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

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ABSTRACT

Objective: The present study envisage a series of sparfloxacin derivatives were synthesized (Q1-Q10) with added derivatives such as aminomethyl benzensulfenyl, methyl (methyleneamino)benzenesulfenyl, aminomethyl benzyol chloride, nitromethyl benzyol chloride, dimethyl phenylamino, methoxymethyl phenylamino, dimethyl oxopyrazol, methyl dioxypropyrolidine, methyl oxopyrrolidone, and N-Boc amino methyl methylypyrrolidine through N-Piperazinyl linkage.

Methods: All the newly synthesized compounds were characterized by infrared, 1H 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 Q7 showed good antibacterial activity against Gram-positive (Staphylococcus aureus) and compound Q9 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-Piperazinyl 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, photosensitivity, tendinitis, 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 generation of fluoroquinolones achieved significant improvement in potency, spectrum and physicochemical properties [5,6].

The structure-activity relationship studies revealed that the fluorine atom of fluoroquinolones is 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 1R and 1H NMR, mass spectrometry and their biological activities of N-piperazinyl derivatives of sparfloxacin (Q1-Q10).

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 (Q1-Q10) 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 (compound Q7) 7-[4-(4-amino-3-methyl benzenesulfenyl)-3,5-diethylpyraperazin-1-yl]-5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid, (compound Q8) 7-[4-(3-methyl-4-(methylamino) benzenesulfenyl)-3,5-diethyl pyraperazin-1-yl]-5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid, (compound Q9) 5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-[4-(4,5-
To an equimolar mixture of finely powdered amino methyl benzoyl chloride (derivative of Q), nitromethyl benzoyl chloride (derivative of Q) 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), 7-(4-(3,4-dimethylphenyl)-1H-pyrazol-1-yl)-5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydroquinoline-3-carboxylic acid, (compound Q), 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-dimethylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic acid):

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

Spectral data

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

IR $\nu$ cm$^{-1}$: 3530.6 (N-H), 3051.18 (CH Ar.), 2920.20 (CH), 2805 (O-H carboxyl), 1622.98 (C=O carboxyl), 1367.51 (SO2). NMR (300 MHz; DMSO-d6) $\delta$: 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 555.18 (M+1), elemental analysis (%): C, 57.24; H, 5.36; F, 6.96; N, 12.84; O, 11.73; S, 5.88.

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

IR $\nu$ cm$^{-1}$: 3302.34 (N-H), 2920.20 (CH Ar.), 1442.49 (C=C), 1342.61 (SO2-Piperazine), 1256.41 (C-N). NMR (300 MHz; DMSO-d6) $\delta$: 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, 57.24; H, 5.36; F, 6.96; N, 12.84; O, 11.73; S, 5.88.

Q: 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 $\nu$ cm$^{-1}$: 3370.87 (N-H), 3033.93 (CH Ar.), 2978.10 (O-H carboxyl), 1627.27 (C-C), 1367.51 (SO2), 1256.41 (C-N). NMR (300 MHz; DMSO-d6) $\delta$: 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, 57.24; H, 5.36; F, 6.96; N, 12.84; O, 11.73; S, 5.88.

Q: 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 $\nu$ cm$^{-1}$: 3530.6 (N-H), 3051.18 (CH Ar.), 2920.20 (CH), 1704.80 (C=O carboxyl), 1627.27 (C=C), 1367.51 (SO2). NMR (300 MHz; DMSO-d6) $\delta$: 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 555.18 (M+1), elemental analysis (%): C, 57.24; H, 5.36; F, 6.96; N, 12.84; O, 11.73; S, 5.88.

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3-carboxylic acid

IR Vmax (cm⁻¹-ATR): 3069.64 (CH Ar), 2871.71 (CH cyclop propane), 2735.23 (O-H carboxy), 1679.00 (C=O carboxy), 1416.37 (C=C), 1322.22 (C-F), 1272.36 (C-N). H'NMR (300 MHz; DMSO-d₆) δ: 1.075–1.33 (3H, cyclopropane), 1.31 (3H, methyl), 2.64 (3H, methyl), 3.465–3.71 (3H, CH₄, methylene), 3.65 (m, 2H, methine), 4.12 (m, 1H, cyclop propane), 6.62 (s, 2H, amino), 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 (%): C₁₂H₁₄F₂N₂O₄; C, 58.59; H, 5.97; F, 7.76; N, 13.69; O, 9.38.

**Q.5:** 5-amino-1-cyclopropyl-7-(4-(3,4-dimethylphenyl) amino)-3,5-dimethylpiperazin-1-yl)-6,8-difluoro-1,4-di hydroy-3-carboxylic acid

IR Vmax (cm⁻¹-ATR): 3370.32 (CH Ar), 3093.32 (CH Ar), 2916.52 (CH cyclop propane), 2652.54 (O-H carboxy), 1665.33 (C=C), 1628.25 (C=O carboxy), 1332.30 (C-F), 1271.09 (C-N). H'NMR (300 MHz; DMSO-d₆) δ: 1.075–1.33 (3H, CH₄, cyclopropane), 1.27 (3H, methyl), 2.19–2.21 (6H, methyl), 2.88 (m, 2H, methine), 3.285–3.54 (4H, CH₂, methylene), 4.12 (m, 1H, cyclop propane), 6.62 (s, 2H, amino), 6.75–6.91 (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 512.14 (M+1), elemental analysis (%): C₁₅H₁₄F₂N₂O₄; C, 61.47; H, 5.92; F, 7.76; N, 14.31; O, 9.38.

**Q.6:** 5-amino-1-cyclopropyl-6,8-difluoro-7-(4-(methoxy-3 amino)-methoxy-3-aminomethyl)piperazin-1-yl)-3,5-dimethylpiperazin-1-yl)-6,8-difluoro-1,4-di hydroy-4-oxo-1,4-diazaquinoline-3-carboxylic acid

IR Vmax (cm⁻¹-ATR): 3069.44 (CH cyclop propane), 2946.43 (CH Ar), 2871.73(C-H carboxy), 2842.41 (OCH₃), 1678.12 (C=O carboxy), 1415.77 (C=C), 1272.00 (C-N), 1069.39 (C-F). H'NMR (300 MHz; DMSO-d₆) δ: 0.75–1.33 (3H, CH₄, cyclopropane), 1.27 (3H, methyl), 2.15 (3H, methyl), 2.88 (m, 2H, methine), 3.28–3.54 (4H, CH₂, methylene), 4.12 (m, 1H, cyclop propane), 6.62 (s, 2H, amino), 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 (%): C₁₅H₂₁F₂N₂O₄; C, 58.38; H, 4.90; F, 7.64; N, 12.61; O, 17.28.

**Q.7:** 5-amino-1-cyclopropyl-7-(4-(4-(3-methyl-2,5-dioxopyrrolidin-1-yl)-4-methylpiperazin-1-yl)-dihydroy-4-oxoquinoline-3-carboxylic acid

IR Vmax (cm⁻¹-ATR): 3382.18 (CH Ar), 3100 (CH cyclop propane), 2915.29 (CH=O carboxy), 1660.31 (C=O carboxy), 1405.09 (C=C), 1332.37 (C-F), 1271.72 (C-N). H'NMR (300 MHz; DMSO-d₆) δ: 0.93 (d, 3H, methyl), 1.075–1.33 (4H, cyclopropane), 1.27 (m, 6H, methyl), 1.5 (m, 2H, amino), 1.44 (s, 1H, pyrrolidine), 1.59 (m, 1H, pyrrolidine), 2.44–2.69 (q, 4H, methylene), 2.6–2.81 (q, 4H, pyrrolidine), 2.88 (m, 2H, methine), 3.28–3.54 (4H, CH₂, methylene), 4.12 (m, 1H, cyclop propane), 6.62 (s, 2H, amino), 8.66 (s, 1H, ethylene), 14.93 (s, 1H, carboxylic acid). MS-ESI: m/z 504.27 (M+1), elemental analysis (%): C₁₅H₂₂F₂N₂O₄; C, 59.51; H, 6.79; F, 7.75; 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 6538 and S. pneumoniae ATCC 29665) using nutrient agar medium. The antifungal activities of the compounds were tested against two fungi, namely Aspergillus niger ATCC 6323 and Aspergillus fumigatus ATCC 60645 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 (10⁵ 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 10⁵ cfu/ml and applied to plates with serially diluted compounds in dimethylformamide to be tested and incubated at 37°C for 24 h and...
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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 Q₁-Q₁₀ with well-established structure of S. aureus and E. coli were performed using AutoDock 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 (Q₁-Q₁₀) were obtained with the help of various derivatives and reagents such as formaline (37%), 5% NaOH, Na₂CO₃, and acetonitrile. All reactions of synthesized compounds occurred with optimum temperature. The structure of all synthesized compounds was confirmed by IR, ¹HNMR, and mass spectral elemental analysis techniques.

Antibacterial activity

All the synthesized compounds (Q₁-Q₁₀) 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 Q₅, Q₆, Q₄, and Q₃ were found to possess significant antibacterial activity against Gram-positive organisms when compared to standard drugs (ciprofloxacin and sparfloxacin). Compounds Q₄, Q₈, Q₉, and Q₁₀ 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 Q₅ exhibited mild antibacterial activity with MIC value in the range of (1.12 µg/ml) when compared to standard sparfloxacin.

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) |
|-----------|----------------------------------------------------------|
| S. aureus | S. epidermidis | E. coli | K. pneumonia | 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<sup>a</sup> | 34 (0.7) | 33 (0.8) | 34 (0.2) | 33 (0.1) | - | - |
| Ciprofloxacin<sup>a</sup> | 37 (0.5) | 35 (0.12) | 36 (0.06) | 36 (0.06) | - | - |
| Ketoconazole<sup>b</sup> | - | - | - | - | 29 (10.8) | 33 (11.4) |
| DMF<sup>c</sup> | - | - | - | - | - | - |

Sparfloxacin<sup>a</sup>, Ciprofloxacin<sup>a</sup>: Standard antibacterial drugs, Ketoconazole<sup>b</sup>: Standard antifungal drug and DMF<sup>c</sup>: 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-Piperazinyl 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.

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Compound Q₅ 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 (Q1-Q10) with MIC values (µg/ml)

| Compounds | Gram-positive bacteria (S. aureus) | Gram-negative bacteria (E. coli) |
|-----------|----------------------------------|---------------------------------|
|           | Docking Score | MIC (µg/ml) | H bonds with 5IWM enzyme | Docking Score | 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) benzene sulfonyl 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 benzyol hydrogen with LYS28 | -7.0          | 1.9         | 5th position amino hydrogen 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 benzyl 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 diox pyrrolidine Piperazine Oxygen with ILE1154 | -7.4          | 1.1         | Methyl diox 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 ox 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 methyl pyrrolidine hydrogen with LEU75 |
| Sparflo-xacin | -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 | -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 |

Based on Auto Dock Vina score, Sparfloacin, ciprofloxacin (standard drugs). MIC: Minimum inhibitory concentrations, S. aureus: Staphylococcus aureus, E.Coli: Escherichia coli

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

Compound Q₄ 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 may be due to the addition of new derivative nitromethyl benzoyl group at the 7th position of piperazinyl ring.

Antifungal activity
All the synthesized compounds (Q₁-Q₁₀) 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 Q₉, Q₉, and Q₇ were found to possess significant antifungal activity against A. niger when compared to standard ketoconazole. Compounds Q₉, Q₇, and Q₇, 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 Q₉ 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 piperazinyl ring.
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 (Q1-Q10) 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, Q5, Q6, Q4, and Q3 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 Q5 at 3rd position carboxylic oxygen forms hydrogen bond with MET294, 5th position amino functional group forms hydrogen bond with ALA275, 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 Q5 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.

Fig. 3: H-bonds interactions between compound (Q5) with topoisomerase-II DNA gyrase enzyme of Gram-positive Staphylococcus aureus bacteria (5IWM)

Fig. 4: Two-dimensional ligand interaction diagram of compounds Q5 with DNA gyrase and Q4 with Escherichia coli, the amino acid residues at the binding site are tagged in circles

Fig. 5: H-bonds interactions between compound (Q4) with topoisomerase-IV enzyme of Gram-negative Escherichia coli bacteria (3FV5)

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 piperazinyl 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 Q4 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, Q4, Q9, and Q10 show good binding affinity and interaction with topoisomerase-IV enzyme (3FV5) with reference to standard drugs ciprofloxacin and sparfloxacin. Compound Q4 at 7th position piperazinyl ring attachment nitromethyl benzoyl oxygen forms hydrogen bond with ALA47 as shown in Fig. 5 and interactions are shown in Fig. 4.

Compound Q4 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 sparflloxacin 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 r² and 0.071 r² (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 sparflloxacin. 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 Q₆, Q₇, Q₈, and Q₉ exhibited good docking score for Gram-positive bacteria and Compounds Q₄, Q₅, Q₆, and Q₇ for Gram-negative bacteria. In vitro, antibacterial activity of tested compounds shows mild activity against microorganisms used. In particular, Compounds Q₂, Q₇, and Q₉ possess significant Gram-positive activity and Compounds Q₂, Q₄, Q₅, and Q₇ 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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