Antibacterial and Antifungal Activities of Some E. oisomerase and compound O6 showed good antibacterial di trachomatis and urea plasma bounds (O1 compounds. The correlation between experimental data (minimum inhibition 134 31 3 18,19 luteus, and Staphylococcus aureus, like other quinolones, 20,21 It has recently been reported 36 Gatifloxacin, Ciprofloxacin, Levofloxacin inhibited members of the family Enterobacteriaceae comparably. 25,26 Gatifloxacin is more potent than ciprofloxacin against methicillin-resistant S. Aureus (MRSA), 26 although clinical significance of this still needs to be investigated. Gatifloxacin was recently investigated for the treatment of non-gonococcal urethritis. 27 Gatifloxacin is usually similar to moxifloxacin and levofloxacin and more active than ciprofloxacin against susceptible streptococci. 28,30 Dysglycemia has been noted as the life-threatening adverse effect of gatifloxacin, which led to its withdrawal from the market in the united states in 2006. 31 Gatifloxacin is reportedly highly active against atypical genitourinary pathogens such as chlamydia trachomatis and urea plasma urealyticum. 32,33 It is extensively distributed into many tissues and body fluids. 34-36 Due to structural characteristics, of gatifloxacin mechanism of killing action is not dependent on bacterial life cycle. 37

The present study reports on the synthesis, spectroscopic analysis including IR and 1H NMR, mass spectrometry and their biological activities of N-piperazinyl derivatives of gatifloxacin (O1-O10). Molecular docking was performed,
Molecular docking plays an important role in the rational design of drugs.

![Figure 1: Gatifloxacin [1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid]](image)

**Figure 1**: Gatifloxacin [1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid]

**Figure 2**: Gatifloxacin (ball and stick 3D model)

In the field of molecular modelling, docking 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. Docking the goal of ligand and protein docking was mainly used to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure, with invitro antimicrobial results in hand, we thought it worthwhile to perform studies to support the result. The main aim was to evaluate the possible relationship between docking activity of the synthesized compounds (O1-O5) along with interaction with crystal structure of DNA gyrase of S.Aureus [PDB:5IWM] and topoisomerase-IV of E.Coli [PDB ID: 3FVS].

**MATERIAL AND METHODS**

**Chemistry**

Chemicals, solvents and thin layer chromatography used in our work were obtained from chemical company E-Merck. The melting points were determined using the melting point apparatus (Bio-techno lab) in opened glass capillary tubes and were uncorrected.Infra-red spectra was listed as KBR powder using attenuated total reflectance (ATR) (Brucker). Mass spectroscopy was carried out to determine molecular weight of compound using GCMC QP5000 (shimadzu). 1HNMR was carried out to assign structure of synthesized compound and were run on JEOL GX400 spectrometer in DMSO-d6.

**Experimental**

Procedure for synthesis of (compound O3) 7-(4-(4-amino-3-methylphenyl)sulfinyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, (compound O2) 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-((3-methyl-4-(methylamino)phenyl)sulfinyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoiline-3-carboxylic acid, (compound O1) 1-cyclopropyl-7-(4-(3,4-dimethyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, (compound O5) 1-cyclopropl-6-fluoro-8-methoxy-7-(3-methyl-4-(3-methyl-2,5-dioxo pyrrolidin-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, (compound O4) 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-(3-methyl-2-oxopyrrolidin-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid and (compound O6) 7-(4-(3-(aminomethyl)-4-methyl pyrrolidin-1-yl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid from gatifloxacin [1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid]:

To an equimolar amino methyl benzenesulfenyl (Derivative O1) methyl (methylamino)benzenesulfenyl (Derivative of O2), dimethyl oxopyrazol (Derivative of O3), methyl dioxopyrrolidine (Derivative of O4), methyl oxopyrrolidine (Derivative of O5), N-Boc amino methyl methyl pyrrolidine (Derivative of O6) and gatifloxacin were added to ethanol, required quantity of formaline (37%) was added and the reaction mixture was heated at reflux overnight, cooled. The resulting precipitate solid was filtered and recrystallized from 95% ethanol (100ml) to give the product.

Procedure for synthesis of (compound O1) 7-(4-(4-amino-3-methylbenzoyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid and (compound O2) 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-(3-methyl-4-nitrobenzoyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid from gatifloxacin [1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid]:

To an equimolar mixture of finely powdered amino methyl benzyol chloride (Derivative of O3), nitro methyl benzoyl chloride (Derivative of O4) and Gatifloxacin was dissolved in 5% NaOH solution and was mixed 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. Gatifloxacin reacted with derivatives in the presence of THF and TEA.

Procedure for synthesis of (compound O3) 1-cyclopropyl-7-(4-((3,4-dimethylphenyl)amino)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, (compound O4) 1-cyclopropyl-6-fluoro-8-methoxy-7-(4-((4-methoxy-3-methylphenyl)amino)-3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid from gatifloxacin [1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid]:

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To an equimolar mixture of Gatifloxacin and sodium bicarbonate in 10ml of acetoneitrile was stirred at 50°C for 4hrs. Reaction mixture was cooled to 0°C and dimethyl phenyl amino (Derivatve of Oxi) and methoxy methyl phenyl amino (Derivative of Oxi) was added. The mixture was stirred at magnetic stirrer for 5hrs at 0-5°C. Acetonitrile was removed, the precipitate was dried by sodium sulphate, recrystallized in hexane acetone mixture.

**Spectral data**

7-(4-((4-amino-3-methylphenyl)sulfinyl)-3-methyl piperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo, 1,4-dihydroquinoline-3-carboxylic acid (Oxi): yield: 83%; m.p: 242-245°C. IR V_max (cm⁻¹): 3376 (N-H), 1633 (C=C), 1634 (C-F), 242-245°C. NMR (300 MHz; DMSO-d₆): δ: 2.06, 2.01 (2H, methylene), 2.73-2.83 (2H, methylene), 3.13-3.17 (2H, methylene), 3.28-3.54 (q, 2H, methylene), 3.83 (3H, methylene), 4.12 (m, 1H, cyclopropane), 6.72 (s, 1H, sec. amine), 6.83 (d, 1H, benzene), 7.29 (q, 2H, benzene), 7.69 (d, 1H, benzene), 8.01 (s, 1H, ethylene), 15.12 (s, 1H, Carbonylic acid). MS-EI: m/z 542.20 (M+1), Elemental analysis (%): C, 72.2%; H, 6.75; F, 2.88; N, 11.33; O, 9.62.

1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-(3,4-dimethylphenylamino)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo,1,4-dihydroquinoline-3-carboxylic acid (Oxi): yield: 68%; m.p: 250-272°C. IR V_max (cm⁻¹): 3301 (C=O carbonyl), 2545(OH Carbonyl), 1737(C=O carbonyl), 1628(C=C), 1332(C-F), 1271(C=N Piperazine). NMR (300 MHz; DMSO-d₆): δ: 1.075-1.33 (3H,4H, Cyclopropane), 1.11 (m,3H, methyl), 2.12(s,3H, methyl), 2.12(s,3H, methyl), 2.66 (m, 2H, methyne), 2.73-2.8 (m, 2H, methylene), 3.32-3.17 (m,2H, methylene), 3.73-3.54 (q, 2H, methylene), 3.83 (3H, methylene), 4.12 (m, 1H, cyclopropane), 6.96 (d, 1H, benzene), 7.24 (d, 1H, benzene), 7.51 (s, 1H, amine), 7.69 (d, 1H, benzene), 8.66 (s, 1H, ethylene), 14.93 (s, 1H, Carbonylic acid). MS-EI: m/z 508.16 (M+1), Elemental analysis (%): C, 72.2%; H, 6.75; F, 2.88; N, 11.33; O, 9.62.

1-cyclopropyl-7-(4-((3,4-dimethylphenylamino)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo,1,4-dihydroquinoline-3-carboxylic acid (Oxi): yield: 68%; m.p: 250-272°C. IR V_max (cm⁻¹): 3301 (C=O carbonyl), 2545(OH Carbonyl), 1737(C=O carbonyl), 1628(C=C), 1332(C-F), 1271(C=N Piperazine). NMR (300 MHz; DMSO-d₆): δ: 1.075-1.33 (3H,4H, Cyclopropane), 1.27 (s,3H, methyl), 2.12(s,3H, methyl), 2.88 (m,1H, methyne), 3.13-3.17 (m,2H, methylene), 3.28-3.54 (q, 2H, methylene), 3.83 (3H, methylene), 4.12 (m, 1H, cyclopropane), 6.75 (d, 1H, benzene), 6.91 (d, 1H, benzene), 6.92 (d, 1H, benzene), 7.69 (d, 1H, benzene), 8.66 (s,1H, ethylene), 8.78 (s,1H, sec. amine), 14.93 (s, 1H, Carbonylic acid).MS-EI: m/z 494.23 (M+1), Elemental analysis (%): C, 72.2%; H, 6.75; F, 2.88; N, 11.33; O, 9.62.

1-cyclopropyl-6-fluoro-8-methoxy-7-(3-(methyl-4-(3,4-dimethylphenylamino)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo,1,4-dihydroquinoline-3-carboxylic acid (Oxi): yield: 58%; m.p: 286-288°C. IR V_max (cm⁻¹): 3069(CH Ar.), 2870(CH Cyclopropane), 2821(OH Cyclopropane), 1657(C=O carbonyl), 1598(C=C), 1322(C-F), 1284(Sec Aminc), 1271(C=N Piperazine). NMR (300 MHz; DMSO-d₆): δ: 1.075-1.33 (3H,4H, Cyclopropane), 1.27 (s,3H, methyl), 2.15(s,3H, methyl), 2.73-2.83 (m, 2H, methylene), 3.13-3.17 (m,2H, methylene), 3.28-3.54 (q, 2H, methylene), 3.83 (3H, methylene), 4.12 (m, 1H, cyclopropane), 6.80 (d, 1H, benzene), 6.80 (d, 1H, benzene), 6.97 (s, 1H, benzene), 7.69 (d, 1H, benzene), 8.66 (s, 1H, ethylene), 8.78 (s, 1H, sec. amine), 14.93 (s, 1H, Carbonylic acid).MS-EI: m/z 510.23 (M+1), Elemental analysis (%): C, 72.2%; H, 6.75; F, 2.88; N, 11.33; O, 9.62.

1-cyclopropyl-7-(4-((3,4-dimethyl-5-oxo-4,5-dihydro-1H-pyrrol-1-yl)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (Oxi): yield: 66%; m.p: 236-239°C. IR V_max (cm⁻¹): 3301(NH), 3050(CH Ar.), 2920(CH Cyclopropane), 2844(OCH₃),
1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-(3-methyl-2-dioxopyrrolidin-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (O2): yield: 74%; m.p: 242-245°C. IR\textsubscript{max} (cm\textsuperscript{-1}): 3371(NH), 3069(CH Ar.), 2915(CH Cyclopropane), 2820(OCH\textsubscript{3}), 2661(OH Carboxyl), 1720(C=O carboxyl), 1628(C=C), 1332(C=O carboxyl), 1271(C=N Piperazine).\textsuperscript{1}H NMR (300 MHz; DMSO-d\textsubscript{6}) \delta: 1.075-1.33(m,4H, Cyclopropane), 1.17(d,3H, methyl), 1.27(m,3H, methyl), 2.33(s,3H, methyl), 2.37-2.83(m,2H, methylene), 2.83(s,3H, methyl), 4.12(m, 1H, cyclopropane), 7.69(d,1H,benzene), 8.66(s,1H, ethylene), 14.93 (1, 1H, Carboxylic acid). MS-ESI: m/z 486.17 (M+1), Elemental analysis (%): C\textsubscript{22}H\textsubscript{27}FN\textsubscript{6}O\textsubscript{5}: C, 59.37; H, 5.81; F, 3.91; N, 14.42; O, 19.73.

- Antibacterial and antifungal activity

All the title compounds were screened for their antibacterial and antifungal activities by paper disc diffusion method. The strain and test organisms were gram-positive bacteria (A)- such as (Staphylococcus Aureus ATCC-6483P, Staphylococcus Epidermidis ATCC 15) and two-gram negative bacteria (Escherichia Coli ATCC 2922 and Klebsiella pneumoniae ATCC 292665) using nutrient agar medium. Antifungal activities were tested against two fungal organism namely Aspergillus fumigatus ATCC 46645 using sabouraud dextrose agar (Apex laboratories Pvt. limited, Chennai, India). For primary screening antimicrobial test were carried out by paper disc diffusion method.

**Paper-Disc Diffusion Technique**

The sterilized medium was autoclaved at 120°C for 30min at a temperature of 40-50°C was inoculated (1ml/100ml of medium) with the suspension (10\textsuperscript{5} cfu/ml) of the micro-organisms (matched to McFarland barium sulphate standard) and was poured into a Petri dish to give a depth of 3-4mm. The paper impregnated with the test compounds (100 µg/ml in dimethyl formamide) was placed on solidified medium. The plates were pre-incubated for 1hr at room temperature and incubated at 37°C for 24 and 48hr under aseptic conditions for antibacterial and antifungal activities against standard antibacterial and antifungal antibiotics. Ciprofloxacin (100 µg/disc) was used as positive control over gram positive and gram-negative bacteria and Ketoconazole (100 µg/disc) was used for control over fungi MIC was also determined by agar streak dilution method and is presented in table-1.

**Minimum inhibitory concentration (MIC)**

MIC of the compound was determined by agar streak dilution method.\textsuperscript{45,46} A stock solution of the synthesized compounds(100 µg/ml) in dimethyl formamide was prepared and graded quantities of the test compounds were incorporated in specified quantity of molten sterile agar (nutrient agar for anti-bacterial activity and sabouraud dextrose agar medium for anti-fungal 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 was allowed to solidify. Suspension of the microorganism were to contain approximately 10\textsuperscript{5}cfu/ml and applied to plates with serially diluted compounds in dimethyl formamide to be tested and incubated at 37 °C for 24 h and 48 h for bacterial 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.

Docking study

Molecular docking studies of synthesized compounds O₁-O₁₀ with well established structure of S. aureus and E. coli was 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 second step required protein was downloaded from PDB, preparation and validation of macromolecule by x-ray crystallography. Third step is identification of binding affinity by the extent of binding of ligand to the protein of molecule.

RESULTS AND DISCUSSION

Chemistry

Syntheses of desired compounds O₁-O₁₀ were obtained with the help of various derivatives. The compounds O₁, O₂, O₃, O₆, O₈ and O₁₀ were obtained by solvents like, formalin 37% and ethanol by reflux method. The compounds O₁, O₂ were obtained by Schotten Baumann method by using reagents like 5% NaOH, tetrahydrofolate (THF) and Tri-ethylamine (TEA). The compounds O₃ and O₆ were obtained by Na₂CO₃, acetonitrile, and ethanol by reflux method as shown in scheme. IR of all compounds showed absorption bonds at 3250-3500cm⁻¹ due to NH₂ group in addition of hydroxy groups at 2500-3000cm⁻¹, carboxyl groups at 1680-1744 and C-F functional group at 1321-1350cm⁻¹, respectively. ¹H NMR of all compounds showed singlets at δ 1.0075-1.33 of CH₂ and δ 8.66 of ethylene group respectively. Their mass spectra were matched with the designed structures.

Antibacterial activity

All the synthesized compounds (O₁-O₁₀) 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 good activity against both gram-positive and gram-negative bacteria. Compounds O₅, O₂, O₆ and O₁ were found to possess significant antibacterial activity against gram positive organisms when compared to standard drugs (ciprofloxacin and gatifloxacin). Compounds O₆ and O₅ were found to possess significant antibacterial activity against gram negative organisms when compared to standard drugs (ciprofloxacin and gatifloxacin). The synthesized compounds exhibited MIC values in the range of 0.2-4.0 µg/ml shown in Table 1.

Compound O₅ exhibited excellent antibacterial activity with MIC value in range of (0.6µg/ml) when compared to standard drug Gatifloxacin (0.8µg/ml) and Ciprofloxacin (0.6µg/ml). This excellent antibacterial activity was may be due to addition of new derivative 3,4-dimethylbenzenamine group at 7th position of piperazinyl ring.

Compound O₆ exhibited good gram negative antibacterial with MIC values in range of (0.2µg/ml) when compared to standard drug Gatifloxacin (0.19µg/ml) and Ciprofloxacin (0.13µg/ml). This good antibacterial activity was may be due to addition of new derivative 4-methoxy-3-methylbenzenamine at 7th position of piperazinyl ring.

Scheme: Synthesis of N-Piperazinyl derivatives of Gatifloxacin
Anti-fungal activity

All the synthesized compounds (O₁-O₁₀) were tested zone of inhibition and MIC values against two fungi organisms (A. Niger and A. fumigatus). All the compounds exhibited mild activity. Compound O₉ was found to possess significant anti-fungal activity against A. Niger and A. fumigatus when compared to standard drug ketoconazole. The synthesized compounds exhibited MIC values in the range of 12.7-17.2µg/ml shown in Table 1. Compound O₉ exhibited mild antifungal activity with MIC values of (12.8µg/ml) and (12.7µg/ml) when compared to standard drug Ketoconazole (11.2µg/ml) and (10.8µg/ml). This mild antifungal activity was maybe due to addition of new derivative 3-methylpyrrolidin-2-one group at 7th position of Piperazinyl ring.

Table 1: Anti-microbial activity of the synthesized compounds (O₁-O₁₀) (100 µg/ml)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Invitro activity - Zone of inhibition in mm (MIC in µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram-Positive bacteria</td>
</tr>
<tr>
<td></td>
<td>S.aureus</td>
</tr>
<tr>
<td>O1</td>
<td>30(1.1)</td>
</tr>
<tr>
<td>O2</td>
<td>33(0.8)</td>
</tr>
<tr>
<td>O3</td>
<td>27(1.9)</td>
</tr>
<tr>
<td>O4</td>
<td>29(1.3)</td>
</tr>
<tr>
<td>O5</td>
<td>36(0.6)</td>
</tr>
<tr>
<td>O6</td>
<td>32(0.8)</td>
</tr>
<tr>
<td>O7</td>
<td>28(1.4)</td>
</tr>
<tr>
<td>O8</td>
<td>27(1.8)</td>
</tr>
<tr>
<td>O9</td>
<td>24(2.1)</td>
</tr>
<tr>
<td>O10</td>
<td>25(2.0)</td>
</tr>
<tr>
<td>Gatifloxacinα</td>
<td>34(0.8)</td>
</tr>
<tr>
<td>Ciprofloxacinα</td>
<td>35(0.6)</td>
</tr>
<tr>
<td>Ketoconazoleβ</td>
<td>-</td>
</tr>
<tr>
<td>DMFβ</td>
<td>-</td>
</tr>
</tbody>
</table>


Docking study

Molecular docking studies was employed for the analysis with training set composed of our synthesized compounds whose inhibitory activity was unknown. In order to find out the molecular facilities responsible for biological activity molecular docking studies were performed. From the docking studies, we predicted that all the synthesized compounds (O₁-O₁₀) possessed better antibacterial activity than the standard drugs (Ciprofloxacin and Gatifloxacin). By having good binding affinity with target protein and it could be used as potential drug as antibacterial.

Gram positive bacteria Docking Studies

Among all the docked compounds O₅, O₂, O₆ and O₁ showed good binding affinity and interaction with topoisomerase-II (DNA gyrase enzyme) (5IWM) with reference to standard drugs Ciprofloxacin and Gatifloxacin. The interactions of H bonds with ligands and bacterial enzymes explained, Compound O₅ at 3rd position carboxylic hydrogen forms hydrogen bond with GLY960 and 4th position oxygen forms hydrogen bond with GLY958 as shown in figure 3 & interactions are shown in figure 5.

Compound O₅ having higher dock score (-9.5) towards bacterial S. Aureus enzyme than the standard Ciprofloxacin (-7.8) and Gatifloxacin (-7.8) drugs. We may declare that the higher docking score was due to addition of 3,4-dimethylbenzenamine group at 7th position of gatifloxacin 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 O₅ and O₉ showed good binding affinity and interaction with topoisomerase-IV enzyme (3FV5) with reference to standard drugs ciprofloxacin and gatifloxacin. Compound O₅ at 3rd position carboxylic hydrogen forms two hydrogen bonds with LEU80 & ARG79 as shown in figure 4 & interactions are shown in figure 5. Compound O₅ was having higher affinity (-7.4) towards E. Coli enzyme than the standard Ciprofloxacin (-7.3) and
Gatifloxacin (-6.7) drugs. We may declare that the higher docking score was due to addition of 4-methoxy-3-methylbenzenamine group at 7th position of gatifloxacin structure. The remaining compounds docking score and hydrogen bond interactions were described in Table 2. The correlation between experimental data (MIC) versus docking score of S.aureus and E.coli are displayed 0.928 $r^2$ and 0.935 $r^2$ (fig 6 & 7) which suggests that, parameters for docking simulation were good for both S.aureus and E.coli in reproducing experimental orientation of synthesized compounds.

Figure 3: H-bonds interactions between compound (O₃) with topoisomerase-II DNA gyrase enzyme of gram positive S. aureus bacteria (5IWM).

Figure 4: H-bonds interactions between compound (O₆) with Topoisomerase-IV enzyme of gram negative E.coli bacteria (3FV5)

Figure 5: Two-Dimensional (2D) ligand interaction diagram of compounds O₃ with DNA gyrase and O₆ with E.Coli, the amino acid residues at the binding site are tagged in circles.

Figure 6: Gram Positive (S.aureus) Correlation plot between MIC (µg/ml) and Docking Scores (Kcal/mol)

Figure 7: Gram Negative (E.coli) Correlation plot between MIC (µg/ml) and Docking Scores (Kcal/mol)
### Table 2: Docking result of Synthesized Compounds (O1-O10) with MIC Values (µg/ml)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Docking Score&lt;sup&gt;a&lt;/sup&gt;</th>
<th>MIC µg/ml</th>
<th>H Bonds with SIWM Protein</th>
<th>Docking Score&lt;sup&gt;a&lt;/sup&gt;</th>
<th>MIC µg/ml</th>
<th>Interactions with 3FVS Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1</td>
<td>-9.1</td>
<td>1.1</td>
<td>Amino methyl benzene sulfenyl Oxygen with GLY934 &amp; Amino hydrogen with GLU691.</td>
<td>-6.7</td>
<td>1.5</td>
<td>4&lt;sup&gt;th&lt;/sup&gt; Position Oxygen with ILE81.</td>
</tr>
<tr>
<td>O2</td>
<td>-9.4</td>
<td>0.8</td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; position COOH oxygen with ALA138 &amp; hydrogen with GLY960.</td>
<td>-6.6</td>
<td>1.6</td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; position COOH oxygen with SER82 &amp; THR13.</td>
</tr>
<tr>
<td>O3</td>
<td>-8.6</td>
<td>1.9</td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; position COOH hydrogen with ILE983 &amp; Amino methyl benzoyl hydrogen with ASP139.</td>
<td>-6.8</td>
<td>1.2</td>
<td>Amino methyl benzoyl oxygen with SER82.</td>
</tr>
<tr>
<td>O4</td>
<td>-8.8</td>
<td>1.3</td>
<td>Nitro methyl benzoyl oxygen with ASP139 &amp; 3&lt;sup&gt;rd&lt;/sup&gt; position COOH oxygen with ILE983.</td>
<td>-6.9</td>
<td>0.9</td>
<td>Nitro methyl benzoyl oxygen forms hydrogen bond with SER82.</td>
</tr>
<tr>
<td>O5</td>
<td>-9.5</td>
<td>0.6</td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; position COOH hydrogen with GLY960 &amp; 4&lt;sup&gt;th&lt;/sup&gt; Position Oxygen with GLY958.</td>
<td>-7.1</td>
<td>0.3</td>
<td>No interactions.</td>
</tr>
<tr>
<td>O6</td>
<td>-9.4</td>
<td>0.8</td>
<td>4&lt;sup&gt;th&lt;/sup&gt; Position Oxygen with ALA138 &amp; Methoxy (methyl phenyl) amino oxygen with LYS982.</td>
<td>-7.4</td>
<td>0.2</td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; position COOH hydrogen forms two hydrogen bonds with LEU80 &amp; ARG79.</td>
</tr>
<tr>
<td>O7</td>
<td>-8.8</td>
<td>1.4</td>
<td>Dimethyl oxopyrazol oxygen with ASP693.</td>
<td>-6.3</td>
<td>2.1</td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; position COOH Oxygen with THR126 &amp; Hydrogen with ARG124.</td>
</tr>
<tr>
<td>O8</td>
<td>-8.6</td>
<td>1.8</td>
<td>Methyl dioxo pyrroloidine Piperazine Oxygen with LEU473.</td>
<td>-6.8</td>
<td>1.2</td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; position COOH Oxygen with THR126 &amp; Hydrogen with THR126</td>
</tr>
<tr>
<td>O9</td>
<td>-7.9</td>
<td>2.1</td>
<td>Methyl oxo pyrolidine Piperazine oxygen with SER694.</td>
<td>-6.7</td>
<td>1.7</td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; position COOH Oxygen with THR126 &amp; Hydrogen with ARG124.</td>
</tr>
<tr>
<td>O10</td>
<td>-8.3</td>
<td>2.0</td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; position COOH hydrogen with LEU843 &amp; Aminomethylmethylpyrroloidine hydrogen with ARG885.</td>
<td>-6.3</td>
<td>2.1</td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; position COOH Oxygen with SER82 &amp; Hydrogen with ILE76, 4&lt;sup&gt;th&lt;/sup&gt; Position oxygen with SER82 &amp; Aminomethylmethylpyrroloidine Two hydrogen bonds with GLY10 &amp; THR13</td>
</tr>
<tr>
<td>Gatiflo-xacin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-7.8</td>
<td>0.8</td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; position COOH oxygen with PHE962 &amp; 4&lt;sup&gt;th&lt;/sup&gt; Position Oxygen with PHE982.</td>
<td>-6.7</td>
<td>0.19</td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; position COOH oxygens with ILE81 &amp; SER82.</td>
</tr>
<tr>
<td>Ciproflo-xacin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-7.8</td>
<td>0.6</td>
<td>7&lt;sup&gt;th&lt;/sup&gt; Position Piperazine hydrogen with VAL1120 &amp; 3&lt;sup&gt;rd&lt;/sup&gt; position COOH oxygen with ALA790.</td>
<td>-7.3</td>
<td>0.13</td>
<td>7&lt;sup&gt;th&lt;/sup&gt; Position Piperazine hydrogen with ASP56.</td>
</tr>
</tbody>
</table>

<sup>a</sup>Based on Auto Dock Vina Score, <sup>b</sup>Gatifloxacin, Ciprofloxacin (Standard Drugs), S. aureus: Staphylococcus aureus, E. Coli: Escherichia coli
CONCLUSION

In conclusion we have synthesized and characterized ten new derivatives of gatifloxacin. All the compounds were fully characterized by spectral data and elemental analysis. All the compounds were studied for their interactions with DNA gyrase (topoisomerase-II) and topoisomerase-IV enzymes by molecular docking protocol. Among tested compounds O5, O2, O5 and O2 exhibited good docking score for gram positive bacteria and compounds like O5 and O5 for gram negative bacteria. Invitro antibacterial activity of tested compounds showed good activity against microorganisms. In particular compounds O5, O2 and O5 possessed significant gram-positive activity and compound O5 and O5 possessed significant gram-negative activity. The results of antibacterial activity were supported by docking analysis. These results provided the essential information for developing new derivatives of Gatifloxacin with improved activity.

REFERENCES


