Abstract

Background: Plant diseases seriously threaten food security, it is urgent to discover efficient and low-risk chemical pesticides. 1,2,4-Oxadiazole derivatives exhibit broad spectrum of agricultural biological activities. For discovering novel molecules with excellent agricultural activities, novel 1,2,4-oxadiazole derivatives were synthesized and evaluated for their agricultural activities.

Result: Bioassays results showed that the title compounds exhibited moderate nematocidal activity against Meloidogyne incognita and anti-fungal activity to Rhizoctonia solani. It’s worth noting that compounds 5m, 5r, 5u, 5v, 5x and 5y showed strong antibacterial effects on Xanthomonas oryzae pv. oryzae (Xoo), with EC50 values of 36.25, 24.14, 28.82, 19.44, 25.37 and 28.52 μg/mL, respectively, superior to bismerthiazol (BMT, EC50 = 77.46 μg/mL) and thiodiazole copper (TDC, EC50 = 99.31 μg/mL). Compounds 5p, 5u and 5v exhibited excellent antibacterial ability against Xanthomonas oryzae pv. oryzicola (Xoc), with EC50 values of 31.40, 19.04 and 21.78 μg/mL, respectively, better than that of BMT (EC50 = 68.50 μg/mL) and TDC (EC50 = 91.05 μg/mL). In addition, compound 5v exerted moderate antibacterial effects on rice bacterial leaf blight.

Conclusions: Twenty-six novel 1,2,4-oxadiazole derivatives were obtained and their biological activities were evaluated. Compound 5u and 5v exhibited excellent antibacterial activity Xoo and Xoc. These results indicated that 1,2,4-oxadiazole derivatives containing a trifluoromethyl pyridine moiety could be as potential alternative templates for discovering novel antibacterial agents.

Keywords: Synthesis, 1,2,4-Oxadiazole derivatives, Trifluoromethyl pyridine, Antibacterial activity, Nematocidal activity

Introduction

Crop plants are constantly challenged by a wide variety of pathogens which threaten their growth and survival, such as bacteria, fungus, and plant-parasitic nematodes. As two rice bacterial diseases, rice bacterial leaf blight and rice bacterial leaf streaks caused by Xanthomonas oryzae pv. oryzae (Xoo) and Xanthomonas oryzae pv. oryzicola (Xoc), respectively, serious impact on every stage of plant growth and development. These diseases may result in a loss of up to 80% of the crop and cause severe economic damage [1–4]. Meanwhile, fungal diseases, for example, rice sheath wilt caused by Rhizoctonia solani, still pose a huge threat to global agriculture [5]. In addition, over 3000 plant species are affected by nematodes worldwide, including ornamental flowers, fruit trees, cereals and vegetables [6–8]. The diseases caused by nematodes infecting plants are a serious threat to crop security, causing over $157 billion in economic losses to farmers worldwide [9, 10]. Root-knot nematode is a plant parasitic nematode that affects plant growth by essentially damaging the plant roots [11, 12]. There is an urgent need to
devise a method for the effective manual control of these plant diseases, as plants cannot quickly and effectively resist them [13]. Presently, pesticides are often used for agricultural control due to their rapid response to plant diseases [14, 15], however, the long-term abuse of pesticides has led to the emergence of resistance in pathogenic organisms and may pose a risk to human health [16–18]. Therefore, developing novel, highly-efficient, and environmentally benign agents against plant diseases remains a daunting task in pesticide sciences.

Heterocyclic structures are widely used in molecular design, and many commodity medicines have been developed [19], such as tioxazafen (Fig. 1), bismerthiazol and fluopyram. As an important five-membered heterocyclic scaffold, 1,2,4-oxadiazoles, with good potent biological properties [20, 21] have been extensively used in pesticide and medicine [22–24] molecule design. Moreover, the 1,2,4-oxadiazole heterocycle is a bioisostere of amide but shows better hydrolytic and metabolic stability [22], it is still used as an important pharmacophore to create novel drug molecules. Meanwhile, 1,3,4-thiadiazol and 1,3,4-oxadiazole have been reported to have good biological activities and were used to design drug molecules in pesticide. In our previous work, we designed and synthesized a series of novel 1,3,4-thiadiazol and 1,3,4-oxadiazole derivatives with effective control of bacterial [25, 26], fungal [27–29] and plant-parasitic nematodes [30] diseases. In addition, trifluoromethyl pyridine is an important heterocyclic structure containing fluorine, and also a common group in the current commercial pesticides. Fluopyram, containing a trifluoromethyl pyridine moiety, is not only used for control of fungal diseases, but also is used for control plant-parasitic nematodes disease [31, 32].

From the above standpoints, the compounds containing an 1,2,4-oxadiazole heterocycle, 1,3,4-thiadiazol (1,3,4-oxadiazole), or trifluoromethyl pyridine moiety exhibit broad-spectrum agricultural biological activities, which can be used as pharmacophore to design the novel pesticide. Encouraged by these promising results, and in order to obtain compounds with higher biological activity, we employed the structure-based bioisostere strategy, an excellent tool for lead were introduced into 1,2,4-optimisation, 1,3,4-thiadiazol (1,3,4-oxadiazole) and trifluoromethyl pyridine pharmacoophores oxadiazole skeleton to design and synthesize a series of novel 1,2,4-oxadiazole derivatives. Meanwhile their agricultural biological activities, including nematocidal, anti-fungal, and antibacterial activity were roundly evaluated. We aimed to discovery novel structure diversity molecules with broad-spectrum activity for development of new pesticides.

### Methods

#### Chemistry

All reagents and chemical materials of the analytically pure were purchased from chemical companies. The reactions were monitored by thin-layer chromatography analysis and the ZF7 ultraviolet analyzer (Yuhua Instrument Co., Ltd. Gong Yi, China). 1H and 13C NMR spectra were obtained on the JEOL-ECX-500 spectrometer (JEOL, Tokyo, Japan) or 400 MHz spectrometer (JEOL, Tokyo, Japan). The melting points of the compounds were measurement by the X-4B melting point instrument of readings were uncorrected (Yidian Physical Optical Instrument Co., Ltd. Shanghai, China). High-resolution mass spectra (ESI TOF (+)) were obtained on the LTQ Orbitrap XL (Thermo Scientific, MO, USA).

#### General synthesis procedure for compounds 5a–5i

The procedure for synthesizing the target compounds 5a–5i was described in Scheme 1. A mixture of substituted benzonitrile (5.0 mmol), NaOH (3.0 mmol) and hydroxylamine hydrochloride (7.5 mmol) in ethanol/water (30 mL, V:V=5:1) was refluxed for 4 h. Then, ethanol was removed and the mixture was extracted with ethyl acetate and removed the solvent to obtain intermediate 1. Then chloroacetyl chloride (2.0 mmol) was added to a solution of intermediate 1 (2.0 mmol) in toluene and resulting mixture stirred for 6–8 h at 110–120 °C. After complication of the reaction, the solvent was removed and the residue was recrystallized from ethyl acetate and removed the solvent to obtain intermediate 1. Then chloroacetyl chloride (2.0 mmol) was added to a solution of intermediate 1 (2.0 mmol) in toluene and resulting mixture stirred for 6–8 h at 110–120 °C. After complication of the reaction, the solvent was removed and the residue was recrystallized from ethanol to obtain intermediate 2. Finally, K$_2$CO$_3$ (1.0 mmol) was added to a solution of corresponding 1,3,4-oxadiazole/thiadiazole thiol intermediate (1.0 mmol) in MeCN (20 mL) and stirred at room temperature for 0.5 h. Then intermediate 2 (1.0 mmol) was added and the mixture was refluxed. After complication
of the reaction, the solvent was removed and the residue was recrystallized from ethanol to obtain the target compounds 5a–5i with 34.8–62.3% yields.

**General synthesis procedure for compounds 5j–5r**

A mixture of 2,3-dichloro-5-(trifluoromethyl) pyridine (5.0 mmol) and 4-hydroxybenzonitrile (5.0 mmol) in DMF (8 mL) was first stirred at room temperature for 0.5 h. Then K2CO3 (10.0 mmol) was added and the mixture was refluxed for 8 h. After completion of the reaction, the mixture was poured into 100 mL of ethanol. The precipitate was filtered off and to obtain intermediate 3. Then, a mixture of intermediate 3 (3.0 mmol), NaHCO3 (3.0 mmol) and hydroxylamine hydrochloride (4.5 mmol) in ethanol (20 mL) was refluxed for 2 h. The solvent was removed and the residue was poured into water. The mixture was extracted with ethyl acetate and removed the solvent to obtain intermediate 4. Then substituted acyl chloride (2.0 mmol) was added to a solution of intermediate 4 (2.0 mmol) in toluene and resulting mixture stirred for 6–8 h at 110–120 °C. After completion of the reaction, the solvent was removed and the residue was recrystallized from ethanol to afford the target compounds 5j–5r with 31.7–62.9% yields (Scheme 2).

**General synthesis procedure for compounds 5s–5z**

K2CO3 (1.0 mmol) was added to a solution of substituted phenol (1.0 mmol) in MeCN (20 mL) and stirred at room temperature for 0.5 h. Then compound 5r (1.0 mmol) was added and the mixture was refluxed for 4–6 h. After completion of the reaction, the solvent was removed and the residue was recrystallized from ethanol to obtain the target compounds 5s–5z with 41.8–61.8% yields (Scheme 3).

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**Scheme 1** Synthesis of target compounds 5a–5i

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**Scheme 2** Synthesis of target compounds 5j–5r

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**Scheme 3** Synthesis of target compounds 5s–5z

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5-((5-(4-fluorobenzyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-3-phenyl-1,2,4-oxadiazole (5a) Yellow solid; yield 55.0%; mp: 106.5–107.1 °C; 1H NMR (400 MHz, CDCl3) δ 7.47 (d, J = 7.6 Hz, 3H, Ph-H), 7.47 (d, J = 7.6 Hz, 3H, Ph-H) 7.19 (d, J = 8.6 Hz, 2H, Ph-H), 4.75 (s, 2H, –CH2–). 13C NMR (101 MHz, CDCl3) δ 174.47, 168.81, 165.77, 162.59, 161.69, 131.52, 129.33, 129.33, 128.77, 128.77, 127.49, 127.49, 126.13, 119.59, 116.62, 116.40, 31.44; HRMS (ESI) calcd for C17H12N4O2SF [M+H]+: 355.06534, found 355.06595.

5-((5-(4-chlorobenzyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-3-phenyl-1,2,4-oxadiazole (5c) Brown solid; yield 57.3%; mp: 83.5–84.7 °C; 1H NMR (400 MHz, CDCl3) δ 8.06–8.02 (m, 2H, Ph-H), 7.51–7.45 (m, 3H, Ph-H), 7.32–7.27 (m, 2H, Ph-H), 7.22 (t, J = 6.8 Hz, 2H, Ph-H), 4.78 (s, 2H, –CH2–), 4.35 (s, 2H, –CH2–). 13C NMR (101 MHz, CDCl3) δ 174.85, 170.43, 168.70, 168.77, 162.58 161.73, 131.42, 129.57, 129.57, 129.29, 128.89, 128.89, 127.86, 127.86, 127.54, 126.32, 31.46; HRMS (ESI) calcd for C17H11N4O2ClF [M+H]+: 389.02698, found 355.06595.
127.48, 127.48, 126.27, 35.80, 27.65; HRMS (ESI) calcd for C_{18}H_{14}N_{4}O_{2}S_{2}Cl [M+H]^+: 401.02856, found 401.02921.

5-(((5-(4-fluorophenyl)-1,3,4-thiadiazol-2-yl)thio)methyl)-3-(o-tolyl)-1,2,4-oxadiazole (5d) Yellow solid; yield 48.6%; mp: 98.1–98.7 °C; 1H NMR (500 MHz, CDCl₃) δ 7.97–7.92 (m, 3H, Ph-H), 7.47 (d, J = 8.7 Hz, 2H, Ph-H), 7.40–7.36 (m, 1H, Ph-H), 7.32–7.26 (m, 2H, Ph-H), 4.76 (s, 2H, –CH₂–), 2.59 (s, 3H, –CH₃). 13C NMR (126 MHz, CDCl₃) δ 173.38, 169.47, 165.86, 162.06, 138.42, 131.54, 130.97, 130.22, 129.63, 129.63, 128.16, 126.10, 125.47, 121.83, 114.02, 26.85, 22.28; HRMS (ESI) calcd for C_{18}H_{15}N_{4}O_{2}S₂ [M+H]^+: 367.06766, found 367.06818.

5-(((5-(3-chlorobenzyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-3-(o-tolyl)-1,2,4-oxadiazole (5e) Yellow solid; yield 62.3%; mp: 82.6–83.2 °C; 1H NMR (500 MHz, CDCl₃) δ 7.94–7.91 (m, 1H, Ph-H), 7.39 (td, J = 7.6, 1.4 Hz, 1H, Ph-H), 7.32–7.26 (m, 4H, Ph-H), 7.23–7.19 (m, 2H, Ph-H), 4.67 (s, 2H, –CH₂–), 4.15 (s, 2H, –CH₂–), 2.58 (s, 3H, –CH₃). 13C NMR (126 MHz, CDCl₃) δ...
5-((5-(4-chlorobenzyl)-1,3,4-oxadiazol-2-ylthio)methyl)-3-(4-chlorophenyl)-1,2,4-oxadiazole (5g) Yellow solid; yield 62.9%; mp: 95.2–96.0 °C; 1H NMR (400 MHz, DMSO-d$_6$) δ 8.61 (d, J = 2.0 Hz, 1H, Pyridine-H), 8.61 (d, J = 1.1 Hz, 1H, Pyridine-H), 8.25–8.19 (m, 4H, Ph-H), 7.57–7.53 (m, 2H, Ph-H), 7.27 (d, J = 8.9 Hz, 2H, Ph-H), 3.95 (s, 3H, –OCH$_3$). 13C NMR (101 MHz, DMSO-d$_6$) δ 175.83, 167.99, 160.94, 155.53, 143.62, 137.88, 130.47, 130.47, 129.31, 129.31, 124.79, 124.21, 123.07, 123.07, 122.48, 122.15, 119.12, 116.11, 115.51, 115.51, 56.49; HRMS (ESI) calcd for C$_{18}$H$_{13}$N$_4$O$_2$FCl [M+H]$^+$: 444.07135, found 444.07212.

(E)-3-(5-(3-chloro-5-(trifluoromethyl)pyridin-2-yl)oxy)phenyl)-5-styryl-1,2,4-oxadiazole (5m) Light yellow solid; yield 41.7%; mp: 121.0–121.5 °C; 1H NMR (400 MHz, DMSO-d$_6$) δ 8.64 (d, J = 2.1 Hz, 1H, Pyridine-H), 8.56 (dd, J = 2.1, 1.0 Hz, 1H, Pyridine-H), 8.14–8.09 (m, 2H, 2H, –CH$_2$–), 7.98 (d, J = 16.4 Hz, 1H, Ph-H), 7.90–7.86 (m, 2H Ph-H), 7.52–7.46 (m, 6H, Ph-H). 13C NMR (101 MHz, DMSO-d$_6$) δ 175.99, 167.89, 160.93, 155.54, 144.29, 143.34, 134.88, 134.69, 131.20, 129.51, 129.51, 129.26, 129.26, 128.92, 124.79, 124.19, 123.07, 123.07, 122.48, 122.12, 119.14, 110.66; HRMS (ESI) calcd for C$_{22}$H$_{14}$N$_4$O$_2$FCl [M+H]$^+$: 444.07135, found 444.07212.

3-(5-(3-chloro-5-(trifluoromethyl)pyridin-2-yl)oxy)phenyl)-5-(4-nitrophenyl)-1,2,4-oxadiazole (5n) White solid; yield 35.8%; mp: 182.4–183.7 °C; 1H NMR (400 MHz, DMSO-d$_6$) δ 8.64 (d, J = 2.1 Hz, 1H, Pyridine-H), 8.65 (dd, J = 1.9 Hz, 1H, Pyridine-H), 8.49 (d, J = 3.4 Hz, 4H, Ph-H), 8.24–8.19 (m, 2H, Ph-H), 7.56–7.50 (m, 2H, Ph-H). 13C NMR (101 MHz, DMSO-d$_6$) δ 175.1, 168.14, 160.61, 157.93, 155.62, 143.61, 137.82, 132.66, 131.32, 129.75, 129.33, 129.33, 124.78, 123.90, 122.50, 121.84, 120.51, 119.14, 117.25; HRMS (ESI) calcd for C$_{20}$H$_{16}$N$_4$O$_2$FCl [M+H]$^+$: 463.04154, found 463.04025.
3-(4-((3-chloro-5-(trifluoromethyl)pyridin-2-yl)oxy)phenyl)-5-(4-(trifluoromethyl)phenyl)-1,2,4-oxadiazole (5a) White solid; yield 44.3%; mp: 102.8–103.7 °C; $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 8.59 (d, $J$ = 2.2 Hz, 1H, Pyridine-H), 8.51 (d, $J$ = 1.0 Hz, 1H, Pyridine-H), 8.36 (d, $J$ = 8.3 Hz, 2H, Pyridine-H) 8.17–8.13 (m, 2H, Ph-H), 8.00 (d, $J$ = 8.4 Hz, 2H, Ph-H), 7.48–7.45 (m, 2H, Ph-H). $^{13}$C NMR (101 MHz, DMSO-d$_6$) $\delta$ 174.84, 168.40, 160.95, 155.78, 143.68, 137.93, 133.62, 133.36, 133.11, 129.87, 129.87, 128.32, 127.61, 124.57, 123.81, 123.81, 122.73, 122.21, 120.00, 119.22; HRMS (ESI) calcd for C$_{21}$H$_{11}$N$_3$O$_3$F$_6$Cl$_3$ [M+H]$^+$: 486.04385, found 486.04257.

3-(4-((3-chloro-5-(trifluoromethyl)pyridin-2-yl)oxy)phenyl)-5-isopropyl-1,2,4-oxadiazole (5p) White solid; yield 56.1%; mp: 88.5–89.2 °C; $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 8.63 (d, $J$ = 2.1 Hz, 1H, Pyridine-H), 8.55–8.54 (m, 1H, Pyridine-H), 8.09 (d, $J$ = 8.8 Hz, 2H, Ph-H), 7.47–7.43 (m, 2H, Ph-H), 2.81 (d, $J$ = 63.7 Hz, 1H, –CH–), 1.39 (d, $J$ = 7.0 Hz, 6H, –CH$_3$–). $^{13}$C NMR (101 MHz, DMSO-d$_6$) $\delta$ 184.70, 167.30, 160.89, 155.43, 143.63, 137.83, 129.75, 129.19, 126.50, 124.78, 124.22, 123.01, 122.48, 122.11, 119.13, 27.28, 20.29; HRMS (ESI) calcd for C$_{15}$H$_9$N$_3$O$_2$F$_3$Cl$_2$ [M+H]$^+$: 398.63228, found 398.63222.

3-(4-((3-chloro-5-(trifluoromethyl)pyridin-2-yl)oxy)phenyl)-5-ethyl-1,2,4-oxadiazole (5q) White solid; yield 56.1%; mp: 88.5–89.2 °C; $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 8.63 (d, $J$ = 2.1 Hz, 1H, Pyridine-H), 8.55–8.54 (m, 1H, Pyridine-H), 8.09 (d, $J$ = 8.8 Hz, 2H, Ph-H), 7.47–7.43 (m, 2H, Ph-H), 2.81 (d, $J$ = 63.7 Hz, 1H, –CH–), 1.39 (d, $J$ = 7.0 Hz, 6H, –CH$_3$–). $^{13}$C NMR (101 MHz, DMSO-d$_6$) $\delta$ 184.70, 167.30, 160.89, 155.43, 143.63, 137.83, 129.75, 129.19, 126.50, 124.78, 124.22, 123.01, 122.48, 122.11, 119.13, 27.28, 20.29; HRMS (ESI) calcd for C$_{15}$H$_9$N$_3$O$_2$F$_3$Cl$_2$ [M+H]$^+$: 398.63228, found 398.63222.

3-(4-((3-chloro-5-(trifluoromethyl)pyridin-2-yl)oxy)phenyl)-5-ethyl-1,2,4-oxadiazole (5r) White solid; yield 56.1%; mp: 88.5–89.2 °C; $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 8.63 (d, $J$ = 2.1 Hz, 1H, Pyridine-H), 8.55–8.54 (m, 1H, Pyridine-H), 8.09 (d, $J$ = 8.8 Hz, 2H, Ph-H), 7.47–7.43 (m, 2H, Ph-H), 2.81 (d, $J$ = 63.7 Hz, 1H, –CH–), 1.39 (d, $J$ = 7.0 Hz, 6H, –CH$_3$–). $^{13}$C NMR (101 MHz, DMSO-d$_6$) $\delta$ 184.70, 167.30, 160.89, 155.43, 143.63, 137.83, 129.75, 129.19, 126.50, 124.78, 124.22, 123.01, 122.48, 122.11, 119.13, 27.28, 20.29; HRMS (ESI) calcd for C$_{15}$H$_9$N$_3$O$_2$F$_3$Cl$_2$ [M+H]$^+$: 398.63228, found 398.63222.

3-(4-((3-chloro-5-(trifluoromethyl)pyridin-2-yl)oxy)phenyl)-5-ethyl-1,2,4-oxadiazole (5s) White solid; yield 56.1%; mp: 88.5–89.2 °C; $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 8.63 (d, $J$ = 2.1 Hz, 1H, Pyridine-H), 8.55–8.54 (m, 1H, Pyridine-H), 8.09 (d, $J$ = 8.8 Hz, 2H, Ph-H), 7.47–7.43 (m, 2H, Ph-H), 2.81 (d, $J$ = 63.7 Hz, 1H, –CH–), 1.39 (d, $J$ = 7.0 Hz, 6H, –CH$_3$–). $^{13}$C NMR (101 MHz, DMSO-d$_6$) $\delta$ 184.70, 167.30, 160.89, 155.43, 143.63, 137.83, 129.75, 129.19, 126.50, 124.78, 124.22, 123.01, 122.48, 122.11, 119.13, 27.28, 20.29; HRMS (ESI) calcd for C$_{15}$H$_9$N$_3$O$_2$F$_3$Cl$_2$ [M+H]$^+$: 398.63228, found 398.63222.
(126 MHz, DMSO-d$_6$) δ 176.62, 167.65, 160.94, 155.72, 143.68, 137.94, 131.22, 130.55, 130.55, 129.40, 129.40, 124.57, 123.74, 123.21, 123.21, 122.62, 122.2, 119.22, 115.18, 115.18, 61.39, 20.61; HRMS (ESI) calcd for C$_{22}$H$_{16}$N$_3$O$_3$F$_3$Cl; [M+H]$^+$: 462.08268, found 462.08160.

4-((3-chloro-5-((trifluoromethyl)pyridin-2-yl)oxy) phenyl)-5-((o-tolyl)oxy)-1,2,4-oxadiazole (5x) Yellow solid; yield 42.2%; mp: 80.0–80.8 °C; $^1$H NMR (500 MHz, DMSO-d$_6$) δ 8.60 (d, J = 2.0 Hz, 1H, Pyridine-H), 8.51 (s, 1H, Pyridine-H), 7.44 (d, J = 8.7 Hz, 3H, Ph-H), 7.16 (dd, J = 13.7, 7.4 Hz, 2H, Ph-H), 7.05 (d, J = 8.0 Hz, 1H, Ph-H), 6.89 (t, J = 7.3 Hz, 1H, Ph-H), 5.59 (s, 2H, –CH$_2$–), 2.20 (s, 3H, -CH$_3$). 13C NMR (126 MHz, DMSO-d$_6$) δ 176.70, 167.67, 160.95, 155.98, 155.75, 143.69, 137.95, 131.37, 129.40, 129.40, 127.62, 126.80, 124.58, 123.75, 123.23, 122.67, 121.90, 119.21, 112.57, 61.68, 16.45; HRMS (ESI) calcd for C$_{22}$H$_{16}$N$_3$O$_3$F$_3$Cl [M+H]$^+$: 462.08268, found 462.08160.

Nematocidal activity

The nematocidal activity of the target compound was carried out according to the reported method [33]. The tomato grown in the greenhouse for cultivating southern root-knot nematodes were uprooted and washed with water. Then take the eggs of the roots with a toothpick and place them in a petri dish containing distilled water. Second instar larvae were collected after 3–7 days of incubation at 27 °C. All tested compounds were dissolved in DMF and diluted with 1% Tween 80 (final concentration of DMF was 0.5%). 250 μL of the test solution was added to a 48-well biochemical culture dish and tested. Subsequently, approximately 100 nematodes were added to each well. Abamectin was used as a positive control, and a test solution containing no compound was used as a negative control. After 48 h of treatment with the compound, the nematode was transferred to clear water for resuscitation, and the nematode that did not move was considered dead.

Corrected mortality % = [(mortality of treatment % – mortality of negative control %)/ (1 – mortality of negative control %)] × 100

4-((3-((4-chloro-5-((trifluoromethyl)pyridin-2-yl)oxy) phenyl)-1,2,4-oxadiazol-5-yl)ethoxy)-2-fluorobenzonitrile (5y) Red solid; yield 43.7%; mp: 114.7–115.3 °C; $^1$H NMR (500 MHz, DMSO-d$_6$) δ 8.59 (d, J = 2.2 Hz, 1H, Pyridine-H), 8.50 (d, J = 1.0 Hz, 1H, Pyridine-H), 8.08–8.04 (m, 2H, Ph-H), 7.92–7.87 (m, 1H, Ph-H), 7.45–7.42 (m, 2H, Ph-H), 7.38 (dd, J = 11.6, 2.4 Hz, 1H, Ph-H), 7.14 (dd, J = 8.8, 2.4 Hz, 1H, Ph-H), 5.75 (s, 2H, –CH$_2$–). 13C NMR (101 MHz, DMSO-d$_6$) δ 175.34, 167.65, 160.87, 155.72, 143.61, 137.87, 135.49, 129.36, 129.36, 124.77, 123.58, 123.35, 123.07, 119.15, 114.63, 113.14, 110.14, 104.03, 103.59, 93.50, 62.12; HRMS (ESI) calcd for C$_{22}$H$_{16}$N$_3$O$_3$F$_3$Cl [M+H]$^+$: 491.05286, found 491.05167.

Antifungal assay

The mycelium growth rate method was utilized to evaluate in vitro antifungal activities of target compounds against R. solani [34]. The DMSO solution of the test compound was added to a sterilized petri dish containing about 10 mL of molten potato dextrose agar (PDA). Subsequently, a mycelial plug with a diameter of 4 mm was cut from the fungal colony and placed in the center of the PDA plate at 28 ± 1 °C for 4 days. For each compound, antifungal assays were performed in triplicate. In addition, pure DMSO and commercial fungicide (Hymexazol) were also used as negative and positive control agents, respectively.

The inhibition rate (I) of the tested compound was determined based on the following formula:

$$I = (C - T) / (C - 0.4) \times 100\%$$

In the formula, C represents the average mycelial diameter of negative control and T represents the average mycelial diameter of tested compound-treated PDA.

Antibacterial activity in vitro

The previously described method was used for in vitro antibacterial activity testing [35–37]. The 50 μL culture of Xoo or Xoc in logarithmic growth phase were added to the test tubes with 5 mL of NB medium containing different concentrations of the target compound, respectively. The commercial bacteriocide thiodiazole copper
Antibacterial activity in vivo

In vivo biometric against rice bacterial leaf blight. The curative and protection activities of compound 5v against rice bacterial leaf blight were determined by Schaad’s method with some slight modifications [4]. The curative activity of the rice plant bacterial leaf blight-reducing compound 5v in potted plants was determined under controlled conditions in the growth room. About 8 weeks after planting the “Fengyouxiangzhan” rice seeds, Xoo was inoculated on the rice leaves. One day after the inoculation, 200 μg/mL 5v solutions was evenly sprayed onto the rice leaves until dripping, and 200 μg/mL BMT and TDC solutions, and distilled water was evenly sprayed as positive and negative control groups, respectively. Then, all inoculated rice plants were placed in a plant growth chamber (28 °C and 90% relative humidity). On the 14th day after spraying, the disease index of the inoculated rice leaves was measured. Similarly, the protective activity of reducing rice bacterial leaf blight of compound 5v was also evaluated, the difference is that 1 day after spraying the compound solution and distilled water, Xoo was inoculated on rice leaves and the disease index of the inoculated rice leaves was measured on the 14th day after inoculation. First, measure the spot area of each leaf and the entire leaf area, and then calculate the percentage of the spot area in the entire leaf area. Secondly, these leaves were classed according to the following grading standards. Grade 1: the area of disease spot accounts for less than 5% of the whole leaf area; Grade 3: the area of disease spot accounts for 6–10% of the whole leaf area; Grade 5: the area of disease spot accounts for 11–20% of the whole leaf area; Grade 7: the area of disease spot 6 accounts for 21–50% of the whole leaf area; Grade 9: the area of disease spot accounts for more than 50% of the whole leaf area. Finally, the disease index (C or T) was calculated using the following formula: Disease index (C or T) = Σ (the number of leaves at each Grade × the corresponding Grade)/(the total number of leaves × the superlative Grade). The control coefficients I (%) for the curative and protection activities are calculated by the following equation. In the equation, C is the disease index of the negative control and T is the disease index of the treatment group.

\[
\text{Control efficiency } I(\%) = \frac{(C - T)}{C} \times 100
\]

Results and discussion

Design of novel 1,2,4-oxadiazole derivatives

1,2,4-Oxadiazole heterocycle is an important pharmacophore to design novel drug molecules. The compounds containing 1,2,4-oxadiazole skeleton possess various bioactivity in agricultural, including antibacterial, antifungal and nematocidal activities. Of which, oxinazoic is a new nematicide with unique mechanism of action developed by Monsanto. In our previous works, some 1,3,4-thiadiazol or 1,3,4-oxadiazole derivatives were designed and synthesized, and they exhibited good antibacterial, antifungal and nematocidal activities. So, we firstly introduced 1,3,4-thiadiazol or 1,3,4-oxadiazole into 1,2,4-oxadiazole skeleton to find highly active compounds. Meanwhile, the literature survey reveals that fluopyram showed good antibacterial activity and nematocidal activity, and the important pharmacophore is trifluoromethyl pyridine moiety. Encouraged by this results, we designed the novel 1,2,4-oxadiazole derivatives containing a trifluoromethyl pyridine moiety to find new lead compounds.

Chemistry

1H NMR, 13C NMR, and HRMS were used to characterize the physical properties of the target compounds 5a–5z. 1H NMR, 13C NMR, and HRMS data are provided in Additional file 1. In 1H NMR spectra of compound 5v, singlet at δ 8.64–8.55 ppm reveals the presence of Pyridine-H protons, singlet at δ 8.12–7.13 ppm reveals the presence of Ph-H protons. From the analysis of the 13C NMR spectrum of the compound 5v, it can be seen that 176.16 and 167.62 ppm are the absorption peaks of carbon on oxadiazole structure, 160.88, 156.59 and 155.70 ppm are the absorption peaks of carbon on the benzene ring directly connected to the oxygen group, and 61.59 ppm is the absorption peak of methylene carbon.

Nematocidal activity screening of title compounds

The in vitro nematocidal activity of the target compounds 5a–5i was evaluated using the direct strike method against Meloidogyne incognita. The results showed that all of the 1,2,4-oxadiazole derivatives containing 1,3,4-thiadiazol or 1,3,4-oxadiazole moiety have
low nematocidal activities. And then, introducing the trifluoromethyl pyridine moiety can enhance the activity. Of which, compounds 5n and 5v exhibited significant nematocidal activity against M. incognita, with the inhibitory ratio of 63.3% and 55.0% at 100 μg/mL, respectively, which was superior to that of tioxazafen (29.0%). There was no good activity of the 1,2,4-oxadiazole derivatives containing trifluoromethyl pyridine and diether groups (Table 1).

Antifungal activity screening of title compounds
Antifungal activity of target compounds was evaluated by using the mycelium growth method. Unfortunately, the results revealed that almost all the compounds failed to exhibit a noticeable fungicidal activity (≥50.0%) against R. solani at 50 μg/mL (Table 1).

Antibacterial activity screening of title compounds
In vitro bacterial activity test was performed using the turbidity method and the results were listed in Table 2. As shown in Table 2, thioether derivatives containing an 1,2,4-oxadiazole scaffold have low antibacterial activities. Some target compounds introducing the trifluoromethyl pyridine moiety showed better antibacterial activities against Xoo and Xoc at a concentration of 50 μg/mL compared to the control drugs, for example compounds 5m, 5r, 5u, 5v, 5x, and 5y with the values of 65.85, 71.89, 64.97, 85.37, 61.97, and 78.44% against Xoo, respectively. Meanwhile, Compounds 5p, 5u, and 5v with the values of 64.53, 65.10, and 64.59% against Xoo, respectively. The half maximal effective concentration (EC50) value of compounds was further tested. The results clearly showed that some target compounds exhibit better antibacterial activity than that of bismethiazol (BMT) and thiodiazole copper (TDC). Compounds 5m, 5r, 5u, 5v, 5x and 5y showed excellent antibacterial effects on Xoo, with EC50 values of 36.25, 24.14, 28.82, 19.44, 25.37 and 28.52 μg/mL, respectively, stronger than BMT (EC50 = 77.46 μg/mL) and TDC (EC50 = 99.31 μg/mL). Compounds 5n, 5p, 5t, 5u, 5v and 5z exhibited strong antibacterial ability against Xoc, with EC50 values of 50.93, 31.40, 56.50, 19.04, 21.78, and 55.32 μg/mL, respectively, superior to the control agents BMT (EC50 = 68.50 μg/mL) and TDC (EC50 = 91.05 μg/mL). Among these target compounds, compound 5v and 5u showed the best antibacterial activity on Xoo and Xoc, respectively.

Table 1 Nematocidal and antifungal activity of compounds 5a–5z

| Compounds | M. incognita Corrected mortality rate (%)a | R. solani Inhibition rate (%)b |
|-----------|-------------------------------------------|-----------------------------|
| 5a        | 33.4±2.7                                  | 28.7±1.3                    |
| 5b        | 28.9±5.2                                  | 15.6±0.7                    |
| 5c        | 37.6±8.1                                  | 25.2±2.2                    |
| 5d        | 26.6±2.0                                  | 20.4±1.2                    |
| 5e        | 0                                         | 18.8±1.7                    |
| 5f        | 29.5±2.7                                  | 34.6±2.3                    |
| 5g        | 31.3±5.3                                  | 16.1±1.1                    |
| 5h        | 29.4±6.1                                  | 19.1±0.5                    |
| 5i        | 40.3±1.8                                  | 23.5±1.6                    |
| 5j        | 39.7±4.2                                  | 15.0±2.3                    |
| 5k        | 14.7±27                                   | 34.7±0.8                    |
| 5l        | 31.3±3.8                                  | 22.1±0.8                    |
| 5m        | 48.0±3.7                                  | 23.8±3.2                    |
| 5n        | 63.3±7.7                                  | 29.4±1.0                    |
| 5o        | 35.5±2.4                                  | 47.0±1.7                    |
| 5p        | 25.3±4.7                                  | 104±0.6                     |
| 5q        | 44.8±6.7                                  | 26.1±0.8                    |
| 5r        | 36.7±5.0                                  | 29.1±0.5                    |
| 5s        | 27.3±8.8                                  | 13.3±1.6                    |
| 5t        | 35.6±3.4                                  | 25.8±1.3                    |
| 5u        | 40.8±3.4                                  | 34.5±2.3                    |
| 5v        | 55.0±7.7                                  | 34.3±0.3                    |
| 5w        | 26.6±4.0                                  | 32.9±1.1                    |
| 5x        | 47.3±3.8                                  | 13.6±3.2                    |
| 5y        | 34.6±2.4                                  | 39.4±1.4                    |
| 5z        | 36.5±2.6                                  | 27.8±1.6                    |

Tioxazafen 290±45 NT
Fluopicolide 100 NT
Azoxyostrobin NT 100

Average of three replicates
NT not tested

a Target compounds 5a–5z at a concentration of 100 μg/mL against J2 of M. incognita
b Target compounds 5a–5z at a concentration of 50 μg/mL against R. solani

Compound 5v exhibited excellent antibacterial ability to Xoo, with an EC50 value of 19.44 μg/mL. Accordingly, the control effect of compound 5v on rice bacterial leaf blight was evaluated and the results were showed in Table 3 and Fig. 2. Compound 5v exerted moderate control effects on rice bacterial leaf blight at 200 μg/mL, with curative and protective activity values of 37.8%
Table 3 Curative and protective activity of compound 5v against rice bacterial leaf blight

| Treatments | Protective activity | Curative activity |
|------------|---------------------|------------------|
|            | Disease index (%)   | Control effect (%) | Disease index (%) | Control effect (%) |
| 5v         | 60.6                | 27.6 ± 3.7        | 50.1             | 37.8 ± 4.2        |
| BMT        | 54.2                | 36.1 ± 2.5        | 43.3             | 46.7 ± 1.8        |
| TDC        | 56.5                | 30.9 ± 3.4        | 56.2             | 31.8 ± 5.6        |
| Negative control | 84.3           | –                 | 81.2             | –                 |

Compounds 5v at a concentration of 200 µg/mL against rice bacterial leaf blight

* Statistical analysis was conducted using the analysis of variance method.
potential of 1,2,4-oxadiazole ether derivatives as effective nematocidal and antimicrobial agents for crop protection and should serve as a basis for future studies.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13065-020-00722-1.

Additional file 1. The \( ^1 \)H NMR, \( ^{13} \)C NMR, and HRMS data of target compounds.

Abbreviations

Xoo: *Xanthomonas oryzae* pv. *oryzae*; Xoc: *Xanthomonas oryzae* pv. *oryzicola*; BMT: Bismerthiazol; TDC: Thiodiazole copper; \( ^1 \)H NMR: \( ^1 \)H nuclear magnetic resonance; \( ^{13} \)C NMR: \( ^{13} \)C nuclear magnetic resonance; HRMS: High resolution mass spectrum.

Acknowledgements

Not applicable.

Authors’ contributions

The current study is an outcome of constructive discussion with BS and XG. LZ, XG and QW carry out their synthesis and characterization experiments; LZ, HZ, DL, YF, and QW performed the biological activities; LZ, XG, HZ, DL, YF, and QW carried out the \( ^1 \)H NMR, \( ^{13} \)C NMR and HRMS spectral analyses; LZ and XG were involved in the drafting of the manuscript and revising the manuscript. All authors read and approved the final manuscript.

Funding

The authors gratefully acknowledge assistance from the National Key Research Development Program of China (2017YFD0201404 and 2018YFD0200100), the National Nature Science Foundation of China (32060622), the Outstanding Young Scientific and Technological Talents Project of Guizhou Province (QJKY[2019]5646), and the Construction Project of Key Laboratories from the Education Department of Guizhou Province (QJHKY[2018]001).

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its Additional files].

Ethics approval and consent to participate

The experimental research was performed following the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora and it was approved by Subcommittee of Experimental Animal Ethics, and Center for Research and Development of Fine Chemicals of Guizhou University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 24 April 2020   Accepted: 12 November 2020
Published online: 22 November 2020

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