Synthesis, Characterization and Antimicrobial Activity of New Pyrrole Derivatives

Akbar Idhayadhulla, Radhakrishnan Surendra Kumar, and Abdul Jamal Abdul Nasser*

P.G. & Research Dept. of chemistry, Jamal Mohamed College, Tiruchirappalli- 620020, Tamil Nadu, India.
jamal_abdulchem@gmail.com

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Abstract. Here we describe pyrrole derivatives with potent antibacterial and antifungal activity. A new series of pyrrole 3a-e derivatives were synthesized using standard amination reactions. All the compounds presented here were obtained with high yields and under easy experimental conditions. Synthesized compounds were characterized by IR, 1H NMR, 13C NMR, mass spectra and mass spectral fragmentation. The above title compounds have not been reported previously. These title compounds have been screened against E. coli and S. aureus for antibacterial activity, as well as against A. niger and C. albicans for antifungal activity. We were able to obtain compounds with higher or equal potency to the reference compounds (Ciprofloxacin and Clotrimazole). The data shows that a 4-hydroxyphenyl ring in our most potent compound seems to be responsible for antifungal activity against C. albicans. Incorporation of a 4-hydroxyphenyl ring as a pharmacophoric feature of compound C. albicans is a promising prospect.

Key words: Pyrrole derivative, antimicrobial activity, structure-activity relationship.

Introduction

Pyrrole heterocyclic derivatives were reported as having important synthetic and biological activities [1, 2] such as COX-1/COX-2 inhibitors [3] and cytotoxic activity against a variety of marine and human tumour models [4]. Thiazole derivatives also display a wide range of biological activities such as anesthetic [5] and anti-inflammatory [6] properties. Based on this observation, we are interested in preparing the biological behaviours of these title compounds. The above title compounds have not been published before, but previously reported methods appeared to meet our requirements. Therefore we synthesized novel pyrrole derivatives and screened their antimicrobial activity.

Results and Discussion

Chemistry

The general procedure for the synthesis of compounds 3a-e and the compound 4-phenyl-1,3-thiazol-2-amine (1a) was prepared according to the method shown in the literature [7, 8].

Diethyl 3,5-dimethyl-1H-pyrrole-2,4-dicarboxylate 2 is prepared from the Fischer and Noller condensation method [9]. The general procedure for the synthesis of compounds 3a-e, 3,5-dimethyl-N2,N4-bis(4-phenylthiazol-2-yl)-1H-pyrrole-2,4-dicarboxamide (3a) is prepared from amination method [10, 11]. The reaction sequence is outlined in Scheme 1. Physicochemical data of compounds 3a-e are summarized in Table.

Resumen. Se describe la síntesis de una nueva serie de derivados pirrolícos con potente actividad antibacteriana y antifúngica. La serie de compuestos 3a-e se sintetizó empleando reacciones de afinación estándar. Todos los compuestos preparados se obtuvieron en elevados rendimientos y bajo fáciles condiciones experimentales. Estos compuestos se caracterizaron por IR, RMN 1H, RMN 13C, y su estudio de fragmentación en espectrometría de masas. Los compuestos sintetizados se evaluaron en su actividad antibacterial contra E. coli y S. aureus, y en su actividad antifúngica contra A. niger y C. albicans. Se logró obtener compuestos con una potencia mayor o igual a los compuestos de referencia: ciprofloxacina y clotrimazol. Los datos muestran que el anillo 4-hidroxifenilo en el compuesto más activo parece ser el grupo farmacofóro responsable de la actividad antifúngica contra C. albicans.

Palabras clave: Derivados pirrolícos, actividad antimicrobiana, relación estructura-actividad.

1. The IR spectrum of 3a shows absorption bands at 3350, 1680.16 and 619.11 cm⁻¹, corresponding to NH in pyrrole ring, CONH and the C-S-C group, respectively. The ¹H NMR spectrum (Figure 3) of compound 3a shows the signals at δ11.59 and 12.93, corresponding to the NH proton in the pyrrole ring and CONH protons, respectively. The ¹³C NMR spectrum (Figure 4) of compound 3a shows the carbon signals at 6168.50 and 103.86, corresponding to CONH and CH carbons in the thiazole ring. The mass spectrum (EI-MS) of compound 3a shows the molecular ion peak at m/z 499.82(M⁺, 87%), which is confirmed as the molecular weight of compound 3a. Figure 5 and Figure 6 indicate the mass spectrum and mass spectral fragmentation of compound 3a.

Scheme 1. Synthetic route of compounds 3a-e.
Biological activities

Antibacterial activity. Compounds 2 and 3a-e were screened for antibacterial activity. The synthesized compound 3d has equipotent activity compared with Ciprofloxacin against E. coli and S. aureus at a concentration of 100 μg/mL. Compound 2 has low activity compared with compounds 3a-e and the reference compound at a concentration of 100 μg/mL. The bacterial zones of inhibition (mm) values are summarized in Table 2. Figure 1 indicated the antibacterial activity variation of compounds 2 and 3a-e.

Antifungal activity. Compounds 2, 3a-e were screened for antifungal activity. The compound 3e has equipotent activity compared with the reference drug (Clotrimazole) against A. niger and C. albicans. The compound 3c and 3d are highly active compared with the reference compound against C. albicans at a concentration of 100 μg/mL. The fungal zones of inhibition (mm) values are summarized in Table 3. Figure 2 indicates the antifungal activity variation of compounds 2 and 3a-e.

Structure-activity relationship

A key structural feature that differentiates our active compound, 3c, from its inactive analogues is the presence of a 4-hydroxyphenyl ring. This functional group seems to be essential for antifungal activity against C. albicans at a concentration of 100 mg/mL. Compound 3c is highly active compared with standard Clotrimazole. Incorporation of a 4-hydroxyphenyl ring into the next generation of compounds, with the aim of antifungal activity against C. albicans, appears attractive to define its pharmacophoric importance.

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>R</th>
<th>mp °C</th>
<th>mw</th>
<th>Yield %</th>
<th>Color</th>
<th>Solvent</th>
<th>Reaction Time</th>
<th>Reaction Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>—H</td>
<td>113</td>
<td>499.11</td>
<td>87</td>
<td>Yellow solid</td>
<td>EtOH</td>
<td>5h</td>
<td>Reflux,65</td>
</tr>
<tr>
<td>3b</td>
<td>—Cl</td>
<td>124</td>
<td>568.64</td>
<td>89</td>
<td>Pale yellow solid</td>
<td>EtOH</td>
<td>6h</td>
<td>Reflux,65</td>
</tr>
<tr>
<td>3c</td>
<td>—OH</td>
<td>120</td>
<td>531.10</td>
<td>81</td>
<td>Pale yellow solid</td>
<td>EtOH</td>
<td>5h</td>
<td>Reflux,65</td>
</tr>
<tr>
<td>3d</td>
<td>—NO₂</td>
<td>123</td>
<td>589.08</td>
<td>86</td>
<td>Light brown solid</td>
<td>EtOH</td>
<td>7h</td>
<td>Reflux,65</td>
</tr>
<tr>
<td>3e</td>
<td>—OCH₃</td>
<td>118</td>
<td>559.65</td>
<td>88</td>
<td>Yellow solid</td>
<td>EtOH</td>
<td>5h</td>
<td>Reflux,65</td>
</tr>
</tbody>
</table>

Table 1. Physicochemical data of the compounds 3a-e.

**Table 2. Antibacterial data of the synthesized compounds 2, 3a-e.**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>E. coli</th>
<th>S. aureus</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>3a</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>3b</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>3c</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>3d</td>
<td>22</td>
<td>15</td>
</tr>
<tr>
<td>3e</td>
<td>20</td>
<td>23</td>
</tr>
<tr>
<td>Standard</td>
<td>22</td>
<td>24</td>
</tr>
</tbody>
</table>

*a Zone of inhibition was measured at (mm) at concentration of 100μg/mL. Ciprofloxacin is used as the standard.

**Table 3. Antifungal data of the synthesized compounds 2, 3a-e.**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>A. niger</th>
<th>C. albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>3a</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>3b</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>3e</td>
<td>18</td>
<td>24</td>
</tr>
<tr>
<td>3d</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>3e</td>
<td>22</td>
<td>17</td>
</tr>
<tr>
<td>Standard</td>
<td>22</td>
<td>18</td>
</tr>
</tbody>
</table>

*a Zone of inhibition was measured at (mm) at concentration of 100μg/mL. Clotrimazole was used as a standard.
Conclusion

This paper describes the synthesis of a new series of pyrrole-connected with thiazole derivatives through a cyclization method and in one-pot reaction procedure. The methodology was previously reported, but the target molecules are newly synthesized compounds at a concentration of 100 μg/mL. Compound 3d had equipotent activity compared with the standard against E. coli at a concentration of 100 μg/mL, and compound 3c was highly active against standard Clotrimazole against C. albicans at a concentration of 100 μg/mL.

Experimental

Melting points were recorded in open capillary tubes and were uncorrected. The IR spectra (KBr) were recorded in KBr on a shimadzu 8201 pc (4000-400 cm\(^{-1}\)). The \(^1\)H NMR and \(^{13}\)C NMR spectra were recorded on a Bruker DRX-300 MHz. The Elemental analysis (C, H, and N,) were recorded using an Elementer analyzer model (Varian EL III). The purity of the compounds was checked by thin layer chromatography (TLC) with silica gel plates.

4-Phenyl-1,3-thiazol-2-amine (1a)

To prepared the mixture of resublimed iodine (2.54 g, 0.01 mol), acetophenone (0.01 mol) and thiourea (3.19 g, 0.02 mol), followed by heating the mixture overnight in an oil bath at 100 °C. After cooling, the mixture was triturated with diethyl ether (50 mL) to remove any unreactant iodine and acetophenone. The solid residue was poured in cold distilled water (200 mL) and treated with 25 % aqueous ammonium hydroxide (pH 9-10). The precipitate was collected and purified from.
hot ethanol. Above procedure was followed by all remaining compounds 1b-1e.

3,5-Dimethyl-\textit{N}²,\textit{N}⁴-bis(4-phenylthiazol-2-yl)-1\textit{H}-pyrrole-2,4-dicarboxamide (3a)

A mixture of 2,4-dimethyl-3,5-dicarbethoxypyrrole 2 (2.39 g, 0.01 mol), 2-amino-4-phenyl-thiazole 1a (3.5 g, 0.02 mol) in ethanol (30 mL), the reaction mixture was heated and refluxed for 5 h, then cooled and poured into water. The resulting solid was filtered off, washed with water, dried and recrystallized from ethanol. Above procedure was followed by all remaining compounds 3b-e.

IR (cm⁻¹): 3350.2 (NH), 3028.38 (Ph-CHstr), 2752.23 (CH₃), 1680.16 (OCNH), 1510.42 (C=N in Thiazole ring), 837.32 (C-Cl), 619.11 (C-S-C);

\textit{¹H} NMR (DMSO-d₆): 12.84 (s, 1H, NH), 7.79 (s, 1H, CH in thiazole ring), 7.26-7.40 (m, 5H, Ph-H), 2.37 (s, 3H, C3-CH₃), 2.14 (s, 3H, C5-CH₃);

\textit{¹³C} NMR (DMSO-d₆): 168.36 (CONH), 166.66 (NH-C), 151.86 (Ph-C in Thiazole), 144.68 (C3,C5-CH₃), 141.13-128.34 (Ph), 103.86 (CH-Thiazole), 14.03 (C5-CH₃), 10.94 (C3-CH₃).

EI-Ms, m/z (M⁺, Relative intensity %): 499.82 (M⁺, 83%), 375.46, 347.41, 181.19 (100%), 151.16. Elemental Analysis:

C_{26}H_{21}N_{5}O_{2}S_{2}, Calc. C 62.50, H 4.24, N 14.02, S 12.84, Found C 62.51, H 4.21, N 12.88, S 12.82.

\textit{N}²,\textit{N}⁴-bis(4-(4-chlorophenyl)thiazol-2-yl)-3,5-dimethyl-1\textit{H}-pyrrole-2,4-dicarboxamide (3b)

IR (cm⁻¹): 3350.21 (NH), 3028.38 (Ph-CHstr), 2752.23 (2-CH₃), 1680.16 (OCNH), 1510.42 (C=N in Thiazole ring), 837.32 (C-Cl), 619.11 (C-S-C);

\textit{¹H} NMR (DMSO-d₆): 12.84 (s, 1H, OCNH), 11.59 (s, 1H, NH), 7.79 (s, 1H, CH in thiazole ring), 7.26-7.40 (m, 5H, Ph-H), 2.37 (s, 3H, C3-CH₃), 2.14 (s, 3H, C5-CH₃);

Figure 5. Mass spectra of compound 3a.

Figure 6. Mass spectral fragmentation of compound 3a.
13C NMR (CDCl3), 1519.09 (C=N in thiazole ring), 7.53-7.89 (dd, 4H, Ph-H), 2.29 (s, 3H, C5-CH3), 2.10 (s, 3H, C3-CH3); 13C NMR (CDCl3): 163.36 (CONH), 164.66 (NH-C), 152.86 (Ph-C in thiazole), 143.68 (C3, C5-CH3), 134.86 (C-Cl), 130.77-128.34 (Ph), 105.33 (CH-Thiazole), 14.55 (C5-CH3), 10.94 (C3-CH3). El-Ms, m/z (M+, Relative intensity %): 568.20 (M+, 13%), 247.41, 264.30, 181.19 (100%), 167.16, 153.13, 67.08. Elemental Analysis: C26H19Cl2N4O2S, Calc. C 54.90, H 3.36, N 3.37, S 11.28, Found C 54.90, H 3.36, N 12.32, S 11.27.

N2,N4-bis(4-(4-hydroxyphenyl)thiazol-2-yl)-3,5-dimethyl-1H-pyrrole-2,4-dicarboxamide (3c)

IR (cm⁻¹): 3350.2 (NH), 3028.38 (Ph-CHstr), 2752.23 (CH3), 1680.16 (OCN), 1465.21 (OH), 1510.28 (C=N in thiazole ring), 619.11 (C-S-C); 1H NMR (DMSO-d6): 12.90 (s,1H, OCNH), 11.57 (s,1H, NH), 9.40 (C-OC), 7.75 (s,1H, CH in thiazole ring), 7.26-7.40 (dd, 4H, Ph-H), 2.36 (s, 3H, C3-CH3), 2.16 (s, 3H, C5-CH3); 13C NMR (CDCl3): 168.30 (CONH), 166.61 (NH-C), 151.86 (Ph-C in thiazole), 158.27 (C-OC), 143.98 (C3, C5-CH3), 141.12-128.24 (Ph), 103.80 (CH-Thiazole), 14.13 (C3-CH3), 10.91 (C5-CH3). El-Ms, m/z (M+, Relative intensity %): 531.22 (M+, 21%), 419.60, 347.41, 319.16 (100%), 123.10, 67.08. Elemental Analysis: C26H19N2O2S2, Calc. C 58.70, H 2.94, N 13.18, S 12.09.

3,5-Dimethyl-N2,N4-bis(4-(4-nitrophenyl)thiazol-2-yl)-1H-pyrrole-2,4-dicarboxamide (3d)

IR (cm⁻¹): 3351.32 (NH), 3027.30 (Ph-CHstr), 2753.20 (CH3), 1682.10 (OCN), 1527 (NO2), 1512 (C=N in thiazole ring), 619.09 (C-S-C); 1H NMR (DMSO-d6): 12.92 (s,1H, OCNH), 11.57 (s,1H, NH), 7.69 (s, 1H, CH in thiazole ring), 7.31-7.52 (m,5H, Ph-H), 2.38 (s, 3H, C3-CH3), 2.10 (s, 3H, C5-CH3); 13C NMR (CDCl3): 168.30 (CONH), 166.54 (NH-C), 151.79 (Ph-C in thiazole), 144.60 (C3, C5-CH3), 141.32-128.21 (Ph), 103.77 (CH-Thiazole), 14.23 (C3-CH3), 10.91 (C5-CH3). El-Ms, m/z (M+, Relative intensity %): 589.59 (M+, 14%), 499.28, 347.41, 333.38, 319.36 (100%), 221.23, 85.10. Elemental Analysis: C26H19N2O2S2, Calc. C 58.70, H 2.94, N 13.18, S 12.09.

N2,N4-bis(4-(4-methoxyphenyl)thiazol-2-yl)-3,5-dimethyl-1H-pyrrole-2,4-dicarboxamide (3e)

IR (cm⁻¹): 3326.33 (NH), 3026.23 (Ph-CHstr), 2750.30 (CH3), 1676.95 (OCN), 1513.44 (C=N in thiazole ring), 617.10 (C-S-C); 1H NMR (DMSO-d6): 12.90 (s,1H, OCNH), 11.51 (s,1H, NH), 7.76 (s,1H, CH in thiazole ring), 7.11-7.32 (m,5H, Ph-H), 2.41 (s, 3H, C3-CH3), 2.23 (s, 3H, C5-CH3); 13C NMR (CDCl3): 168.56 (CONH), 166.60 (NH-C), 151.81 (Ph-C in thiazole), 144.02 (C3, C5-CH3), 161.32 (C-OCH3), 142.31-128.41 (Ph), 103.80 (CH-thiazole), 55.45 (C-OCH3), 14.16 (C5-CH3), 10.94 (C3-CH3). El-Ms, m/z (M+, Relative intensity %): 559.81 (M+, 83%), 499.01, 423.51, 151.16 (100%), 123.15, 95.14. Elemental Analysis: C26H19N2O2S2, Calc. C 60.10, H 4.50, N 12.51, S 11.46, Found C 60.10, H 4.54, N 12.53, S 11.49.

### Biological evaluation

**In vitro antibacterial screening**

The compounds 2 and 3a-e were evaluated for their in vitro antibacterial activity against *Escherichia coli* (MTCC-739) and *Staphylococcus aureus* (MTCC-96), by disc diffusion method [12]. The bioassay was performed using Mueller–Hinton agar (Hi-Media) medium. Ciprofloxacin was used as a reference drug. Each compound was tested at concentration 100μg/mL in DMSO. The zone of inhibition (mm) was measured after 24h incubation at 37°C.

**In vitro antifungal screening**

The compounds 2, 3a-e were evaluated for their in vitro antifungal activity such as *Aspergillus niger*, *Candida albicans* (recultured) using disc diffusion method [13] with sabouraud’s dextrose agar (Hi-Media). Clotrimazole was used as a reference drug. Each compound was tested at a concentration of 100μg/mL in DMSO. The zone of inhibition (mm) was measured incubated at 37°C.

### Acknowledgements

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### References

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