SYNTHESIS, MOLECULAR DOCKING AND ANTIBACTERIAL EVALUATION OF SOME NOVEL N-4 PIPERIDINYL DERIVATIVES OF SPARFLOXACIN

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ABSTARCT

Objective: The present study envisage a series of sparfloxacin derivatives were synthesized (Q_1-Q_{10}) with added derivatives such as aminomethyl benzenesulfenyl, methyl (methylamino)benzenesulfenyl, amino methyl benzoyl chloride, nitromethyl benzoyl chloride, dimethyl phenylamino, methoxymethyl phenylamino, dimethyl oxopyrazol, methyl dioxopyrrolidine, methyl oxopyrrolidine, and N-Boc amino methyl methylpyrrolidine through N-Piperzinyl linkage.

Methods: All the newly synthesized compounds were characterized by infrared,[1]H nuclear magnetic resonance, mass spectrometry, and elemental analysis technique, screened for docking stimulation to find out binding modes of synthesized derivatives with 3FV5 and 3IMW, and evaluated for *in vitro* antimicrobial activity.

Results: From this study, it was found that the compound Q_5 showed good antibacterial activity against Gram-positive (*Staphylococcus aureus*) and compound Q_4 showed good antibacterial activity against Gram-negative (*Escherichia coli*) in comparison with standard drugs (ciprofloxacin and sparfloxacin). The zone of inhibition and minimum inhibitory concentrations studies performed to synthesized compounds. The correlation between experimental data (minimum inhibitory concentrations) and docking score suggests that penetration for docking simulation is good to mild in reproducing experimental orientation of these synthesized compounds.

Conclusion: The analogs of sparfloxacin are suggested to be potent inhibitors with sufficient scope for further exploration.

Keywords: N-Piperzinyl derivatives, Ciprofloxacin, Sparfloxacin, DNA gyrase, Topoisomerase-IV, Docking studies.

INTRODUCTION

Quinolones have become a major class of antibacterial agents; they have an attraction because of their extremely potent activity, rapid bactericidal effects, and low incidence of resistance development.The main disadvantage of the quinolones is their limited activity against Gram-positive pathogens and methicillin-resistant *Staphylococcus aureus* [1,2]. In addition, quinolones can cause adverse effects, such as central nervous system effects, phototoxicity, tendonitis, hypoglycemia, and serious cardiac dysrhythmias [3,4]. Thus, despite many advances in the fluoroquinolones field, there exists continuous need for novel quinolones with better activity profile, pharmacokinetics, and tolerability, to overcome the limitation of existing drugs.The new generations of fluoroquinolones achieved significant improvement in potency, spectrum and physicochemical properties [5,6]. The structure-activity relationship studies revealed that the fluorine atom and the 1-alkyl, 1,4-dihydro-4-oxo-quinolone-3-carboxylic acid skeleton of fluoroquinolones are responsible for potency represented in binding with topoisomerase-II DNA gyrase and topoisomerase IV enzymes [7-9]. Topoisomerase II is a target for a variety of quinolones-based drugs. High activity against the eukaryotic type II enzyme is exhibited by drugs contains aromatic substituents at their C-7 position [10].

Sparfloxacin is a fluoroquinolones antibacterial agent active against Gram-positive and Gram-negative bacteria [11]. It has a controversial safety profile and about 37–45% bound to protein in the blood [12,13]. Its structure and ball-stick three-dimensional model have shown in Figs.1 and 2, respectively.

Skin penetration of sparfloxacin is good. The skin/Plasma ratio was about 1.00 at 4h (time of peak plasma concentration) and 1.39 at 5h [14]. The compound is used for treating community-acquired lower respiratory tract infection (acute sinusitis, exacerbations of chronic bronchitis caused by susceptible bacteria, and community-acquired pneumonia) [15-17].

The present study reports on the synthesis, spectroscopic analysis including IR and[1]H NMR, mass spectrometry and their biological activities of N-piperazinyl derivatives of sparfloxacin (Q_1-Q_{10}).

Molecular docking plays an important role in the rational design of drugs. In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Molecular docking can be defined as an optimization problem, which would describe the "best-fit" orientation of a ligand that binds to a particular protein of interest [18,19]. The main aim is to evaluate the possible relationship between docking activity of the synthesized compounds (Q_1-Q_{10}) along with interaction with the crystal structure of DNA gyrase of *S. aureus* [PDB:5IWM] and topoisomerase-IV of *Escherichia coli* [PDB ID: 3FV5].

METHODS

Experimental

Procedure for synthesis of (compoundQ_1) 7-(4-(4-amino-3-methyl benzenesulfenyl)-3,5-dimethylpiperazin-1-yl)-5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid, (compoundQ_2) 7-(4-(3-methyl-4-(methylamino) benzenesulfenyl)-3,5-dimethylpiperazin-1-yl)-5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid, (compound Q_7) 5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-(4-(4,5-
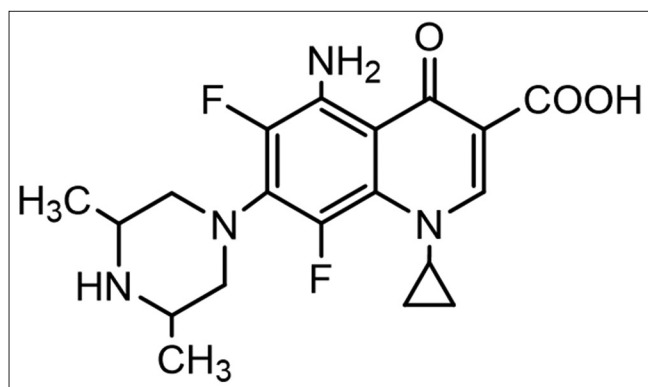
Fig. 1: Sparfloxacin [5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-(3,5-dimethyl piperazin-1-yi)-4-oxoquinoline-3-carboxylic acid]
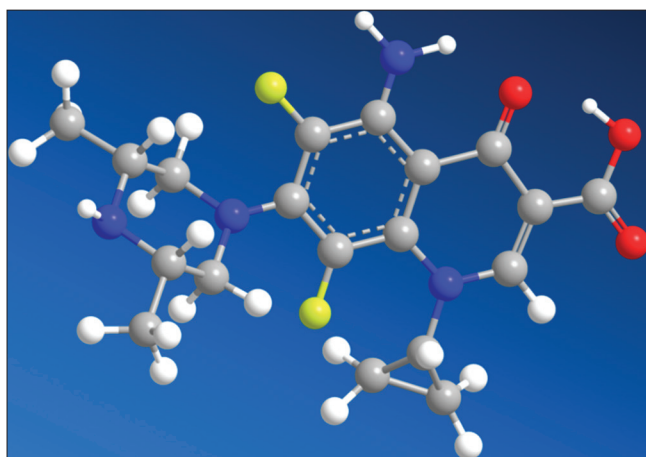

Fig. 2: Sparfloxacin (ball and stick three-dimensional model)

dihydro-3,4-dimethyl-5-oxopyrazol-1-yl)-3,5-dimethylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic acid, (compound Q_8) 5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-(3,5-dimethyl-4-(3-methyl-2,5-dioxopyrrolidin-1-yl)piperazin-1-yl)-4-oxoquinoline-3-carboxylic acid, (compound Q_9) 5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-(3,5-dimethyl-4-(3-methyl-2-oxopyrrolidin-1-yl)piperazin-1-yl)-4-oxoquinoline-3-carboxylic acid and (compound Q_{10}) 5-amino-7-(4-(3-(aminomethyl)-4-methylpyrrolidin-1-yl)-3,5-dimethylpiperazin-1-yl)-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid from Sparfloxacin (5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-(3,5-dimethyl piperazin-1-yi)-4-oxoquinoline-3-carboxylic acid):

To an equimolar amino methyl benzenesulfenyl (derivative Q_1) methyl (methylamino)benzenesulfenyl (derivative of Q_2), dimethyl oxopyrazol (derivative of Q_7), methyl dioxopyrrolidine (derivative of Q_8), methyl oxopyrrolidine (derivative of Q_9), N-Boc amino methyl methylpyrrolidine (derivative of Q_{10}), and sparfloxacin were added to ethanol, required quantity of formaline (37%) was added, and the reaction mixture was heated at reflux overnight, cooled. The resulting precipitated solid was filtered and recrystallized from 95% ethanol (100 ml) to give the product.

Procedure for synthesis of (compound Q_3) 7-(4-(4-amino-3-methylbenzoyl)-3,5-dimethylpiperazin-1-yl)-5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid and (compound Q_4) 7-(4-(4-nitro-3-methylbenzoyl)-3,5-dimethylpiperazin-1-yl)-5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid from Sparfloxacin (5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-(3,5-dimethyl piperazin-1-yi)-4-oxoquinoline-3-carboxylic acid):

To an equimolar mixture of finely powdered amino methyl benzoyl chloride (derivative of Q_3), nitromethyl benzoyl chloride (derivative of Q_4) and sparfloxacin were dissolved in 5% NaOH solution mix vigorously. The reaction mixture was warmed for an hour and allowed to cool for crystallization. The precipitate was filtered off, washed and dried under vacuum in a desiccator. Sparfloxacin reacts with derivatives in the presence of THF and TEA.

Procedure for synthesis of (compound Q_5) 5-amino-1-cyclopropyl-7-(4-((3,4-dimethylphenyl) amino)-3,5-dimethylpiperazin-1-yl)-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, (compound Q_6) 5-amino-1-cyclopropyl-6,8-difluoro-7-(4-((4-methoxy-3-methylphenyl) amino)-3,5-dimethylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid from Sparfloxacin (5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-(3,5-dimethyl piperazin-1-yi)-4-oxoquinoline-3-carboxylic acid):

To an equimolar mixture of sparfloxacin and sodium bicarbonate in 10ml of acetonitrile was stirred at 50°C for 4 h reaction mixture was cooled to 0°C and dimethyl phenyl amino (derivative of Q_5) and methoxy methyl phenyl amino (derivative of Q_6) were added. The mixture was stirred at magnetic stirrer for 5 h at 0–5°C acetonitrile was removed, the precipitate was dried by sodium sulfate, recrystallized in hexane acetone mixture.

Spectral data

Q_1: 7-(4-(4-amino-3-methylbenzenesulfenyl)-3,5-dimethylpiperazin-1-yl)-5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid

IR V_{max} (cm⁻¹ATR): 3530.6 (N-H), 3051.18 (CH Ar.), 3034.27 (CH cyclopropane), 2619.52 (O-H carboxyl), 1704.80 (C=O carboxyl), 1622.98 (C=C), 1383 (SO_2-Piperazine), 1342.61 (C-F), 1270.93 (C-N).¹H NMR (300 MHz; DMSO-d_6) δ:1.075–1.33 (m,4H, Cyclopropane), 1.11 (m,6H, methyl), 2.12(s,3H,methyl), 2.68(m,2H,methine), 3.2-3.5(q, 4H, methylene), 4.12(m, 1H, cyclopropane), 6.62 (s, 2H, amine), 6.96–7.24 (d, 3H, benzene), 7.51 (s, 2H,amine), 8.01 (s, 1H, ethylene), 15.12 (s, 1H, Carboxylic acid). MS-ESI: m/z 545.19 (M+1), elemental analysis (%): $C_{26}H_{29}F_2N_5O_4S$: C, 57.24; H, 5.36; F, 6.96; N, 12.84; O, 11.73; S, 5.88.

Q_2: 7-(4-(3-methyl-4-(methylamino)benzenesulfenyl)-3,5-dimethylpiperazin-1-yl)-5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid

IR V_{max} (cm⁻¹ATR): 3302.34(N-H), 2920.20 (CH), 2805 (O-H carboxyl), 1442.49 (C=C), 1342.61 (SO_2-Piperazine), 1256.41 (C-N), 1088.94 (C-F).¹H NMR (300 MHz; DMSO-d_6) δ:1.075–1.33 (m,4H, Cyclopropane), 1.11 (m,6H, methyl), 2.12 (s,3H, methyl), 2.68 (m,2H, methine), 3.2–3.5 (q, 4H, methylene), 4.12 (m, 1H, cyclopropane), 6.62 (s, 2H, amine), 6.96–7.24 (d, 3H, benzene), 7.24 (m, 2H, benzene), 7.51 (s, 2H, amine), 8.01 (s, 1H, ethylene), 15.12 (s, 1H, carboxylic acid). MS-ESI: m/z 555.18 (M+1), elemental analysis (%): $C_{27}H_{31}F_2N_5O_4S$: C, 57.24; H, 5.36; F, 6.96; N, 12.84; O, 11.73; S, 5.88.

Q_3: 7-(4-(4-amino-3-methylbenzoyl)-3,5-dimethylpiperazin-1-yl)-5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid

IR V_{max} (cm⁻¹ATR): 3370.87 (N-H), 3033.93 (CH Ar.), 2978.10 (O-H carboxyl), 2923.14 (C-H cyclopropane), 1703.94 (C=O carboxyl), 1627.27 (C=C), 1367.51 (SO_2-Piperazine), 1288 (N-H sec amine), 1265.08 (C-N), 1088.93 (C-F).¹H NMR (300 MHz; DMSO-d_6) δ:1.075-1.33 (m,4H, Cyclopropane), 1.31 (m,6H, methyl), 2.12 (s,3H, methyl), 3.465–3.71 (m,4H, methylene), 3.65 (m, 2H, methine), 4.12 (m, 1H, cyclopropane), 6.62 (s, 2H, amine), 6.93 (d, 1H, benzene),7.44 (d, 1H, benzene), 7.51 (s, 2H, amine), 7.66 (s,1H, benzene), 8.66 (s, 1H, ethylene), 14.93 (s, 1H, carboxylic acid). MS-ESI: m/z 525.22 (M+1), elemental analysis (%): $C_{27}H_{29}F_2N_5O_4$: C, 61.71; H, 5.56; F, 7.23; N, 13.33; O, 12.18.

Q_4: 7-(4-(4-nitro-3-methylbenzoyl)-3,5-dimethylpiperazin-1-yl)-5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxoquinoline-

3-carboxylic acid

IR V$_{max}$ (cm^{-1}ATR): 3069.64 (CH Ar.), 2871.71 (CH cyclopropane), 2725.33 (O-H carboxyl), 1679.00 (C=O carboxyl), 1416.37 (C=C), 1322.22 (C-F), 1272.36 (C-N).^{1}H NMR (300 MHz; DMSO-d$_6$) δ:1.075–1.33 (m,4H, Cyclopropane), 1.31 (m,6H, methyl), 2.64 (s,3H, methyl), 3.465-3.71 (m,4H, methylene), 3.65 (m, 2H, methine), 4.12 (m, 1H, cyclopropane), 6.62(s, 2H, amine), 8.01 (d, 1H, benzene), 8.07 (s, 1H, benzene), 8.30 (d, 1H, benzene), 8.66 (s, 1H, ethylene), 14.93 (s, 1H, carboxylic acid). MS-ESI: m/z 559.19 (M+1), elemental analysis(%): C$_{27}$H$_{27}$F$_2$N$_5$O$_6$: C, 58.38; H, 4.90; F, 6.84; N,12.61; O, 17.28.

Q$_5$: 5-amino-1-cyclopropyl-7-(4-((3,4-dimethylphenyl) amino)-3,5-dimethylpiperazin-1-yl)-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid

IR V$_{max}$ (cm^{-1}ATR): 3370.32(N-H), 3095.32 (CH Ar.), 2916.52 (C-H cyclopropane), 2652.54 (O-H carboxyl), 1665.33 (C=C), 1628.25 (C=O carboxyl), 1332.30 (C-F), 1271.09 (C-N).^{1}H NMR (300 MHz; DMSO-d$_6$) δ:1.075–1.33 (m,4H, Cyclopropane), 1.27 (m,6H, methyl), 2.19–2.21 (d,6H, methyl), 2.88 (m,2H, methine), 3.285–3.54 (q, 4H, methylene), 4.12 (m, 1H, cyclopropane), 6.62 (s, 2H, amine), 6.75–691 (d, 2H, benzene), 6.92 (d, 1H, benzene), 8.66 (s, 1H, ethylene), 8.78 (s,1H, sec amine), 14.93 (s, 1H, carboxylic acid). MS-ESI: m/z 511.24 (M+1), elemental analysis (%): C$_{27}$H$_{31}$F$_2$N$_5$O$_3$: C, 63.69; H, 6.11; F, 7.43; N, 13.69; O, 9.38.

Q$_6$: 5-amino-1-cyclopropyl-6,8-difluoro-7-(4-((4-methoxy-3-methylphenyl)amino)-3,5-dimethylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid

IR V$_{max}$ (cm^{-1}ATR): 3069.44 (C-H cyclopropane), 2946.43 (CH Ar.), 2871.73(O-H carboxyl), 2842.41 (OCH3), 1678.12 (C=O carboxyl), 1415.77 (C=C), 1272.00 (C-N), 1069.39 (C-F).^{1}H NMR (300 MHz; DMSO-d$_6$) δ: 1.075–1.33 (m,4H, Cyclopropane), 1.27 (m,6H, methyl), 2.15 (s,3H, methyl), 2.88 (m,2H, methine), 3.28–3.54 (q, 4H, methylene), 3.72 (s,3H, methyl), 4.12 (q, 1H, cyclopropane), 6.62(t, 2H, amine), 6.65–6.80 (d, 2H, benzene), 6.92(s, 1H, benzene), 8.66 (s, 1H, ethylene), 8.78 (s,1H, sec amine), 14.93 (s, 1H, carboxylic acid). MS-ESI: m/z 527.23 (M+1), elemental analysis (%): C$_{27}$H$_{31}$F$_2$N$_5$O$_4$: C,61.47; H, 5.92; F, 7.20; N, 13.27; O, 12.13.

Q$_7$: 5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-(4-(4,5-dihydro-3,4-dimethyl-5-oxopyrazol-1-yl)-3,5-dimethylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic acid

IR V$_{max}$ (cm^{-1}ATR): 3311.85 (N-H), 3057.23 (CH Ar.), 2933.28 (C-H cyclopropane), 2844.14(O-H carboxyl), 1625.38(C=O carboxyl), 1477.71(C=C), 1256.33(C-N), 1089.42(C-F).^{1}H NMR (300 MHz; DMSO-d$_6$) δ: 0.70 (d,3H, methyl), 1.075–1.33 (m,4H, Cyclopropane), 1.27 (m,6H, methyl), 1.94 (s,3H, methyl), 2.5 (q,1H, methine), 2.88 (m, 2H, methine), 3.28–3.54 (q,4H, methylene), 4.12 (m, 1H, cyclopropane), 6.62 (s, 2H, amine), 8.66 (s, 1H, ethylene), 14.93 (s, 1H, carboxylic acid). MS-ESI: m/z 502.41 (M+1), elemental analysis (%): C$_{24}$H$_{28}$F$_2$N$_6$O$_3$: C,57.36; H, 5.62; F, 7.56; N, 16.72; O, 12.73.

Q$_8$: 5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-(3,5-dimethyl-4-(3-methyl-2,5-dioxopyrrolidin-1-yl)piperazin-1-yl)-4-oxoquinoline-3-carboxylic acid

IR V$_{max}$ (cm^{-1}ATR): 3373.50 (N-H), 3084.24 (CH Ar.), 2915.38 (O-H carboxyl), 1628.46 (C=O carboxyl), 1470.32 (C=C), 1217.17 (C-N), 1025.73 (C-F).^{1}H NMR (300 MHz; DMSO-d$_6$) δ: 1.075–1.33 (m,4H, Cyclopropane), 1.17 (d,3H, methyl), 1.27 (m,6H, methyl), 2.61–2.85 (m,3H, succinimide), 2.88 (m,2H, methane), 3.28–3.54 (q,4H, methylene), 4.12 (m, 1H, cyclopropane), 6.62 (s, 2H, amine), 8.66 (s, 1H, ethylene), 14.93 (s, 1H, carboxylic acid). MS-ESI: m/z 505.90 (M+1), elemental analysis (%): C$_{24}$H$_{27}$F$_2$N$_5$O$_5$: C,57.25; H, 5.4; F, 7.55; N, 13.91; O, 15.89.

Q$_9$: 5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-(3,5-dimethyl-4-(3-methyl-2-oxopyrrolidin-1-yl)piperazin-1-yl)-4-

oxoquinoline-carboxylic acid

IR V$_{max}$ (cm^{-1}ATR): 3377.55 (N-H), 3084.48 (CH Ar.), 2849.11 (C-H cyclopropane), 2687.43 (O-H carboxyl), 1703.90 (C=O carboxyl), 1622.59 (C=C), 1342.49 (C-F), 1270.55 (C-N).^{1}H NMR (300 MHz; DMSO-d$_6$) δ: 1.075–1.33 (m,4H, Cyclopropane), 1.12 (d,3H, methyl), 1.27 (m,6H, methyl), 1.91–2.17 (m,2H,pyrrolidine), 2.33 (m,1H, pyrrolidine-2-one), 2.88 (m, 2H, methine), 3.24–3.34 (m,2H,pyrrolidine-2-one), 3.28–3.54 (q,4H, methylene), 4.12 (m, 1H, cyclopropane), 6.62 (s, 2H, amine), 8.66 (s, 1H, ethylene), 14.93 (s, 1H, carboxylic acid).MS-ESI: m/z 489.22 (M+1), elemental analysis(%): C$_{24}$H$_{29}$F$_2$N$_5$O$_4$: C,58.59; H, 5.97; F, 7.76; N, 14.31; O, 13.07.

Q$_{10}$: 5-amino-7-(4-(3-(aminomethyl)-4-methylpyrrolidin-1-yl)-3,5-dimethylpiperazin-1-yl)-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid

IR V$_{max}$ (cm^{-1}ATR): 3382.18 (N-H), 3084.70 (CH Ar.), 3100 (C-H cyclopropane), 2915.29 (O-H carboxyl), 1660.31 (C=O carboxyl), 1405.09 (C=C), 1332.37 (C-F), 1271.72 (C-N).^{1}H NMR (300 MHz; DMSO-d$_6$) δ: 0.93 (d,3H, methyl), 1.075–1.33 (m,4H, Cyclopropane), 1.27 (m,6H, methyl), 1.5 (m,2H,amine), 1.44 (s,1H,pyrrolidine), 1.59 (m,1H,pyrrolidine), 2.44–2.69 (q,2H, methylene), 2.6–2.81 (q,4H,pyrrolidine), 2.88 (m, 2H, methine), 3.28–3.54 (q,4H,methylene), 4.12 (m, 1H, cyclopropane), 6.62 (s, 2H, amine), 8.66 (s, 1H, ethylene), 14.93 (s, 1H, carboxylic acid). MS-ESI: m/z 504.27 (M+1), elemental analysis(%): C$_{25}$H$_{34}$F$_2$N$_6$O$_8$: C,59.51; H, 6.79; F, 7.53; N, 16.66; O, 9.51.

Biological evaluations

Antimicrobial activity

All the title compounds were screened for their antibacterial and antifungal activities. The antibacterial activity of the synthesized compounds was tested against two Gram-positive bacteria (*S. aureus* ATCC 6438P and *Staphylococcus epidermidis* ATCC 155) and two Gram-negative bacteria (*E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 29665) using nutrient agar medium. The antifungal activities of the compounds were tested against two fungi, namely *Aspergillus niger* ATCC 9029 and *Aspergillus fumigatus* ATCC 46645 using Sabouraud dextrose agar. For preliminary screening, the antimicrobial tests were carried out by the paper disc diffusion method. The minimum inhibitory concentrations (MIC) of the compounds were also determined by agar streak dilution method.

Paper disc diffusion technique

The sterilized [20] (autoclaved at 120°C for 30 min) medium (40–50°C) was inoculated (1 ml/100 ml of medium) with the suspension (10^5 cfu/ml) of the microorganism (matched to McFarland barium sulfate standard) and poured into a Petri dish to give a depth of 3–4 mm. The paper impregnated with the test compounds (100 μg/ml in dimethylformamide) was placed on the solidified medium. The plates were pre-incubated for 1 h at room temperature and incubated at 37°C for 24 and 48 h for antibacterial and antifungal activities, respectively. Ciprofloxacin (100 μg/disc) and ketoconazole (100 μg/disc) were used as a standard for antibacterial and antifungal activities, respectively. The observed zone of inhibition is presented in Table 1.

MIC

MIC [21] of the compound was determined by agar streak dilution method. A stock solution of the synthesized compound (100 μg/ml) in dimethylformamide was prepared, and graded quantities of the test compounds were incorporated in a specified quantity of molten sterile agar (nutrient agar for antibacterial activity and Sabouraud dextrose agar medium for antifungal activity). A specified quantity of the medium (40–50°C) containing the compound was poured into a Petri dish to give a depth of 3–4 mm and allowed to solidify. Suspension of the microorganism was prepared to contain approximately 10^5 cfu/ml and applied to plates with serially diluted compounds in dimethylformamide to be tested and incubated at 37°C for 24 h and

Table 1: Antimicrobial activity of the synthesized compounds (Q1-Q10) (100 μg/ml)

Compounds	*In vitro* activity - zone of inhibition in mm (MIC in μg/ml)					
	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	S. aureus	*S. epidermidis*	*E. coli*	*K. pneumoniae*	*A. niger*	*A. fumigatus*
Q1	28 (1.2)	25 (3.4)	23 (1.2)	24 (4.2)	18 (14.1)	23 (15.6)
Q2	29 (1.3)	24 (3.3)	26 (0.8)	24 (2.8)	19 (13.6)	24 (14.9)
Q3	29 (1.6)	26 (3.8)	26 (1.9)	22 (3.5)	17 (13.8)	23 (15.2)
Q4	30 (1.4)	26 (2.6)	30 (0.9)	31 (2.2)	18 (13.9)	20 (14.8)
Q5	32 (1.1)	31 (2.2)	22 (2.2)	20 (3.9)	23 (14.2)	23 (15.6)
Q6	32 (1.2)	28 (2.9)	23 (1.0)	22 (3.8)	21 (14.7)	21 (16.2)
Q7	24 (2.4)	24 (3.1)	27 (1.8)	24 (4.1)	25 (13.2)	25 (14.3)
Q8	21 (2.0)	25 (3.3)	29 (1.1)	27 (2.3)	25 (12.9)	24 (13.1)
Q9	29 (1.4)	27 (2.5)	28 (1.7)	26 (3.5)	26 (12.7)	27 (13.8)
Q10	28 (2.5)	21 (3.1)	28 (1.9)	23 (2.6)	22 (14.1)	21 (14.8)
Sparfloxacin[a]	34 (0.7)	33 (0.8)	34 (0.2)	33 (0.1)	-	-
Ciprofloxacin[a]	37 (0.5)	35 (0.12)	36 (0.06)	36 (0.06)	-	-
Ketoconazole[b]	-	-	-	-	29 (10.8)	33 (11.4)
DMF[c]	-	-	-	-	-	-

Sparfloxacin[a], Ciprofloxacin[a]: Standard antibacterial drugs, Ketoconazole[b]: Standard antifungal drug and DMF[c]: Control. *S. aureus: Staphylococcus aureus, S. epidermidis: Staphylococcus epidermidis, E. coli: Escherichia coli, K. pneumonia: Klebsiella pneumonia, A. niger: Aspergillus niger, A. fumigatus: Aspergillus fumigatus*. MIC: Minimum inhibitory concentrations
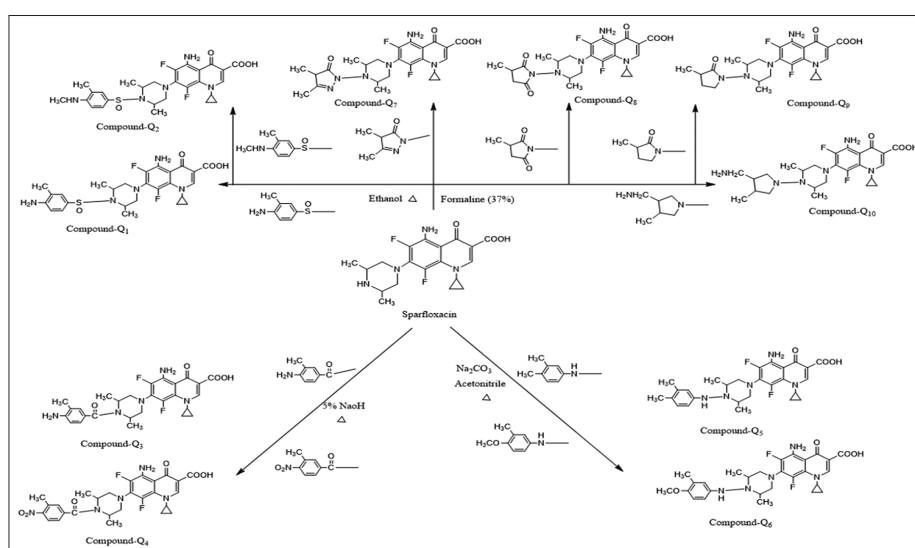
Scheme 1: Synthesis of N-Piperzinyl derivatives of sparfloxacin

48 h for bacteria and fungi, respectively. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate. The observed MIC is presented in Table 1.

Molecular docking studies of sparfloxacin
Molecular docking studies of synthesized compounds Q_1-Q_{10} with well-established structure of *S. aureus* and *E. coli* were performed using Auto Dock vina 1.12 version and chimera 1.12 version. The binding pocket of the active site of DNA gyrase (PDB: 5IWM) for Gram-positive bacteria like *S. aureus* and (PDB: 3FV5) for Gram-negative bacteria like *E. coli*. Docking method involves the following steps. First, the ligand molecule was build, in the second step required protein was downloaded from PDB, preparation, and validation of macromolecule by X-ray crystallography. Third step is the identification of binding affinity by the extent of binding of a ligand to the protein of molecule.

RESULTS AND DISCUSSION

Chemistry
The synthetic route to obtain the necessary derivative from commercially available reagent is briefly outlined in Scheme 1. The synthesized

compounds $(Q_1$-$Q_{10})$ were obtained with the help of various derivatives and reagents such as formaline (37%), 5% NaOH, Na_2CO_3, and acetonitrile. All reactions of synthesized compounds occurred with optimum temperature. The structure of all synthesized compounds was confirmed by IR,^{1}HNMR, and mass spectral elemental analysis techniques.

Antibacterial activity
All the synthesized compounds $(Q_1$-$Q_{10})$ were tested zone of inhibition and MIC values against two Gram-positive (*S. aureus* and *S. epidermidis*) and two Gram-negative (*E. coli* and K. Pneumonia) bacteria. All the compounds exhibited mild to moderate activity against both Gram-positive and Gram-negative bacteria. Compounds Q_5, Q_6, Q_4, and Q_3 were found to possess significant antibacterial activity against Gram-positive organisms when compared to standard drugs (ciprofloxacin and sparfloxacin). Compounds Q_4, Q_8, Q_9, and Q_{10} were found to possess significant antibacterial activity against Gram-negative organisms when compared to standard drugs (ciprofloxacin and sparfloxacin). The synthesized compounds exhibited MIC values in the range of 0.8–4.2 μg/ml shown in Table 1.

Compound Q_5 exhibited mild antibacterial activity with MIC value in the range of (1.12 μg/ml) when compared to standard sparfloxacin

Table 2: Docking result of synthesized compounds (Q$_1$-Q$_{10}$) with MIC values (µg/ml)

Compounds	Gram-positive bacteria (*S. aureus*)			Gram-negative bacteria (*E. coli*)		
	Docking Score[a]	MIC (µg/ml)	H bonds with 5IWM enzyme	Docking Score[a]	MIC (µg/ml)	Interactions with 3FV5 enzyme
Q1	−9.2	1.2	3rd position COOH oxygen with ALA138 and 5th position amino hydrogen with GLY963	−7.1	1.2	5th position amino hydrogen with ARG79
Q2	−9.2	1.3	5th position amino hydrogen with ASP475 and methyl (methylamino) benzenesulfenyl amino hydrogen with ALA459	−7.2	0.8	5th position amino nitrogen with ILE81
Q3	−9.4	1.6	3rd position COOH Oxygen with GLY958 and Amino methyl benzoyl hydrogen with LYS28	−7.0	1.9	5th position amino hydrogens with ARG124 And ASP56 and nitrogen with THR126
Q4	−9.5	1.4	3rd position COOH hydrogen with GLY287, 5th position amino hydrogens with ALA275 and GLY287 nitromethyl benzoyl oxygen with ILE312	−7.6	0.9	Nitromethyl benzoyl oxygen forms hydrogen bond with ALA47
Q5	−9.8	1.1	3rd position COOH Oxygen with MET294, 5th position amino hydrogen with GLY292 and dimethyl phenylamino hydrogen with GLY287	−7.2	2.2	5th position amino hydrogen with THR126
Q6	−9.6	1.2	4th Position Oxygen with GLY958 and 5th position amino hydrogens with GLU997 and PRO365 and amino nitrogen with GLY958	−7.1	1.0	3rd position COOH hydrogen with GLY59
Q7	−8.8	2.4	5th position amino hydrogen with GLY958 and Dimethyl oxopyrazol oxygen with ILE983	−7.2	1.8	Dimethyl oxopyrazol oxygen with ILE81
Q8	−8.6	2.0	3rd position COOH oxygen with PHE962 and Methyl dioxo pyrrolidine Piperazine Oxygen with ILE1154	−7.4	1.1	Methyl dioxo pyrrolidine Piperazine oxygen with ILE81
Q9	−8.6	1.4	5th position amino hydrogen with GLY958 and 3rd position COOH oxygen with ALA138	−7.4	1.7	Methyl oxo pyrrolidine Piperazine oxygen with SER82
Q10	−8.2	2.5	3rd position COOH oxygen with SER22 and ASP21 and Aminomethyl methyl pyrrolidine hydrogens with DA1349 and DG1369	−7.3	1.9	Aminomethyl methylpyrrolidine hydrogen with LEU75
Sparflo-xacin[b]	−8.2	0.7	3rd position COOH oxygen with ALA970 and 5th position amino hydrogens with ASP968 and SER964	−7.2	0.2	3rd position COOH hydrogen with HIS37, 5TH Position amino nitrogen with THR126 and hydrogen with THR126
Ciproflo-xacin[b]	−7.8	0.5	7th position Piperazine hydrogen with VAL1120 and 3rd position COOH oxygen with ALA790	−7.3	0.06	7th position Piperazine hydrogen with ASP56

[a]Based on Auto Dock Vina score, [b]Sparfloxacin, ciprofloxacin (standard drugs). MIC: Minimum inhibitory concentrations, *S. aureus: Staphylococcus aureus, E.Coli: Escheriachia coli*

(0.7 µg/ml) and ciprofloxacin (0.5 µg/ml). This mild antibacterial activity is maybe due to addition of new derivative dimethyl phenylamino group at the 7th position of piperzinyl ring.

Compound Q$_4$ exhibited mild Gram-negative antibacterial with MIC values in the range of (0.9 µg/ml) when compared to standard sparfloxacin (0.2 µg/ml) and ciprofloxacin (0.06 µg/ml). This mild antibacterial activity is maybe due to the addition of new derivative nitromethyl benzoyl group at the 7th position of piperzinyl ring.

Antifungal activity

All the synthesized compounds (Q$_1$-Q$_{10}$) were tested zone of inhibition and MIC values against two fungi organisms (*A. niger* and *A. fumigatus*).

All the compounds exhibited mild to moderate activity. Compounds Q$_9$, Q$_8$, and Q$_7$ were found to possess significant antifungal activity against *A. niger* when compared to standard ketoconazole. Compounds Q$_9$, Q$_2$, and Q$_7$ were found to possess significant antifungal activity against *A. fumigatus* when compared to standard drug ketoconazole. The synthesized compounds exhibited MIC values in the range of 12.7–16.2 µg/ml shown in Table 1.

Compound Q$_9$ exhibited mild antifungal activity with MIC values of (12.7 µg/ml) and (13.8 µg/ml) when compared to standard ketoconazole (10.8 µg/ml) and (11.4 µg/ml). This mild antifungal activity is maybe due to the addition of new derivative methyl oxopyrrolidine group at the 7th position of piperzinyl ring.
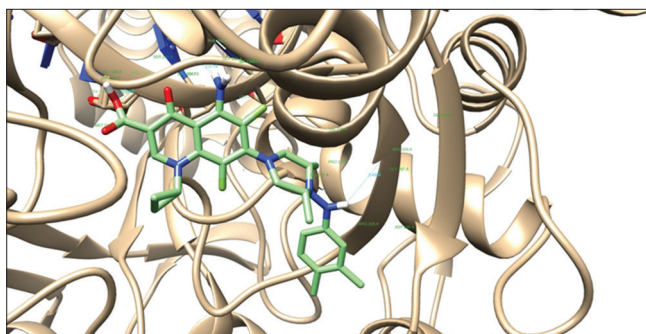
Fig. 3: H-bonds interactions between compound (Q$_5$) with topoisomerase-II DNA gyrase enzyme of Gram-positive *Staphylococcus aureus* bacteria (5IWM)
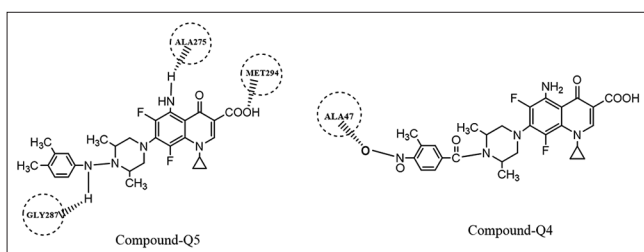

Fig. 4: Two-dimensional ligand interaction diagram of compounds Q$_5$ with DNA gyrase and Q$_4$ with *Escherichia coli*, the amino acid residues at the binding site are tagged in circles
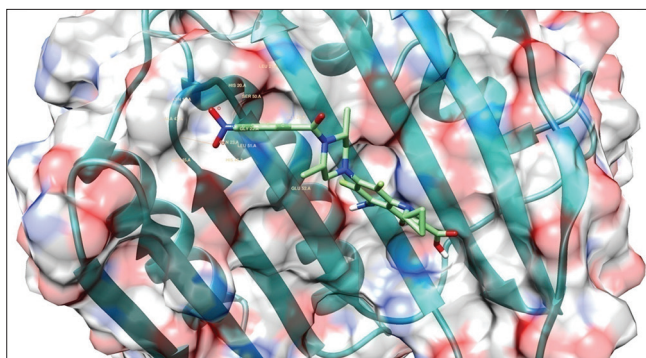

Fig. 5: H-bonds interactions between compound (Q$_4$) with topoisomerase-IV enzyme of Gram-negative *Escherichia coli* bacteria (3FV5)

Docking study

Molecular docking studies were employed for the analysis with a training set composed of our synthesized compounds whose inhibitory activity is unknown. To find out the molecular facilities responsible for biological activity molecular docking studies was performed. From the docking studies, we predicted that all the synthesized compounds (Q$_1$-Q$_{10}$) possess better antibacterial activity than the standard drugs (ciprofloxacin and sparfloxacin). By having good binding affinity with target protein, it could be used as a potential drug as antibacterial.

Gram-positive bacteria docking studies

Among all the docked compounds, Q$_5$, Q$_6$, Q$_4$, and Q$_3$ show good binding affinity and interaction with topoisomerase-II DNA gyrase enzyme (5IWM) with reference to standard drugs ciprofloxacin and sparfloxacin.

The interactions of H bonds with ligands and bacterial enzymes explained that Compound Q$_5$ at 3rd position carboxylic oxygen forms hydrogen bond with MET294, 5th position amino functional group forms
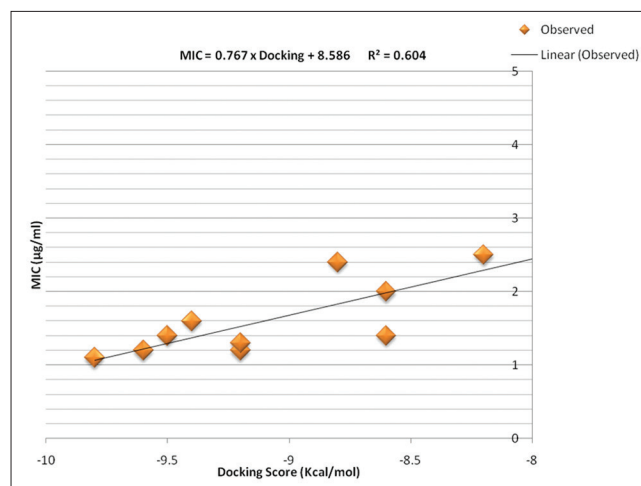

Fig. 6: Gram-positive (*Staphylococcus aureus*) correlation plot between minimum inhibitory concentrations (µg/ml) and docking scores (Kcal/mol)
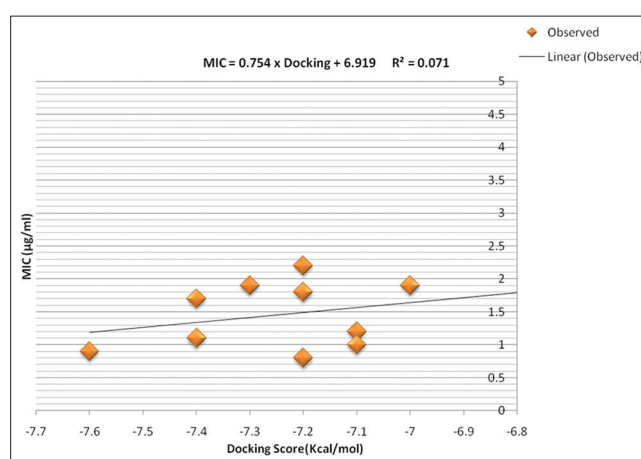

Fig. 7 Gram-negative (*Escherichia coli*) correlation plot between minimum inhibitory concentrations (µg/ml) and docking scores (Kcal/mol)

hydrogen bond with ALA275, 7th position piperzinyl ring attachment dimethyl phenylamino group, and one of the methyl group hydrogen forms hydrogen bond with GLY287 as shown in Fig. 3 and interactions are shown in Fig. 4.

Compound Q$_5$ having higher dock score (−9.8) toward bacterial *S. aureus* enzyme than the standard ciprofloxacin (−7.8) and sparfloxacin (−8.2) drugs. We may declare that the higher docking score is due addition of dimethyl phenylamino group at the 7th position of sparfloxacin structure. The remaining compounds docking score and hydrogen bond interactions are described in Table 2.

Gram-negative bacteria docking studies

Among all the docked compounds, Q$_4$, Q$_8$, Q$_9$, and Q$_{10}$ show good binding affinity and interaction with topoisomerase-IV enzyme (3FV5) with reference to standard drugs ciprofloxacin and sparfloxacin.

Compound Q$_4$ at 7th position piperzinyl ring attachment nitromethyl benzoyl oxygen forms hydrogen bond with ALA47 as shown in Fig. 5 and interactions are shown in Fig. 4.

Compound Q$_4$ is having higher affinity (−7.6) toward *E. coli* enzyme than the standard ciprofloxacin (−7.3) and sparfloxacin (−7.2) drugs. We may declare that the higher docking score is due addition

of nitromethyl benzoyl group at the 7th position of sparfloxacin structure. The remaining compounds docking score and hydrogen bond interactions are described in Table 2. The correlation between experimental data (MIC) and docking score of *S. aureus* and *E. coli* is displayed 0.604 r^2 and 0.071 r^2 (Figs. 6 and 7) which suggests that parameters for docking simulation are good for *S. aureus* and mild for *E. coli* in reproducing experimental orientation of synthesized compounds.

CONCLUSION

We have synthesized and characterized 10 new derivatives of sparfloxacin. All the molecules were studied for their interactions with topoisomerase-II DNA gyrase and topoisomerase-IV enzymes by molecular docking protocol. Among the tested molecules, Compounds Q_5, Q_6, Q_4, and Q_3 exhibited good docking score for Gram-positive bacteria and Compounds Q_4, Q_8, Q_9, and Q_{10} for Gram-negative bacteria. *In vitro,* antibacterial activity of tested compounds shows mild activity against microorganisms used. In particular, Compounds Q_5, Q_6, Q_4, and Q_3 possess significant Gram-positive activity and Compounds Q_4, Q_8, Q_9, and Q_{10} possess significant Gram-negative activity. The results of antibacterial activity are supported by docking analysis only for *S. aureus.*

AUTHOR'S CONTRIBUTION

Hemanth K Sudheer Kumar make contributions to the conception, design and implementation of the research, to the analysis of the results and to the writing of the manuscript. Parameshwar H helped to supervise the project and gave final approval of the written manuscript.

CONFLICTS OF INTEREST

The author declares that they have no conflicts of interest.

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