NOVEL SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF 7-SUBSTITUTED 1-CYCLOPROPYL-6-FLUORO-1, 4-DIHYDRO-8-METHOXY-4-OXOQUINOLINE-3-CARBOXAMIDE DERIVATIVES

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ABSTRACT

Here in we report synthesis of 1-cyclopropyl-6, 7-difluoro-1, 4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide. The target compound was synthesized from 1-cyclopropyl-6, 7-difluoro-1, 4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid by one pot synthesis using simple reagent and condition. Then different novel derivatives of 7-substituted 1-cyclopropyl-6-fluoro-1, 4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide were synthesized by substituting fluoro group at C-7 position with different cyclo amine or cyclo amine with different hetero atoms. The novel synthesized compounds were characterized then examined for their antibacterial and antifungal activities.

KEYWORDS: Antimicrobial, Quinoline-3-carboxamide, Moxifloxacin, Gatifloxacin, 1-(3-Dimethylaminopropyl)-3-ethylcarboadiimide Hydrochloride and 1-Hydroxybenzotriazole.

INTRODUCTION

The fluoroquinolones are totally synthetic broad spectrum antibacterial agents and many of these drugs are used in the treatment of various bacterial infections.[1-2] The first quinolone compound showing antimicrobial activity was serendipitously discovered in the early 1960s as “byproduct” 7-chloro-1,4-dihydro-1-ethyl-quinoline-4-oxo 3-carboxylic acid (Ib) during synthesis of antimalerial drug chloroquinoline (Ia)[3-5] showing antimicrobial activity against gram-negative bacteria. Its potency and antimicrobial spectrum was very narrow. So it cannot
be used for therapy and was considered as lead compound. After doing different changes on this lead compound and studying their microbial activity.

Nalidixic acid (Ic) (1-ethyl-1, 4-dihydro-7-methyl-4-oxoquinoline-3-carboxylic acid) was launched as drug in 1960s as parent compound of quinolones class of antibiotics.\[^6\] It was discovered by Lescher and colleagues for the treatment of urinary tract infections caused by Escherichia coli and a few other Gram negative pathogens.\[^7\] Its use was limited due to its narrow spectrum of activity, its high protein binding (approximately 90%) and little half life (about 1.5 h). It was active against some Gram negative bacteria. Unfortunately, bacteria could develop a rapid resistance to it.\[^8,\,1\] This rapid emergence of drug resistance pathogens like, penicillin-resistant Streptococcus pneumonia, vancomycin-resistant Enterococci, methicillin-resistant Staphylococcus aureus and multi-resistant Salmonellae has now become a serious public health problem so new classes having effective antimicrobial agents against VRSA, MRSA and K. pneumoniae infections are urgently required.\[^9,\,10\] There was a significant breakthrough after 20 years in 1980 it was observed that by modifications at C6 and C7 position of lead compound (IIa) there was improvement in absorption and activity.

The addition of fluorine molecule at C6 position of quinolone is also known as fluoroquinolones\[^11\] which had increased more than 10-fold in gyrase inhibition and up to 100-fold improvement in MIC.\[^9\] Flumequine (IVa) was the first mono fluoroquinolone drug with a fluoro group at position C-6 gave the first indications that modifications of the basic 1,4-dihydro-4-oxoquinoline-3-carboxylic acid core could improve Gram-positive activity.\[^12\]

From 2005-2010 more focus was given on modification at C-7 position of 4-Quinolone because this position strongly influenced the potency, pharmacokinetics and safety profiles of quinolones.\[^11\] Piperazine at position C-7 led to increased spectrum and potency. Ciprofloxacin (IIa) and Norfloxacin (IIb)\[^13\]\[^2\] are widely used antibiotics in bulk quantities have piperazine at C-7 position.
Pipemidic acid (III), Enoxacin (IV) and Sarafloxain (V) also have piperazine ring at C-7 position. There are data which shows that a piperazine ring may play an important role in inhibiting efflux mechanisms, thereby improving the potency of these drugs.

A methoxy group at position C-8 is found in Moxifloxacin (Va), Gatifloxacin (VIa) and Balofloxacin (VIIIa) which show good Gram-negative activity and in addition further improved activity against Gram-positive and atypical anaerobic bacteria.\cite{12, 14} They are active against all the primary pathogens that cause typical respiratory disease, e.g. *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*. But Gatifloxacin has been removed from the market because of severe dysglycaemia in some patients.

It has being found that introduction of a cyclopropyl group at N-1 position in ciprofloxacin increased 2-8 fold in potency\cite{9} against Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* when compared with Norfloxacin\cite{15} and increased in the volume of distribution and reduce the resistance selection.

But Carboxylic acid group at C-3 and Carbonyl group at C-4 position are very important for antimicrobial property of compound and position C-3 and C-4, having a link between the carboxylic acid group and the keto group are generally considered necessary for binding of quinolones to DNA gyrase.\cite{12, 16} Hydrogen atom at C-2 position is very important and very close to this important binding site, and any modification leads to complete lost in antimicrobial activity.

Therefore, we thought to synthesis the fluorinated quinolones with cyclo amine with different hetero atoms at the C-7 position and carboxamide at C-3 position. This present work
describes the synthesis of different novel derivatives of 7-substituted 1-cyclopropyl-6-fluoro-1, 4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide and their subsequent evaluation for antibacterial and antifungal activities.[19-20]

MATERIALS AND METHODS
All the raw materials used for synthesis are obtained from commercial suppliers and was purified as per requirement. The intermediate ethyl 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate is commercially available and synthesis of this intermediate is also reported in literature. Mass spectra were recorded on ‘LCMS-Qp2010s’ instrument by direct injection method. Nuclear Magnetic Resonance spectra (1HNMR) were recorded on Bruker advance spectrometer (400 MHz) using DMSO-d_6 or CDCl_3 solvents. Tetramethylsilane was used as internal standard. Chemical shift (δ) are reported in parts per million. Reactions were monitored and its purity was checked by Merck pre-coated plate (silica gel 60 F254) Thin Layer Chromatography was visualized with UV light. Melting points were determined in open capillary tube and are uncorrected.

RESULT AND DISCUSSIONS
The outline for synthesis of target compounds is given in Scheme-1. Compound (VII) 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid was synthesized by hydrolysis of ethyl 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (VI) with lithium hydroxide. THF and water was used as solvent. Compound (VIII) 1-cyclopropyl-6, 7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide was synthesized by reacting 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid with coupling reagent 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide Hydrochloride and1-Hydroxybenzotriazole. Reaction carried out in aprotic N,N-Dimethylformamide solvent. Ammonium chloride was used instead of ammonium gas or solution for amidation which is cheaper and safe to handle on large scale. N,N-Diisopropylethylamine used as base. Reaction carried out at low temperature to avoid side reaction. Substitution at C-7 position with different cycloamine was carried out by conventional method. In literature 4-oxoquinoline-3-carboxamide derivatives are synthesized by different methods such that making acylimidazole derivative of 3-carboxylic acid by reacting with 1,1’ carbonyldiimidazole in DMF at 100°C to 150°C and then treating its acylimidazole derivative with anhydrous ammonia.[17, 18] Reacting 4-oxoquinoline-3-carboxylate ester with aqueous ammonia and capryl alcohol (Catalytic
amount) was stirred on a steam bath till reaction get completed or with saturated methanolic solution of ammonia in a stainless steel vessel pressure vessel at 150 °C for 23 hours or refluxed with hydrazine hydrate for overnight then cool to ambient temperature and this hydrazide is refluxed with Raney nickel in DMF solvent for 36 h. Reacting 4-oxoquinoline-3-carboxylic acid derivatives is reacted with ethyl chloroformate and organic base at 0 °C in DMF solvent and then treated with concentrated aqueous ammonia and then stirred for overnight at room temperature.

**General procedure for synthesis of VII to IXa- IXn**

![Chemical structures for synthesis](image)

(a) LiOH, THF, H2O, (b) DMF, EDAPC, HOBT, DIPEA, NH4Cl; (c) RH DMSO or DMF, Base, 80 °C. R= cyclo amine or cyclo amine with different hetero atoms.

**Scheme 1: Synthesis of target compound IX**

**Procedure; Synthesis of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid (VII)**

To the suspension of ethyl 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (VI) (15.4mmol) in THF (50 ml) was added the lithium hydroxide (16.94mmol) solution (25ml water) drop wise at 0 °C in 30 minutes. Reaction mass was stirred at room temperature for 4 hour. Solvent was removed under the reduced pressure at 35 °C. Cool the reaction mass to 0 °C and pH was adjusted (between 5 to 6) by 2 N HCl solution. Reaction mass was filtered and obtained cake was washed with water (3 x 20ml) and dried under reduced pressure at 35 °C. Compound (VII) obtained yield 85%; mp 188-190 °C; 1H NMR (CDCl3) (400 MHz) δ: 14.39 (s, 1H), 8.875 (s, 1H), 8.075-8.029 (t, 1H, J= 8.4 Hz), 4.151 (s, 3H), 4-145-4.127(m, 1H), 1.303-1.284 (m, 2H), 1.144-1.129 (m, 2H); MS (ESI) m/z 296 (M+1).

**Synthesis of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide (VIII):** To the suspension of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid (VII) (16.9mmol) in N,N-Dimethylformamide (50mL) were added 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide HCl (25.4mmol) and 1-
Hydroxybenzotriazole (18.6 mmol) at 0°C and stirred for 1h. Ammonium chloride (119mmol) and N,N-Diisopropylethylamine (169mmol) were added. After stirring the reaction mixture for 1h at 0°C, allow the mixture to stir at room temperature for 16h, the reaction was quenched to 500mL chilled water, filter and wash the cake with 50mL water. Dry the solid mass under reduced pressure at 40°C. The obtain crude product was further purified and dried. Yield 63%; mp >220°C; 1H NMR (DMSO-d6, 400 MHz) δ: 8.974 (s, 1H), 8.710 (s, 1H), 7.939 (t, 1H), 7.584 (s, 1H), 4.145-4.111 (m, 1H), 4.067 (s, 3H), 1.178-1.076 (m, 4H), MS (ESI): m/z 315.10 (M+1).

Synthesis of 1-cyclopropyl-6-fluoro-7-(heterocyclic amino)-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide (IXa-IXk).

To a suspension of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide (VIII) (1.53mmol) in dimethylsulfoxide (2mL) were heterocyclic amine (3.3mmole) was added. Reaction mass stirred at 80°C. Concentrate the reaction mass under reduced pressure. Add ice water and stir the reaction mass for 1h. Reaction mass was filtered and dried.

Synthesis of 1-cyclopropyl-6-fluoro-7-(heterocyclic amino)-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide (IXl&IXn).

To a suspension of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide (VIII) (1.53mmol) in N,N-Dimethylformamide (5mL) were heterocyclic amine (1.681 mmole) and N,N-Diisopropylethylamine (7.31mmol) was added. Reaction mass stirred at 80°C. Concentrate the reaction mass under reduced pressure. Add ice water and stir the reaction mass for 1h. Reaction mass was filtered and dried.

Synthesis of 1-cyclopropyl-6-fluoro-7-(heterocyclic amino)-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide (IXm).

To a suspension of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide (VIII) (1.53mmol) in N,N Dimethylformamide (5ml) were heterocyclic amine (1.688 mmole) and cesium carbonate (Cs2CO3) (1.688mmol) was added. Reaction mass was at 80°C. Concentrate the reaction mass under reduced pressure. Add ice water and stir the reaction mass for 1h. Reaction mass was filtered and dried.
Table: 1- Physical data of synthesized compounds (IXa- IXn).

<table>
<thead>
<tr>
<th>No</th>
<th>R1</th>
<th>MF</th>
<th>Mwt</th>
<th>% yield</th>
<th>M.P (°C)</th>
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<tbody>
<tr>
<td>IXa</td>
<td></td>
<td>C_{19}H_{22}F_{3}N_{3}O_{3}</td>
<td>359.40</td>
<td>41%</td>
<td>224-226</td>
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<tr>
<td>IXb</td>
<td></td>
<td>C_{18}H_{20}F_{3}N_{3}O_{3}</td>
<td>345.38</td>
<td>75%</td>
<td>220-222</td>
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<tr>
<td>IXc</td>
<td></td>
<td>C_{18}H_{20}F_{3}N_{4}O_{3}</td>
<td>361.38</td>
<td>67%</td>
<td>190-192</td>
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<tr>
<td>IXd</td>
<td></td>
<td>C_{20}H_{24}F_{3}N_{3}O_{3}</td>
<td>373.43</td>
<td>61%</td>
<td>218-220</td>
</tr>
<tr>
<td>IXe</td>
<td></td>
<td>C_{20}H_{24}F_{3}N_{3}O_{3}</td>
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<td>46%</td>
<td>&gt;230</td>
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<tr>
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<td>C_{21}H_{26}F_{3}N_{3}O_{3}</td>
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<td>65%</td>
<td>&gt;230</td>
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<tr>
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<td>66%</td>
<td>&gt;230</td>
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<tr>
<td>IXh</td>
<td></td>
<td>C_{17}H_{18}F_{3}N_{3}O_{3}</td>
<td>331</td>
<td>79%</td>
<td>&gt;230</td>
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<td>IXi</td>
<td></td>
<td>C_{19}H_{22}F_{3}N_{4}O_{3}</td>
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<td>70%</td>
<td>224-226</td>
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<tr>
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<td>42%</td>
<td>192-194</td>
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<tr>
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<td>188-190</td>
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<tr>
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<td>21%</td>
<td>222-224</td>
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<td>C_{22}H_{26}F_{3}N_{3}O_{5}</td>
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<td>20%</td>
<td>216-218</td>
</tr>
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<td>IXn</td>
<td></td>
<td>C_{17}H_{18}F_{3}N_{4}O_{4}</td>
<td>347</td>
<td>44%</td>
<td>216-218</td>
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</table>
Preparation of 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxo-7-(piperidin-1-yl)quinoline-3-carboxamide (IXa): A off white colour solid isolated was crystallized from methanol: yield 41%; mp 224-226 °C; 1H NMR (DMSO-D₆) (400 MHz) δ: 9.104 (s, 1H), 8.653 (s, 1H), 7.682 (d, 1H, J=12.4 Hz), 7.471 (s,1H), 4.087-4.067 (m, 1H), 3.762 (s, 3H), 3.255 (m,4H), 1.659 (m, 6H), 1.0925 (d, 2H, J= 8 Hz), 0.933 (m, 2H); MS (ESI) m/z 360 (M⁺).

1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxo-7-(pyrrolidin-1-yl)quinoline-3-carboxamide (IXb): A off white colour solid isolated was crystallized from methanol: yield 75%; mp 220-222 °C; 1H NMR (DMSO-D₆) (400 MHz) δ: 9.146 (s,1H), 8.594 (s,1H), 7.594 (d,1H, J= 14.4 Hz), 7.409 (s,1H), 4.048-4.022 (m,1H),3.527-3.521 (m, 4H), 3.508 (s, 3H), 1.901 (s,4H), 1.062-1.046 (d, 2H, J= 6.4Hz), 0.889 (m, 2H); MS (ESI) m/z 346(M⁺).

1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-morpholino-4-oxoquinoline-3-carboxamide (IXc): A off white colour solid isolated was crystallized from methanol: yield 67%; mp 190-192 °C; 1H NMR (CDCl₃)(400 MHz) δ:9.57 (s, 1H), 8.844 (s, 1H), 7.895 (d, 1H, J=12.8 Hz), 5.664 (s, 1H), 3.959-3.950 (m, 1H), 3.942-3.850 (m,4H), 3.005 (s, 3H), 3.400 (m,4H), 1.179-1.1444 (m, 2H), 0.957-0.950 (m, 2H); MS (ESI) m/z 362(M⁺).

1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(4-methylpiperidin-1-yl)-4-oxoquinoline-3-carboxamide (IXd): A off white color solid isolated was crystallized from methanol; yield 61%; mp 218-220 °C; 1H NMR (CDCl₃)(400 MHz) δ: 9.636(s, 1H), 8.829 (s, 1H), 7.848 (d, 1H, J= 12.4Hz), 5.647 (s, 1H), 3.978-3.943 (s, 1H), 3.757 (s, 3H), 3.461-3.196 (m,2H), 3.166 (t, 2H, J= 12 Hz), 1.746-1.719 (m, 2H), 1.436-1.366 (m, 3H), 1.179-1.128 (m, 2H), 0.995 (d, 3H), 0.961-0.935(m, 2H); MS (ESI) m/z 374(M⁺).

1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methylpiperidin-1-yl)-4-oxoquinoline-3-carboxamide (IXe): A off white color solid isolated was crystallized from methanol; yield 46%; mp >230 °C; 1H NMR (CDCl₃)(400 MHz) δ:9.637 (s, 1H), 8.829 (s, 1H), 7.860-7.834 (d, 1H, J= 10.4Hz), 5.654 (s, 1H), 3.980-3.945(m, 1H), 3.747 (s, 3H), 3.448-3.385(m, 2H), 3.121-3.090 (m, 1H), 2.809-2.748 (m, 1H), 1.894-1.719 (m, 4H), 1.181-1.105 (m, 3H), 0.979-0.928 (m, 5H), MS (ESI) m/z 374(M⁺).

1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7(3,5-dimethylpiperidin-1-yl)-4-oxoquinoline-3-carboxamide (IXf): A off white color solid isolated was crystallized from
methanol; yield 65\%; mp >230^\circ C; \textsuperscript{1}H NMR (CDCl\textsubscript{3})(400 MHz) \(\delta\): 9.631 (s, 1H), 8.828 (s, 1H), 7.848 (d, 1H, J=12.4 Hz), 5.658 (s, 1H), 3.983-3.947 (m, 1H), 3.729 (s, 1H), 3.400-3.370 (m, 2H), 2.703-2.640 (m, 2H), 1.880-1.831 (m, 2H), 1.169-1.134 (m, 2H), 0.975-0.960 (m, 2H), 0.926-0.910 (d, 6H), 0.786-0.751 (m, 2H); MS (ESI) m/z 388(M\textsuperscript{+}).

1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(4-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxamide (IXg): A off white color solid isolated was crystallized from methanol; yield 66\%; mp >230\^\circ C; \textsuperscript{1}H NMR (CDCl\textsubscript{3})(400 MHz) \(\delta\): 9.598 (s, 1H), 8.835 (s, 1H), 7.881-7.850 (d, 1H, J=12.4 Hz), 5.654 (s, 1H), 3.965-3.939 (m, 1H), 3.754 (s, 3H), 2.612-2.579 (m, 4H), 2.383 (s, 3H), 1.160-1.099 (m, 2H); MS (ESI) m/z 375(M\textsuperscript{+}).

7-(azetidin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide (IXh): A off white colour solid isolated was crystallized from methanol; yield 79\%; mp >230\^\circ C; \textsuperscript{1}H NMR (CDCl\textsubscript{3})(400 MHz) \(\delta\): 9.693 (s, 1H), 8.771 (s, 1H), 7.794-7.761 (d, 1H, J=12.4 Hz), 3.897-3.860 (m, 1H), 3.571 (s, 3H), 2.430-2.355 (m, 2H), 1.150-1.099 (m, 2H), 0.985-0.960 (m, 2H), MS (ESI) m/z 332(M\textsuperscript{+}).

1-cyclopropyl-6-fluoro-1,4-dihydro-7-(4-hydroxypiperidin-1-yl)-8-methoxy-4-oxoquinoline-3-carboxamide (IXi): A off white colour solid isolated was crystallized from methanol; yield 70\%; mp 224-226\^\circ C; \textsuperscript{1}H NMR (CDCl\textsubscript{3})(400 MHz) \(\delta\): 9.611 (s, 1H), 8.839 (s, 1H), 7.907-7.876 (d, 1H, J=12.4 Hz), 3.969 (s, 1H), 3.979-3.914 (m, 1H), 3.775 (s, 3H), 3.568-3.536 (m, 2H), 2.624-2.308 (m, 2H), 2.071-2.030 (m, 2H), 1.751-1.760 (m, 2H), 1.170-1.135 (m, 2H), 0.98-0.956 (m, 2H); MS (ESI) m/z 376(M\textsuperscript{+}).

1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxamide (IXj): A off white colour solid isolated was crystallized from methanol; yield 42\%; mp 192-194\^\circ C; \textsuperscript{1}H NMR (CDCl\textsubscript{3})(400 MHz) \(\delta\): 9.5805 (s, 1H), 8.847 (s, 1H), 7.907-7.876 (d, 1H, J=12.4 Hz), 3.679-3.934 (m, 1H), 3.790 (s, 3H), 3.470-3.459 (m, 2H), 3.165-3.141 (m, 2H), 2.409-2.318 (m, 4H), 1.255-0.938 (m, 4H); MS (ESI) m/z 361(M\textsuperscript{+}).

1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxamide (IXk): A off white colour solid isolated was crystallized from methanol; yield 57\%; mp 188-190\^\circ C; \textsuperscript{1}H NMR (CDCl\textsubscript{3})(400 MHz) \(\delta\): 9.600 (s, 1H), 8.847 (s,
1H), 7.903-7.872 (d, 1H, J=12.4Hz), 5.740 (s, 1H), 3.965-3.947 (m, 1H), 3.773 (s, 3H), 3.380 (s, 1H), 3.143 (m, 3H), 2.418-2.321 (m, 4H), 1.228-1.213(m, 3H), 1.175-0.896 (m, 4H). MS (ESI) m/z 375(M⁺).

1-cyclopropyl-6-fluoro-7-(4-fluoropiperidin-1-yl)-1,4-dihydro-8-methoxy-4-o xoquinoline-3-carboxamide (IXl): yield 21 %; mp 188-190°C; ¹H NMR (DMSO-D₆) (400 MHz) δ: 8.984(s, 1H), 7.989 (s, 1H), 7.684-7.653 (d, 1H, J= 12.4 Hz), 7.584(s, 1H), 4.972-4.955 (m, 1H), 4.853-4.843(m, 1H), 3.495-3.443 (m, 2H), 3.280-3.213(m, 2H), 3.001 (s, 3H), 2.080-2.001(m, 1H), 1.880-1.688(m, 3H), 1.143-1.051 (m, 2H), 0.943 (m, 2H); MS (ESI) m/z 378(M⁺).

Ethyl 1-(3-carbamoyl-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-o xoquinolin-7-yl)piperidine-3-carboxylate (IXm)
yield 20 %; mp 216-218°C; ¹H NMR (CDCl₃)(400 MHz) δ: 9.600 (s, 1H), 8.839 (s, 1H), 7.888-7.857  (d, 1H, J=12.4Hz), 5.655 (s, 1H), 4.339-4.323 (m, 2H), 4.175-4.129 (m, 1H), 4.022-3.948 (m, 2H), 3.749 (s, 3H), 3.702-3.637(m, 2H), 3.383-3.364(m, 2H), 3.220-3.194(m, 1H), 2.721 (m, 1H), 2.128 (m, 1H), 1.870-1.718 (m, 3H), 1.214-1.165 (m, 2H), 0.978-0.955 (m, 2H); MS (ESI) m/z 432(M⁺).

1-cyclopropyl-6-fluoro-1,4-dihydro-7-(3-hydroxyazetidin-1-yl)-8-methoxy-4- oxoquinoline-3-carboxamide (IXn): A off white colour solid isolated was crystallized from methanol; yield 44 %; mp 216-218°C; (M⁺)+ 348),¹H NMR (DMSO-D₆) (400 MHz) δ: 9.136(s, 1H), 8.569 (s, 1H), 7.604-7.571 (d, 1H, J= 13.2Hz), 7.404(s, 1H), 5.400 (s, 1H), 4.491-4.473(m, 3H), 3.971(m, 3H), 3.540 (s, 3H), 1.063-1.046 (m, 2H), 0.899 (m, 2H), MS (ESI) m/z 348(M⁺).

RESULT AND DISCUSSIONS
The outline for synthesis of target compounds is given in Scheme-1. Compound (VII) 1-cyclopropyl-6, 7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid was synthesized by hydrolysis of ethyl 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4- oxoquinoline-3-carboxylate (VI) with lithium hydroxide. THF and water was used as solvent. Compound (VIII) 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide was synthesized by reacting 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid with coupling reagent 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide Hydrochloride and1-Hydroxybenzotriazole.
Reaction carried out in aprotic N,N-Dimethylformamide solvent. Ammonium chloride was used instead of ammonium gas or solution for amidation which is cheaper and safe to handle on large scale. N,N-Diisopropylethylamine used as base. Reaction carried out at low temperature to avoid side reaction. Substitution at C-7 position with different cycloamine was carried out by conventional method. In literature 4-oxoquinoline-3-carboxamide derivatives are synthesized by different methods such that making acylimidazole derivative of 3-carboxylic acid by reacting with 1,1' carbonyldiimidazole in DMF at 100°C to 150°C and then treating its acylimidazole derivative with anhydrous ammonia.\[17\]\[18\] Reacting 4-oxoquinoline-3-carboxylate ester with aqueous ammonia and capryl alcohol (Catalytic amount) was stirred on a steam bath till reaction get completed or with saturated methanolic solution of ammonia in a stainless steel vessel pressure vessel at 150°C for 23 hours\[17\]\[18\] or refluxed with hydrazine hydrate for overnight then cool to ambient temperature and this hydrazide is refluxed with Raney nickel in DMF solvent for 36 h.\[17\] Reacting 4-oxoquinoline-3-carboxylic acid derivatives is reacted with ethyl chloroformate and organic base at 0°C in DMF solvent and then treated with concentrated aqueous ammonia and then stirred for overnight at room temperature.

**Antimicrobial testing (Disc diffusion assay):** Various bacterial strains - *Staphylococcus aureus* (NCIM 2079), *Escherichia coli* (NCIM 2109) and fungal strain *Candida albicans* (NCIM 3471) were used as test microorganism to evaluate the antimicrobial testing of newly synthesized compounds (Table-1) IX (a-n), IIIIV and compound X (a-b).

Pure culture of test bacterial strain was picked with a loop, and the growth was transferred into a tube containing 5 ml of a nutrient broth medium, while pure culture of test fungal strain was transferred into a tube containing 5 ml of a MGYP medium. The broth culture was incubated at 37°C until it achieves or exceeds the turbidity of the 0.5 McFarland standard (usually to 6 hours). The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain a turbidity optically comparable to that of the 0.5 McFarland standard. This results in a suspension contains 2 x 10^8 CFU/ml of microbial cells.

Within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. The surface of a nutrient agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated.
by streaking several times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum.

Stock solution [1000 microgram per ml] of each newly synthesized compounds were prepared in dimethylsulfoxide (DMSO). The sterile discs of 6 mm diameter were used in this assay. The disc diffusion assay was carried out by taking concentration 100 microorganism per disc. The discs immersed with compounds were dispensed onto the surface of the inoculated agar plate. Also, Ciprofloxacin (10 microgram/disk, Amphotericin-B (100 units/disk) [Hi-media, Mumbai, disc diameter 6 mm] moistened with DMSO were placed on agar plate as standard. Each disc was pressed down to ensure complete contact with the agar surface. The plates were placed in a refrigerator at to 8°C for 30 minutes after the discs are applied. Then the plates were incubated in incubator at 37°C for 24 hours.

After 24 hours of incubation, each plate was examined. The diameters of the zones of complete inhibition including the diameter of the disc was measured using Vernier caliper, which is held on the back of the inverted petri plate. The results were summarized in Table 1.

Table: 1: Antibacterial and Antifungal activity data of compounds (IXa - IXn).

<table>
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<tr>
<th></th>
<th>R</th>
<th>R1</th>
<th>R2</th>
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<th>R4</th>
<th>S.aureus</th>
<th>E. coli</th>
<th>C.albicans</th>
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</table>

**Diameter in mm calculated by Vernier Caliper** ‘-’ means no zone of inhibition, NA Not applicable

Microbial Cultures Used to test antimicrobial Activity, Fungi (Yeast), *Candida albicans*, Gram Positive Bacteria: *Staphylococcus aureus*. Gram Negative Bacteria: *Escherichia coli*.

**BIOLOGICAL ACTIVITY**

**Antifungal studies.** The antifungal activity was studied against with *Candida albicans* pathogenic fungi. Amphotericin was used as reference for inhibitory activity against fungi.
Compounds (Table-1) IX (a-n), VIII and compound X (a-b) show no activity against *Candida albicans* pathogenic fungi.

**Antibacterial studies.** All synthesized compounds (Table-1) IX (a-n), VIII and compound X (a-b) were tested against *Staphylococcus aureus* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria). Ciprofloxacin used as a reference standard.

It is widely believed that carboxylic acid at C-3 and Carbonyl at C-4 position is necessary for antimicrobial activity. So compound (Xa) have slightly better activity as compared to compound VIII (Table-1) which have fluoro group at C-7 position and carboxamide at C-3 position against *Staphylococcus aureus* and *Escherichia coli* pathogens. So compound IX (a-n) (Table-1) were synthesized to increase the activity against both pathogens by substituting different cyclo amine or cyclo amine with different hetero atoms at C-7 position.

Antibacterial screening data (Table-1) shows that all the tested compounds depict moderate to good bacterial inhibition capabilities. As per Structure Activity Relation (SAR) from Table-1 it has been observed that group present at C-7 position play important role in bacterial inhibition capabilities.

Compound IX(a) showed better activity (piperidine ring at C-7 position) against *S. aureus and E. coli* have better activity against *S. aureus and E. coli* than compound IX(b) (pyrolidine) and IX(h) (azitidine) respectively.

When methyl group is introduced on piperidine ring at C-7 position then there is decrease in activity for example compound IX(a) have better activity against *S. aureus and E. coli* then a compound IX(d), IX(e) and IX(f) respectively which shows activity against *S. aureus* pathogen and show no activity against *E. coli* pathogen. Activity of methyl piperidene at C-7 position depends upon the position and number of methyl group on piperidine ring if we compare the activity of compound IX(d), IX(e) and IX(f) with compound IX(a). So when methyl group is introduced at piperidine ring there is decrease in activity against *S. aureus* pathogen and shows no activity against *E. coli* pathogen.

But when fluoro group is present at 4- position of piperidine ring then compound IX(l) shows better activity than compound IX(n) and IX(i) against *S. aureus and E. coli* pathogen but if the hydroxyl group is at 4-position of piperidine ring in compound IX(i) then there is no activity against *E. coli* pathogen.
When hetero atom is introduced in piperidine ring such as oxygen or nitrogen atom then there is change in the activity of compound. When piperizine group is introduced at C-7 position in compound IX(j) then it shows good activity against *S. aureus* and *E. coli* and when morpholine group is introduced at C-7 position in compound IX(c) then it shows good activity against *S. aureus* pathogen and show no activity against *E. coli* pathogen. This shows that piperizine ring at C-7 position play a very important role in increasing the activity of compound against *S. aureus* and *E. coli*. But when methyl group is introduced at 4-position of piperazine ring in compound IX(g) showed very good activity against *Staphylococcus aureus* pathogens and *Escherichia coli* pathogens as compared to all other synthesized molecule in Table -1 but when methyl group was introduced at 3-position of piperizine in compound IX(k) then there was slight decrease in activity when we compared with compound IX(g). This show that when methyl group is introduced on nitrogen atom of piperazine ring there is drastic increase in activity against both pathogens and when methyl group is introduced at carbon atom of piperazine ring there is decrease in activity against both pathogens. When ethyl ester group introduces at C-3 position of piperidine ring in compound IX(m) it shows better activity against *Staphylococcus aureus* pathogens and *Escherichia coli* pathogens.

**CONCLUSION**

Developed a very efficient, simple and safe method for the synthesis of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide. In previous reports ammonium gas (pressure reaction) or solution at higher temperature for amidation. We introduced ammonium chloride for amidation which is cheaper and safe to handle on large scale also.

As per Table-1 Compounds VII, VIII and IX (a-n) were synthesized and characterized by $^1$H NMR and mass. All compounds (Table-1) were screened against *Staphylococcus aureus pathogen* which shows moderate to very good antimicrobial activity. While some compounds shows moderate to very good antimicrobial activity against *Escherichia coli pathogen*. But all compounds show no antifungal activity. Compound IX(g) had shown very good activity against both pathogens. Above data in Table-1 shows that carboxamide at C-3 position, Carbonyl group at C-4 position with different heterocycles at C-7 position can improve the antimicrobial activity of compounds with less side effects by doing suitable modification on quinolone core at different position.
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REFERENCES


