RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2022, **12**, 2399

Synthesis of novel antibacterial and antifungal quinoxaline derivatives†

Xuemei Tang,‡[a] Qing Zhou,‡[a] Wenliang Zhan,[a] Die Hu,[a] Ran Zhou,[a] Nan Sun,[a] Shuai Chen,[a] Wenneng Wu*[b] and Wei Xue *[a]

Received 13th October 2021
Accepted 27th December 2021

DOI: 10.1039/d1ra07559d

rsc.li/rsc-advances

A series of quinoxaline derivatives were designed, synthesized and evaluated as antimicrobial agents against plant pathogenic bacteria and fungi. Some of these compounds exhibited significant antibacterial and antifungal activities *in vitro*. Compound **5k** displayed good antibacterial activity against *Acidovorax citrulli* (*Ac*). Compounds **5j** and **5t** exhibited the most potent anti-*RS* (*Rhizoctonia solani*) activity, with the corresponding EC_{50} values of 8.54 and 12.01 μg mL^{-1}, respectively, which are superior to that of the commercial azoxystrobin (26.17 μg mL^{-1}). Further, the scanning electron microscopy results proved that compound **5j** had certain effects on the cell morphology of *RS*. Moreover, an *in vivo* bioassay also demonstrated that the anti-*RS* activity of compound **5j** could effectively control rice sheath blight. These results indicate that quinoxaline derivatives could be promising agricultural bactericides and fungicides.

1. Introduction

A variety of diseases caused by plant pathogen infections seriously threaten the quality and yields of crops, and thereby also lead to a large number of economic losses in agricultural production.[1,2] For example, rice sheath blight, bacterial fruit spot of melon and bacterial blight of rice, and so forth, are difficult to control in agricultural production.[3–5] Rice sheath blight is one of the most widely distributed in rice and caused by *RS* infection. It mainly infects the leaf sheath and leaf of rice, and its symptom change from an early water damage disease spot to a moire shape. It is also one of the main reasons for the decline of rice yields and quality globally, and meanwhile has caused huge economic losses.[6,7] The importance of rice is self-evident as one of the main food crops in the world.[8] Bacterial fruit spot melon is caused by *Ac*, which could infect the leaf veins, extend along the leaf veins causing irregular spots of light brown to red on the leaves, and softening and rotting the fruits.[5,9] At present, the main prevention and control methods are chemical drugs, such as bismerthiazol (BT), thiodiazole-copper (TC), azoxystrobin, *etc.*[10] However, due to the ecological pollution caused by drug abuse and the emergence of drug resistance, many adverse effects have become increasingly prominent.[11,12] Therefore, the discovery of new pesticides with low toxicity, high-efficiency and good environment compatibility is very critical to ensure agriculture production.

Quinoxaline derivatives, a class of N-containing heterocyclic compounds, which is an important chemical intermediate, have played an important role in the design and synthesis of new heterocyclic compounds,[13] and are of great significance for drug discovery.[14] Quinoxaline derivatives exhibit broad-spectrum bioactivities, such as antibacterial,[15,16] antiviral,[17,18] antitumor,[19–21] anti-tuberculous,[22] anticancer,[23,24] and so on.[25,26] Therefore, quinoxaline moieties widely exist in pesticides and medicines, and some commercialized pesticides used in agricultural production, including quintiofos, quizalofop-*p*-ethyl, chloramphenicol, olaquindox, and so forth (Fig. 1). Among them, quintiofos is an organophosphorus insecticide, developed by the Bayer company, that became a more widely used insecticide because of its low toxicity and replaced many highly toxic and harmful insecticides including parathion, methyl parathion, methamidophos, *etc.*[27] Similarly, quizalofop-*p*-ethyl is also a low toxicity molecule, developed by the Nissan Chemical Company, and is widely used to control gramineous weeds.[28] The above survey suggests that quinoxoline derivatives have huge application prospects in agrochemicals. However, few studies have been done on quinoxoline derivatives with strong antibacterial and antifungal activities.[29] Therefore, the study of quinoxaline derivatives as antibacterial and antifungal agents has an important significance for the diversification of pesticide development.

Based on these considerations, a class of quinoxaline derivatives was designed (Fig. 2), synthesized and evaluated for their anti-microbial activities. The bioassay results displayed that

[a]*State Key Laboratory Breeding Base of Green Pesticide and Agricultural Bioengineering, Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Research and Development Center for Fine Chemicals, Guizhou University, Guiyang 550025, P. R. China. E-mail: wxue@gzu.edu.cn; Fax: +86-851-88292090; Tel: +86-851-88292090*

[b]*Food and Pharmaceutical Engineering Institute, Guiyang University, Guiyang 550003, P. R. China. E-mail: wuwenneng123@126.com*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/d1ra07559d

‡ These authors contributed equally to this work.

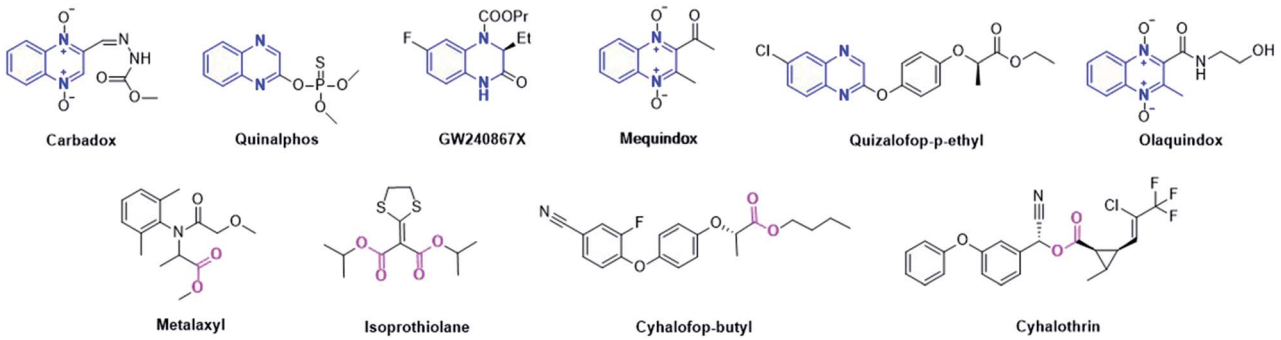


Fig. 1 Structure of some commercial agents.

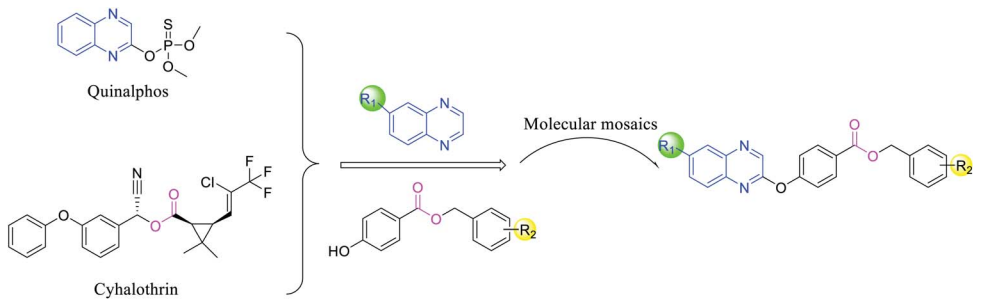


Fig. 2 Design strategy of target compounds.

some of the target compounds exhibited obviously superior antifungal and antibacterial activities to those of the commercial agents bismerthiazol, thiodiazole copper and azoxystrobin. In addition, the mechanisms of action of these compounds were preliminarily studied, and an *in vivo* biological activity study of compound **5j** against *RS* was performed.

2. Experimental

2.1 Instruments and chemicals

The melting points were measured on an XT-4 binocular microscope (Beijing Tech. Instrument Co., China) and left uncorrected. ^{1}H NMR, ^{13}C NMR, and ^{19}F NMR spectra were completed in a chloroform-*d* solution on an ASCEND 400 NMR (Swiss Bruker). High-resolution mass spectrometry (HRMS) was conducted using a Thermo Scientific Q Exactive (Thermo Scientific, Missouri, USA). All of the reactions were monitored by TLC. All the chemical materials and reagents involved in the reactions were purchased from commercial suppliers and the reagents were chemically or analytically pure. The plant pathogenic fungi were provided by the Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Science.

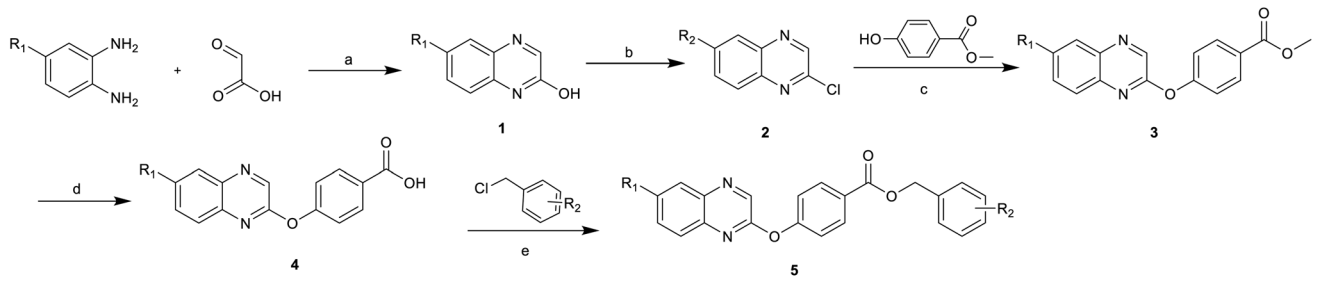
2.2 General procedures for preparing intermediates 1–4 and the target compounds 5a–5t

Compounds **5a–5t** were synthesized according to the designed route shown in Scheme 1. Intermediates **1**, **2**, **3**, and **4** were synthesized by literature methods.[30,31] The intermediate **4** and K_2CO_3 were heated in acetonitrile for 30 min, then **2** was dissolved in acetonitrile and added dropwise, to give compounds **5a–5t** after the complete reaction. The reaction was monitored using TLC. The target compounds **5a–5t** were purified by column chromatography (V/V, petroleum ether : ethyl acetate = 20 : 1 to 12 : 1).

2.3. Biological activities tests

2.3.1 ***In vitro*** **antibacterial activity tests.** The *in vitro* antibacterial activities of target compounds **5a–5t** against the five plant pathogenic bacteria *Xanthomonas oryzae pv. Oryzae* (*Xoo*), *Xanthomonas campestris pv. Mangiferae indicae* (*Xcm*), *Pectobacterium carotovorum* subsp. *Brasiliense* (*Pcb*), *Ralstonia solanocearum* (*Rs*) and *Acidovorax citrulli* (*Ac*) were evaluated by a slightly modified 96-well plate method.[32] Bismerthiazol (BT) and thiodiazole-copper (TC) were used as positive control agents. The details are listed in the ESI.†

2.3.2 ***In vitro*** **antifungal tests.** The *in vitro* antifungal effects of the target compounds against *Alternaria brassicae* (*AB*), *Fusarium fujikuroi* (*FF*), *Fusarium oxysporum f.* sp. *cucumerinum* (*FO*), *Colletotrichum truncatum* (*CT*), *Phytophthora capsici* (*PC*), *Colletotrichum gloeosporioides* (*CG*), *Rhizoctonia solani* (*RS*), *Fusarium graminearum* (*FG*), *Phytophthora sojae* (*PS*), *Phytophthora palmivora* (*PP*), *Botrytis cinerea* (*BC*), and *Phytophthora litchii* (*PL*) were evaluated by a mycelial growth rate method.[33–35] The experimental details for the twelve fungi are presented in the ESI.†



(a) EtOH; (b) $POCl_3$, DMF; (c) Cs_2CO_3, acetone; (d) KOH, H_2O, tetrahydrofuran; (e) DMF, K_2CO_3.

5a:R_1=H, R_2=2,4-di-Cl	**5f**:R_1=H, R_2=2-Cl	**5k**:R_1=Cl, R_2=2,4-di-Cl	**5p**:R_1=Cl, R_2=2-Cl
5b:R_1=H, R_2=4-NO_2	**5g**:R_1=H, R_2=3-F	**5l**:R_1=Cl, R_2=4-NO_2	**5q**:R_1=Cl, R_2=3-F
5c:R_1=H, R_2=2-CH_3	**5h**:R_1=H, R_2=4-Cl	**5m**:R_1=Cl, R_2=2-CH_3	**5r**:R_1=Cl, R_2=4-Cl
5d:R_1=H, R_2=4-F	**5i**:R_1=H, R_2=2-Cl-6-F	**5n**:R_1=Cl, R_2=4-F	**5s**:R_1=Cl, R_2=2-Cl-6-F
5e:R_1=H, R_2=3-OCH_3	**5j**:R_1=H, R_2=4-CN	**5o**:R_1=Cl, R_2=3-OCH_3	**5t**:R_1=Cl, R_2=4-CN

Scheme 1 Synthetic route of the target compounds **5a–5t**.

2.3.3 *In vivo* antifungal tests. The *in vivo* biological activity of compound **5j** against rice sheath blight was carried out by a reported detached leaf assay and greenhouse experiment[36–38] with slight modifications, and the variety of rice was Fengyouxiangzhan.

2.3.4 Sclerotia germination inhibition tests. The sclerotia of *RS* were obtained after 21 days of culture in PDA (potato dextrose agar) medium, and medium with concentrations of 0, 10, 50 and 100 μg mL^{-1} containing **5j** were prepared. Fifteen sclerotia were placed in a Petri dish with three replicates for each concentration. Azoxystrobin was used as a positive control. All the treatments were incubated at 25 °C for 24 h, and the inhibition rate was calculated.[39]

2.4 Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) experiments were performed according to a reported method.[40–43]

3 Results and discussion

3.1 Chemistry

Compounds **5a–5t** were synthesized according to the design route in Scheme 1. All the target compounds were characterized by ^{1}H NMR, ^{13}C NMR, ^{19}F NMR and HRMS, and the specific data are presented in the ESI.†

3.2 Biological activities test

3.2.1 *In vitro* antibacterial test. As can be seen in Table S1,† some compounds exhibited good inhibitory activities against the pathogenic bacteria at concentrations of 200 μg mL^{-1}. Compound **5k** (86.28%) displayed a good inhibitory effect against *Ac*, and its inhibitory rate was significantly better than those of TC (57.67%) and BT (41.07%). Meanwhile, compound **5o** (72.64%) showed a moderate inhibitory activity on *Pcb*, which was better than those of TC and BT (51.09 and 49.61%, respectively). Moreover, the inhibition rates of **5o** and **5p** to *Xoo* were 72.84 and 76.15%, respectively, higher than those of TC (60.11%) and BT (52.13%). In addition, the antibacterial activity of **5b** (71.33%) and **5c** (70.45%) against *Rs in vitro* was similar to that of TC (66.01%) and slightly better than that of BT (42.33%), and the inhibition rates of **5p** (75.17%) and **5q** (74.23%) against *Xcm* were higher than those of TC (67.82%) and BT (46.82%).

Further, EC_{50} tests were carried out and the toxicity curve and EC_{50} values were calculated (Table 1). The results display that compound **5k** had a good anti-*Ac* ability with an EC_{50} value of 35.18 μg mL^{-1}, which was significantly better than those of TC (198.51 μg mL^{-1}) and BT (295.15 μg mL^{-1}). Meanwhile, the EC_{50} value of compound **5p** for *Xoo* was 72.21 μg mL^{-1}, superior to those of TC (182.85 μg mL^{-1}) and BT (230.23 μg mL^{-1}). Furthermore, the EC_{50} value for compound **5p** (86.88 μg mL^{-1}) was analogous to that of TC (80.14 μg mL^{-1}) against *Xcm*, which was better than that of BT (232.82 μg

Table 1 Virulence curves and EC_{50} values of the target compounds against five kinds of bacteria[a,b]

Pathogen	Chemical	Toxic regression equation	r	EC_{50} (μg mL^{-1})
Ac	**5k**	$y = 1.1818x + 3.1726$	0.9570	35.18
	TC	$y = 1.4286x + 1.7174$	0.9724	198.51
	BT	$y = 1.6492x + 0.9264$	0.9935	295.15
Pcb	**5o**	$y = 1.6514x + 1.6444$	0.9801	107.64
	TC	$y = 1.8284x + 0.7780$	0.9899	203.76
	BT	$y = 1.3964x + 1.7517$	0.9966	211.93
Xoo	**5o**	$y = 1.5922x$+1.7321	0.9749	112.83
	5p	$y = 1.1848x + 2.8051$	0.9698	72.211
	TC	$y = 1.4216x + 1.7842$	0.9675	182.85
	BT	$y = 1.8337x + 0.6685$	0.9853	230.23
Rs	**5b**	$y = 0.7773x + 3.6717$	0.9804	51.15
	5c	$y = 1.3088x + 2.3014$	0.9551	115.31
	TC	$y = 1.0061x + 3.1290$	0.9975	72.38
	BT	$y = 1.7183x + 0.8514$	0.9966	259.63
Xcm	**5p**	$y = 1.2743x + 2.5292$	0.9618	86.88
	5q	$y = 1.5969x + 1.7239$	0.9654	112.59
	TC	$y = 1.0849x + 2.9345$	0.9976	80.14
	BT	$y = 1.7293x + 0.9067$	0.9859	232.82

[a] The experiments were repeated three times. [b] Commercial bactericides bismerthiazol (BT) and thiodiazole-copper (TC) were used as positive control agents.

Table 2 Virulence curves and EC_{50} values of the target compounds against four fungal pathogens[a]

Pathogen	Chemical	Toxic regression equation	r	EC_{50} ($\mu g\ mL^{-1}$)
FF	**5k**	$y = 2.5936x + 0.7048$	0.9747	45.29
	Azoxystrobin	$y = 1.0678x + 3.2400$	0.9828	44.48
PS	**5k**	$y = 2.20480 + 1.2841$	0.9505	48.45
	Azoxystrobin	$y = 1.6159x + 2.1092$	0.9781	61.51
PP	**5k**	$y = 2.3750x + 1.0053$	0.9517	48.08
	Azoxystrobin	$y = 1.3961x + 2.9045$	0.9978	31.69
RS	**5j**	$y = 0.7190x + 4.3299$	0.9989	8.54
	5t	$y = 1.8452x + 3.0077$	0.9850	12.01
	Azoxystrobin	$y = 1.2996x + 3.1574$	0.9885	26.17

[a] The experiments were repeated three times.

mL^{-1}). According to Table 1, all the compounds tested had certain antibacterial effects, and **5k** had the best antibacterial activity against *Ac*.

Some compounds had a good inhibitory activity against *Xoo*. When R_1 was the electron-donating group H, the inhibition rates of compounds **5a**, **5e**, **5g** and **5i** against *Xoo* were 62.53, 66.81, 65.49 and 61.35%, respectively, and **5p** (R_2 = 2-Cl) > **5o** (R_2 = 3-OCH_3) > **5n** (R_2 = 2-F) > **5l** (R_2 = 4-NO_2), which indicates that R_1 and R_2 are electron-donating groups, which were beneficial to improve the inhibitory activity of compounds against *Xoo*. When the R_1 group was the electron withdrawing group Cl, the inhibition rates of compounds **5l**, **5n**, **5o** and **5p** against Xoo were 67.25, 67.28, 72.84 and 76.15%, respectively, with **5p** (R_2 = 2-Cl) > **5o** (R_2 = 3-OCH_3) > **5n** (R_2 = 2-F) > **5l** (R_2 = 4-NO_2), and it is speculated that when the R_2 group was an electron withdrawing group, the weaker the electron withdrawing ability, the stronger the compound's ability to inhibit *Xoo*. When R_2 = 3-OCH_3, the activity against *Xoo* with R_1 an electron withdrawing group was better than that with R_1 being an electron donating group, for example, **5o** (R_1 = Cl) > **5e** (R_1 = H).

In terms of the inhibitory activity of the target compounds against *Rs*, the inhibition rate of R_1 = H was generally better than that of R_2 = Cl; for example, **5b** (R_1 = H, R_2 = 4-NO_2) > **5l** (R_1 = Cl, R_2 = 4-NO_2), **5c** (R_1 = H, R_2 = 2-CH_3) > **5m** (R_1 = Cl, R_2 = 2- CH_3), **5e** (R_1 = H, R_2 = 3-OCH_3) > **5o** (R_1 = Cl, R_2 = 3-OCH_3), indicating that an R_1 group electron donor group conducive for the target compound to inhibit the growth of *Rs*. When R_1 = H, **5b** (R_2 = 4-NO_2) has the best inhibitory activity against *Rs*; when R_1 = Cl, **5l** (R_2 = 4-NO_2) has a good inhibitory activity against *Rs*, **5b** > **5l**. It is speculated that when the R_1 group is an electron donor group and R_2 is an electron strong group, the inhibitory activity of the target compounds against *Rs* could be improved.

3.2.2 *In vitro* antifungal test. The results shown in Table S2† indicate that some compounds exhibited good antifungal activities. Among them, compounds **5j** and **5t** showed a good control effect on *RS*, with inhibition rates of 89.56 and 95.17%, respectively, which were significantly better than that of azoxystrobin (76.43%). Meanwhile, the inhibitory rates of compound **5k** on *FF*, *PS* and *PP* were 89.38, 89.92 and 89.38%, respectively, which were better than those of commercial azoxystrobin (51.34, 55.70 and 77.19%, respectively). Furthermore, the toxicological curves and EC_{50} values shown in Table 2 and Fig. 4 indicate that compounds **5j** and **5t** had an exceptionally significant antifungal activity against *RS* with EC_{50} values of 8.54 and 12.01 $\mu g\ mL^{-1}$, respectively, which were better than that of azoxystrobin (26.17 $\mu g\ mL^{-1}$). The structure of compound **5j** shown in Fig. 3.

On the basis of *in vitro* antifungal bioassay shown in Table S2†, the preliminary analysis of structure activity relationships could be generalized, as below. Obviously, comparing

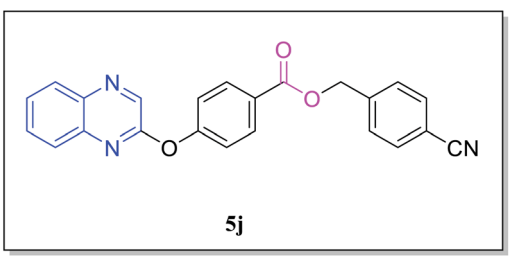


Fig. 3 Structures of compound **5j**.

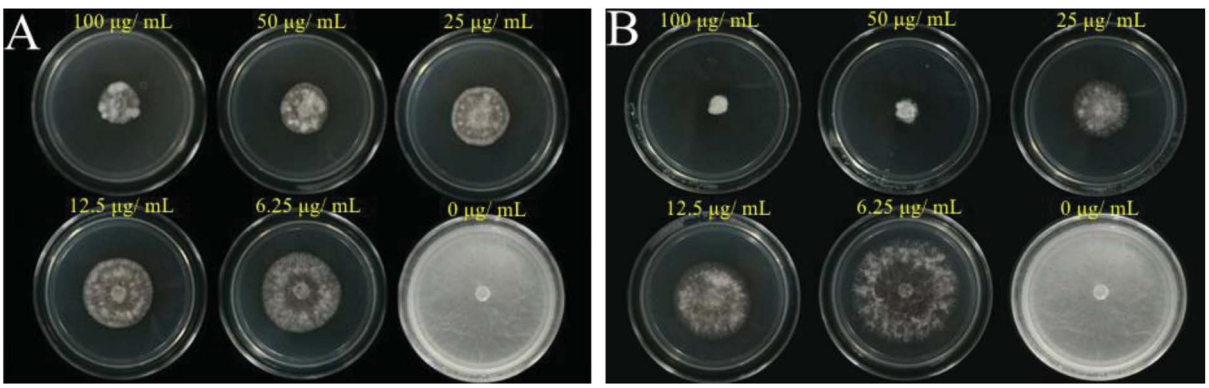


Fig. 4 *In vitro* anti-*RS* activity of **5j**(A) and **5t**(B).

Table 3 Anti-*RS* protective activity data of compound **5j** on detached rice leaves[a]

Chemical	Treatment (μg mL^{-1})	Lesion length (cm)	Control efficacy (%)
5j	100	2.07 ± 0.31	66.17
	200	0.41 ± 0.16	93.30
	500	0.00 ± 0.00	100.00
Azoxystrobin	100	0.35 ± 0.12	94.28
	200	0.18 ± 0.11	97.06
Carbendazim	100	3.57 ± 0.19	41.67
	200	2.85 ± 0.22	53.43
Negative control	—	6.12 ± 0.58	—

[a] Values are the average of 15 replicates.

compounds **5a–5j** with **5k–5t**, the *in vitro* inhibitory activity against *FF*, *RS* of most compounds at 100 μg mL^{-1} with R_1 = Cl was better than of R_1 = H. For example, **5k** (89.38%, R_1 = Cl) > **5a** (22.12%, R_1 = H), **5t** (95.17%, R_1 = Cl) > **5j** (89.56%, R_1 = H).

3.2.3 *In vivo* antifungal test. *In vitro* antifungal activity suggested that compound **5j** had remarkable effects against *RS*. Further, compound **5j** was evaluated for its activity *in vivo*, and the results were in Table 3 and Fig. 5. Compound **5j** displayed a good protective activity *in vivo* on detached leaves at 5 days after inoculating with a control efficacy of 66.17% at 100 μg mL^{-1} and 93.3% at 200 μg mL^{-1}, superior to those of carbendazim at 100 and 200 μg mL^{-1} (41.67 and 55.43%, respectively). The control efficacy reached 100% when the concentration was increased to 500 μg mL^{-1}. The control efficacy of **5j** (93.30%) at 200 μg mL^{-1} was equivalent to that of azoxystrobin (94.28%) at 100 μg mL^{-1}.

Moreover, for the anti-*RS* results of compound **5j** in the greenhouse experiment shown in Table 4 and Fig. 6, treatment with compound **5j** at 100 μg mL^{-1} proved that the protective efficacy was 85.99% in the greenhouse experiment, similar to that of azoxystrobin (84.75%) at 200 μg mL^{-1}. In contrast, the curative activity of compound **5j** at the concentrations of 200 and 100 μg mL^{-1} were 68.43 and 49.72%, respectively, which were better than those of azoxystrobin (65.64 and 46.50%, respectively).

3.2.4 Sclerotia germination inhibition of 5j. As shown in Fig. 7, compound **5j** had a certain inhibitory effect on the germination of *RS* sclerotia in a dose-dependent manner. At a concentration of 100 μg mL^{-1}, the inhibitory rate of compound **5j** could reach 40.00%, which was better than that of azoxystrobin (28.89%). As can be seen, a high concentration of azoxystrobin has a good inhibitory effect on the outward growth of mycelia, the mycelia growth circle was small, and the growth trend of mycelia was similar to that of the negative control. At high and low concentrations of **5j**, the edge of the sclerotia hyphae growth is close to the circle, but the hyphae grow totally differently to those with the negative control, with the negative control sclerotium near the hyphae appearing more transparent, and with a medium growth of mycelial attachment, whereas the **5j** processed sclerotium hyphae were whiter in color, and the hyphae gathered to grow.

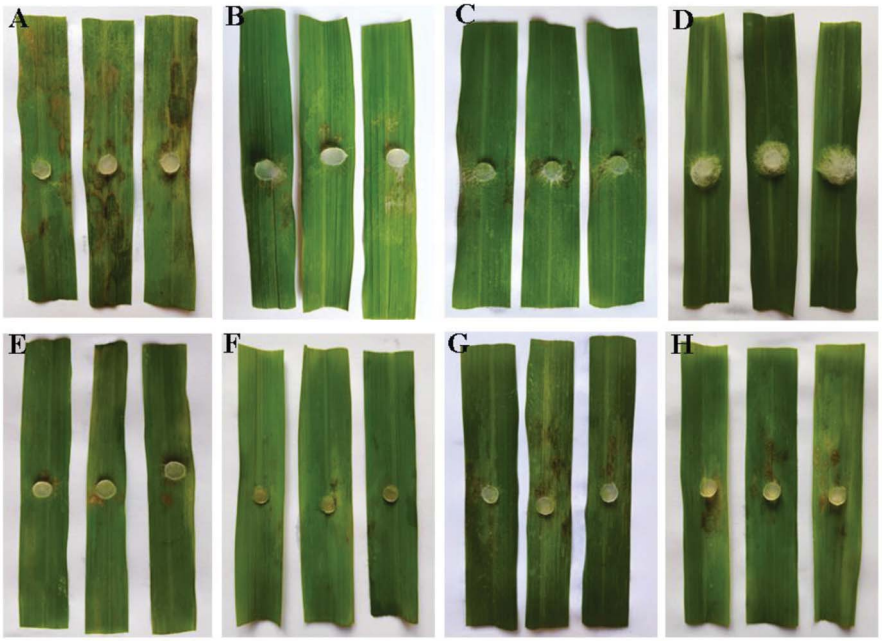


Fig. 5 Anti-*RS* protection efficacy photographs of compound **5j** on detached rice leaves. (A) Negative control; (B) **5j** at 100 μg mL^{-1}; (C) **5j** at 200 μg mL^{-1}; (D) **5j** at 500 μg mL^{-1}; (E) azoxystrobin at 100 μg mL^{-1}; (F) azoxystrobin at 200 μg mL^{-1}; (G) carbendazim at 100 μg mL^{-1}; (H) carbendazim at 200 μg mL^{-1}.

Table 4 *In vivo* control efficacy of compound **5j** against rice sheath blight under greenhouse conditions[a]

		Protective activity		Curative activity	
Chemical	Treatment (μg mL^{-1})	Lesion length (cm)	Control efficacy (%)	Lesion length (cm)	Control efficacy (%)
5j	200	1.13 ± 0.28	85.99	2.26 ± 0.11	68.43
	100	2.33 ± 0.17	71.12	3.60 ± 0.43	49.72
Azoxystrobin	200	0.80 ± 0.31	90.08	2.46 ± 0.55	65.64
	100	1.23 ± 0.59	84.75	3.83 ± 0.27	46.50
Negative control		8.07 ± 0.13	—	7.16 ± 0.25	—

[a] Values are the average of 15 replicates.

3.3 Scanning electron microscopy (SEM)

The effect of compound **5k** on the morphology of *Ac* cells was observed by SEM as illustrated in Fig. 8. The image shows that the degree of cell membrane damage and the concentration of compound **5k** in a dose-dependent manner. To be specific, the cell membrane changed from a simple deformation to depression and rupture when the concentration ranged from 100 to 200 μg mL^{-1}. In contrast, the control group was intact and full. Through the analysis of the SEM results, the antibacterial mechanism of compound **5k** for *Ac* might be that it destroyed the cell membrane and led to cell death, thus achieving the antibacterial effect.

The morphological changes of *RS* hyphae were observed by SEM, as shown in Fig. 9. The untreated mycelial surface was smooth and presents a full cylindrical shape, while the mycelial growth was abnormal after treatment with compound **5j** for 48 h. A large number of folds were generated on the mycelial surface, with many short folds that shrink inward, but a cylindrical shape could still be seen when the concentration was 50 μg mL^{-1}. There were a few long fold marks on the smooth mycelial surface when the concentration increased to 100 μg mL^{-1}, while the hyphae were of a flake shape. We speculate that the initial antifungal mechanism of **5j** on *RS* was that with the increase of drug concentration, the mycelial epidermis gradually concave inward, squeezing the internal material of mycelium and inactivating it, leading to the mycelium changes from a full columnar to sheet, so as to inhibit the growth of *RS*.

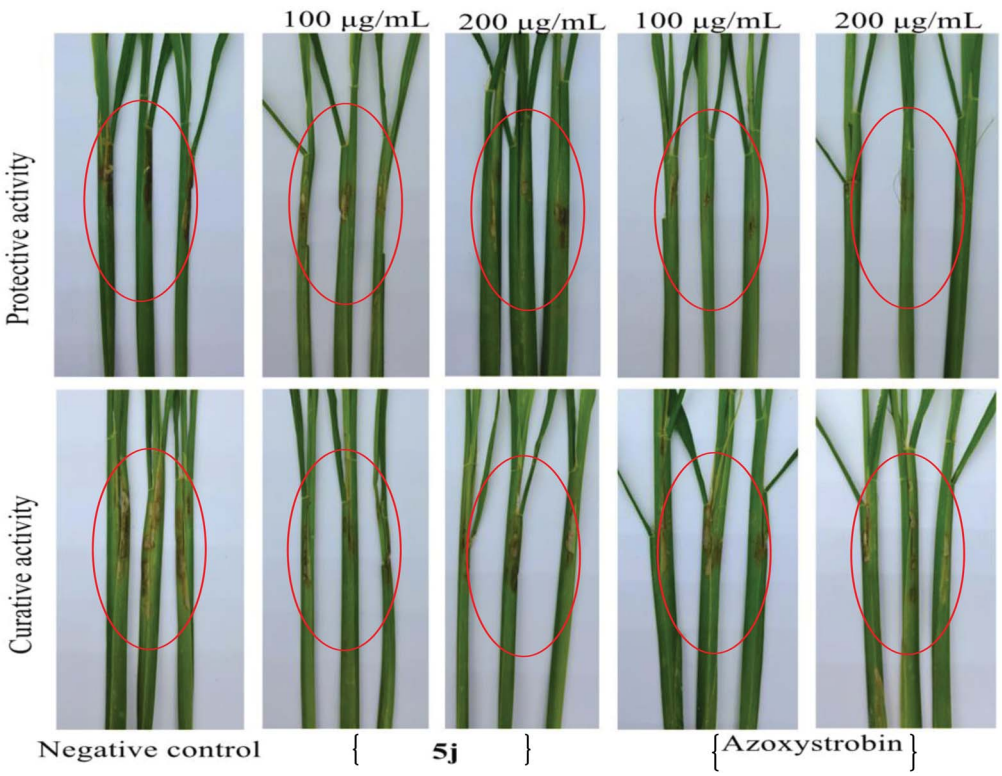


Fig. 6 Anti-*RS* efficacy photographs of compound **5j** in greenhouse experiment.

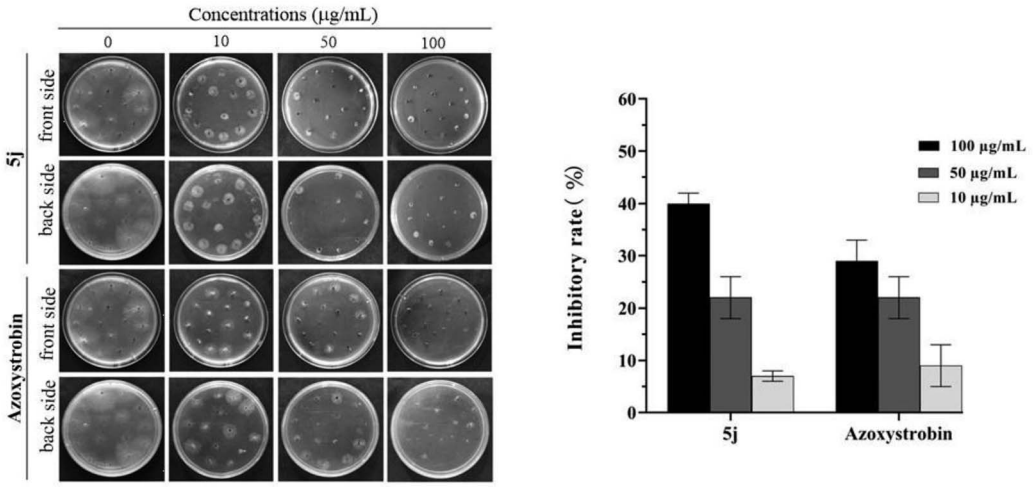


Fig. 7 Inhibitory activity of compound **5j** on the germination of the sclerotia of *RS*.

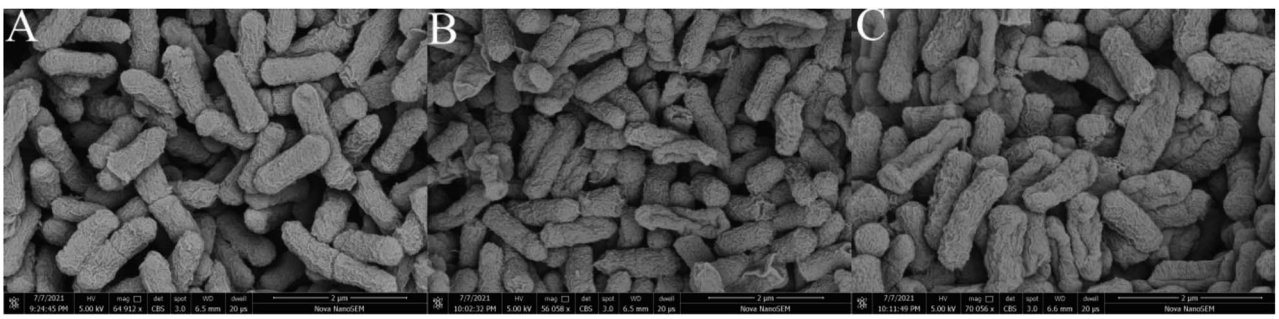


Fig. 8 SEM images for *Ac*. after incubation in different concentrations of compound **5k**. (A) 0 μg mL^{-1}; (B) 100 μg mL^{-1} and (C) 200 μgmL^{-1}. Scale bar for is 2 μm.

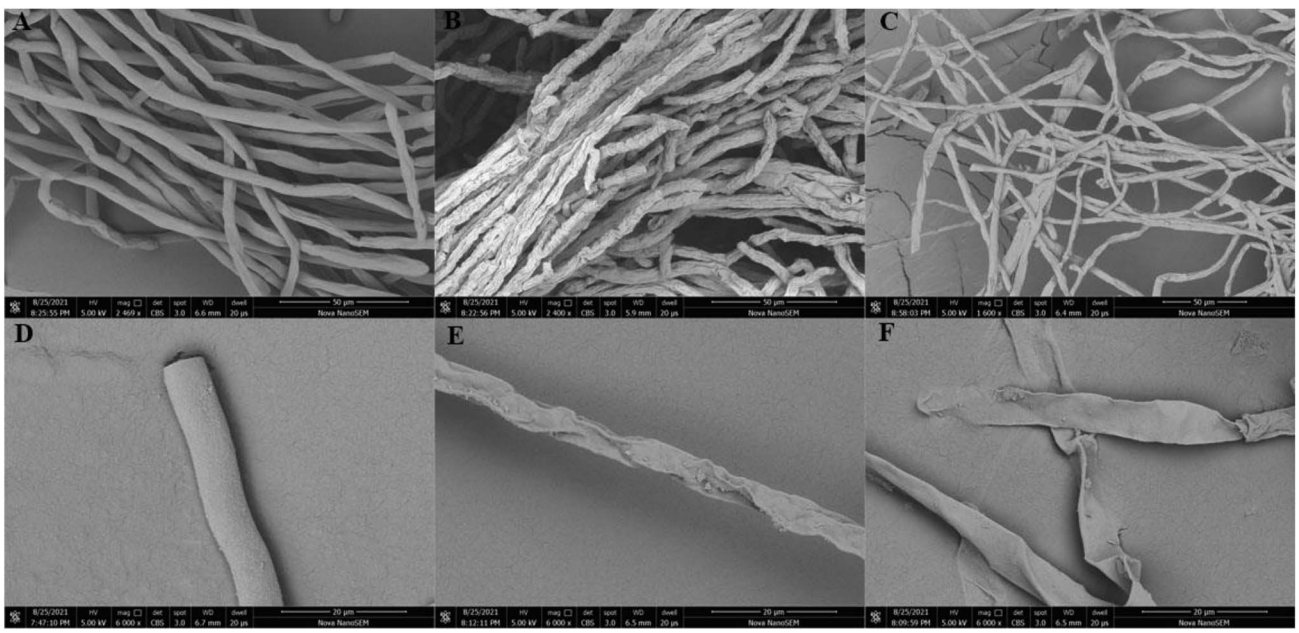


Fig. 9 Scanning electron micrographs of the hyphae of *RS* grown on PDA plates with **5j** at 28 °C. (A and D) control (DMSO); (B and E) **5j** (50 μg mL^{-1}) and (C and F) **5j** (100 μg mL^{-1}).

4. Conclusions

In summary, a series of quinoxaline derivatives was designed, synthesized, and evaluated for their biological activities (five bacteria and twelve fungi). The preliminary experiment showed that some of the designed compounds were identified with an excellent antimicrobial competence. Antibacterial assays discovered that compound **5k** had better activities than those of BT and TC against *Ac*. It is worth noting that **5j** showed a highlighted fungicidal activity against *RS*, and an *in vivo* assay further proved that it could effectively control rice sheath blight. Moreover, the SEM result confirmed that this series of compounds had the competence to change and destroy the bacteria and fungi cell morphologies. By and large, all the findings suggest that quinoxaline derivatives are of research value for agricultural bactericides and fungicides, which could be used to develop potential agrochemicals in the future.

Conflicts of interest

The authors declare no competing financial interest.

Acknowledgements

The authors gratefully acknowledge the National Nature Science Foundation of China (No. 31701821), the Science Foundation of Guizhou Province (No. 20192452), the Natural Science research project of Guizhou Education Department (No. 2018009), Frontiers Science Centre for Asymmetric Synthesis and Medicinal Molecules, Department of Education, Guizhou Province (No. 2020004), and Program of Introducing Talents of Discipline to Universities of China (111 Program, D20023).

References

1 X. B. Wang, X. C. Fu, J. H. Yan, A. Wang, M. Q. Wang, M. Chen, C. L. Yang and Y. M. Song, *Mol. Diversity*, 2019, **23**, 573–583.
2 S. C. Jiang, X. Tang, M. Chen, J. He, S. J. Su, L. W. Liu, M. He and W. Xue, *Pest Manage. Sci.*, 2020, **76**, 853–860.
3 J. Shi, M. H. Ding, N. Luo, S. R. Wan, P. J. Li, J. H. Li and X. P. Bao, *J. Agric. Food Chem.*, 2020, **68**, 9613–9623.
4 Q. Q. Tao, L. W. Liu, P. Y. Wang, Q. S. Long, Y. L. Zhao, L. H. Jin, W. M. Xu, Y. Chen, Z. Li and S. Yang, *J. Agric. Food Chem.*, 2019, **67**, 7626–7639.
5 B. Saul and W. Ron, *Mol. Plant Pathol.*, 2012, **13**, 805–815.
6 X. F. Shang, L. X. Dai, Z. J. Zhang, C. J. Yang, S. S. Du, T. L. Wu, Y. H. He, J. K. Zhu, Y. Q. Liu, Y. F. Yan, X. L. Miao and J. Y. Zhang, *J. Agric. Food Chem.*, 2021, **69**, 6455–6464.
7 N. A. Slaton, R. D. Cartwright, J. Meng, E. E. Gbur and R. J. Norman, *Agron. J.*, 2003, **95**, 1489–1496.
8 D. Durgadevi, S. Harish, R. Manikandan, S. R. Prabhukarthikeyan, D. Alice and T. Raguchander, *Physiol. Mol. Plant Pathol.*, 2021, **115**, 101669.
9 S. Horuz, *J. Plant Pathol.*, 2021, **103**, 751–758.
10 X. Tang, S. J. Su, M. Chen, J. He, R. J. Xia, T. Guo, Y. Chen, C. Zhang, J. Wang and W. Xue, *RSC Adv.*, 2019, **9**, 6011–6020.
11 X. B. Wang, X. C. Fu, M. Chen, A. Wang, J. H. Yan, Y. D. Mei, M. Q. Wang and C. L. Yang, *Chin. Chem. Lett.*, 2019, **30**, 1419–1422.
12 T. Guo, R. J. Xia, M. Chen, S. J. Su, J. He, M. He, H. Wang and W. Xue, *RSC Adv.*, 2019, **9**, 24942–24950.
13 A. Keivanloo, S. Abbaspour, S. Sepehri and M. Bakherad, *Polycyclic Aromat. Compd.*, 2020, 1–14.
14 H. Behzadi, P. Roonasi, K. A. taghipour, D. V. Spoel and S. Manzetti, *J. Mol. Struct.*, 2015, **1091**, 196–202.
15 A. Soozani, A. Keivanloo and M. Bakherad, *ChemistrySelect*, 2017, **2**, 9701–9705.
16 S. Alavi, M. H. Mosslemin, R. Mohebat and A. R. Massah, *Res. Chem. Intermed.*, 2017, **43**, 4549–4559.
17 R. J. Xia, T. Guo, M. Chen, S. J. Su, J. He, X. Tang, S. C. Jiang and W. Xue, *New J. Chem.*, 2019, **43**, 16461–16467.
18 X. Y. Li, Y. H. Wang, K. Chen, D. Gao, D. M. Wang and W. Xue, *Pestic. Biochem. Physiol.*, 2019, **155**, 45–50.
19 X. M. Zhao, C. C. Xia, X. D. Wang, H. Wang, M. Xin, Y. L. Xin and Y. L. Liang, *Front. Pharmacol.*, 2018, **9**, 126–136.
20 A. G. Giuglio-Tonolo, C. Curti, T. Terme and P. Vanelle, *Molecules*, 2020, **25**, 5922.
21 B. Galina, S. Alexander, K. Alexander and D. Pavel, *Curr. Org. Synth.*, 2020, **17**, 29–39.
22 R. S. Keri, S. S. Pandule, S. Budagumpi and B. M. Nagaraja, *Arch. Pharm.*, 2018, **351**, e1700325.
23 M. A. Sibiya, L. Raphoko, D. Mangokoana, R. Makola, W. Nxumalo and T. M. Matsebatlela, *Molecules*, 2019, **24**, 407–423.
24 A. M. Newahie, Y. M. Nissan, N. M. Ismail, D. A. Ella, S. M. Khojah and K. A. Abouzid, *Molecules*, 2019, **24**, 1175–1197.
25 M. K. Ibrahim, I. H. Eissa, A. E. Abdallah, A. M. Metwaly, M. M. Radwan and M. A. ElSohly, *Bioorg. Med. Chem.*, 2017, **25**, 1496–1513.
26 S. Tariq, O. Alam and M. Amir, *Bioorg. Chem.*, 2017, **76**, 343–358.
27 C. Gonçalves, A. Dimou, V. Sakkas, M. F. Alpendurada and T. A. Albanis, *Chemosphere*, 2006, **64**, 1375–1382.
28 X. Y. Yu, C. Huang, L. Wu, S. J. Hua, J. Ye, L. Meng and C. Xu, *Sci. Total Environ.*, 2021, **776**, 146036.
29 R. J. Xia, T. Guo, J. He, M. Chen, S. J. Su, S. C. Jiang, X. Tang, Y. Chen and W. Xue, *Monatsh. Chem.*, 2019, **150**, 1325–1334.
30 M. I. Shahin, D. E. Ella, N. M. Ismail and K. A. Abouzid, *Bioorg. Chem.*, 2014, **56**, 16–26.
31 B. T. Fahr, T. O'Brien, P. Pham, N. D. Waal, S. Baskaran, B. C. Raimundo, J. W. Lam, M. M. Sopko, H. E. Purkey and M. J. Romanowski, *Bioorg. Med. Chem. Lett.*, 2005, **16**, 559–562.
32 M. Moghaddam, M. R. Alymanesh, L. Mehdizadeh, H. Mirzaei and A. G. Pirbalouti, *Ind. Crops Prod.*, 2014, **59**, 144–148.
33 X. B. Wang, A. Wang, L. L. Qiu, M. Chen, A. M. Lu, G. H. Li, C. L. Yang and W. Xue, *J. Agric. Food Chem.*, 2020, **68**, 14426–14437.

34 M. Zhang, M. L. Dou, Y. Y. Xia, Z. Hu, B. J. Zhang, Y. X. Bai, J. Xie, Q. F. Liu, C. P. Xie, D. D. Lu, S. Hou, J. G. He, J. Tao and R. F Sun, *J. Agric. Food Chem.*, 2021, **69**, 5435–5445.
35 Y. H. Ye, L. Ma, Z. C. Dai, Y. Xiao, Y. Y. Zhang, D. D. Li, J. X. Wang and H. L. Zhu, *J. Agric. Food Chem.*, 2014, **62**, 4063–4071.
36 Z. J. Zhang, Y. Zeng, Z. Y. Jiang, B. S. Shu, V. Sethuraman and G. H. Zhong, *Pest Manage. Sci.*, 2018, **74**, 1736–1746.
37 X. Wang, Z. C. Dai, Y. F. Chen, L. L. Cao, W. Yan, S. K. Li, J. X. Wang, Z. G. Zhang and Y. H. Ye, *Eur. J. Med. Chem.*, 2017, **126**, 171–182.
38 D. Peng, S. D. Li, J. X. Wang, C. J. Chen and M. G. Zhou, *Pest Manage. Sci.*, 2014, **70**, 258–263.
39 Z. J. Zhang, Z. Y. Jiang, Q. Zhu and G. H. Zhong, *J. Agric. Food Chem.*, 2018, **66**, 9598–9607.
40 S. C Jiang, S. J. Su, M. Chen, F. Peng, Q. Zhou, T. T. Liu, L. W. Liu and W. Xue, *J. Agric. Food Chem.*, 2020, **68**, 5641–5647.
41 M. Chen, X. M. Tang, T. T. Liu, F. Peng, Q. Zhou, H. Luo, M. He and W. Xue, *Fitoterapia*, 2021, **149**, 104804.
42 J. Zhang, L. T. Yan, E. L. Yuan, H. X. Ding, H. C. Ye, Z. K. Zhang, C. Yan, Y. Q. Liu and G. Feng, *J. Agric. Food Chem.*, 2014, **62**, 4905–4910.
43 L. Y. Feng, F. W. Wu, J. Li, Y. M. Jiang and X. W. Duan, *Postharvest Biol. Technol.*, 2011, **61**, 160–164.