SHORT COMMUNICATION

Synthesis, characterization and biological evaluation of dihydropyrimidine derivatives

Adithya Adhikari a, Balakrishna Kalluraya a,*, K.V. Sujith a, Gouthamchandra b, Riaz Mahmod b

a Department of Studies in Chemistry, Mangalore University, Mangalagangothri 574 199, Karnataka, India
b Department of P.G. Studies and Research in Biotechnology and Bioinformatics, Jnanasahyadri, Kuvempu University, Shankaraghatta 577 451, Karnataka, India

Received 22 March 2011; accepted 14 April 2011
Available online 23 April 2011

KEYWORDS
Quinolinodihydropyrimidines;
MW assisted synthesis; Three component reaction; Antioxidant activity; Antibacterial activity; Antifungal activity

Abstract A series of dihydropyrimidines containing quinoline were prepared under conventional heating and microwave irradiation. The structures of newly synthesized compounds were established based on analytical and spectral studies. Further these compounds were evaluated for their antioxidant, antifungal and antibacterial activities. Most of the compounds showed moderate to good activity when compared with standard.

1. Introduction
Dihydropyrimidinones are an important class of compounds due to their therapeutic and pharmacological properties, such as anti-inflammatory (Sadanandam et al., 1992), antihypertension (Chikhale et al., 2009), anticancer (Kumar et al., 2009) and antimicrobial (Chitra et al., 2010) activities. They also act as inhibitors for the propagation of the malarial parasite (Chiang et al., 2009), calcium channel blocking agents (Manjula et al., 2004; Singh et al., 2009), inhibitor of rho kinase (ROCK1) (Sehon et al., 2008), inhibitor of human kinesin (Eg5) (Kaan et al., 2010) and as anti-HBV agents (Zhu et al., 2010).

The common synthetic routes to these compounds generally involve multi-step transformations that are essentially based on the Biginelli condensation methodology. Multicomponent reactions constitute major importance in organic synthesis because of its diversity, efficiency and quick access to highly functionalized organic molecules which make them significant in drug discovery process (Sujith et al., 2009; Isloor et al., 2009).

Prompted by these observations and in continuation of our search for green technique (Hegde et al., 2007; Priya et al., 2007; Rai et al., 2009; Gowda et al., 2010) we herein report the synthesis of title compounds using microwave (MW) energy. Recently a major development that had a profound impact on heterogeneous reactions is the use of MW irradiation...
techniques. This approach has been used for a variety of applications in organic synthesis and functional group transformations. MW assisted heating under controlled conditions is an invaluable technology because it often dramatically reduces reaction times, typically from days or hours to minutes or even seconds (Larhed and Hallberg, 2001; Lidström et al., 2001).

2. Experimental

2.1. General

Purity of the newly synthesized compounds was checked by TLC on silica gel plates (Merck, Silica gel 60F254). Melting points were determined in open capillary tubes and are uncorrected. Elemental analysis was carried out in Vario EL III Elementa model. IR spectra were recorded by dispersing the compounds in KBr pellets on a Schimadzu FT-IR 157 spectrophotometer. $^1$H NMR spectra were recorded on a Bruker Avance II 400 MHz NMR spectrometer and all the chemical shift values were reported as δ (ppm). $^{13}$C NMR spectra were recorded on a Bruker Avance II 400 MHz NMR spectrometer. Mass spectra were recorded on a JEOL JMS – D300 mass spectrometer operating at 70 eV. Microwave reactions were carried out in Godrej (GMC 20E 08 SSGX) microwave oven. Title compounds are prepared as shown in Scheme 1. The precursor 6-substituted-2-chloro-3-formylquinoline (I) was prepared from substituted acetanilides (Meth-Cohn et al., 1981).

2.2. General method for the preparation of 6-substituted-2-hydroxyquinoline-3-carbaldehyde (2a–c)

We modified our earlier procedure (Kalluraya et al., 2003) for the synthesis of compounds 2a–c. In this method in a 250 mL beaker, 6-substituted-2-chloro-3-formylquinolines (I) (0.01 mol) was taken along with a solution of HCl (35 mL, 4 M) and subjected to MW irradiation (120 W) for 6 min. As the beaker was allowed to cool, yellow solid precipitates out. This was poured into a beaker containing crushed ice (100 g), filtered, dried and recrystallised from acetic acid.

2.3. General method for the preparation of 1-substituted-4-(6-substituted-2-hydroxyquinoline-3-yl)-5-acetyl/carboxyethyl-6-methyl-pyrimidine-2-one/thiones (3a–l)

2.3.1. Conventional method

In a 50 mL R.B. flask 6-substituted-2-hydroxyquinoline-3-carbaldehyde (0.01 mol), ethylacetacetaldehyde/acetylelaceton (0.012 mol), urea/thiourea/phenylthiourea (0.01 mol) and DMF (10 mL) were taken. Added two drops of conc. H$_2$SO$_4$ and refluxed on an oil bath. Completion of the reaction was monitored by TLC. The solid precipitated was filtered, washed with ethanol, dried and recrystallised using DMF.

2.3.2. Microwave method

6-Substituted-2-hydroxyquinoline-3-carbaldehyde (0.005 mol), ethylacetacetaldehyde/acetylelaceton (0.005 mol), urea/thiourea/phenylthiourea (0.005 mol) and DMF (5 mL) were taken in a 100 mL beaker. Added two drops of conc. H$_2$SO$_4$ and subjected to MW irradiation (160 W). The completion of the reaction was monitored using TLC. The solid precipitated was filtered, washed with ethanol, dried and recrystallised using DMF. M.P., time required and yield data of the newly synthesised compounds are summarized in Table 1.

2.3.2.1. 4–(2-Hydroxyquinoline-3-yl)-5-carboxyethyl-6-methyl–pyrimidine-2-one (3a).

IR (KBr) $\gamma$/cm$^{-1}$: 3106.7 (O–H), 2982.1 (C–H), 1705.8 (ester C=O), 1664.5 (amide

\begin{align*}
\text{R} = & H, \text{CH}_3, \text{OCH}_3 \\
\text{R}_1 = & \text{CH}_3, \text{OC}_2\text{H}_5 \\
\text{R}_2 = & H, \text{Ph} \\
\text{X} = & O, S
\end{align*}

Scheme 1
1. 1-Phenyl-4-[(2-hydroxyquinoline-3-yl)-5-carboxethyl-6-methyl-pyrimidine-2-thione (3a)]. IR (KBr) cm⁻¹: 3159.5 (O–H), 2969.5 (C–H), 1719.1 (ester C=O), 1634.5 (amide C=O). [H NMR (DMSO-d₆): 1.03 (t, 3H, CH₃), 2.39 (s, 3H, CH₃), 2.36 (s, 3H, CH₃), 3.73 (q, 2H, CH₂), 5.04 (d, 1H, CH), 7.03–7.8 (m, 5H, Ar-H), 8.5 (s, 1H, N-H), 9.01 (s, 1H, N-H), 12.02 (s, 1H, OH); MS: m/z = 328 [M⁺ + 1]. Anal. calcd for C₁₇H₁₇N₃O₃S (%): C, 62.38; H, 5.23; N, 12.84. Found: C, 62.25; H, 5.18; N, 12.58.

2. 3.2.2. 1-Phenyl-4-[(2-hydroxyquinoline-3-yl)-5-carboxethyl-6-methyl-pyrimidine-2-thione (3b)]. IR (KBr) cm⁻¹: 3211.4 (O–H), 2952.3 (C–H), 1712.9 (ester C=O), 1641.5 (amide C=O). [H NMR (DMSO-d₆): 1.05 (t, 3H, CH₃), 2.21 (s, 3H, CH₃), 2.61 (s, 3H, CH₃), 2.6 (q, 2H, CH₂), 5.34 (d, 1H, CH), 7.07–7.49 (m, 4H, Ar-H), 7.5 (s, 1H, NH), 9.2 (s, 1H, N-H), 11.81 (s, 1H, OH); MS: m/z = 342 [M⁺ + 1]. Anal. calcd for C₁₇H₁₇N₃O₃S (%): C, 59.46; H, 4.99; N, 12.24. Found: C, 59.37; H, 4.98; N, 12.18.

3. 2.3.2.4. 4-(6-Methyl-2-hydroxyquinoline-3-yl)-5-carboxethyl-6-methyl-pyrimidine-2-thione (3d)]. IR (KBr) cm⁻¹: 3190.7 (O–H), 1703.5 (ester C=O), 1625.1 (amide C=O). [H NMR (DMSO-d₆): 1.05 (t, 3H, CH₃), 2.31 (s, 3H, CH₃), 2.33 (s, 3H, CH₃), 3.97 (q, 2H, CH₂), 5.34 (d, 1H, CH), 7.07–7.49 (m, 4H, Ar-H), 7.5 (s, 1H, NH), 9.2 (s, 1H, N-H), 11.81 (s, 1H, OH); MS: m/z = 343 [M⁺ + 1]. Anal. calcd for C₁₇H₁₇N₃O₃S (%): C, 59.46; H, 4.99; N, 12.23. Found: C, 59.37; H, 4.98; N, 12.18.

4. 2.3.2.5. 1-Phenyl-4-(6-methyl-2-hydroxyquinoline-3-yl)-5-carboxethyl-6-methyl-pyrimidine-2-thione (3e)]. IR (KBr) cm⁻¹: 3159.5 (O–H), 2969.5 (C–H), 1719.1 (ester C=O), 1649.9 (amide C=O). [H NMR (DMSO-d₆): 1.38 (s, 3H, CH₃), 2.43 (s, 3H, CH₃), 3.33 (s, 3H, CH₃), 4.38 (q, 2H, CH₂), 5.15 (d, 1H, CH), 7.1–7.59 (m, 9H, Ar-H), 8.43 (s, 1H, N-H), 11.91 (s, 1H, OH); MS: m/z = 434 [M⁺ + 1]. Anal. calcd for C₂₄H₂₃N₃O₃S (%): C, 66.49; H, 5.35; N, 9.69. Found: C, 66.46; H, 5.26; N, 9.57.

5. 2.3.2.6. 4-(6-Methyl-2-hydroxyquinoline-3-yl)-5-carboxethyl-6-methyl-pyrimidine-2-thione (3f)]. IR (KBr) cm⁻¹: 3235.6 (O–H), 2969.4 (C–H), 1710.4 (ester C=O), 1628.2 (amide C=O); [H NMR (DMSO-d₆): 1.13 (t, 3H, CH₃), 2.39 (s, 3H, CH₃), 3.31 (s, 3H, CH₃), 4.21 (q, 2H, CH₂), 5.73 (d, 1H, CH), 7.1–7.68 (m, 4H, Ar-H), 8.37 (s, 1H, N-H), 8.9 (s, 1H, N-H), 11.93 (s, 1H, OH); MS: m/z = 358 [M⁺ + 1]. Anal. calcd for C₁₈H₁₉N₃O₅S (%): C, 60.49; H, 5.36; N, 11.76. Found: C, 60.37; H, 5.28; N, 11.68.

6. 2.3.2.7. 4-(6-Methyl-2-hydroxyquinoline-3-yl)-5-acetyl-6-methyl-pyrimidine-2-one (3g)]. IR (KBr) cm⁻¹: 3210.1 (O–H), 2899.6 (C–H), 1688.6 (acetyl C=O), 1634.5 (amide C=O); [H NMR (DMSO-d₆): 2.14 (s, 3H, CH₃), 2.31 (s, 3H, CH₃), 3.34 (s, 3H, CH₃), 5.41 (d, 1H, CH), 7.07–7.48 (m, 5H, Ar-H), 9.2 (s, 1H, N-H), 11.9 (s, 1H, OH); MS: m/z = 312 [M⁺ + 1]. Anal. calcd for C₁₉H₁₉N₃O₃ (%): C, 65.58; H, 5.50; N, 13.5. Found: C, 65.47; H, 5.38; N, 13.41.

7. 2.3.2.8. 1-Phenyl-4-(2-hydroxyquinoline-3-yl)-5-acetyl-6-methyl-pyrimidine-2-thione (3h)]. IR (KBr) cm⁻¹: 3272.5 (O–H), 2899.6 (C–H), 1694.7 (acetyl C=O), 1652.5 (amide C=O); [H NMR (DMSO-d₆): 2.14 (s, 3H, CH₃), 3.34 (s, 3H, CH₃), 5.38 (d, 1H, CH), 6.9–7.9 (m, 10H, Ar-H), 8.28 (s, 1H, N-H), 12.06 (s, 1H, OH); MS: m/z = 374 [M⁺ + 1]. Anal. calcd for C₂₀H₂₁N₃O₄ (%): C, 67.84; H, 4.92; N, 10.79. Found: C, 67.76; H, 4.88; N, 10.71.

8. 2.3.2.9. 4-(2-Hydroxyquinoline-3-yl)-5-acetyl-6-methyl-pyrimidine-2-thione (3i)]. IR (KBr) cm⁻¹: 3169.3 (O–H), 2973.5 (C–H), 1689.1 (acetyl C=O), 1627.3 (amide C=O); [H NMR (DMSO-d₆): 2.16 (s, 3H, CH₃), 3.31 (s, 3H, CH₃), 5.43 (d, 1H, CH), 7.1–7.8 (m, 5H, Ar-H), 8.31 (s, 1H, N-H), 8.92 (s, 1H, N-H), 11.98 (s, 1H, OH); MS: m/z = 314 [M⁺ + 1]. Anal. calcd for C₁₉H₁₉N₃O₃ (%): C, 61.32; H, 4.82; N, 13.41. Found: C, 61.27; H, 4.78; N, 13.28.

9. 2.3.2.10. 4-(2-Hydroxyquinoline-3-yl)-5-acetyl-6-methyl-pyrimidine-2-one (3j)]. IR (KBr) cm⁻¹: 3128.8 (O–H), 2945.9 (C–H), 1684.7 (acetyl C=O), 1635.7 (amide C=O);
1H NMR (DMSO-d$_6$) δ: 2.18 (s, 3H, CH$_3$), 3.34 (s, 3H, CH$_3$), 5.37 (d, 1H, CH), 7.1–7.79 (m, 5H, Ar-H), 8.29 (s, 1H, N–H), 8.89 (s, 1H, N–H), 12.02 (s, 1H, OH); MS: m/z = 298 [M$^+$ + 1], Anal. calcd for C$_{16}$H$_{15}$N$_3$O$_3$ (%): C, 64.64; H, 5.09; N, 14.13. Found: C, 64.57; H, 5.01; N, 14.08.

2.3.2.11. 4–(6-Methyl-2-hydroxyquinoline-3-yl)-5-acetyl-6-methyl–pyrimidine-2-thione (3k). IR (KBr) $\tilde{\nu}$/cm$^{-1}$: 3224.7 (O–H), 2904.8 (C–H), 1685.9 (acetyl C=O), 1638.8 (amide C=O); 1H NMR (DMSO-d$_6$) δ: 2.15 (s, 3H, CH$_3$), 2.33 (s, 3H, CH$_3$), 3.34 (s, 3H, CH$_3$), 5.44 (d, 1H, CH), 7.03–7.62 (m, 5H, Ar-H), 8.92 (s, 1H, N–H), 11.97 (s, 1H, OH); MS: m/z = 328 [M$^+$ + 1], Anal. calcd for C$_{17}$H$_{17}$N$_3$O$_2$S (%): C, 62.36; H, 5.23; N, 12.83. Found: C, 62.27; H, 5.18; N, 12.71.

2.3.2.12. 1-Phenyl-4-(6-methyl-2-hydroxyquinoline-3-yl)-5-acetyl-6-methyl–pyrimidine-2-thione (3l). IR (KBr) $\tilde{\nu}$/cm$^{-1}$: 3235.1 (O–H), 2926.5 (C–H), 1684.8 (acetyl C=O), 1642.2 (amide C=O); 1H NMR (DMSO-d$_6$) δ: 2.15 (s, 3H, CH$_3$), 2.34 (s, 3H, CH$_3$), 3.35 (s, 3H, CH$_3$), 5.43d, 1H, CH), 7.1–7.9 (m, 9H, Ar-H), 8.51 (s, 1H, N–H), 11.95 (s, 1H, OH); MS: m/z = 404 [M$^+$ + 1], Anal. calcd for C$_{23}$H$_{21}$N$_3$O$_2$S (%): C, 68.46; H, 5.25; N, 10.41. Found: C, 68.39; H, 5.18; N, 10.31.

3. Results and discussion

3.1. Chemistry

Our modified method describes the successful conversion of 6-substituted-2-chloro-3-formylquinolines (1) into 6-substituted-2-hydroxyquinoline-3-carbaldehyde (2) under MW irradiation. This method is rapid and convenient over conventional heating method. Also when these (2) were subjected to MW irradiation along with substituted urea/thiourea and active methylene compound underwent rapid Biginelli reaction to afford 1-substituted-4-(6-substituted-2-hydroxyquinoline-3-yl)-5-acetyl/ carboxyethyl-6-methylpyrimidine-2-one/thiones (3) than conventional heating method. These reactions are summarized in Scheme 1 and results are presented in Table 1.

3.2. DPPH radical scavenging activity

Literature procedure (Williams et al., 1995) was followed for evaluation of the free radical-scavenging capacity. Briefly, 1 mL of the test sample (100 µg/mL) was mixed with the methanolic 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (2 mL, 0.2 mM). The absorbance was measured at 517 nm immediately after standing at room temperature for 30 min. The percentage of scavenging has been calculated as the ratio of the absorption of the sample relative to the control DPPH (0.2 mM) solution without test samples. DPPH radical-scavenging activity was expressed as the inhibition percentage. Results are shown in Table 2.

3.3. Antibacterial activity

The bacteria namely Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa were screened for their sensitivity towards synthesized compounds by Agar well diffusion method (Tepe et al., 2004). In this method, 24 h old Muller–Hinton broth cultures of test bacteria were swabbed uniformly on solidified sterile Muller–Hinton agar plates using sterile cotton swab. Then, aseptically wells of 6 mm diameter were bored in the inoculated plates with the help of gel puncher and the samples (100 µL; 10 mg/mL of DMSO), standard (Chloramphenicol, Table 2 DPPH radical scavenging activity of compounds 3a–l.

<table>
<thead>
<tr>
<th>Compound no.</th>
<th>3a</th>
<th>3b</th>
<th>3c</th>
<th>3d</th>
<th>3e</th>
<th>3f</th>
<th>3g</th>
<th>3h</th>
<th>3i</th>
<th>3j</th>
<th>3k</th>
<th>3l</th>
<th>BHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Inhibition</td>
<td>83.27</td>
<td>86.61</td>
<td>81.71</td>
<td>85.33</td>
<td>89.54</td>
<td>80.14</td>
<td>81.11</td>
<td>89.10</td>
<td>81.23</td>
<td>82.89</td>
<td>82.61</td>
<td>81.30</td>
<td>91.64</td>
</tr>
</tbody>
</table>

Table 3 Antibacterial data of compounds 3a–l.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Zone of inhibition in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. coli 50 µg/mL</td>
</tr>
<tr>
<td>100 µg/mL</td>
<td>50 µg/mL</td>
</tr>
<tr>
<td>3a</td>
<td>17</td>
</tr>
<tr>
<td>3b</td>
<td>16</td>
</tr>
<tr>
<td>3c</td>
<td>20</td>
</tr>
<tr>
<td>3d</td>
<td>21</td>
</tr>
<tr>
<td>3e</td>
<td>17</td>
</tr>
<tr>
<td>3f</td>
<td>18</td>
</tr>
<tr>
<td>3g</td>
<td>17</td>
</tr>
<tr>
<td>3h</td>
<td>15</td>
</tr>
<tr>
<td>3i</td>
<td>20</td>
</tr>
<tr>
<td>3j</td>
<td>17</td>
</tr>
<tr>
<td>3k</td>
<td>18</td>
</tr>
<tr>
<td>3l</td>
<td>15</td>
</tr>
<tr>
<td>Standard</td>
<td>19</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
</tbody>
</table>
the results are shown in Table 4.

Most of the compounds tested showed moderate to good pharmacological activities when compared to the standard. Thus on the basis of the results obtained it can be concluded that the dihydropyrimidines bearing quinoline moiety demonstrated good antioxidant, antibacterial and antifungal activities.

### Acknowledgement

The author A.A. thanks University Grant Commission for the financial assistance in the form of fellowship.

### References


### Table 4 Antifungal data of compounds 3a–l.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Zone of inhibition in mm at 100 mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. albicans</td>
</tr>
<tr>
<td>3a</td>
<td>15</td>
</tr>
<tr>
<td>3b</td>
<td>17</td>
</tr>
<tr>
<td>3c</td>
<td>17</td>
</tr>
<tr>
<td>3d</td>
<td>19</td>
</tr>
<tr>
<td>3e</td>
<td>16</td>
</tr>
<tr>
<td>3f</td>
<td>12</td>
</tr>
<tr>
<td>3g</td>
<td>13</td>
</tr>
<tr>
<td>3h</td>
<td>20</td>
</tr>
<tr>
<td>3i</td>
<td>18</td>
</tr>
<tr>
<td>3j</td>
<td>17</td>
</tr>
<tr>
<td>3k</td>
<td>14</td>
</tr>
<tr>
<td>3l</td>
<td>16</td>
</tr>
<tr>
<td>Standard</td>
<td>20</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
</tbody>
</table>

1 mg/mL of antifungal activity by agar well diffusion method (Perez et al., 1990) with sterile cork borer of size 6.0 mm. The cultures of 48 h old grown on potato dextrose agar (PDA) were used for inoculation of fungal strain on PDA plates. An aliquot (0.02 mL) of inoculum was introduced to molten PDA and poured into a petri dish. After solidification, the appropriate wells were made on agar plate by using cork borer. Incubation period of 24–48 h at 28 °C was maintained for observation of antifungal activity of the compounds. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth. The complete antifungal analysis was carried out under strict aseptic conditions. The zones of inhibition were measured. Among the compounds tested 3c, 3d, 3i, 3l showed significant activity. All the results are shown in Table 4.

### 3.4. Antifungal activity

Synthesized compounds were screened for antifungal activity by agar well diffusion method (Perez et al., 1990) with sterile cork borer of size 6.0 mm. The cultures of 48 h old grown on potato dextrose agar (PDA) were used for inoculation of fungal strain on PDA plates. An aliquot (0.02 mL) of inoculum was introduced to molten PDA and poured into a petri dish. After solidification, the appropriate wells were made on agar plate by using cork borer. Incubation period of 24–48 h at 28 °C was maintained for observation of antifungal activity of the compounds. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth. The complete antifungal analysis was carried out under strict aseptic conditions. The zones of inhibition were measured. Among the compounds tested 3c, 3d, 3i, 3l showed significant activity. All the results are shown in Table 4.

### 4. Conclusion

Synthesis of heterocyclic compounds like pyrimidines bearing quinoline moiety generally involves prolonged heating conditions. Therefore, researchers continue to discover simple, rapid and convenient routes for the synthesis of these heterocycles. Therefore, on the basis of the results obtained it can be concluded that our approach to the synthesis of dihydropyrimidines bearing quinoline moiety is rapid and useful over conventional heating technique. In short a convenient and high-yielding methodology to synthesize pyrimidines bearing quinoline moiety has been developed.

Furthermore the results obtained from antioxidant, antifungal and antibacterial screening of the novel compounds are encouraging. Most of the compounds tested showed moderate to good pharmacological activities when compared to the standard. Thus on the basis of the results obtained it can be concluded that the dihydropyrimidines bearing quinoline moiety demonstrated good antioxidant, antibacterial and antifungal activities.