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Research Article

Synthesis of *N*-Methylmorpholinium Derivatives Possessing a 1,3,4-Oxadiazole Core as Feasible Antibacterial Agents against Plant Bacterial Diseases

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To develop a kind of quaternary ammonium compounds that can safely apply in agriculture for managing the plant bacterial diseases, herein, a series of *N*-methylmorpholinium derivatives possessing a classical 1,3,4-oxadiazole core were prepared and the antibacterial activities both *in vitro* and *in vivo* were screened. Bioassay results revealed that compounds **3l** and **3i** showed the strongest antibacterial activity toward pathogens *Xanthomonas oryzae* pv. *oryzae* and *X. axonopodis* pv. *citri* with the lowest EC_{50} values of 1.40 and 0.90 μg/mL, respectively. Phytotoxicity test trials indicated that target compounds bearing a bulky *N*-methylmorpholinium pendant are safe for plants. The following *in vivo* bioassays showed that compound **3l** could control the rice bacterial blight disease, thereby affording good control efficiencies of 55.95% (curative activity) and 53.09% (protective activity) at the dose of 200 μg/mL. Preliminary antibacterial mechanism studies suggested that target compounds had strong interactions with the cell membrane of bacteria via scanning electron microscopy imaging. Additionally, this kind of framework also displayed certain antifungal activity toward *Fusarium oxysporum* and *Phytophthora cinnamomi*. Given the above privileged characteristics, this kind of 1,3,4-oxadiazole-tailored *N*-methylmorpholinium derivatives could stimulate the design of safe quaternary ammonium bactericides for controlling plant bacterial diseases.

1. Introduction

Phytopathogens are a group of aggressive microorganisms that can invade all sorts of plants for nutrient competition and/or self-reproduction, thereby seriously threatening the quality and yield of agricultural products [1–4]. The representative notorious bacterial strains, namely, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *X. axonopodis* pv. *citri* (*Xac*), are the widely distributed pathogens worldwide and can lead to the development of severe diseases on major crops, exemplified by the bacterial leaf blight on rice and canker on citrus [5–9]. As for the bacterial blight disease, it can occur in all stages of rice growth, thereby reducing approximately 10 to 50 percent rice production annually in rice-growing countries [10–14]. This outcome has strongly strained the demand for food supply. Currently, the long-term use and/or abuse of traditional bactericides have contributed to the development of immeasurable microorganic resistance and ineffective control efficiency toward plant infections [15–19]. Therefore, novel and safe frameworks with highly efficient bioactivity should be urgently excavated and developed [20–23].

In the development of bactericides, quaternary ammonium compounds possessing the typical positive charge on the nitrogen atom have been intensively highlighted in consideration of their versatile biological profiles, especially in the antibacterial and antifungal aspects [24–30]. Investigations found that these kinds of quaternary ammonium salts (QASs) displayed strong interactions with the cell membrane of microorganisms, thereby leading to cell membrane destruction and finally bacterial death [31–38]. Given this feature, many QASs were commercialized and

exploited to sterilize medical instruments, hospital protective clothing, medical implants, wound dressings, food packaging materials, and daily consumer goods [39]. Although this kind of substrates have many prominent properties, most of which are only used in industries. To date, rare QASs are applied in agriculture due to the possible phytotoxicity. Thus, exploration and development of a kind of quaternary ammonium compounds that can safely apply in agriculture for managing the plant bacterial diseases is highly pursued and approved.

On the other hand, morpholinium salts, owning a large steric hindrance scaffold, as a member of QASs, were used in medicine and pesticides (Figure 1). The representative structure of *N,N*-dimethylmorpholine chloride was used as a type of plant growth regulator that can increase the yield of cotton [40–42]. Another morpholinium salt, namely, pinaverium bromide has been applied as a gastrointestinal crystallizer that can inhibit the flow of calcium ions into intestinal smooth muscle cells. Based on this, the associated pinaverium bromide tablets are safely used for intestinal-related pain [43–45]. Meanwhile, novel biological ingredients possessing the typical morpholinium moiety were constantly prepared and evaluated. For instance, Bakhite et al. screened the insecticidal activity of a kind of morpholinium derivatives and found that compound **1** showed good insecticidal activities against nymphs of cowpea aphid, which was superior to that of the insecticide acetamiprid [46]. Yang et al. prepared the morpholinium-tailored substrate soyaethyl morpholinium ethosulfate (SME) that could self-assemble into micelles with excellent antibacterial effects toward both the *Staphylococcus aureus* and methicillin-resistant *S. aureus*. Additionally, SME presented a relatively low toxicity to mammalian cells [47]. Given the unique superiority of this morpholinium scaffold, it should be introduced into the final target framework for the development of safe agricultural chemicals.

In our previous work, we developed and evaluated the antibacterial functions of an array of pyridinium-tailored compounds and found that they showed excellent antibacterial activities but high phytotoxicity toward rice leaves [15, 48]. To sequentially develop a kind of safe quaternary ammonium compounds that can safely apply in agriculture for managing the plant bacterial diseases, herein, the morpholinium scaffold was used to replace the planar pyridinium group to yield 1,3,4-oxadiazole-tailored *N*-methylmorpholinium derivatives. The following *in vitro* and *in vivo* bioassays against *Xoo* and *Xac* and the relevant phytotoxicity test were evaluated. Simultaneously, the antifungal activity toward *Fusarium oxysporum* (*F. oxysporum*) and *Phytophthora cinnamomi* (*P. cinnamomi*) was also screened.

2. Materials and Methods

2.1. Instruments and Chemicals. The NMR spectra of synthesized compounds were measured using the Bruker Biospin-AG-500 apparatus (BRUKEROPTICS, Switzerland). DMSO and TMS were used as the solvent and internal standard, respectively. All chemicals were purchased from Energy Chemical and used without further purification. All solvents meet the standard of analytical purity. The reaction process was monitored by using thin-layer chromatography.

2.2. In Vitro Antibacterial Bioassays. The antibacterial activity against pathogens *Xoo* and *Xac* was evaluated by the turbidimeter test [3, 22, 48]. All the synthesized compounds were tested under different concentrations (such as 50.0, 25.0, 12.5, 6.25, 3.125, and 1.5625 μg/mL). Bismerthiazol and thiadiazole copper served as positive controls, whereas dimethyl sulfoxide (DMSO) in sterile distilled water was the blank control. Absorbing 40 μL bacteria solution ($OD_{595} = 0.8$, logarithmic phase of growth) was added into a 5 mL nutrient broth medium (components: 3 g beef extract, 5 g peptone, 1 g yeast powder, 10 g glucose, 18 g agar in 1 L of distilled water, pH 7.0–7.2) containing different dosages of target compounds and controls. Then, these samples were incubated in a constant shaker (180 rpm) at $28 \pm 1^{\circ}$C for about 24–48 h until the DMSO control reached the logarithmic growth phase. After that, 200 μL samples were tested the optical density at 595 nm (OD_{595}) through a microplate reader. The turbidity corrected values = $OD_{\text{bacterial wilt}} - OD_{\text{no bacterial wilt}}$, and the inhibition rate I was calculated by $I = (C - T)/C \times 100\%$. C is the corrected turbidity value of bacterial growth on untreated NB (blank control), and T is the corrected turbidity value of bacterial growth on treated NB. By using SPSS 17.0 software and the obtained inhibition rates at different concentrations, a regression equation was provided. The results of antibacterial activities (expressed by EC_{50}) against *Xoo* and *Xac* were calculated from the equation. The experiment was repeated three times.

2.3. In Vivo Bioassays against Rice Bacterial Blight. Compound **3l**, thiadiazole copper (TC, 20% suspending agent) as the positive control, and an equivalent DMSO as a blank control in sterile distilled water were used to test the bioactivity against rice bacterial blight [22]. The rice variety "Fengyouxiangzhan" was planted for about 8 weeks before use. For the curative activity, an aseptic scissor dipped with *Xoo* cells was used to infect rice leaves. One day later, compound **3l** and TC with a concentration of 200 μg/mL were evenly sprayed on leaves. Meanwhile, the drug-free solution was used for the negative group. All the treated plants were cultured in the constant temperature (28°C) and humidity (90% RH) incubator for 14 days before testing the disease index. For the protective effects, the difference was that the drug solution with the same dosage was firstly sprayed before inoculation with *Xoo* cells. The related disease index could be obtained after 14 days. The corresponding control efficiencies I were provided according to the formula: $I = (\text{CK} - \text{T})/\text{CK} \times 100\%$. CK and T represent the disease index of negative and drug-treated controls, respectively.

2.4. Scanning Electron Microscopy (SEM) Imaging. Scanning electron microscopy (SEM) imaging of *Xoo* cells triggered by compound **3l**. 1.5 mL *Xoo* cells incubated at the logarithmic phase were centrifuged and washed with PBS

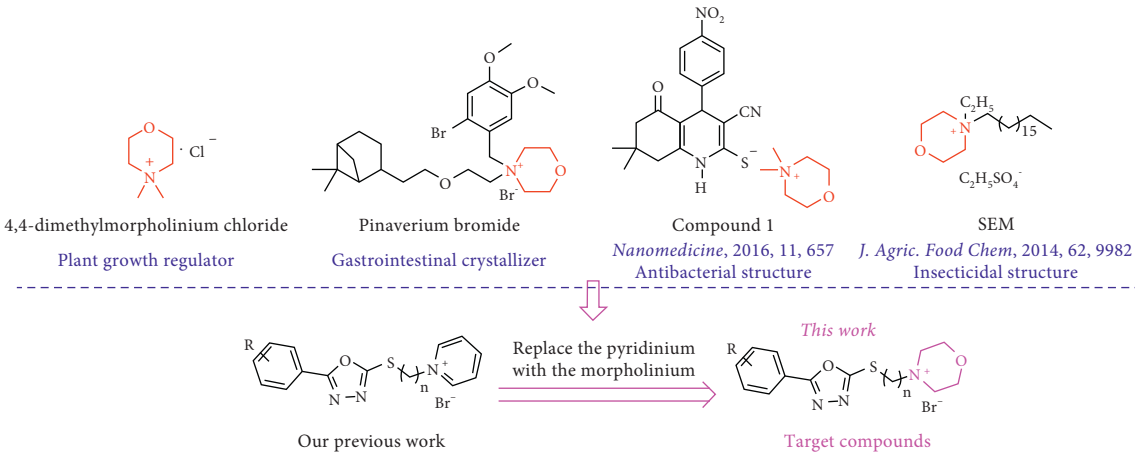


Figure 1: Bioactive structures containing the morpholinium moiety and the design strategy for target molecules in this work.

(pH = 7.2) and resuspended in 1.5 mL of PBS buffer (pH = 7.2). Then, these *Xoo* cells were incubated with compound **3l** at a dose of 14.0 μg/mL and an equivalent volume of DMSO (solvent control) for 8 h in a shaker under 180 rpm at 28°C. After incubation, these samples were washed 3 times with PBS (pH = 7.2). Subsequently, the bacterial cells were fixed for 8 h at 4°C with 2.5% glutaraldehyde and then dehydrated with graded ethanol series and pure tert-butanol (2 times with 10 min/time). Following dehydration, samples were freeze-dried and coated with gold and visualized using Nova Nano SEM 450 [3,22].

2.5. In Vitro Antifungal Bioassay against F. oxysporum and P. cinnamomi. All target molecules were dissolved in DMSO (1.0 mL) and then added into 9.0 mL sterilized water containing Tween 20 (volume ratio 0.1%) before mixing with potato dextrose agar (PDA, 90.0 mL). These compounds were tested at a concentration of 25 μg/mL. The stock solution was transferred into three 9 cm diameter Petri dishes evenly. Then, mycelia dishes of approximately 4 mm diameter were cut from the culture medium and inoculated in the middle of the PDA plate aseptically. The inoculated plates were incubated at 28 ± 1°C for 3–5 days. DMSO in sterile distilled water was used as the negative control, whereas hymexazol served as the positive control. Each treatment condition contained three replicates. Radial growth of the fungal colonies was measured, and the data were statistically analyzed. Inhibitory effects toward these fungi were calculated by the formula $I = [(C - T)/(C - 0.4)] \times 100\%$, where C represents the diameter of fungal growth on untreated PDA, T represents the diameter of fungi on treated PDA, and I represents the inhibition rate [49].

*2.6. General Procedures for Preparing Intermediate **2**.* Intermediate **1** was prepared by following our previous works [48, 49]. Then, intermediate **1** (1 mmol) was dissolved in 8 mL DMF containing K_2CO_3 (1.3 mmol) and 1,8-dibromooctane, 1,10-dibromodecane, or 1,12-dibromododecane (1.3 mmol). After stirring the mixture for 2 h at 25°C, the organic matter was extracted with EtOAc, washed twice with purified water, and dried with anhydrous sodium sulfate. After that, the solvent was removed under a reduced pressure. Finally, intermediate **2** was obtained by silica gel column chromatography using petroleum ether/EtOAc (10/1, V/V) as the eluent.

*2.7. General Procedures for Preparing Target Compounds **3a–3o**.* Intermediate **2** (0.15 g) and 4-methylmorpholine (0.5 mL) in 4 mL CH_3CN were refluxed at 85°C for 8 h. After that, the solvent was removed under a reduced pressure. Finally, target compounds **3a–3o** were obtained by silica gel column chromatography using CH_2Cl_2/CH_3OH (20/1, V/V) as the eluent.

*2.8. 4-Methyl-4-(8-((5-phenyl-1,3,4-oxadiazol-2-yl)thio)octyl)morpholin-4-ium Bromide (**3a**).* A yellow solid, m. p. 102.5~104.6°C, yield 63.2%; ^{1}H NMR (500 MHz, CD_3OD) δ 7.96 (dd, J = 7.8, 1.3 Hz, 2H, Ben-H), 7.52–7.45 (m, 3H, Ben-H), 4.16–4.01 (m, 4H, morpholine-H), 3.89–3.78 (m, 4H, morpholine-H), 3.66 (t, J = 9.3 Hz, 2H, N^+-CH_2), 3.54 (s, 3H, CH_3), 3.25 (t, J = 7.3 Hz, 2H, S-CH_2), 1.81 (dt, J = 14.5, 7.4 Hz, 4H, S-CH_2CH_2 and N^+-CH_2CH_2), 1.48–1.33 (m, 8H, S-$(CH_2)_2CH_2CH_2CH_2CH_2$); ^{13}C NMR (126 MHz, CD_3OD) δ 165.8, 164.8, 131.8, 129.2, 126.7, 123.7, 60.9, 59.8, 32.5, 29.1, 28.8, 28.5, 28.2, 26.1, 21.9; HRMS (ESI) $[M\text{-}Br]^+$ calcd for $C_{21}H_{32}N_3O_2S$: 390.2210, found: 390.2209.

*2.9. 4-Methyl-4-(10-((5-phenyl-1,3,4-oxadiazol-2-yl)thio)octyl)morpholin-4-ium Bromide (**3b**).* A white solid, m. p. 144.1~146.4°C, yield 65%; ^{1}H NMR (500 MHz, CD_3OD) δ 7.98 (t, J = 1.5 Hz, 1H, Ben-H), 7.97 (t, J = 1.9 Hz, 1H, Ben-H), 7.61–7.53 (m, 4H, Ben-H), 3.98 (s, 4H, morpholine-H), 3.45 (dd, J = 14.6, 6.9 Hz, 6H, morpholine-H and N^+-CH_2), 3.29 (t, J = 1.6 Hz, 2H, S-CH_2), 3.19 (s, 3H, CH_3), 1.82 (dt, J = 17.1, 8.5 Hz, 4H, S-CH_2CH_2 and N^+-CH_2CH_2), 1.49–1.45 (m, 2H, S-$(CH_2)_2CH_2$), 1.36 (d, J = 18.2 Hz, 10H, S-$(CH_2)_3CH_2CH_2CH_2CH_2CH_2$); ^{13}C NMR (126 MHz, CD_3OD) δ 165.9, 165.3, 131.9, 129.1, 126.3, 123.3, 60.3, 59.7,

32.1, 29.2, 29.0, 29.0, 28.8, 28.6, 28.1, 26.0, 21.2; HRMS (ESI) $[M\text{-}Br]^+$ calcd for $C_{23}H_{36}N_3O_2S$: 418.2523, found: 418.2522.

*2.10. 4-Methyl-4-(12-((5-phenyl-1,3,4-oxadiazol-2-yl)thio) octyl)morpholin-4-ium Bromide (**3c**).* A white solid, m. p. 111.2~113.8°C, yield 67%; ^{1}H NMR (500 MHz, CD_3OD) δ 7.98 (dd, J = 8.2, 1.5 Hz, 2H, Ben-H), 7.59–7.53 (m, 3H, Ben-H), 3.98 (s, 4H, morpholine-H), 3.49–3.42 (m, 6H, morpholine-H and N^+-CH_2), 3.29–3.28 (m, 2H, S-CH_2), 3.19 (s, 3H, CH_3), 1.82 (dt, J = 15.0, 7.4 Hz, 4H, S-CH_2CH_2 and N^+-CH_2CH_2), 1.50–1.44 (m, 2H, S-$(CH_2)_2CH_2$), 1.34 (d, J = 36.0 Hz, 14H, S-$(CH_2)_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2$); ^{13}C NMR (126 MHz, CD_3OD) δ 165.9, 165.3, 131.9, 129.1, 126.3, 123.3, 60.3, 59.7, 59.7, 59.7, 32.1, 29.2, 29.2, 29.2, 28.9, 28.7, 28.1, 26.1, 21.2; HRMS (ESI) $[M\text{-}Br]^+$ calcd for $C_{25}H_{40}N_3O_2S$: 446.2836, found: 446.2835.

*2.11. 4-(8-((5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl)thio) octyl)-4-methylmorpholin-4-ium Bromide (**3d**).* A brown oily liquid, yield 60%; ^{1}H NMR (500 MHz, CD_3OD) δ 7.91 (d, J = 7.8 Hz, 1H, Ben-H), 7.62 (d, J = 8.0 Hz, 1H, Ben-H), 7.57 (t, J = 7.7 Hz, 1H, Ben-H), 7.49 (t, J = 7.5 Hz, 1H, Ben-H), 3.98 (s, 4H, morpholine-H), 3.49 (dd, J = 11.0, 6.1 Hz, 6H, morpholine-H and N^+-CH_2), 3.34–3.28 (m, 2H, S-CH_2), 3.21 (s, 3H, CH_3), 1.87–1.75 (m, 4H, S-CH_2CH_2 and N^+-CH_2CH_2), 1.53–1.44 (m, 2H, S-$(CH_2)_2CH_2$), 1.43–1.35 (m, 6H, S-$(CH_2)_3CH_2CH_2CH_2$); ^{13}C NMR (126 MHz, CD_3OD) δ 165.9, 164.0, 132.9, 132.5, 131.1, 130.9, 127.5, 122.5, 60.3, 59.7, 59.7, 47.6, 32.1, 29.2, 28.6, 28.4, 28.0, 25.9, 21.2; HRMS (ESI) $[M\text{-}Br]^+$ calcd for $C_{21}H_{31}ClN_3O_2S$: 424.1820, found: 424.1820.

*2.12. 4-(10-((5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl)thio) octyl)-4-methylmorpholin-4-ium Bromide (**3e**).* A brown solid, m. p. 93.6–94.5°C, yield 64%; ^{1}H NMR (500 MHz, CD_3OD) δ 7.92 (dd, J = 7.8, 1.5 Hz, 1H, Ben-H), 7.62 (dd, J = 8.0, 1.1 Hz, 1H, Ben-H), 7.59–7.55 (m, 1H, Ben-H), 7.52–7.47 (m, 1H, Ben-H), 3.99 (d, J = 9.9 Hz, 4H, morpholine-H), 3.53–3.43 (m, 6H, morpholine-H and N^+-CH_2), 3.31 (d, J = 7.3 Hz, 2H, CH_2, S-CH_2), 3.20 (s, 3H, CH_3), 1.87–1.76 (m, 4H, S-CH_2CH_2 and N^+-CH_2CH_2), 1.47 (dt, J = 14.8, 7.4 Hz, 2H, S-$(CH_2)_2CH_2$), 1.36 (dd, J = 18.3, 6.0 Hz, 10H, S-$(CH_2)_3CH_2CH_2CH_2CH_2CH_2$); ^{13}C NMR (126 MHz, CD_3OD) δ 165.9, 164.0, 132.9, 132.6, 131.1, 130.9, 127.4, 122.5, 60.3, 59.7, 32.1, 29.3, 29.0, 29.0, 28.8, 28.7, 28.1, 26.0, 21.2; HRMS (ESI) $[M\text{-}Br]^+$ calcd for $C_{23}H_{35}ClN_3O_2S$: 452.2133, found: 452.2133.

*2.13. 4-(12-((5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl)thio) octyl)-4-methylmorpholin-4-ium Bromide (**3f**).* A brown solid, m. p. 79.6–81.5°C, yield 66%; ^{1}H NMR (500 MHz, CD_3OD) δ 7.92 (dd, J = 7.8, 1.7 Hz, 1H, Ben-H), 7.62 (dd, J = 8.1, 1.3 Hz, 1H, Ben-H), 7.60–7.54 (m, 1H, Ben-H), 7.48–7.51 (m, 1H, Ben-H), 4.03–3.94 (m, 4H, morpholine-H), 3.53–3.41 (m, 6H, morpholine-H and N^+-CH_2), 3.34–3.27 (m, 2H, S-CH_2), 3.20 (s, 3H, CH_3), 1.89–1.72 (m, 4H, S-CH_2CH_2 and N^+-CH_2CH_2), 1.51–1.41 (m, 2H, S-$(CH_2)_2CH_2$), 1.35 (dd, J = 34.1, 10.6 Hz, 14H, S-$(CH_2)_3CH_2CH_2CH_2CH_2CH_2CH_2$); ^{13}C NMR (126 MHz, CD_3OD) δ 165.9, 164.0, 132.9, 132.6, 131.0, 130.9, 127.4, 122.6, 120.0, 65.4, 63.9, 60.3, 60.1, 59.7, 53.4, 32.2, 29.3, 29.2, 29.2, 28.9, 28.7, 28.2, 26.1, 21.3; HRMS (ESI) $[M\text{-}Br]^+$ calcd for $C_{25}H_{39}ClN_3O_2S$: 480.2446, found: 480.2446.

*2.14. 4-(8-((5-(3-Chlorophenyl)-1,3,4-oxadiazol-2-yl)thio) octyl)-4-methylmorpholin-4-ium Bromide (**3g**).* A brown solid, m. p. 131.4~132.6°C, yield 58.2%; ^{1}H NMR (500 MHz, CD_3OD) δ 7.92 (t, J = 1.7 Hz, 1H, Ben-H), 7.87 (dt, J = 7.5, 1.5 Hz, 1H, Ben-H), 7.59–7.56 (m, 1H, Ben-H), 7.53 (t, J = 7.7 Hz, 1H, Ben-H), 4.01–3.96 (m, 4H, morpholine-H), 3.50 (dd, J = 17.5, 7.3 Hz, 6H, morpholine-H and N^+-CH_2), 3.30 (d, J = 7.3 Hz, 2H, S-CH_2), 3.22 (s, 3H, CH_3), 1.82 (dd, J = 14.8, 7.4 Hz, 4H, S-CH_2CH_2 and N^+-CH_2CH_2), 1.51–1.46 (m, 2H, S-$(CH_2)_2CH_2$), 1.41 (s, 6H, S-$(CH_2)_3CH_2CH_2CH_2$); ^{13}C NMR (126 MHz, CD_3OD) δ 165.8, 164.6, 135.0, 131.7, 130.9, 126.0, 125.1, 124.7, 60.4, 59.8, 32.1, 29.1, 28.6, 28.4, 28.0, 25.9, 21.2; HRMS (ESI) $[M\text{-}Br]^+$ calcd for $C_{21}H_{31}ClN_3O_2S$: 424.1820, found: 424.1820.

*2.15. 4-(10-((5-(3-Chlorophenyl)-1,3,4-oxadiazol-2-yl)thio) octyl)-4-methylmorpholin-4-ium Bromide (**3h**).* A yellow solid, m. p. 54.9~56.3°C, yield 60%; ^{1}H NMR (500 MHz, CD_3OD) δ 7.96 (t, J = 1.8 Hz, 1H, Ben-H), 7.91–7.88 (m, 1H, Ben-H), 7.60–7.57 (m, 1H, Ben-H), 7.54 (t, J = 7.9 Hz, 1H, Ben-H), 3.98 (s, 4H, morpholine-H), 3.47 (dd, J = 17.6, 9.5 Hz, 6H, morpholine-H and N^+-CH_2), 3.30–3.28 (m, 2H, S-CH_2), 3.20 (s, 3H, CH_3), 1.81 (dd, J = 15.0, 7.1 Hz, 4H, S-CH_2CH_2 and N^+-CH_2CH_2), 1.47 (dd, J = 14.4, 6.8 Hz, 2H, S-$(CH_2)_2CH_2$), 1.36 (d, J = 20.9 Hz, 10H, S-$(CH_2)_3CH_2CH_2CH_2CH_2CH_2$); ^{13}C NMR (126 MHz, CD_3OD) δ 165.8, 164.6, 135.0, 131.7, 130.9, 126.0, 125.1, 124.7, 60.3, 59.7, 59.7, 32.1, 29.2, 29.1, 29.0, 28.9, 28.7, 28.2, 26.0, 21.2; HRMS (ESI) $[M\text{-}Br]^+$ calcd for $C_{23}H_{35}ClN_3O_2S$: 452.2133, found: 452.2133.

*2.16. 4-(12-((5-(3-Chlorophenyl)-1,3,4-oxadiazol-2-yl)thio) octyl)-4-methylmorpholin-4-ium Bromide (**3i**).* A white solid, m. p. 114.5~115.1°C, yield 62%; ^{1}H NMR (500 MHz, CD_3OD) δ 7.96 (t, J = 1.7 Hz, 1H, Ben-H), 7.91–7.89 (m, 1H, Ben-H), 7.59 (m, J = 8.1, 2.1, 1.2 Hz, 1H, Ben-H), 7.54 (t, J = 7.8 Hz, 1H, Ben-H), 3.99 (d, J = 9.8 Hz, 4H, morpholine-H), 3.50–3.44 (m, 6H, morpholine-H and N^+-CH_2), 3.29 (dt, J = 3.2, 1.7 Hz, 2H, S-CH_2), 3.20 (s, 3H, CH_3), 1.81 (dt, J = 14.9, 7.4 Hz, 4H, S-CH_2CH_2 and N^+-CH_2CH_2), 1.46 (dd, J = 15.1, 7.6 Hz, 2H, S-$(CH_2)_2CH_2$), 1.34 (d, J = 40.9 Hz, 14H, S-$(CH_2)_3CH_2CH_2CH_2CH_2CH_2CH_2$); ^{13}C NMR (126 MHz, CD_3OD) δ 165.8, 164.6, 135.0, 131.7, 130.9, 126.0, 125.1, 124.7, 65.4, 63.6, 60.3, 59.7, 45.9, 43.4, 32.1, 29.3, 29.2, 28.9, 28.8, 28.2, 26.1, 21.3; HRMS (ESI) $[M\text{-}Br]^+$ calcd for $C_{25}H_{39}ClN_3O_2S$: 480.2446, found: 480.2446.

*2.17. 4-Methyl-4-(8-((5-(p-tolyl)-1,3,4-oxadiazol-2-yl)thio) octyl)morpholin-4-ium Bromide (**3j**).* A white solid, m. p. 113.1–114.6°C, yield 64.8%; ^{1}H NMR (500 MHz, $CDCl_3$) δ

7.80 (d, $J = 8.2$ Hz, 2H, Ben-H), 7.24 (dd, $J = 8.5$, 0.5 Hz, 2H, Ben-H), 4.16–4.10 (m, 2H, morpholine-H), 4.02 (d, $J = 14.1$ Hz, 2H, morpholine-H), 3.80 (d, $J = 13.2$ Hz, 2H, morpholine-H), 3.75–3.70 (m, 2H, morpholine-H), 3.64 (t, $J = 10.8$ Hz, 2H, N^+-CH_2), 3.48 (s, 3H, N-CH_3), 3.23–3.18 (m, 2H, S-CH_2), 2.36 (s, 3H, Ben-CH_3), 1.76 (dt, $J = 14.8$, 7.4 Hz, 4H, S-CH_2CH_2 and N^+-CH_2CH_2), 1.44–1.28 (m, 8H, S-$(CH_2)_2CH_2CH_2CH_2CH_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.9, 164.3, 142.3, 129.9, 126.6, 120.8, 120.0, 65.5, 60.9, 59.9, 47.5, 32.5, 29.2, 28.9, 28.6, 28.3, 26.2, 21.9, 21.7; HRMS (ESI) $[M\text{-}Br]^+$ calcd for $C_{22}H_{34}N_3O_2S$: 404.2366, found: 404.2366.

*2.18. 4-Methyl-4-(10-((5-(p-tolyl)-1,3,4-oxadiazol-2-yl)thio) octyl)morpholin-4-ium Bromide (**3k**).* A white solid, m. p. 136.5–137.4°C, yield 66%; ^{1}H NMR (500 MHz, CD_3OD) δ 7.83 (d, $J = 8.1$ Hz, 2H, Ben-H), 7.35 (d, $J = 7.9$ Hz, 2H, Ben-H), 4.02–3.95 (m, 4H, morpholine-H), 3.47 (m, $J = 8.8$, 3.7 Hz, 6H, morpholine-H and N^+-CH_2), 3.28 (dd, $J = 7.6$, 4.5 Hz, 2H, S-CH_2), 3.21 (s, 3H, N-CH_3), 2.40 (s, 3H, Ben-CH_3), 1.80 (dt, $J = 14.8$, 7.4 Hz, 4H, S-CH_2CH_2 and N^+-CH_2CH_2), 1.45 (dd, $J = 14.0$, 6.6 Hz, 2H, S-$(CH_2)_2CH_2$), 1.35 (d, $J = 24.5$ Hz, 10H, S-$(CH_2)_2CH_2CH_2CH_2CH_2CH_2$); ^{13}C NMR (126 MHz, CD_3OD) δ 166.0, 164.8, 142.8, 129.7, 126.3, 120.5, 65.4, 60.3, 59.7, 46.0, 32.1, 29.2, 29.0, 28.8, 28.7, 28.1, 26.0, 21.3, 20.3; HRMS (ESI) $[M\text{-}Br]^+$ calcd for $C_{24}H_{38}N_3O_2S$: 432.2679, found: 432.2679.

*2.19. 4-Methyl-4-(12-((5-(p-tolyl)-1,3,4-oxadiazol-2-yl)thio) octyl)morpholin-4-ium Bromide (**3l**).* A white solid, m. p. 141.5–144.1°C, yield 65%; ^{1}H NMR (500 MHz, CD_3OD) δ 7.83 (d, $J = 8.2$ Hz, 2H, Ben-H), 7.35 (d, $J = 7.9$ Hz, 2H, Ben-H), 3.99 (d, $J = 10.5$ Hz, 4H, morpholine-H), 3.51–3.44 (m, 6H, morpholine-H and N^+-CH_2), 3.29–3.26 (m, 2H, S-CH_2), 3.21 (s, 3H, N-CH_3), 2.40 (s, 3H, Ben-CH_3), 1.79 (dd, $J = 14.7$, 7.3 Hz, 4H, S-CH_2CH_2 and N^+-CH_2CH_2), 1.44 (dd, $J = 15.0$, 7.4 Hz, 2H, S-$(CH_2)_2CH_2$), 1.39–1.27 (m, 14H, S-$(CH_2)_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2$); ^{13}C NMR (126 MHz, CD_3OD) δ 165.9, 164.8, 142.8, 129.7, 126.3, 120.4, 60.3, 59.7, 32.1, 29.3, 29.2, 29.2, 28.9, 28.8, 28.2, 26.1, 21.3, 20.4; HRMS (ESI) $[M\text{-}Br]^+$ calcd for $C_{26}H_{42}N_3O_2S$: 460.2992, found: 460.2992.

*2.20. 4-Methyl-4-(8-((5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl) thio)octyl)morpholin-4-ium Bromide (**3m**).* A brown oily liquid, yield 64%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.82 (t, $J = 1.9$ Hz, 1H, Ben-H), 8.37 (dd, $J = 6.4$, 1.6 Hz, 1H, Ben-H), 8.36 (d, $J = 4.1$ Hz, 1H, Ben-H), 7.72 (t, $J = 8.0$ Hz, 1H, Ben-H), 3.40 (t, $J = 6.8$ Hz, 3H, morpholine-H), 3.35–3.29 (m, 3H, morpholine-H), 1.92–1.86 (m, 2H, morpholine-H), 3.64 (t, $J = 10.8$ Hz, 2H, N^+-CH_2), 1.84 (dd, $J = 9.7$, 4.6 Hz, 3H, N-CH_3), 1.49 (d, $J = 7.5$ Hz, 2H, S-CH_2), 1.44 (dd, $J = 15.7$, 9.1 Hz, 4H, S-CH_2CH_2 and N^+-CH_2CH_2), 1.36 (dt, $J = 9.5$, 6.5 Hz, 8H, S-$(CH_2)_3CH_2CH_2CH_2CH_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.0, 163.8, 148.7, 147.1, 132.2, 130.5, 126.1, 125.4, 121.6, 34.1, 32.8, 32.7, 29.2, 28.9, 28.7, 28.6, 28.1; HRMS (ESI) $[M\text{-}Br]^+$ calcd for $C_{21}H_{31}N_4O_4S$: 435.2061, found: 435.2061.

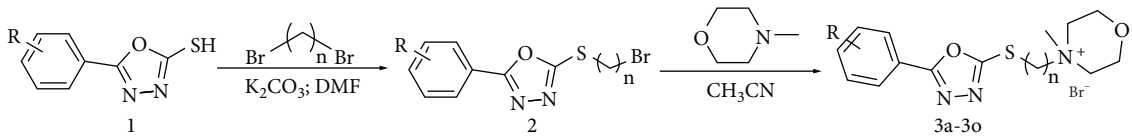
*2.21. 4-Methyl-4-(10-((5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl)thio)octyl)morpholin-4-ium Bromide (**3n**).* A white solid, m. p. 72.7–74.9°C, yield 64%; ^{1}H NMR (500 MHz, CD_3OD) δ 8.75–8.74 (m, 1H, Ben-H), 8.42 (dd, $J = 9.2$, 2.3 Hz, 1H, Ben-H), 8.36–8.34 (m, 1H, Ben-H), 7.83 (t, $J = 8.1$ Hz, 1H, Ben-H), 3.99 (d, $J = 9.5$ Hz, 4H, morpholine-H), 3.51–3.45 (m, 6H, morpholine-H and N^+-CH_2), 3.34–3.31 (m, 2H, S-CH_2), 3.21 (s, 3H, CH_3), 1.88–1.79 (m, 4H, S-CH_2CH_2 and N^+-CH_2CH_2), 1.51–1.46 (m, 2H, S-$(CH_2)_2CH_2$), 1.37 (dd, $J = 11.9$, 8.5 Hz, 10H, S-$(CH_2)_3CH_2CH_2CH_2CH_2CH_2$); ^{13}C NMR (126 MHz, CD_3OD) δ 166.3, 164.1, 131.9, 130.8, 126.0, 125.0, 120.9, 120.0, 60.3, 59.7, 59.7, 59.5, 32.1, 29.2, 29.1, 29.0, 28.9, 28.7, 28.2, 26.0, 21.2; HRMS (ESI) $[M\text{-}Br]^+$ calcd for $C_{23}H_{35}N_4O_4S$: 463.2374, found: 463.2373.

*2.22. 4-Methyl-4-(12-((5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl)thio)octyl)morpholin-4-ium Bromide (**3o**).* A white solid, m. p. 72.7–74.9°C, yield 64%; ^{1}H NMR (500 MHz, CD_3OD) δ 8.76 (d, $J = 1.8$ Hz, 1H, Ben-H), 8.45–8.41 (m, 1H, Ben-H), 8.36 (d, $J = 7.9$ Hz, 1H, Ben-H), 7.83 (t, $J = 8.1$ Hz, 1H, Ben-H), 3.98 (s, 4H, morpholine-H), 3.47 (m, $J = 12.7$, 8.3, 5.0 Hz, 6H, morpholine-H and N^+-CH_2), 3.29 (dd, $J = 2.9$, 1.4 Hz, 2H, S-CH_2), 3.19 (s, 3H, CH_3), 1.84 (dd, $J = 14.9$, 7.4 Hz, 4H, S-CH_2CH_2 and N^+-CH_2CH_2), 1.50–1.45 (m, 2H, S-$(CH_2)_2CH_2$), 1.31 (s, 14H, S-$(CH_2)_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2$); ^{13}C NMR (126 MHz, CD_3OD) δ 166.3, 164.1, 148.8, 131.9, 130.8, 126.0, 125.0, 120.9, 65.4, 60.3, 59.7, 45.9, 32.2, 29.3, 29.2, 28.9, 28.8, 28.2, 26.1, 21.2; HRMS (ESI) $[M\text{-}Br]^+$ calcd for $C_{25}H_{39}N_4O_4S$: 491.2687, found: 491.2679.

3. Results and Discussion

*3.1. Synthesis of Target Compounds **3a–3o**.* As shown in Figure 2, the starting material **1** was reacted with the dibromo alkane to give a key intermediate **2** bearing a bromine atom at the tail. Later, the target compounds **3a–3o** were synthesized through the substitution reaction between intermediate **2** and 4-methylmorpholine in the solvent CH_3CN at 85°C for 8 hours. Finally, all the molecular structures were characterized by using the correlative NMR and HRMS analyses (Figures S1–S45, supporting information).

*3.2. Antibacterial Activities of Compounds **3a–3o** and Structure-Activity Relationship (SAR).* The turbidimetric method was used to screen the antibacterial activity against pathogens *Xoo* and *Xac* [41–43]. Meanwhile, the commercial agrochemicals bismerthiazol (BT) and thiadiazole copper (TC) were used as the positive controls. Bioassay results showed that most of these designed molecules displayed good to excellent antibacterial functions (Table 1), indicating that the introduction of *N*-methylmorpholinium pattern could still endow the final target compounds with appreciable bactericidal effects. Among them, compounds **3l** and **3i** displayed the strongest antibacterial activity toward pathogens *Xoo* and *Xac* with the lowest EC_{50} values of 1.40 and 0.90 μg/mL, respectively. Those values were quite



3a: n=8, R=H
3b: n=10, R=H
3c: n=12, R=H
3d: n=8, R=2-Cl
3e: n=10, R=2-Cl
3f: n=12, R=2-Cl
3g: n=8, R=3-Cl
3h: n=10, R=3-Cl
3i: n=12, R=3-Cl
3j: n=8, R=4-CH_3
3k: n=10, R=4-CH_3
3l: n=12, R=4-CH_3
3m: n=8, R=3-NO_2
3n: n=10, R=3-NO_2
3o: n=12, R=3-NO_2

Figure 2: Synthetic route for target compounds **3a–3o**.

Table 1: *In vitro* antibacterial effects of compounds **3a–3o** against *Xoo* and *Xac*.

Compd.	*Xoo*			*Xac*		
	Regression equation	R^2	EC_{50} (μg/mL)	Regression equation	R^2	EC_{50} (μg/mL)
3a	$y = 7.381x - 3.59$	0.88	14.56 ± 1.03	$y = 1.281x + 3.60$	0.97	12.41 ± 0.57
3b	$y = 1.632x + 3.78$	0.95	5.65 ± 0.88	$y = 1.300x + 4.38$	0.95	2.99 ± 0.26
3c	$y = 6.824x + 3.43$	0.84	1.70 ± 0.01	$y = 4.201x + 4.13$	0.87	1.61 ± 0.06
3d	$y = 4.435x + 1.40$	0.84	6.50 ± 0.30	$y = 1.183x + 4.01$	0.98	6.98 ± 1.33
3e	$y = 4.424x + 3.77$	0.91	1.90 ± 0.10	$y = 2.481x + 3.87$	0.92	2.86 ± 0.17
3f	$y = 7.873x + 3.00$	0.98	1.80 ± 0.14	$y = 2.881x + 4.40$	0.95	1.61 ± 0.10
3g	$y = 13.262x - 8.20$	0.98	9.90 ± 0.18	$y = 2.972x + 2.26$	0.92	8.36 ± 0.44
3h	$y = 9.761x + 0.11$	0.97	3.17 ± 0.42	$y = 3.600x + 3.09$	0.95	3.39 ± 0.14
3i	$y = 10.972x + 0.12$	0.95	2.80 ± 0.24	$y = 6.021x + 5.26$	0.83	0.90 ± 0.01
3j	$y = 2.661x + 2.47$	0.87	8.90 ± 0.81	$y = 4.173x + 0.47$	0.92	12.24 ± 0.42
3k	$y = 4.711x + 2.58$	0.95	3.27 ± 0.34	$y = 0.731x + 4.39$	0.99	6.92 ± 0.93
3l	$y = 6.352x + 4.07$	0.96	1.40 ± 0.06	$y = 6.572x + 3.94$	0.97	1.45 ± 0.04
3m	$y = 2.632x + 0.62$	0.94	45.98 ± 4.70	$y = 1.200x + 2.69$	0.99	84.52 ± 5.04
3n	$y = 7.884x - 2.02$	0.89	7.79 ± 0.33	$y = 3.300x + 2.69$	0.97	5.03 ± 0.57
3o	$y = 7.532x + 3.12$	0.94	1.78 ± 0.05	$y = 5.321x + 3.79$	0.99	1.69 ± 0.04
BT	$y = 4.783x - 2.367$	0.94	34.69 ± 2.15	$y = 2.241x + 0.57$	0.94	94.96 ± 7.29
TC	$y = 2.675x + 0.107$	0.93	67.45 ± 3.6	$y = 2.152x + 0.94$	0.96	77.04 ± 1.96

superior to those of BT (34.69 and 94.96 μg/mL) and TC (67.45 and 77.04 μg/mL). Meanwhile, compounds **3c**, **3e**, **3f**, **3h**, **3i**, **3k**, and **3o** gave excellent anti-*Xoo* activity with EC_{50} values within 1.70–3.27 μg/mL. For the anti-*Xac* activity, compounds **3b**, **3c**, **3e**, **3f**, **3h**, **3l**, and **3o** provided the EC_{50} values ranging from 1.45 to 3.39 μg/mL. The SAR was summarized as follows: (1) Increasing the alkyl chain lengths can enhance the anti-*Xoo* and anti-*Xac* activity, illustrated by the bioactivity comparison of compounds **3a–3c**, **3d–3f**, **3g–3i**, **3j–3l**, and **3m–3o** with different alkyl chains. (2) Introducing an electron-donating group (4-CH_3, **3l**) provides the best anti-*Xoo* activity than those compounds bearing electron-withdrawing groups (2-Cl, **3f**; 3-Cl, **3i**; 3-NO_2, **3o**). (3) Fusion of a meta-position chlorine atom (**3i**) displays better anti-*Xac* activity than that of compounds with an *ortho*-position chlorine atom (**3f**) and a *meta*-position nitro group (**3o**). Given the above analysis, compounds **3l** and **3i** with the best antibacterial ability should be further studied.

3.3. Phytotoxicity Study, In Vivo Antibacterial Activity, and Antibacterial Mechanism. Phytotoxicity test trials at 200 μg/mL showed that the bioactive compound **3l** gave a lower phytotoxicity against rice leaves (Figure 3), indicating that this kind of compounds bearing a bulky *N*-methylmorpholinium pendant are safe for rice plants. The following *in vivo* bioassays showed that compound **3l** could control the rice bacterial leaf blight disease and thus providing good control efficiencies of 55.95% (curative activity) and 53.09% (protective activity) at the dose of 200 μg/mL (Table 2 and Figure 4). Those data were superior to those of TC (37.53% and 36.68%), suggesting that this kind of 1,3,4-oxadiazole-tailored *N*-methylmorpholinium derivatives can serve as safe quaternary ammonium bactericides for controlling plant bacterial diseases. The preliminary antibacterial mechanism was investigated via scanning electron microscopy imaging. As displayed in Figure 5, the morphology of *Xoo* cells were transformed from well shaped to corrugated and/or broken after treating *Xoo* with compound **3l** (14 μg/mL, 10 × EC_{50}), suggesting that target compounds had strong interactions with the cell membrane of bacteria.

3.4. Antifungal Activities. The antifungal activity against two kinds of plant fungal pathogens was screened by the mycelium growth rate method [49], while hymexazol was coassayed for comparison. Table 3 shows that some target compounds were identified with good antifungal activities at 25.0 μg/mL. Among them, the inhibition rate of compounds

(a) (b)

Figure 3: Phytotoxicity studies and images after incubation with different concentrations of compound **3l** (treatment for 7 days): (a) 0 μg/mL and (b) 200 μg/mL.

Table 2: *In vivo* bioactivity of compound **3l** against rice bacterial blight at 200 μg/mL (14 days after spraying).

Chemicals	Protective activity			Curative activity		
	Morbidity (%)	Disease index (%)	Control efficiency (%)[b]	Morbidity (%)	Disease index (%)	Control efficiency (%)[b]
3l	100	41.06	53.09A	100	39.56	55.95A
TC	100	55.42	36.68B	100	54.68	37.53B
CK[a]	100	87.53		100	87.53	

[a]Negative control. [b]Statistical analysis was conducted using ANOVA under the condition of equal variances assumed ($P > 0.05$) and equal variances not assumed ($P < 0.05$). Different uppercase letters indicate the values of bioactivity with significant differences among different treatment groups at $P < 0.05$.

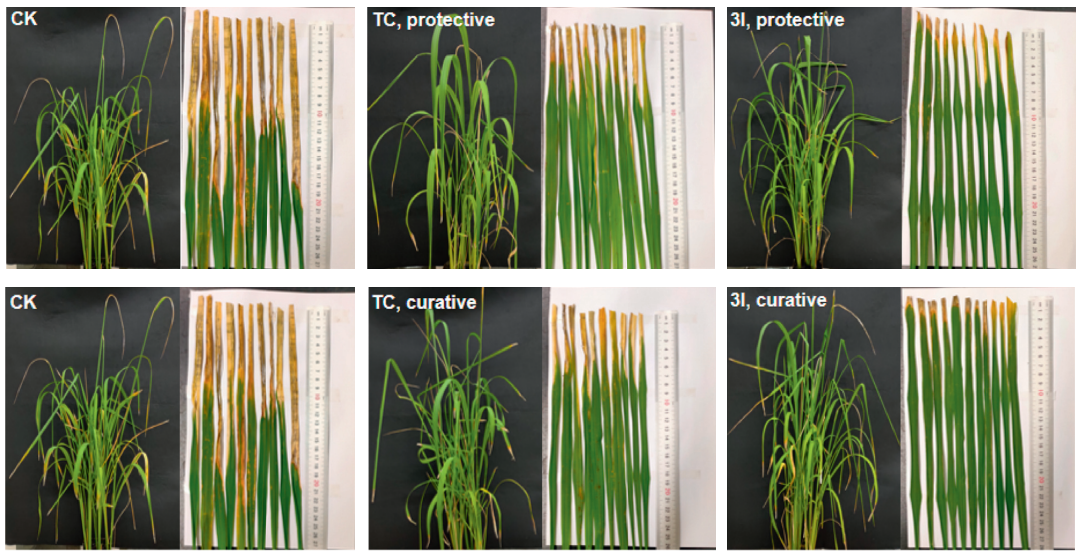


Figure 4: Protective and curative effects of compound **3l** against rice bacterial blight at 200 μg/mL.

3b, **3c**, **3f**, **3h**, **3i**, and **3o** against the *F. oxysporum* strain was 43.45–59.52%, which was superior to the positive drug hymexazol (39.22%). For the anti-*P. cinnamomi* activity, compounds **3e** and **3f** exhibited the inhibitory effects of 50.16% and 44.98%, respectively. This outcome manifests that this kind of 1,3,4-oxadiazole-tailored *N*-methylmorpholinium derivatives also can serve as antifungal leads for the future design of novel fungicides.

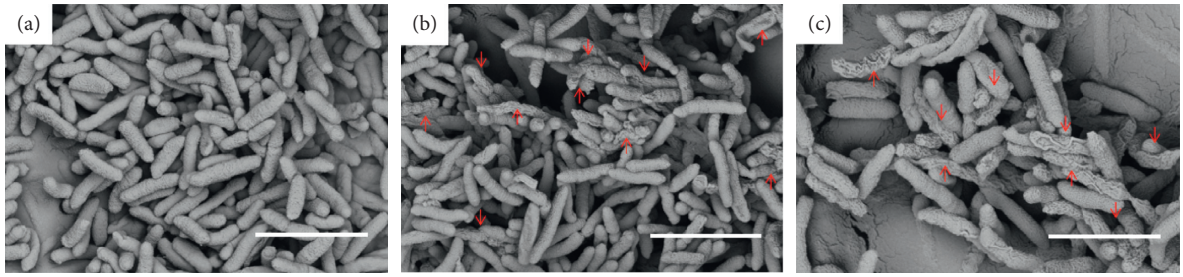


Figure 5: SEM images of *Xoo* after incubation with different concentrations of compound **3l**: (a) 0 μg/mL and (b, c) 14 μg/mL; scale bars for (a-b) are 3 μm and scale bar for (c) is 2 μm.

Table 3: Inhibition rates of **3a–3o** against two fungal strains at 25.0 μg/mL.

Compd.	Inhibition rate (%)	
	F. oxysporum	*P. cinnamomi*
3a	21.13 ± 1.87	0
3b	43.45 ± 5.18	0
3c	49.70 ± 0.63	0
3d	17.86 ± 0.90	13.11 ± 0.69
3e	32.14 ± 1.80	50.16 ± 7.29
3f	54.46 ± 3.11	44.98 ± 1.48
3g	27.23 ± 0.63	16.18 ± 4.98
3h	50.60 ± 3.63	37.86 ± 8.24
3i	51.79 ± 4.66	36.25 ± 4.79
3j	24.11 ± 0.10	0
3k	31.55 ± 1.37	15.86 ± 0.56
3l	21.43 ± 0.90	0
3m	13.39 ± 1.80	0
3n	26.19 ± 2.26	14.24 ± 0.56
3o	59.52 ± 1.87	28.16 ± 2.57
Hymexazol	39.22 ± 1.01	41.50 ± 1.21

4. Conclusions

In summary, a novel type of *N*-methylmorpholinium derivatives bearing a typical 1,3,4-oxadiazole scaffold was prepared to explore a kind of quaternary ammonium compounds that could safely apply in agriculture for controlling the plant bacterial diseases. *In vitro* bioassay screening results showed that compounds **3l** and **3i** displayed the best antibacterial activity toward pathogens *Xoo* and *Xac* with the minimal EC_{50} values of 1.40 and 0.90 μg/mL, respectively. Interestingly, this kind of *N*-methylmorpholinium salts gave the lower phytotoxicity toward rice plants. *In vivo* bioassays revealed that the bioactive compound **3l** could control the rice bacterial blight disease and thus providing good curative activity and protective activity of 55.95% and 53.09% at the dose of 200 μg/mL, respectively. Given the above superiority, 1,3,4-oxadiazole-tailored *N*-methylmorpholinium compounds could be further investigated as safe quaternary ammonium bactericides for managing plant bacterial diseases.

Data Availability

The data supporting these results are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Xinxin Tuo contributed to investigation, data curation, formal analysis, and methodology and prepared the original draft. Jie Yang and Yedong Zhang contributed to data curation, formal analysis, and methodology. Peiyi Wang contributed to data curation, supervision, and formal analysis and reviewed and edit the manuscript.

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Supplementary Materials

The supplementary data contain the related NMR and HRMS spectra of the target compounds **3a–3o** and statistical results for the bioactivity by using ANOVA and post hoc analysis. . (*Supplementary Materials*)

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