SYNTHESIS AND ANTIVIRAL ACTIVITY OF SOME NOVEL 2-SUBSTITUTED, 3-(6-ETHYL, 4-AMINO, 5-(4-CHLOROPHENYL)–PYRIMIDIN–2-YL)] QUINAZOLIN–4 (3H)–ONES

P. SELVAM* B. CHENNAMAa, N. MURUGESHb, M. CHANDRAMOHANC and E. DE CLERCSQd

A. K. College of Pharmacy, Anand nagar, KRISHNANKOIL – 626 190 (TN) INDIA
aPeriyar College of Pharmaceutical Science for Girls, TIRUCHIRAPPALLI – 620 021 (TN) INDIA
bInstitute of Pharmacology, Madurai Medical College, MADURA1 – 625 001 (TN) INDIA
cBharat Ratna Kamarajar Liver Hospital and Research Centre, MADURA1 – 625 001 (TN) INDIA
dRega Institute of Medical Research, Katholieke University – Leuven, Minder Broedersstraat, B–3000, LEUVEN, BELGIUM

ABSTRACT

2-substituted benzoxazin-4-ones were condensed with pyrimethamine to form the 2,3-disubstituted quinazolin-4(3H)-ones. Their chemical structures were elucidated by means of spectral analysis (IR, NMR and MS). Synthesized compounds were evaluated for antiviral activity against HSV-1 and 2 in HEL cells, influenza B virus in MDCK cells and SARS virus in Vero E6 cells. The compound 2-phenyl, 3-(6-ethyl, 4-amino, 5-(4-chlorophenyl) pyrimidin-2-yl)] quinazolin-4(3H)-one (3) have minimum inhibitory concentration of 48 μg/mL against herpes simplex virus-1. Compounds (1) and (3) were active against influenza B virus in MDCK cells.

Key words: Quinazolin-4(3H)-one, Pyrimethamine, Antiviral activity.

INTRODUCTION

Quinazolin-4(3H)-one is a versatile lead molecule for the designing of potential bioactive agents. Quinazolin-4(3H)-one derivatives were reported to posses sedative1, anticonvulsant2, antifungal and antibacterial2, anti-HIV4,5 and anticancer5 activities. Schiff and Mannich bases of isatin with trimethoprim and pyrimethamine were synthesized and screened for anti-HIV activity. Some of their derivatives showed significant anti-HIV activity6,7. In recent years, much attention has been devoted in search of effective chemotherapeutic agents for the treatment of fatal diseases like AIDS, SARS etc. We have synthesized some novel heterocyclic compounds and evaluated for antiviral activity. Some of these derivatives exhibited significant activity against HIV and vaccinia viruses9–12.

*For correspondence email – periyamasamyselvam2001@yahoo.co.in
The present work involves the synthesis of some 2,3-disubstituted quinazolin-4(3H)-one derivatives by condensation of 2-substituted 1,3-benzoxazin-4-ones and pyrimethamine (Scheme 1). The synthesized compounds were evaluated for the antiviral activities against Herpes simplex virus-1 and 2 in HEL cells, influenza virus B in MDCK cells and SARS virus Vero E6 cells.

**EXPERIMENTAL**

Melting points were determined by using Thomas Hoover melting point apparatus and are uncorrected. The structures of the synthesized compounds were elucidated by using DOMEM MV 102 FT–IR in KBr disc and $^1$H NMR was taken on a Bruker AMX (500 MHz) FT–NMR. Mass spectra were obtained on a Varian Atlas CH–7 mass spectrometer at 70 eV. The purity was checked by TLC using silica gel G as stationary phase and CHCl$_3$ : CH$_3$OH (9 : 1) as mobile phase.

**Synthesis of 2–substituted, 3–[(6–ethyl, 4–amino, 5–(4–chlorophenyl) pyrimidin–2–yl)] quinazolin–4(3H)–ones**

An equimolar (0.01 mol) mixture of 2–substituted–1,3–benzoxazin–4–one and pyrimethamine was refluxed for 6 h in presence of 10 mL glacial acetic acid. The mixture was then cooled at room temperature and poured into crushed ice and solid thus obtained was recrystallised from ethanol.
2-methyl, 3-[4-amino-5-(4-chlorophenyl)-6-ethylpyrimidin-2-yl] quinazolin-4(3H)-one (1)

Yield = 84%, mp 211°C, $^1$H-NMR (DMSO-$d_6$) : 0.1 (t, 3H, CH$_3$), 2.5 (q, 2H, J=6.8 Hz, CH$_2$), 3.5 (b, 2H, NH$_2$), 7.0 (t, 1H, H-7), 7.32 (t, 1H, H-6), 7.5-7.6 (m, 4H, Ar-H), 8.2 (d, 1H, H-5), 8.5 (d, 1H, J=6.8 Hz, H-8); IR (KBr) cm$^{-1}$: 3455 (NH), 1645 (C=N), 3204 (C-H), 1669 (C=O); EI-MS m/z: 391, 859.

2-ethyl,3-[4-amino-5-(4-chlorophenyl)-6-ethylpyrimidin-2-yl]quinazolin-4 (3H)-one (2)

Yield = 87%, mp 210°C, $^1$H-NMR (DMSO-$d_6$) : 1.1 (t, 3H, CH$_3$), 2.5 (q, 2H, J=5.9 Hz, CH$_2$), 3.5 (b, 2H, NH$_2$), 7.0 (t, 1H, J=7 Hz, H-7), 7.32 (t, 1H, H-6), 7.5-7.6 (m, 4H, Ar-H), 8.2 (d, 1H, H-5), 8.5 (d, 1H, J=6.8 Hz, H-8); IR (KBr) cm$^{-1}$: 3465 (NH) 1645 (C=N), 3210 (C-H) 1672 (C=O); EI-MS m/z: 405, 886.

2-phenyl, 3-[4-amino-5-(4-chlorophenyl)-6-ethyl]pyrimidin-2-yl]quinazolin-4(3H)-one (3)

Yield = 88%, mp 169°C, $^1$H-NMR (DMSO-$d_6$) : 1.0 (t, 3H, CH$_3$), 2.5 (q, 2H, J=5Hz, CH$_2$), 3.5 (b, 2H, NH$_2$), 7.0 (t, 1H, J=7 Hz, H-7), 7.32 (t, 1H, H-6), 7.5 (m, 4H, Ar-H), 7.9 (m, 5H, Ar-H), 8.2 (d, 1H, H-5), 8.7 (d, 1H, H-8); IR (KBr) cm$^{-1}$: 3393 (NH), 1657 (C=N), 3119 (C-H), 1681 (C=O); EI-MS m/z: 453, 93.

**Antiviral screening**

Antiviral activity of test compounds was tested against HSV-1 and HSV-2 in HEL cell culture. Parameters such as Minimum Inhibitory Concentration (MIC), a concentration of substance required to reduce virus-induced cytopathogenicity to 50% and Minimum Cytotoxic Concentration (MCC), a concentration of substance required to cause microscopically detectable alteration of normal cell were determined.

**Table I. Cytotoxicity and Antiviral activity of compounds in HEL cell**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Minimum Cytotoxic Concentration$^a$ (µg/mL)</th>
<th>Minimum Inhibitory Concentration$^b$ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Herpes Simplex Virus-1 (KOS)</td>
</tr>
<tr>
<td>(1)</td>
<td>&gt; 400</td>
<td>&gt; 400</td>
</tr>
<tr>
<td>(2)</td>
<td>400</td>
<td>&gt; 80</td>
</tr>
<tr>
<td>(3)</td>
<td>400</td>
<td>48</td>
</tr>
<tr>
<td>Brivudin</td>
<td>&gt; 400</td>
<td>0.0153</td>
</tr>
</tbody>
</table>

$^a$Required to cause a microscopically detectable alteration of normal cell morphology.

$^b$Required to reduce a virus-induced cytopathogenicity by 50%.
Table 2. Cytotoxicity and Antiviral activity of compounds in MDCK cell

<table>
<thead>
<tr>
<th>Compound</th>
<th>Methods</th>
<th>Virus</th>
<th>EC$_{50}a$</th>
<th>IC$_{50}b$</th>
<th>SI$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>CPE inhibition</td>
<td>IVB</td>
<td>5.5</td>
<td>&gt; 100</td>
<td>&gt; 18</td>
</tr>
<tr>
<td>(2)</td>
<td>CPE inhibition</td>
<td>IVB</td>
<td>6</td>
<td>32</td>
<td>5.3</td>
</tr>
<tr>
<td>(3)</td>
<td>CPE inhibition</td>
<td>IVB</td>
<td>6</td>
<td>&gt; 100</td>
<td>&gt; 17</td>
</tr>
<tr>
<td>RIBAVIRIN (STD)</td>
<td>CPE inhibition</td>
<td>IVB</td>
<td>1.8</td>
<td>&gt; 100</td>
<td>&gt; 56</td>
</tr>
</tbody>
</table>

$^a$Required to reduce a virus-induced cytopathogenicity by 50%.
$^b$Required to cause a microscopically detectable alteration of normal cell morphology.
$^c$Selectivity index—ratio of IC$_{50}$ to EC$_{50}$

Table 3. Cytotoxicity and Antiviral activity of compounds in Vero cell

<table>
<thead>
<tr>
<th>Compound</th>
<th>Methods</th>
<th>Virus</th>
<th>EC$_{50}a$</th>
<th>IC$_{50}b$</th>
<th>SI$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>CPE inhibition</td>
<td>SARS</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>0</td>
</tr>
<tr>
<td>(2)</td>
<td>CPE inhibition</td>
<td>SARS</td>
<td>20</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>(3)</td>
<td>CPE inhibition</td>
<td>SARS</td>
<td>20</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>RIBAVIRIN (STD)</td>
<td>CPE inhibition</td>
<td>SARS</td>
<td>5</td>
<td>20</td>
<td>4</td>
</tr>
</tbody>
</table>

$^a$Required to reduce a virus-induced cytopathogenicity by 50%.
$^b$Required to cause a microscopically detectable alteration of normal cell morphology.
$^c$Selectivity index—ratio of IC$_{50}$ to EC$_{50}$

REFERENCES


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