Synthesis of N’-(7-Chloroquinolin-4-yl)-6-Methyl-2-Oxo-4-Phenyl-1,2,3,4-
Tetrahydropyrimidine-5-Carbohydrazide Derivatives As Potent
Antibacterial Agents

Manoj N. Bhoi\textsuperscript{a}, Mayuri A. Borad\textsuperscript{b}, Edwin. A. Pithawala\textsuperscript{b},
Shweta Modi\textsuperscript{a}, and Hitesh D. Patel\textsuperscript{a}

\textsuperscript{a}Department of Chemistry, School of Sciences, Gujarat University, Ahmedabad, India.
\textsuperscript{b}Department of Life Sciences, School of Sciences, Gujarat University, Ahmedabad, India.

E-mail address: hitesh13chem@rediffmail.com Tel.: +91-079-26300969; fax: +91-079-26308545

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ABSTRACT. Some novel N’-(7-chloroquinolin-4-yl)-6-methyl-2-oxo-4-phenyl-1,2,3,4-
tetrahydropyrimidine-5-carbohydrazide derivatives were synthesized via three step reactions by
conventional method. The structures of all the compounds have been confirmed by FT-IR, NMR,
and MASS and by elemental analysis. We have been evaluated it inhibition capacity for various
gram positive and gram negative bacterial strain. All compounds were found to be good to excellent
active against all four bacterial strains.

1. INTRODUCTION

The synthesis of heterocyclic compounds has become one of the key areas of research in the
field, both synthetic as well as medicinal chemistry. The most of the compounds with biological
activity are derived from heterocyclic structures. Dihydropyrimidines derivative have a diverse and
unique place in the medicinal chemistry owing to their wide therapeutic application scenario
containing anti-inflammatory agents,[1] anti-bacterial agents,[2] calcium-channel antagonists,[3]
agents,[7] etc.

Quinolines is a more important heterocyclic scaffold of paramount importance to the human race.
Several quinoline derivatives isolated from natural resources or prepared synthetically are
significant with respect to medicinal chemistry and biomedical use. Certainly quinolines derivatives
are some of the oldest compounds which have been utilized for the treatment of a variety of
diseases. Quinolines have caught the attention of researchers owing to their broad range of activities
and of course for their wide applications such as antimalarial,[8] Antibacterial,[9] antifungals,[10]
anticancer agents,[11] Anti-inflammatory activity, Antiviral, Anti-protozoal, CNS effects,
Cardiovascular activity, Analgesic activity, Antineoplastic, Anthelmintic, Hypoglycaemic activity,
Reproductive System etc.[12]

Encouraged by their promising medicinal properties of Dihydropyrimidines and Quinoline
derivative, we design new compound which contains dihydropyrimidines derivative which linkage
with quinolines. So we have synthesised new N’-(7-chloroquinolin-4-yl)-6-methyl-2-oxo-4-phenyl-
1,2,3,4-tetrahydropyrimidine-5-carbohydrazide derivative. Biological activities of newly
synthesised dihydropyrimidines derivatives were evaluated under in vitro Antibacterial activity
against gram positive and gram negative bacterial strain.

2. MATERIALS AND METHODS

All Starting materials and other reagents were purchased from commercial suppliers and were
used without any further purification unless otherwise indicated. The reactions were assayed by thin
layer chromatography (TLC) and terminated as judged by the consumption of starting material.
Analytical thin-layer chromatography (TLC) was performed on silica gel G 60 F\textsubscript{254} (Merck) plates.
The melting points were recorded on optimelt automated melting point system and were uncorrected. IR spectra was recorded on a Perkin–Elmer 377 spectrophotometer, $^1$H NMR spectra was measured on Bruker AV 400 MHz using DMSO as a solvent and TMS as an internal standard. Mass spectra was recorded on Advion Expression CMS, USA, using Methanol: Water: Formic acid (80: 20: 0.1) as mobile phase. Elemental analysis were performed on the vario MICRO cube, elementar CHN analyser serial no.: 15084053.

General Procedure for the synthesis of compound (4a-4g):
To a stirred mixture of aldehydes derivative (1a-1g) (2.0 mmol), ethyl acetoacetate 2 and urea 3 (1.5 mmol) in ethanol solvent in the presence of Alumino silica (10 mol %). The solvent was gradually evaporated by a heating reaction mixture was heated with stirring for 40-55 min. at reflux temperature. Progress of reaction monitored by TLC. After completion of the reaction, cooling the solid precipitate was filtered and washed with cold water and ethanol under reduced pressure and the residue was crystallized from ethanol or ethyl acetate-hexane (1:3) to afford the pure product (1a-1g) with very good yields.

General Procedure for the synthesis of compound (5a-5g):
Derivative of 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide (5a-5g) was prepared by refluxing a mixture of compound 4a-4g (0.01 mmol) and hydrazine hydrate 99 % (0.01 mmol) in ethanol for 14 h. The reaction mixture was cooled, dump in cold water and the precipitated solid was collected by filtration, washed with water, dried, and crystallized it.

General Procedure for the synthesis of compound (6a-6g):
6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide derivative (5a-5g) (0.01 mmol) and 4,7-dichloroquinoline 6 (0.01 mmol) dissolved in 5 ml CHCl$_3$ in 25 ml flat bottom flask by using Triethyl amine as a base. The resulting mixture was put in the heated oil bath for 6-8 hrs at reflux temperature. After confirmation of each reaction using TLC analysis (30 % Ethyl acetate: hexane), the reaction mixture was allowed to cool at room temperature and poured into cold water. The mixture was filtered continuously along with a wash of water. The solid crude product was simply purified by column chromatography over silica gel using a solvent system (20 % ethyl acetate: hexane) to obtain subsequent pure product (7a-7g) with 70-80 % yield.

N’-(7-chloroquinolin-4-yl)-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide (7a): IR (KBr) u max (cm$^{-1}$): 3056 (C-H Aromatic), 3034 (C-H alkene), 1675 (C=C alkene), 1545 (C=C aromatic), 1569 (C=O amide), 1345 (C-N Aromatic); $^1$H NMR (400 MHz, DMSO) δ 8.86 – 8.76 (m, 2H), 8.15 (s, 1H), 8.03 (d, 1H), 7.79 (s, 1H), 7.58 (d, 1H), 7.43 (dd, 1H), 7.29 – 6.75 (m, 5H), 6.72 (d, 1H), 5.56 (s, 1H), 2.42 (s, 1H), 2.32 (s, 3H); ESI-MS: m/z Calculated 407.8, found [M+H]$^+$ 408.8; Anal. Calcd for C$_{18}$H$_{18}$ClN$_5$O$_2$: C, 61.84; H, 4.45; Cl, 8.69; N, 17.17; O, 7.85 %; found C, 61.79; H, 4.65; N, 17.34 %.

4-(4-CHLORO-3-METHYLPHENYL)-N’-(7-chloroquinolin-4-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide (7b): IR (KBr) u max (cm$^{-1}$): 3065 (C-H Aromatic), 3075 (C-H alkene), 1634 (C=C alkene), 1509 (C=C aromatic), 1565 (C=O amide), 1350 (C-N Aromatic); $^1$H NMR (400 MHz, DMSO) δ 8.93 (s, 1H), 8.51 – 8.43 (m, 2H), 7.98 (d, 1H), 7.86 (s, 1H), 7.51 (d, 1H), 7.42 (dd, 1H), 7.27 (d, 2H), 7.19 (d, 2H), 5.56 (s, 1H), 4.02 (s, 1H), 2.37 (s, 3H); ESI-MS: m/z Calculated 441.08, found [M+H]$^+$ 442.0; Anal. Calcd for C$_{21}$H$_{18}$ClN$_5$O$_2$: C, 57.03; H, 3.87; Cl, 16.03; N, 15.83; O, 7.23 %; found C, 57.23; H, 3.74; N, 15.65 %.

N’-(7-chloroquinolin-4-yl)-4-(4-(dimethylamino)phenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydro pyrimidine-5-carbohydrazide (7c): IR (KBr) u max (cm$^{-1}$): 3025 (C-H Aromatic), 3046 (C-H alkene), 1656 (C=C alkene), 1486 (C=C aromatic), 1665 (C=O amide), 1354 (C-N Aromatic); $^1$H NMR (400 MHz, DMSO) δ 8.90 (s, 1H), 8.66 (s, 1H), 8.48 (d, 1H), 7.97 (d, 1H), 7.90 (s, 1H), 7.52 (d, 1H), 7.41 (dd, 1H), 7.06 (d, 2H), 6.59 (d, 2H), 6.21 (d, 1H), 5.56 (s, 1H), 4.03 (s, 1H), 2.88 (s,
6H), 2.35 (s, 3H); ESI-MS: m/z Calculated 450.92, found [M+H]+ 451.9; Anal. Calcd for C23H23ClN6O2: C, 61.26; H, 5.14; Cl, 7.86; N, 18.64; O, 7.10 %; found C, 61.36; H, 5.26; N, 18.54 %.

N'-7-chloroquinolin-4-yl)-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide (7d): IR (KBr) umax (cm⁻¹): 3077 (C-H Aromatic), 3035 (C-H alkene), 1674 (C=C alkene), 1541 (C=C aromatic), 1671 (C=O amide), 1285 (C-N Aromatic); 1H NMR (400 MHz, DMSO) δ 8.88 (s, 1H), 8.75 (d, 1H), 8.61 (s, 1H), 8.11 (dt, 1H), 8.02 (d, 2H), 7.76 – 7.63 (m, 3H), 7.53 – 7.44 (m, 2H), 6.63 (d, 1H), 5.56 (s, 1H), 2.76 (s, 1H), 2.36 (s, 3H); ESI-MS: m/z Calculated 452.8, found [M+H]+ 453.8; Anal. Calcd for C21H17ClN6O4: C, 55.70; H, 3.78; Cl, 7.83; N, 18.56; O, 14.13 C, 55.55; H, 3.69; N, 18.45 %.

4-(3-chlorophenyl)-N'-7-chloroquinolin-4-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide (7f): IR (KBr) umax (cm⁻¹): 3037 (C-H Aromatic), 3025 (C-H alkene), 1651 (C=C alkene), 1489 (C=C aromatic), 1674 (C=O amide), 1365 (C-N Aromatic); 1H NMR (400 MHz, DMSO) δ 8.69 (d, 2H), 8.06 – 7.95 (m, 2H), 7.87 (s, 1H), 7.69 (d, 1H), 7.46 (dd, 1H), 7.41 – 7.24 (m, 4H), 6.43 (d, 1H), 5.27 (s, 1H), 2.13 (s, 3H), 1.38 (s, 1H); ESI-MS: m/z Calculated 442.3, found [M+H]+ 443.3; Anal. Calcd for C21H17ClN2O2: C, 57.03; H, 3.87; Cl, 16.03; N, 15.83; O, 7.23 %; found C, 57.19; H, 3.78; N, 15.86 %.

Antibacterial Activity

Antibacterial activities of 7a-7g were carried out in Nutrient-agar plates by well diffusion assay. Cultures were activated in Nutrient broth. Isolates were inoculated in Nutrient broth and incubated at 37 °C for 24 hours for activation of cultures and then centrifuged at 3000 rpm for 15 min and the supernatant was collected to study antibacterial activity.

Using in-vitro agar well diffusion method, antimicrobial activity experiments were carried out. The activity of 7a-7g against test microorganisms (1000 µl volume with 1000 µg/ml concentration) of activated test cultures 2 Gram negative and 2 Gram positive; viz. Enterobacter aerogens MTCC No. 8558, Escherichia coli MTCC No. 1610, Micrococcus luteus MTCC No. 11948 and Bacillus cereus MTCC No. 8557 was inoculated in molten agar and poured into sterile plates than allowed to solidify. Wells with 5mm diameter were prepared at equal distance in solidified agar plates using cup-borer. Various derivatives 7a-7g with 1000 µgm/ml concentration were inoculated in the wells of nutrient agar whereas test microorganisms were inoculated by pour plate technique. The plates were incubated at 37 °C for 24 hours. The inhibition zones were measured at the end of the incubation period.
3. RESULT AND DISCUSSION

3.1. Chemistry

The title compounds, \( \text{N'}-(7\text{-chloroquinolin-4-yl})-6\text{-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydro pyrimidine-5-carbohydrazide derivative} (7a–7g) \) were synthesized via three step reactions from biginelli reaction reaction, hydrazine hydrate and 4,7-dichloro Quinoline as outlined in scheme 1.

Biginelli reaction is a versatile method for the preparation of Dihydropyrimidines derivative by reaction between aldehydes derivative (1a-1g), ethyl acetoacetate 2 and urea 3 in ethanol solvent in the presence of Alumino silica (10 mol %) as prompt catalyst. As per literature survive, here we first time reported Alumino silica catalysed synthesis of Dihydropyrimidines derivative.

\[
\begin{align*}
\text{CHO} & \quad \text{2} \\
\text{H}_2\text{N} & \quad \text{NH}_2
\end{align*}
\]

Scheme 1 synthesis of targeted compound 7a-7g

As shown in Table 1, it was found that this method works with a wide variety of substrates. A series of different position substituted benzaldehyde including either electron-withdrawing or electron-donating groups were used in this reaction, in good yields (70–80%).

<table>
<thead>
<tr>
<th>Entry</th>
<th>2-amino benzothiazole derivative</th>
<th>Product</th>
<th>yield %</th>
<th>mp °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1a</td>
<td>7a</td>
<td>75.8</td>
<td>177-178</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>2</th>
<th>CHO</th>
<th>Cl</th>
<th>Cl</th>
<th>73.6</th>
<th>174-177</th>
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<tbody>
<tr>
<td>3</td>
<td>CHO</td>
<td>N</td>
<td>1c</td>
<td>76.4</td>
<td>173-175</td>
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<tr>
<td>4</td>
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<td>NO₂</td>
<td>1d</td>
<td>80.1</td>
<td>175-176</td>
</tr>
<tr>
<td>5</td>
<td>CHO</td>
<td>HO</td>
<td>HO</td>
<td>1e</td>
<td>73.4</td>
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<tr>
<td>6</td>
<td>CHO</td>
<td>Cl</td>
<td>1f</td>
<td>74.8</td>
<td>175-179</td>
</tr>
<tr>
<td>7</td>
<td>CHO</td>
<td>NO₂</td>
<td>1g</td>
<td>70.5</td>
<td>172-176</td>
</tr>
</tbody>
</table>

The structure of the compound 7a was explained with the help of IR, ^1^H NMR, mass spectral data, and elemental analysis. The mass spectra of these compounds displayed molecular ion peaks at the appropriate m/z values at 408.8 mass unit. The IR spectra of compounds 7a exhibited peaks at strong amide (C=O) stretching band at 1659 υ (cm⁻¹). C=C and C–H aromatic stretching band was observed at 1675 and 3056 cm⁻¹ respectively. ^1^H NMR spectrum of 7a shows peaks at 8.86, δ value resulting from -NH of dihydropyrimidine ring. Amide type proton resonate in ^1^H NMR at
8.15 δ ppm. Aromatic proton of targeted compound resonates at 7.5–6.72 δ ppm. Rest of all proton resonated at 5.56, 2.42, 2.32 δ ppm.  

3.2. Biology  

Antibacterial Activity  

All the compounds (7a–7g) were screened for their antibacterial activity out on Nutrient-agar plates by well–diffusion assay against test culture. Cultures were stimulated in Nutrient broth. Isolates inhibits the above mentioned organisms or not were studied. A zone of inhibition was measured in terms of zone diameter and with the help of that zone index was calculated by using streptomycin as a standard drug. Compounds 7a–7g were screened against Enterobacter aerogens (MTCC 8558), Escherichia coli (MTCC 1610), Micrococcus luteus (MTCC 11948), and Bacillus cereus (MTCC 8558). The results are illustrated in Table 3.

<table>
<thead>
<tr>
<th>Derivatives</th>
<th>Enterobacter aerogens MTCC No. 8558</th>
<th>Escherichia coli MTCC No. 1610</th>
<th>Micrococcus luteus MTCC No. 11948</th>
<th>Bacillus cereus MTCC No. 8558</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean value for Zone of Inhibition (mm)</td>
<td>Activity Index (A.I.)</td>
<td>Mean value for Zone of Inhibition (mm)</td>
<td>Activity Index (A.I.)</td>
</tr>
<tr>
<td>7a</td>
<td>21</td>
<td>0.875</td>
<td>22</td>
<td>0.916</td>
</tr>
<tr>
<td>7b</td>
<td>19</td>
<td>0.791</td>
<td>24</td>
<td>1.000</td>
</tr>
<tr>
<td>7c</td>
<td>20</td>
<td>0.833</td>
<td>23</td>
<td>0.958</td>
</tr>
<tr>
<td>7d</td>
<td>24</td>
<td>1.000</td>
<td>24</td>
<td>1.000</td>
</tr>
<tr>
<td>7e</td>
<td>25</td>
<td>1.041</td>
<td>20</td>
<td>0.833</td>
</tr>
<tr>
<td>7f</td>
<td>21</td>
<td>0.875</td>
<td>21</td>
<td>0.875</td>
</tr>
<tr>
<td>7g</td>
<td>18</td>
<td>0.750</td>
<td>22</td>
<td>0.916</td>
</tr>
<tr>
<td>Std drug</td>
<td>24</td>
<td>---</td>
<td>24</td>
<td>---</td>
</tr>
</tbody>
</table>

**Determination of activity index**  

The activity index of the probiotic culture was calculated as:  

\[
\text{Activity index (A.I.)} = \frac{\text{Mean of zone of inhibition of derivative}}{\text{Zone of inhibition obtained for standard antibiotic drug}}
\]

**Note:** Standard drug used was Streptomycin with 1000 µg/ml concentration

The antibacterial activity data revealed that almost all the compounds 7a–7g exhibited promising antibacterial activity against gram negative and gram positive bacteria compared to streptomycin standard drug. Compounds 7d–7f exhibited better zone of inhibition against Enterobacter aerogens gram negative bacteria. When compound 7a–7g shows 87.5 % zone of inhibition to standard drug
against Escherichia coli. For Micrococcus luteus, compounds 7d-7f found to be very active than all other compounds in the series. Moreover, the compounds 7c, 7d, and 7f displayed good activity against Bacillus cereus gram positive bacteria.

4. CONCLUSION

In this article, we have presented the initial efforts made toward the synthesis of novel, potentially active N’-(7-chloroquinolin-4-yl)-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxyhydrazide derivatives. These derivatives were prepared through three step reaction of biginelli reaction, hydrazine hydrate and 4,7-dichloro Quinoline. When compound 7a-7g shows 87.5 % zone of inhibition to standard drug against Escherichia coli. Compounds 7d-7f exhibited better zone of inhibition against Enterobacter aerogens gram negative bacteria. For Micrococcus luteus, compounds 7d-7f found to be very active than all other compounds in the series. Moreover, the compounds 7c, 7d, and 7f displayed good activity against Bacillus cereus gram positive bacteria. Finally, it is conceivable that further derivatization of such compounds will be of interest with good hope to get more selective antibacterial agents.

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References


