Synthesis and pesticidal activity of new 1,3,4-oxadiazole thioether compounds containing a trifluoromethylpyrazolyl moiety

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Abstract
In order to find new lead compounds with high pesticidal activity, a series of 1,3,4-oxadiazole thioether compounds (5 series) were designed by using penthiopyrad as a synthon. They were synthesized easily via five steps by using ethyl 4,4,4-trifluoro-3-oxobutanoate and triethyl orthoformate as starting materials. The synthesized compounds were characterized by 1H NMR, 13C NMR and HRMS. The compound 2-(benzylthio)-5-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (5a) was further determined by X-ray single-crystal diffraction. It crystallized in the monoclinic system, space group P21/c, Z=4. All the 1,3,4-oxadiazole thioether derivatives were screened for fungicidal activity against ten fungi and herbicidal activity against two weeds. The bioassay results indicated that some of the synthesized 1,3,4-oxadiazole compounds exhibited good fungicidal activity (> 50% inhibition) against the plant pathogens Sclerotinia sclerotiorum and Rhizoctonia solani at 50 μg/mL. Some of them exhibited certain herbicidal activity, and compounds 2-((3-chlorobenzyl)thio)-5-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (5e) and 2-((4-bromobenzyl)thio)-5-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (5 g) had bleach effect. Molecular docking is to find the best fit orientation of the 2-((4-bromobenzyl)thio)-5-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (5 g) molecule with the SDH protein (PDB: 2FBW). The docking results indicate that the compound 5 g and the lead compound penthiopyrad have similar binding interactions with SDH and carbonyl is a key group for these compounds.

Keywords Synthesis · Crystal structure · Oxadiazole fungicides · Herbicides · Molecular docking

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Introduction

Heterocycles play important roles in natural products [1], medicines [2], pesticides [3], and other chemical products [4]. Pyrazole nucleus can be regarded a significant skeleton for the design of biologically active compounds in synthetic pesticides and medicines [5–7]. For instance, the recent succinate dehydrogenase inhibitor (SDHI) fungicides pydiflumetofen, iso flu cyp ram, and pyrapropoyne have pyrazole amide groups. Numerous biological activities can be performed by modifying the pyrazole scaffold, such as nematocidal [8–10], herbicidal [11, 12], antimicrobial [13], antioxidant [14], fungicidal [15–17], anticancer [18], and insecticidal activities [19].

In the last two decades, 1,3,4-oxadiazoles have emerged as the one of the most promising pharmacophores exhibiting important biological activities like anti-tumor [20], ketol-acid reductoisomerase inhibitors [21], antiproliferative [22], anti-SARS-CoV-2 [23], insecticidal [24], fungicidal activities [25], cardioprotective agents [26], nematocidal activity [27], anti-TMV activity [28] and anti-Alzheimer agents [29]. The hybridization of 1,3,4-oxadiazole unit with several other heterocycles like benzimidazole [30], coumarin [31], pyridine [32], glycosyl [33], and triazole [34] has empowered to develop various molecular hybrid displaying excellent activity.

In our previous work, many pyrazole amide compounds were designed and synthesized as SDHIs [35–37], which possessed good fungicidal activity. In order to discover new lead compounds, penthiopyrad was used as a lead compound and a scaffold hopping strategy is used in this paper. The acyl amide group was cyclized as 1,3,4-oxadiazole ring, and the thiophene ring was opened (Fig. 1). It is possible that pyrazole-linked 1,3,4-oxadiazole derivatives possess fungicidal activity. Most of them exhibited no herbicidal activity against bentgrass and lettuce.

Experimental

Instruments

Melting points were determined using an X-4 digital microscopic melting point detector (Taike, Beijing, China) and uncorrected. 1H NMR spectra were measured on a Bruker AV-500 instruments using TMS as an internal standard and CDCl₃ as
the solvent. $^{13}$C NMR spectra were measured on a Bruker AV-400 instruments using TMS as an internal standard and CDCl$_3$ as the solvent. HRMS was determined on an Agilent 1200RRRLC-6520 instrument. All the reagents are of analytical grade or freshly prepared before use. Analytical TLC (Huanghai, Qingdao, China) was performed on silica gel GF$_{254}$.

**General procedure**

**ethyl 1-methyl-3-(trifluoromethyl)-1H-pyrazole-4-carboxylate (2)**

In a 1000-mL, four-necked flask, ethyl 4,4,4-trifluoro-3-oxobutanoate (7.36 g, 40 mmol), triethyl orthoformate (8.88 g, 60 mmol) and acetic anhydride (12.24 g, 0.12 mol) were added and refluxed for 8 h. Then, the solvent was removed and the product was then distilled under vacuum over a column to give a brown liquid. Then, the CH$_3$NHNH$_2$ (6.9 g, 60 mmol) was added into the solution of brown liquid in EtOH (20 mL) under ice bath, and the mixture was stirred at 60 °C for 8 h. The solvent was evaporated, and the resulting intermediate 2 was given. Mp. 55–56 °C, white solid, yield 69%; $^1$H NMR (CDCl$_3$, 500 MHz), $\delta$: 1.35 (t, 3H, $J$= 7.32 Hz, CH$_3$), 3.97 (s, 3H, CH$_3$), 4.31 (m, 2H, CH$_2$), 7.92 (s, 1H, CH).

**1-Methyl-3-(trifluoromethyl)-1H-pyrazole-4-carbohydrazide (3)**

To a solution of ethyl 1,3-dimethyl-1H-pyrazole-4-carboxylate (1.1 g, 5 mmol) in EtOH (10 mL), 80% hydrazine hydrate (0.63 g, 10 mmol) was added dropwise. Then the mixture solution was stirred and refluxed for 10 h. The solvent was evaporated and cooled, and the intermediate 3 was given. Mp. 145–146 °C, white solid, yield 85%; $^1$H NMR (CDCl$_3$, 500 MHz), $\delta$: 3.94 (s, 3H, CH$_3$), 4.46 (s, 2H, NH$_2$), 8.24 (s, 1H, CH), 9.46 (s, 1H, NH).

**5-(1-Methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole-2-thiol (4)**

The solution of 1,3-dimethyl-1H-pyrazole-4-carbohydrazide (1.04 g, 5 mmol) and KOH (0.34 g, 6 mmol) in EtOH (10 mL) were stirred at 0 °C for 15 min, and then the CS$_2$ (0.46 g, 6 mmol) was added dropwise. The mixture was refluxed for 8 h. The EtOH was evaporated, and the residue was dissolved in water. The solution was acidified by conc. HCl, and a final intermediate 4 was produced and filtered, giving a yield of 89%, mp. 132–133 °C, white solid, $^1$H NMR (CDCl$_3$, 500 MHz), $\delta$: 3.95 (s, 3H, CH$_3$), 7.93 (s, 1H, CH), 13.54 (s, 1H, SH).

**General procedure for the synthesis of compounds 5a–5 m.**

N,N-dimethylformamide (5 mL), 5-(1,3-dimethyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole-2-thiol (0.25 g, 1 mmol), K$_2$CO$_3$ (0.16 g, 1.2 mmol), and RCH$_2$Cl (1.1 mmol) were put into a round bottom. Then the mixture was stirred at room temperature
overnight. It was poured into crushed ice, and the expected 1,3,4-oxiadiazole derivative was collected and recrystallized in EtOH.

2-(benzylthio)-5-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (5a).

M.p. 97–98 °C, white solid, yield 89.8%. 1H NMR (CDCl3, 500 MHz), δ: 4.05 (s, 3H, CH3), 4.51 (s, 2H, CH2), 7.32–7.38 (m, 3H, Ph), 7.46 (d, J = 7.2 Hz, 2H, Ph), 8.10 (s, 1H, CH); 13C NMR (CDCl3, 100 MHz), δ: 36.76, 39.98, 105.01, 118.97(q, J = 269.75 Hz, CF3), 128.10, 128.78(2C), 129.08(2C), 133.28, 135.35, 139.11(q, J = 37.72 Hz, Pyrazole), 158.74, 163.87; HRMS (ESI) for C14H11F3N4OS m/z: Calculated, 341.0678, Found, 341.0680 [M + H]+.

2-((3,4-dichlorobenzyl)thio)-5-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (5b).

M.p. 106–107 °C, white solid, yield 83.1%. 1H NMR (CDCl3, 500 MHz), δ: 4.06 (s, 3H, CH3), 4.44 (s, 2H, CH2), 7.32–7.43 (m, 2H, Ph), 7.58 (s, 1H, Ph), 8.09 (s, 1H, CH); 13C NMR (CDCl3, 100 MHz), δ: 35.38, 40.02, 104.88, 118.92(q, J = 269.65 Hz, CF3), 128.46, 130.66, 130.93, 132.27, 132.72, 133.27, 135.93, 139.15(q, J = 39.08 Hz, Pyrazole), 158.97, 163.14; HRMS (ESI) for C14H9Cl2F3N4OS m/z: Calculated, 408.9899, Found, 408.9882 [M + H]+.

2-((2,4-Dichlorobenzyl)thio)-5-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (5c).

M.p. 101–103 °C, white solid, yield 92.9%. 1H NMR (CDCl3, 500 MHz), δ: 4.05 (s, 3H, CH3), 4.57 (d, J = 8.0 Hz, 1H, Ph), 7.23 (d, J = 8.0 Hz, 1H, Ph), 7.45 (s, 1H, Ph), 7.60 (d, J = 8.0 Hz, 1H, Ph); 13C NMR (CDCl3, 100 MHz), δ: 33.91, 40.01, 104.95, 118.91(q, J = 267.47 Hz, CF3), 127.35, 129.58, 132.23(2C), 133.21, 134.82, 135.00, 138.58(q, J = 39.25 Hz, Pyrazole), 158.94, 163.59; HRMS (ESI) for C14H9Cl2F3N4OS m/z: Calculated, 408.9899, Found, 408.9901 [M + H]+.

2-((4-Chlorobenzyl)thio)-5-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (5d).

M.p. 102–103 °C, white solid, yield 92.9%. 1H NMR (CDCl3, 500 MHz), δ: 4.05 (s, 3H, CH3), 4.46 (s, 2H, CH2), 7.31–7.42 (m, 4H, Ph), 8.09 (s, 1H, CH); 13C NMR (CDCl3, 100 MHz), δ: 35.97, 40.01, 104.97, 118.93(q, J = 269.85 Hz, CF3), 128.92(2C), 130.45, 133.24(2C), 133.99, 134.08, 139.54(q, J = 39.09 Hz, Pyrazole), 158.85, 163.50; HRMS (ESI) for C14H10ClF3N4OS m/z: Calculated, 375.0289, Found, 375.0290 [M + H]+.

2-((3-Chlorobenzyl)thio)-5-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (5e).

M.p. 95–96 °C, white solid, yield 88.1%. 1HNMR (CDCl3, 500 MHz), δ: 4.06 (s, 3H, CH3), 4.47 (s, 2H, CH2), 7.30–7.36 (m, 3H, Ph), 7.47 (s, 1H, Ph), 8.09 (s, 1H, CH); 13C NMR (CDCl3, 100 MHz), δ: 36.01, 39.99, 104.93, 118.94(q, J = 269.45 Hz, CF3), 127.27, 128.30, 129.09, 130.02, 133.28, 134.48, 137.54, 139.13(q, J = 38.75 Hz, Pyrazole), 158.88, 153.42; HRMS (ESI) for C14H10ClF3N4OS m/z: Calculated, 375.0289, Found, 375.0290 [M + H]+.

2-((2-Chlorobenzyl)thio)-5-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (5f).

M.p. 103–104 °C, white solid, yield 93.5%. 1H NMR (CDCl3, 500 MHz), δ: 4.05 (s, 3H, CH3), 4.62 (s, 2H, CH2), 7.24–7.30 (m, 2H, Ph), 7.43 (d, J = 7.6 Hz, Pyrazole), 158.82, 163.75; HRMS (ESI) for C14H10ClF3N4OS m/z: Calculated, 375.0289, Found, 375.0291 [M + H]+.
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2H, Ph), 8.09 (s, 1H, CH); $^{13}$C NMR (CDCl$_3$, 100 MHz), $\delta$: 34.67, 39.99, 105.01, 118.94 (q, $J=262.56$ Hz, CF$_3$), 127.09, 129.65, 129.76, 131.39, 133.21, 133.49, 134.31, 139.14 (q, $J=38.78$ Hz, Pyrazole), 158.84, 163.86; HRMS (ESI) for C$_{14}$H$_{10}$ClF$_3$N$_4$OS $m/z$: Calculated, 375.0289, Found, 375.0293 [M + H]$^+$. 2-((4-Bromobenzyl)thio)-5-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (S 5)

Mp. 113–114 °C, white solid, yield 83.3%, $^1$H NMR (CDCl$_3$, 500 MHz), $\delta$: 4.05 (s, 3H, CH$_3$), 4.44 (s, 2H, CH$_2$), 7.34 (d, $J=8.4$ Hz, 2H, Ph), 7.46–7.49 (m, 2H, Ph), 8.08 (s, 1H, CH); $^{13}$C NMR (CDCl$_3$, 100 MHz), $\delta$: 34.67, 39.99, 105.01, 118.94 (q, $J=262.56$ Hz, CF$_3$), 127.09, 129.65, 129.76, 131.39, 133.21, 133.49, 134.31, 139.14 (q, $J=38.78$ Hz, Pyrazole), 158.84, 163.86; HRMS (ESI) for C$_{14}$H$_{10}$ClF$_3$N$_4$OS $m/z$: Calculated, 375.0289, Found, 375.0293 [M + H]$^+$. 2-((5-(1-Methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazol-2-yl)thio)acetonitrile (S 5 h)

Mp. 81–82 °C, brown solid, yield 27.7%, $^1$H NMR (CDCl$_3$, 500 MHz), $\delta$: 4.07 (s, 3H, CH$_3$), 4.11 (s, 2H, CH$_2$), 8.17 (s, 1H, CH); $^{13}$C NMR (CDCl$_3$, 100 MHz), $\delta$: 17.75, 40.09, 104.42, 114.55, 118.86 (q, $J=269.70$ Hz, CF$_3$), 133.58, 139.31 (q, $J=38.75$ Hz, Pyrazole), 159.86, 160.50; HRMS (ESI) for C$_9$H$_6$F$_3$N$_5$OS $m/z$: Calculated, 290.0318, Found, 290.0321 [M + H]$^+$. 2-(Allylthio)-5-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (S 5 i)

Mp. 77–78 °C, white solid, yield 78.4%, $^1$H NMR (CDCl$_3$, 500 MHz), $\delta$: 3.91 (d, $J=7.2$ Hz, 2H, CH$_2$), 4.06 (s, 3H, CH$_3$), 5.31 (m, 2H, CH$_2$), 6.03 (m, 1H, CH), 8.12 (s, 1H, CH); $^{13}$C NMR (CDCl$_3$, 100 MHz), $\delta$: 35.19, 39.99, 105.04, 118.96 (q, $J=269.89$ Hz, CF$_3$), 119.76, 131.57, 133.27, 139.49 (q, $J=39.95$ Hz, Pyrazole), 158.81, 163.69; HRMS (ESI) for C$_{10}$H$_9$F$_3$N$_4$OS $m/z$: Calculated, 291.0522, Found, 291.0524 [M + H]$^+$. 2-(((2-Chlorothiazol-5-yl)methyl)thio)-5-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (S 5 j)

Mp. 94–95 °C, yellow solid, yield 81.3%, $^1$H NMR (CDCl$_3$, 500 MHz), $\delta$: 4.06 (s, 3H, CH$_3$), 4.64 (s, 2H, CH$_2$), 7.57 (s, 1H, CH), 8.05 (s, 1H, CH); $^{13}$C NMR (CDCl$_3$, 100 MHz), $\delta$: 28.52, 40.05, 104.73, 118.90 (q, $J=269.57$ Hz, CF$_3$), 133.37, 135.58, 139.58 (q, $J=39.38$ Hz, Pyrazole), 152.45, 159.31, 162.64; HRMS (ESI) for C$_{11}$H$_7$ClF$_3$N$_5$OS$_2$ $m/z$: Calculated, 381.9805, Found, 381.9804 [M + H]$^+$. 2-(((6-Chloropyridin-3-yl)methyl)thio)-5-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (S 5 k)

Mp. 111–112 °C, light yellow solid, yield 79.9%, $^1$H NMR (CDCl$_3$, 500 MHz), $\delta$: 4.05 (s, 3H, CH$_3$), 4.46 (s, 2H, CH$_2$), 7.32 (d, $J=8.0$ Hz, 1H, Py), 7.84 (d, $J=8.0$ Hz, 1H, Py), 8.09 (s, 1H, CH), 8.48 (s, 1H, Py); $^{13}$C NMR (CDCl$_3$, 100 MHz), $\delta$: 32.84, 40.03, 104.78, 118.90 (q, $J=269.79$ Hz, CF$_3$), 124.24, 130.91, 133.30, 139.49, 139.56 (q, $J=37.51$ Hz, Pyrazole), 149.92, 151.08, 159.10, 162.81; HRMS (ESI) for C$_{13}$H$_8$ClF$_3$N$_5$OS $m/z$: Calculated, 376.0241, Found, 376.0242 [M + H]$^+$. 2-((2-Fluorobenzyl)thio)-5-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (S 5 l)

Mp. 101–101 °C, white solid, yield 78.2%, $^1$H NMR (CDCl$_3$, 500 MHz), $\delta$: 4.05 (s, 3H, CH$_3$), 4.54 (s, 2H, CH$_2$), 7.07–7.14 (m, 2H, Ph), 7.30–7.34 (m, 1H, Ph), 7.54
(t, J = 7.6 Hz, 1H, Ph), 8.09 (s, 1H, CH); $^{13}$C NMR (CDCl$_3$, 100 MHz), δ: 30.08(d, $J = 3.55$ Hz), 39.98, 104.98, 115.48(d, $J = 21.12$ Hz), 118.96(q, $J = 269.51$ Hz, CF$_3$), 122.85(d, $J = 14.47$ Hz), 124.30(d, $J = 3.69$ Hz), 130.08(d, $J = 8.22$ Hz), 131.27(d, $J = 3.21$ Hz), 132.29, 139.50(q, $J = 39.14$ Hz, Pyrazole), 158.87, 159.68(d, $J = 248.44$ Hz), 163.66; HRMS (ESI) for C$_{14}$H$_{10}$F$_4$N$_4$OS m/z: Calculated, 359.0584, Found, 359.0581 [M + H]$^+$.  

4-(((5-(1-Methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazol-2-yl)thio)methyl)benzonitrile (5 m)

Mp. 126–127 °C, white solid, yield 38.4%. $^1$H NMR (CDCl$_3$, 500 MHz), δ: 4.05 (s, 3H, CH$_3$), 4.51 (s, 2H, CH$_2$), 7.60–7.66 (m, 4H, Ph), 8.09 (s, 1H, CH); $^{13}$C NMR (CDCl$_3$, 100 MHz), δ: 35.96, 40.04, 111.97, 118.74(q, $J = 265.65$ Hz, CF$_3$), 118.82, 129.84(2C), 132.50(2C), 133.24, 133.33, 139.72(q, $J = 39.37$ Hz, Pyrazole), 141.19, 159.07, 162.99; HRMS (ESI) for C$_{15}$H$_{10}$F$_3$N$_5$OS m/z: Calculated, 366.0631, Found, 366.0637 [M + H]$^+$.  

Structure determination

The crystal of compound 5a with dimensions of 0.36 mm×0.34 mm×0.24 mm was mounted on a CCD area detector with a graphite-monochromated MoKα radiation ($λ = 0.71073$ Å) by using phi and scan modes at 296 (2) K in the range of 4.636° ≤ θ ≤ 50.036°. The crystal belongs to monoclinic system with space group P2$_1$/n and crystal parameters of $a = 4.7008(4)$ Å, $b = 28.244(2)$ Å, $c = 11.2817(9)$ Å, $α = 90°$, $β = 95.705(2)^o$, $γ = 90°$, $V = 1490.4(2)$ Å$^3$, $Dc = 1.517$ g/cm$^3$. The absorption coefficient $μ = 0.259$ mm$^{-1}$ and $Z = 4$. The structure was solved by direct methods with SHELXS-97[38] and refined by the full-matrix least squares method on F$^2$ data using SHELXL-97. The empirical absorption corrections were applied to all intensity data. The H atom of N–H was initially located in a difference Fourier map and was refined with the restraint Uiso (H) = 1.2Ueq (N). Other H atoms were positioned geometrically and refined using a riding model, with d(C—H) = 0.93–0.97 Å and Uiso(H) = 1.2Ueq(C) or 1.5Ueq(Cmethyl). The detailed parameters are listed in Table 1.  

Fungicidal activity

The fungicidal activity of compounds 5a–5 m was tested in vitro against G. zeae, P. infestans, P. capsici, S. sclerotiorum, R. solani, A. solani, B. cinerea, F. oxysporum, C. arachidicola, P. Piricola, and their relative percent inhibition (%) has been determined using the mycelium growth rate method according to the previous work [39], and fluxapyroxad was used as positive control. Each compound was dissolved in DMSO with 1% tween to prepare the 10,000 mg/L stock solution. The ten fungal species were inoculated into a petri dish containing 50 μg/mL stock solution and incubated in a 25 °C biochemical incubator in darkness. The solvent DMSO with 1% tween was used as a blank assay. The fungicidal effect was investigated 3 days
later. Each process (species and compound) was repeated three times. The inhibition of compounds compared to the blank assay was calculated via the following equation:

\[
\text{inhibition (\%)} = \frac{(\text{CK} - \text{AI})}{\text{CK}} \times 100\%
\]

where CK is the average diameter of mycelia in the blank test and AI is the average diameter of mycelia in the presence of those compounds.

**Phytotoxicity studies**

The method of Dayan et al. [40] was used. Briefly, seeds of lettuce (*Lactuca sativa*—Iceberg A Crisphead cultivar from Burpee Seeds, Warminster, PA, USA) and bentgrass (*Agrostis stolonifera*—Penncross variety obtained from Turf-Seed, Inc. of Hubbard, OR, USA) were surface-sterilized with a 0.5% to 1% (v/v) sodium hypochlorite solution for approximately 10 min, rinsed with deionized water and dried in a sterile environment. A filter paper disk (Whatman Grade 1, 1.5 cm) was placed in each well of a 24-well plate. The control wells contained 200 μL of deionized water. The control + solvent well contained 180 μL of water and 20 μL of the solvent. All sample wells contained 180 μL of water and 20 μL of the appropriate dilution of the sample. Water was pipetted into the well before the sample or solvent. Test samples were dissolved in acetone, and the final concentration of acetone in the wells was 10%. For the bioassay, five lettuce seeds or 20 mg of bentgrass seeds were placed in each well before sealing the plate with Parafilm. The plates were incubated for seven days in a Percival Scientific (Perry, IA, USA) CU-36L5 incubator under...
continuous light conditions at 26 °C and 120 μmol·s⁻¹·m⁻² average photosynthetically active radiation (PAR). A qualitative estimate of phytotoxicity was made by assigning a rating of 0 for no effect (sample well plants looked identical to the control + solvent well plants; seeds had germinated and resulting seedlings had grown normally), 2 for less than 20–40% germination inhibition, 3 for about 40–60% germination inhibition, 4 for more than 60% germination inhibition, and 5 for no germination of the seeds. Each experiment was repeated two times.

Molecular docking

Molecular docking between the compound 5g and SDH was done using Discovery Studio 2.5 software. The binding sites were generated from the SDH (PDB code: 2FBW). The detailed method was done according to our previous work [41].

Results and discussion

Synthesis and spectra analysis

Synthesis and spectra of target 1,3,4-oxadiazole compounds

The synthetic route of title 1,3,4-oxadiazole compounds is outlined in Scheme 1. The key intermediate trifluoromethylpyrazole ring was cyclized by using ethyl-2-(ethoxymethylene)-4,4,4-trifluoro-3-oxobutanoate and CH₃NHNH₂.
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according to the classic Knorr pyrazole synthesis method. We also successfully used NH₂NH₂•H₂O instead of CH₃NHNH₂ to synthesize the pyrazole ring. Unfortunately, when CH₃I was used as the methylation reagent to synthesize intermediate 2, the yield was low. Then trifluoromethylpyrazole carbohydrazide was prepared easily from trifluoromethylpyrazole ester and NH₂NH₂•H₂O. The 1,3,4-oxadiazole ring was cyclized using pyrazole carbohydrazide in CS₂ and KOH under reflux conditions with high yield. The 1,3,4-oxadiazole-2-thiol 4 can tautomerize, in a thiol/thione equilibrium. At last, the 1,3,4-oxadiazole-2-thiol reacted with different RCH₂Cl compounds, and the final products were easily produced. Compounds 5a–5m had 27.7–93.5% yields.

All the trifluoromethylpyrazole compounds were confirmed by ¹H-NMR, ¹³C-NMR and HRMS. From the ¹H-NMR results, a SCH₂ signal was observed at 4.11–5.31 ppm, and the CH proton signal of pyrazole ring was found around 8.18–8.17 ppm as a single peak. The N–CH₃ on the pyrazole ring appeared at about 4.05–4.07 ppm. In the ¹³C NMR spectra, the CF₃ can be found at 118 ppm as quartet with the coupling constant 269 Hz. The carbon atom of pyrazole linked with CF₃ group also found at 139 ppm as quartet with the coupling constant 40 Hz, due to the carbon is split by fluorine atoms. The carbon atom of N–CH₃ on the pyrazole ring appeared at about 40 ppm. The carbon atom of SCH₂ is at around 35 ppm. All the pyrazole derivatives showed the expected [M + H]⁺ peak in the HRMS data.

Crystal structure

The target compound 5a was further elucidated by X-ray crystal diffraction. The molecular structure and packing diagram of compound 5a are illustrated in Fig. 2. The general bond angles and bond lengths of the pyrazole ring, 1,3,4-oxadiazole ring and phenyl ring were in normal ranges. For the 1,3,4-oxadiazole ring, N(2)-C(9) [1.279(3) Å] and N(1)-C(8) [1.282(3) Å] bonds were a little longer than the normal C = N (1.27 Å), which indicated electron delocalized on 1,3,4-oxadiazole ring. The bond length of C(8)-O(1) and C(9)-O(1) is 1.362(2) Å, which is longer than C = O group. The torsion angle of C(1)-C(7)-S(1)-C(8) was 76.71(18)°, which indicated that the phenyl ring was vertical with the 1,3,4-oxadiazole ring. The torsion angle of C(11)-C(10)-C(9)-N(2) was 11.8(4)°, which indicated that the pyrazole ring and 1,3,4-oxadiazole ring are in the same plane (Fig. 2A). There is one intermolecular face-to-face π-π stacking between the pyrazole ring and 1,3,4-oxadiazole ring in the crystal. Also, there is a face-to-edge π-π stacking between the SCH₂ and phenyl ring in the crystal. It is worth noting that the two π-π stackings were centrosymmetric: the centroid distance of pyrazole ring-1,3,4-oxadiazole ring was 3.872 Å and the SCH₂-phenyl ring was 2.538 Å. These π-π stackings formed infinite one-dimensional chain structures to form a zigzag structure (Fig. 2B).

Fungicidal activity

Fungicidal activities of compound 5a–5m against Gibberella zeae (GZ), Phytophthora infestans (PI), Phytophthora capsici (PC), Sclerotinia sclerotiorum
(SS), *Rhizoctonia solani* (RS), *Alternaria solani* (AS), *Botrytis cinerea* (BC), *Fusarium oxysporum* (FO), *Cercospora arachidicola* (CA), and *Physalospora piricola* (PP) were evaluated at 50 μg/mL. Fluxapyroxad was used as a positive control. The results of antifungal activity against these ten different fungi are shown in Table 2. Most of the title compounds exhibited low activity (< 50%) against *G. zeae*, *P. infestans*, *P. capsici*, *A. solani*, *F. oxysporum*, *C. arachidicola*, and *P. piricola* at 50 μg/mL, except for *S. sclerotiorum*, *B. cinerea*, and *R. solani*. For the fungus *S. sclerotiorum*, all of the title compounds exhibited good activity (around 50%). Among them, the compounds 5d (69.2%) and 5h (76.9%) possessed the best activity, which is better than that of the positive control (63.6%). For *B. cinerea*, compounds 5h (61.3%) and 5i (67.7%) exhibited good activity, which is a little weaker than that of the positive control (88.4%). For *R. Solani*, compounds 5g (55.6%) and 5i (53.1%) exhibited good activity, which is better than that of the positive control (44.4%). The synthesis of these new fungicides provides a basis for preparing lead compounds with higher activity in the future.

![Molecular structure (A) and packing diagram (B) of the title compound 5a](image-url)
The phytotoxicity of the compound

The phytotoxicity of the 1,3,4-oxadiazole compounds are listed in Table 3. As shown in Table 3, most of the 1,3,4-oxadiazole compounds had low phytotoxicity against lettuce (Lactuca sativa) and bentgrass (Agrostis stolonifera) at 1 mM, except compound 5 g. Compounds 5 h and 5 i were moderately phytotoxic (ranking 3) against bentgrass at 1 mM. Compounds 5 e, 5 k, 5 l, and 5 m possessed weak phytotoxicity (ranking 1–2) against bentgrass at 1 mM. Interestingly, 5 e had a bleaching effect against bentgrass, although the phytotoxicity is low. For the dicot lettuce, compounds 5 a, 5 b, 5 e, 5 f, 5 h, and 5 j, and 5 m possessed weak phytotoxicity (ranking 1–2) at 1 mM. Compound 5 l exhibited moderate phytotoxicity (ranking 3) against lettuce at 1 mM. The most herbicidal compound 5 g exhibited a little lower activity (ranking 3 & 4) against both plant species than that of aminotriazole (ranking 4 & 5), but we also found the bleaching phenomenon against lettuce.

SAR study by molecular docking

The target of 1,3,4-oxadiazole fungicides may be SDH; therefore, molecular docking between compound 5 g and SDH was performed and carried out by using Discovery Studio 2.5 software. The crystal structure of SDH (PDB: 2FBW) was downloaded from the PDB bank (http://www.rcsb.org). As shown in Fig. 3, the compound 5 g (Fig. 3A) and penthiopyrad (Fig. 3B) are well matched to the active pocket of SDH. There were six strong hydrogen bonds (3.6, 3.3, 2.5, 3.0, 2.6 and 2.3 Å). The N–H···O and N–H···S hydrogen bonds were formed by Trp173 of SDH and the O and S atoms in the compound 5 g, the O–H···N and O–H···Br hydrogen bonds were formed by Tyr58 of SDH and the Br and N atoms of compound 5 g, and the N–H···F

Table 2 The fungicidal activity of compound 5a–5o at 50 mg/mL.

| No | AA | GZ | PI | PC | CA | SS | BC | RS | FO | PP |
|----|----|----|----|----|----|----|----|----|----|----|
| 5a | 41.2 | 8.8 | 10.5 | 10.7 | 14.3 | 50.0 | 9.7 | 12.3 | 23.5 | 7.8 |
| 5b | 17.6 | 20.6 | 5.3 | 7.1 | 7.1 | 40.4 | 25.8 | 12.3 | 17.6 | 17.6 |
| 5c | 23.5 | 8.8 | 10.5 | 17.9 | 7.1 | 40.4 | 12.9 | 12.3 | 23.5 | 9.8 |
| 5d | 35.3 | 41.2 | 5.3 | 25.0 | 21.4 | 69.2 | 25.8 | 43.2 | 11.8 | 43.1 |
| 5e | 17.6 | 8.8 | 5.3 | 17.9 | 7.1 | 51.9 | 3.2 | 35.8 | 17.6 | 29.4 |
| 5f | 29.4 | 8.8 | 15.8 | 7.1 | 7.1 | 46.2 | 25.8 | 37.0 | 17.6 | 7.8 |
| 5g | 29.4 | 14.7 | 21.1 | 35.7 | 21.4 | 55.8 | 48.4 | 55.6 | 35.3 | 5.9 |
| 5h | 29.4 | 29.4 | 15.8 | 17.9 | 21.4 | 76.9 | 61.3 | 43.2 | 23.5 | 39.2 |
| 5i | 29.4 | 41.2 | 21.1 | 28.6 | 28.6 | 51.9 | 67.7 | 53.1 | 29.4 | 5.9 |
| 5j | 17.6 | 23.5 | 5.3 | 10.7 | 7.1 | 50.0 | 41.9 | 30.9 | 17.6 | 5.9 |
| 5k | 23.5 | 11.8 | 10.5 | 10.7 | 14.3 | 46.2 | 12.9 | 30.9 | 29.4 | 9.8 |
| 5l | 17.6 | 5.9 | 5.3 | 10.7 | 14.3 | 50.0 | 12.9 | 24.7 | 17.6 | 9.8 |
| 5m | 17.6 | 17.6 | 5.3 | 3.6 | 7.1 | 50.0 | 25.8 | 12.3 | 17.6 | 5.9 |
| Water | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Fluxapyroxad | 88.9 | 30.3 | 29.7 | 38.1 | 96.4 | 63.6 | 88.4 | 44.4 | 100 | 84.6 |
hydrogen bond was formed by Arg43 of SDH and the F atom of compound 5 g, respectively. Meanwhile, there is a π-cation interaction between the Arg43 and the pyrazole ring. On the other hand, the commercial SDH inhibitor penthiopyrad had three hydrogen bonds (3.8, 2.5 and 2.8 Å). The O–H···O and O–H···N hydrogen bonds were formed by Try58 of SDH and the O atom of C=O and the N atom of the amide in penthiopyrad, and the N–H···F hydrogen bond was formed by Trp173

![Fig. 3](image-url)
of SDH and the F atom of pentiopyrad, respectively. There is a \( \pi \)-cation interaction between the Agr43 and the pyrazole ring. It is indicated that carbonyl is a key group in these compounds. This is in agreement with the reported references [30].

**Conclusion**

In conclusion, thirteen new 1,3,4-oxadiazole derivatives containing a trifluoromethylpyrazole moiety were evaluated for fungicidal activity. Low activity levels were found, but none of the activities approached those of commercial fungicides. All of the tested compounds had little to moderate growth inhibition on dicot plant, and some has slightly more activity on monocots. The complete lack of phytotoxicity of 5c coupled with moderate fungicidal activity indicates that it could be applied directly to plants for control of some fungal diseases. The antifungal activities reported here are sufficient for further structure manipulation to improve the antifungal activity. The structure–activity relationship was studied using molecular docking.

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**Author contributions** Conceptualization: N-BS and X-HL; Software L-JM; Investigation: H-BS, Z-WZ, CLC, and JB-H; Data Curation: H-BS, Z-WZ, CLC, and JB-H; Writing—Original Draft: H-BS; Writing—Review & Editing: L-JM, LH, CLC, JB-H, and SOD; Supervision: X-HL and N-BS.

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**Declarations**

**Competing interests** The authors declare no competing interests.

**Data availability** The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interest** The authors declare no conflict of interest.

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