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ORIGINAL ARTICLE

Synthesis and antibacterial evaluation of novel analogs of fluoroquinolones annulated with 6-substituted-2-aminobenzothiazoles

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Abstract Keeping in view the immense biological importance of fluoroquinolones and aminobenzothiazoles as antimicrobials and in search for better antibacterial agents, design and synthesis of new fluoroquinolone derivatives having substituted piperazine rings at the C-7 position are described in the present communication. The synthesized compounds were characterized by suitable spectroscopic methods. Most of the new compounds (**4a–l**) demonstrated high *in vitro* antibacterial activity with some compounds exhibiting more potent activities against Gram-positive organisms than those of ciprofloxacin, norfloxacin and gatifloxacin. The results of the present study reveal that the compounds have significant antibacterial potential and are suitable candidates for further exploration.

1. Introduction

Fluoroquinolones have been the landmark discovery in the treatment of bacterial infections (Srivastava et al., 2007). During recent years much attention has been devoted to the synthesis of new fluoroquinolones for their antibacterial activity (Jazayeri et al., 2009). Despite a large number of approved fluoroquinolones for treatment of various infections, there have been unabated efforts for the discovery of fluoroquinolones with specific properties, such as desired pharmacokinetic profile, therapeutic index and more importantly to overcome the problems of growing bacterial resistance (Srivastava et al.,

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2007). The major concern has been growing incidence of resistance especially to *Staphylococci* and *Enterococci*. Some of the side effects, such as central nervous system effects, phototoxicity, tendonitis, hypoglycemia and serious cardiac dysarrythmias of quinolone antibacterials are unacceptable, for example, grepafloxacin was withdrawn from market due to increased cases of heart problems in clinical findings. Similarly trovafloxacin was removed from the market due to liver toxicity (Graul and Rabasseda, 1999). Hence, there is an utmost need to develop newer fluoroquinolone analogs with enhanced potency and broad spectrum of activity.

Most of the quinolone antibacterial research has been focused on the functionality at C-7 position since the introduction of norfloxacin **1** and ciprofloxacin **2** (Figs. 1 and 2) (Foroumadi et al., 2005, 2006). Moreover, C-7 substituent is the most adaptable site for chemical intervention and is an area that determines the potency and target preferences. This area also controls the pharmacokinetic properties of the drug with basic nitrogen. A five or six membered ring is the most commonly employed substitution at position C-7, for example, gemifloxacin and trovafloxacin having aminopyrrolidine substituent at C-7 (Sharma et al., 2009, 2010; Domagala et al., 1988). Piperazine substituents at C-7 position have resulted in a wide range of clinically useful fluoroquinolone antibacterial agents namely norfloxacin, ciprofloxacin, perfloxacin, pefloxacin, ofloxacin, amifloxacin, fleroxacin, lomefloxacin, sparfloxacin, difloxacin, enoxacin, enrofloxacin, levofloxacin, marbofloxacin and orbifloxacin. Fluoroquinolones with 7-piperazinyl moiety have been reported to possess potent antibacterial activity (Foroumadi et al., 2005).

Mechanism of action and structure activity relationship studies of quinolones reveals that the site near the C-7 substituent is regarded as the drug enzyme interaction domain. In addition, it is also reported that the cell permeability is dominantly controlled by C-7 substituents. These facts motivated our concerns to develop some C-7 substituted analogs of quinolone. The piperazine moiety of fluoroquinolones possesses enough structural flexibility to allow product optimization (Foroumadi et al., 2006). Thus, we anticipated that safer and superior antibacterial compounds could be developed by attaching appropriate moiety through *N*-atom of the substituents at C-7 position. Hence, in the present communication we report the synthesis, characterization and antibacterial activity of some *N*-substituted piperazinyl quinolones by introducing specific substituents in the piperazine unit of 7-piperazinyl quinolones.

Small ring heterocycles bearing nitrogen, sulfur and oxygen have been under investigation for long time because of their important medicinal activities (Kumar et al., 2011). Moreover, 2-amino-6-substituted benzothiazoles are reported to show

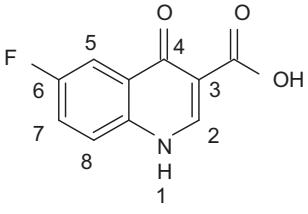


Figure 1 Quinolone core with main substitution sites.

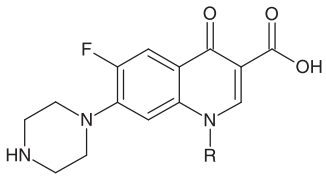


Figure 2 Structure of norfloxacin and ciprofloxacin. **1**, Norfloxacin R = ethyl **2**, Ciprofloxacin R = cyclopropyl.

cytotoxic, antibacterial, fungicidal, analgesic, antiinflammatory, antioxidant activities and quinolone derivatives are also found to possess antibacterial, antimycobacterial, antimalarial, antioxidant, analgesic and antiviral activities (Chu et al., 1985; Senthilkumar et al., 2009; Jayashree et al., 2009; El-Gazzar et al., 2009; Dinakaran et al., 2008; Winter et al., 2008; German et al., 2008; Sharma and Jain, 2008). Thus keeping in view these facts, the present study was aimed to achieve better antimicrobial profile at lower concentrations, by preparing *N*-substituted piperazinyl quinolone derivatives carrying benzothiazolyl substituents.

2. Experimental

2.1. General

All the chemicals and solvents used in this study were laboratory grade and procured from E. Merck (Germany), S. D. Fine Chemicals (India). Melting points were determined on a Labindia MR-VIS visual melting point apparatus and are uncorrected. The thin layer chromatography (TLC) plates (Silica Gel G) were used to confirm the purity of commercial reagents used, compounds synthesized and to monitor the reactions as well. Absorbance values against wavelength were taken on a Systronic double beam UV-166 spectrophotometer. The IR spectra were obtained on a Perkin Elmer IR spectrophotometer (KBr pellet). ^{1}H NMR spectra were recorded using Bruker 400 spectrometer and chemical shifts are expressed as δ (ppm) using tetramethylsilane as an internal standard in DMSO-d_6. Mass spectra of some selected compounds were obtained using a micromass-Q-Tof-micro spectrometer.

2.2. Chemistry

*2.2.1. General procedure for synthesis of 2-amino-6-substituted benzothiazole (**2a**–**e**)*

The 2-amino-6-substituted benzothiazoles (**2a**–**e**) were prepared by mixing *p*-substituted aniline (0.05 mol) (**1a**–**e**) and potassium thiocyanate (0.2 mol) in 90 ml of 96% acetic acid. To this was added drop wise, with stirring, a solution of bromine (0.05 mol) in glacial acetic acid (37.5 ml) and temperature was maintained below 35°C. After all the bromine solution was added, the mixture was stirred for another 10 h at room temperature and was filtered, the residue so obtained was washed with water. The combined filtrate and washings were neutralized with ammonium hydroxide solution. The precipitate thus obtained was collected and dried. Further purification was carried out by crystallization from benzene. Adopting the above procedure, five different 2-amino-6-substi-

tuted benzothiazoles (**2a**–**e**) have been synthesized. The purity of the benzothiazoles was checked by TLC.

*2.2.2. General procedure for synthesis of fluoroquinolone derivatives (**4a**–**l**)*

For synthesis of target fluoroquinolone derivatives (**4a**–**l**), a mixture of 2-amino-6-substituted benzothiazole (0.05 mmol) (**2a**–**e**), respective fluoroquinolone (0.05 mmol) (**3a**–**c**) and formalin (0.05 mmol) in acetic acid (10 ml), was refluxed for 10 h. After complete consumption of fluoroquinolone (monitored by thin layer chromatography), ammonia solution was added drop wise till neutralization and the precipitate was filtered and washed with water to yield the crude product. The purity of final compounds was ascertained by thin layer chromatography. By adopting the captioned procedure, twelve compounds (**4a**–**l**) were synthesized.

*2.2.2.1. 1-Ethyl-6-fluoro-7-(4-(N-(6-methoxy-1,3-benzothiazol-2-yl)aminomethyl)piperazin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**4a**)*. Mp 190–193 °C (63% yield). ^{1}H NMR (DMSO-d_6): δ/ppm = 1.37 (3H, J = 6.6, t, CH_3 ethyl), 2.47 (2H, J = 6.6, q, CH_2 ethyl), 3.12–3.57 (8H, m, piperazine), 3.76 (3H, s, OCH_3 benzothiazole), 5.68 (2H, s, CH_2 methylene bridge), 5.06 (1H, s, NH), 6.87–7.90 {5H, m, aromatic proton (H_5, H_8-quinolone and $H_{4'}$, $H_{5'}$, $H_{7'}$-benzothiazole)}, 8.91 (1H, s, H_2-quinolone), 15.12 (1H, s, COOH); IR (KBr): cm^{-1} = 1585 (C═C), 1710 (C═O), 1257 (C–O), 1057 (C–N), 2939–2831 (C–H), 3302 (COOH); MS: m/z = 511.6 (M^+).

*2.2.2.2. 1-Ethyl-6-fluoro-7-(4-(N-(6-nitro-1,3-benzothiazol-2-yl)aminomethyl)piperazin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**4b**)*. Mp 235–237 °C (66% yield). ^{1}H NMR (DMSO-d_6): δ/ppm = 1.39 (3H, J = 6.9, t, CH_3 ethyl), 2.29 (2H, J = 6.9, q, CH_2 ethyl), 3.17–3.62 (8H, m, piperazine), 5.32 (2H, s, CH_2 methylene bridge), 4.87 (1H, s, NH), 6.98–7.94 {5H, m, aromatic proton (H_5, H_8-quinolone and $H_{4'}$, $H_{5'}$, $H_{7'}$-benzothiazole)}, 8.68 (1H, s, H_2-quinolone), 15.01 (1H, s, COOH); IR (KBr): cm^{-1} = 1574 (C═C), 1718 (C═O), 1296 (C–O), 1126 (C–N), 3101-2854 (C–H), 3317 (COOH); MS: m/z = 526.5 (M^+).

*2.2.2.3. 1-Ethyl-6-fluoro-7-(4-(N-(6-methyl-1,3-benzothiazol-2-yl)aminomethyl)piperazin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**4c**)*. Mp 211–213 °C (68% yield). ^{1}H NMR (DMSO-d_6): δ/ppm = 1.35 (3H, J = 5.7, t, CH_3 ethyl), 2.31 (2H, J = 5.7, q, CH_2 ethyl), 3.01–3.64 (8H, m, piperazine), 2.46 (3H, s, CH_3 benzothiazole), 5.71 (2H, s, CH_2 methylene bridge), 5.10 (1H, s, NH), 6.98–7.89 {5H, m, aromatic proton (H_5, H_8-quinolone and $H_{4'}$, $H_{5'}$, $H_{7'}$-benzothiazole)}, 8.92 (1H, s, H_2 quinolone), 14.94 (1H, s, COOH); IR (KBr): cm^{-1} = 1585 (C═C), 1706 (C═O), 1257 (C–O), 1103 (C–N), 3024-2862 (C–H), 3294 (COOH); MS: m/z = 495.6 (M^+).

*2.2.2.4. 1-Ethyl-6-fluoro-7-(4-(N-(6-fluoro-1,3-benzothiazol-2-yl)aminomethyl)piperazin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**4d**)*. Mp 222–225 °C (70% yield). ^{1}H NMR (DMSO-d_6): δ/ppm = 1.38 (3H, J = 6.3, t, CH_3 ethyl), 1.89 (2H, J = 6.3, q, CH_2 ethyl), 3.03–3.61 (8H, m, piperazine), 5.75 (2H, s, CH_2 methylene bridge), 5.12 (1H, s, NH), 7.17–7.78 {5H, m, aromatic proton (H_5, H_8-quinolone and $H_{4'}$, $H_{5'}$, $H_{7'}$-benzothiazole)}, 8.90 (1H, s, H_2-quinolone), 14.88 (1H, s, COOH); IR (KBr): cm^{-1} = 1588 (C═C), 1718 (C═O), 1265 (C–O), 1134 (C–N), 3155–2839 (C–H), 3294 (COOH).

*2.2.2.5. 7-(4-(N-(6-Bromo-1,3-benzothiazol-2-yl)aminomethyl)piperazin-1-yl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**4e**)*. Mp 198–200 °C (59% yield). ^{1}H NMR (DMSO-d_6): δ/ppm = 1.29 (3H, J = 5.4, t, CH_3 ethyl), 1.96 (2H, J = 5.4, q, CH_2 ethyl), 2.94–3.59 (8H, m, piperazine), 5.62 (2H, s, CH_2 methylene bridge), 5.07 (1H, s, NH), 7.09–8.20 {5H, m, aromatic proton (H_5, H_8-quinolone and $H_{4'}$, $H_{5'}$, $H_{7'}$-benzothiazole)}, 9.03 (1H, s, H_2-quinolone), 14.98 (1H, s, COOH); IR (KBr): cm^{-1} = 1585 (C═C), 1682 (C═O), 1273 (C–O), 1080 (C–N), 3063 (C–H), 3271 (COOH).

*2.2.2.6. 1-Cyclopropyl-6-fluoro-7-(4-(N-(6-methoxy-1,3-benzothiazol-2-yl)amino methyl)piperazin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**4f**)*. Mp 267–270 °C (62% yield). ^{1}H NMR (DMSO-d_6): δ/ppm = 1.17 (4H, J = 7.2, d, CH_2–CH_2– cyclopropyl), 3.02–3.11 (1H, m, CH cyclopropyl), 3.37–3.80 (8H, m, piperazine), 3.89 (3H, s, OCH_3 benzothiazole), 5.71 (2H, s, CH_2 methylene bridge), 5.10 (1H, s, NH), 6.89–7.49 {5H, m, aromatic proton (H_5, H_8-quinolone and $H_{4'}$, $H_{5'}$, $H_{7'}$-benzothiazole)}, 8.68 (1H, s, H_2-quinolone), 14.89 (1H, s, COOH); IR (KBr): cm^{-1} = 1585 (C═C), 1710 (C═O), 1257 (C–O), 1057 (C–N), 2939–2831 (C–H), 3302 (COOH); MS: m/z = 523.6 (M^+).

*2.2.2.7. 1-Cyclopropyl-6-fluoro-7-(4-(N-(6-nitro-1,3-benzothiazol-2-yl)amino methyl)piperazin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**4g**)*. Mp 242–245 °C (63% yield). ^{1}H NMR (DMSO-d_6): δ/ppm = 1.27 (4H, J = 6.0, d, CH_2–CH_2– cyclopropyl), 3.07–3.15 (1H, m, CH cyclopropyl), 3.30–3.73 (8H, m, piperazine), 5.32 (2H, s, CH_2 methylene bridge), 5.00 (1H, s, NH), 7.21–7.87 {5H, m, aromatic proton (H_5, H_8-quinolone and $H_{4'}$, $H_{5'}$, $H_{7'}$-benzothiazole)}, 8.79 (1H, s, H_2-quinolone), 14.82 (1H, s, COOH); IR (KBr): cm^{-1} = 1584 (C═C), 1718 (C═O), 1260 (C–O), 1003 (C–N), 2916 (C–H), 3415 (COOH); MS: m/z = 538.6 (M^+).

*2.2.2.8. 1-Cyclopropyl-6-fluoro-7-(4-(N-(6-methyl-1,3-benzothiazol-2-yl)amino methyl)piperazin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**4h**)*. Mp 302–305 °C (71% yield). ^{1}H NMR (DMSO-d_6): δ/ppm = 1.17 (4H, J = 5.4, d, CH_2–CH_2– cyclopropyl), 3.06–3.14 (1H, m, CH cyclopropyl), 3.36–3.81 (8H, m, piperazine), 1.90 (3H, s, CH_3 benzothiazole), 5.74 (2H, s, CH_2 methylene bridge), 5.13 (1H, s, NH), 6.98–7.57 {5H, m, aromatic proton (H_5, H_8-quinolone and $H_{4'}$, $H_{5'}$, $H_{7'}$-benzothiazole)}, 8.69 (1H, s, H_2-quinolone), 15.00 (1H, s, COOH); IR (KBr): cm^{-1} = 1585 (C═C), 1710 (C═O), 1250 (C–O), 1095 (C–N), 2916 (C–H), 3279 (COOH).

*2.2.2.9. 1-Cyclopropyl-6-fluoro-7-(4-(N-(6-fluoro-1,3-benzothiazol-2-yl)amino methyl)piperazin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid **(4i)***. Mp 275–278 °C (66% yield). ^{1}H NMR (DMSO-d_6): δ/ppm = 1.13 (4H, J = 6.6, d, CH_2–CH_2– cyclopropyl), 3.09–3.18 (1H, m, CH cyclopropyl), 3.29–3.77 (8H, m, piperazine), 5.73 (2H, s, CH_2 methylene bridge), 5.12 (1H, s, NH), 6.97–7.76 {5H, m, aromatic proton (H_5, H_8-quinolone and $H_{4'}$, $H_{5'}$, $H_{7'}$-benzothiazole)}, 8.65 (1H, s, H_2-quinolone), 14.86 (1H, s, COOH); IR (KBr): cm^{-1} = 1583 (C═C), 1710 (C═O), 1211 (C–O), 1041 (C–N), 3070–2831 (C–H), 3410 (COOH).

*2.2.2.10. 7-(4-(N-(6-bromo-1,3-benzothiazol-2-yl)aminomethyl) piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**4j**)*. Mp 257-260 °C (65% yield). ^{1}H NMR (DMSO-d_6): δ/ppm = 1.18 (4H, J = 7.5, d, CH_2–CH_2– cyclopropyl), 3.08–3.18 (1H, m, CH cyclopropyl), 3.30–3.73 (8H, m, piperazine), 5.76 (2H, s, CH_2 methylene bridge), 5.01 (1H, s, NH), 7.02–7.98 {5H, m, aromatic proton (H_5, H_8-quinolone and $H_{4'}$, $H_{5'}$, $H_{7'}$-benzothiazole)}, 8.65 (1H, s, H_2-quinolone), 15.19 (1H, s, COOH); IR (KBr): cm^{-1} = 1586 (C═C), 1709 (C═O), 1258 (C–O), 1052 (C–N), 2932 (C–H), 3415 (COOH).

*2.2.2.11. 1-Cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-(N-(6-nitro-1,3-benzothiazol-2-yl)aminomethyl)piperazin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**4k**)*. Mp 226–229 °C (66% yield). ^{1}H NMR (DMSO-d_6): δ/ppm = 0.94 (4H, J = 7.2, d, CH_2–CH_2– cyclopropyl), 2.96–3.06 (1H, m, CH cyclopropyl), 3.29–3.65 (7H, m, piperazine), 2.47 (3H, s, CH_3 piperazine), 3.92 (3H, s, OCH_3), 5.73 (2H, s, CH_2 methylene bridge), 5.12 (1H, s, NH), 7.14–7.93 {4H, m, aromatic proton (H_5-quinolone and $H_{4'}$, $H_{5'}$, $H_{7'}$-benzothiazole)}, 8.76 (1H, s, H_2-quinolone), 14.86 (1H, s, COOH); IR (KBr): cm^{-1} = 1583 (C═C), 1710 (C═O), 1250 (C–O), 1196 (C–N), 3086 (C–H), 3387 (COOH); MS: m/z = 582.6 (M^+).

*2.2.2.12. 1-Cyclopropyl-6-fluoro-7-(4-(N-(6-fluoro-1,3-benzothiazol-2-yl)aminomethyl)-3-methylpiperazin-1-yl)-8-methoxy-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**4l**)*. Mp 182–184 °C (61% yield). ^{1}H NMR (DMSO-d_6): δ/ppm = 1.21 (4H, J = 7.8, d, CH_2–CH_2– cyclopropyl), 3.01–3.12 (1H, m, CH cyclopropyl), 3.26–3.72 (7H, m, piperazine), 2.39 (3H, s, CH_3 piperazine), 3.89 (3H, s, OCH_3), 5.73 (2H, s, CH_2 methylene bridge), 5.13 (1H, s, NH), 6.99–7.83 {4H, m, aromatic proton (H_5-quinolone and $H_{4'}$, $H_{5'}$, $H_{7'}$-benzothiazole)}, 8.69 (1H, s, H_2-quinolone), 15.13 (1H, s, COOH); IR (KBr): cm^{-1} = 1583 (C═C), 1712 (C═O), 1250 (C–O), 1196 (C–N), 3086 (C–H), 3279 (COOH).

2.3. Biological evaluation

2.3.1. Antibacterial activity assay

The newly synthesized compounds (**4a**–**l**) were evaluated for antibacterial activities using agar well diffusion method and by minimum inhibitory concentration (MIC) method Kumar et al., 2009; McFarland, 1907. Nutrient agar media and King's B media were used for the biological assay as per the following composition: Nutrient agar media (NAM) made up of peptone 5 g, beef extract 3 g, NaCl 5 g, nutrient agar 2% and the final volume of media was adjusted to 1000 ml with double distilled water (pH 7.0). King's B media containing peptone 2%, glycerol 1%, KH_2PO_4 0.15%, $MgSO_4$ 0.15%, agar 2% and the final volume of media was adjusted to 1000 ml with distilled water (pH 7.0). Synthesized compounds were screened for antibacterial activities against two Gram-positive bacteria, that is, *Staphylococcus auerus* (NCDC 110), *Bacillus subtilis* (NCDC 71) and two Gram-negative bacteria, that is, *Escherichia coli* (NCDC 134), *Pseudomonas aeruginosa* (NCDC 105). The bacterial cultures were revived as per the protocol provided by National Collection of Dairy Cultures (NCDC), Karnal, India. *P. aeruginosa* culture was maintained on King's B media while all other cultures were maintained on nutrient agar media. Suspension of each test organism was prepared to evaluate antibacterial activity of the synthetic compounds. All stock cultures were stored at 4 °C.

Each petri plate was prepared by pouring 40 ml of appropriate agar media. A fixed volume (100 µl) of respective microorganism was spread on each petri plate with the help of a spreader. In each seeded agar plate, wells were bored using a borer of 6 mm diameter. Three concentrations (100, 50, 10 µg/ml) of each compound reconstituted in dimethyl sulphoxide (DMSO) were added to the wells of seeded plates. DMSO was used as a control for all the experiments. The plates were kept in laminar air flow for 15 min to allow diffusion of the compounds into agar. The plates were incubated at 37 °C for 16 h and antibacterial activity was determined by measuring the diameter of inhibition zone. Each test was performed in triplicates and mean diameter of zone of inhibition was calculated. The results obtained were compared against three standard drugs, that is, ciprofloxacin, norfloxacin, gatifloxacin.

MIC of the synthesized compounds was determined using the method described by Kumar et al. (2009). A stock solution of 3 mg/ml of each compound was prepared in DMSO and further diluted to get a final concentration ranging from 200–0.05 µg/ml. Optical density was measured at 600 nm using UV-visible spectrophotometer. The minimum concentration, where no microbial growth was observed is called as MIC of the compound.

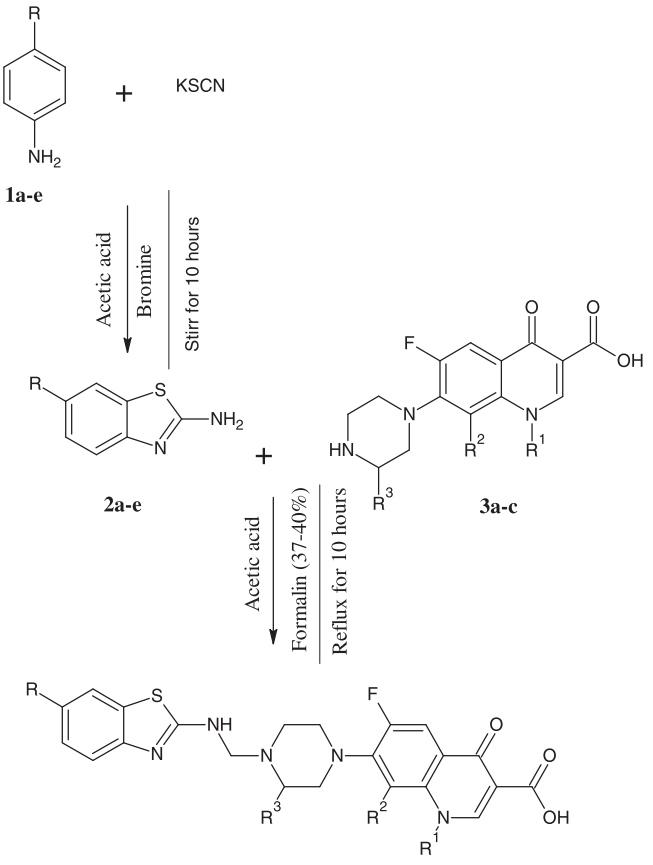
3. Results and discussion

Our synthetic route to target compounds is presented in Scheme 1. The requisite 2-amino-6-substituted benzothiazoles (**2a**–**e**) were prepared according to the method described by Stuckwisch (Stuckwisch, 1949). Reaction of fluoroquinolones, benzothiazole and formalin in the presence of glacial acetic acid afforded target compounds.

The newly synthesized compounds are characterized by spectral analysis (IR, ^{1}H NMR and mass). The ^{1}H NMR spectra showed characteristic peak in DMSO-d_6 at 5.3–5.7 δ (singlet 2H) –CH_2 methylene bridge, 4.8–5.2 δ (singlet 1H) of -NH, 8.6–8.9 δ (singlet 1H) of H_2 quinolone and 14.7–15.3 δ (broad singlet 1H) of –COOH group. Characteristic peaks in IR spectra were obtained at (KBr) ν cm^{-1} at approximately 1710, 1300, 3400, 2900, 3000 and 1600.

All the synthesized fluoroquinolone derivatives were screened for antibacterial activities against two Gram positive bacterial strains, that is, *S. auerus* (NCDC 110), *B. subtilis* (NCDC 71) and two Gram negative strains, that is, *E. coli* (NCDC 134), *P. aeruginosa* (NCDC 105). The antibacterial activity was determined by measuring the diameter of zone of inhibition and minimum inhibitory concentration (MIC). The results of zone of inhibition and MIC screening are summarized in Tables 1 and 2 respectively.

These novel derivatives demonstrated varying antibacterial activities (zone of inhibition) against different strains. Compound 1-cyclopropyl-6-fluoro-7-(4-(*N*-(6-fluoro-1,3-benzothiazol-2-yl)amino methyl)piperazin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**4i**), possessing fluoro functionality at 6-position of benzothiazole nucleus depicted the significant zone of inhibition of 9.8, 10.0 and 15.3 mm at 10, 50 and



Compound No.	R	R^1	R^2	R^3	Compound No.	R	R^1	R^2	R^3
4a	$-OCH_3$	$-C_2H_5$	-H	-H	**4g**	$-NO_2$		-H	-H
4b	$-NO_2$	$-C_2H_5$	-H	-H	**4h**	$-CH_3$		-H	-H
4c	$-CH_3$	$-C_2H_5$	-H	-H	**4i**	-F		-H	-H
4d	-F	$-C_2H_5$	-H	-H	**4j**	-Br		-H	-H
4e	-Br	$-C_2H_5$	-H	-H	**4k**	$-NO_2$		$-OCH_3$	$-CH_3$
4f	$-OCH_3$		-H	-H	**4l**	-F		$-OCH_3$	$-CH_3$

Scheme 1 Synthetic route of target compounds.

100 μg/ml concentrations, respectively, against *S. auerus*. Compounds **4g** and **4k** both have the nitro functional group at 6-position of benzothiazole moiety, also showed significantly potent antibacterial activities against *S. auerus*. However, compound 1-cyclopropyl-6-fluoro-7-(4-(*N*-(6-methyl-1,3-benzothiazol-2-yl)amino methyl)piperazin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**4h**) showed moderate activity. 6-Fluoro substituted benzothiazole containing derivatives of ciprofloxacin (**4i**) and gatifloxacin (**4l**) showed zone of inhibition of 4.5 mm at concentration 10 μg/ml against *B. subtilis*. Compounds **4f**, **4h**, **4j** and **4k** showed good antibacterial activities against *B. subtilis*. Analog 1-cyclopropyl-6-fluoro-7-(4-(*N*-(6-fluoro-1,3-benzothiazol-2-yl)aminomethyl)piperazin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**4i**) and 1-cyclopropyl-6-fluoro-7-(4-(*N*-(6-fluoro-1,3-benzothiazol-2-yl)aminomethyl)-3-methylpiperazin-1-yl)-8-methoxy-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**4l**) were found to possess even better antibacterial activities than the standard antibiotics norfloxacin and gatifloxacin when tested against *E. coli* and **4f** showed comparable activities at higher concentration (50 μg/ml and 100 μg/ml). Compounds **4d** and **4g** depicted superior antibacterial activities as compared to the standard antibiotic norfloxacin when tested against *P. aeruginosa*.

When tested against *S. auerus* ciprofloxacin annulated with 6-methoxy benzothiazole (**4f**) derivative showed MIC of 08 μg/ml, which reveals that it is approximately eight times more potent as compared to standard drug ciprofloxacin (MIC value 50 μg/ml). While some norfloxacin (**4a**, **4c**

Table 1 Zone of inhibition in mm at 10, 50 and 100 μg/ml concentrations.

Product	*S. auerus* (NCDC 110)			*B. subtilis* (NCDC 71)			*E. coli* (NCDC 134)			*P. aeruginosa* (NCDC 105)		
	10	50	100	10	50	100	10	50	100	10	50	100
4a	—	3.5	6.8	—	3.5	5.0	—	3.0	4.8	—	3.3	4.4
4b	—	—	—	—	—	—	—	3.8	3.8	—	—	—
4c	—	4	5	1.0	1.5	2.0	—	4.8	5.8	—	—	—
4d	—	—	4	—	—	—	—	2.0	2.8	3.3	3.8	4.8
4e	—	—	—	—	—	4.5	—	2.8	3.8	—	—	4.5
4f	2.3	7.5	8.8	3.0	6.0	6.5	3.3	7.8	10.0	—	5.3	8.0
4g	4.5	8.8	9.3	—	4.8	5.4	4.3	8.0	8.5	3.5	5.5	6.3
4h	5.0	8.8	11.5	3.5	7.5	8.0	4.3	7.5	7.5	—	3.0	7.3
4i	9.8	10.0	15.3	4.5	6.5	8.3	6.8	9.5	13.8	—	3.5	8.5
4j	2.0	6.3	9.0	3.2	5.5	8.0	4.0	7.0	9.0	—	—	—
4k	6.8	9.0	11.0	3.8	7.5	8.8	3.8	8.0	10.0	—	—	3.0
4l	—	7.5	10.5	4.5	8.0	9.0	6.5	10.0	11.5	—	—	—
Norfloxacin	9.2	10.5	12.5	5.0	8.0	10.2	4.2	8.5	10.8	1.0	2.0	7.2
Ciprofloxacin	8.0	11.0	13.0	8.0	11.0	12.2	8.2	10.5	11.3	4.5	8.0	10.9
Gatifloxacin	8.0	11.0	11.3	6.5	10.2	11.5	6.0	9.7	9.8	2.7	4.2	4.7
Control	—	—	—	—	—	—	—	—	—	—	—	—

Table 2 Minimum inhibitory concentration (MIC) in μg/ml against *S. auerus*, *B. subtilis*, *E. coli*, *P. aeruginosa* bacterial strains.

Product	*S. auerus* (NCDC 110)	*B. subtilis* (NCDC 71)	*E. coli* (NCDC 134)	*P. aeruginosa* (NCDC 105)
4a	25	50	30	30
4b	110	50	50	130
4c	20	90	05	80
4d	100	170	110	130
4e	10	110	01	20
4f	08	70	25	50
4g	150	80	100	20
4h	70	25	60	50
4i	80	130	50	50
4j	15	100	05	175
4k	50	50	30	35
4l	10	110	30	50
Norfloxacin	10	05	70	15
Ciprofloxacin	50	20	25	60
Gatifloxacin	05	100	100	50
Control	—	—	—	—

and **4e**) and gatifloxacin derivatives (**4k** and **4l**) showed a comparable activity. Compounds **4a**, **4b**, **4h** and **4k** showed significant MICs against *B. subtilis* when compared with standards. Moreover, compound **4h** possessing methyl group at 6-position of benzothiazole part of novel derivative showed better MIC (25 μg/ml) against *B. subtilis* than standard antibiotic gatifloxacin (MIC value 100 μg/ml). Derivative **4e** bearing bromo substitution at 6-position of benzothiazole nucleus, exhibited 25 times more potent activity with MIC value 01 μg/ml against strain *E. coli*, when compared to the standard drug ciprofloxacin (MIC value 25 μg/ml) and was 70 times more potent than standard drug norfloxacin (MIC value 70 μg/ml). Interestingly the same compound exhibited 100 times more potent MIC than standard drug gatifloxacin having MIC 100 μg/ml when tested against *E. coli*. Analog **4c**, **4e**, **4f** and **4j** showed superior MIC than the standard antibiotics ciprofloxacin, norfloxacin and gatifloxacin when tested against *E. coli*. Most of the all compounds showed comparable MIC against the standard antibiotics norfloxacin and gatifloxacin when tested against *P. aeruginosa*.

4. Conclusion

In conclusion, we have synthesised twelve novel derivatives of three fluoroquinolone drugs, that is, norfloxacin, ciprofloxacin and gatifloxacin. Fluoroquinolone drugs norfloxacin, ciprofloxacin and gatifloxacin have been linked to different 2-amino-6-substituted benzothiazoles via methylene bridge linkage. The derivatives are characterized by physicochemical and spectral analysis such as IR, ^{1}H NMR and mass. The spectral data obtained was in full agreement with proposed structures. The *in vitro* evaluation of newly synthesized compounds revealed improved therapeutic effectiveness as compared to the parent drugs. Some derivatives showed more

potent or equipotent antibacterial activities against the selected strains. Experimental data of the present studies reveal that the synthesized novel derivatives of fluoroquinolones have remarkable antibacterial potential.

Conflicts of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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