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Synthesis and Biological Evaluation of Novel Sulfone Derivatives Containing 1,3,4-Oxadiazole Moiety

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Abstract A series of novel sulfone derivatives containing 1,3,4-oxadiazole moiety were synthesized. All the target com-pounds were characterized by ^{1}H and ^{13}C nuclear magnetic resonance, infrared spectroscopy and elemental analysis. Their antifungal activities were tested *in vitro* with six important phytopathogenic fungi, namely, *Gibberella zeae, Fusarium oxysporum, Cytospora mandshurica, Phytophthora infestans, Paralepetopsis sasakii* and *Sclerotinia sclerotiorum* using the mycelium growth inhibition method. Their antibacterial activities were tested *in vitro* with two important phytopathogenic bacteria, namely, *Xanthomonas oryzae* and *Ralstonia solanacearum* from tobacco bacterial by the turbid meter test. Remarkably, compounds **5h**, **5j**, **5u** and **5v** exhibited the most potent inhibition against *R. solanacearum* and *X. oryzae* with 50% inhibition concentration (EC_{50}) from 1.97 to 7.75 μg/mL and 0.45 to 0.52 μg/mL, respectively. Their antifungal tests indicated that among target compounds exhibited good antifungal activities against six kinds of fungi, especially against *S. sclerotiorum* with EC*50* from 3.71 to 17.44 μg/mL. *In vivo* antibacterial activities tests demonstrated that the controlling effect of compounds **5u** (81.9%) against rice bacterial leaf blight were better than that of bismerthiazol (50.8%) and thiodiazole-copper (44.7%). Our results also demonstrated that com-pounds **5h**, **5u** and **5v** have a better antifungal and antibacterial activity, with good characteristics of broad spectrum. The structure−activity relationship (SAR) was also discussed.

Keywords: *antibacterial activity, antifungal activities, sulfone derivatives, 1,3,4-Oxadiazole*

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1. Introduction

Agricultural security and food security are always an issue of supreme importance. Ensuring the global food security has long been a big challenge in the agricultural sector. The constantly outbreak of fungal and bacterial pathogens of the genera *Gibberella zeae, Cytospora mandshurica, Cytospora mandshurica, Cytospora mandshurica, Sclerotinia Chinasclerotiorum, Fusarium oxysporum, Xanthomonas oryzae* and *Ralstonia solanacearum* have become a matter of great attention throughout the world [1]. The application of current commercial pesticides was not effective as expected, while it was accompanied with high resistance and high residue level or negative impact on the environment. Therefore, the search for new antifungal and antibacterial agents, which are more effective, environment-friendly and lower toxic, still remains a difficult task and greatly needed in the field of agricultural fungicide.

Sulfone derivatives have a wide range of biological activities including antibacterial, antifungal, insecticidal, herbicidal in pesticide chemistry [2,3,4,5], and anticancer, anti-HIV-1, antihepatitis, antitumor, and anti-inflammatory activities in medicinal chemistry [6-10]. 2, 5-disubstituted-1,3,4-oxadiazole derivatives are reported to exhibit a wide range of biological especially antifungal and antibacterial activity [11,12]. 2-(Benylsulfonyl)-5-(m-chlorophenoxymethyl)-1,3,4-oxadiazole, which was prepared according to Giri *et al* [13], exhibits high activity against *A. niger* and *A. flavus*. Kleefeld [14] reported that methyl-5-(4-chlorophenyl)-1,3,4-oxadiazole sulfones had antifungal activity against *B. cinerea* at the concentration of 100 μg/mL. Moreover, 2-(5-ethyl-1-methyl-1H-pyrazol-3-yl)-5-(methylsulfonyl)-1,3,4-oxadiazole exhibited medium inhibitory activity against *P. asparagi* [15].

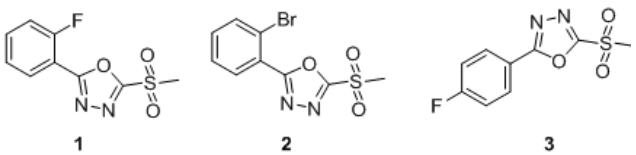
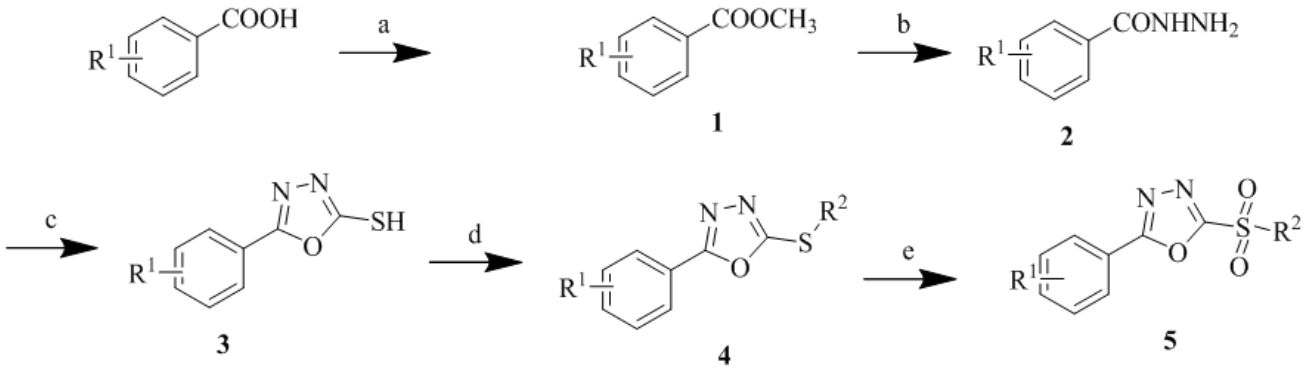


Figure 1. Compounds strunture

In our previous work, a series of sulfone derivatives of 2-(sulfonyl)-5-(mono-substituted benzene)-1,3,4-oxadiazole demonstrated antifungal and antibacterial activities. *In vitro* bioactivity indicated that compound 1 and 2 [16] possess high antifungal activities against eight kinds of

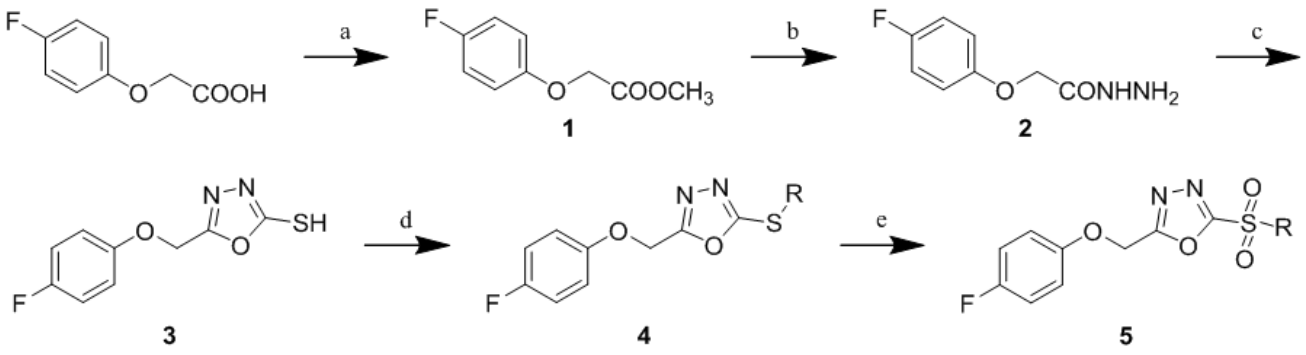
fungi at the concentration of 50 μg/mL, and the EC_{50} of 3 [17] against Tobacco Bacterial was 32.1 μg/mL (Figure 1). Structure-activity relationship (SAR) analyses suggested compounds with 2-(methylsulfonyl)-5-(mono-substituted benzene)-1,3,4-oxadiazole moiety had higher activities than those with poly-substituents benzene.

The SAR of early synthesized compounds suggested that 2-(methylsulfonyl)-1,3,4-oxadiazole or 2-(ethylsulfonyl)-1,3,4-oxadiazole affords antifungal activities higher than 2-(phenylsulfonyl) -1,3,4-oxadiazole or 2-(yl)-1,3,4-thiadiazole [18,19]. The further research for novel sulfone derivatives, with illustration of SAR, a series of novel sulfone derivatives containing 1,3,4-oxadiazole moiety were synthesized from disubstituted and trifluoromethyl-substituted benzoic acid (Figure 2) and 4-fluorophenoxyacetic acid (Figure 3) via reactivity-selectivity principle. Their antibacterial and antifungal activities on selected target phytopathogenic fungi and bacteria were investigated, and their SAR was also discussed. To our knowledge, the antibacterial and antifungal activities of all the synthetic derivatives including known compounds were reported for the first time.



5a: R^1=4-F-2-CH_3, R^2=CH_3; **5b**: R^1=4-F-2-CH_3, R^2=CH_2CH_3; **5c**: R^1=2,5-di-Cl, R^2=CH_3;
5d: R^1=2,5-di-Cl, R^2=CH_2CH_3; **5e**: R^1=2,5-di-Cl, R^2=$CH(CH_3)_2$; **5f**: R^1=2-Br-4-F, R^2=CH_3;
5g: R^1=2-Br-4-F, R^2=CH_2CH_3; **5h**: R^1=2-Cl-4-F, R^2=CH_3; **5i**: R^1=2-Cl-4-F, R^2=CH_2CH_3;
5j: R^1=4-CF_3, R^2=CH_3; **5k**: R^1=4-CF_3, R^2=CH_2CH_3; **5l**: R^1=4-Br-2-F, R^2=CH_3;;
5m: R^1=4-Br-2-F, R^2=CH_2CH_3; **5n**: R^1=2-Br-5-F, R^2=CH_3; **5o**: R^1=2-Br-5-F, R^2=CH_2CH_3;
5p: R^1=2-Br-5-F, R^2=$CH_2CH_2CH_3$; **5q**: R^1=5-Br-2-F, R^2=CH_3; **5r**: R^1=5-Br-2-F, R^2=CH_2CH_3;
5s: R^1=3-Br-5-CF_3, R^2=CH_3; **5t**: R^1=3-Br-5-CF_3, R^2=CH_2CH_3;

Figure 2. Synthesis routes of sulfone derivatives containing 1,3,4-oxadiazole moiety. Reaction conditions and reagents: (a) MeOH, 98%H_2SO_4, reflux 5h; (b) NH_2NH_2•H_2O, EtOH, 25°C–reflux, 4h; (c) KOH, CS_2, EtOH, 25-46-76°C, 7h; (d) NaOH, H_2O, dimethyl(diethyl) sulfate or halide (RX), rt 4 h, 90%; (e) $KMnO_4$, AcOH, rt, 1 h



5u: R=CH_3; **5v**: R=CH_2CH_3; **5w**: R= Benzyl;

Figure 3. Synthesis routes of sulfone derivatives containing phenoxymethyl and 1,3,4-oxadiazole moiety. Reaction conditions and reagents: (a) MeOH, 98%H_2SO_4, reflux 5h; (b) NH_2NH_2•H_2O, EtOH, 25°C–reflux, 1h; (c)KOH, CS_2, EtOH, 25-46-76°C, 7h; (d) NaOH, H_2O, dimethyl(diethyl) sulfate or halide (RX), rt 4 h, 90%; (e) $KMnO_4$, AcOH, rt, 1 h

2. Experimental

2.1. Materials and Instrumentation

^1H and ^{13}C NMR (solvent $CDCl_3$ or DMSO-d_6) spectral analyses were recorded on a JEOL-ECX 500 NMR spectrometer at ambient temperature using TMS as an internal standard. The IR spectra were obtained from a KBr pellet using a Bruker VECTOR 22 spectrometer. The melting points of the compounds were determined on an XT-4 digital microscope apparatus (Beijing Tech Instrument Co.) and were uncorrected. Elemental analysis was performed on an Elemental Vario-III CHN analyzer.

Analytical thin-layer chromatography was performed on silica gel 60 GF_{254} (Qingdao Haiyang Chemical Co.). All reagents were of analytical grade or chemically pure.

2.2. General Synthetic Procedures for Target Molecules

Target compounds 5a-t were synthesized as shown in Figure 2 using previously reported methods [13], and compounds 5u-w were synthesized as shown in Figure 3 [16]. Substituted methyl benzoates 1 were produced when substituted benzoic acid was heated with absolute methanol in the presence of 98% H_2SO_4. Then substituted methyl benzoates 1 was treated with hydrazine hydrate in methanol under reflux condition to form substituted benzoyl hydrazines 2, 2-mercapto-5-substituted-1,3,4-oxadiazoles 3 was easily prepared by the reaction of substituted benzoyl hydrazines 2, potassium hydroxide, and carbon disulfide in ethanol under reflux conditions, then, 2-mercapto-5-substituted-1,3,4-oxadiazoles 3 was converted to thioether derivatives 4 by a thioetherification reaction with dimethyl(diethyl) sulfate or halide (RX). Treatment of thioether derivatives 4 by $KMnO_4$ afforded the target compounds 5. The compounds were synthesized from substituted benzoic acid by five steps following esterification, hydrazidation, cyclization, thioetherification and oxidation. The structures of the target compounds were confirmed by ^1H and ^{13}C nuclear magnetic resonance, infrared spectroscopy and elemental analysis.

2.3. General Procedure for the Preparation of 4

To a solution of 2-thiol-5-substituted-1,3,4-oxadiazole 3 (4 mmol) and sodium hydroxide (0.16 g, 4 mmol) in water (10 mL), dimethyl sulfate (0.64 g, 5 mmol) was added dropwise. After stirring for 2 h at room temperature, for solid products, the precipitate was filtered off, washed with distilled water, dried to afford the white solid products 4; for liquid products, the reaction mixture was washed with saturation sodium chloride solution, and then extracted with ethyl ether (3 × 20 mL), the ethyl ether layer was dried with anhydrous Na_2SO_4, after removal of the organic solvent under reduced pressure, and oily products 4 were thus obtained.

2.4. General Procedure for the Preparation of 5a-w

The parent compound 4 (5 mmol) and acetic acid (15 mL) were added to a 50 mL three-neck round-bottom flask equipped with a magnetic stirrer. The resulting solution was stirred for 10 min, and then 7% $KMnO_4$ solution (5 mmol) was slowly added. The unreacted potassium permanganate was deoxidized by adding 10% sodium bisulfite solution, and reaction mixtures were subsequently dried under vacuum. Compounds 5a-w was obtained by recrystallized from ethanol.

2.4.1. 2-(methylsulfonyl)-5-(4-fluoro-2-methylphenyl)-1,3,4-oxadiazole (5a)

White acicular crystal; Yield: 65%; m.p.: 120-121 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ: 2.75 (s, 3H), 3.54 (s, 3H),. 7.16-7.01 (m, 2H), 8.03 (dd, J = 8.7, 5.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 22.41, 43.01, 114.10 (d, J = 22.1 Hz), 117.59 (d, J = 3.0 Hz), 119.17 (d, J = 21.8 Hz), 132.19 (d, J = 9.6 Hz), 143.03 (d, J = 9.1 Hz), 162.89 (d, J = 279.2 Hz), 166.03, 166.16; IR (KBr) ν: 3030, 2927, 1527, 1456, 1338, 1107 cm^{-1}; Anal. Calcd for $C_{10}H_9FN_2O_3S$: C, 46.87; H, 3.54; N, 10.93; found: C, 46.79; H, 3.98; N, 11.01.

2.4.2. 2-(ethylsulfonyl)-5-(4-fluoro-2-methylphenyl)-1,3,4-oxadiazole (5b)

White acicular crystal; Yield: 49%; m.p.: 80-82 °C. ^1H NMR ($CDCl_3$,500 MHz) δ: 1.67-1.28 (t, J = 7.4 Hz, 3H), 2.76 (s, 3H), 3.63 (q, J = 7.4, 2.2 Hz, 2H), 7.17-7.03 (m, 2H), 8.09-7.89 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 6.97, 22.43, 50.12, 114.18, 117.64, 119.08, 132.16, 142.97, 160.91, 166.01, 166.20; IR (KBr) ν: 3030, 2972, 2927, 1507, 1388, 1134 cm^{-1}; Anal. Calcd for $C_{11}H_{11}FN_2O_3S$: C, 48.88; H, 4.10; N, 10.36; found: C, 48.58; H, 3.74; N, 10.20.

2.4.3. 2-(methylsulfonyl)-5-(2,5-Dichlorophenyl)-1,3,4-oxadiazole (5c)

White acicular crystal; Yield: 45%; m.p.: 117-119 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ: 3.56 (s, 3H), 7.54 (dd, J = 3.9, 1.3 Hz, 2H), 8.06 (d, J = 2.1 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 43.09, 122.71, 131.32, 132.26, 132.89, 133.64, 133.83, 162.69, 163.89; IR (KBr) ν: 3030, 2927, 1527, 1456, 1338, 1107 cm^{-1}; Anal. Calcd for $C_9H_6Cl_2N_2O_3S$: C, 36.88; H, 2.06; N, 9.56; found: C, 36.87; H, 2.31; N, 9.85.

2.4.4. 2-(ethylsulfonyl)-5-(2,5-Dichlorophenyl)-1,3,4-oxadiazole (5d)

White acicular crystal; Yield: 72%; m.p.: 101-103 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ: 1.72-1.35 (t, 3H), 3.63 (q, J = 14.9, 2H), 7.74-7.41 (m, 2H), 8.09-7.94 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 6.94, 50.26, 122.77, 131.31, 132.14, 132.87, 133.55, 133.79, 161.91, 163.94; IR (KBr) ν: 3064, 2926, 1543, 1473, 1334, 1157, 1041 cm^{-1}. Anal. Calcd for $C_{10}H_8Cl_2N_2O_3S$: C, 39.10; H, 2.63; N, 9.12; found: C, 38.98; H, 2.87; N, 9.37.

2.4.5. 2-(i-propylsulfonyl)-5-(2,5-Dichlorophenyl)-1,3,4-oxadiazole (5e)

White acicular crystal; Yield: 68%; m.p.: 108-110 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ: 3.72 (hept, J = 6.9 Hz, 1H), 1.55 (d, J = 6.9 Hz, 6H), 7.27 (d, J = 0.7 Hz, 1H), 7.59-7.48 (m, 1H), 8.09-8.04 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 15.13, 56.67, 122.86, 131.36, 132.18, 132.84, 133.65, 133.79, 161.44, 164.11; IR (KBr): 3091, 2895, 1531, 1456, 1361, 1134 cm^{-1}. Anal. Calcd for $C_{11}H_{10}Cl_2N_2O_3S$: C, 41.04; H, 3.14; N, 8.72; found: C: 40.52; H, 3.20; N: 8.76.

2.4.6. 2-(methylsulfonyl)-5-(2-bromo-4-fluorophenyl)-1,3,4-oxadiazole (5f)

White acicular crystal; Yield: 74%; m.p.: 135-136 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ: 3.55 (s, 3H), 7.26-7.23 (m, 1H), 7.56 (dd, J = 8.0, 2.5 Hz, 1H), 8.04 (dd, J = 8.8, 5.7 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 43.06, 115.79 (d, J = 21.9 Hz), 120.02 (d, J = 3.8 Hz), 122.65 (d, J = 24.8 Hz), 123.40 (d, J = 10.0 Hz), 133.94 (d, J = 9.6 Hz), 163.02 (d, J = 122.7 Hz), 164.82, 165.58; IR (KBr) ν: 3080, 2926, 1602, 1483, 1337, 1220, 1159 cm^{-1}. Anal. Calcd for $C_9H_6BrFN_2O_3S$: C, 33.66; H, 1.88; N, 8.72; found: C, 33.72; H, 2.10; N, 8.95.

2.4.7. 2-(ethylsulfonyl)-5-(2-bromo-4-fluorophenyl)-1,3,4-oxadiazole (5g)

White acicular crystal; Yield: 36%; m.p.: 76-77 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.56 (t, J = 7.4 Hz, 3H), 3.63 (q, J = 7.4 Hz, 2H), 7.30-7.20 (m, 1H), 7.56 (dd, J = 8.1, 2.4 Hz, 1H), 8.04 (dd, J = 8.7, 5.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 6.98, 50.22, 115.78 (d, J = 21.9 Hz), 120.09 (d, J = 4.2 Hz), 122.61 (d, J = 25.0 Hz), 123.35 (d, J = 10.1 Hz), 133.95 (d, J = 9.7 Hz), 162.62 (d, J = 219.8 Hz), 164.88, 165.56; IR (KBr) ν: 3078, 2945, 1597, 1337, 1269, 1139 cm^{-1}. Anal. calcd for $C_{10}H_8BrFN_2O_3S$: C, 35.84; H, 2.41; N, 8.36; found: C, 35.72; H, 2. 97; N, 8.83.

2.4.8. 2-(methylsulfonyl)-5-(2-chloro-4-fluorophenyl)-1,3,4-oxadiazole (5h)

White acicular crystal; Yield: 79%; m.p.: 116-117 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 3.63 (s, 3H), 7.30-7.20 (m, 1H), 7.56 (dd, J = 8.1, 2.4 Hz, 1H), 8.04 (dd, J = 8.7, 5.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 43.06, 115.32, 117.98, 119.38 (d, J = 25.4 Hz), 133.58 (d, J = 9.8 Hz), 135.73 (d, J = 10.3 Hz), 162.49, 163.82, 164.30; IR (KBr) ν: 3095, 2931, 1600, 1479, 1358, 1267, 1151 cm^{-1}; Anal. Calcd for $C_9H_6ClFN_2O_3S$: C, 39.07; H, 2.19; N, 10.13; found: C, 38.62; H, 2.53; N, 10.11.

2.4.9. 2-(ethylsulfonyl)-5-(2-chloro-4-fluorophenyl)-1,3,4-oxadiazole (5i)

White acicular crystal; Yield: 73%; m.p.: 74-76 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.59 – 1.52 (t, 3H), 3.62 (q, J = 7.5 Hz, 2H), 7.24 – 7.15 (m, 1H), 7.36 (dd, J = 8.3, 2.5 Hz, 1H), 8.08 (dt, J = 10.1, 5.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 6.96, 50.21, 115.40 (d, J = 22.0 Hz), 118.07 (d, J = 3.7 Hz), 119.36 (d, J = 25.2 Hz), 133.58 (d, J = 9.8 Hz), 135.69 (d, J = 10.9 Hz), 162.76 (d, J = 264.6 Hz), 164.37, 165.87; IR (KBr) ν: 3078, 2985, 1597, 1348, 1269, 1138 cm^{-1}. Anal. calcd for $C_{10}H_8ClFN_2O_3S$: C, 41.32; H, 2.77; N, 9.64; found: C, 40.85; H, 3.05; N, 9.75.

2.4.10. 2-(methylsulfonyl)-5-(4-(trifluoromethyl)phenyl)-1,3,4-oxadiazole (5j)

White acicular crystal; Yield: 82%; m.p.: 156-157 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 3.56 (s, 3H), 7.85 (d, J = 8.5 Hz, 2H), 8.28 (d, J = 8.1 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 43.08, 122.28, 124.45, 125.38, 126.56 (q, J = 3.8 Hz), 128.26, 134.77, 135.03, 162.68, 165.43; IR (KBr) ν: 3012, 2927, 1558, 1417, 1324, 1157 cm^{-1}; Anal. Calcd for $C_{10}H_7F_3N_2O_3S$: C, 41.10; H, 2.41; N, 9.59; found: C, 40.68; H, 2.72; N, 9.56.

2.4.11. 2-(ethylsulfonyl)-5-(4-(trifluoromethyl)phenyl)-1,3,4-oxadiazole (5k)

White acicular crystal; Yield: 85%; m.p.: 74-76 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.57 (t, J = 7.4 Hz, 3H), 3.65 (q, J = 7.4 Hz, 2H), 7.85 (d, J = 8.1 Hz, 2H), 8.29 (d, J = 8.1 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 6.95, 50.19, 126.54, 126.57, 128.25, 134.85, 135.05, 136.66, 161.92, 165.46; IR (KBr) ν: 3022, 2935, 1558, 1417, 1328, 1126 cm^{-1}. Anal. calcd for $C_{11}H_9F_3N_2O_3S$: C, 43.14; H, 2.96; N, 9.15; found: C, 42.62; H, 3.35; N,9.31.

2.4.12. 2-(methylsulfonyl)-5-(4-bromo-2-fluorophenyl)-1,3,4-oxadiazole (5l)

White acicular crystal; Yield: 73%; m.p.: 126-127 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 3.54 (s, 3H), 7.53 (d, J = 8.8 Hz, 2H), 8.00 (t, J = 7.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 43.10, 109.91 (d, J = 11.8 Hz), 121.23 (d, J = 23.5 Hz), 128.79 (d, J = 3.8 Hz), 128.96 (d, J = 9.2 Hz), 131.03 (d, J = 1.3 Hz 162.87), 160.08 (d, J = 265.8 Hz), 162.48; IR (KBr) ν: 3010, 2927, 1610, 1475, 1348, 1155 cm^{-1}. Anal. calcd for $C_9H_6BrFN_2O_3S$: C, 33.66; H, 1.88; N, 8.72; found: C: 33.87; H, 1.95; N, 9.10.

2.4.13. 2-(ethylsulfonyl)-5-(4-bromo-2-fluorophenyl)-1,3,4-oxadiazole (5m)

White acicular crystal; Yield: 66%; m.p.: 76-77 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.56 (t, J = 7.5 Hz, 3H), 3.63 (q, J = 7.4 Hz, 2H), 7.53 (d, J = 8.6 Hz, 2H), 8.01 (t, J = 7.7 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 6.94, 50.21, 109.99 (d, J = 11.6 Hz), 121.22 (d, J = 23.7 Hz), 128.77 (d, J = 3.9 Hz), 128.91 (d, J = 9.2 Hz), 131.03 (d, J = 1.6 Hz), 160.08 (d, J = 265.7 Hz), 161.69, 162.91; IR (KBr) ν: 3097, 2937, 1608, 1469, 1226, 1148 cm^{-1};Anal. calcd for $C_{10}H_8BrFN_2O_3S$: C, 35.84; H, 2.41; N, 8.36; found: C,35.88; H, 2.61; N, 8.27.

2.4.14. 2-(methylsulfonyl)-5-(2-bromo-5-fluorophenyl)-1,3,4-oxadiazole (5n)

White acicular crystal; Yield: 48%; m.p.: 132-133 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 3.56 (s, 3H), 7.23 (dd, J = 11.3, 4.5 Hz, 1H), 7.85-7.71 (m, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 43.08, 116.71 (d, J = 3.8 Hz), 119.21 (d, J = 25.7 Hz), 121.41 (d, J = 22.2 Hz), 124.85 (d, J = 8.2 Hz), 136.74 (d, J = 8.0 Hz), 161.54 (d, J = 250.4 Hz), 162.70, 164.48; IR (KBr) ν: 3061, 2922, 1541, 1475, 1224, 1161 cm^{-1}; Anal. calcd for $C_9H_6BrFN_2O_3S$: C, 33.66; H, 1.88; N, 8.72; found: C, 33.14; H, 2.03; N, 8.75.

2.4.15. 2-(ethylsulfonyl)-5-(2-bromo-5-fluorophenyl)-1,3,4-oxadiazole (5o)

White acicular crystal; Yield: 65%; m.p.: 76-77 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.56-1.55 (m, 3H), 3.65-3.61 (m, 2H), 7.55 (s, 1H), 8.03 (d, J = 5.7 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 6.93, 50.23, 112.60 (d, J = 12.8 Hz), 117.58 (d, J = 3.7 Hz), 119.23 (d, J = 22.2 Hz), 132.68, 138.02 (d, J = 8.7 Hz), 159.48 (d, J = 261.9 Hz), 161.84, 162.26; IR (KBr) ν: 3028, 2989, 1573, 1485, 1263, 1128 cm^{-1}; Anal. calcd for $C_{10}H_8BrFN_2O_3S$: C, 35.84; H, 2.41; N, 8.36; found: C, 35.76; H, 2.46; N,8.55.

2.4.16. 2-(n-propylsulfonyl)-5-(2-bromo-5-fluorophenyl)-1,3,4-oxadiazole (5p)

White acicular crystal; Yield: 45%; m.p.: 111-113 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.16 (t, J = 7.4 Hz, 3H), 2.03 (dq, J = 15.1, 7.4 Hz, 2H), 3.63-3.52 (m, 2H), 7.38-7.13 (m, 1H), 7.85-7.67 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 12.94, 16.10, 57.11, 116.67 (d, J = 3.7 Hz), 119.22 (d, J = 25.6 Hz), 120.00, 121.36 (d, J = 22.2 Hz), 124.95 (d, J = 8.5 Hz), 136.70 (d, J = 7.8 Hz), 161.42 (d, J = 220.8 Hz), 162.54, 164.49; IR (KBr) ν: 3086, 2956, 1544, 1475, 1259, 1150 cm^{-1}; Anal. Calcd for $C_{11}H_{10}BrFN_2O_3S$: C, 37.84; H, 2.89; N, 8.02; found: C, 37.41; H, 3.18; N, 8.15.

2.4.17. 2-(methylsulfonyl)-5-(5-bromo-2-fluorophenyl)-1,3,4-oxadiazole (5q)

White acicular crystal; Yield: 76%; m.p.: 163-164 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 3.55 (s, 3H), 7.21 (d, J = 9.1 Hz, 1H), 7.75-7.73 (m, 1H), 8.25 (d, J = 2.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 43.11, 117.60 (d, J = 3.7 Hz), 119.25 (d, J = 22.2 Hz), 120.01, 132.69, 138.07 (d, J = 8.7 Hz), 159.51 (d, J = 262.1 Hz), 162.21, 162.66; IR (KBr) ν: 3091, 2933, 1610, 1465, 1230, 1150 cm^{-1}; Anal. calcd for $C_9H_6BrFN_2O_3S$: C, 33.66; H, 1.88; N, 8.72; found: C, 33.27; H, 2.2; N, 8.93.

2.4.18. 2-(ethylsulfonyl)-5-(5-bromo-2-fluorophenyl)-1,3,4-oxadiazole (5r)

White acicular crystal; Yield: 65%; m.p.: 141-142 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.56 (t, J = 7.5 Hz, 3H), 3.63 (q, J = 7.4 Hz, 2H), 7.26 – 7.17 (m, 1H), 7.74 (ddd, J = 8.9, 4.4, 2.5 Hz, 1H), 8.26 (dd, J = 6.0, 2.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 6.93, 50.23, 112.60 (d, J = 12.8 Hz), 117.58 (d, J = 3.7 Hz), 119.23 (d, J = 22.2 Hz), 138.02 (d, J = 8.7 Hz), 132.68, 159.48 (d, J = 261.9 Hz), 161.84,162.26; IR (KBr) ν: 3072, 2980, 1533, 1469, 1228, 1154 cm^{-1}; Anal. calcd for $C_{10}H_8BrFN_2O_3S$: C, 35.84; H, 2.41; N, 8.36; found: C, 35.54; H, 2.396; N, 8.46.

2.4.19. 2-(methylsulfonyl)-5-(5-bromo-3-(trifluoromethyl)phenyl)-1,3,4-oxadiazole (5s)

Yellow acicular crystal; Yield: 44%; m.p.: 120-121 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 3.56 (s, 3H), 8.03 (s, 1H), 8.33 (s, 1H), 8.48 (s, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 43.10, 121.33, 123.29 (q, J = 3.8 Hz), 123.50, 124.14, 124.69, 133.01 (q, J = 4.0 Hz), 133.74, 162.81, 164.19. IR (KBr) ν: 3008, 2916, 1541, 1344, 1303, 1152 cm^{-1}; Anal. calcd for $C_{10}H_6BrF_3N_2O_3S$: C, 32.36; H, 1.63; N, 7.55; found: C, 31.92; H, 1.68; N, 7.60.

2.4.20. 2-(ethylsulfonyl)-5-(5-bromo-3-(trifluoromethyl) phenyl)-1,3,4-oxadiazole (5t)

Pale yellow acicular crystal; Yield: 38%; m.p.: 120-121 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.58 (t, J = 7.5 Hz, 3H), 3.68-3.63 (q, 2H), 8.03 (s, 1H), 8.34 (s, 1H), 8.48 (s, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 6.93, 50.22, 121.34, 123.29 (q, J = 3.7 Hz), 123.51, 124.12, 124.76, 133.05 – 132.83 (m), 133.71 (d, J = 4.4 Hz), 162.02, 164.22; IR (KBr) ν: 3089, 2947, 1543, 1429, 1303, 1150 cm^{-1}; Anal. calcd for $C_{11}H_8BrF_3N_2O_3S$: C, 34.30; H, 2.09; N, 7.27; found: C, 33.95; H, 2.36; N, 7.39.

2.4.21. 2-(methylsulfonyl)-5-(4-fluorophenoxymethyl)-1,3,4-oxadiazole (5u)

White acicular crystal; Yield: 73%; m.p.: 108-110 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 3.50 (s, 3H), 5.32 (s, 2H), 7.12-6.89 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 42.15, 61.56, 112.67 (d, J = 46.1 Hz), 116.23, 116.42 (d, J = 1.9 Hz), 116.49, 120.00 (d, J = 1.0 Hz), 155.45 (d, J = 468.7 Hz), 173.84, 178.98; IR (KBr) ν: 3034, 2933, 1575, 1346, 1244, 1147 cm^{-1}; Anal. calcd for $C_{10}H_9FN_2O_4S$: C, 44.12; H, 3.33; N, 10.29; found: C,43.51; H, 3.53; N, 10.33.

2.4.22. 2-(ethylsulfonyl)-5-(4-fluorophenoxymethyl) -1,3,4-oxadiazole (5v)

White acicular crystal; Yield: 75%; m.p.: 106-108 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.51 (t, J = 7.4 Hz, 3H), 3.58 (q, J = 7.4 Hz, 2H), 5.32 (s, 2H), 7.12-6.90 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 6.85, 50.15, 60.60, 115.87, 116.37, 116.40, 116.46, 116.55, 153.35, 162.67, 164.56; IR (KBr) ν: 2926, 1575, 1500, 1207, 1145 cm^{-1}; Anal. calcd for $C_{11}H_{11}FN_2O_4S$: C, 46.15; H, 3.87; N, 9.79; found: C, 45.65; H, 3.84; N, 9.85.

2.4.23. 2-(benzylsulfonyl)-5-(4-fluorophenoxymethyl) -1,3,4-oxadiazole (5w)

White acicular crystal; Yield: 88%; m.p.: 94-96 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 4.75 (s, 4H), 5.21 (s, 4H), 7.06-6.88 (m, 4H), 7.42-7.23 (m, 5H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 60.37, 62.11, 116.32 (t, J = 4.1 Hz), 116.52, 124.55, 129.33, 129.99, 131.21, 153.29, 158.36 (d, J = 240.9 Hz), 162.61, 164.66; IR (KBr) ν: 2983, 2926, 1558, 1350, 1205, 1153 cm^{-1}; Anal. calcd for $C_{15}H_{13}FN_2O_4S$: C, 55.17; H, 3.76; N, 8.04; found: C, 55.17; H, 3.66; N, 8.29.

2.5. Biological Assays

2.5.1. *In Vitro* Antifungal Bioassay

The antifungal activities of the target compounds were screened and evaluated in vitro against six phyto-pathogenic fungi, namely, *G. zeae*, *F. oxysporum*, *C. mandshurica*, *P. sasakii*, *P. infestans* and *S. sclerotiorum* using the mycelial growth rate method. All target compounds were dissolved in DMSO (1 mL) and diluted with sterile distilled water containing 0.1% Tween-20 (9mL) to prepare the 500 μg/mL stock solution before mixing with molten potato dextrose agar (PDA; 90mL) below 60°C. The compounds were tested at a concentration of 50 μg/mL for the initial screening. All fungi were poured into sterilized Petri dishes in PDA at 26.5 ± 0.5 °C for 4 days to make new mycelium for antifungal activity test. Then, mycelia dishes of approximately 4 mm diameter were cut from the culture medium. A mycelium was obtained using a germ-free inoculation needle and inoculated in the center of the PDA plate aseptically. The inoculated plates were incubated at 26.5 ± 0.5 °C for 4-5 days. DMSO in sterile distilled water containing 0.1% tween served as negative control, whereas epoxiconazol served as positive control. Each treatment condition consisted of three replicates. Radial growth diameter of the three replicate of the fungal colonies was measured twice by cross bracketing method then the data was statistically analyzed. Inhibitory effects of the test compounds *in vitro* on these fungi were calculated by the formula:

$$\text{Inhibition rate}\ (\%) = \left[(\text{CK} - \text{T}) / (\text{CK} - 4\text{mm})\right] \times 100$$

where "CK" means the average diameter of fungal growth in the negative control, "T" means the average diameter of fungi on treated PDA.

2.5.2. *In Vitro* Antibacterial Bioassay

The antibacterial activities of target compounds against *R. solanacearum* and *X. oryzae* were evaluated by the turbidimeter test. The target compounds were dissolved in 150 μL of DMSO and diluted with sterile distilled water containing 0.1% Tween-20 (4 mL) to prepare 1000 and 500 μg/mL stock solution. DMSO in sterile distilled water containing 0.1% Tween-20 served as the negative control, whereas Kocide 3000 served as positive control. Approximately 1 mL of stock solution was added to 4mL nontoxic nutrient broth liquid medium (NB, 3 g of beef

extract, 5 g of peptone, 1 g of yeast powder, 10 g of glucose, and 1000 mL of distilled water, pH 7.0 to 7.2) in tubes. Then, approximately 40 μL of NB containing tobacco bacterial wilt was added to 5 mL of solvent NB containing compounds, DMSO or Kocide 3000. Then, the inoculated test tubes were incubated with continuous shaking at 180 rpm for 24 h at 30 ± 1 °C. The growth of bacterial culture was monitored with a spectrophotometer by measuring the optical density at 600 nm (OD600). The inhibitory rate of bacterial culture growth was calculated which formula follows:

$$I(\%) = (CK - T)/CK \times 100.$$

Where "CK" means the value of corrected optical density of bacterial growth on untreated NB (negative control), and "T" means the value of corrected optical density of bacterial growth on treated NB. "I" means the inhibition rate. Similarly, the solvent for *X. oryzae* was M210 (8 g of casein acid hydrolysate, 5 g of saccharose, 4 g of yeast powder, 3 g of K_2HPO_4, 0.3 g of $MgSO_4 \cdot 7H_2O$, 1000 mL of distilled water, pH 7.0-7.2), and bismerthiazol served as positive control. The inoculated test tubes were incubated with continuous shaking at 180 rpm for 36 h at 28 ± 1 °C.

2.5.3. Protective Bioassay (*in vivo*)

Compounds with the most potential *in vitro* bioassay were conducted for biological activity test *in vivo* by proceeding on rice plant. 10-15 rice seeds soaked with water were sown in each bucket and cultivated at 25 °C for 15-20 d. After 15-20 d, the uniformly grown rice plants will have three to four leaves and are ready to test. Three compounds were used in this study, compounds 5u (6 mg), commercially available bismerthiazol (6 mg, 97%) and thiodiazole-copper (25.5 mg, 20%). All compounds were dissolved in 120 μL DMSO and diluted with 30 ml 0.1% Tween-20 to the final concentration of 200 μg/mL. *X. oryzae* in NB culture media, inoculated for 12 h at 30 °C, 180 rpm, was used as bacterial working suspension for infection. Compound solution was evenly sprayed on the rice leaves. After 1 d, the rice leaves were intentionally damaged via the leaf-cutting method and then soaked in water or the prepared bacteria working suspension for 10 s. Water without either compound or bacteria was set as blank control (CK1), while bacteria treatment without following compound treatment was set as negative control (CK2). Three replicates were set for each treatment. The incidence of disease of rice plants were investigated at 15^{th}-21^{th} days after treatment. The data (Table 6) were statistically analyzed via ANOVA (least significant difference), and the results showed that no significant differences ($p > 0.05$) and equal variances not assumed ($p < 0.05$) exist among the compounds treatments.

2.6. Chemistry

The general synthetic route of target compounds 5a-w is shown in Figure 2 and Figure 3. Target compounds were obtained from disubstituted benzoic acid, 4-(trifluoromethyl)-benzoic acid and 4-fluorophenoxyacetic acid by previously reported methods [13,16]. Substituted methyl benzoates 1 were produced when substituted benzoic acid was heated with absolute methanol in the presence of an acid catalyst. Then substituted methyl benzoates 1 was treated with hydrazine hydrate to form substituted benzoyl hydrazines 2, 2-mercapto-5-substituted-1,3,4-oxadiazoles 3 was easily prepared by cyclization, then, 2-mercapto-5-substituted-1,3,4-oxadiazoles 3 was converted to thioether derivatives 4 by a thioetherification reaction with dimethyl(diethyl) sulfate or halide (RX). Treatment of thioether derivatives 4 by $KMnO_4$ afforded the target compounds 5. It is generally known that there are lots of studies of oxidation of sulfides to sulfone by different oxidant, such as, H_2O_2-ammonium molybdate, H_2O_2-sodium tungstate and AcOH-$KMnO_4$. In our research, sulfone was prepared by the oxidation of sulfide with AcOH-$KMnO_4$ at room temperament. Through the research of different experimental conditions during oxidation of sulfides to sulfone, we found out that the yield and rate of reaction were closely related to the proportion of V(AcOH) to V(H_2O). The key point of oxidation reaction is the volume ratio of V(AcOH) and V(H_2O), hence, any improper volume of V(AcOH) or V(H_2O) can lead to incomplete reaction or no reaction. It was demonstrated that the best proportion of volume of V(AcOH) and V(H_2O) is 1.5:1.

3. Results and Discussion

3.1. Biological Results

3.1.1. Antifungal Activity

The antifungal activities of the target compounds were determined using the mycelial growth rate method. The results, of the compounds are expressed as EC_{50} values, are shown in Table 1 and Table 2. As summarized in Table 1, some of the target compounds displayed inhibition rates ranging from 36.48 to 89.94% against *G. zeae*, 42.14 to 96.05% against *F. oxysporum*, 32.46 to 80.95% against *C. mandshurica*, 27.59 to 70.76% against *P. infestans*, 19.08 to 78.95% against *P. sasakii*, and 22.68 to 100% against *S. sclerotiorum* at 50 μg/mL, respectively, compare to 100% inhibition rate of epoxiconazol as positive control against six fungi at 50 μg/mL. All the target compounds possessed good activities against *G. zeae*, *F. oxysporum* and *S. sclerotiorum* among six targeted fungi.

Some target compounds which showed excellent activity against *S. sclerotiorum* in previous bioassays were further tested for EC_{50} values. As summarized in Table 2, EC_{50} of compound **5** ranges from 3.71 to 17.44 μg/mL, and compounds **5a** (3.80 μg/mL) and **5b** (3.71 μg/mL), show superiority over other target compounds.

SAR Analysis of Antifungal Activities

To examine the SAR, different substituent groups and its positions on the phenyl group were designed and discussed. The structure−activity relationship was discussed according to activity result in Table 1. When R^1 were 2-substitued methyl and 4-substitued F on the benzene ring, the corresponding target compounds had good activities, as indicated by **5a** and **5b** on the antifungal activity. When R^1 was fixed, and R^2 = methyl or ethyl, no significant activity changes were observed against *G. zeae*, *P. infestans* and *P. sasakii*, but methyl compounds were superior active against other three kinds of fungi than ethyl one. As indicated in Table 1,

bioactivity of methyl substituted compound was superior to that of compounds with n-propyl, i-propyl and benzyl moiety. The relationships of the antifungal activities with the different aryl groups were systemly analyzed based on activity results in Table 1 and Table 2. For 2, 4-disubstituted, when R^1 was substituted with 4-F-2-Me (**5a**), the corresponding target compounds exhibited higher activity than 2-Br-4-F (**5f**), 2-Cl-4-F (**5h**) and 4-Br-2-F (**5l**), against six fungi. For 2-substituted in R^1, the target compounds with electron-donating group 2-methyl were observed with higher inhibition activity than those with electron-withdrawing groups (2-Cl, 2-Br). For example, inhibition rates of compound **5a** (77.20%, 96.05%, 80.95%, 57.84%, 77.30%, 100%) against six fungi was crucial for antifungal activities of compound **5f** (58.19%, 67.43%, 62.27%, 44.25%, 36.17%, 50.51%), **5h** (89.94%, 87.10%, 60.57%, 48.98%, 49.67%, 100%) and **5l** (62.26%, 95.62%, 54.84%, 70.76%, 43.42%, 82.47%) at 50 μg/mL. For 2, 5-disubstituted, when R^1 was substituted with 2, 5-di-Cl, the target compounds exhibited remarkable activity than the others. For example, inhibition rates against *S. sclerotiorum* of compound **5c** (R^1 = 2, 5-di-Cl) was 100%, whereas that of compound **5q** (R^1 =2-Br-5-F) was 67.12% at 50 μg/mL, whereas EC_{50} of compound **5c** (R^1 = 2, 5-di-Cl) was 7.99 μg/mL.

Table 1. Antifungal activities of compounds 5a-w against six phytopathogenic fungi at 50 μg/mL

Compd	Average values of inhibition rate (%)[a]					
	G. zeae	*F. oxysporum*	*C. mandshurica*	*P. infestans*	*P. sasakii*	*S. sclerotiorum*
5a	77.20±2.52	96.05±3.95	80.95±5.24	57.84±2.74	77.30±8.56	100.00±3.96
5b	75.16±1.32	76.09±3.44	57.71±1.57	58.28±1.88	78.95±1.97	100.00±3.96
5c	65.22±1.33	93.75±3.71	70.33±2.82	51.22±3.12	44.68±0.71	100.00±3.74
5d	61.87±1.03	72.37±1.38	69.60±1.94	48.78±1.08	50.00±1.00	78.40±5.75
5e	58.81±1.63	49.48±0.89	46.59±1.66	46.90±2.38	65.46±1.92	50.51±1.15
5f	58.19±1.10	67.43±2.92	62.27±6.71	44.25±2.08	36.17±0.65	100.00±3.96
5g	64.78±3.20	71.38±2.64	54.84±1.78	59.31±2.59	58.88±1.63	93.07±3.01
5h	89.94±3.49	87.10±1.61	60.57±2.43	48.96±0.63	49.67±1.92	100.00±3.96
5i	66.04±0.89	77.42±12.32	56.27±1.94	52.07±2.74	68.75±1.98	100.00±3.96
5j	79.88±5.67	94.06±3.48	65.95±4.22	65.12±1.92	70.39±1.66	86.11±4.51
5k	72.96±3.25	77.78±1.09	55.20±1.46	54.14±2.48	42.43±1.13	67.35±1.06
5l	62.26±2.33	95.62±3.22	54.84±1.78	70.76±1.89	43.42±1.19	82.47±1.84
5m	66.67±2.15	81.48±5.92	62.01±2.50	53.79±2.10	49.67±1.48	81.44±2.40
5n	52.37±3.82	88.22±3.85	45.88±1.64	46.90±2.16	43.75±1.78	100.00±3.96
5o	61.64±3.12	71.72±11.71	45.52±1.68	44.48±1.55	47.70±1.44	100.00±3.96
5p	58.81±1.63	61.62±2.83	53.05±2.13	40.34±1.62	50.66±1.70	65.64±2.59
5q	65.09±4.16	73.74±1.87	50.90±1.37	47.18±1.44	40.79±2.07	67.12±2.70
5r	60.38±2.11	55.89±1.40	35.48±1.41	29.66±1.53	40.35±1.04	45.02±2.12
5s	54.09±0.93	71.72±2.78	67.74±1.99	64.48±2.29	53.95±0.86	86.60±2.17
5t	62.89±1.36	59.93±0.91	56.63±1.49	56.90±1.83	60.53±1.36	67.70±2.51
5u	54.73±2.22	87.14±1.52	35.07±1.97	36.11±1.03	37.19±0.95	100.00±8.77
5v	72.19±8.36	56.79±3.66	32.46±1.97	63.46±2.98	62.46±1.34	100.00±8.77
5w	48.52±1.03	42.14±1.13	32.46±1.61	36.88±1.42	57.89±1.39	45.89±1.60
Ep[b]	100.00±5.45	100.00±4.58	100.00±5.91	100.00±4.58	100.00±3.74	100.00±3.96

[a] Average of three replicates. [b] The commercial agricultural fungicide epoxiconazole was used for the positive control.

In other aspect, compounds, with F and Br substituents in different position of benzene, cause no apparent difference in six fungi except *S. sclerotiorum*. For example, antifungal activities of compounds with 5f (2-Br-4-F), 5l (4- Br-2-F), 5n (2-Br-5-F) and 5q (5-Br-2-F) were similarity. It is worth to note that when R^1 was substituted with 2-Br-4-F, the corresponding target compounds exhibited excellent activity than others against *S. sclerotiorum*. For example, EC_{50} of compound 5f (R^1 = 2-Br-4-F) was 6.55 μg/mL, to that of compound 5n (R^1 = 2-Br-5-F) was 17.44 μg/mL. For trifluoromethyl substituted, the corresponding disubstituted target compounds exhibited lower activity than 4-CF_3. For example, experiments on *G. zeae* and *F. oxysporum* indicated that inhibition rates of compound 5j (R^1 = 4-CF_3) were 79.88% and 94.06%, however compound 5s (R^1=3-Br - 5 - CF_3) were 54.09% and 71.72%

Table 2. Antifungal activity of some compounds against *S. sclerotiorum*[a]

compd	EC_{50} (μg/mL)	toxic regression eq	r
5a	3.80	y = 1.636x + 4.052	0.9944
5b	3.71	y = 0.822x + 4.532	0.9767
5c	7.99	y = 1.108x + 4.000	0.9602
5f	6.55	y = 1.585x + 3.706	0.9980
5h	6.98	y = 2.648x + 2.765	0.9592
5i	6.77	y = 1.147x + 4.047	0.9854
5n	17.44	y = 1.313x + 3.370	0.9518
5o	13.68	y = 1.638x + 3.139	0.9808
5u	6.45	y = 1.316x + 3.935	0.9555
5v	10.34	y = 1.402x + 3.547	0.9965
Ep[b]	0.15	y = 2.1486x + 6.7648	0.9883

[a] Average of three replicates. [b] The commercial agricultural fungicide epoxiconazole was used as positive control.

3.1.2. Antibacterial Activity

Newly synthesized **5a-w** were also tested for their *in vitro* antibacterial activity against *R. solanacearum* and *X. oryzae* by turbidmeter test. Commercial agricultural antibacterial Kocide 3000 and bismerthiazol served as the positive control. The antibacterial activity data was listed in Table 3. The relation of antibacterial activity and structure of target compounds were also discussed.

First, for antibacterial activity against *R. solanacearum*, some of the target compounds exhibited excellent antibacterial activity at 100 and 200 μg/mL. In particular, the EC_{50} of compounds **5h**, **5l**, **5u** and **5v** against *R. solanacearum* were 5.89, 4.42, 1.97 and 7.75 μg/mL, respectively, exceeding that of Kocide 3000 (45.91 μg/mL). Moreover, compounds **5c** (57%), **5f** (73%), **5g** (55%), **5i** (73%), **5j** (87%), and **5n** (70%) showed moderate antibacterial activity against *R. solanacearum* at 100 μg/mL.

Second, as summarized in Table 3, all the target compounds showed promising potency against *X. oryzae*. The antibacterial activities of the target compounds a showed excellent activity than that of bismerthiazol (72%) at 100 and 200 μg/mL. All the target compounds showed a great inhibition rate for a longer research at 5 μg/mL. Most importantly, the EC_{50} of compounds **5u** and **5v** against *X. oryzae* were 0.45 and 0.52 μg/mL, respectively, overwhelmingly exceeding that of bismerthiazol (92.61 μg/mL).

Table 3. Antibacterial activities of compounds 5a-w against two phytopathogenic bacteria

compd	Average values of inhibition rate (%)[a]					
	X. oryzae				*R. solanacearum*	
	200 μg/mL	100 μg/mL	5 μg/mL	2.5 μg/mL	200 μg/mL	100 μg/mL
5a	100	100	100	87	30	21
5b	100	100	100	60	23	19
5c	100	100	100	70	100	57
5d	100	100	90	58	0	0
5e	100	100	59	42	0	0
5f	100	100	95	81	85	73
5g	100	100	99	96	78	55
5h	100	100	100	96	100	98
5i	100	100	100	94	89	73
5j	100	100	99	87	100	87
5k	100	100	98	70	38	20
5l	100	100	100	80	100	100
5m	100	100	100	77	49	50
5n	100	100	100	81	80	70
5o	100	100	100	77	49	37
5p	100	100	100	88	24	20
5q	100	100	100	95	46	23
5r	100	100	100	90	34	14
5s	100	100	96	74	36	24
5t	100	100	90	72	36	33
5u	100	100	100	100	100	99
5v	100	100	100	100	100	99
5w	100	100	97	87	33	25
Kocide 3000[b]	-[c]	-	-	-	100	100
bismerthiazol[b]	72	54	0	0	-	-

[a] Average of three replicates. [b] The commercial agricultural antibacterial agents bismerthiazol and Kocide 3000 were used as positive control. [c] "-" means not test.

SAR Analysis of Antibacterial Activities

The *in vitro* results of the target compounds against *R. solanacearum* and *X. oryzae* are listed in Table 3. Commercial agricultural antibacterial agents Kocide 3000 and bismerthiazol, were used as positive control for treatment of *R. solanacearum* and *X. oryzae*, respectively. The SAR based on activity against two phytopathogenic bacteria was similar to the target compounds against six fungi. While four target compounds (**5h**, **5l**, **5u**, **5v**) were superior to Kocide 3000 against *R. solanacearum*, and all of them surpassed bismerthiazol against *X. oryzae*. When R^2 = methyl or ethyl, no significant activity changes was observed from Table 3 against *X. oryzae*, and both were superior to n-propyl, i-propyl and benzyl. But there were very large gap in the aspect of against *R. solanacearum*. When R^2 = methyl, target compounds was superior to ethyl, for example, **5h** (100%) was higher than **5i** (89%) at 200 μg/mL.

For R^1, the relationship between the antibacterial activities with the different aryl group (type and position) could be summarizing that the activity of 2, 4-disubstituted is exceeding that of 2, 5-disubstituted compounds. For example, the inhibition rate of compound **5l** (R1 = 4-Br-2-F) against *R. solanacearum* was 100% at 100 μg/mL against *R. solanacearum*, while compounds **5n** (R^1 = 2-Br-5-F) and **5q** (R^1 = 5-Br-2-F) were 70% and 23% at the same concentration. For the activity of resistance to *X. oryzae*, all the target compounds showed excellent activity, without significant differences. Just like antifungal and antibacterial activities, **5u** and **5v** showed unique activity.

Table 4. Antibacterial activity of some compounds against *R. solanacearum*[a]

Compd.	EC_{50} (μg/mL)	Toxic regression eq	r
5h	5.99	y = 2.5708x + 3.002	0.9707
5l	4.42	y = 1.6237x + 3.9523	0.9534
5u	1.97	y = 2.2502x + 4.3381	0.9840
5v	7.75	y = 1.0364x + 4.0784	0.9904
Kocide 3000[b]	45.91	y = 4.8739x−3.1000	0.9792

[a] Average of three replicates. [b] The commercial agricultural fungicide Kocide 3000 was used as positive control.

Table 5. Antibacterial activity of some compounds against *X. oryzae*[a]

Compd.	EC_{50} (μg/mL)	toxic regression eq	r
5u	0.45	y = 0.9007x + 5.3160	0.8779
5v	0.52	y = 0.9866x + 5.2806	0.9555
bismerthiazol[b]	92.61	y = 1.4990x + 2.0520	0.9800

[a] Average of three replicates. [b] The commercial agricultural fungicide bismerthiazol was used as positive control.

3.1.3. Antibacterial Activities of compounds I3, II6 (*in vivo*)

All bacteria suspension treatments (CK2) were seriously infected in 3 weeks indicating that the plant was successfully infected. At the same time, CK1 was not infected throughout the whole month. Control efficiency of compound **5u** was 81.9%, which were much higher than that of bismerthiazol (50.8%) and thiodiazole-copper (44.7%). It is obvious that the new synthesized compound was much more potent against rice bacterial leaf blight than bismerthiazol and thiodiazole-copper from Table 6.

In this paper, we introduce flexible segment on rigid perssad, for search drugs which have high biological activities on antibacterial aspects. We add a $–OCH_2-$ between benzene and oxadiazole. As we'd expect, target compounds containing phenoxymethyl showed higher activity than those without. The inhibition rate of **5u** was higher than others against *R. solanacearum* and *X. oryzae*. For example, EC_{50} of compound **5u** (1.97 μg/mL) was higher than that of compounds **5h** (5.99 μg/mL), **5l** (4.42 μg/mL) against *R. solanacearum*, the inhibition rate of compound **5u** (100%) and **5v** (100%) were higher than that of others (42-96%) against *X. oryzae*. Compounds **5a**, **5f**, **5g**, **5h**, **5i**, **5j**, **5l**, **5n**, **5r**, **5w**, **5t** still have the same lower EC_{50} values as **5u** and **5v** against *X. oryzae*, even if we didn't.

Table 6. Controlling Effect of Testing Compounds against rice bacterial leaf blight at 200 μg/mL

Compd.	21 Days after spraying	
	Morbidity (%)	Control efficiency[c] (%)
5u	100.0	81.9±19.4a
thiodiazole-copper	100.0	44.7±12.4b
Bismerthiazol	100.0	50.8±15.9b
CK1[a]	0.0	100.0±0.0a
CK2[b]	100.0	/

[a]CK1: blank control sample. [b]CK2: negative control sample. [c]Statistical analysis was conducted via the ANOVA method at a condition of equal variances assumed ($p > 0.05$) and equal variances not assumed ($p < 0.05$). Different lowercase letters indicate the values of inhibition and EC_{50} with significant difference among different treatment groups at $p < 0.05$.

4. Conclusions

In summary, a series of sulfone derivatives containing 1,3,4-oxadiazole moiety were synthesized from substituted benzoic acid. The target compounds **5a**, **5b** and **5u** exhibited excellent activity against six fungi as well as two phytopathogenic bacteria compared to the commercial fungicides bactericides epoxiconazole, Kocide 3000 and bismerthiazol, respectively. The antibacterial tests showed that when 4-F substituted benzene combined with 2-substitued electron withdrawing group (Br, Cl), the corresponding compounds had presented good antibacterial activities. Moreover, introduction of $–OCH_2-$, compounds resulted in excellent to good activities against *S. sclerotiorum* and two phytopathogenic bacteria, but not increase against five other fungi. In addition, the antifungal and antibacterial assays demonstrated that the inhibition activity of compounds **5u** was the best among all target compounds and even superior to the commercial agents, Kocide 3000 and bismerthiazol. To our knowledge, this is the first report of sulfone derivatives containing 1,3,4-oxadiazole moieties containing disubstituted benzene and phenoxymethyl with potent controlling effect against *R. solanacearum* and *X. oryzae*. Further evaluation of their biological efficacy, crop safety, and toxicity is conducted before them as bactericide candidates adopted for widespread use.

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