6.57 (d, 1H, $J = 4$ Hz, H_3-furan), 7.30 (d, 1H, $J = 4.0$ Hz, H_4-furan).

Synthesis of 2-bromomethyl-5-nitrofuran ***(3)***

Compound **2** (483 mg, 3.8 mmol) was dissolved in dichloromethane (4 mL) and the solution was cooled to 0 °C. Then, phosphorus tribromide (250 mg, 5 mmol) in dichloromethane (3 mL) was added dropwise and the solution was stirred for a further 1 h. After completion of the reaction, dichloromethane (25 mL) was added and the solution was washed with water (2 × 25 mL) and dried with Na_2SO_4. After evaporation of solvent, the residue was a pale yellow oil which was left in the fridge. The solid product **3** thus obtained on cooling was used without further purification. Yield 70 %; mp 43–45 °C; IR (KBr, cm^{-1}): ν_{max} 1526 and 1345 (NO_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.49 (s, 2H, CH_2), 6.64 (d, 1H, $J = 4.0$ Hz, H_3-furan), 7.28 (d, 1H, $J = 4.0$ Hz, H_4-furan).

General procedure for the synthesis of N-[(5-nitrofuran-2-yl)methyl] piperazinylquinolones ***(4a–f)***

To a mixture of 7-piperazinylquinolone (0.33 mmol) and sodium bicarbonate (28 mg, 0.33 mmol) in DMF (4 mL), 2-bromomethyl-5-nitrofuran (**3**, 69 mg, 0.33 mmol) was added and the mixture was stirred at room temperature for 5 days. After completion of the reaction, water (15 mL) was added and the mixture was left in the refrigerator overnight. The precipitated solid was collected by filtration, washed with water, and dried. The product was recrystallized from ethanol-chloroform to give pure compound.

1-Ethyl-6-fluoro-1,4-dihydro-7-[4-((5-nitrofuran-2-yl) methyl)piperazin-1-yl]-4-oxoquinoline-3-carboxylic acid ***(4a)*** Yield 91 %; mp 206–207 °C; IR (KBr, cm^{-1}): ν_{max} 3444 (OH), 1722 and 1626 (C=O), 1488 and 1359 (NO_2); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.40 (t, 3H, $J = 6.95$ Hz, $-CH_3$), 2.67 (br s, 4H, piperazine), 3.34 (br s, 4H, piperazine), 3.76 (s, 2H, nitrofuran–CH_2–N), 4.57 (q, 2H, $J = 7.05$ Hz, $-CH_2$ ethyl), 6.81 (d, 1H, $J = 3.55$ Hz, H_3-furan), 7.15 (d, 1H, $J_{H,F} = 7.05$ Hz, H_8-quinoline), 7.68 (d, 1H, $J = 3.2$ Hz, H_4-furan), 7.88 (d, 1H, $J_{H,F} = 13.1$ Hz, H_5-quinoline), 8.93 (s, 1H, H_2-quinoline), 15.33 (br s, 1H, COOH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.29, 49.02, 49.34, 51.78, 53.46, 105.87, 106.99, 110.99, 111.18 (d, $J_{C,F} = 22.72$ Hz), 113.15, 113.87, 119.19, 137.08, 145.36, 148.44, 151.37, 151.81, 153.80, 156.46, 166.08, 176.08.

1-Cyclopropyl-6-fluoro-1,4-dihydro-7-[4-((5-nitrofuran-2-yl) methyl)piperazin-1-yl]-4-oxoquinoline-3-carboxylic acid ***(4b)*** Yield 56 %; mp 212–213 °C; IR (KBr, cm^{-1}): ν_{max} 3445 (OH), 1725 and 1629 (C=O), 1491 and 1354 (NO_2); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.11–1.23 (m, 2H, cyclopropyl), 1.25–1.38 (m, 2H, cyclopropyl), 2.61–2.74 (m, 4H, piperazine), 3.30–3.43 (m, 4H, piperazine), 3.77 (s, 2H, nitrofuran-CH_2–N), 3.82 (br s, 1H, cyclopropyl), 6.82 (d, 1H, $J = 3.15$ Hz, H_3-furan), 7.54 (d, 1H, $J_{H,F} = 6.9$ Hz, H_8-quinoline), 7.68 (d, 1H, $J = 3.05$ Hz, H_4-furan), 7.85 (d, 1H, $J_{H,F} = 13.1$ Hz, H_5-quinoline), 8.64 (s, 1H, H_2-quinoline), 15.09 (br s, 1H, COOH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 7.52, 35.78, 49.27, 51.77, 53.46, 106.38, 110.76, 110.94, 113.18, 113.88, 118.55, 139.05, 145.04, 147.91, 151.38, 151.94, 153.91, 156.45, 165.89, 176.24.

1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[3-methyl-4-((5-nitrofuran-2-yl)methyl)piperazin-1-yl]-4-oxoquinoline-3-carboxylic acid ***(4c)*** Yield 91 %; mp 133–134 °C; IR (KBr, cm^{-1}): ν_{max} 3444 (OH), 1728 and 1619 (C=O), 1498 and 1354 (NO_2); ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.97–1.06 (m, 2H, cyclopropyl), 1.09–1.11 (m, 2H, cyclopropyl), 1.14 (d, 3H, $J = 5.4$ Hz, CH_3), 2.52–2.55 (m, 2H, piperazine), 2.85–2.90 (m, 1H, piperazine), 3.01 (t, 1H, $J = 10.15$ Hz, piperazine), 3.71 (s, 3H, OCH_3), 3.81 (d, 1H, $J = 15.4$ Hz, piperazine), 3.98 (d, 1H, $J = 15.4$ Hz, piperazine), 4.11–4.18 (m, 1H, cyclopropyl), 6.82 (d, 1H, $J = 3.7$ Hz, H_3-furan), 7.68 (d, 1H, $J = 3.6$ Hz, H_4-furan), 7.70 (d, 1H, $J_{H,F} = 12.8$ Hz, H_5-quinoline), 8.68 (s, 1H, H_2-quinoline), 14.75 (br s, 1H, COOH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 8.89, 8.95, 15.73, 40.72, 49.32, 50.43, 51.49, 54.43, 56.93, 62.90, 106.42, 106.60, 113.20, 113.91, 120.76, 134.06, 138.84, 138.93, 145.68, 150.48, 151.31, 153.19, 154.37, 156.35, 157.13, 165.63, 176.23.

*(RS)-1-Ethyl-6,8-difluoro-1,4-dihydro-7-[3-methyl-4-((5-nitrofuran-2-yl)methyl)piperazin-1-yl]-4-oxo-quinoline-3-carboxylic acid (**4d**)* Yield 88 %; IR (KBr, cm^{-1}): ν_{max} 3444 (OH), 1727 and 1619 (C=O), 1488 and 1354 (NO_2); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.15 (br s, 3H, –CH_3 ethyl), 1.43 (br s, 3H, CH_3-piperazine), 2.50–2.64 (br s, 1H, piperazine) 2.80–2.90 (m, 1H, piperazine), 2.91–3.07 (m, 1H, piperazine), 3.33–3.37 (m, 2H, piperazine), 3.40 (s, 2H, nitrofuran-CH_2), 3.82 (d, 1H, $J = 15.35$ Hz, piperazine), 3.95 (d, 1H, $J = 15.4$ Hz, piperazine), 4.57 (br s, 2H, CH_2 ethyl), 6.82 (br s, 1H, H_3-furan), 7.69 (br s, 1H, H_4-furan), 7.80–7.92 (m, 1H, H_5-quinoline), 8.92 (s, 1H, H_2-quinoline), 14.87 (br s, 1H, COOH).

*1-Ethyl-6-fluoro-1,4-dihydro-7-[4-((5-nitrofuran-2-yl)methyl) piperazin-1-yl]-4-oxo-1,8-naphthyridine-3-carboxylic acid (**4e**)* Yield 81 %; mp 190–191 °C; IR (KBr, cm^{-1}): ν_{max} 3444 (OH), 1716 and 1630 (C=O), 1473 and 1355 (NO_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.38 (t, 3H, $J = 7.0$ Hz, –CH_3), 2.64 (br s, 4H, piperazine), 3.74 (s, 2H, nitrofuran-CH_2), 3.85 (br s, 4H, piperazine), 4.78 (q, 2H, $J = 6.8$ Hz, –CH_2 ethyl), 6.79 (d, 1H, $J = 3.2$ Hz, H_3-furan), 7.64 (d, 1H, $J = 4.0$ Hz, H_4-furan), 8.06 (d, 1H, $J_{H,F} = 13.6$ Hz, H_5-quinoline), 8.96 (s, 1H, H_2-quinoline), 15.25 (br s, 1H, COOH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.62, 46.49, 47.12, 51.84, 53.35, 107.97, 112.54, 113.22, 113.85, 119.24, 119.41, 144.67, 147.61, 149.73, 151.37, 156.30, 165.78, 176.19.

*9-Fluoro-3-methyl-10-[4-((5-nitrofuran-2-yl)methyl)piperazin-1-yl]-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de][1,4] benzoxazine-6-carboxylic acid (**4f**)* Yield 60 %; mp 175–177 °C; IR (KBr, cm^{-1}): ν_{max} 3437 (OH), 1723 and 1621 (C=O), 1498 and 1353 (NO_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.44 (d, 3H, $J = 6.4$ Hz, CH_3), 2.50–2.68 (m, 4H, piperazine), 3.34 (br s, 4H, piperazine), 3.73 (s, 2H, nitrofuran-CH_2–N), 4.35 (d, 1H, $J = 10.4$ Hz, OCH_a), 4.56 (d, 1H, $J = 11.2$ Hz, OCH_b), 4.85–4.98 (m, 1H, N_1–CH), 6.78 (d, 1H, $J = 3.6$ Hz, H_3-furan), 7.57 (d, 1H, $J = 12.4$ Hz, H_5-quinoline), 7.66 (d, 1H, $J = 3.6$ Hz, H_4-furan), 8.95 (s, 1H, H_2-quinoline), 15.16 (s, 1H, COOH).

*Synthesis of 1-cyclopropyl-6-fluoro-1,4-dihydro-7-[4-(4-nitrobenzyl)piperazin-1-yl]-4-oxoquinoline-3-carboxylic acid (**4g**)* To a mixture of ciprofloxacin (110 mg, 0.33 mmol) and sodium bicarbonate (28 mg, 0.33 mmol) in DMF (4 mL), 4-nitrobenzyl chloride (57 mg, 0.33 mmol) was added and the mixture was stirred at room temperature for 5 days. After completion of the reaction, water (15 mL) was added and the mixture was left in the refrigerator overnight. The precipitated solid was collected by filtration, washed with water, and dried to give pure compound **4g**. Yield 90 %; mp 252–253 °C; IR (KBr, cm^{-1}): ν_{max} 3441 (OH), 1721 and 1627 (C=O), 1520 and 1348 (NO_2); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.18 (br s, 2H, cyclopropyl), 1.31 (br s, 2H, cyclopropyl), 2.64 (br s, 4H, piperazine), 3.36 (s, 4H, piperazine), 3.73 (s, 2H, CH_2), 3.82 (br s, 1H, cyclopropyl), 7.57 (m, 1H, H_8-quinoline), 7.67 (d, 2H, $J = 7.7$ Hz, Ph), 7.89 (d, 1H, $J_{H,F} = 12.8$ Hz, H_5-quinoline), 8.22 (d, 2H, $J = 7.7$ Hz, Ph), 8.66 (s, 1H, H_2-quinoline), 15.18 (s, 1H, COOH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 7.52, 35.79, 49.38, 52.17, 60.77, 106.36, 106.65, 110.75, 110.93, 123.37, 129.75, 139.07, 145.06, 146.36, 146.58, 147.90, 151.97, 153.95, 165.89, 176.25.

Antibacterial activity

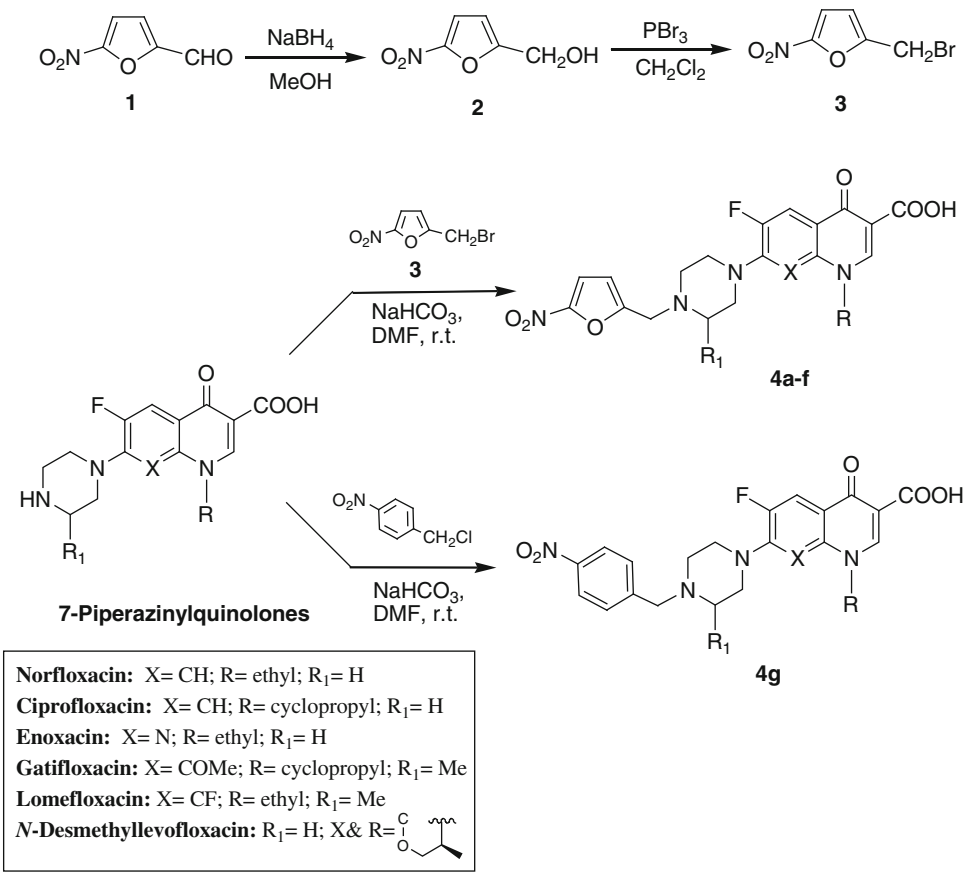
The minimum inhibitory concentrations (MICs) of the newly synthesized compounds **4a-g** were determined against Gram-positive (*S. aureus* ATCC 6538, *S. epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633) and Gram-negative (*E. coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Klebsiella pneumoniae* ATCC 10031) bacteria by the conventional agar dilution method (Baron and Finegold, 2002). Two-fold serial dilutions of the compounds and parent piperazinylquinolones were prepared in Mueller–Hinton agar. For preparation of stock solution, the test compounds (10.0 mg) were dissolved in DMSO (1 mL) and then diluted with water (9 mL). Further progressive double dilution with molten sterile 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, and 0.025 μg/mL. The medium containing the test compounds was dispensed into a sterile Petri dish. Then, the medium was allowed to solidify. Petridishes were inoculated with 1–5 × 10^4 CFU (colony-forming units) and incubated at 37 °C for 18 h. The MIC was defined as the lowest concentration of the test compound, which resulted in no visible growth on the plate.

Results and discussion

Chemistry

The synthesis of compounds **4a–g** is based on the synthetic routes shown in Scheme 1. Commercial available 5-nitro-2-furfural (**1**) was converted into 5-nitrofurfuryl alcohol (**2**) by sodium borohydride reduction in methanol. The reaction of alcohol **2** with phosphorus tribromide in dichloromethane afforded 2-(bromomethyl)-5-nitrofuran (**3**). The final compounds **4a–f** were prepared by the nucleophilic reaction of 7-piperazinylquinolones with 2-(bromomethyl)-5-nitrofuran (**3**) in the presence of $NaHCO_3$ in DMF. Similarly, the 4-nitrobenzyl analog **4g** was obtained by the reaction of ciprofloxacin with 4-nitrobenzyl chloride.

Scheme 1 Synthetic routes to intermediate 2-(bromomethyl)-5-nitrofuran (**3**) and target compounds **4a–g**



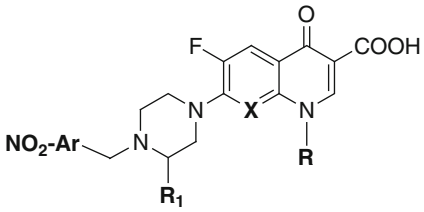
Antibacterial activity

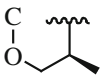
The growth inhibitory activities of target compounds **4a–g** were evaluated by the determination of their minimum inhibitory concentrations in comparison with reference quinolones. Table 2 presents MIC values of tested compounds against Gram-positive (*S. aureus* ATCC 6538, *S. epidermidis* ATCC 12228, *B. subtilis* ATCC 6633) and Gram-negative (*E. coli* ATCC 8739, *P. aeruginosa* ATCC 9027, *K. pneumonia* ATCC 10031) bacteria. In general, all compounds exhibited significant antibacterial activity against staphylococci at concentrations equal or less than 3.13 μg/mL.

Compounds **4a** and **4e** with MIC values of 0.39 μg/mL showed the best inhibitory activity against *S. aureus,* while compound **4f** was the most potent compound at the same concentration against *S. epidermidis*. In these cases, the observed activities were comparable to those of norfloxacin, enoxacin, and gatifloxacin. The MIC values of compounds against *B. subtilis* revealed that compound **4d** exhibited the highest activity (MIC = 0.097 μg/mL). Also, compounds **4b** and **4f** had good activity against *B. subtilis* as Gram-positive bacteria. Their activities were comparable to that of gatifloxacin. In the case of *E. coli*, again compound **4d** followed by compounds **4b** and **4f** showed better growth inhibitory activity. The comparison of MIC values against tested strains demonstrated that less susceptibilities were observed against *P. aeruginosa* (MICs = 3.13–50 μg/mL). Among the compounds, lower MIC value was observed for ciprofloxacin analog **4b** against the former Gram-negative strain. Furthermore, compound **4b** with MIC value of 0.39 μg/mL was the most potent compound against another Gram-negative bacteria *K. pneumonia*.

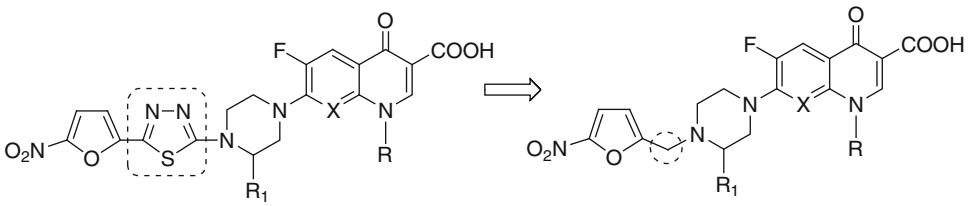
By comparing the MIC values of nitrofuran analog **4b** with its nitrophenyl counterpart **4g**, it is revealed that nitrofuran derivative **4b** has stronger activities against all tested microorganisms. As appeared from antibacterial data, the (5-nitrofuran-2-yl) attachment to the 7-piperazinylquinolones cannot improve the antibacterial profile with respect to the parent quinolones. However, the influence of this substitution against different bacterial species depends on the type of substituents at the N-1 and C-8 positions. For example, better results were obtained with ethyl at N-1 and CF at C-8 in the term of activity against *B. subtilis* and *E. coli*. While, optimum activity against *S. aureus*, *P. aeruginosa*, and *K. pneumonia* was provided by a molecule possessing cyclopropyl at N-1 and CH at C-8. The differences between SARs of the designed molecules and parent piperazinylquinolones, as well as particular effect of substituents pattern

Table 2 Minimum inhibitory concentrations (MICs, μg/mL) of compounds **4a–g** in comparison with standard quinolones against a panel of microorganisms



Compounds	NO_2–Ar	X	R	R_1	Microorganisms					
					S. a.	*S. e.*	*B. s.*	*E. c.*	*P. a.*	*K. p.*
4a	5-NO_2-Furan-2-yl-	CH	Et	H	1.56	3.13	12.5	6.25	25	6.25
4b	5-NO_2-Furan-2-yl-	CH	*c*-Pr	H	0.39	1.56	0.78	1.56	3.13	0.39
4c	5-NO_2-Furan-2-yl-	COMe	*c*-Pr	Me	0.78	1.56	3.13	3.13	6.25	1.56
4d	5-NO_2-Furan-2-yl-	CF	Et	Me	1.56	3.13	0.097	0.39	25	6.25
4e	5-NO_2-Furan-2-yl-	N	Et	H	0.39	3.13	6.25	6.25	12.5	3.13
4f	5-NO_2-Furan-2-yl-			H	1.56	0.39	0.78	0.78	12.5	1.56
4g	4-NO_2-Phenyl-	CH	*c*-Pr	H	3.13	3.13	3.13	3.13	50	12.5
Ciprofloxacin					0.195	0.195	0.195	0.097	0.39	0.049
Norfloxacin					0.39	0.39	0.195	0.195	1.56	0.39
Enoxacin					0.39	0.78	0.195	0.39	1.56	0.78
Gatifloxacin					0.78	0.78	0.78	1.56	0.78	0.195
Levofloxacin					0.39	0.39	0.78	–	3.13	0.39
N-Desmethyl levofloxacin					3.13	1.56	1.56	–	>6.25	0.39

S. a. Staphylococcus aureus ATCC 6538, *S. e.Staphylococcus epidermidis* ATCC 12228, *B. s. Bacillus subtilis* ATCC 6633, *E. c. E. coli* ATCC 8739, *P. a.Pseudomonas aeruginosa* ATCC 9027, *K. p. Klebsiella pneumonia* ATCC 10031



1,3,4-Thiadiazole-bridged nitrofuran **Methylene-bridged nitrofuran**

Fig. 3 Structural simplification of 1,3,4-thiadiazole-bridged nitrofuran to the methylene-bridged nitrofuran

on antibacterial activity serve them as new lead compounds for further optimization.

In the recent years, we have described several hybrids of 5-(nitroaryl)-1,3,4-thiadiazoles and different quinolones including ciprofloxacin, norfloxacin, enoxacin, gatifloxacin, and levofloxacin (Fig. 3), with enhanced antibacterial activity against some Gram-positive organisms (Foroumadi *et al.*, 2003, 2006b; Jazayeri *et al.*, 2009). In these hybrids, the enhancement of activity against Gram-positive was generally at the expense of activity against Gram-negative bacteria. Indeed, in the present study we simplified the former hybrids by replacing 1,3,4-thiadiazole with simple methylene bridge. This modification results in new molecules with lower molecular weight and preserving the basicity of the piperazine ring. Although, (5-nitrofuran-2-yl) methyl-piperazinylquinolones were not as active as 5-(nitroaryl)-1,3,4-thiadiazole hybrids, but the activity against Gram-negative bacteria was maintained.

Conclusions

In order to find new antibacterial agents, we have focused on introducing appropriate functional groups to the

piperazine ring of 7-piperazinylquinolones. For introducing new functionality through N-atom of 7-piperazinylquinolones, we selected methylene-bridged nitrofuran scaffold instead of former thiadiazole-bridged nitroheterocyles. The target compounds were directly prepared by the reaction of 7-piperazinylquinolones with the key intermediate 2-(bromomethyl)-5-nitrofuran. The results of antibacterial evaluation revealed that the influence of (5-nitrofuran-2-yl) attachment to the 7-piperazinylquinolones against different bacterial species depends on the type of substituents at the N-1 and C-8 positions. Better results were obtained with ethyl at N-1 and CF at C-8 in the term of activity against *B. subtilis* and *E. coli*. While, the optimum activity against *S. aureus*, *P. aeruginosa*, and *K. pneumonia* was entailed by a molecule possessing cyclopropyl at N-1 and CH at C-8. Consideration of the above findings suggests that further studies probing the in vivo utility of prototype molecules **4** as new antibacterial agents are promised.

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