Mohamed, Hanan A., Abdel-Wahab, Bakr F., Sabry, Eman, Kariuki, Benson M. and El-Hiti, Gamal A. 2022. Synthesis and antimicrobial activity of 2,5-bis(Pyrazol-3-yl or Triazol-4-yl)-1,3,4-oxadiazoles. Heterocycles 104 (7), pp. 1293-1302. 10.3987/COM-22-14676 file

Publishers page: http://dx.doi.org/10.3987/COM-22-14676

Please note: Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher’s version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See http://orca.cf.ac.uk/policies.html for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.
SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF 2,5-BIS(PYRAZOL-3-YL OR TRIAZOL-4-YL)-1,3,4-OXADIAZOLE

Hanan A. Mohamed,1 Bakr F. Abdel-Wahab,1 Eman Sabry,1 Benson M. Kariuki,2 and Gamal A. El-Hiti3*

1 Chemical Industries Research Institute, National Research Centre, Dokki, Giza 12622, Egypt; 2 School of Chemistry, Cardiff University, Main Building, Park Place, Cardiff CF10 3AT, UK; 3 Department of Optometry, College of Applied Medical Sciences, King Saud University, Riyadh 11433, Saudi Arabia. E-mail: gelhiti@ksu.edu.sa (Gamal A. El-Hiti).

Abstract – The reaction of pyrazole-3-carbohydrazide (1) or 1,2,3-triazole-4-carbohydrazides 5a,b and 2-(ethoxymethylene)malononitrile (2) in ethanol under reflux conditions afforded the corresponding N,N'-diacylhydrazines 3 or 6a,b, respectively in high yields. Ring closure of 3 or 6a,b in the presence of phosphorus oxychloride furnished the corresponding 2,5-bis(heterocyclic)-1,3,4-oxadiazoles 4 or 7a,b, respectively in good yields. The synthesized heterocyclic compounds showed moderate activity against Staphylococcus aureus, Listeria monocytogenes, and Escherichia coli.

INTRODUCTION

Diacylhydrazines are common insecticides and have been used as a biologically active component in insect growth regulators.1–3 1,2-Diacylhydrazines are commonly synthesized through coupling between carbohydrazides and acyl chlorides, dimerization of carbohydrazides, and reactions between carboxylic acids or isocyanates and hydrazine hydrate.4–7 Diacylhydrazines are common precursors for the production of many heterocyclic ring systems.4 1,3,4-Oxadiazole represents an important class of heterocycles that exhibits pesticidal, antibacterial, and antiviral activities.8–10 Various medications containing 1,3,4-oxadiazole ring systems are available and can be used as antiretroviral and anticancer agents.11–13 1,2-Diacylhydrazines have been used as precursors for the production of 1,3,4-oxadiazoles using a dehydrating agent (e.g., phosphorus pentoxide, polyphosphoric acid, phosphorus oxychloride, thionyl chloride, iodine or bromine, triflic anhydride, or
4-methylbenzenesulfonyl chloride) in the presence of a base (e.g., triethylamine, potassium carbonate, or pyridine) or Vilsmeier reagent.\textsuperscript{14-16} 1,2,3-Triazoles exhibit a variety of biological activities and act as antibacterial, antitubercular, and antiviral agents.\textsuperscript{17-22} In addition, pyrazoles act as inhibitors of protein glycation, antibacterial, antifungal, and antiviral agents.\textsuperscript{4,8} Therefore, the synthesis of novel heterocycles containing 1,3,4-oxadiazole, 1,2,3-triazole, or pyrazoles is of interest.\textsuperscript{23,24} Here, we report the synthesis of novel heterocycles containing 1,3,4-oxadiazole moiety using a simple procedure as a continuation of our long-term interest in the synthesis of bioactive molecules.\textsuperscript{25-27}

**RESULTS AND DISCUSSION**

Reaction of 5-(4-methoxyphenyl)-1-phenyl-1\textsubscript{H}-pyrazole-3-carbohydrazide (1) and \(2\)-(ethoxymethylene)malononitrile (2) in ethanol (EtOH) under reflux conditions for 6 h afforded 5-(4-methoxyphenyl)-1-phenyl-1\textsubscript{H}-pyrazole-3-carboxylic acid 2-[[5-(4-methoxyphenyl)-1-phenyl-1\textsubscript{H}-pyrazol-3-yl]carbonyl]hydrazide (3) in 85\% yield. Dehydration of 3 took place in the presence of boiling phosphorus oxychloride (POCl\textsubscript{3}) for 8 h to give 2,5-\textit{bis}(5-(4-methoxyphenyl)-1-phenyl-1\textsubscript{H}-pyrazol-3-yl)-1,3,4-oxadiazole (4) in 77\% yield (Scheme 1).

\[ 
\text{EtO} \quad \text{CN} \quad \text{CN} \\
\text{EtOH, reflux, 6 h} \\
\text{MeO} \quad \text{MeO} \quad \text{MeO} \\
\text{POCl\textsubscript{3}} \quad \text{reflux, 8 h} \\
\text{4 (77\%)} \\
\]

\[ 
\text{Scheme 1. Synthesis of 3 and 4} \\
\]

The \(1\)H NMR spectrum of 3 showed two characteristic singlet singles at 10.07 and 10.69 ppm corresponding to the two NH protons. The NH protons were absent in the \(1\)H NMR spectrum of 4. In addition, it shows two singlets at 3.71 and 3.78 ppm corresponding to the two OMe groups. The structures of 3 and 4 were confirmed further using the \(13\)C NMR spectra and single-crystal X-ray crystallography.
The independent part of the crystal structure of 3 is half a molecule with the rest being generated by symmetry (Figure 1). The molecule comprises three unique rings, 3A (C2—C7), 3B (C8—C10, N1, and N2) and 3C (C12—C17), with twist angles between the planes of neighboring rings in the molecule between 44–49° (Table 1).

Figure 1. A 50% probability ellipsoid ORTEP representation of the molecule of 3 showing atom numbering

The molecule in the structure of 4 is shown in Figure 2. The molecule comprises seven rings, 4A (C2—C7), 4B (C8—C10, N1, and N2), 4C (C11—C16), 4D (C17, C18, N3, and N4, O2), 4E (C19—C21, N5, and N6), 4F (C22—C27), 4G (C28—C33). The twist angles between the planes of adjacent rings are between 5–54° (Table 1).

Figure 2. A 50% probability ellipsoid ORTEP representation of the molecule of 4 showing atom numbering
Table 1. Twist angles (°) between adjacent planes in the structures of 3, 4, and 7a. The geometry after local optimization of geometry. The planes are defined in the discussion.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Experimental</th>
<th>Optimized</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3A/3B</td>
<td>44.78(9)</td>
<td>61.90</td>
</tr>
<tr>
<td>3B/3C</td>
<td>48.57(8)</td>
<td>16.57</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4A/4B</td>
<td>44.60(9)</td>
<td>63.03</td>
</tr>
<tr>
<td>4B/4C</td>
<td>50.72(8)</td>
<td>16.02</td>
</tr>
<tr>
<td>4B/4D</td>
<td>18.32(13)</td>
<td>0</td>
</tr>
<tr>
<td>4D/4E</td>
<td>5.24(16)</td>
<td>0</td>
</tr>
<tr>
<td>4E/4F</td>
<td>53.82(8)</td>
<td>16.23</td>
</tr>
<tr>
<td>4E/4G</td>
<td>33.55(10)</td>
<td>62.41</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7A/7B</td>
<td>30.68(8)</td>
<td>19.08</td>
</tr>
<tr>
<td>7B/7C</td>
<td>7.51(11)</td>
<td>3.80</td>
</tr>
<tr>
<td>7C/7D</td>
<td>21.14(9)</td>
<td>3.73</td>
</tr>
<tr>
<td>7D/7E</td>
<td>42.96(5)</td>
<td>18.92</td>
</tr>
</tbody>
</table>

The synthesis of 5-methyl-1-phenyl-1H-1,2,3-triazole-4-carboxylic acid 2-[(5-methyl-1-phenyl-1H-1,2,3-triazole-4-yl)carbonyl]hydrazide (6a) in 72% from reaction of 5-methyl-1-phenyl-1H-1,2,3-triazole-4-carbohydrazide (5a) and 2-(ethoxymethylene)malononitrile (2) has been reported. Similarly, N,N'-diacylhydrazine 6b was synthesized in 87% yield (Scheme 2) from reaction of 5-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazole-4-carbohydrazide (5b). Ring closure of both 6a and 6b in boiling in POCl3 for 8 h gave the corresponding 1,3,4-oxadiazoles 7a and 7b in 77 and 72% yields, respectively (Scheme 2).

The ¹H NMR spectrum of 6b showed a characteristic singlet at 10.59 ppm corresponding to the two NH protons. The carbonyl carbon appears at 160.5 ppm in its ¹³C NMR spectrum. The ¹H NMR spectra of both 7a and 7b showed the absence of NH protons.
The structure of 7a was confirmed further using crystal X-ray crystallography. The molecule in the structure of 7a is shown in Figure 3. The molecule comprises five rings, 7A (C1—C6), 7B (N1—N3 and C7—C9), 7C (O1, N4, N5, C10, and C11), 7D (N6, N7, N8, and C12—C14), 7E (C15—C20). The twist angles between the planes of adjacent rings are between 7–43° (Table 1).

Figure 3. A 50% probability ellipsoid ORTEP representation of the molecule of 7a showing atom numbering

In addition to the elemental composition, the biological activity of a molecule may be influenced by its flexibility. Local optimization of molecules isolated from the crystal structures of 3, 4 and 7a using Avogadro\textsuperscript{29} gave the results shown in Table 1. The calculations showed that the molecules in the crystal structure were not at the lowest energy conformations, indicating that the geometry may adopt to that of the pathogen.

The antimicrobial activities of 3, 4, 6a, 6b, 7a, and 7b were assessed against some pathogenic microorganisms obtained from the American type culture collection (ATCC; Rockville, MD, USA). The organisms used were \textit{Staphylococcus aureus} ATCC-47077 (\textit{S. aureus}), \textit{Listeria monocytogenes} ATCC-35152 (\textit{L. monocytogenes}), \textit{Escherichia coli} ATCC-25922 (\textit{E. coli}), \textit{Salmonella typhi} ATCC-15566, and \textit{Candida albicans} ATCC-10231 (\textit{C. albicans}). Ampicillin and vancomycin were used reference antibiotics for comparison. Compared with the reference drugs, the tested compounds showed moderate activity against the microorganisms (Table 2).
Table 2. Antimicrobial activity of the synthesized heterocycles

<table>
<thead>
<tr>
<th>Compound</th>
<th>Gram-positive bacteria</th>
<th>Gram-negative bacteria</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus</td>
<td>L. monocytogenes</td>
<td>E. coli</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>6a</td>
<td>12</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>6b</td>
<td>13</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>7a</td>
<td>10</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>7b</td>
<td>12</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>15</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>14</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

CONCLUSIONS

Novel 2,5-bis(pyrazol-3-yl)-1,3,4-oxadiazoles and 2,5-bis(1,2,3-triazol-4-yl)-1,3,4-oxadiazoles were synthesized in high yields from the appropriate carbohydrazide using simple procedures. The synthesized heterocycles showed moderate activity against *Staphylococcus aureus*, *Listeria monocytogenes*, and *Escherichia coli*.

EXPERIMENTAL

General Melting points were determined using an Electrothermal (variable heater) melting point apparatus. The NMR spectra were measured on a JEOLNMR 500 MHz spectrometer. $^1$H (500 MHz) and $^{13}$C NMR (125 MHz) spectra were recorded in deuterated dimethyl sulfoxide (DMSO-$_d_6$) using tetramethylsilane as a standard. The chemical shift (δ) is reported in ppm and the coupling constant (J) in Hz. Compounds 1$^{30}$, 2$^{31}$, 5a,b$^{32,33}$ and 6a$^{28}$ were prepared following literature procedures. 

Synthesis of N,N'-diacylhydrazines 3 and 6b. A mixture of 1 or 5b (5 mmol) 2 (0.61 g, 5 mmol) in dry EtOH (25 mL) was heated under reflux conditions for 6 h. The mixture was left overnight and the product obtained was collected by filtration, washed with EtOH, and dried to give a colorless solid. 

5-(4-Methoxyphenyl)-1-phenyl-1H-pyrazole-3-carboxylic acid 2-[[5-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-3-yl]carbonyl]hydrazide (3) Yield 85%, mp 218–220 °C. $^1$H NMR: 10.69 (s, exch., 1H, NH), 10.07 (s, exch., 1H, NH), 7.71–7.68 (m, 12H, Ar), 7.25 (d, 4H, J = 8.5 Hz, Ar), 6.90–6.89 (m, 4H, Ar), 3.78 (s, 3H, OMe), 3.71 (s, 3H, OMe). $^{13}$C NMR: 161.4, 160.6, 154.8, 154.8, 137.8, 137.8, 135.3, 134.6, 130.3, 127.7, 127.3, 122.8, 115.6, 115.1, 55.8. Anal. Calcd for C$_{34}$H$_{28}$N$_6$O$_4$ (584.22): C, 69.85; H, 4.83; N, 14.38; Found: C, 69.99; H, 4.92; N, 14.49%.

5-Methyl-1-(4-nitrophenyl)-1H-1,2,3-triazole-4-carboxylic acid 2-[(5-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)carbonyl]hydrazide (6b) Yield 87%, mp > 300 °C. $^1$H NMR: 10.59 (s, exch., 2H, 2 NH), 8.46 (d, 4H, J = 8.6 Hz, Ar), 7.99 (d, 4H, J = 8.6 Hz, Ar), 2.46 (s, 6H, 2 Me). $^{13}$C NMR: 160.5, 148.4, 140.7,
Anal. Calcd for C_{20}H_{16}N_{10}O_{6} (492.12): C, 48.78; H, 3.28; N, 28.45; Found: C, 48.87; H, 3.40; N, 28.66%.

### 2,5-Bis(heterocyclic)-1,3,4-oxadiazoles 4 and 7a,b

A mixture of 3 or 6a,b (2 mmol) and POCl$_3$ (20 mL) was refluxed for 8 h. The product obtained on cooling was collected by filtration, washed with EtOH, and dried to give the corresponding product 4 or 7a,b.

#### 2,5-Bis[5-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-3-yl]-1,3,4-oxadiazole (4)

Yield 77%, mp 233–235 ºC. $^1$H NMR: 7.49–36 (m 8H, Ar), 7.26 (s, 2H, Ar), 7.22 (d, 4H, $J = 8.5$ Hz, Ar), 6.91 (d, 4H, $J = 9.5$ H, Ar),3.72 (s, 6H, 2 OMe). $^{13}$C NMR: 162.8, 160.3, 160.0, 139.8, 137.7, 130.6, 129.8, 129.2, 126.2, 121.4, 114.7, 107.7, 55.8. Anal. Calcd for C$_{34}$H$_{26}$N$_6$O$_3$ (566.21): C, 72.07; H, 4.63; N, 14.83; Found: C, 72.37; H, 4.72; N, 14.95%.

#### 2,5-Bis[5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl]-1,3,4-oxadiazole (7a)

Yield 72%, mp 220–222 ºC. $^1$H NMR: 7.70–7.63 (m, 10H, Ar), 2.46 (s, 6H, 2 Me). $^{13}$C NMR: 160.7, 158.4, 137.2, 130.8, 130.3, 126.0, 125.9, 10.3. Anal. Calcd for C$_{20}$H$_{16}$N$_8$O (384.14): C, 62.49; H, 4.20; N, 29.15; Found: C, 62.52; H, 4.33; N, 29.33%.

#### 2,5-Bis[5-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl]-1,3,4-oxadiazole (7b)

Yield 75%, mp > 300 ºC. $^1$H NMR: 8.49 (d, 4H, $J = 8.6$ Hz, Ar), 7.99 (d, 4H, $J = 8.6$ Hz, Ar), 2.45 (s, 6H, 2 Me). $^{13}$C NMR: 160.5, 148.4, 140.4, 138.6, 137.8, 127.0, 125.6, 10.0. Anal. Calcd for C$_{20}$H$_{14}$N$_{10}$O$_5$ (474.11): C, 50.64; H, 2.97; N, 29.53; Found: C, 20.73; H, 3.08; N, 29.66%.

### Antimicrobial Activity

The agar well diffusion procedure was employed to investigate the antimicrobial activities of 3, 4, 6a, 6b, 7a, and 7b.$^{34,35}$ Ampicillin and vancomycin were used as standards for comparison. Bacterial (70 µL) and yeast (106 CFU/mL) cells were spread on plates containing nutrient agar. The wells (6 mm diameter) were excavated on the injected agar plates then each sample (200 mg) in DMSO (1 mL) was added. The reference antibiotics disks (10 and 30 µg/disk of ampicillin and vancomycin, respectively) were introduced on the surface of agar inoculated plates. The plates were kept at 4 ºC for 2 h before incubation to permit diffusion to occur. The plates were kept at 37 ºC for 24 h except for yeast strains that were incubated at 28 ºC for 24 h. The diameter of the inhibition zone (mm) was measured. The tests were replicated five times and the averages were calculated.

### Crystal Structure Determination

Single-crystal XRD data were collected at room temperature on an Agilent SuperNova Dual Atlas diffractometer with a mirror monochromator using Mo radiation. The crystal structures were solved by SHELXS$^{36}$ and refined using SHELXL.$^{37}$ Non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were inserted in idealized positions, and a riding model was used with Uiso set at 1.2 or 1.5 times the value of Ueq for the atom to which they are bonded. 3: C$_{17}$H$_{14}$N$_3$O$_2$, FW = 292.31, T = 293(2) K, $\lambda = 0.71073$ Å, monoclinic, P2$_1$/n, a = 6.4199(4) Å, b =
9.6695(5) Å, c = 23.5094(15) Å, β = 93.691(6)°, V = 1456.37(15) Å³, Z = 4, density (cal) = 1.333 mg/m³, absorption coefficient = 0.090 mm⁻¹, F(000) = 612, crystal size = 0.320 × 0.146 × 0.069 mm³, reflections collected = 13237, independent reflections = 3660, R(int) = 0.0293, parameters = 201, goodness-of-fit on F² = 1.052, R1 = 0.0586, wR2 = 0.1472 based on (I>2σ(I)), R1 = 0.0881, wR2 = 0.1684 based on all data, largest diff. peak and hole = 0.219 and −0.178 e.Å⁻³. 4: C₃₄H₂₆N₆O₃, FW = 566.61, T = 293(2) K, λ = 0.71073 Å, triclinic, P[I], a = 8.3728(6) Å, b = 13.3452(9) Å, c = 13.6662(9) Å, α = 75.407(6)°, β = 77.290(6)°, γ = 89.585(5)°. V = 1439.66(18) Å³, Z = 2, density (cal) = 1.307 mg/m³, absorption coefficient = 0.086 mm⁻¹, crystal size = 0.319 × 0.203 × 0.050 mm³, reflections collected = 12111, independent reflections = 6838, R(int) = 0.0327, parameters = 391, goodness-of-fit on F² = 1.038, R1 = 0.0614, wR2 = 0.1382 based on (I>2σ(I)), R1 = 0.1006, wR2 = 0.1656 based on all data, largest diff. peak and hole = 0.265 and −0.199 e.Å⁻³. 7a: C₂₀H₁₆N₈O, FW = 384.41, T = 296(2) K, λ = 0.71073 Å, monoclinic, P2₁/n, a = 11.9990(8) Å, b = 7.8858(5) Å, c = 19.4028(12) Å, β = 98.952(6)°, V = 1813.6(2) Å³, Z = 4, density (cal) = 1.408 mg/m³, absorption coefficient = 0.095 mm⁻¹, F(000) = 800, crystal size = 0.400 × 0.280 × 0.196 mm³, reflections collected = 15595, independent reflections = 4562, R(int) = 0.0422, parameters = 265, goodness-of-fit on F² = 1.051, R1 = 0.0546, wR2 = 0.1103 based on (I>2σ(I)), R1 = 0.1113, wR2 = 0.1387 based on all data, largest diff. peak and hole = 0.165 and −0.157 e.Å⁻³. The crystal structures have been deposited in the Cambridge Structural Database under reference CCDC 2162219–2162221.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

ACKNOWLEDGEMENTS
H.A. Mohamed, B.F. Abdel-Wahab and E. Sabry thank the National Research Center, Dokki, Giza, Egypt for support. G.A. El-Hiti acknowledges the support received from the Researchers Supporting Project number (RSP-2021/404), King Saud University, Riyadh, Saudi Arabia.

REFERENCES


