Synthesis and Antibacterial Evaluation of Novel Fluoroquinolone Derivatives

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Abstract—A series of six novel 1-ethyl-6-fluoro-7-[4-(1-alkyl-1,4-dihydropyridine-3-carbonyl)-piperazin-1-yl]-4-oxo-1,4-dihydropyridine-3-carboxylates has been synthesized and evaluated for antibacterial activity. Norfloxacin was reacted with thionyl chloride, to yield norfloxacin acid chloride which was used immediately in next step by reacting with respective alcohols to furnish the corresponding esters i.e. 1-ethyl-6-fluoro-1,4-dihydro-7-piperazin-1-yl-4-oxoquinoline-3-carboxylates. Nicotinoyl chloride was prepared by adopting reported procedures and was reacted appropriately with previously synthesized esters to yield the amides. Amides were converted into the corresponding quaternary compounds by reacting with suitable alkyl halides. The quaternary compounds were reduced in the inert atmosphere to yield the title compounds. The structures of synthesized compounds were established on the basis of analytical and spectral studies. All the synthesized compounds were evaluated for antibacterial activity against five different strains of bacteria. Compounds exhibited moderate to significant minimum inhibitory concentration.

Keywords—Fluoroquinolones, norfloxacin, anti-bacterial activity, MIC

I. INTRODUCTION

Infectious diseases caused by bacteria affect millions of people and are leading causes of death worldwide [1,2]. Treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including emerging and increasing number of multidrug resistant microbial pathogens [3]. Considering the antimicrobial resistance phenomenon as one of the greatest challenges in 21st century facing the modern medicine system, discovery of new substances with potential effectiveness against several pathogenic microorganisms becomes highly desirable [4].

Anti-infective chemotherapy is the science of administering chemical agents to treat infectious diseases. Historically, the use of anti-infective agents can be credited with saving more human lives than any other area of medicinal therapy discovered to date [5]. Since their introduction, antimicrobials are one of the most significant weapons in fighting bacterial infections. They have extremely benefited the health related quality of human life [6]. One of the main objectives of organic and medicinal chemistry is the design, synthesis and production of molecules having value as human therapeutic agents [7]. During the past three decades, antimicrobial agents have been introduced at a rate exceeding our ability to integrate them into clinical practice [8].

Development of novel chemotherapeutic agents is an important and challenging task for the medicinal chemists and many research programs are directed towards the design and synthesis of new drugs for their chemotherapeutic usage [9]. Development of new antibiotics has been achieved from derivatives of known antimicrobial agents or by identification of novel agents active against previously unexploited targets [10].

Quinolones and fluoroquinolones are a relatively new class of synthetic antibiotics with potent bactericidal, broad spectrum activity against many clinically important pathogens which are responsible for variety of infections including urinary tract infections (UTI), gastrointestinal infections, respiratory tract infections (RTI), sexually transmitted diseases (STD) and skin infections. They are primarily used against urinary tract infections and are also clinically useful against prostatitis, infections of skin and bones and penicillin resistant sexually transmitted disease. These agents are also employed against bacterial enteric infections, prophyaxis in the immuno compromised neutropenic host. New quinolones provide a valid alternative antibacterial therapy, especially in areas where the prevalence of penicillin resistant and macrolide resistant organisms exist [5].

Since their introduction, fluoroquinolones have become a mainstay in the treatment of serious bacterial infections. These are synthetic antibacterial agents structurally related to nalidixic acid. They depict several favorable properties such as excellent bioavailability, good tissue penetrability and a relatively low incidence of adverse and toxic effects. These drugs are potentially used in the treatment of urinary tract infections and prostatitis. They are also employed against bacterial enteric infections, biliary tract infections, sexually transmitted diseases and prophyaxis in the immuno compromised neutropenic host [8].

On the other hand, despite of much significant advancement in the antimicrobial therapy, many problems remain to be solved for most of the antimicrobial drugs available. It is emphasized that further developments are required in order to combat with the infectious conditions. Hence, discovery of novel antimicrobial agents with better
pharmacological profile is still highly desirable [11]. A potential approach to overcome the resistance problem is to design novel and innovative agents [12]. The development of new antibiotics can be achieved from derivatives of known antimicrobial agents or by identification of novel agents active against previously unexploited targets [13].

Thus, keeping in view the need of novel antibacterial agents with improved antimicrobial profile, and as a part of our ongoing programme on design and discovery of novel pharmacotherapeutic and antimicrobial agents [14-18], it was also decided to evaluate the newly synthesized compounds for their antibacterial potential.

II. SYNTHETIC CHEMISTRY

The entire chemicals were supplied by E. Merck (Germany) and S.D. Fine Chemicals (India). The melting points of synthesized compounds were determined in open capillary tubes using Veego VMP-1 melting point apparatus, expressed in °C and are uncorrected. The IR spectra of compounds were recorded on Perkin Elmer Infra Red Spectrophotometer in KBr disc and absorption bands are expressed in cm⁻¹. 1H NMR spectra were recorded on Bruker Avance 700 MHz NMR Spectrometer (Chemical shift in δ ppm) using TMS as internal standard.

A. Synthesis of Norfloxacin Alkyl Esters and Amides

Syntheses of alkyl esters and amides were carried out by our earlier reported procedure [13].

B. Synthesis of pyridinium halides

Appropriately synthesized amide (0.025 mol) was dissolved in a mixture of acetone (20 mL), triethylamine (10 mL) and ethanol (5 mL). Appropriate quantity of alkyl halide (methyl iodide or ethyl bromide) was added at different time intervals during the 66 to 72 h stirring at room temperature. The solvents were removed under reduced pressure. The product (pyridinium halide) was recrystallized in methanol and yellowish crystals were obtained.

C. Synthesis of Dihydropyridine Derivatives

The quaternary compounds were reduced in a mixture of triethylamine/pyridine/diethylamine and/or ethyl acetate (Figure 4.5). The solution was cooled to about 5 °C. Sodium trimethylsilylacetamide (2g) and sodium dithionate (1g) were added to the above solution. The reduction was carried out in an atmosphere of nitrogen gas while stirring for 4h to 5h. The organic layer was separated and solvent was removed under reduced pressure. The product (pyridinium halide) was recrystallized in methanol and yellowish crystals were obtained [19,20]. Physical constants of synthesized compounds is presented in Table I.

Spectral characterization

**1-H NMR, δ ppm (DMSO-d₆):** 14.70 (s, 1H, -COOH); 8.50 (s, 1H, H₂-quinolone); 7.58-7.24 (s, 1H, C₂ dihydropyridine); 7.03-6.85 {m, 2H, aromatic proton (H₂ and H₂-quinolone)}; 5.88-5.74 (d, 1H, C₁ dihydropyridine); 4.72-4.56 {m, 3H, 1H(C₁ dihydropyridine) and 2H (N-CH₂CH₃ quinolone)}; 3.46-3.18 (m, 8H, piperazine); 3.08 (d, 1H, C₄ dihydropyridine); 2.94 (s, 3H, N-CH₃CH₃ quinolone).

1-Ethyl-7-[4-(1-ethyl-1,4-dihydropyridine-3-carbonyl)-piperazin-1-yl]-6-flouro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (VIIInb)

**IR (cm⁻¹, KBr):** 3152 (Ar-C-H), 3078-3050 and 2949, 2927-2853, 1740, 1714, 1737 and 1739, 1648, 1649, 1152, 1154, 8.46 (s, 1H, H₂-quinolone); 5.85-5.78 (d, 1H, C₁ dihydropyridine); 4.72-4.54 {m, 3H, 1H(C₁ dihydropyridine) and 2H (N-CH₂CH₃ quinolone)}; 3.45-3.12 {m, 1O, 8H (piperazine) and 2H (N-CH₂CH₃)}; 3.11 (d, 1H, C₄ dihydropyridine); 1.42-1.29 {t, 6H, 3H (N-CH₂CH₃) and 3H (N-CH₂CH₃ quinolone)}.

1-Ethyl-6-fluoro-7-[4-(1-methyl-1,4-dihydropyridine-3-carbonyl)-piperazin-1-yl]-6-flouro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid methyl ester (VIIIna)

**IR (cm⁻¹, KBr):** 3157 (Ar-C-H), 1649, 1718, 1737, (C=O), 1221 (C-O), 1156 (C-F).

1-H NMR, δ ppm (DMSO-d₆): 8.18 (s, 1H, H₂-quinolone); 7.32-7.19 (s, 1H, C₂ dihydropyridine); 7.12-6.86 {m, 2H, aromatic proton (H₂ and H₂-quinolone)}; 5.85-5.78 (d, 1H, C₁ dihydropyridine); 4.69-4.53 {m, 3H, 1H (C₄ dihydropyridine) and 2H (N-CH₂CH₃ quinolone)}; 3.61 (s, 3H, CH₃); 3.39-3.20 (m, 8H, piperazine); 3.04 (d, 1H, C₄ dihydropyridine); 2.87 (s, 3H, N-CH₃); 1.45 (t, 3H, N-CH₂CH₃ quinolone).

1-Ethyl-7-[4-(1-ethyl-1,4-dihydropyridine-3-carbonyl)-piperazin-1-yl]-6-flouro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid methyl ester (VIIInb)

**IR (cm⁻¹, KBr):** 3152 (Ar-C-H), 1649, 1718, 1737, (C=O), 1222 (C-O), 1153 (C-F).

1-H NMR, δ ppm (DMSO-d₆): 8.18 (s, 1H, H₂-quinolone); 7.32-7.15 (s, 1H, C₂ dihydropyridine); 7.08-6.85 {m, 2H, aromatic proton (H₂ and H₂-quinolone)}; 5.82-5.71 (d, 1H, C₁ dihydropyridine); 4.74-4.58 {m, 3H, 1H (C₄ dihydropyridine) and 2H (N-CH₂CH₃ quinolone)}; 3.64 (s, 3H, CH₃); 3.44-3.15 (m, 10H, 8H(piperazine) and 2H (N-CH₂CH₃)); 3.07 (d, 1H, C₄ dihydropyridine); 1.50-1.25 (t, 6H, 3H (N-CH₂CH₃) and 3H (N-CH₂CH₃ quinolone)).

**1-H NMR, δ ppm (DMSO-d₆):** 8.46 (s, 1H, H₂-quinolone); 7.34-7.25 (s, 1H, C₂ dihydropyridine); 7.13-6.84 {m, 2H, aromatic proton (H₂ and H₂-quinolone)}; 5.85-5.76 (d, 1H, C₁ dihydropyridine); 4.72-4.51 {m, 3H, 1H (C₄ dihydropyridine) and 2H (N-CH₂CH₃ quinolone)}; 3.15 (q, 2H, -CH₂CH₃); 3.42-3.21 (m, 8H, piperazine); 3.04 (d, 1H, H⁻)}.
positive strains (*Bacillus subtilis* and *Staphylococcus aureus*). Norfloxacin was used as standard drugs for antibacterial activity. When the title compounds (VIIIIn-f) were tested against *E. coli*, compounds without substitution at C-3 carboxylic acid group i.e. compounds VIIIIna and VIIIInb were found most active with MIC of 0.20 µg/mL. All the compounds exhibited good antibacterial activity with MIC ranging from 0.20 µg/mL to 0.30 µg/mL. When screened against another Gram negative bacterial strain *P. aeruginosa*, Compound VIIIIna bearing free carboxylic acid group and methyl group at dihydropyridine was most active among norfloxacin derivatives (VIIIIn-a) demonstrating good activity (i.e. 20 µg/mL).

All the synthesized norfloxacin derivatives (VIIIIn-f) were also screened against these Gram positive bacterial strains. Compound VIIIIna with free carboxylic acid group at quinolone ring and and methyl group at dihydropyridine portion was found to be most active against *S. aureus* with MIC of 1.30 µg/mL when tested against standard drug norfloxacin (MIC 1.5 µg/mL). Second most active compounds of series were compound VIIIInb and VIIIInc (MIC 1.35 µg/mL). Antibacterial screening results against *B. subtilis* depicted that compound VIIIInb and VIIIInc were most active (MIC 1.35 µg/mL).

V. CONCLUSION

In conclusion, we have synthesized six new derivatives of fluoroquinoline antibacterial drug norfloxacin and evaluated them for their antimicrobial activities against selected bacterial strains. The compounds have demonstrated moderate to significant activities.

REFERENCES


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**TABLE I.** PHYSICAL CONSTANTS OF THE SYNTHESIZED COMPOUNDS

<table>
<thead>
<tr>
<th>Compound</th>
<th>R'R</th>
<th>R</th>
<th>M.P.(°C)</th>
<th>Yield (%)</th>
<th>Mol Formula</th>
<th>Mol Wt</th>
<th>RF value</th>
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<tr>
<td>VIIINa</td>
<td>CH₃</td>
<td>H</td>
<td>141-143</td>
<td>63.6</td>
<td>C₄H₈F₉N₄O₄</td>
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<td>VIIINb</td>
<td>C₂H₅</td>
<td>H</td>
<td>164-165</td>
<td>56.5</td>
<td>C₅H₁₀F₈N₄O₄</td>
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<td>0.52</td>
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<td>VIIINc</td>
<td>CH₃</td>
<td>CH₃</td>
<td>136-137</td>
<td>61.2</td>
<td>C₅H₁₀F₈N₄O₄</td>
<td>454.49</td>
<td>0.56</td>
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<tr>
<td>VIIInd</td>
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<td>CH₃</td>
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<td>58.3</td>
<td>C₆H₁₂F₈N₄O₄</td>
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<td>VIIINe</td>
<td>CH₃</td>
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<td>VIIINf</td>
<td>C₂H₅</td>
<td>C₂H₅</td>
<td>158-159</td>
<td>56.6</td>
<td>C₈H₁₆F₈N₄O₄</td>
<td>482.55</td>
<td>0.62</td>
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TABLE II.  MINIMUM INHIBITORY CONCENTRATION IN MG/ML OF COMPOUNDS VIIIN-NA AGAINST SELECTED BACTERIAL STRAINS.

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Gram negative bacteria</th>
<th>Gram positive bacteria</th>
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<tr>
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<tr>
<td></td>
<td>E. coli (NCDC 134)</td>
<td>S. dysentry (NCDC 63)</td>
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<td></td>
<td>P. aeruginosa (NCDC 105)</td>
<td>B. subtilis (NCDC 71)</td>
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<td></td>
<td>S. auerus (NCDC 110)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MIC (µg/mL)</td>
<td>MIC (µg/mL)</td>
</tr>
<tr>
<td>VInI</td>
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<td>0.20</td>
</tr>
<tr>
<td>VInII</td>
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</tr>
<tr>
<td>VInIII</td>
<td>0.25</td>
<td>0.30</td>
</tr>
<tr>
<td>VInIV</td>
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<tr>
<td>VInV</td>
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<td>0.35</td>
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<td>VInVI</td>
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<td>Norfloxacin</td>
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