Design, synthesis and antimicrobial activities of novel 1,3,5-thiadiazine-2-thione derivatives containing a 1,3,4-thiadiazole group

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Abstract

A series of 1,3,5-thiadiazine-2-thione derivatives containing a 1,3,4-thiadiazole group were designed, synthesized and screened for their antibacterial effects against *Xanthomonas oryzae pv. oryzicola* (*Xoc*) and *Xanthomonas oryzae pv. oryzae* (*Xoo*) as well as their antifungal effects against *Rhizoctonia solani* (*Rs*) and *Fusarium graminearum* (*Fg*). The *in vitro* antimicrobial bioassays indicated that some title compounds exhibited impressive antimicrobial effects against the above tested strains. Notably, the anti-*Rs* EC_{50} value of *N*-(5-(ethylthio)-1,3,4-thiadiazol-2-yl)-2-(5-methyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acetamide (**8a**) reached 33.70 μg/mL, which is about double more effective than the commercial fungicide hymexazol (67.10 μg/mL). In addition, compound **8a** also displayed the obvious antibacterial effects against *Xoc* and *Xoo* at 100 μg/mL, with the inhibition rates of 30% and 56%, respectively, which are better than a commercialized bactericide thiodiazole-copper (18% and 40%). Given the above results, bioorganic molecules with antimicrobial activities against phytopathogenic microorganisms

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might be obtained via the further structural modification of 1,3,5-thiadiazine-2-thione derivatives containing a 1,3,4-thiadiazole group.

Keywords: 1,3,5-thiadiazine-2-thione, 1,3,4-thiadiazole, crop protection, antibacterial activity, antifungal activity

Introduction

A variety of plant diseases caused by pathogenic organisms seriously affect crop productions, leading tremendous losses to agricultural economy every year (Wilson et al., 2009; Liu et al., 2013). Besides, the rapid emergence of resistant strains against traditional antimicrobial agents has become a huge challenge in agricultural industry (Wang et al., 2013). In the last decades, researchers have found a large number of bioactive molecules with strong inhibitory effects on phytopathogenic bacteria and fungi. However, these compounds are rarely used in crop production due to structural instability or poor control in farmland. Therefore, it is tardy to search the highly-effective and eco-friendly agrochemicals for fighting against agricultural pathogenic microorganisms (Qian et al., 2010; Li et al., 2018).

1,3,5-Thiadiazine-2-thione derivatives are attractive bioactive molecules that exhibited antibacterial (Mao et al., 2017), antifungal (Vicentini et al., 2002), herbicidal (Vicentini et al., 2005), anticancer (El-Shorbagi et al., 2018), antileishmanial (Arshad et al., 2018), antiepileptic (Semreen et al., 2010), antimalarial (Coro et al., 2006), antioxidant (Ji et al., 2004), antitubercular (Katiyar et al., 2003) and trypanocidal (Coro et al., 2005) activities. Noticeably, the agricultural application of 1,3,5-thiadiazine-2-thione derivatives has attracted great attentions from chemists and biologists in the last three decades. For example, dazomet and milneb (**Fig. 1**) were respectively developed as the important agricultural nematicide and fungicide that contains a 1,3,5-thiadiazine-2-thione moiety (Lam et al., 1993; Nakamura et al., 2010). Recently, Mao et al. found that dazomet could be applied as a promising agricultural bactericide to effectively control ginger blast in field trials (Mao et al., 2017). Meanwhile, our previous work found that 1,3,5-thiadiazine-2-thione derivatives with an acylhydrazide group displayed obvious antifungal activity *in vitro* and *in vivo* (Wang et al., 2018).

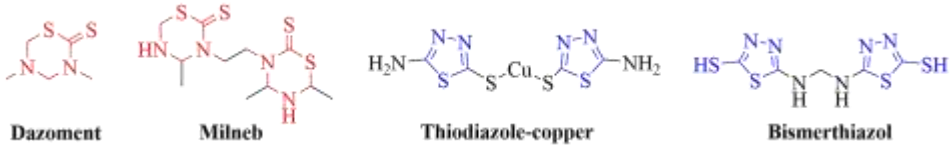


Figure 1. Bioactive compounds containing a 1,3,5-thiadiazine-2-thione or 1,3,4-thiadiazole fragment.

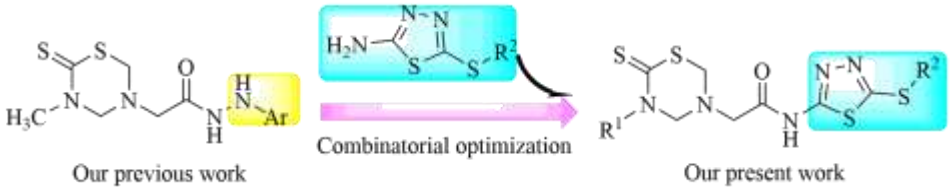
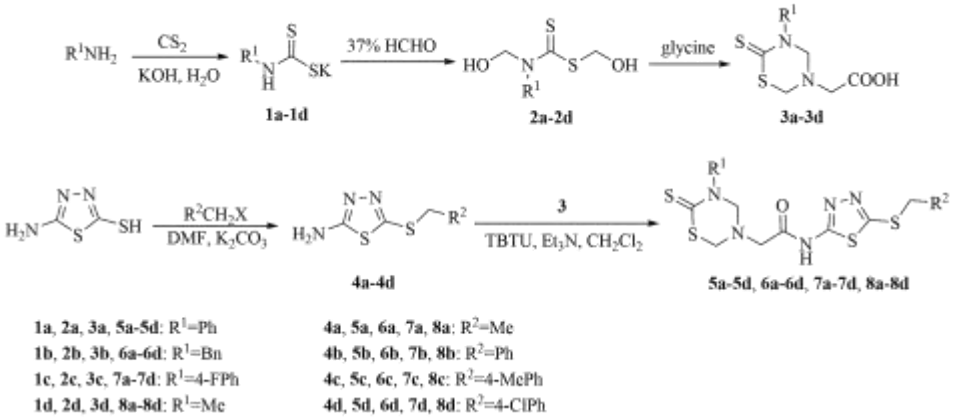


Figure 2. Design strategy for title compounds.



Scheme.1 Synthetic route to title compounds **5**–**8.**

1,3,4-thiadiazole derivatives attract great attention from biochemists due to their various bioactivities including antibacterial (Zhong et al., 2017), antifungal (Chen et al., 2000), insecticidal (Luo et al. 2007), antiviral (Chen et al., 2010), herbicidal (Cummings et al., 2009), anticancer (Casey et al., 2004), anti-tubercular (Foroumadi et al., 2003), antiparasitic (Coura et al., 2002), antidepressant (Siddiqui et al., 2011), antioxidant (Khan et al., 2010) and anti-inflammatory (Kumar et al., 2008) activities. Among the above biological activities, the remarkable antimicrobial activity of 1,3,4-thiadiazole derivatives were well reported during the last decades. As representative agrochemicals containing 1,3,4-thiadiazole group, thiodiazole-copper and bismerthiazol (**Fig. 1**) were widely used to control crop bacterial diseases. In addition,

researchers recently found that 1,3,4-thiadiazole derivatives bearing a sulfone moiety could effectively inhibit various agricultural fungi and bacteria (Xu et al., 2011; Li et al., 2014).

In order to find novel antimicrobial candidates and combining the above studies, the 1,3,4-thiadiazole fragment was introduced to the 5-position of the 1,3,5-thiadiazine-2-thione according to the method of "combinatorial optimization" (**Fig. 2**) to obtain a series of 1,3,5-Thiadiazine-2-thione derivatives containing 1,3,4-thiadiazole scaffold (**Scheme 1**). Their antibacterial activities against *Xanthomonas oryzae pv. oryzae (Xoo)* and *Xanthomonas oryzae pv. oryzicola (Xoc)* and their antifungal activity against *Rhizoctonia solani (Rs)* and *Fusarium graminearum (Fg)* were evaluated. Furthermore, preliminary biological assay showed that certain of the title compounds exhibited good antibacterial and antifungal activities. To the best of our knowledge, this is the first reported that synthesis and biological activity study of 1,3,5-thiadiazine-2-thione derivatives containing a 1,3,4-thiadiazole moiety in agrochemicals.

Materials and Methods

Materials

All solvents and reagents were purchased from commercial suppliers and used without further purification. The reaction processes were monitored by analytical thin-layer chromatography (TLC) on silica gel GF_{254}. The melting points were measured on a SMP50 automatic melting point apparatus (Cole-Parmer, England). ^{1}H and ^{13}C NMR spectra were recorded on a Brucker 400 spectrometer (Brucker, Germany) at room temperature with DMSO-d_6 as a solvent and TMS as an internal standard. HRMS data were measured on a TRACE 2000 spectrometer (Finnigan, America).

General procedures for substituted 2-(6-thioxo-1,3,5-thiadiazinan-3-yl) acetic acids 3

Carbon disulfide (7.61 g, 100 mmol) was added dropwise to the 18% aqueous potassium hydroxide solution (100 mL, 100 mmol – this stoichiometric calculations does not make sense) containing substituted phenylamine (9.31 g, 100 mmol). After stirred for 4 h at room temperature, the reaction mixture turned from colorless to orange and appeared white solid. Then, 37% formaldehyde solution (18.66 g, 230 mmol) was added in the reaction mixture, and the above solution was stirred for another 1 h at room temperature. After filtered, the obtained filtrate was slowly dropped into a phosphate buffer solution (pH 7.8, 100 mL) containing glycine (7.51 g, 100 mmol). After stirred for 2 h at room temperature and filtered, the filtrate was washed with

diethyl ether until the color of organic phase was changed to colorless. Under ice bath conditions, the obtained water phase was acidified with dilute hydrochloric acid to generate white precipitates. The formed precipitates were filtered, washed with iced ethanol, and dried to acquire the key intermediates **3a**. This method was suitable for the synthesis of compounds **3b**–**3d**.

General procedures for substituted 5-(ethylthio)-1,3,4-thiadiazol-2-amines 4

The DMF (20 mL) solution containing 5-amino-1,3,4-thiadiazole-2-thiol (2.0 g, 15 mmol) and K_2CO_3 (2.76 g, 20 mmol) was stirred for 15 minutes at room temperature. A substituted ethyl bromide (2.18 g, 20 mmol) was added dropwise in the above mixture. After stirred for 8 h at room temperature and poured into cold water (10 mL), the obtained mixture containing white solids was filtered and recrystallized with ethanol and water to gain pale yellow solids **4a**. This method was suitable for the synthesis of compounds **4b**–**4d**.

General synthetic procedure for title compounds 5–8.

The intermediates **3a** (0.50 g, 1.86 mmol), *O*-(Benzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU, 0.72 g, 2.24 mmol), triethylamine (0.38 g, 3.76 mmol) were added into dichloromethane (30 mL) and the mixture was stirred for 0.5 h at room temperature. Then, the intermediates **4a** (0.45 g, 2.80 mmol) was added and stirred for another 2 h at room temperature. The resulting precipitate was filtered, washed with dichloromethane, and dried to give the desired product **5a**. This method was suitable for the synthesis of title compounds **5**–**8.**（Si et al., 2019)

Antibacterial activities test *in vitro*

The antibacterial activities of title compounds against *Xanthomonas oryzae pv. oryzae (Xoo)* and *Xanthomonas oryzae pv. oryzicola (Xoc)* were evaluated by the turbidimeter test (Li et al.,2014; Wang et al., 2013; Xu et al., 2012). The compounds were dissolved in dimethylsulfoxide (DMSO) and diluted with water (containing 0.1% Tween-20) to obtain a solution with a final concentration of 100 and 50 μg/mL by adding different amounts water. DMSO in sterile distilled water served as a blank control, thiodiazole-copper served as positive controls. Approximately 1 mL of sample liquid was added to the nontoxic nutrient broth (NB, 3.0 g of beef extract, 5.0 g of peptone, 1.0 g of yeast powder, 10.0 g of glucose, and 1000 mL of distilled water, pH 7.0 to 7.2) liquid medium in 4 mL tubes. Then, about 40 μL of solvent NB containing *Xoo* or *Xoc* was added to 5 mL of solvent NB containing the test compounds and positive controls. The inoculated test tubes were incubated at 28 ± 1°C and continuously cultured shakily at 180 rpm for 2-3 days. The

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growth of the cultures was monitored on a microplate reader by measuring the optical density at 600 nm (OD_{600}) given by $turbidity_{corrected\ values} = OD_{bacterium} - OD_{no\ bacterium}$, and then the inhibition rate I was calculated by $I\ (\%) = (C_{tur} - T_{tur})/C_{tur} \times 100$. C_{tur} is the corrected turbidity values of bacterial growth on untreated NB (blank control), and T_{tur} is the corrected turbidity values of bacterial growth on treated NB.

Antifungal activities test *in vitro*

Rhizoctonia solani and *Fusarium graminearum* were chosen as the test strains. The antifungal activities of title compounds against *Rs* and *Fg in vitro* were tested by the mycelium growth rate method (Wang et al., 2017; Chen et al., 2012). The compounds were dissolved in DMSO and mixed with sterile molten potato dextrose agar (PDA) medium to obtain a final concentration of 100 and 50 μg/mL. Each treatment condition was produced in three replicates. DMSO in sterile distilled water was used as the negative control, commercial fungicide hymexazol was selected as a positive control. Transfer disks of mycelia felt (4 mm diameter) of fungi to the center of Petri dishes in a sterile environment and the treatment was incubated in the dark at 25 ± 1°C. The diameters of the sample colonies were measured, after the colonies in the control experiment covered two-thirds of the culture dishes. Inhibitory percentages of the title compounds *in vitro* on these fungi were calculated as $I\ (\%) = [(C - T) / (C - 4)] \times 100$, where I was the inhibition rate, C was the diameter of fungal growth on untreated PDA (mm), and T was the diameter of fungal growth on treated PDA (mm).

Crystal structure determination

The title compound was recrystallized from a mixture of DMF and methanol (V: V=1:1) to obtain a suitable single crystal. The X-ray single crystal diffraction date was collected on an Agilent Super Nova (Dual, Cu at zero, AtlasS2) single crystal diffractometer at 100.00 (10) K using the monochromatized MoKα radiation (λ = 0.71073 Å) using w scan mode. The CrysAlisPro program was used to integrate the diffraction profile. The structure was solved directly and optimized by using full matrix least square method *via* SHELXL (Sheldrick, 1997). All the non-hydrogen atoms were refined by full-matrix least-squares technique on F^2 with anisotropic thermal parameters. All the hydrogen atoms were positioned geometrically and refined using a riding model. PLATON program was used for structure analysis and drawings preparation (Spek, 2003).

Results and discussion

Design and synthesis of novel compounds 5–8

The physical properties and HRMS of title compound **5**–**8** are listed in **Table 1**, and their ^{1}H NMR and ^{13}C NMR data are presented in **Table 2**. In the ^{1}H NMR spectra of title compound (**5a**), t-The broad singlet proton peak at 12.73 ppm of amide group and (-NCH_2N-, -SCH_2N-) proton of the 1,3,5-thiadiazinethione are about 4.76 ppm and 4.69 ppm, respectively. Other (-CH_2C=O) proton at around 4.02 ppm. The ^{13}C NMR peak of the thiophenone group (-C=S) emerged between 194.03 and 190.48 ppm, and carbonyl group (-C=O) peak in the range of 168.65–167.83 ppm. In addition, the carbon atom peak at 2 or 5 position of the 1,3,4-thiadiazole at 159.47–158.02 ppm. In the HRMS spectra exhibited that the structure of the title compounds is stable by the greater abundances of the [M+H] + or [M+Na] + ions.

Table 1. Physical properties and HRMS data of title compounds **5**–**8**

Compd	R^1	R^2	Yield	Appearance	M. P. (°C)	HRMS, m/z (calcd.)
5a	Ph	CH_3	60.9%	White solid	158-160	412.0388(412.0388[M+H] $^+$)
5b	Ph	Ph	66.3%	White solid	175-176	496.0365(496.0366[M+Na] $^+$)
5c	Ph	4-MePh	67.0%	White solid	169-171	488.0702(488.0696[M+H] $^+$)
5d	Ph	4-ClPh	68.5%	White solid	179-181	529.9975(529.9970[M+Na] $^+$)
6a	Bn	CH_3	78.7%	White solid	179-181	426.0545(426.0542[M+H] $^+$)
6b	Bn	Ph	79.0%	White cotton	160-162	488.0702(488.0695[M+H] $^+$)
6c	Bn	4-MePh	70.5%	White cotton	179-181	502.0858(502.0850[M+H] $^+$)
6d	Bn	4-ClPh	71.7%	White cotton	175-177	544.0132(544.0125[M+Na] $^+$)
7a	4-FPh	CH_3	41.1%	White solid	173-175	430.0295(430.0291[M+H] $^+$)
7b	4-FPh	Ph	57.1%	White solid	163-165	492.0451(492.0444[M+H] $^+$)
7c	4-FPh	4-MePh	48.9%	White solid	170-171	506.0608(506.0598[M+H] $^+$)
7d	4-FPh	4-ClPh	48.4%	White solid	174-175	526.0061(526.0049[M+H] $^+$)
8a	CH_3	CH_3	45.0%	White cotton	159-161	350.0232(350.0230[M+H] $^+$)
8b	CH_3	Ph	66.0%	White cotton	166-168	434.0208(434.0204[M+Na] $^+$)
8c	CH_3	4-MePh	56.3%	White cotton	168-170	426.0545(426.0541[M+H] $^+$)
8d	CH_3	4-ClPh	55.6%	White cotton	161-163	467.9819(467.9814[M+Na] $^+$)

Table 2. Spectral data of title compounds **5**–**8**

Compd	^{1}H NMR (400MHz, DMSO-d_6) δ	^{13}C NMR (101MHz, DMSO-d_6) δ
5a	12.73 (s, 1H,CONH), 7.47 (t, *J* = 7.6 Hz, 2H,ArH), 7.35 (t, *J* = 7.4 Hz, 1H,ArH), 7.25 (d, *J* = 7.5 Hz, 2H,ArH), 4.76 (s, 2H,NCH_2N), 4.69 (s, 2H,SCH_2N), 4.02 (s, 2H,$COCH_2$), 3.23 (q, *J* = 7.3 Hz, 2H,CH_2CH_3), 1.33 (t, *J* = 7.3 Hz, 3H, CH_3).	193.43, 168.36, 159.30, 158.74, 129.95, 128.34, 127.71, 74.08, 59.26, 53.18, 28.52, 15.20.
5b	7.46 (t, *J* = 7.7 Hz, 2H, ArH), 7.40 (d, *J* = 7.3 Hz, 2H, ArH), 7.34 (dd, *J* = 17.5, 7.6 Hz, 3H, ArH), 7.28 (d, *J* = 7.1 Hz, 1H, ArH), 7.24 (d, *J* = 7.6 Hz, 2H, ArH), 4.75 (s, 2H,NCH_2N), 4.68 (s, 2H,SCH_2N), 4.48 (s, 2H,SCH_2Ph), 4.00 (s, 2H,$COCH_2$).	193.42, 168.40, 159.11, 158.70, 144.71, 137.16, 129.94, 129.45, 129.02, 128.33, 128.05, 127.70, 74.10, 59.28, 53.20, 37.99.
5c	7.46 (t, *J* = 7.2 Hz, 2H,ArH), 7.38 – 7.32 (m, 1H,ArH), 7.26 (dd, *J* = 14.8, 7.7 Hz, 4H,ArH), 7.13 (d, *J* = 7.5 Hz, 2H,ArH), 4.75 (s, 2H,NCH_2N), 4.68 (s, 2H,SCH_2N), 4.44 (s, 2H,SCH_2Ph), 4.00 (s, 2H,$COCH_2$), 2.27 (s, 3H,CH_3).	193.44, 168.42, 159.10, 158.80, 144.73, 137.31, 134.02, 129.94, 129.58, 129.37, 128.32, 127.71, 74.10, 59.28, 53.22, 37.84, 21.18.
5d	7.49 – 7.32 (m, 7H, ArH), 7.24 (d, *J* = 7.5 Hz, 2H, ArH), 4.75 (s, 2H, NCH_2N), 4.68 (s, 2H, SCH_2N), 4.49 (d, *J* = 11.2 Hz, 2H, SCH_2Ph), 4.00 (s, 2H, $COCH_2$).	193.44, 168.47, 159.33, 158.28, 144.73, 136.52, 132.61, 131.31, 129.94, 128.96, 128.32, 127.71, 74.10, 59.27, 53.24, 37.12.
6a	12.50 (s, 1H,CONH), 7.35 (d, *J* = 7.5 Hz, 2H, ArH), 7.27 (t, *J* = 7.6 Hz, 2H,ArH), 7.12 (t, *J* = 7.3 Hz, 1H,ArH), 5.28 (s, 2H,NCH_2Ph), 4.58 (s, 2H,NCH_2N), 4.50 (s, 2H,SCH_2N), 3.69 (s, 2H,$COCH_2$), 3.22 (q, *J* = 7.3 Hz, 2H,CH_2CH_3), 1.34 (t, *J* = 7.3 Hz, 3H, CH_3).	192.15, 167.83, 159.06, 158.62, 135.98, 129.02, 128.43, 127.91, 68.88, 59.25, 53.34, 52.65, 28.57, 15.20.
6b	12.46 (s, 1H), 7.41 (d, *J* = 7.4 Hz, 2H), 7.33 (s, 4H), 7.30 – 7.21 (m, 3H), 7.08 (t, *J* = 7.3 Hz, 1H), 5.27 (s, 2H, NCH_2Ph), 4.57 (s, 2H, SCH_2Ph), 4.48 (s, 4H, NCH_2NCH2S), 3.66 (s, 2H, $COCH_2$).	192.16, 167.89, 159.04, 158.42, 137.21, 135.97, 129.44, 129.01, 128.44, 128.05, 127.92, 68.89, 59.28, 53.35, 52.69, 38.04.
6c	12.43 (s, 1H, ArH), 7.34 (d, *J* = 7.4 Hz, 2H,ArH), 7.31 – 7.21 (m, 4H,ArH), 7.14 (d, *J* = 7.2 Hz, 2H,ArH), 7.09 (t, *J* = 7.0 Hz, 1H,ArH), 5.28 (s, 2H,NCH_2Ph), 4.57 (s, 2H,SCH_2Ph), 4.48 (s, 2H,NCH_2N), 4.44 (s, 2H,SCH_2N), 3.66 (s, 2H,$COCH_2$), 2.27 (s, 3H,CH_3).	192.16, 167.87, 158.95, 158.57, 137.30, 135.97, 134.05, 129.57, 129.35, 129.00, 128.45, 127.92, 68.89, 59.28, 53.35, 52.68, 37.88, 21.19.
6d	7.41 (dd, *J* = 16.2, 7.8 Hz, 4H, ArH), 7.34 (d, *J* = 7.4 Hz, 2H, ArH), 7.24 (t, *J* = 7.3 Hz, 2H, ArH), 7.07 (t, *J* = 7.3 Hz, 1H, ArH), 5.28 (s, 2H, NCH_2Ph), 4.57 (s, 2H, SCH_2Ph), 4.48 (s, 4H, NCH_2NCH_2S), 3.66 (s, 2H, $COCH_2$).	192.16, 167.93, 159.19, 158.02, 136.54, 135.97, 132.62, 131.29, 128.99, 128.95, 128.44, 127.91, 68.89, 59.28, 53.35, 52.70, 37.15.
7a	7.30 (d, *J* = 6.9 Hz, 4H, ArH), 4.76 (s, 2H, NCH_2N), 4.69 (s, 2H, SCH_2N), 4.01 (s, 2H, $COCH_2$), 3.22 (q, *J* = 7.3 Hz, 2H, CH_2CH_3), 1.33 (t, *J* = 7.3 Hz, 3H, CH_3).	194.03, 168.39, 159.30, 158.73, 140.82, 129.99, 129.90, 116.87, 116.64, 74.07, 59.34, 53.23, 28.54, 15.20.
7b	12.73 (s, 1H), 7.44 (d, *J* = 7.0 Hz, 2H, ArH), 7.40 – 7.30 (m, 7H, ArH),	194.03, 168.40, 159.07, 158.75, 140.81, 137.16,

	4.79 (s, 2H, NCH_2N), 4.72 (s, 2H, SCH_2N), 4.53 (s, 2H, SCH_2Ph), 4.05 (s, 2H, $COCH_2$).	129.99, 129.90, 129.45, 129.02, 128.06, 116.87, 116.64, 74.09, 59.35, 53.24, 38.01.
7c	7.29 (t, *J* = 7.8 Hz, 6H, ArH), 7.13 (d, *J* = 7.6 Hz, 2H, ArH), 4.75 (s, 2H, NCH_2N), 4.68 (s, 2H, SCH_2N), 4.44 (s, 2H, SCH_2Ph), 4.00 (s, 2H, $COCH_2$), 2.27 (s, 3H, CH_3).	194.03, 168.46, 159.13, 158.79, 140.79, 137.30, 134.02, 129.99, 129.90, 129.58, 129.36, 116.87, 116.64, 74.08, 59.36, 53.27, 37.83, 21.18.
7d	7.40 (dd, *J* = 16.1, 7.9 Hz, 4H, ArH), 7.30 (d, *J* = 6.5 Hz, 4H, ArH), 4.75 (s, 2H, NCH_2N), 4.68 (s, 2H, SCH_2N), 4.48 (s, 2H, SCH_2Ph), 4.00 (s, 2H, $COCH_2$).	194.03, 168.50, 159.34, 158.28, 140.81, 136.52, 132.61, 131.31, 129.99, 129.90, 128.96, 116.87, 116.64, 74.08, 59.35, 53.29, 37.12.
8a	4.55 (s, 4H, NCH_2NCH_2S), 3.83 (s, 2H, $COCH_2$), 3.37 (s, 3H, NCH_3), 3.23 (q, *J* = 7.3 Hz, 2H, CH_2CH_3), 1.35 (t, *J* = 7.3 Hz, 3H, CH_2CH_3).	190.48, 168.52, 159.24, 158.83, 71.78, 59.30, 58.79, 53.31, 28.55, 15.20.
8b	12.45 (s, 1H), 7.41 (d, *J* = 7.4 Hz, 2H, ArH), 7.34 (t, *J* = 7.3 Hz, 2H, ArH), 7.31 – 7.24 (m, 1H, ArH), 4.54 (s, 4H, NCH_2NCH_2S), 4.49 (s, 2H, SCH_2Ph), 3.82 (s, 2H, $COCH_2$), 3.37 (s, 3H, NCH_3).	190.48, 168.58, 159.23, 158.62, 137.18, 129.45, 129.03, 128.06, 71.79, 58.82, 53.34, 38.01
8c	7.28 (d, *J* = 7.5 Hz, 2H, ArH), 7.13 (d, *J* = 7.7 Hz, 2H, ArH), 4.53 (s, 4H, NCH_2NCH_2S), 4.44 (s, 2H, SCH_2Ph), 3.81 (s, 2H, $COCH_2$), 3.36 (s, 3H, NCH_3), 2.27 (s, 3H, $PhCH_3$).	190.48, 168.57, 159.17, 158.75, 137.31, 134.03, 129.58, 129.37, 71.80, 58.81, 53.34, 37.84, 21.19.
8d	12.49 (s, 1H, CONH), 7.41 (q, *J* = 8.1 Hz, 4H, ArH), 4.53 (s, 4H, NCH_2NCH_2S), 4.48 (s, 2H, SCH_2Ph), 3.81 (s, 2H, $COCH_2$), 3.36 (s, 3H, CH_3).	190.48, 168.65, 159.47, 158.17, 136.53, 132.61, 131.31, 128.96, 71.80, 58.82, 53.37, 37.14.

Antibacterial activity screening of title compounds *in vitro*

As listed in **Table 3**, the preliminary bioassay results *in vitro* demonstrated that some title compounds possessed of the impressive antibacterial activities against *Xoo* and *Xoc* at 100 and 50 μg/mL. For example, the inhibitory rates of compounds **5b**, **5c**, **8c** and **8a** against *Xoc* respectively were 22%, 25%, 18% and 30% at 100 μg/mL, which are better than that of thiodiazole-copper (18%). Otherwise, compounds **5a**–**5d**, **6a**–**6c**, **7a** and **8a**–**8c** exhibited certain inhibitory activities against *Xoc* comparing with the thiodiazole-copper at 50 μg/mL. In addition, the title compounds **5a**, **6a**, **7a**, **8a** and **8b** also showed certain activities against *Xoo* at 50 μg/mL and 100 μg/mL. Among them, **8a** exhibited better antibacterial activities (56%) than thiodiazole-copper (40%). As can be seen, compound **8a** was the best inhibitor among all the compounds, not only has antibacterial activity against *Xoo*, but also has a certain inhibitory effect against *Xoc*.

Antifungal bioassays of title compounds *in vitro*

The antifungal activities of title compounds against *Rs* and *Fg in vitro* were tested by the

mycelium growth rate method and the results are exhibited in **Table 3**. From the biological activity data sheet, it could be found that compounds **5a**, **6d**, **7d** and **8b** possessed of certain activities against both *Rs* and *Fg*. At the concentration of 100 μg/mL, compound **7d** and **8b** showed better activity against *Rs* than hymexazol. Whether at 100 μg/mL or 50 μg/mL, the inhibitory activity of compound **8a** against *Rs* was better than that of the corresponding concentration of hymexazol. Meanwhile, the compound **8a** also demonstrated equivalently inhibitory rate (67%, 43%) to the hymexazol (66%, 43%) against *Fg* at the corresponding concentrations, respectively. Afterwards, the EC_{50} values of compound **8a** against *Rs* and *Fg* were tested, which were shown in **Table 4** and **Fig. 3**. The compound **8a** showed remarkable activity against *Rs* with an EC_{50} value of 33.70 μg/mL, which was superior to that of hymexazol (67.10 μg/mL).

Table 3. Inhibition rates of title compounds against phytopathogenic microorganisms [a].

	Xoc		*Xoo*		*Rs*		*Fg*	
Compd	100 μg/mL	50 μg/mL	100 μg/mL	50 μg/mL	100 μg/mL	50 μg/mL	100 μg/mL	50 μg/mL
5a	17 ± 0.47	14 ± 3.08	30 ± 2.02	17 ± 1.66	35 ± 3.68	10 ± 2.24	34 ± 4.10	18 ± 3.05
5b	22 ± 1.41	18 ± 4.13	24 ± 4.10	15 ± 3.48	14 ± 1.76	6 ± 4.65	15 ± 3.35	7 ± 3.55
5c	25 ± 3.54	20 ± 1.71	22 ± 0.11	14 ± 2.55	21 ± 1.94	5 ± 1.72	17 ± 3.69	6 ± 1.76
5d	13 ± 2.15	10 ± 2.02	24 ± 0.34	10 ± 0.10	27 ± 2.69	15 ± 3.40	20 ± 4.18	7 ± 2.29
6a	17 ± 4.18	10 ± 1.15	38 ± 1.06	26 ± 0.55	23 ± 1.69	13 ± 2.74	23 ± 1.39	12 ± 2.96
6b	14 ± 3.31	11 ± 2.11	29 ± 4.18	19 ± 0.69	18 ± 1.47	14 ± 3.27	26 ± 4.44	18 ± 2.61
6c	17 ± 0.92	11 ± 1.52	20 ± 4.11	13 ± 0.43	29 ± 2.44	22 ± 4.10	26 ± 4.71	12 ± 1.88
6d	9 ± 4.74	5 ± 1.13	25 ± 0.43	16 ± 0.55	30 ± 2.20	15 ± 1.83	32 ± 3.41	15 ± 2.79
7a	14 ± 0.01	12 ± 1.35	35 ± 0.91	25 ± 0.02	21 ± 1.69	10 ± 2.24	15 ± 3.66	11 ± 2.69
7b	13 ± 3.42	6 ± 3.77	26 ± 2.97	20 ± 0.70	22 ± 1.67	9 ± 1.70	19 ± 3.51	6 ± 2.43
7c	15 ± 3.85	9 ± 2.10	24 ± 0.36	17 ± 4.63	8 ± 1.67	0	14 ± 3.47	6 ± 3.03
7d	10 ± 7.54	5 ± 4.44	25 ± 0.91	13 ± 2.30	46 ± 2.44	28 ± 4.10	14 ± 1.75	7 ± 2.04
8a	30 ± 2.58	25 ± 5.87	56 ± 2.02	29 ± 2.03	100 ± 1.0	62 ± 6.37	67 ± 7.14	43 ± 5.05
8b	16 ± 4.13	12 ± 3.61	32 ± 0.36	26 ± 4.63	50 ± 5.89	36 ± 3.00	30 ± 5.80	16 ± 3.39
8c	18 ± 4.68	15 ± 4.40	20 ± 5.12	12 ± 3.49	19 ± 4.76	13 ± 2.26	20 ± 1.47	9 ± 2.59
8d	13 ± 2.80	10 ± 4.00	25 ± 4.12	19 ± 4.05	22 ± 6.15	7 ± 2.59	21 ± 1.52	12 ± 3.49
TC [b]	18 ± 4.77	10 ± 2.79	40 ± 1.02	29 ± 4.43	–	–	–	–
HY [b]	–	–	–	–	47 ± 6.94	37 ± 7.70	66 ± 4.09	43 ± 1.89

Notes: [a] Average of three replicates.

[b] A commercial microbicides thiodiazole-copper (TC) and hymexazol (HY) were used to compare the inhibition effects of title

compounds.

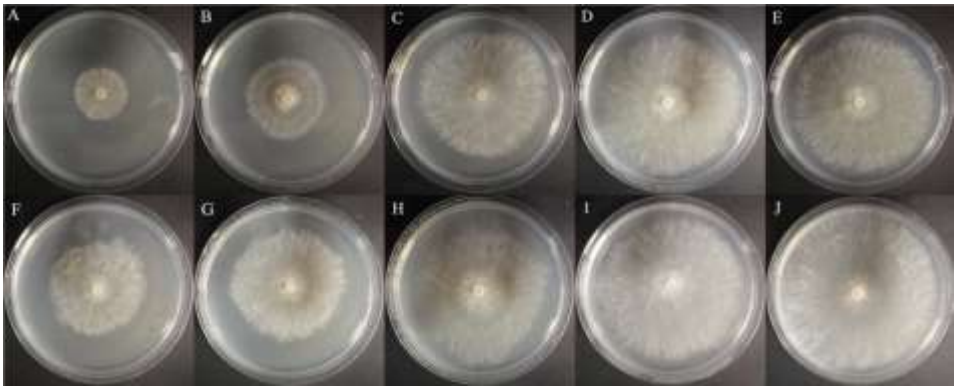

Figure 3. Anti-*Rs* effects of the bioactive compounds **8a** and hymexazol.
(A) **8a** at 50 μg/mL, (B) **8a** at 25 μg/mL, (C) **8a** at 12.5 μg/mL, (D) **8a** at 6.25 μg/mL, (E) **8a** at 3.125 μg/mL, (F) hymexazol at 50 μg/mL, (G) hymexazol at 25 μg/mL, (H) hymexazol at 12.5 μg/mL, (I) hymexazol at 6.25 μg/mL and (J) hymexazol at 3.125 μg/mL.

Table 4. EC_{50} values of the title compound **8a** against *Rs* and *Fg*

Compd	Strains	Regression equation	r	EC_{50} (μg/mL)
8a	*Rs* [a]	y=2.1218x + 1.7578	0.9925	33.70 ± 0.24
8a	*Fg* [a]	y=1.2648x + 2.5363	0.9654	88.70 ± 0.49
Hymexazol [b]	*Rs* [a]	y=1.5892x + 2.0968	0.9869	67.10 ± 0.24
Hymexazol [b]	Fg [a]	y=1.8967x + 1.6815	0.9986	56.19 ± 1.68

Notes: [a] Average of three replicates.

[b] The agricultural fungicide hymexazol was used for comparison of antifungal effects.

X-ray crystal structure of compound 8d

The structure of compound **8d** was further studied using the single crystal X-ray analysis. The corresponding crystal structure and crystal packing diagrams are shown by **Fig. 4** and **Fig. 5**, respectively. The crystallographic data and hydrogen bonds are given in **Table 5** and **Table 6**. As shown in **Fig. 4**, the intramolecular hydrogen bond N (3)–H (3) ⋯N (4) formed a new five-membered ring with two other C atoms. In addition, the intramolecular hydrogen bond C (3)–H

(3B) ⋯N (5), together with thiadiazinthion ring constituted a new bridge ring. In the packing diagram of the compound **8d** (**Fig. 5**), the molecules connected each other through intermolecular hydrogen bonds N (3)–H (3) ⋯O (1) and C (12)–H (12) ⋯S (3) (**Table 6**). Among them, intermolecular hydrogen bond C (12)–H (12) ⋯S (3) connected different molecules to form chains, while N (3)–H (3) ⋯O (1) connected different chains to form planes, eventually the spatial network was formed. Crystallographic data were deposited with the Cambridge Crystallographic Data Centre. The deposition number was CCDC 1912576.

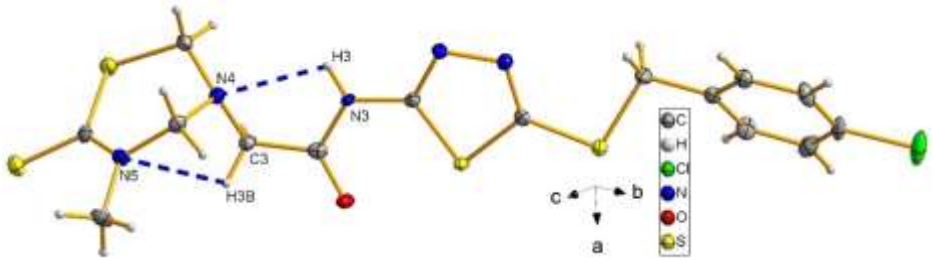

Fig. 4 The molecular ellipsoid of compound **8d**.

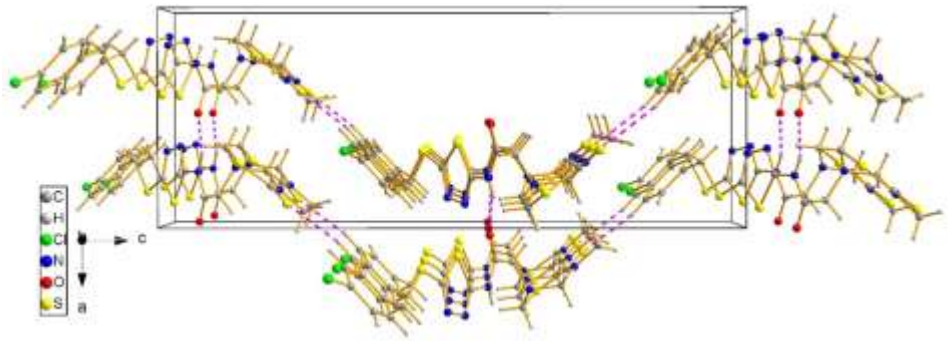

Fig. 5 Crystal packing diagram of compound **8d**.

Table 5 Crystal data of compound **8d**

Empirical formula	$C_{15}H_{16}ClN_5OS_4$
Formula weight	446.02

Temperature/K	100.00(10)
Crystal system	Orthorhombic
Space group	P$na2_1$ (No. 33)
a(Å)	9.8513(5)
b(Å)	7.3392(4)
c(Å)	26.2975(15)
α(°)	90
β(°)	90
γ(°)	90
V (Å^3)	1901.33(18)
Z	4
ρ_{calc}(g/cm^3)	1.558
μ(mm^{-1})	0.656
F(000)	920.0
Crystal size(mm^3)	0.10 × 0.11 × 0.12
Radiation	MoKα (λ = 0.71073)
2θ range for data collection (°)	5.8 to 56.6
Index ranges	-13≤h≤10, -6≤k≤9, -33≤l≤31
Reflections collected	12108
Independent reflections	4013 [R_{int}= 0.039]
Data/restraints/parameters	4343/1/240
Goodness-of-fit on F^2	1.044
Final R indexes [I>=2σ (I)]	R_1= 0.0361, wR_1= 0.0676
Final R indexes [all data]	R_2= 0.0413, wR_2= 0.0706
Largest diff. peak/hole (e Å^{-3})	0.28 and 0.31

Table 6 Hydrogen bond distances (Å) and angles (°) of compound **8d**

D–H···A	d(D–H)	d(H···A)	d(D···A)	∠(DHA)
N (3)–H (3) ···N (4)	0.84(4)	2.41(4)	2.759(4)	106(3)
C (3)–H (3B) ···N (5)	0.9700	2.6100	3.008(5)	105.00
N (3)–H (3) ···O (1)[a]	0.84(4)	2.00(4)	2.809(4)	160(4)
C (12)–H (12) ···S (3)[b]	0.9300	2.8600	3.769(4)	165.00

Notes: [a] Symmetry code: -1/2+x,1/2-y, z; [b] Symmetry code: 2-x,1-y, -1/2+z.

Structure–activity relationships analysis of antibacterial activities

Based on preliminary bioactivity results, the structure–activity relationships showed two general rules. First, in general, the target compounds have a better inhibitory activity against *Xoo* than *Xoc* at a concentration of 100 μg/mL, except for compound **5c**. For example, when the test concentration was 100 μg/mL, the compound **5a** fell into order by inhibitory rate as 30% (against *Xoo*) > 17% (against *Xoc*). Second, as seen from the bioassays results in **Table 3**, title compounds bearing a methyl group at R^2 position exhibited more obvious anti-*Xoo* effects than the remaining homologues containing a Ph, 4-MePh or 4-ClPh moiety at R^2 position. For example, at the tested concentration 100 μg/mL, the antibacterial effects of title compounds against *Xoo* follow the below orders: **5a** (R^2=Me, 30%) > **5b** (R^2=Ph, 24%) = **5d** (R^2=4-ClPh, 24%) > **5c** (R^2=4-MePh, 22%), **6a** (R^2=Me, 38%) > **6b** (R^2=Ph, 29%) > **6d** (R^2=4-ClPh, 25%) > **6c** (R^2=4-MePh, 20%), **7a** (R^2=Me, 35%) > **7b** (R^2=Ph, 26%) > **7d** (R^2=4-ClPh, 25%) > **7c** (R^2=4-MePh, 24%), **8a** (R^2=Me, 56%) > **8b** (R^2=Ph, 32%) > **8d** (R^2=4-ClPh, 25%) > **8c** (R^2=4-MePh, 20%).

Structure–activity relationships of antifungal activities

From **Table 3** and **Table 4**, it can be found that the changes in substituent groups of title compounds greatly influenced their antifungal effects against *Rs* and *Fg*. The structure–activity relationships showed three general rules. First, overall, the inhibitory activities of the title compounds against *Rs* were higher than that of *Fg*, except for compounds **6b** and **7c**. Second, when R^1 was Me, that was to say **8a**–**8d**, exhibited better antifungal effects than those compounds when R^1 was Ph (**5a**–**5d**), 4-FPh (**7a**–**7d**) or Bn (**6a**–**6b**). Third, when R^2 was 4-Clphenyl, the activities of the corresponding compounds were superior to these compounds with R^2 was 4-Mephenyl. For example, the compounds fell into order by inhibitory rate as **5d** > **5c**, **6d** > **6c**, **7d** > **7c** and **8d** > **8c**, against both fungi at the concentration of 100 μg/mL.

Conclusions

In order to find new compounds with excellent antimicrobial activities against phytopathogenic microorganisms, a 1,3,5-thiadiazine-2-thione scaffold and a 1,3,4-thiadiazole group were integrated in a single molecular architecture to obtain a series of 1,3,5-thiadiazine-2-thione derivatives containing a 1,3,4-thiadiazole group. Their structures were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS, and their antimicrobial activities against *Xoc*, *Xoo*, *Rs* and *Fg* were evaluated. The antimicrobial bioassays shown that some title compounds displayed valuable

antibacterial and antifungal activities. Among them, compounds **5b**, **5c** and **8a** possessed of important anti-*Xoc* effects, with the corresponding inhibition rates of 22%, 25% and 30% at 100 μg/mL, which are more effective than thiodiazole-copper (18%). In addition, the compound **8a** exhibited more meaningful antifungal activity against *Rs* (EC_{50}=33.70 μg/mL) than hymexazol (EC_{50}=67.10 μg/mL). Given the above results, bioorganic molecules with antimicrobial activities against phytopathogenic microorganisms might be obtained via the further structural modification of 1,3,5-thiadiazine-2-thione derivatives containing a 1,3,4-thiadiazole group.

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