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Synthesis and biological activity of novel 1,3,4-oxadiazole derivatives containing a pyrazole moiety

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Abstract

Several new 1,3,4-oxadiazole derivatives containing a pyrazole ring were designed and synthesized from ethyl acetoacetate and triethyl orthoformate as starting materials via multi-step reactions. The compound structures were confirmed by melting point, ^{1}H NMR and HRMS. They were evaluated for fungicidal and herbicidal activities. Four of the compounds exhibited moderate fungicidal activity against *Colletotrichum* species. Most of the compounds had moderate-to-good activity as a herbicide.

Keywords Oxadiazole · Pyrazole · Synthesis · Fungicide · Herbicide

Introduction

Heterocycles play an important role in natural product chemistry [1–3], medicinal chemistry [4–6], organic synthetic chemistry [7, 8] and pesticidal chemistry [9]. The pyrazole ring, a five-membered heterocyclic compound, has been used considerably in synthetic medicines and pesticides [10–13]. Pyrazole compounds possess diverse biological activities, such as herbicidal [14, 15], antioxidant [16], antimicrobial [17], anticancer [18], nematocidal [10, 19–27], fungicidal [28–37] and insecticidal

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activities [38]. Most of the succinate dehydrogenase inhibitor (SDHI) fungicides, an important fungicide class, have pyrazole rings. In addition, many references report 1,3,4-oxadiazole compounds with diverse biological activities, such as ketol-acid reductoisomerase inhibitors [39] and antiviral [40], anti-tumor [41], fungicidal [42] and antiproliferative activities [43].

Penflufen has good activity against *Rhizoctonia solani* and was developed commercially as a seed treatment, potato tuber treatment and soil application to protect seeds and seedlings against a wide range of fungal pathogens in field crops. Our interest in this study was to evaluate this group of pyrazole derivatives for possible activity against any of the three *Colletotrichum* species common to another high-value field crop, strawberry (*Fragaria*×*ananassa* Duchesne). In line with our continued efforts on the synthesis of novel lead compounds for pesticides [44–55], the commercial carboxamide fungicide penflufen was selected as a lead compound. The scaffold of this lead compound is maintained at the 1-methyl-3-methyl-1*H*-pyrazole moiety of the molecule. The fluorine atom was replaced by hydrogen atom, and the carboxamide group was cyclized by 1,3,4-oxidiazole ring. Our original strategy is depicted in Fig. 1. It is possible that pyrazole amide derivatives possess fungicidal activity. Most of them exhibited good herbicidal activity against a monocot, creeping bentgrass (*Agrostis stolonifera*).

Experimental

General information

The chemical intermediates were prepared in our laboratory, and reagents were of analytical grade. Melting points were recorded on an X-4 apparatus and were uncorrected. ^{1}H-NMR spectra were determined on an Avance 500 MHz spectrometer using $CDCl_3$ as solvent. HRMS were recorded on an Agilent TOF–MS instrument.

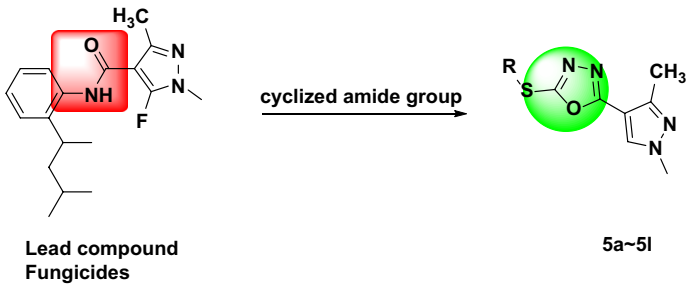


Fig. 1 Design strategy of the pyrazole compounds with 1,3,4-oxadiazole moiety

Synthesis

Ethyl 1,3-dimethyl-1*H*-pyrazole-4-carboxylate (**2**)

In a 1000-mL four-necked flask, ethyl 3-oxobutanoate (130 g, 1 mol), triethyl orthoformate (296 g, 2 mol) and acetic anhydride (255 g, 2.5 mol) were added and heated at 120 °C for 10 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_3NHNH_2 was added into the solution of brown liquid in EtOH (550 mL) at 0 °C, and the mixture was stirred at room temperature for 3 h. The solvent was evaporated, and the resulting white solid was produced with a yield of 51.2%, 86.1 g, m.p. 48–50 °C.

1,3-Dimethyl-1*H*-pyrazole-4-carbohydrazide (**3**)

To a solution of ethyl 1,3-dimethyl-1*H*-pyrazole-4-carboxylate (1.68 g, 10 mmol) in EtOH (10 mL), 80% hydrazine hydrate (5.63 g, 90 mmol) was added dropwise. Then, the mixture solution was stirred and refluxed for 8 h. The solvent was evaporated and cooled, and a white solid was given with a yield of 92.1%, 1.42 g, m.p. 140–141 °C.

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

The solution of 1,3-dimethyl-1*H*-pyrazole-4-carbohydrazide (15.4 g) and KOH (7 g) in EtOH (300 mL) was stirred at 0 °C for 15 min; then, the CS_2 (22.8 g) 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 84%, 16.56 g, m.p. 237–238 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ: ppm 7.84 (s, 1H, pyrazole-H), 3.92 (s, 3H, N-CH_3), 2.48 (s, 3H, pyrazole-CH_3).

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

N,N-Dimethylformamide (5 mL), 5-(1,3-dimethyl-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole-2-thiol (0.25 g, 1 mmol), NaOH (0.05 g, 1.2 mmol) and RCH_2Cl (1.1 mmol) were put into a pressure-rated vial (10 mL). Then, the mixture was reacted at 90 °C for 15 min under a microwave synthesizer. After the mixture temperature was below 50 °C, it was poured into crushed ice, and the expected 1,3,4-oxadiazole was collected and recrystallized.

2-(Benzylthio)-5-(1,3-dimethyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole **5a**

White solid, m.p. 82–85 °C, yield 28.0%, ^{1}H NMR (500 MHz, $CDCl_3$) δ: ppm 7.82 (s, 1H, pyrazole-H), 7.45–7.44(m, 2H, Ar–H), 7.36–7.33(m, 2H, Ar–H), 7.31–7.29(m, 1H, Ar–H), 4.59(s, 2H, –CH_2–), 3.91(s, 3H, N-CH_3), 2.49(s, 3H,

pyrazole-CH_3); ESI-HRMS calcd for $C_{14}H_{14}N_4OS$ 287.0961, found 287.0961 $[M+H]^+$.

2-((4-Bromobenzyl)thio)-5-(1,3-dimethyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole **5b**

Light yellow solid, m.p. 94–98 °C, yield 70.1%, ^{1}H NMR (500 MHz, $CDCl_3$) δ: ppm 7.79 (s, 1H, pyrazole-H), 7.45 (d, $J=8.3$ Hz, 2H, Ar–H), 7.33 (d, $J=8.3$ Hz, 2H, Ar–H), 4.41 (s, 2H, –CH_2–), 3.88 (s, 3H, N-CH_3), 2.51 (s, 3H, pyrazole-CH_3); ESI-HRMS calcd for $C_{14}H_{13}BrN_4OS$ 365.0066, found 365.0066 $[M+H]^+$.

2-((3,4-Dichlorobenzyl)thio)-5-(1,3-dimethyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole **5c**

White solid, m.p. 124–126 °C, yield 89.0%, ^{1}H NMR (500 MHz, $CDCl_3$) δ: ppm 7.82 (s, 1H, pyrazole-H), 7.58 (d, $J=2.01$ Hz, 1H, Ar–H), 7.42 (d, $J=8.3$ Hz, 1H, Ar–H), 7.34 (dd, $J=8.26$, 2.01 Hz, 1H, Ar–H), 4.43 (s, 2H, –CH_2–), 3.91 (s, 3H, N-CH_3), 2.53 (s, 3H, pyrazole-CH_3); ESI-HRMS calcd for $C_{14}H_{12}Cl_2N_4OS$ 355.0182, found 355.0178 $[M+H]^+$.

2-((2-Chlorobenzyl)thio)-5-(1,3-dimethyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole **5d**

White solid, m.p. 105–106 °C, yield 85.9%, ^{1}H NMR (500 MHz, $CDCl_3$) δ: ppm 7.82 (s, 1H, pyrazole-H), 7.64 (d, $J=7.0$ Hz, 1H, Ar–H), 7.42 (d, $J=7.5$ Hz, 1H, Ar–H), 7.31–7.21 (m, 2H, Ar–H), 4.61 (s, 2H, –CH_2–), 3.91 (s, 3H, N-CH_3), 2.53 (s, 3H, pyrazole-CH_3); ESI-HRMS calcd for $C_{14}H_{13}ClN_4OS$ 321.0571, found 321.0571 $[M+H]^+$.

2-((3-Chlorobenzyl)thio)-5-(1,3-dimethyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole **5e**

White solid, m.p. 70–71 °C, yield 75.0%, ^{1}H NMR (500 MHz, $CDCl_3$) δ: ppm 7.77 (s, 1H, pyrazole-H), 7.45–7.18 (m, 4H, Ar–H), 4.40 (s, 2H, –CH_2–), 3.86 (s, 3H, N-CH_3), 2.48 (s, 3H, pyrazole-CH_3); ESI-HRMS calcd for $C_{14}H_{13}ClN_4OS$ 321.0571, found 321.0570 $[M+H]^+$.

2-((4-Chlorobenzyl)thio)-5-(1,3-dimethyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole **5f**

White solid, m.p. 88–89 °C, yield 67.2%, ^{1}H NMR (500 MHz, $CDCl_3$) δ: ppm 7.81 (s, 1H, pyrazole-H), 7.42 (d, $J=8.1$ Hz, 2H, Ar–H), 7.34–7.26 (m, 2H, Ar–H), 4.45 (s, 2H, –CH_2–), 3.91 (s, 3H, N-CH_3), 2.53 (s, 3H, pyrazole-CH_3); ESI-HRMS calcd for $C_{14}H_{13}ClN_4OS$ 321.0571, found 321.0569 $[M+H]^+$.

2-((2,4-Dichlorobenzyl)thio)-5-(1,3-dimethyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole **5g**

Light yellow solid, m.p. 109–110 °C, yield 94.6%, ^{1}H NMR (500 MHz, $CDCl_3$) δ: ppm 7.80 (s, 1H, pyrazole-H), 7.60 (d, $J=8.3$ Hz, 1H, Ar–H)), 7.41 (s, 1H, Ar–H)), 7.20 (d, $J=7.3$ Hz, 1H, Ar–H), 4.54 (s, 2H, –CH_2–), 3.89 (s, 3H, N-CH_3),

2.51 (s, 3H, pyrazole-CH_3); ESI-HRMS calcd for $C_{14}H_{12}Cl_2N_4OS$ 355.0182, found 355.0182 $[M+H]^+$.

***2-(((6-Chloropyridin-3-yl)methyl) thio)-5-(1,3-dimethyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole* 5h**

Light yellow solid, m.p. 130–132 °C, yield 31.2%, ^{1}H NMR (500 MHz, $CDCl_3$) δ: ppm 8.50 (s, 1H, pyrazole-H), 7.90–7.79 (m, 2H, pyridine-H), 7.32 (d, $J=8.2$ Hz, 1H, pyridine-H), 4.45 (s, 2H, –CH_2–), 3.91 (s, 3H, N-CH_3), 2.53 (s, 3H, pyrazole-CH_3); ESI-HRMS calcd for $C_{14}H_{14}N_4OS$ 322.0524, found 322.0518 $[M+H]^+$.

***2-(1,3-Dimethyl-1H-pyrazol-4-yl)-5-((2-fluorobenzyl)thio)-1,3,4-oxadiazole* 5i**

White solid, m.p. 120–123 °C, yield 82.2%, ^{1}H NMR (500 MHz, $CDCl_3$) δ: ppm 7.82 (s, 1H, pyrazole-H), 7.58–7.53 (m, 1H, Ar–H), 7.34–7.27 (m, 1H, Ar–H), 7.14–7.05 (m, 2H, Ar–H), 4.52 (s, 2H, –CH_2–), 3.91 (s, 3H, N-CH_3), 2.53 (s, 3H, pyrazole-CH_3); ESI-HRMS calcd for $C_{14}H_{13}FN_4OS$ 305.0867, found 305.0868 $[M+H]^+$.

***4-(((5-(1,3-Dimethyl-1H-pyrazol-4-yl)-1,3,4-oxadiazol-2-yl)thio)methyl)benzonitrile* 5j**

Light yellow solid, m.p. 118–119 °C, yield 72.3%, ^{1}H NMR (500 MHz, $CDCl_3$) δ: ppm 7.81 (s, 1H, pyrazole-H), 7.68–7.58 (m, 4H, Ar–H), 4.50 (s, 2H, –CH_2–), 3.90 (s, 3H, N-CH_3), 2.51 (s, 3H, pyrazole-CH_3); ESI-HRMS calcd for $C_{14}H_{14}N_4OS$ 312.0914, found 312.0914 $[M+H]^+$.

***2-((5-(1,3-Dimethyl-1H-pyrazol-4-yl)-1,3,4-oxadiazol-2-yl)thio)acetonitrile* 5k**

Light yellow solid, m.p. 158–160 °C, yield 27.7%, ^{1}H NMR (500 MHz, $CDCl_3$) δ: ppm 7.88 (s, 1H, pyrazole-H), 4.09 (s, 2H, –CH_2–), 3.93 (s, 3H, N-CH_3), 2.56 (s, 3H, pyrazole-CH_3); ESI-HRMS calcd for $C_{15}H_{13}N_5OS$ 236.0601, found 236.0600 $[M+H]^+$.

***2-(((2-Chlorothiazol-5-yl)methyl) thio)-5-(1,3-dimethyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole* 5l**

Brown solid, m.p. 115–117 °C, yield 47.4%, ^{1}H NMR (500 MHz, $CDCl_3$) δ: ppm 7.84 (s, 1H, pyrazole-H), 7.57 (s, 1H, thiazole-H), 4.63 (s, 2H, –CH_2–), 3.92 (s, 3H, N-CH_3), 2.55 (s, 3H, pyrazole-CH_3); ESI-HRMS calcd for $C_{11}H_{10}ClN_5OS_2$ 328.0088, found 328.0088 $[M+H]^+$.

Fungicidal activity

Fungal pathogen production

Isolates of *Colletotrichum acutatum* Simmonds, *C. fragariae* Brooks, *C. gloeosporioides* (Penz.) Penz. & Sacc. in Penz. were obtained from B. J. Smith, USDA–ARS,

Small Fruit Research Station, Poplarville, MS. The three *Colletotrichum* species were isolated from strawberry (*Fragaria*×*ananassa* Duchesne).

Bioautography assay

Direct bioautography is a technique to screen large numbers of crude extracts, or pure compounds. Bioautography procedures were described in our previous studies [56, 57]. Pure compounds were evaluated for antifungal activity against strawberry anthracnose-causing plant pathogens, *Colletotrichum acutatum*, *C. fragariae* and *C. gloeosporioides* using the direct overlay bioautography. Technical grade commercial fungicide standards benomyl, cyprodinil, azoxystrobin and captan (with different modes of action) were used at 0.9–1.61 µg/µL concentrations in 95% ethanol. After sample application, each TLC plate was subsequently sprayed with a spore suspension (3.0×10^5 spores/mL) of the fungus of interest and incubated in a moisture chamber for 4 days at 26 °C with a 12-h photoperiod. Clear zones of fungal growth inhibition on the TLC plate indicate the presence of antifungal constituents in each extract or of the pure compounds.

Micro-dilution antifungal assay

A standardized 96-well micro-dilution broth assay developed by Wedge and Kahajek [58] was used to evaluate antifungal activity of pure compounds from extracts that were identified as active by bioautography [59]. This assay was used to evaluate antifungal activity of pure compounds toward *Colletotrichum acutatum* Simmonds, *C. fragariae* Brooks, *C. gloeosporioides* (Penz.) Penz. & Sacc. in Penz., in comparison with known fungicide standards [60–62]. Commercial, technical grade azoxystrobin and captan (without formulation) were used as validation controls in all micro-dilution broth assays. Each fungus was treated with 50, 75 and 150 µM of each compound. Captan and azoxystrobin standards were run at 0.3, 3.0 and 30.0 µM. Microtiter plates (Nunc MicroWell, untreated; Roskilde, Denmark) were covered with a plastic lid and incubated in a growth chamber as described previously for fungal growth. Fungal growth was then evaluated by measuring absorbance of each well at 620 nm using a microplate photometer (Packard Spectra Count, Packard Instrument Co., Downers Grove, IL). Mean absorbance values with standard errors were used to evaluate fungal growth at 48 and 72 h. Means for percent inhibition/stimulation of each fungus at each dose of test compound relative to the untreated positive growth controls were used to evaluate fungal growth. The SAS Proc ANOVA was used to identify significant factors, and Fisher's protected LSD was used to separate means. All experiments were repeated at least once in time.

Herbicidal activity

The method of Dayan et al. [63] was used for bioassay of a monocot and dicot plant species. Bioassays were done in duplicate in sterile non-pyrogenic polystyrene 24-well cell culture plates (CoStar 3524, Corning Incorporated). One filter paper

disk (Whatman Grade 1, 1.5 cm) was placed in each well. The control wells contained 200 µL of distilled 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 mL of the appropriate dilution of the sample. Water was always pipetted into the well before the sample or solvent. All plate preparation was done in a sterile environment to reduce chances of microbial contamination.

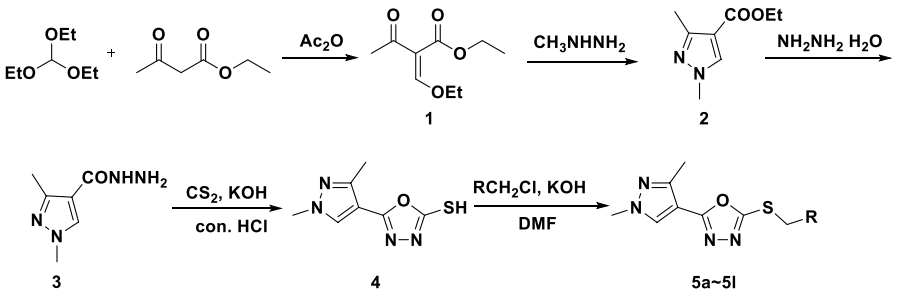
For lettuce (*Lactuca sativa* L.cv. Iceberg A Crisphead; Burpee seeds, W. Atlee Burpee & Co., Warminster, PA, USA), five seeds were placed in each well, and 20–30 seeds per well of creeping bentgrass (*Agrostis stolonifera* L. cv. Penncross; Turf Seed Inc., Hubbard, OR, USA) were used. Lids were sealed with Parafilm. The plates were incubated in a Percival Scientific CU-36L5 incubator under continuous light conditions at 26 °C and 120 µmol/s m^2 photon flux of photosynthetically active radiation average light intensity. Plates were incubated for at least 7 days. Ranking of plant growth was subjective.

On a scale of 0–5, a ranking of 0 indicated no apparent inhibition (sample well plants looked identical to the control + solvent well plants). A ranking of 5 indicated no growth or complete inhibition. A ranking of 5 was given only if no seeds germinated.

Results and discussion

Synthesis and spectra

The studied 1,3,4-oxadiazole derivatives are outlined in Scheme 1. The key intermediate pyrazole ring was synthesized using ethyl 2-(ethoxymethylene)-3-oxobutanoate and CH_3NHNH_2 according to the reference. Also, we also successfully used $NH_2NH_2{\cdot}H_2O$ instead of CH_3NHNH_2 to synthesize the pyrazole ring. Unfortunately, when we used CH_3I as the methylation reagent to synthesize intermediate **2**, it did not work well. The pyrazole carbohydrazide was prepared from a pyrazole ester and $NH_2NH_2{\cdot}H_2O$. The 1,3,4-oxadiazole ring was cyclized using pyrazole carbohydrazide in CS_2 and KOH under reflux conditions with high yield. Lastly, the



Scheme 1 Synthetic route of compounds **5a**–**5l**

1,3,4-oxadiazole thione reacted with different RCH_2Cl compounds in water under microwave irradiation, and the final products were easily produced. Compounds **5a–5l** had 28–89% yields.

All the pyrazole compounds were confirmed by ^{1}H-NMR and HRMS. From the ^{1}H-NMR results, the SCH_2 single was observed at 4.09–4.63 ppm, and the CH proton signal of pyrazole ring was found around 7.8 ppm as a single peak. The two methyl groups on the pyrazole ring appeared at 3.9 and 2.5 ppm, respectively. Meanwhile, all the pyrazole derivatives showed the $M+H^+$ peak in the HRMS data.

Antifungal activity

The bioautography assay results are listed in Table 1. The micro-dilution broth assay of test compounds demonstrated that compound **5b** was the most active against *C. acutatum* (Fig. 2). However, inhibitory activity of this compound decreased between 48 and 72 h 45% to 26%, respectively (72 h data not shown). Loss of fungal inhibitory activity over time is usually due to compound instability in the test solution or to metabolic degradation within the fungus. At the highest concentration of 150 μM, none of the test compounds possessed antifungal activity near that of azoxystrobin or captan standards that were tested at lower concentrations. As seen for compound **5l**, growth stimulation by fungicides at subtoxic concentrations (hormesis) in fungi is common [64]. Compounds **5b**, **5c** and **5f** were the most active analogs against *C. gloeosporioides*. Compound **5f** was the most active analog against *C. fragariae*; however, it only produced 28% inhibition. The structure–activity relationship of tested compounds indicated that when the halogen was substituted on the para- or meta-position of the benzene ring, the compounds had good antifungal activity, such as compounds **5b**, **5c**, **5e** and **5f**. Also, if there was no substitution on the benzene ring, the activity was still good.

Phytotoxicity

The herbicidal activity of the 1,3,4-oxadiazole compounds is listed in Table 1. As shown in Table 2, most of the 1,3,4-oxadiazole compounds had varying degrees of phytotoxicity against lettuce and Agrostis at 1 mM, except compound **5c**. Compound **5c** was not phytotoxic against lettuce and bentgrass at 1 mM. All the active compounds were more phytotoxic to Agrostis than to lettuce, with **5k** showing high phytotoxicity. The most herbicidal compound **5k** exhibited the same activity against bentgrass as control aminotriazole (ranking 5), but it had lower activity (ranking 3) against lettuce than that of aminotriazole (ranking 4). Compounds **5b**, **5h**, **5i**, **5j** and **5l** also had good herbicidal activity against bentgrass (ranking 4). Only compounds

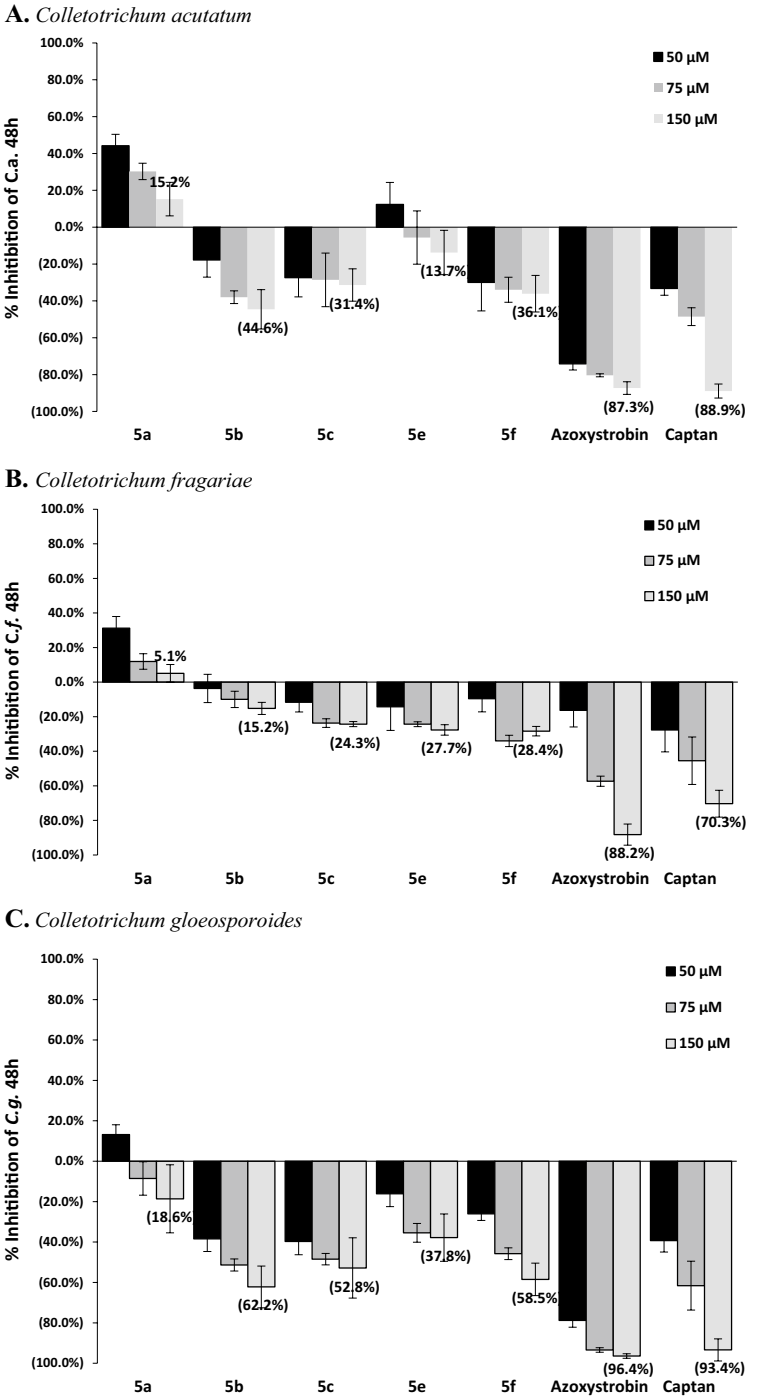


Fig. 2 Micro-dilution broth assay of five compounds against three *Colletotrichum* species

Table 1 Antifungal activity of five pyrazole derivatives against *Colletotrichum* test species using direct bioautography at 12 mM with 8 µL

Compounds	Mean zone diameter of fungal growth inhibition in mm		
	C. acutatum	*C. fragariae*	*C. gloeosporioides*
5a	13	6	Diffuse
5b	6	5	Diffuse
5c	9	6	Diffuse
5e	15	12	10
5f	Diffuse	Diffuse	Diffuse
Benomyl*	Diffuse	Diffuse	Diffuse
Captan*	12	15	16
Cyprodinil*	Diffuse	Diffuse	Diffuse
Azoxystrobin*	Diffuse	21	17

* is the note the control concentration is 2 mM and applied 2 µL

Table 2 Phytotoxicity of title compounds at 1 mM

No.	R	Lettuce	Agrostis
5a	Ph	2	3
5b	4-BrPh	1	4
5c	3,4-Cl_2Ph	0	0
5d	2-ClPh	3	3
5e	3-ClPh	2	3
5f	4-ClPh	2	3
5g	2,4-Cl_2Ph	0	2
5h	2-ClPy	4	4
5i	2-FPh	4	4
5j	4-CNPh	1	4
5k	CN	3	5
5l	2-Cl-thiazole	3	4
Aminotriazole		4	5

0 = no effect, 5 = no growth

5d, **5e** and **5f** displayed moderate herbicidal activity against bentgrass (ranking 3). For lettuce, only compounds **5h** and **5i** exhibited good herbicidal activity (ranking 4), which is the same as the positive control aminotriazole (ranking 4). Comparing herbicidal activity among **5a–5l** in Table 2, the compounds with electron withdrawing group at para-position of benzene ring exhibited good herbicidal activity against bentgrass. The compound with a cyanide group had the best activity against the two species. When the R is a heterocycle, the herbicidal activity is higher than that of phenyl compounds, such as **5h** and **5l**.

Conclusions

Twelve new 1,3,4-oxadiazole derivatives containing a pyrazole moiety were evaluated for fungicidal activity. Low levels of both activities were found, but none of the activities approached those of commercial fungicides. All of the tested compounds had little-to-moderate growth inhibition on dicots and major 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 diseases. The antifungal activities reported here are sufficient for further structure manipulation to improve the antifungal activity.

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