Microwave assisted synthesis of 1,3,4-oxadiazoles carrying benzimidazole moiety and their antimicrobial properties

Janardhana Gowda¹, A M A Khadar*¹, Balakrishna Kalluraya¹ & Nalilu Suchetha Kumari²
¹Department of Studies in Chemistry, Mangalore University, Mangalagangothri 574 199, India
²Department of Biochemistry, Justice K.S. Hegde Academy, Deralakatte 575 018, India
E-mail: amakhader@yahoo.com

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A new series of 2-substituted-1-[(5-substituted phenyl-1,3,4-oxadiazol-2-yl)methyl]-1H-benzimidazole have been synthesized. The structures of new compounds have been confirmed by spectral and analytical data. The newly synthesized compounds have been evaluated for their antifungal and antibacterial activity.

Keywords: 1,3,4-oxadiazoles, benzimidazoles, antimicrobial activities

Benzimidazole and its derivatives are of great importance in medicinal chemistry because of their wide variety of biological and pharmacological applications.² A large number of benzimidazole derivatives have been found to exhibit various biological activities such as anti-inflammatory, antifungal, antibacterial and anthelmintic activities, etc. Similarly, a number of oxadiazole derivatives were also reported to possess varied biological activities such as anti-inflammatory, antibacterial, fungicidal, analgesic, muscle relaxant and tranquillising properties. Fascinated by the varied biological activity of benzimidazole and oxadiazole derivatives it was contemplated to synthesize a new series of 1,3,4-oxadiazoles carrying benzimidazole moiety.

Results and Discussion

The synthetic route followed for obtaining the title compounds is outlined in Scheme I. The 2-substituted-1H-benzimidazole 2 on reaction with ethyl chloroacetate in presence of potassium carbonate in dry acetone gave ethyl (2-substituted-1H-benzimidazol-1-yl)acetate 3, which on reaction with hydrazine hydrate gave 2-(2-substituted-1H-benzimidazol-1-yl)acethydrazide 4. Reaction of 2-(2-substituted-1H-benzimidazol-1-yl)acethydrazide 4 with different aromatic acids in presence of phosphorus oxychloride gave 2-substituted-1-[(5-substituted phenyl-1,3,4-oxadiazole-2-yl)methyl]-1H-benzimidazole 5a-j. The structures of the newly synthesized compounds 5a-j were established on the basis of analytical and spectral data. Their characterization data are given in Table I.

The IR spectra of 2-substituted-1-[(5-substituted phenyl-1,3,4-oxadiazol-2-yl)methyl]-1H-benzimidazole 5a-j showed absorption bands in the region of 3066-2963 cm⁻¹ characteristic of the C-H stretching. The C=N absorption band was observed around 1580-1612 cm⁻¹. In a typical example the ¹H NMR spectra of 2-propyl-1-[(5-(p-anisyl)-1,3,4-oxadiazole-2-yl)-methyl]-1H-benzimidazole 5e the propyl protons came into resonance as a triplet at δ 1.06 integrating for three protons of the methyl group, a multiplet at δ 1.92 integrating for two protons and another triplet at δ 3.04 integrating for two protons. The methoxy protons appeared as a singlet at δ 3.82 integrating for three protons while the N-CH₂ protons appeared as a singlet at δ 5.83 integrating for two protons. The ortho and meta protons of the p-anisyl moiety appeared as two doublets centred at δ 7.0 and 7.84 each integrating for two protons with a coupling constant of J = 8.79 Hz. The benzimidazole protons appeared as multiplets in the region of δ 7.25 - 7.62 integrating for four protons. Further, in the mass spectrum of this compound, the molecular ion peak was observed at m/z 349.2 (M⁺+1) (Molecular formula C₂₀H₂₁N₄O₂) which is also the base peak thereby indicating the stability of the molecule. Similarly, the spectral details for a few other compounds are given below.

1-[(5-Phenyl-1,3,4-oxadiazol-2-yl)methyl]-2-propyl-1H-benzimidazole 5a. ¹H NMR (DMSO-d₆): δ 1.06 (t, 3H, CH₃ of propyl), 1.90 (m, 2H, -CH₂- of propyl), 3.03 (t, 2H, -CH₂- of propyl), 5.84 (s, 2H, N-CH₂), 7.21-7.92 (m, 9H, C-H of aromatic); LC-MS: m/z 319.2 (M⁺+1), Mol. formula C₁₉H₁₈N₄O.

1-[(5-(4-Bromophenyl)-1,3,4-oxadiazol-2-yl)methyl]-2-propyl-1H-benzimidazole 5c. ¹H NMR (DMSO-d₆): δ 0.98 (t, 3H, CH₃ of propyl), 1.84 (m, 2H, -CH₂- of propyl), 2.95 (t, 2H, -CH₂- of propyl),
5.89 (s, 2H, N-CH$_2$), 7.17-7.84 (m, 8H, C-H of aromatic); LC-MS: $m/z$ 397.1 and 398.9 in 1:1 ratio (M$^+$+1), Mol. formula C$_{19}$H$_{17}$BrN$_4$O.

2-Benzyl-1-[(5-phenyl-1, 3, 4-oxadiazol-2-yl) methyl]-1H-benzimidazole 5f. $^1$H NMR (DMSO-$d_6$): $\delta$ 4.41 (s, 2H, CH$_2$ of benzyl), 5.91 (s, 2H, N-CH$_2$), 7.11-7.78 (m, 14H, C-H of aromatic); LC-MS: $m/z$ 367.2 (M$^+$+1), Mol. formula C$_{23}$H$_{20}$N$_4$O.

Scheme I

5a: R$_1$ = Propyl & R$_2$ = H, 5f: R$_1$ = Benzyl & R$_2$ = H,
5b: R$_1$ = Propyl & R$_2$ = Cl, 5g: R$_1$ = Benzyl & R$_2$ = Cl,
5c: R$_1$ = Propyl & R$_2$ = Br, 5h: R$_1$ = Benzyl & R$_2$ = Br,
5d: R$_1$ = Propyl & R$_2$ = Me, 5i: R$_1$ = Benzyl & R$_2$ = Me,
5e: R$_1$ = Propyl & R$_2$ = OMe, 5j: R$_1$ = Benzyl & R$_2$ = OMe,
δ 3.81 (s, 3H, O-CH$_3$) 4.40 (s, 2H, CH$_2$ of benzyl), 5.88 (s, 2H, N-CH$_2$), 7.0-7.71 (m, 13H, C-H of aromatic); LC-MS: m/z 397.2 (M$^+$+1), Mol. formula C$_{24}$H$_{20}$N$_4$O$_2$.

Antimicrobial Studies

Antibacterial Activity

The newly synthesized compounds were screened for their antibacterial activity in vitro against Gram-positive bacteria namely *Escherichia coli*, *Staphylococcus aureus* and Gram-negative bacteria namely *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The anti-bacterial activity of the newly synthesized compounds in the present investigation was assessed by Minimum Inhibitory Concentration (MIC) by serial dilution method$^{17}$. The test compounds were dissolved in N,N-dimethyl formamide to obtain solutions of different concentrations. The concentration at which there was no turbidity was taken as Minimum Inhibitory Concentration (MIC). The results are tabulated in Table II. Compounds carrying benzyl group at the 2-position of the benzimidazole moiety and electron releasing group on the phenyl groups namely, 5f, 5i and 5j showed a MIC of 12.5 µg/mL and all other compounds did not show any significant activity.

<table>
<thead>
<tr>
<th>Compd</th>
<th>R$_1$</th>
<th>R$_2$</th>
<th>m.p. (°C) (Yield %)</th>
<th>Mol. Formula (Mol. Wt)</th>
<th>Found % (Calcd)</th>
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<tr>
<td>5a</td>
<td>Propyl</td>
<td>H</td>
<td>145-50 (75)</td>
<td>C$<em>{24}$H$</em>{32}$N$_4$O</td>
<td>71.60 7.57 17.63</td>
</tr>
<tr>
<td>5b</td>
<td>Propyl</td>
<td>Cl</td>
<td>205 (65)</td>
<td>C$<em>{24}$H$</em>{27}$ClN$_4$O</td>
<td>64.60 4.89 15.87</td>
</tr>
<tr>
<td>5c</td>
<td>Propyl</td>
<td>Br</td>
<td>195-200 (81)</td>
<td>C$<em>{24}$H$</em>{27}$BrN$_4$O</td>
<td>57.40 4.30 14.15</td>
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<tr>
<td>5d</td>
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<td>CH$_3$</td>
<td>125-30 (79)</td>
<td>C$<em>{24}$H$</em>{32}$N$_4$O</td>
<td>72.24 6.05 16.89</td>
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<tr>
<td>5e</td>
<td>Propyl</td>
<td>OCH$_3$</td>
<td>130-32 (89)</td>
<td>C$<em>{24}$H$</em>{27}$N$_4$O$_2$</td>
<td>68.96 5.78 16.07</td>
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<tr>
<td>5f</td>
<td>Benzyl</td>
<td>H</td>
<td>168-70 (74)</td>
<td>C$<em>{24}$H$</em>{27}$N$_4$O</td>
<td>75.40 4.94 15.32</td>
</tr>
<tr>
<td>5g</td>
<td>Benzyl</td>
<td>Cl</td>
<td>170-72 (77)</td>
<td>C$<em>{24}$H$</em>{27}$ClN$_4$O</td>
<td>68.91 4.27 13.98</td>
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<tr>
<td>5h</td>
<td>Benzyl</td>
<td>Br</td>
<td>180-82 (83)</td>
<td>C$<em>{24}$H$</em>{27}$BrN$_4$O</td>
<td>62.03 3.85 12.58</td>
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<tr>
<td>5i</td>
<td>Benzyl</td>
<td>CH$_3$</td>
<td>160-62 (72)</td>
<td>C$<em>{24}$H$</em>{32}$N$_4$O</td>
<td>75.76 5.31 14.75</td>
</tr>
<tr>
<td>5j</td>
<td>Benzyl</td>
<td>OCH$_3$</td>
<td>155-58 (75)</td>
<td>C$<em>{24}$H$</em>{32}$N$_4$O$_2$</td>
<td>72.75 5.08 14.10</td>
</tr>
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Solvent for crystallisation: Ethanol-DMF mixture (2:1)

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Table II — Characterization data of 2-substituted-1-[[5-substituted phenyl-1,3,4-oxadiazol-2-yl)methyl]-1H-benzimidazoles 5a-j

<table>
<thead>
<tr>
<th>Compd</th>
<th>Y</th>
<th>m.p. (°C) (Yield %)</th>
<th>Mol. Formula (Mol. Wt)</th>
<th>Found % (Calcd)</th>
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<tr>
<td>5a</td>
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<td>5b</td>
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<td>Cl</td>
<td>C$<em>{24}$H$</em>{27}$ClN$_4$O</td>
<td>64.60 4.89 15.87</td>
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<tr>
<td>5c</td>
<td>Propyl</td>
<td>Br</td>
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<td>5d</td>
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<td>CH$_3$</td>
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<tr>
<td>5e</td>
<td>Propyl</td>
<td>OCH$_3$</td>
<td>C$<em>{24}$H$</em>{27}$N$_4$O$_2$</td>
<td>68.96 5.78 16.07</td>
</tr>
<tr>
<td>5f</td>
<td>Benzyl</td>
<td>H</td>
<td>C$<em>{24}$H$</em>{27}$N$_4$O</td>
<td>75.40 4.94 15.32</td>
</tr>
<tr>
<td>5g</td>
<td>Benzyl</td>
<td>Cl</td>
<td>C$<em>{24}$H$</em>{27}$ClN$_4$O</td>
<td>68.91 4.27 13.98</td>
</tr>
<tr>
<td>5h</td>
<td>Benzyl</td>
<td>Br</td>
<td>C$<em>{24}$H$</em>{27}$BrN$_4$O</td>
<td>62.03 3.85 12.58</td>
</tr>
<tr>
<td>5i</td>
<td>Benzyl</td>
<td>CH$_3$</td>
<td>C$<em>{24}$H$</em>{32}$N$_4$O</td>
<td>75.76 5.31 14.75</td>
</tr>
<tr>
<td>5j</td>
<td>Benzyl</td>
<td>OCH$_3$</td>
<td>C$<em>{24}$H$</em>{32}$N$_4$O$_2$</td>
<td>72.75 5.08 14.10</td>
</tr>
</tbody>
</table>

Antifungal Activity

Screening for antifungal activity was carried out against four different fungi namely *Penicillium marneffei*, *Trichophyton mentagrophytes*, *Aspergillus flavus* and *Aspergillus fumigatus* using the same procedure as described before and fluconazol as standard drug. The results are presented in the
Table III. Only 5b, 5c, 5f, 5i and 5j showed a MIC of 12.5 µg/mL and all other compounds were found to be inactive. So it is evident that the presence of a benzyl group at the 2-position of the benzimidazole moiety increases the antibacterial and antifungal activity of the compounds significantly.

**Experimental Section**

Melting points of the newly synthesized compounds were determined in open capillary tubes and are uncorrected. IR spectra (cm−1) were recorded on a Perkin Elmer 577 spectrophotometer in KBr pellets. 1H NMR spectra were recorded on a Bruker AMX -400 (400 MHz) spectrometer using DMSO-d6 as solvent and TMS as an internal standard. All chemical shifts values are reported in δ scale downfield from TMS. Mass spectra were recorded on a Jeol model JMS-D300 mass spectrometer operating at 70 eV. CHN analysis was carried out on a Jeol model JMS-D300 mass spectrometer using DMSO-d6 as solvent and TMS as an internal standard. All chemical shifts values are reported in δ scale downfield from TMS. Mass spectrum was recorded on a Varian-EL (Elementar-III) model. Homogeneity of the compound was checked by TLC on silica gel plates.

**General procedure for the preparation of 2-substituted benzimidazoles 2.** A mixture of o-phenylenediamine (10 g, 0.092 mole) and aliphatic/aromatic acid (0.11 mole) was dissolved in 4N HCl (10 mL) and refluxed at 100°C for 12 hr. Completion of the reaction was monitored by TLC. The contents were cooled to RT and neutralized with saturated solution of NaHCO3. The solid separated was filtered, dried and taken as such for the synthesis of ethyl(2-substituted-1H-benzimidazol-1-yl)acetate 3.

Compounds prepared as per this procedure are:

2a: 2-Propylbenzimidazole: m.p. 148°C, Yield 90%.
2b: 2-Benzylbenzimidazole: m.p. 175°C Yield 93%.

**General procedure for the preparation of ethyl- (2-substituted-1H-benzimidazol-1-yl)acetate 3.** The solution of 2-substituted benzimidazole 2 (0.062 mole) in acetone (20 mL) was mixed with ethyl chloroacetate (7.9 mL, 0.074 mole) and potassium carbonate (16.5 g, 0.12 mole) and refluxed for 6 hr. Completion of the reaction was monitored by TLC. The reaction mixture was filtered. From the clear filtrate, excess acetone was removed by distillation and then was added to water. The solid product separated was collected by filtration and dried. Further purification was done by crystallization from ethyl acetate to give ethyl(2-substituted-1H-benzimidazol-1-yl)acetate 3.

Compounds prepared as per this procedure are:

3a: Ethyl(2-propyl-1H-benzimidazol-1-yl)acetate: m.p. 85-90°C, Yield 97%.
3b: Ethyl(2-benzyl-1H-benzimidazol-1-yl)acetate: m.p. 90-95°C, Yield 95%.

**Procedure for the preparation of 2-(2-substituted-1H-benzimidazol-1-yl)acethydrazide 4.** The solution of ethyl(2-substituted-1H-benzimidazol-1-yl)acetate 3 (0.04 mole) in ethanol (15 mL) was mixed with hydrazine hydrate (99%) (2.2 mL, 0.044 mole) and refluxed for 4 hr. Completion of the reaction was monitored by TLC. The excess of solvent was removed by distillation and the contents were added to excess of water. The solid separated was collected by filtration. The crude product was purified by recrystallization from ethanol.

Compounds prepared as per this procedure are:

4a: 2-(2-propyl-1H-benzimidazol-1-yl) acethydra- zide: m.p. 176-79°C, Yield 90%.
4b: 2-(2-Benzyl-1H-benzimidazol-1-yl) acethydra- zide: m.p. 170-75°C, Yield 92%.

**General procedure for the preparation of oxadiazoles 5a-j.** The mixture of 2-(2-substituted-1H-benzimidazol-1-yl)acethydrazide 4 (0.002 mole), aromatic acid (0.003 mole) and phosphorus oxychloride (1 mL) was ground to get a homogeneous mixture and then heated in a beaker under microwave irradiation at 160 W for 5-10 min. Completion of the reaction was monitored by TLC. The contents were cooled to RT and added to excess ice-cold water. The solid product separated was collected by filtration. Further purification was done by recrystallization.
from ethanol-DMF mixture (2:1). The yield, melting point and other characterization data of the compounds are given in Table I.

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References