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.
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 Quick 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. The intermediate 4 and K$_2$CO$_3$ 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. Brasilienne (Pcb), Ralstonia solanacearum (Rs) and Acidovorax citrulli (Ac) were evaluated by a slightly modified 96-well plate method. 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 brassicace (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. The experimental details for the twelve fungi are presented in the ESI.†
### 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 ESL.†

#### 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 mL$^{-1}$).

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**Table 1** Virulence curves and EC$_{50}$ values of the target compounds against five kinds of bacteria

| Pathogen | Chemical | Toxic regression equation $r$ | EC$_{50}$ (μg mL$^{-1}$) |
|----------|----------|------------------------------|--------------------------|
| Ac       | 5k       | $y = 1.1818x + 3.1726$       | 0.9570 35.18             |
| TC       |          | $y = 1.4268x + 1.7174$       | 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             |
|          | 5p       | $y = 1.8284x + 0.7780$       | 0.9899 203.76             |
| Xoo      | 5o       | $y = 1.5922x + 1.7321$       | 0.9749 112.83             |
|          | 5p       | $y = 1.1848x + 2.8051$       | 0.9698 72.211             |
| Rs       | 5b       | $y = 0.7773x + 3.6717$       | 0.9804 51.15              |
|          | 5c       | $y = 1.3088x + 2.3014$       | 0.9551 115.31             |
|          | 5p       | $y = 1.0061x + 3.1290$       | 0.9975 72.38              |
|          | 5q       | $y = 1.7183x + 0.8514$       | 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             |
|          | BT       | $y = 1.0849x + 2.9345$       | 0.9976 80.14              |
|          |          | $y = 1.7293x + 0.9067$       | 0.9859 232.82             |

$^a$ The experiments were repeated three times. $^b$ Commercial bactericides bismethiazol (BT) and thiadiazole-copper (TC) were used as positive control agents.
mlL−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 $R_2$ 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 μg mL$^{-1}$, respectively, which were better than that of azoxystrobin (26.17 μg 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

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**Table 2** Virulence curves and EC$_{50}$ values of the target compounds against four fungal pathogens

| Pathogen | Chemical | Toxic regression equation | $r$ | EC$_{50}$ (μg mL$^{-1}$) |
|----------|----------|---------------------------|----|------------------------|
| $FF$     | $5k$     | $y = 2.5936x + 0.7048$    | 0.9747 | 45.29                 |
| Azoxystrobin | $y = 1.0678x + 3.2400$    | 0.9828 | 44.48                |
| $PS$     | $5k$     | $y = 2.2048x + 1.2841$    | 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                |

*The experiments were repeated three times.*

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

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

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compounds 5a–5j with 5k–5t, the in vitro inhibitory activity against FF, RS of most compounds at 100 μg mL⁻¹ with R₁ = Cl was better than of R₁ = H. For example, 5k (89.38%, R₁ = Cl) > 5a (22.12%, R₁ = H), 5t (95.17%, R₁ = Cl) > 5j (89.56%, R₁ = 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 grew 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.

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

| 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        | —                    |

*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.

### Table 4  In vivo control efficacy of compound 5j against rice sheath blight under greenhouse conditions

| Chemical | Treatment (μg mL⁻¹) | Protective activity | Curative activity |
|----------|---------------------|---------------------|------------------|
|          | 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 |
| Azoxyostrobine | 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      | 8.07 ± 0.13         | —                | 7.16 ± 0.25 | —    |

*Values are the average of 15 replicates.*
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 μg mL⁻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).

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