Synthesis and antifungal activity of novel 2,5-disubstituted-1,3,4-oxadiazoles containing benzimidazole moiety

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(Received June 18, 2012; Accepted July 30, 2012)

Eighteen novel compounds of 2,5-disubstituted-1,3,4-oxadiazoles containing benzimidazole moiety were synthesized from 1H-benzimidazole-2-carbohydrazide, carbon disulfide and alkyl halide or benzyl halide by multi-step reactions. The structures of the target compounds were confirmed by IR, 1H-NMR spectra and elemental analyses. Preliminary antifungal activities against Botrytis cinerea and Sclerotinia sclerotiorum were also evaluated by the mycelium growth rate method, and the results indicated that many target compounds possess excellent antifungal activity, even higher than the control fungicide (carbendazim).

Keywords: 1,3,4-oxadiazole, benzimidazole, synthesis, antifungal activity.

Introduction

1,3,4-Oxadiazole derivatives have been reported to have a wide range of biological activities, including antiinflammatory, anticancer, antiviral, antibacterial, antifungal and herbicidal activities. Currently, many attentions are directed toward the discovery and development of novel 1,3,4-oxadiazole fungicides.

On the other hand, benzimidazole derivatives also exhibit a broad spectrum of biological activities, such as antifungal, antibacterial, antiviral, antitumor and antiparasitic activities, which are important substances in agricultural and pharmaceutical fields. Among benzimidazole fungicides, carbendazim, benomyl and thiabenazol are still used in fungicide market due to their high efficiency, broad spectrum and good systemic property. However, owing to the enhancement of the resistance of many fungal pathogens, their antifungal activities are becoming lower. Therefore, it is necessary to develop some novel benzimidazole derivatives with low resistance. With the aim of searching for excellent benzimidazole fungicide, herein we linked a benzimidazole moiety to the 1,3,4-oxadiazole ring, designed and synthesized two series of novel 2,5-disubstituted-1,3,4-oxadiazoles containing benzimidazole moiety, and the synthetic pathway is shown in Fig. 1. Consequently, their antifungal activities have been evaluated against two selected plant pathogens, and the result shows that some target compounds possess excellent antifungal activity.

Materials and Methods

1. Instruments and reagents

Melting points were recorded on an X-5 microscopic melting point apparatus and are uncorrected. IR spectra were recorded on a Nicolet-380 infrared spectrophotometer. 1H-NMR spectra were performed in CDCl3 or DMSO-d6 solvent on a Varian Mercury Plus 400 spectrophotometer using tetramethylsilane as an internal standard. Elemental analyses were carried out using a Vario EL III Elemental Analyzer.

1H-Benzimidazole-2-carbohydrazide (1) was prepared according to the reference. Other chemical reagents were purchased from J&K Chemical Ltd. or Aladdin Reagent Co., Ltd. and were of analytical grade.

2. Chemical synthesis

2.1. 5-(1H-Benzimidazol-2-yl)-1,3,4-oxadiazole-2-thiol (3)

The intermediate was synthesized from carbohydrazide (1) and carbon disulfide by addition and cyclization reactions, which was a yellow solid, m.p. 278.5–280.3°C.

2.2. General procedure for synthesizing compounds 4a–4i

To a stirred solution of 3 (1.10 g, 5 mmol) and sodium hydroxide (0.24 g, 6 mmol) in water (30 mL) was added a solution of corresponding alkyl halide or benzyl halide (6 mmol) in methanol (10 mL) dropwise within 15 min. The mixture was stirred for 2–8 hr at room temperature, giving a white or yellow precipitate. Then the mixture was filtered under reduced pressure, and the solid residue was recrystallized from N,N-dimethylformamide to afford the target compounds 4a–4i as white solids.

2-(1H-benzimidazol-2-yl)-5-methylthio-1,3,4-oxadiazole (4a)

Yield: 60%. m.p. 240.5–241.3°C. IR νmax (KBr) cm−1: 1627, 1502, 1327. 1H-NMR δH (CDCl3): 2.87 (s, 3H, J = 7.2 Hz), 7.32–7.68 (m, 4H, PhH), 11.75 (s, 1H, NH). Anal. Found: C, 51.46; H, 3.40; N, 23.88%. Calcd. for C10H8N4OS: C, 51.71; H, 3.47; N, 24.12%.

2-(1H-benzimidazol-2-yl)-5-ethylthio-1,3,4-oxadiazole (4b)

Yield: 51%. m.p. 245.4–246.1°C. IR νmax (KBr) cm−1: 1676, 1632, 1502, 1327. 1H-NMR δH (DMSO-d6): 1.47 (t, 3H, CH3J = 7.2 Hz), 3.36 (q, 2H, SCH2J = 7.2 Hz), 7.32–7.68

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Published online November 2, 2012
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(m, 4H, PhH), 13.81 (s, 1H, NH). Anal. Found: C, 53.94; H, 4.26; N, 22.49%. Calcd. for C_{16}H_{11}FN_{4}OS: C, 53.64; H, 4.09; N, 22.75%.

2-(1H-benimidazol-2-yl)-5-propylthio-1,3,4-oxadiazole (4c) Yield: 43%. m.p. 263.1–263.9°C. IR ν_{max} (KBr) cm\(^{-1}\): 3188, 3065, 2947, 1670, 1629, 1491, 1323. \(^1\)H-NMR δ_{H} (CDCl\(_3\)) 1.14 (t, 3H, CH\(_3\), J = 7.2 Hz), 1.97 (m, 2H, CH\(_2\)), 3.38 (t, 2H, SCH\(_2\), J = 7.2 Hz), 7.26–7.92 (m, 4H, PhH), 12.26 (s, 1H, NH). Anal. Found: C, 55.18; H, 4.37; N, 21.32%. Calcd. for C\(_{16}\)H\(_{12}\)N\(_4\)OS: C, 55.37; H, 4.65; N, 21.52%.

2-(1H-benimidazol-2-yl)-5-isopropylthio-1,3,4-oxadiazole (4d) Yield: 47%. m.p. 260.4–261.5°C. IR ν_{max} (KBr) cm\(^{-1}\): 3183, 3058, 2924, 1655, 1618, 1477, 1328. \(^1\)H-NMR δ_{H} (CDCl\(_3\)) 0.98 (t, 3H, CH\(_3\), J = 7.2 Hz), 1.53–1.59 (m, 2H, CH\(_2\)), 1.83–1.95 (m, 2H, CH\(_2\)), 3.39 (t, 2H, SCH\(_2\), J = 7.2 Hz), 7.26–7.95 (m, 4H, PhH), 12.55 (s, 1H, NH). Anal. Found: C, 57.25; H, 4.98; N, 20.14%. Calcd. for C\(_{16}\)H\(_{12}\)N\(_4\)OS: C, 55.91; H, 5.14; N, 20.42%.

2-(1H-benimidazol-2-yl)-5-butythio-1,3,4-oxadiazole (4e) Yield: 40%. m.p. 267.5–268.5°C. IR ν_{max} (KBr) cm\(^{-1}\): 3188, 3058, 2924, 1655, 1618, 1477, 1328. \(^1\)H-NMR δ_{H} (CDCl\(_3\)) 0.98 (t, 3H, CH\(_3\), J = 7.2 Hz), 1.53–1.59 (m, 2H, CH\(_2\)), 1.83–1.95 (m, 2H, CH\(_2\)), 3.39 (t, 2H, SCH\(_2\), J = 7.2 Hz), 7.26–7.95 (m, 4H, PhH), 12.55 (s, 1H, NH). Anal. Found: C, 57.25; H, 4.98; N, 20.14%. Calcd. for C\(_{16}\)H\(_{12}\)N\(_4\)OS: C, 55.91; H, 5.14; N, 20.42%.

2-(1H-benimidazol-2-yl)-5-(4-chlorobenzylthio)-1,3,4-oxadiazole 4i Yield: 88%. m.p. 248.6–249.1°C. IR ν_{max} (KBr) cm\(^{-1}\): 3186, 3060, 2930, 1671, 1643, 1489, 1333. \(^1\)H-NMR δ_{H} (CDCl\(_3\)) 1.42 (s, 1H, NH). Anal. Found: C, 50.68; H, 2.53; N, 14.56%. Calcd. for C\(_{16}\)H\(_{12}\)Cl\(_2\)N\(_2\)O: C, 50.94; H, 2.67; N, 14.85%.

2.3. General procedure for synthesizing compounds 5a–5i

A mixture of 4 (5 mmol), sodium hydroxide (0.24 g, 6 mmol) and a catalytic amount of tetrabutyl ammonium iodide (TBAI, 0.05 g) in acetonitrile (20 mL) was stirred at 40–50°C, and then 4-fluorobenzyl bromide (1.13 g, 6 mmol) in acetonitrile (10 mL) was added dropwise. The reaction mixture was refluxed with stirring for 1.5–3.5 hr, and then the reaction was cooled. After evaporating the reaction mixture on a rotary evaporator, the residue was dissolved in dichloromethane (20 mL). The insoluble substance was removed by filtration, and the filtrate was dried over anhydrous magnesium sulfate. The solvent was removed and the crude product was obtained, which was purified by recrystallization from ethanol to give the target compounds 5a–5i as white solids.

2-[1-(4-fluorobenzyl)-1H-benimidazol-2-yl]-5-methylthio-1,3,4-oxadiazole (5a) Yield: 84%. m.p. 180.2–181.3°C. IR ν_{max} (KBr) cm\(^{-1}\): 3059, 3023, 2931, 2877, 1617, 1461, 1327, 1159. \(^1\)H-NMR δ_{H} (CDCl\(_3\)) 2.81 (s, 3H, SCH\(_3\)), 6.02 (s, 2H, NCH\(_2\)), 6.92–7.93 (m, 8H, PhH). Anal. Found: C, 60.25; H, 4.02; N, 16.21%. Calcd. for C\(_{16}\)H\(_{12}\)FN\(_2\)O: C, 59.99; H, 3.85; N, 16.46%.

2-[1-(4-fluorobenzyl)-1H-benzimidazol-2-yl]-5-ethylthio-1,3,4-oxadiazole (5b) Yield: 82%. m.p. 146.2–148.3°C. IR ν_{max} (KBr) cm\(^{-1}\): 3058, 3033, 2977, 2854, 1612, 1459, 1321, 1160. \(^1\)H-NMR δ_{H} (CDCl\(_3\)) 1.54 (t, 3H, CH\(_3\), J = 7.6 Hz), 3.36 (q, 2H, SCH\(_2\), J = 7.6 Hz), 6.02 (s, 2H, NCH\(_2\)), 6.92–7.93 (m, 8H, PhH). Anal. Found: C, 61.28; H, 4.15; N, 15.69%. Calcd. for C\(_{16}\)H\(_{12}\)FN\(_2\)O: C, 61.00; H, 4.27; N, 15.81%.

2-[1-(4-fluorobenzyl)-1H-benzimidazol-2-yl]-5-propylthio-1,3,4-oxadiazole (5c) Yield: 86%. m.p. 241.7–243.3°C. IR ν_{max} (KBr) cm\(^{-1}\): 3188, 3065, 2939, 1670, 1495, 1323. \(^1\)H-NMR δ_{H} (CDCl\(_3\)) 1.46 (s, 1H, NH). Anal. Found: C, 56.38; H, 3.05; N, 16.10%. Calcd. for C\(_{16}\)H\(_{12}\)Cl\(_2\)N\(_2\)O: C, 56.06; H, 3.23; N, 16.34%.
4.05 (m, 1H, SCH, \(J = 7.0\) Hz), 6.03 (s, 2H, NCH₂), 6.94–7.95 (m, 8H, PhH). Anal. Found: C, 61.94; H, 4.65; N, 15.52%. Calcd. for C₁₉H₁₇FN₄OS: C, 61.94; H, 4.65; N, 15.21%.

2-[1-(4-fluorobenzyl)-1H-benimidazol-2-yl]-5-butythio-1,3,4-oxadiazole (5d) Yield: 82%. m.p. 130.6–132.1°C. IRν max (KBr) cm⁻¹: 3051, 3032, 2943, 2810, 1629, 1469, 1324, 1175. ¹H-NMR δ H (CDCl₃): 1.65 (6H, 2xCH₃, J = 7.0 Hz), 4.05 (m, 1H, SCH, J = 7.0 Hz), 6.03 (s, 2H, NCH₂), 6.94–7.95 (m, 8H, PhH). Anal. Found: C, 61.94; H, 4.65; N, 15.21%.

2-[1-(4-fluorobenzyl)-1H-benimidazol-2-yl]-5-butythio-1,3,4-oxadiazole (5e) Yield: 86%. m.p. 131.5–133.3°C. IRν max (KBr) cm⁻¹: 3065, 3033, 2942, 2864, 1629, 1430, 1323, 1164. ¹H-NMR δ H (CDCl₃): 0.98 (t, 3H, CH₃), 1.83–1.95 (m, 2H, CH₂), 3.38 (t, 2H, SCH₂, J = 7.0 Hz), 6.03 (s, 2H, NCH₂), 6.94–7.94 (m, 8H, PhH). Anal. Found: C, 63.12; H, 5.16; N, 14.39%. Calcd. for C₂₀H₁₉FN₄OS: C, 63.58; H, 3.71; N, 12.90%.

2-[1-(4-fluorobenzyl)-1H-benimidazol-2-yl]-5-benzylthio-1,3,4-oxadiazole (5f) Yield: 84%. m.p. 137.0–138.1°C. IRν max (KBr) cm⁻¹: 3065, 3032, 2942, 2864, 1629, 1430, 1323, 1164. ¹H-NMR δ H (CDCl₃): 4.54 (s, 2H, SCH₂), 6.01 (s, 2H, NCH₂), 6.97–7.93 (m, 13H, PhH). Anal. Found: C, 66.52; H, 4.07; N, 12.63%. Calcd. for C₂₃H₁₇FN₄OS: C, 66.52; H, 3.12; N, 11.54%.

2-[1-(4-fluorobenzyl)-1H-benimidazol-2-yl]-5-(4-chlorobenzylthio)-1,3,4-oxadiazole (5i) Yield: 84%. m.p. 134.1–144.8°C. IRν max (KBr) cm⁻¹: 3060, 3029, 2937, 2846, 1623, 1433, 1346, 1168. ¹H-NMR δ H (CDCl₃): 4.53 (s, 2H, SCH₂), 5.93 (s, 2H, NCH₂), 7.01–8.25 (m, 11H, PhH). Anal. Found: C, 56.73; H, 3.36; N, 11.75%. Calcd. for C₂₃H₁₈ClFN₄OS: C, 56.92; H, 3.12; N, 11.54%.

2.4. Antifungal activity assays

The in vitro antifungal activities of the target compounds 4 and 5 against Botrytis cinerea and Sclerotinia sclerotiorum were evaluated using the mycelium growth rate method. The tested pathogens, Botrytis cinerea (BC-JfC02) and Sclerotinia sclerotiorum (SS-JSZ01) were both collected from Tai'an city, China, and stored in Key Laboratory of Pesticide Toxicology and Application Technique of Shandong Province. Moreover, the S. sclerotiorum is benzimidazole-sensitive strain, while the B. cinerea is benzimidazole moderately-resistant strain. The culture media, with known concentrations (10, 50, 100 and 200 mg/L) of the test compounds, were obtained by mixing the water suspension.

<table>
<thead>
<tr>
<th>Compd.</th>
<th>R</th>
<th>EC₅₀ (mg/L), 95% CL</th>
</tr>
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<tbody>
<tr>
<td>4a</td>
<td>CH₂</td>
<td>10.57 (6.90–16.20)</td>
</tr>
<tr>
<td>4b</td>
<td>CH₂CH₃</td>
<td>2.70 (2.20–3.32)</td>
</tr>
<tr>
<td>4c</td>
<td>n-C₃H₇</td>
<td>16.56 (12.68–21.63)</td>
</tr>
<tr>
<td>4d</td>
<td>i-C₃H₇</td>
<td>24.15 (20.41–28.58)</td>
</tr>
<tr>
<td>4e</td>
<td>n-C₃H₇</td>
<td>9.86 (7.45–13.05)</td>
</tr>
<tr>
<td>4f</td>
<td>C₂H₅CH₂</td>
<td>18.38 (15.03–22.49)</td>
</tr>
<tr>
<td>4g</td>
<td>4-FC₂H₅CH₂</td>
<td>12.76 (8.89–18.32)</td>
</tr>
<tr>
<td>4h</td>
<td>4-ClC₂H₅CH₂</td>
<td>14.84 (13.08–16.85)</td>
</tr>
<tr>
<td>4i</td>
<td>2,4-Cl₂C₂H₅CH₂</td>
<td>16.39 (14.48–18.54)</td>
</tr>
<tr>
<td>5a</td>
<td>CH₂</td>
<td>13.96 (9.34–20.87)</td>
</tr>
<tr>
<td>5b</td>
<td>CH₂CH₃</td>
<td>3.08 (0.91–10.44)</td>
</tr>
<tr>
<td>5c</td>
<td>n-C₃H₇</td>
<td>5.63 (4.97–6.39)</td>
</tr>
<tr>
<td>5d</td>
<td>i-C₃H₇</td>
<td>11.38 (8.87–14.61)</td>
</tr>
<tr>
<td>5e</td>
<td>n-C₃H₇</td>
<td>6.26 (5.70–6.88)</td>
</tr>
<tr>
<td>5f</td>
<td>C₂H₅CH₂</td>
<td>1.31 (0.56–3.06)</td>
</tr>
<tr>
<td>5g</td>
<td>4-FC₂H₅CH₂</td>
<td>13.95 (8.84–22.01)</td>
</tr>
<tr>
<td>5h</td>
<td>4-ClC₂H₅CH₂</td>
<td>6.07 (5.81–6.33)</td>
</tr>
<tr>
<td>5i</td>
<td>2,4-Cl₂C₂H₅CH₂</td>
<td>7.81 (2.99–20.41)</td>
</tr>
</tbody>
</table>

EC₅₀: Concentration that inhibited mycelium growth by 50%; 95% CL: 95% confidence interval of EC₅₀.
(1 mL) of 4 or 5 with potato dextrose agar (PDA, 9 mL) at 50°C. After cooling to the room temperature, the fungus cake which was cultivated for 3 days was placed on each culture medium. The cultures were placed in a light incubator at 25±1°C for 96 hr. Three replications were performed for each concentration. Moreover, carbendazim, a commercial benzimidazole fungicide, was used as a control, and sterile water was used as a blank. The inhibition rate was expressed as the mean of values obtained in three independent experiments. Lastly, effective concentrations (EC_{50}) that inhibited mycelium growth by 50% were obtained according to Finney’s probability value method (Table 1). The inhibition rate was calculated according to the formula:

\[
\text{Inhibition rate} = \left(\frac{D_0 - D_1}{D_0}\right) \times 100\%
\]

Where, \(D_0\) is the 6 expansion diameter of mycelia in the blank test, and \(D_1\) is the expansion diameter of mycelia in the presence of tested compounds.

**Results and Discussion**

1. **Synthesis**

The target compound 4 was synthesized by the reaction of 5-(1H-benzimidazol-2-yl)-1,3,4-oxadiazole-2-thiol (3) with R–X. In compound 3, there are two active moieties, NH in benzimidazole ring and SH at the 2-position of oxadiazole, both reacting with R–X. However, only SH reacted with R–X in methanol-water solution at room temperature due to its higher reactivity. In addition, the reaction was in yields around 50% when the substituent group R is alkyl, while it was in better yields (over 80%) when R is substituted benzyl. On the other hand, the target compound 5 was conveniently synthesized from 4 and 4-fluorobenzyl bromide in the presence of he phase transfer catalyst TBAD, with yields of 82–86%.

2. **Antifungal activity**

As shown in Table 1, nine target compounds exhibited high antifungal activities against B. cinerea, in which EC_{50} values were 1.31 to 10.57 mg/L. Particularly, the activities of 4b, 5b, 5c, 5d, 5e, 5f and 5h were higher than that of the control fungicide (carbendazim), with an EC_{50} value of 2.70, 3.08, 5.63, 6.26, 1.31 and 6.07 mg/L, respectively. In addition, the title compounds 4b, 5e, 5f, 5h and 5i displayed excellent activities against S. sclerotiorum, and their EC_{50} values ranged from 0.04 to 1.31 mg/L. Among the target compounds, 5f possessed the highest activity with an EC_{50} value of 0.04 mg/L. On the other hand, the activity of compound 5 was higher than that of compound 4 in general, which revealed that the introduction of a 4-fluorobenzyl group to benzimidazole ring can greatly increase the antifungal activity of this kind of 1,3,4-oxadiazole derivative.

**Acknowledgments**

This project was supported by Shandong Province Natural Science Foundation (No. ZR2009BM044).

**References**