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Synthesis and biological evaluation of novel 2-(substituted isoxazol-4-yl)-5-arylamino-1,3,4-oxadiazoles

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Abstract A series of 2-(5-methyl-3-(4-chloro/trifluoromethylphenyl)isoxazol-4-yl)-5-arylamino-1,3,4-oxadiazoles were synthesized from 4-chloro/trifluoromethyl benzaldehyde, ethyl acetoacetate, hydroxylamine hydrochloride, hydrazine hydrate, and aryl isocyanate by multi-step reactions. The structures of the target compounds were elucidated by IR, ^{1}H NMR, MS, and elemental analysis. All these compounds were tested for in vitro antifungal activities against *Botrytis cinerea* and *Rhizoctonia cerealis* by the mycelium growth rate method, and the results indicated that some compounds displayed high antifungal activity against *Botrytis cinerea.*

Keywords 1,3,4-Oxadiazole · Isoxazole · Synthesis · Antifungal activity

Introduction

Oxadiazole derivatives are an important class of heterocyclic compounds due to their remarkable biological and pharmacological properties, such as anti-inflammatory [1], anticancer [2], antiviral [3], antibacterial [4], antifungal [5], insecticidal [6], and herbicidal [7] activities. For example, metoxadiazone, oxadiazon, and oxadiargyl have been developed as agrochemicals. Therefore, the synthesis and biological activity of oxadiazole derivatives are a research focus. On the other hand, many isoxazole compounds display antiviral [8], antifungal [9], insecticidal [10], and herbicidal [11] activities, and more than ten pesticides and drugs have been developed, e.g., clomazone, sulfamethoxazole and oxacillin. The isoxazole

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derivatives have drawn much attention because of their wide applications in pesticide and medicinal chemistry.

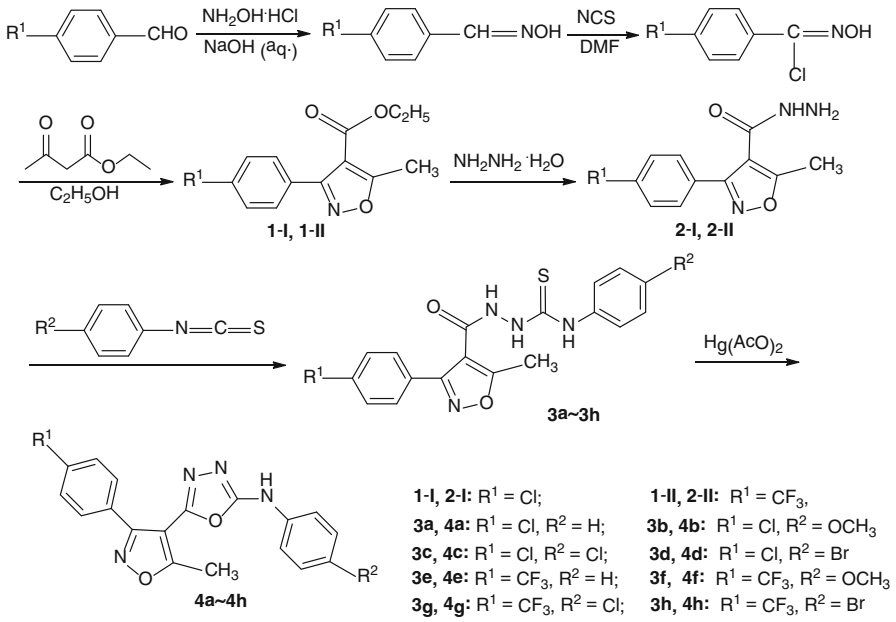
In view of the facts mentioned above, we here introduced a substituted isoxazole moiety to the oxadiazole ring, synthesized a series of novel 2-(5-methyl-3-(4-chlorophenyl)isoxazol-4-yl)-5-arylamino-1,3,4-oxadiazoles (**4a–d**) and 2-(5-methyl-3-(4-trifluoromethylphenyl)isoxazol-4-yl)-5-arylamino-1,3,4-oxadiazoles (**4e–h**), and also evaluated their in vitro antifungal activity. The synthetic route is shown in Scheme 1.

Experimental

General

Melting points were measured on a Tech X-5 microscopic melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu IR-440 infrared spectrophotometer using KBr disks. ^{1}H NMR spectra were determined on a Bruker AM-400 spectrometer with $CDCl_3$ as solvent and TMS as internal standard, Chemical shifts are reported in δ (parts per million) values. ESI–MS spectra were acquired on an Agilent 1200 spectrometer. The elemental analysis was measured by an Elementary Vario EL III analyzer.

Chemical reagents were purchased from J&K Chemical or Aladdin Reagent, and are of analytical grade.



Scheme 1 Synthetic route for the target compounds **4a–4h**

Synthesis

The key intermediates, (3-(4-chlorophenyl)-5-methyl)isoxazole-4-caroxylic acid ethyl ester (**1-I**) and (3-(4-trifluoromethylphenyl)-5-methyl)isoxazole-4-carboxylic acid ethyl ester (**1-II**), were synthesized from 4-chloro/trifluoromethyl benzaldehyde, hydroxylamine hydrochloride, *N*-chlorosuccinimide (NCS), and ethyl acetoacetate by three steps [12]. Compounds (3-(4-chlorophenyl)-5-methyl)isoxazole-4-carbohydrazide (**2-I**) and (3-(4-trifluoromethylphenyl)-5-methyl)isoxazole-4-carbohydrazide (**2-II**) were prepared by the reaction of **1-I** (or **1-II**) with 80 % hydrazine hydrate [13].

General procedure for the synthesis of 2-(5-methyl-3-(4-substituted phenyl)isoxazol-4-yl)-5-arylamino-1,3,4-oxadiazoles (**4**)

To a mixture of the substituted isoxazole-4-carbohydrazide **2-I** or **2-II** (5 mmol) in ethanol (50 mL) was added aryl isothiocyanate (5 mmol) at room temperature, and then the reaction mixture was refluxed with stirring for 3–4 h. Upon disappearance of the starting materials as monitored by thin layer chromatography, the reaction was cooled. The solid was filtered and recrystallized from 95 % ethanol to afford 5-methyl-3-(4-substituted phenyl)-isoxazole-4-carbonyl thiosemicarbazide **3** in yields of 69–90 %.

Mercuric acetate (0.64 g, 2 mmol) was added to a mixture of the thiosemicarbazide **3** (2 mmol) in ethanol (50 mL). The reaction mixture was refluxed for 3 h, and then concentrated under reduced pressure [14]. The residue was dissolved in hot *N,N*-dimethylformamide (DMF) and filtered. The filtrate was removed the solvent under reduced pressure, and the obtained solid was recrystallized from ethanol/DMF to give the target compound **4**.

*2-(5-Methyl-3-(4-chlorophenyl)isoxazol-4-yl)-5-phenylamino-1,3,4-oxdiazole (**4a**)*

Yield: 68 %, m.p. 235–237 °C; ^{1}H NMR (DMSO-d_6, 400 Hz): δ 2.78 (s, 3H, CH_3), 6.97–7.54 (m, 5H, Ar–H), 7.50–7.52 (d, $J = 8.4$ Hz, 2H, Ar–H), 7.71–7.73 (d, $J = 8.4$ Hz, 2H, Ar–H), 10.38 (s, 1H, NH); IR (KBr): ν (cm^{-1}) 3,174 (N–H), 1,635 (C=N), 1,051 (C–O–C); Anal. calcd. for $C_{18}H_{13}ClN_4O_2$: C 61.29, H 3.72, N 15.88; found: C 61.40, H 3.35, N 15.74; ESI–MS *m/z* calcd. for $C_{18}H_{13}ClN_4O_2$ $(M+H)^+$ 353.7, obsd. 353.3.

*2-(5-Methyl-3-(4-chlorophenyl)isoxazol-4-yl)-5-(4-methoxyphenylamino)-1,3,4-oxdiazole (**4b**)*

Yield: 54 %, m.p. 246–247 °C; ^{1}H NMR (DMSO-d_6, 400 Hz): δ 2.77 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 6.83–6.85 (d, $J = 8.8$ Hz, 2H, Ar–H), 7.40–7.43 (d, $J = 8.8$ Hz, 2H, Ar–H), 7.52–7.54 (d, $J = 8.4$ Hz, 2H, Ar–H), 7.71–7.73 (d, $J = 8.4$ Hz, 2H, Ar–H), 10.23 (s, 1H, NH); IR (KBr): ν (cm^{-1}) 3,174 (N–H), 1,634 (C=N), 1,051 (C–O–C); Anal. calcd. for $C_{19}H_{15}ClN_4O_3$: C 59.62, H 3.95, N 14.63; found: C 59.32, H 4.00, N 14.52; ESI–MS *m/z* calcd. for $C_{19}H_{15}ClN_4O_3$ $(M+H)^+$ 383.7, obsd. 383.3.

*2-(5-Methyl-3-(4-chlorophenyl)isoxazol-4-yl)-5-(4-chlorophenylamino)-1,3,4-oxdiazole (**4c**)*

Yield: 87 %, m.p. 236–238 °C; ^{1}HNMR (DMSO-d_6, 400 Hz): δ 2.77 (s, 3H, CH_3), 7.27–7.29 (d, $J = 8.8$ Hz, 2H, Ar–H), 7.52–7.55 (m, 4H, Ar–H), 7.71–7.73 (d, $J = 8.8$ Hz, 2H, Ar–H), 10.58 (s, 1H, NH); IR (KBr): v (cm^{-1}) 3,170 (N–H), 1,632 (C=N), 1,050 (C–O–C); Anal. calcd. for $C_{18}H_{12}Cl_2N_4O_2$: C 55.83, H 3.12, N 14.46; found: C 55.60, H 3.28, N 14.85; ESI–MS m/z calcd. for $C_{18}H_{12}Cl_2N_4O_2$ $(M+H)^+$ 388.2, obsd. 388.4.

*2-(5-Methyl-3-(4-chlorophenyl)isoxazol-4-yl)-5-(4-bromophenylamino)-1,3,4-oxdiazole (**4d**)*

Yield: 76 %, m.p. 229–231 °C; ^{1}H NMR (DMSO-d_6, 400 Hz): δ 2.78 (s, 3H, CH_3), 7.39–7.42 (d, $J = 8.8$ Hz, 2H, Ar–H), 7.48–7.50 (d, $J = 8.8$ Hz, 2H, Ar–H), 7.52–7.54 (d, $J = 8.4$ Hz, 2H, Ar–H), 7.71–7.73 (d, $J = 8.4$ Hz, 2H, Ar–H), 10.59 (s, 1H, NH); IR (KBr): v (cm^{-1}) 3,174 (N–H), 1,632 (C=N), 1,050 (C–O–C); Anal. calcd. for $C_{18}H_{12}BrClN_4O_2$: C 50.08, H 2.80, N 12.97; found: C 50.45, H 2.59, N 12.68; ESI–MS m/z calcd. for $C_{18}H_{12}BrClN_4O_2$ $(M+H)^+$ 432.6, obsd. 432.9.

*2-(5-Methyl-3-(4-trifluoromethylphenyl)isoxazol-4-yl)-5-phenylamino-1,3,4-oxdiazole (**4e**)*

Yield: 79 %, m.p. 237–239 °C; ^{1}H NMR (DMSO-d_6, 400 Hz): δ 2.80 (s, 3H, CH_3), 6.97–7.52 (m, 5H, Ar–H), 7.82–7.84 (d, $J = 8.4$ Hz, 2H, Ar–H), 7.94–7.96 (d, $J = 8.4$ Hz, 2H, Ar–H), 10.45 (s, 1H, NH); IR (KBr): v (cm^{-1}) 3,178 (N–H), 1,634 (C=N), 1,051 (C–O–C); Anal. calcd. for $C_{19}H_{13}F_3N_4O_2$: C 59.08, H 3.39, N 14.50; found: C 59.55, H 3.03, N 14.99; ESI–MS m/z calcd. for $C_{19}H_{13}F_3N_4O_2$ $(M+H)^+$ 387.2, obsd. 387.3.

*2-(5-Methyl-3-(4-trifluoromethylphenyl)isoxazol-4-yl)-5-(4-methoxyphenylamino)-1,3,4-oxdiazole (**4f**)*

Yield: 91 %, m.p. 258–260 °C; ^{1}H NMR (DMSO-d_6, 400 Hz): δ 2.79 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 6.82–6.84 (d, $J = 8.8$ Hz, 2H, Ar–H), 7.40–7.43 (d, $J = 8.8$ Hz, 2H, Ar–H), 7.81–7.83 (d, $J = 8.4$ Hz, 2H, Ar–H), 7.93–7.95 (d, $J = 8.4$ Hz, 2H, Ar–H), 10.20 (s, H, NH); IR (KBr): v (cm^{-1}) 3,177 (N–H), 1,629 (C=N), 1,050 (C–O–C); Anal. calcd. for $C_{20}H_{15}F_3N_4O_3$: C 57.70, H 3.63, N 13.45; found: C 57.25, H 3.35, N 13.51; ESI–MS m/z calcd. for $C_{20}H_{15}F_3N_4O_3$ $(M+H)^+$ 417.3, obsd. 417.4.

*2-(5-Methyl-3-(4-trifluoromethylphenyl)isoxazol-4-yl)-5-(4-chlorophenylamino)-1,3,4-oxdiazole (**4g**)*

Yield: 80 %, m.p. 241–243 °C; ^{1}H NMR (DMSO-d_6, 400 Hz): δ 2.80 (s, 3H, CH_3), 7.25–7.27 (d, $J = 8.8$ Hz, 2H, Ar–H), 7.53–7.55 (d, $J = 8.8$ Hz, 2H, Ar–H),

7.80–7.82 (d, $J = 8.4$ Hz, 2H, Ar–H), 7.93–7.95 (d, $J = 8.4$ Hz, 2H, Ar–H), 10.56 (s, H, NH); IR (KBr): v (cm^{-1}) 3,170 (N–H), 1,627 (C=N), 1,049 (C–O–C); Anal. calcd. for $C_{19}H_{12}ClF_3N_4O_2$: C 54.24, H 2.88, N 13.31; found: C 54.75, H 2.99, N 13.07; ESI–MS m/z calcd. for $C_{19}H_{12}ClF_3N_4O_2$ $(M+H)^+$ 421.7, obsd. 421.3.

*2-(5-Methyl-3-(4-trifluoromethylphenyl)isoxazol-4-yl)-5-(4-bromophenylamino)-1,3,4-oxdiazole (**4h**)*

Yield: 89 %, m.p. 240–241 °C; ^{1}H NMR (DMSO-d_6, 400 Hz): δ 2.80 (s, 3H, CH_3), 7.26–7.28 (d, $J = 8.8$ Hz, 2H, Ar–H), 7.49–7.52 (d, $J = 8.8$ Hz, 2H, Ar–H), 7.84–7.86 (d, $J = 8.4$ Hz, 2H, Ar–H), 7.92–7.94 (d, $J = 8.8$ Hz, 2H, Ar–H), 10.58 (s, 1H, NH); IR (KBr): v (cm^{-1}) 3,160 (N–H), 1,628 (C=N), 1,050 (C–O–C); Anal. calcd. for $C_{19}H_{12}BrF_3N_4O_2$: C 49.05, H 2.60, N 12.04; found: C 49.73, H 2.25, N 12.41; ESI–MS m/z calcd. for $C_{19}H_{12}BrF_3N_4O_2$ $(M+H)^+$ 466.2, obsd. 466.0.

Bioassays of antifungal activity

The in vitro fungicidal activities of the target compounds **4a**–**h** against *Botrytis cinerea* and *Rhizoctonia cerealis* were evaluated using the mycelium growth rate method [15, 16]. The culture media, with known concentrations (10, 50, 100, and 200 μg/mL) of the test compounds, were obtained by mixing the water suspension (1 mL) of **4** with potato dextrose agar (PDA, 9 mL) at 50 °C. The medium was poured onto 9-cm Petri dishes. When the medium solidified, it was inoculated onto a 5-mm-diameter agar disc which was grown with mycelium and cultivated for 3 days. The discs were placed in the center of the dish and in a light incubator at 25 ± 1 °C for 48–96 h, and then assessed by measurement of the colony diameter. A commercial fungicide carbendazim was used as the positive control, and sterile water was used as the blank. Three replications were used per treatment. The inhibition rate was expressed as the mean of values obtained in three independent experiments. Effective concentrations (EC_{50}) that inhibited mycelium growth by 50 % were obtained using log-probit analysis (Table 1). The inhibition rate was calculated according to the formula:

$$\text{Inhibition rate } (\%) = [(D_0 - D_1)/D_0] \times 100$$

where D_0 is the 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

Spectra

The signals of the CH_3 protons connected to the isoxazole ring were observed in the ^{1}H NMR spectra of target compounds at δ 2.77–2.80 ppm, and singlet signals at δ 10.20–10.59 ppm were assigned to NH proton. ArH protons appeared at δ 6.82–7.95 ppm. Moreover, owing to the strong electron-withdrawing effect of CF_3,

Table 1 Antifungal activity for the target compounds **4a–h**

Compds	R^1	R^2	EC_{50}, (μg/mL)*	
			B. cinerea pers	*R. cerealis*
4a	Cl	H	46.36 ± 1.22	47.02 ± 1.16
4b	Cl	OCH_3	18.15 ± 1.07	58.46 ± 1.14
4c	Cl	Cl	48.63 ± 1.28	80.05 ± 1.46
4d	Cl	Br	24.88 ± 1.15	61.48 ± 1.30
4e	CF_3	H	40.42 ± 1.38	37.39 ± 1.17
4f	CF_3	OCH_3	14.03 ± 1.05	54.44 ± 1.22
4g	CF_3	Cl	16.89 ± 1.13	61.70 ± 1.34
4h	CF_3	Br	36.69 ± 1.19	54.53 ± 1.25
Carbendazim			48.68 ± 1.21	1.52 ± 0.26

*Antifungal activity was expressed as EC_{50}

both the two doublet signals of the 4-trifluoromethylphenyl protons moved downfield (**4e–h**, δ 7.80–7.86 and 7.92–7.96 ppm, respectively). In their IR spectra, the N–H stretching vibrations were observed at 3,160–3,178 cm^{-1} and the C=N absorption bands of the heterocycles were near 1,630 cm^{-1}. The bands around 1,050 cm^{-1} were attributed to the C–O–C absorption of the oxadiazole ring.

Antifungal activity

As shown in Table 1, the EC_{50} values of the target compounds ranged from 14.03 to 48.63 μg/mL against *Botrytis cinerea,* near to or less than that of carbendazim (EC_{50} = 48.68 μg/mL), suggesting moderate to high antifungal activities. Among them, **4b**, **4f** and **4g** displayed high activities due to their low EC_{50} values of less than 20 μg/mL. However, compared with carbendazim, their EC_{50} values against *Rhizoctonia cerealis* were quite large (37.39–80.05 μg/mL), which means that the target compounds displayed low activities against this fungus.

Conclusion

In summary, we have synthesized four 2-(5-methyl-3-(4-chlorophenyl)isoxazol-4-yl)-5-arylamino-1,3,4-oxadiazoles and four 2-(5-methyl-3-(4-trifluoromethylphenyl)isoxazol-4-yl)-5-arylamino-1,3,4-oxadiazoles by multi-step reactions, which were elucidated by IR, ^{1}H NMR, MS, and elemental analysis. All these compounds were tested for in vitro antifungal activities against *Botrytis cinerea* and *Rhizoctonia cerealis* by the mycelium growth rate method, and the results indicated that three compounds displayed high antifungal activity against *Botrytis cinerea*, with EC_{50} values of less than 20 μg/mL.

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