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ORIGINAL ARTICLE

Design and synthesis of novel 2-(6-thioxo-1,3,5-thiadiazinan-3-yl)-*N*′-phenylacethydrazide derivatives as potential fungicides

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Abstract

A series of novel 2-(6-thioxo-1,3,5-thiadiazinan-3-yl)-*N*′-phenylacethydrazide derivatives were designed, synthesized and evaluated for their antifungal activities against *Fusarium graminearum* (Fg), *Rhizoctonia solani* (Rs), *Botrytis cinerea* (Bc) and *Colletotrichum capsici* (Cc). The bioassay results in vitro showed that most of the title compounds exhibited impressive antifungal activities against the above plant fungi. Particularly, the compounds **5c**, **5f**, **5g**, **5i**, **5m** and **5p** displayed desirable anti-Rs activities, with the corresponding EC_{50} values of 0.37, 0.32, 0.49, 0.50, 0.46 and 0.45 μg/mL, respectively, which are superior to the positive control carbendazim (0.55 μg/mL). Further in vivo bioassay results showed that the anti-Rs activity of title compound **5f** at 200 μg/mL reached 95.84% on detached rice leaves and 93.96% on rice plants. Featuring convenient synthesis, novel structures and desirable antifungal activity, these 2-(6-thioxo-1,3,5-thiadiazinan-3-yl)-*N*′-phenylacethydrazide derivatives could be further studied as the potential candidates of novel agricultural fungicides.

Keywords Heterocyclic agrochemical · 1,3,5-thiadiazine-2-thione · Hydrazide · Antifungal activity

Introduction

Plant pathogenic fungi are destructive parasitic organisms that easily generate numerous spores to infect various economic crops and are extremely difficult to control in agriculture [1]. In addition, fungal infections tend to produce various toxins in infected plants, thereby reducing the quality and output of commercial crops [2]. Nowadays, the rational utilization of agricultural fungicides is still a realistic and principal measure to effectively control the fungal infections in agriculture [3]. Unfortunately, some problems related the long-term application of traditional fungicides in agriculture, such as negative impacts on the environment [4], pernicious effects against non-target species [5] and ceaseless evolutions of fungal resistances [6], gradually attract great attentions and vigilances. Therefore, developing novel and highly efficient fungicides is still an urgent demand in agriculture [7].

1,3,5-Thiadiazine-2-thione derivatives have attracted enormous attentions from chemists and biologists due to their various bioactivities, such as anticancer [8], anti-bacterial [9], anti-epileptic [10], antifungal [11], anti-leishmanial [12], antimalarial [13], antioxidant [14], anti-tubercular [15], trypanocidal [16] and herbicidal [17] properties. Recently, pharmacokinetic studies demonstrated that 1,3,5-thiadiazine-2-thione derivatives feature high lipid solubility and desirable enzymatic hydrolyzation that facilitate their absorption and bioavailability within organisms [18, 19]. Besides its extensive applications in pharmaceutical developments, these nitrogenous heterocycles also have important development values in agricultural chemistry. As important examples of 1,3,5-thiadiazine-2-thione derivatives, milneb (Fig. 1a) and

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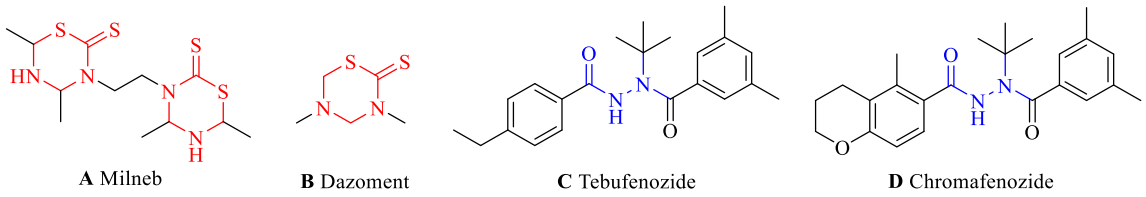
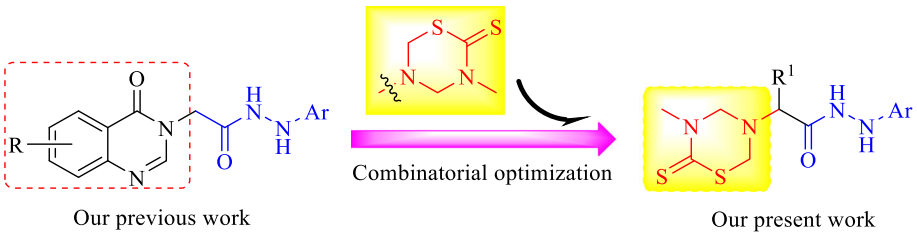
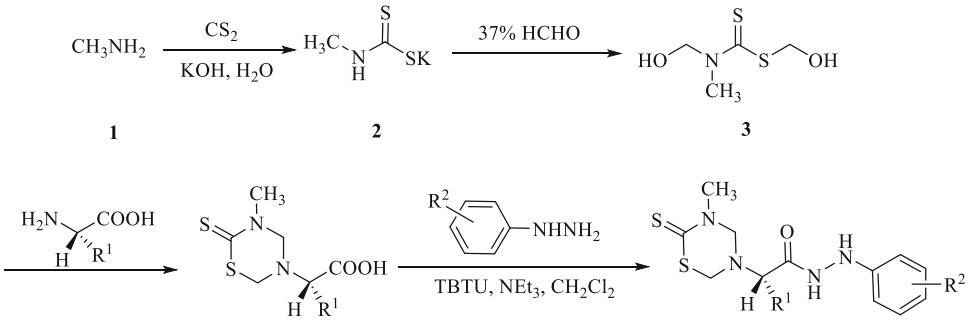


Fig. 1 Bioactive compounds containing a 1,3,5-thiadiazine or hydrazide fragment

Fig. 2 Design strategy of title compounds



Scheme 1 Synthetic route to title compounds **5a–5r**



5a: R^1=H, R^2=H; **5b**: R^1=H, R^2=2-F; **5c**: R^1=H, R^2=4-F; **5d**: R^1=H, R^2=2-Cl;
5e: R^1=H, R^2=3-Cl; **5f**: R^1=H, R^2=4-Cl; **5g**: R^1=H, R^2=2,4-di-Cl; **5h**: R^1=H, R^2=2,4,6-tri-Cl;
5i: R^1=H, R^2=4-Br; **5j**: R^1=H, R^2=4-CH_3; **5k**: R^1=H, R^2=3,4-di-CH_3; **5l**: R^1=H, R^2=4-OCH_3;
5m: R^1=H, R^2=4-CF_3; **5n**: R^1=CH_3, R^2=H; **5o**: R^1=CH_3, R^2=4-F; **5p**: R^1=CH_3, R^2=4-Cl;
5q: R^1=CH_3, R^2=4-Br; **5r**: R^1=CH_3, R^2=4-CH_3.

dazoment (Fig. 1b) have been developed as the agricultural fumigants that are widely used to protect fruit trees, vegetables and ornamental plants [9, 20, 21].

Hydrazide is widely researched as the nitrogenous configuration that exists in many impressive molecules with anticancer [22], anticoagulant [23], anti-inflammatory [24], antimalarial [25], anti-bacterial [26], antifungal [27], antiviral [28], insecticidal [29] and herbicidal [30] properties. As representative compounds bearing a hydrazide group, tebufenozide (Fig. 1c) and chromafenozide (Fig. 1d) were launched as commercial insecticides that target the nonsteroidal ecdysone of agricultural pests [31]. Subsequently, the studies on structural optimizations of hydrazide derivatives documented that introducing an arylhydrazide fragment into a heterocycle nucleus (e.g., pyrazole [32], 1,2,3-triazole [33] and coumarin [34]) could greatly enhance and broaden their inhibition effects against plant fungi. Inspired by the excellent biochemical characteristics of hydrazide substructure, our previous work investigated the antifungal effects of quinazolin-4(3*H*)-one derivatives bearing a hydrazide moiety and found that a *N*′-phenylacethydrazide fragment in molecular structures played a key role in maintaining their biological activity [35].

Aiming to continue our previous works on searching for hydrazide derivatives as novel agricultural fungicides, we theorized that introducing a 1,3,5-thiadiazine-2-thione group into *N*′-phenylacethydrazide scaffolds might generate novel lead molecules with better physicochemical properties. Thus, a series of novel 2-(6-thioxo-1,3,5-thiadiazinan-3-yl)-*N*′-phenylacethydrazide derivatives were designed (Fig. 2), synthesized (Scheme 1) and evaluated for their in vitro and in vivo antifungal activities against *Fusarium graminearum* (Fg), *Rhizoctonia solani* (Rs), *Botrytis cinerea* (Bc) and *Colletotrichum capsici* (Cc) in this work. To the best of our knowledge, it is the first report on the synthesis and antifungal activity of 2-(6-thioxo-1,3,5-thiadiazinan-3-yl)-*N*′-phenylacethydrazide derivatives.

Table 1 Crystal data of title compound **5k**

Crystal data	
$C_{14}H_{20}N_4OS_2$	$\alpha = 90°$
FW = 324.46	$\beta = 93.348\ (2)°$
Monoclinic, P21/n	$\gamma = 90°$
$a = 6.5923(4)$ Å	$V = 1594.63(17)$ Å^3
$b = 19.5021(12)$ Å	$Z = 4$
$c = 12.4246(8)$ Å	$\mu = 0.388$ mm^{-1}
$F\ (000) = 688$	Crystal size (mm^3): $0.11 \times 0.12 \times 0.15$
Data collection	
$T_{min} = 0.6492$, $T_{max} = 0.7456$	4284 observed reflections with $I > 2\sigma(I)$
15399 measured reflections	$R_{int} = 0.036$
3635 independent reflections	$\theta_{min} = 2.1°$, $\theta_{max} = 27.5°$
Refinement	
193 parameters	R indices (all data) $R_1 = 0.0595$, w$R_2 = 0.1237$
Goodness-of-fit: 1.092	R indices $[I > 2\sigma(I)]$ $R_1 = 0.0445$, w$R_2 = 0.1237$
$\Delta\rho_{max} = 0.53$ eÅ^{-3}, $\Delta\rho_{min} = -0.57$ eÅ^{-3}	$w\ [P = (F_o^2 + 2F_c^2)/3] = 1/[(2\backslash s)^2(F_o^2) + (0.0564P)^2 + 0.7380P]$

Results and discussion

Chemistry

The synthetic route to title compounds was shown in Scheme 1. Methylamine **1** was firstly reacted with carbon disulfide in sodium hydroxide solution at room temperature for 4 h to produce a turbid liquid. After filtration by filter paper, the filtrate containing the intermediate **2** was directly reacted with formaldehyde at room temperature for 2 h to obtain a clear liquid. Then, the obtained solution containing the intermediate **3** was reacted with glycine or (*S*)-alanine in phosphate buffer (pH 7.8) at room temperature for 2 h and was subsequently acidified with hydrochloric acid until the pH value reached 2.0 to generate a substituted 2-(6-thioxo-1,3,5-thiadiazinan-3-yl)acetic acid **4**. An intermediate **4** was reacted with substituted phenylhydrazine to produce a corresponding 2-(6-thioxo-1,3,5-thiadiazinan-3-yl)-*N*′-phenylacethydrazide derivative **5** (Scheme 1).

Spectral characteristic of title compounds

The structures of obtained title compounds were confirmed by FTIR, ^{19}F NMR, ^{1}H NMR, ^{13}C NMR and HRMS spectra. In IR spectra, the obvious signals at 3368–3200 cm^{-1} and 1692–1655 cm^{-1} are, respectively, attributed to the stretching vibrations of the NH and C=O fragments. In ^{19}F NMR spectra, the obvious singlets at − 133.24 ppm, (− 126.35)–(− 126.45) ppm and − 59.30 ppm confirm, respectively, the presence of a 2-F atom, a 4-F atom and a 4-CF_3 group at the benzene ring of phenylhydrazine configurations. In ^{1}H NMR spectra, two signal peaks at 10.18–9.83 ppm and 8.40–7.36 ppm confirm the presence of CONHNH fragments. The other two signal peaks at 4.71–4.45 ppm are assigned to the CH_2 protons at the 4- and 6-positions of thiadiazine ring. In ^{13}C NMR spectra, two singlets at 191.15–190.41 ppm and 172.06–168.09 ppm are, respectively, assigned to the characteristic peaks of C=O and C=S groups. The ^{13}C NMR spectra also show multiple signal peaks at 71.94–51.57 ppm, which confirm the existence of CH_2 fragments in the molecular structures of title compounds. Furthermore, in HRMS spectra of title compounds, the obvious absorption signals of $[M+H]^+$ or $[M+Na]^+$ ions are consistent with the corresponding molecular weights.

X-ray crystal structure of compound 5k

Aiming to further understand the structural characteristics of title compounds, the structure of compound **5k** was studied as a representative example by single-crystal X-ray analysis. The evaporation of dimethyl formamide containing the compound **5k** took place slowly at room temperature to crystallize a yellow single crystal that was suitable for X-ray diffraction analyses. The crystal diffraction data collected by the reported methods in our previous works [36, 37] are presented in Table 1. The corresponding crystal structure diagram and crystal packing diagram are shown in Figs. 3 and 4, respectively. Figure 3 shows that the intramolecular hydrogen bond N2–H2···N3 combines with the C9 and C10 atoms to form a

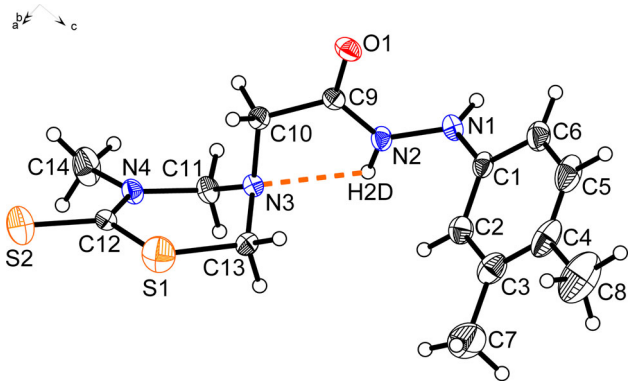


Fig. 3 Crystal structure diagram of compound **5k**

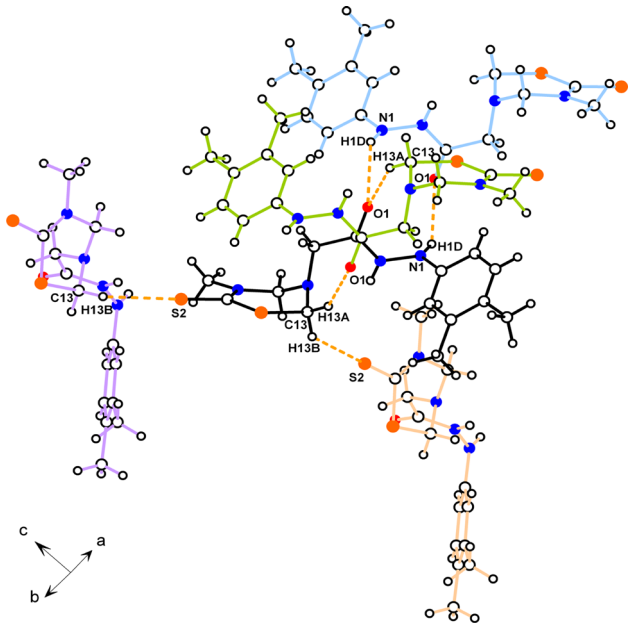


Fig. 4 Crystal packing diagram of compound **5k**

latent penta-heterocycle. The above latent penta-heterocycle unites a benzene ring and a 1,3,5-thiadiazine configuration to construct the structural skeleton of 2-(6-thioxo-1,3,5-thiadiazinan-3-yl)-*N*′-phenylacethydrazide derivatives. As shown in Fig. 4, the three-dimensional structure of title compound **5k** was constructed by three intermolecular hydrogen bonds C13–H13B···S2, C13–H13A···O1 and N1–H1D···O1.

In vitro antifungal activities of title compounds

The antifungal effects of title compounds against plant fungi Fg, Bc, Rs and Cc were tested by a mycelial growth rate method [3, 32–41], and the obtained EC_{50} values are summarized in Table 2. Under the same conditions, the agricultural fungicides carbendazim, penthiopyrad and azoxystrobin were used as the positive controls for evaluating the antifungal effects of title compounds. As shown in Table 2, the title compounds **5a**, **5c**, **5f**, **5o**, **5p**, **5q** and **5r** exhibited desirable anti-Fg activities, with the corresponding EC_{50} values of 3.63, 2.30, 3.77, 1.63, 2.43, 3.47 and 3.23 μg/mL, respectively. Meanwhile, Table 2 also exhibits that the title compounds **5a**, **5c**, **5e**, **5f**, **5i**, **5j**, **5o**, **5p** and **5q** obviously inhibited the mycelian growth of Bc in vitro, with the EC_{50} values of 3.88, 1.37, 2.13, 1.02, 1.59, 3.69, 1.72, 1.50 and 2.08 μg/mL, respectively. In addition, Table 2 shows that the title compounds **5b**, **5c**, **5d**, **5e**, **5g**, **5h**, **5m**, **5o** and **5p** had obvious anti-Cc effects, with the corresponding EC_{50} values of 1.74, 3.92, 1.73, 3.05, 1.21, 0.80, 0.76, 2.72 and 2.58 μg/mL, respectively. Strikingly, the EC_{50} values of title compounds **5c**, **5f**, **5g**, **5i**, **5m** and **5p** against Rs reached 0.37, 0.32, 0.49, 0.50, 0.46 and 0.45 μg/mL, respectively, which are better than that of carbendazim (0.55 μg/mL).

In vivo anti-Rs activity of title compound 5f

Table 2 indicates that 2-(6-thioxo-1,3,5-thiadiazinan-3-yl)-*N*′-phenylacethydrazide derivatives show impressive antifungal activities against Fg, Bc, Rs and Cc. Strikingly, the EC_{50} value of compound **5f** reached 0.32 μg/mL against Rs, which is obviously superior to that of carbendazim (0.55 μg/mL). Aiming to further understand the antifungal activities of title compounds, the in vivo anti-Rs effects of title compound **5f** on detached rice leaves and on rice plants were tested by the reported methods [42]. Five days after inoculating a mycelian cake on detached leaves, Table 3 and Fig. 5 show that the title compound **5f** obviously inhibited the mycelian growth of Rs with the inhibition rates of 31.81% at 100 μg/mL, 61.34% at 150 μg/mL and 95.84% at 200 μg/mL, respectively. As shown in Table 4 and Fig. 6, the title compound **5f** also has significant anti-Rs effects on rice plants at 100 μg/mL and 200 μg/mL, with control effects of 56.32% and 93.96%, respectively. The above research results indicated that 2-(6-thioxo-1,3,5-thiadiazinan-3-yl)-*N*′-phenylacethydrazide derivatives can serve as the potential lead structures in searching for novel agricultural fungicides.

Structure–activity relationships

The results of antifungal assays in Table 2 showed that the structural variations of 2-(6-thioxo-1,3,5-thiadiazinan-3-yl)-*N*′-phenylacethydrazide derivative have greatly affected their biological activity against agricultural fungi. Based on the antifungal effects of title compounds in Table 2, some structure–activity relationships were analyzed and concluded as below. First, the anti-Rs effects of all title compounds are better than their antifungal effects against Fg, Bc and Cc. Second, when the R^1 substituent group was substituted with

Table 2 Antifungal activities of title compounds **5a**–**5r**

Compd.	R^1	R^2	EC_{50} (μg/mL)			
			Fg	Bc	Rs	Cc
5a	H	H	3.63 ± 2.94	3.88 ± 3.37	2.01 ± 1.96	7.39 ± 4.59
5b	H	2-F	25.96 ± 2.04	5.56 ± 0.68	0.99 ± 0.82	1.74 ± 1.94
5c	H	4-F	2.30 ± 1.84	1.37 ± 1.03	0.37 ± 0.48	3.92 ± 0.68
5d	H	2-Cl	74.29 ± 2.71	4.95 ± 4.07	1.13 ± 1.42	1.73 ± 2.14
5e	H	3-Cl	12.56 ± 6.48	2.13 ± 2.66	0.82 ± 1.00	3.05 ± 2.28
5f	H	4-Cl	3.77 ± 2.48	1.02 ± 0.65	0.32 ± 0.51	5.14 ± 5.68
5g	H	2,4-di-Cl	6.59 ± 1.91	6.46 ± 4.14	0.49 ± 0.61	1.21 ± 1.50
5h	H	2,4,6-tri-Cl	25.71 ± 2.93	31.82 ± 7.66	7.12 ± 5.29	0.80 ± 1.19
5i	H	4-Br	4.22 ± 3.76	1.59 ± 2.19	0.50 ± 0.69	4.51 ± 4.76
5j	H	4-Me	6.06 ± 4.87	3.69 ± 2.94	1.25 ± 1.63	35.26 ± 4.01
5k	H	3,4-di-Me	5.92 ± 3.79	5.33 ± 3.71	6.15 ± 3.58	4.88 ± 1.93
5l	H	4-OMe	5.12 ± 4.35	6.30 ± 5.64	3.50 ± 1.24	47.17 ± 6.03
5m	H	4-CF_3	5.46 ± 0.55	4.03 ± 1.44	0.46 ± 0.78	0.76 ± 1.05
5n	Me	H	7.55 ± 3.02	34.01 ± 6.53	2.61 ± 2.80	12.14 ± 5.19
5o	Me	4-F	1.63 ± 1.36	1.72 ± 0.86	0.67 ± 0.90	2.72 ± 1.72
5p	Me	4-Cl	2.43 ± 3.01	1.50 ± 1.83	0.45 ± 0.71	2.58 ± 0.96
5q	Me	4-Br	3.47 ± 4.26	2.08 ± 2.54	0.86 ± 1.01	4.24 ± 4.81
5r	Me	4-Me	3.23 ± 2.68	5.86 ± 1.75	2.05 ± 2.52	39.88 ± 1.09
Carbendazim[a]	–	–	0.54 ± 0.56	–	0.55 ± 0.61	–
Penthiopyrad[a]	–	–	–	0.82 ± 0.86	–	–
Azoxystrobin[a]	–	–	–	–	–	0.44 ± 0.48

The EC_{50} values were calculated according to the inhibitory rate averages with three replicates

[a]The commercial agricultural fungicides carbendazim, penthiopyrad and azoxystrobin were used to evaluate the antifungal activities of title compounds

Table 3 Anti-Rs activity data of compound **5f** on detached rice leaves

Compound	Treatment (μg/mL)	Lesion length[a] (cm)	Control efficacy (%)
5f	100	5.08 ± 0.12	31.81
5f	150	2.88 ± 0.25	61.34
5f	200	0.31 ± 0.28	95.84
Carbendazim[b]	100	0.00 ± 0.00	100.00
Negative control	–	7.45 ± 0.65	–

[a]Values are the average of 10 replicates

[b]The commercial fungicide carbendazim was used to evaluate the anti-*Rs* activity of title compound **5f**

Fig. 5 Anti-Rs efficacy photographs of compound **5f** on detached rice leaves. **a** Negative control; **b** Title compound **5f** at 100 μg/mL; **c** Title compound **5f** at 150 μg/mL; **d** Title compound **5f** at 200 μg/mL; **e** Carbendazim at 100 μg/mL

a methyl, the corresponding target compounds overall exhibited better anti-Fg and anti-Cc effects than those compounds bearing a H atom at the R^1 position. For instance, the antifungal effects of title compounds **5o**, **5p** and **5q** (R^1 = Me; R^2 = 4-F, 4-Cl and 4-Br) were 1.63, 2.43 and 3.47 μg/mL against Fg and 2.72, 2.58 and 4.24 μg/mL against Cc, which are better than that of title compounds **5c**, **5f** and **5i** (R^1 = H; R^2 = 4-F, 4-Cl and 4-Br) against Fg (2.30, 3.77 and 4.22 μg/mL) and Cc (3.92, 5.14 and 4.51 μg/mL). Third, introducing a methyl at the R^1 position is adverse for the antifungal activity of title compounds against Rs and Bc. For example, title compounds bearing a H atom (**5a**, **5c**, **5f**, **5i** and **5j**) have better EC_{50} values against Bc and Rs than those compounds containing a Me group (**5n**, **5o**, **5p**, **5q** and **5r**) at the R^1 position. Fourth, a presence of 4-F, 4-Cl and 4-Br groups at the

Table 4 Anti-Rs activity data of compound **5f** on rice plants

Compound	Treatment (μg/mL)	Lesion length[a] (cm)	Control efficacy (%)
5f	100	3.18 ± 0.53	56.32
5f	200	0.44 ± 0.16	93.96
Carbendazim[b]	100	0.21 ± 0.36	97.12
Negative control	–	7.28 ± 0.12	–

[a]Values are the average of 15 replicates
[b]The commercial fungicide carbendazim was used to evaluate the anti-*Rs* activity of title compound **5f**

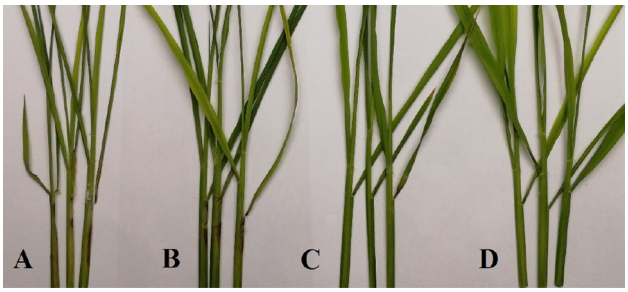


Fig. 6 Anti-Rs efficacy photographs of compound **5f** on rice plants. **a** Negative control; **b** Title compound **5f** at 100 μg/mL; **c** Title compound **5f** at 200 μg/mL; **d** Carbendazim at 100 μg/mL

R^2 position plays a pivotal role in improving the antifungal activities against Fg, Bc and Rs. Table 2 shows that the EC_{50} values against Fg, Bc and Rs of title compounds bearing a 4-F (**5c** and **5o**), 4-Cl (**5f** and **5p**) or 4-Br (**5i** and **5q**) group at R^2 position are obviously better than that of compounds containing a H (**5a** and **5n**), 2-F (**5b**), 2-Cl (**5d**), 3-Cl (**5e**), 2,4-di-Cl (**5g**), 2,4,6-tri-Cl (**5h**), 4-Me (**5j** and **5r**), 3,4-di-Me (**5k**), 4-OMe (**5 l**) or 4-CF_3 (**5m**) group.

Conclusions

A series of novel 2-(6-thioxo-1,3,5-thiadiazinan-3-yl)-*N*′-phenylacethydrazide derivatives were designed, synthesized and well confirmed by FTIR, ^{19}F NMR, ^{1}H NMR, ^{13}C NMR, HRMS and single-crystal X-ray diffraction analyses. The in vitro bioassay results showed that most of the title compounds exhibited impressive antifungal activities against Fg, Rs, Bc and Cc. Strikingly, the EC_{50} values of compounds **5c**, **5f**, **5g**, **5i**, **5m** and **5p** against Rs, respectively, reached 0.37, 0.32, 0.49, 0.50, 0.46 and 0.45 μg/mL, which are superior to that of carbendazim (0.55 μg/mL). Meanwhile, the in vivo bioassay studies on the anti-Rs activity of title compound **5f** indicated that these 2-(6-thioxo-1,3,5-thiadiazinan-3-yl)-*N*′-phenylacethydrazides could be served as potential candidates of novel agricultural fungicides. The present works will lay a significant foundation for the development of novel agricultural fungicides bearing a 1,3,5-thiadiazine or acethydrazide fragment. Further antifungal mechanism and structural modification of 2-(6-thioxo-1,3,5-thiadiazinan-3-yl)-*N*′-phenylacethydrazides are currently underway.

Methods and materials

General methods

Melting points (m.p.) of synthetic compounds were determined by an uncorrected SMP50 Automatic Melting Point Apparatus (Bibby Scientific LTD, Staffordshire, UK). Infrared spectra (IR) were recorded on a Thermo Nicolet 380 FTIR spectrometer (Thermo Nicolet Corporation, America) in a KBr disk. Using DMSO-d_6 as the solvent, ^{1}H, ^{13}C and ^{19}F nuclear magnetic resonance spectra (NMR) were acquired by a BRUKER 400 NMR spectrometer (Bruker Corporation, Germany) at room temperature. Multiplicities of NMR signals were expressed by the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet and m = multiplet. Mass spectral studies were performed on a JMS-AX505HA high-resolution mass spectrometer (JEOL, Japan). The thin-layer chromatography (TLC) on silica gel GF_{254} was used to monitor reactions under ultraviolet light at 254 nm. All reagents and reactants were purchased from commercial suppliers and were analytical reagent grade or chemically pure.

General synthetic procedure for key intermediates 4a and 4b

The intermediates **4a** and **4b** were synthesized according to the synthetic method reported in the literature [43] with small modification. A mixture containing carbon disulfide (10 mmol), methylamine **1** (10 mmol) and potassium hydroxide (10 mmol) in distilled water (40 mmol) was stirred at room temperature for 4 h, and the resulting reaction mixture was then filtered through filter paper. The filtrate containing an intermediate **2** was added into formaldehyde solution (37%, 20 mmol) and subsequently stirred at room temperature for 2 h. After that, the obtained mixture containing an intermediate **3** was slowly added into a stirred solution of glycine or (*S*)-alanine (10 mmol) in phosphate buffer (pH 7.8, 20 mL). After stirring for 6 h at room temperature, the reaction mixture was acidified with 10% hydrochloric acid until the pH value reached 2.0 to generate a white precipitate. The precipitate was filtered and washed with cool diethyl ether to obtain an intermediate **4**. The structures of intermediates **4a** and **4b** were confirmed by ^{1}H NMR and HRMS spectra.

2-(5-Methyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acetic acid (**4a**). White solid; m.p. 129.1–130.4 °C; yield 67.2%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.71 (s, 1H, COOH), 4.54 (s, 2H, thiadiazine-4-2H), 4.52 (s, 2H, thiadiazine-6-2H), 3.57 (s, 2H, CH_2), 3.36 (d, J = 6.5 Hz, 3H, CH_3); HRMS calcd for $C_6H_{10}N_2NaO_2S_2$ $[M+Na]^+$ 229.0076, found 229.0054.

(*S*)-2-(5-Methyl-6-thioxo-1,3,5-thiadiazinan-3-yl)propanoic acid (**4b**). White solid; m.p. 125.1–125.9 °C; yield 68.9%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.81 (s, 1H, COOH), 4.64 (dd, J = 18.6, 13.3 Hz, 2H, thiadiazine-4-2H), 4.55–4.43 (m, 2H, thiadiazine-6-2H), 3.62 (q, J = 7.0 Hz, 1H, CH), 3.36 (d, J = 3.9 Hz, 3H, CH_3), 1.33 (d, J = 7.0 Hz, 3H, CH_3); HRMS calcd for $C_7H_{12}N_2NaO_2S_2$ $[M+Na]^+$ 243.0232, found 243.0219.

General synthetic procedure for title compounds

A mixture of an intermediate **4** (2.00 mmol), *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluroniumtetrafluoroborate (TBTU, 2.40 mmol) and NEt_3 (6.00 mmol) in dichloromethane (30 mL) was stirred at room temperature for 15 min. Then, a substituted phenylhydrazine (3.00 mmol) dissolved in dichloromethane (10 mL) was added in the resulting mixture, and the obtained solution was stirred for 2 h under room temperature. After that, the white precipitate was filtered and washed with dichloromethane to produce a target compound **5** with a good yield. The structures of obtained title compounds **5a**–**5r** were confirmed by FTIR, ^{19}F NMR, ^{1}H NMR, ^{13}C NMR and HRMS spectra.

N′-Phenyl-2-(5-methyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acethydrazide (**5a**). White solid; m.p. 176.2–177.5 °C; yield 64.5%; IR (KBr, cm^{-1}) ν: 3360, 3279, 1672, 1597, 1494, 1473, 1352, 1324, 1198, 1100, 976, 890; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.89 (s, 1H, CONH), 7.75 (d, J = 2.5 Hz, 1H, NH), 7.14 (t, J = 7.7 Hz, 2H, Ar-3,5-2H), 6.72 (q, J = 6.4 Hz, 3H, Ar-1,4,6-3H), 4.55 (s, 4H, thiadiazine-4,6-4H), 3.56 (s, 2H, CH_2), 3.39 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 190.57, 168.61, 149.59, 129.20, 119.06, 112.64, 71.89, 58.90, 52.27, 40.41; HRMS calcd for $C_{12}H_{16}N_4NaOS_2$ $[M+Na]^+$ 319.0658, found 319.0656.

N′-(2-Fluorophenyl)-2-(5-methyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acethydrazide (**5b**). White solid; m.p. 165.0–166.5 °C; yield 73.1%; IR (KBr, cm^{-1}) ν: 3362, 3277, 2971, 1676, 1506, 1487, 1470, 1447, 1321, 1194, 1101, 745; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.95 (s, 1H, CONH), 7.66 (s, 1H, NH), 7.11–7.03 (m, 1H, Ar-3-H), 6.99 (t, J = 7.7 Hz, 1H, Ar-5-H), 6.82–6.69 (m, 2H, Ar-4,6-2H), 4.54 (s, 4H, thiadiazine-4,6-4H), 3.57 (s, 2H, CH_2), 3.39 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 190.54, 168.72, 151.78, 149.41, 137.19, 137.08, 125.02, 119.27, 119.20, 115.41, 115.23, 114.07, 114.04, 71.82, 58.87, 52.26, 40.38; ^{19}F NMR (376 MHz, DMSO-d_6) δ: − 133.24; HRMS calcd for $C_{12}H_{16}FN_4OS_2$ $[M+H]^+$ 315.0744, found 315.0714.

N′-(4-Fluorophenyl)-2-(5-methyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acethydrazide (**5c**). White solid; m.p. 165.8–166.7 °C; yield 85.6%; IR (KBr, cm^{-1}) ν: 3364, 3283, 1674, 1502, 1466, 1349, 1322, 1196, 1094, 980, 831; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.91 (d, J = 2.1 Hz, 1H, CONH), 7.73 (d, J = 2.8 Hz, 1H, NH), 6.98 (t, J = 8.8 Hz, 2H, Ar-3,5-2H), 6.76–6.68 (m, 2H, Ar-2,6-2H), 4.53 (s, 4H, thiadiazine-4,6-4H), 3.55 (s, 2H, CH_2), 3.39 (d, J = 7.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 190.61, 168.70, 157.51, 155.20, 146.19, 115.77, 115.55, 113.94, 113.86, 71.94, 58.94, 52.33, 40.42; ^{19}F NMR (376 MHz, DMSO-d_6) δ: − 126.45; HRMS calcd for $C_{12}H_{16}FN_4OS_2$ $[M+H]^+$ 315.0744, found 315.0744.

N′-(2-Chlorophenyl)-2-(5-methyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acethydrazide (**5d**). White solid; m.p. 170.1–171.4 °C; yield 80.3%; IR (KBr, cm^{-1}) ν: 3280, 2992, 2901, 1667, 1593, 1515, 1476, 1341, 1314, 1199, 1104, 958, 893, 780; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.04 (s, 1H, CONH), 7.44 (s, 1H, NH), 7.29 (d, J = 7.8 Hz, 1H, Ar-3-H), 7.16 (t, J = 7.7 Hz, 1H, Ar-5-H), 6.76 (dd, J = 13.4, 7.6 Hz, 2H, Ar-4,6-2H), 4.55 (s, 4H, thiadiazine-4,6-4H), 3.59 (s, 2H, CH_2), 3.39 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 190.51, 168.65, 144.94, 129.61, 128.23, 120.05, 117.64, 113.40, 71.80, 58.89, 52.35, 40.44; HRMS calcd for $C_{12}H_{16}ClN_4OS_2$ $[M+H]^+$ 331.0449, found 331.0451.

N′-(3-Chlorophenyl)-2-(5-methyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acethydrazide (**5e**). White solid; m.p. 161.1–162.4 °C; yield 75.5%; IR (KBr, cm^{-1}) ν: 3368, 3262, 2987, 2903, 1681, 1601, 1511, 1476, 1353, 1324, 1200, 1100, 893, 842, 776; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.95 (s, 1H, CONH), 8.06 (d, J = 1.9 Hz, 1H, NH), 7.15 (t, J = 8.0 Hz, 1H, Ar-5-H), 6.75–6.63 (m, 3H, Ar-2,4,6-3H), 4.55 (s, 4H, thiadiazine-4,6-4H), 3.58 (s, 2H, CH_2), 3.39 (d, J = 6.3 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 190.55, 168.73, 151.18, 133.98, 130.86, 118.49, 111.88, 111.28, 71.88, 58.90, 52.23, 40.42; HRMS calcd for $C_{12}H_{16}ClN_4OS_2$ $[M+H]^+$ 331.0449, found 331.0450.

N′-(4-Chlorophenyl)-2-(5-methyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acethydrazide (**5f**). White solid; m.p. 166.3–167.6 °C; yield 65.4%; IR (KBr, cm^{-1}) ν: 3366, 3275, 2977, 1909, 1674, 1487, 1465, 1350, 1321, 1095, 893, 827, 811, 703; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.93 (d, J = 2.4 Hz, 1H, CONH), 7.94 (d, J = 2.2 Hz, 1H, NH), 7.17 (d, J = 8.8 Hz, 2H, Ar-3,5-2H), 6.71 (d, J = 8.8 Hz, 2H, Ar-2,6-2H), 4.53 (s, 4H, thiadiazine-4,6-4H), 3.56 (s, 2H, CH_2), 3.39 (d, J = 4.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 190.56, 168.69, 148.55, 128.96, 122.34, 114.14, 71.90, 58.92, 52.29, 40.42; HRMS calcd for $C_{12}H_{16}ClN_4OS_2$ $[M+H]^+$ 331.0449, found 331.0437.

N′-(2,4-Dichlorophenyl)-2-(5-methyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acethydrazide (**5g**). White solid; m.p. 168.2–

169.1 °C; yield 93.2%; IR (KBr, cm^{-1}) ν: 3274, 2978, 2900, 1678, 1518, 1476, 1417, 1198, 1097, 889, 801, 743; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.07 (s, 1H, CONH), 7.65 (s, 1H, NH), 7.43 (d, J = 2.1 Hz, 1H, Ar-3-H), 7.22 (dd, J = 8.8, 2.2 Hz, 1H, Ar-5-H), 6.77 (d, J = 8.8 Hz, 1H, Ar-6-H), 4.54 (d, J = 9.2 Hz, 4H, thiadiazine-4,6-4H), 3.58 (s, 2H, CH_2), 3.40 (d, J = 5.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 190.49, 168.73, 144.20, 128.89, 128.17, 122.44, 118.03, 114.44, 71.79, 58.85, 52.31, 40.42; HRMS calcd for $C_{12}H_{14}Cl_2N_4NaOS_2$ $[M+Na]^+$ 386.9878, found 386.9939.

N′-(2,4,6-Trichlorophenyl)-2-(5-methyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acethydrazide (**5h**). White solid; m.p. 159.5–160.8 °C; yield 52.9%; IR (KBr, cm^{-1}) ν: 3336, 3266, 2978, 2897, 1663, 1505, 1478, 1324, 1315, 1201, 1099, 877, 769; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.18 (d, J = 1.9 Hz, 1H, CONH), 7.50 (s, 2H, Ar-3,5-2H), 7.36 (d, J = 1.8 Hz, 1H, NH), 4.49 (d, J = 3.1 Hz, 4H, thiadiazine-4,6-4H), 3.48 (s, 2H, CH_2), 3.35 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 190.41, 168.09, 141.16, 128.97, 125.39, 124.43, 71.74, 58.77, 51.57, 40.36; HRMS calcd for $C_{12}H_{13}Cl_3N_4NaOS_2$ $[M+Na]^+$ 420.9489, found 420.9486.

N′-(4-Bromophenyl)-2-(5-methyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acethydrazide (**5i**). White solid; m.p. 161.9–162.8 °C; yield 74.3%; IR (KBr, cm^{-1}) ν: 3360, 3272, 2970, 2908, 1672, 1506, 1465, 1350, 1316, 1199, 1099, 894, 806; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.94 (s, 1H, CONH), 7.96 (d, J = 1.9 Hz, 1H, NH), 7.29 (d, J = 8.6 Hz, 2H, Ar-3,5-2H), 6.67 (d, J = 8.7 Hz, 2H, Ar-2,6-2H), 4.53 (s, 4H, thiadiazine-4,6-4H), 3.56 (s, 2H, CH_2), 3.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 190.55, 168.68, 148.95, 131.80, 114.65, 109.86, 71.90, 58.92, 52.28, 40.43; HRMS calcd for $C_{12}H_{15}BrN_4NaOS_2$ $[M+Na]^+$ 396.9763, found 396.9798.

N′-(4-Methylphenyl)-2-(5-methyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acethydrazide (**5j**). White solid; m.p. 164.1–165.4 °C; yield 90.5%; IR (KBr, cm^{-1}) ν: 3362, 3274, 2970, 2901, 1672, 1509, 1465, 1350, 1319, 1200, 1097, 979, 953, 892, 813; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.85 (d, J = 2.2 Hz, 1H, CONH), 7.57 (d, J = 2.6 Hz, 1H, NH), 6.95 (d, J = 8.1 Hz, 2H, Ar-3,5-2H), 6.62 (d, J = 8.1 Hz, 2H, Ar-2,6-2H), 4.53 (s, 4H, thiadiazine-4,6-4H), 3.54 (s, 2H, CH_2), 3.38 (s, 3H, CH_3), 2.17 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 190.57, 168.52, 147.30, 129.60, 127.70, 112.90, 71.86, 58.91, 52.27, 40.41, 20.65; HRMS calcd for $C_{13}H_{18}N_4NaOS_2$ $[M+Na]^+$ 333.0814, found 333.0812.

N′-(3,4-Dimethylphenyl)-2-(5-methyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acethydrazide (**5k**). White solid; m.p. 176.5–177.6 °C; yield 58.2%; IR (KBr, cm^{-1}) ν: 3343, 3286, 2970, 1901, 1671, 1512, 1350, 1317, 1200, 1102, 974, 949, 888, 814; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.83 (d, J = 3.0 Hz, 1H, CONH), 7.48 (d, J = 3.0 Hz, 1H, NH), 6.89 (d, J = 8.1 Hz, 1H, Ar-5-H), 6.52 (s, 1H, Ar-2-H), 6.45 (d, J = 8.0 Hz, 1H, Ar-6-H), 4.53 (s, 4H, thiadiazine-4,6-4H), 3.54 (s, 2H, CH_2), 3.38 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 2.09 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 190.56, 168.48, 147.64, 136.64, 130.13, 126.52, 114.31, 110.31, 71.83, 58.86, 52.21, 20.21, 18.97; HRMS calcd for $C_{14}H_{20}N_4NaOS_2$ $[M+Na]^+$ 347.0971, found 347.0968.

N′-(4-Methoxyphenyl)-2-(5-methyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acethydrazide (**5 l**). White solid; m.p. 167.3–168.4 °C; yield 57.1%; IR (KBr, cm^{-1}) ν: 3356, 3275, 2979, 2903, 1672, 1505, 1475, 1320, 1239, 1097, 891, 827, 725; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.86 (d, J = 3.3 Hz, 1H, CONH), 7.42 (d, J = 3.4 Hz, 1H, NH), 6.76 (d, J = 8.9 Hz, 2H, Ar-2,6-2H), 6.68 (d, J = 8.9 Hz, 2H, Ar-3,5-2H), 4.53 (s, 4H, thiadiazine-4,6-4H), 3.66 (s, 3H, CH_3), 3.53 (s, 2H, CH_2), 3.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 190.56, 168.51, 153.14, 143.44, 114.67, 114.15, 71.87, 58.89, 55.73, 52.29, 40.40; HRMS calcd for $C_{13}H_{19}N_4O_2S_2$ $[M+Na]^+$ 327.0944, found 327.0940.

N′-(4-(Trifluoromethyl)phenyl)-2-(5-methyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acethydrazide (**5m**). White solid; m.p. 177.2–178.6 °C; yield 53.6%; IR (KBr, cm^{-1}) ν: 3356, 3275, 2979, 1672, 1505, 1475, 1350, 1320, 1237, 1193, 1097, 827; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.05 (s, 1H, CONH), 8.40 (s, 1H, NH), 7.47 (d, J = 8.6 Hz, 2H, Ar-3,5-2H), 6.81 (d, J = 8.5 Hz, 2H, Ar-2,6-2H), 4.55 (s, 4H, thiadiazine-4,6-4H), 3.58 (s, 2H, CH_2), 3.39 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 190.56, 168.78, 152.72, 129.51, 126.82, 126.69, 126.65, 126.62, 126.58, 124.13, 121.45, 119.19, 118.87, 118.56, 118.24, 111.95, 71.91, 58.89, 52.26, 40.41; ^{19}F NMR (376 MHz, DMSO-d_6) δ: −59.30; HRMS calcd for $C_{13}H_{16}F_3N_4OS_2$ $[M+H]^+$ 365.0712, found 365.0707.

(*S*)-*N*′-Phenyl-2-(5-methyl-6-thioxo-1,3,5-thiadiazinan-3-yl)propanohydrazide (**5n**). White solid; m.p. 151.2–152.6 °C; yield 47.6%; IR (KBr, cm^{-1}) ν: 3246, 3224, 2986, 2892, 1692, 1505, 1329, 1206, 1094, 943, 893, 832, 696; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.04 (s, 1H, CONH), 7.75 (s, 1H, NH), 7.14 (t, J = 7.6 Hz, 2H, Ar-3,5-2H), 6.71 (t, J = 9.0 Hz, 3H, Ar-2,4,6-3H), 4.71–4.61 (m, 2H, thiadiazine-4-2H), 4.46 (dd, J = 13.2, 5.6 Hz, 2H, thiadiazine-6-2H), 3.68 (q, J = 6.6 Hz, 1H, CH), 3.39 (s, 3H, CH_3), 1.34 (d, J = 6.7 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 191.15, 172.00, 149.70, 129.26, 119.12, 112.60, 69.00, 56.81, 56.18, 40.33, 16.86; HRMS calcd for $C_{13}H_{18}N_4NaOS_2$ $[M+H]^+$ 333.0814, found 333.0820.

(*S*)-*N*′-(4-Fluorophenyl)-2-(5-methyl-6-thioxo-1,3,5-thiadiazinan-3-yl)propanohydrazide (**5o**). White solid; m.p. 184.3–185.6 °C; yield 45.9%; IR (KBr, cm^{-1}) ν: 3329, 3200, 2978, 1678, 1536, 1483, 1325, 1317, 1229, 1134, 1100, 1076, 867; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.05 (d, J = 3.1 Hz, 1H, CONH), 7.74 (d, J = 3.1 Hz, 1H,

NH), 7.03–6.95 (m, 2H, Ar-3,5-2H), 6.72–6.67 (m, 2H, Ar-2,6-2H), 4.70–4.60 (m, 2H, thiadiazine-4-2H), 4.45 (dd, J = 13.3, 3.9 Hz, 2H, thiadiazine-6-2H), 3.66 (q, J = 6.7 Hz, 1H, CH), 3.38 (s, 3H, CH_3), 1.33 (d, J = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 191.14, 172.02, 157.50, 155.18, 146.25, 146.24, 115.82, 115.60, 113.85, 113.77, 68.97, 56.82, 56.20, 40.32, 16.81; ^{19}F NMR (376 MHz, DMSO-d_6) δ: − 126.35; HRMS calcd for $C_{13}H_{17}FN_4NaOS_2$ $[M+Na]^+$ 351.0720, found 351.0723.

(*S*)-*N*′-(4-Chlorophenyl)-2-(5-methyl-6-thioxo-1,3,5-thiadiazinan-3-yl)propanohydrazide (**5p**). White solid; m.p. 186.5–187.8 °C; yield 94.5%; IR (KBr, cm^{-1}) ν: 3224, 2967, 2898, 1658, 1485, 1328, 1312, 1211, 1098, 1079, 961, 891, 826, 701; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.08 (d, J = 2.7 Hz, 1H, CONH), 7.95 (d, J = 2.6 Hz, 1H, NH), 7.18 (d, J = 8.8 Hz, 2H, Ar-3,5-2H), 6.69 (d, J = 8.8 Hz, 2H, Ar-1,6-2H), 4.66 (t, J = 12.4 Hz, 2H, thiadiazine-4-2H), 4.50–4.42 (m, 2H, thiadiazine-6-2H), 3.67 (q, J = 6.7 Hz, 1H, CH), 3.39 (s, 3H, CH_3), 1.33 (d, J = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 191.14, 172.06, 148.68, 129.05, 122.39, 114.09, 68.97, 56.84, 56.23, 40.32, 16.81; HRMS calcd for $C_{13}H_{17}ClN_4NaOS_2$ $[M+Na]^+$ 367.0425, found 367.0420.

(*S*)-*N*′-(4-Bromophenyl)-2-(5-methyl-6-thioxo-1,3,5-thiadiazinan-3-yl)propanohydrazide (**5q**). White solid; m.p. 182.4–183.9 °C; yield 95.4%; IR (KBr, cm^{-1}) ν: 3241, 2985, 2897, 1655, 1510, 1484, 1321, 1209, 1099, 1086, 890, 814; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.08 (d, J = 2.7 Hz, 1H, CONH), 7.96 (d, J = 3.6 Hz, 1H, NH), 7.29 (t, J = 5.9 Hz, 2H, Ar-3,5-2H), 6.65 (t, J = 5.9 Hz, 2H, Ar-2,6-2H), 4.64 (q, J = 12.2 Hz, 2H, thiadiazine-4-2H), 4.50–4.41 (m, 2H, thiadiazine-6-2H), 3.70–3.63 (m, 1H, CH), 3.39 (s, 3H, CH_3), 1.33 (d, J = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 191.14, 172.05, 149.08, 131.89, 114.60, 109.90, 68.97, 56.83, 56.22, 40.33, 16.82; HRMS calcd for $C_{13}H_{17}BrN_4NaOS_2$ $[M+Na]^+$ 410.9919, found 410.9917.

(*S*)-*N*′-(4-Methylphenyl)-2-(5-methyl-6-thioxo-1,3,5-thiadiazinan-3-yl)propanohydrazide (**5r**). White solid; m.p. 186.5–187.8 °C; yield 94.5%; IR (KBr, cm^{-1}) ν: 3240, 2972, 2895, 1691, 1660, 1596, 1519, 1494, 1318, 1208, 1100, 937, 891, 757, 690; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.00 (d, J = 3.2 Hz, 1H, CONH), 7.57 (d, J = 3.2 Hz, 1H, NH), 6.95 (d, J = 8.2 Hz, 2H, Ar-3,5-2H), 6.61 (d, J = 8.4 Hz, 2H, Ar-2,6-2H), 4.65 (dd, J = 13.2, 7.9 Hz, 2H, thiadiazine-4-2H), 4.45 (dd, J = 13.2, 8.7 Hz, 2H, thiadiazine-6-2H), 3.66 (q, J = 6.7 Hz, 1H, CH), 3.38 (s, 3H, CH_3), 2.18 (d, J = 6.9 Hz, 3H, CH_3), 1.32 (d, J = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 191.14, 171.90, 147.45, 129.66, 127.74, 112.85, 69.00, 56.77, 56.12, 40.32, 20.63, 16.85; HRMS calcd for $C_{14}H_{20}N_4NaOS_2$ $[M+Na]^+$ 347.0971, found 347.0969.

Antifungal bioassay in vitro of title compounds

The tested plant pathogenic fungi strains Fg, Bc, Rs and Cc, which were, respectively, isolated from infected wheat, strawberries, rice and peppers from disease outbreak regions in Jiangsu Province, were provided by the Laboratory of Plant Disease Control at Nanjing Agricultural University. The antifungal effects of title compounds **5a**–**5r** against the above fungi were tested by a mycelial growth rate method [3, 32–41]. Under same conditions, the agricultural fungicides carbendazim, penthiopyrad and azoxystrobin were used as positive controls to evaluate the antifungal effects of title compounds. The antifungal effects of all title compounds against the four tested fungi at five double-declining concentrations were tested to calculate the corresponding EC_{50} values.

Anti-Rs activity of title compound 5f on detached rice leaf

Using the agricultural fungicide carbendazim as the positive control, the in vivo anti-*Rs* activity of title compound **5f** was carried out on rice leaves by detached leaf assay [42] with some minor modifications. Appropriate amounts of the title compound **5f** and carbendazim in 200 uL of DMSO were mixed with a solution containing distilled water (50 mL) and Tween 20 (500 uL). At tillering stages of rice plants, fresh leaf pieces with a length of approximate 10 cm were collected, washed by 75% aqueous ethyl alcohol and dipped for 10 min in the above solution. After evaporation under room temperature, the cuticles on the center of rice leaves were punctured with a sterilized needle and inoculated via a mycelia cake with a diameter of approximate 5 mm. Then, all inoculated leaves were placed in an illumination incubator (25 °C and 90% relative humidity) in an environment with a 12-h light/12-h dark photoperiod. Five days after inoculation, the lesion lengths of all treatments were timely measured, and then, the control effects of all treatments were statistically calculated.

Anti-Rs activity of title compound 5f on rice plant

Using carbendazim as the positive control and a rice variety (yiyou 186) as the tested plants, the in vivo protective activity of title compound **5f** against *Rs* was carried out on potted rice plants by the reported method [42] with some minor modifications. The rice seeds (yiyou 186) were sown and grown in pots (18 cm in diameter and 16 cm in height) for approximate 6 weeks under greenhouse conditions. Appropriate amounts of the title compound **5f** and carbendazim in 200 uL of DMSO were mixed with a solution containing distilled water (50 mL) and Tween 20 (500 uL). At tillering stages of rice plants, the obtained solution was uniformly sprayed on

rice plants until the plants were completely wetted. One day later, a mycelia dish with a diameter of approximate 5 mm was placed in the leaf sheaves of rice plant by a sterilized needle. Then, all inoculated plants were placed into an illumination incubator (25 °C and 90% relative humidity) in an environment with a 12-h light/12-h dark photoperiod. Six days after inoculation, the lesion lengths of rice plants were timely measured and statistically calculated to obtain the control effects of all treatments.

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