



Synthesis and Bioactivities of Novel 1,3,4-Thiadiazole Derivatives of Glucosides

Meihang Chen^{1*}, Xun Zhang¹, Daowang Lu¹, Hairong Luo¹, Zengyan Zhou¹, Xufeng Qin¹, Wenneng Wu^{2*} and Guoping Zhang³

¹Colleges of Material and Chemistry Engineering, Tongren University, Tongren, China, ²Colleges of Food and Pharmaceutical Engineering, Guiyang University, Guiyang, China, ³Colleges of Chemistry and Material Science, Huaibei Normal University, Huaibei, China

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*Correspondence:

Meihang Chen
chenmeihang0123@126.com
Wenneng Wu
wuWenneng123@126.com

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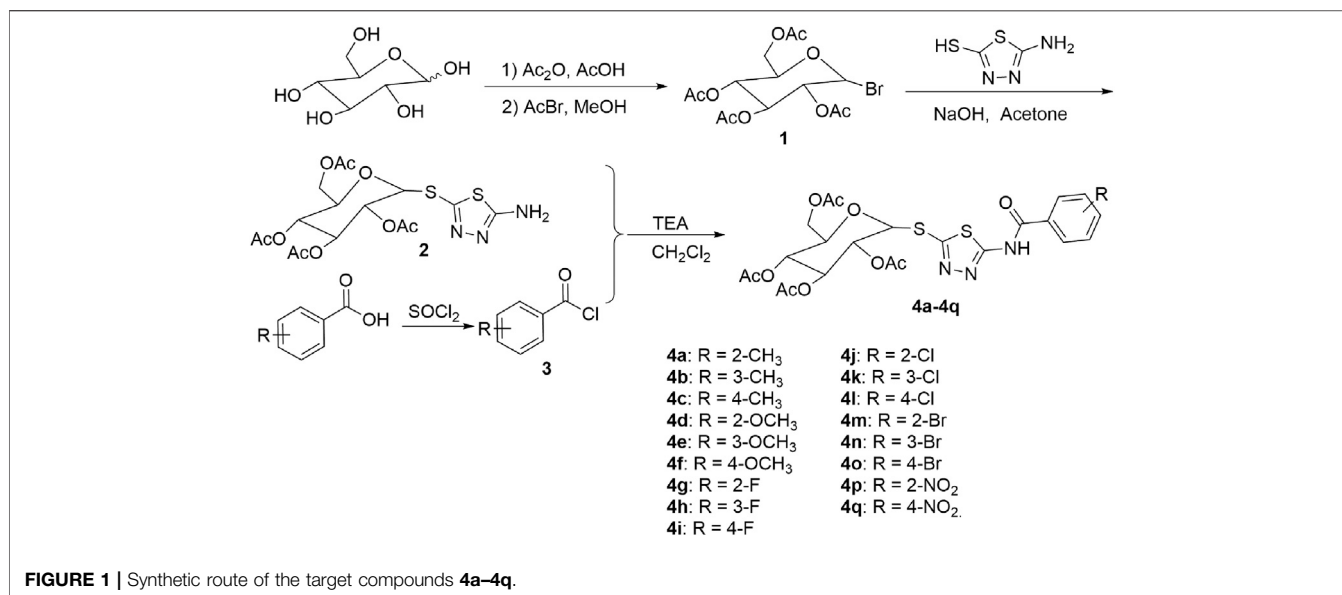
A series of novel 1,3,4-thiadiazole derivatives of glucosides were synthesized by the starting materials *D*-glucose and 5-amino-1,3,4-thiadiazole-2-thiol in good yields with employing a convergent synthetic route. The results of bioactivities showed that some of the target compounds exhibited good antifungal activities. Especially, compounds **4i** showed higher bioactivities against *Phytophthora infestans* (*P. infestans*), with the EC₅₀ values of 3.43, than that of Dimethomorph (5.52 μg/ml). In addition, the target compounds exhibited moderate to poor antibacterial activities against *Xanthomonas oryzae* *pv.* *oryzae* (*Xoo*), *Xanthomonas campestris* *pv.* *citri* (*Xcc*).

Keywords: thiadiazole, amide, glucoside, synthesis, bioactivity

INTRODUCTION

Crop disease, caused by fungi, bacteria, viruses, and nematodes and parasitic seed plants, can effect on the biological or non-biological factors of plants causing the phenomenon of a series of morphological, physiological and biochemical pathologic changes, further blocking the normal growth and the development process and the human economic benefits (Zhan et al., 2015). Nowadays, some of the traditional fungicides and bactericides, such as Carbendazim, Kresoxim-methyl, Streptomycin sulfate, Bismethiazol, etc., have been widely used to prevent and control plant fungal and bacterial diseases. However, long-term using these pesticides could lead to drug resistance, serious ecological, and environmental problem (Aktar et al., 2009). Therefore, development of novel and promising fungicides and bactericides is still an urgent task.

1,3,4-Thiadiazole derivatives have shown extensive biological activities, such as anti-inflammatory (Maddila et al., 2016), anticancer (Yang et al., 2012; Sridhar et al., 2020), antifungal (Alwan et al., 2015; Bhinge et al., 2015; Chudzik et al., 2019), antibacterial (Aggarwal et al., 2012; Taflan et al., 2019; Zhang et al., 2019), and plant growth regulator (Knyazyan et al., 2012) activities. Since 1,3,4-thiadiazole compounds with antibacterial activity was synthesized by Masaki in the 1950s, 1,3,4-thiadiazole pesticides, such as Bismethiazol and Thiodiazole-copper, have been developed and widely used in agriculture. Recent years, a variety of studies found that amide derivatives containing 1,3,4-thiadiazole thioether moiety showed good antifungal activities against *Fusarium oxysporum* (*F. oxysporum*), *Cytospora mandshurica* (*C. mandshurica*), and *Gibberella zeae* (*G. zeae*) at 50 mg/L (Xie et al., 2016) and exhibited exciting antibacterial activities against *Xanthomonas oryzae* *pv.* *oryzae* (*Xoo*), *Xanthomonas campestris* *pv.* *citri* (*Xcc*), and *Ralstonia solanacearum* (*Rs*) (Chen J. et al., 2019).



Glycosides are secondary metabolites that widely exist in all organs of plants, such as flowers, fruits, leaves, skins, and roots, etc (Gruner et al., 2002), and previous studies found that glycosides had a wide range of pharmacological activities, such as antiviral (Chen W. et al., 2019; Khodair et al., 2019), antibacterial (Mohammed et al., 2019), anticancer (Gurung et al., 2018; Rahim et al., 2020), antioxidant (Jiang et al., 2018; Hawas et al., 2019), and anti-HIV (He et al., 2019) activities. Meanwhile, studies also found that glycoside derivatives showed exceeding inhibitory activities against plant pathogens. For example, Ningnanmycin, an important glycoside biological pesticide, is mainly used in rice seedling blight, soybean root rot, rice stripe disease, apple spot deciduous leaf disease and cucumber powdery mildew (Hu et al., 1997). In addition, it was also found that glycosylation is one of the effective ways to improve the functional activity of active lead compounds and develop new drugs. (Gurung et al., 2018; Wu et al., 2014).

In order to develop new lead compounds with highly bioactivity, in this study, we aimed to introduce a 1,3,4-thiadiazole group into glucosides moiety to design a series of novel 1,3,4-thiadiazole derivatives of glucosides and then evaluate for their antifungal and antibacterial activities. Results indicated that some of the target compounds exhibited good antifungal activities. Especially, the compounds **4i** showed higher bioactivities against *Phytophthora infestans* (*P. infestans*), with the EC₅₀ values of 3.43 μg/ml, respectively, than that of Dimethomorph (5.52 μg/ml). In addition, the target compounds showed moderate to poor antibacterial activities against *Xoo* and *Xcc*. As far as we know, this is the first report on the antifungal and antibacterial activities of 1,3,4-thiadiazole derivatives of glucosides.

MATERIALS AND METHODS

Materials and Instruments

Melting points were determined on an XT-4 melting apparatus (Beijing Tech Instrument Co., China). ¹H NMR and ¹³C NMR

spectra were measured on a Bruker AVANCE III TM 400 and HD 600 MHz Digital NMR Spectrometer (Bruker Company, Billerica, MA, US.) in CDCl₃ as solvent and recorded in relative to internal standard tetramethylsilane. High-resolution mass spectrometry (HRMS) was carried out on an Agilent Technologies 6540 UHD Accurate-Mass Q-TOF LC/MS (Agilent Technologies, Palo Alto, CA, United States). The course of the reactions was monitored by thin-layer chromatography (TLC) analysis on silica gel GF254. All reagents and solvents meet the standards of analytical reagent before use.

Chemistry

Preparation of 2,3,4,6-tetra-*O*-acetyl- α -*D*-glucopyranosyl bromide (**1**). As shown in **Figure 1**, acetic anhydride (88 ml, 0.9 mol) was added to a solution of *D*-glucose (29.75 g, 0.15 mol) in glacial acetic acid (300 ml) and stirred at room temperature for 20 min. Then, perchloric acid (0.3 ml) was added to the above reaction mixture at room temperature. After TLC analysis showed complete disappearance of *D*-glucose, a solution of acetyl bromide (34 ml, 0.45 mol) in 50 ml CH₂Cl₂ was added to the resultant reaction mixture and stirred at room temperature. After the completion of the reaction, the reaction mixture was poured into water and extracted with CH₂Cl₂. The organic layer was dried, filtered, and evaporated in vacuo to remove CH₂Cl₂. The crude product was recrystallized by a mixture of petroleum ether and diethyl ether (volume ratio 1:2) to afford intermediate **1**. (Scattolin et al., 2020). ¹H NMR spectral data for intermediate **1** are listed in the Supplementary Material.

Preparation of (2*R*,3*R*,4*S*,5*R*,6*R*)-2-(acetoxymethyl)-6-((5-amino-1,3,4-thiadiazol-2-yl)thio)tetrahydro-2*H*-pyran-3,4,5-triyltriacetate (**2**). A mixture of 2-amino-5-mercapto-1,3,4-thiadiazole (1.33 g, 10.0 mmol), acetone (50 ml), NaOH (0.4 g, 10.0 mmol), and water (10 ml) was stirred for 30 min at room temperature. Then, a solution of intermediate **1** (0.98 g, 10.0 mmol) in 5 ml acetone was added dropwise and continuously stirred at room temperature. After the reaction

completed (monitored by TLC), acetone was evaporated in vacuo, the residues were diluted with water, extracted with CH_2Cl_2 . The combined CH_2Cl_2 extract was dried over anhydrous sodium sulfate, evaporated in vacuo and separated by silica gel column chromatography to afford intermediate **2** (Kamat et al., 2007). ^1H NMR spectral data for intermediate **2** are listed in the Supplementary Material.

General procedure for preparation of the target compounds **4a–4q**. Substituted benzoic acid (1.2 mmol) was added in 2 ml SOCl_2 and refluxed for about 2 h SOCl_2 was distilled off in vacuo to obtain intermediates **3**. And then, a solution of intermediate **3** in 2 ml CH_2Cl_2 was added dropwise to a mixture of the intermediate **2** (1.0 mmol) and triethylamine (TEA, 1.2 mmol) in 10 ml CH_2Cl_2 . After the reaction was completed (monitored by TLC), the mixture was diluted with water, the organic layer was dried over anhydrous sodium sulfate, filtered and distilled off in vacuo, and the crude products were recrystallized with isopropanol to afford title compounds **4a–4q**.

(2*R*,3*R*,4*S*,5*R*,6*R*)-2-(acetoxymethyl)-6-((5-(2-methylbenzamido)-1,3,4-thiadiazol-2-yl)thio)tetrahydro-2*H*-pyran-3,4,5-triyltriacetate (**4a**). White solid; yield 67.1%; m. p. 160–162°C; $R_f = 0.67$ (ethyl acetate: petroleum ether, 1:2); IR (KBr, cm^{-1}): ν : 3,433 (NH), 1747 (COO), 1,678 (CON); ^1H NMR (400 MHz, CDCl_3 , ppm) δ : 7.66 (d, $J = 8.3$ Hz, 1H, Ar-H), 7.49 (d, $J = 7.6$ Hz, 1H, Ar-H), 7.42–7.30 (m, 2H, Ar-H), 5.29 (t, $J = 10.0$ Hz, 1H, H-3'), 5.21–5.06 (m, 3H, H-1', H-2', H-4'), 4.34–4.16 (m, 2H, H-5', H-6'), 3.84–3.80 (m, 1H, H-6''), 2.55 (s, 3H, CH_3), 2.15 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.02 (s, 3H, CH_3); ^{13}C NMR (150 MHz, CDCl_3 , ppm) δ : 170.66 (COCH₃), 170.17 (COCH₃), 169.45 (COCH₃), 169.34 (CONH), 169.28 (thiadiazole-C), 158.58 (thiadiazole-C), 157.90 (Ar-C), 137.27 (Ar-C), 132.05 (Ar-C), 131.56 (Ar-C), 130.75 (Ar-C), 129.18 (Ar-C), 82.52 (C-1'), 75.97 (C-5'), 73.89 (C-3'), 69.28 (C-2'), 67.87 (C-4'), 61.62 (C-6'), 20.74 (CH_3), 20.61 (CH_3), 20.59 (CH_3), 20.02 (CH_3); HRMS [$\text{M} + \text{H}$]⁺ calculated for $\text{C}_{24}\text{H}_{27}\text{N}_3\text{O}_{10}\text{S}_2$: m/z 582.1230, found 582.1209.

(2*R*,3*R*,4*S*,5*R*,6*R*)-2-(acetoxymethyl)-6-((5-(3-methylbenzamido)-1,3,4-thiadiazol-2-yl)thio)tetrahydro-2*H*-pyran-3,4,5-triyltriacetate (**4b**). White solid; yield 65.3%; m. p. 163–165°C; $R_f = 0.45$ (ethyl acetate: petroleum ether, 1:2); IR (KBr, cm^{-1}): ν : 3,468 (NH), 1749 (COO), 1,666 (CON); ^1H NMR (400 MHz, CDCl_3 , ppm) δ : 7.99 (s, 1H, Ar-H), 7.94 (d, $J = 7.5$ Hz, 1H, Ar-H), 7.51–7.43 (m, 2H, Ar-H), 5.28 (t, $J = 9.0$ Hz, 1H, H-3'), 5.21–4.98 (m, 3H, H-1', H-2', H-3'), 4.40–4.05 (m, 2H, H-5', H-6'), 3.74–3.70 (m, 1H, H-6''), 2.48 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.02 (s, 3H, CH_3); ^{13}C NMR (150 MHz, CDCl_3 , ppm) δ : 171.01 (COCH₃), 170.16 (COCH₃), 169.41 (COCH₃), 165.24 (CONH), 162.64 (thiadiazole-C), 155.20 (thiadiazole-C), 139.01 (Ar-C), 134.34 (Ar-C), 130.86 (Ar-C), 128.75 (Ar-C), 125.40 (Ar-C), 84.07 (C-1'), 76.31 (C-5'), 73.58 (C-3'), 69.67 (C-2'), 67.79 (C-4'), 61.77 (C-6'), 21.35 (CH_3), 20.72 (CH_3), 20.64 (CH_3), 20.58 (CH_3); HRMS [$\text{M} + \text{H}$]⁺ calculated for $\text{C}_{24}\text{H}_{27}\text{N}_3\text{O}_{10}\text{S}_2$: m/z 582.1230, found 582.1208.

(2*R*,3*R*,4*S*,5*R*,6*R*)-2-(acetoxymethyl)-6-((5-(4-methylbenzamido)-1,3,4-thiadiazol-2-yl)thio)tetrahydro-2*H*-pyran-3,4,5-triyltriacetate (**4c**). White solid; yield 75.1%; m. p. 159–161°C; $R_f = 0.63$ (ethyl acetate: petroleum ether, 1:2); IR (KBr, cm^{-1}): ν : 3,433 (NH), 1747 (COO), 1,666 (CON); ^1H NMR (400 MHz, CDCl_3 , ppm) δ : 11.76 (s, 1 H, NH), 8.05 (d, $J = 8.1$ Hz, 2 H, Ar-H), 7.36 (d, $J = 8.0$ Hz, 2H, Ar-

H), 5.49 (d, $J = 3.2$ Hz, 1H, H-1'), 5.38 (t, $J = 10.0$ Hz, 1H, H-3'), 5.11 (dd, $J = 9.9, 3.3$ Hz, 1H, H-2'), 5.05 (d, $J = 10.1$ Hz, 1H, H-4'), 4.21 (d, $J = 6.1$ Hz, 2H, H-5', H-6''), 4.05–4.01 (m, 1H, H-6'), 2.47 (s, 3H, CH_3), 2.19 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.00 (s, 3H, CH_3); ^{13}C NMR (150 MHz, CDCl_3 , ppm) δ : 170.93 (COCH₃), 170.10 (COCH₃), 169.35 (COCH₃), 164.84 (CONH), 162.72 (thiadiazole-C), 155.32 (thiadiazole-C), 144.59 (Ar-C), 129.77 (Ar-C), 128.41 (Ar-C), 84.08 (C-1'), 76.36 (C-5'), 73.60 (C-3'), 69.71 (C-2'), 67.79 (C-4'), 61.76 (C-6'), 21.77 (CH_3), 20.72 (CH_3), 20.68 (CH_3), 20.60 (CH_3); HRMS [$\text{M} + \text{H}$]⁺ calculated for $\text{C}_{24}\text{H}_{27}\text{N}_3\text{O}_{10}\text{S}_2$: m/z 582.1230, found 582.1209.

(2*R*,3*R*,4*S*,5*R*,6*R*)-2-(acetoxymethyl)-6-((5-(2-methoxybenzamido)-1,3,4-thiadiazol-2-yl)thio)tetrahydro-2*H*-pyran-3,4,5-triyltriacetate (**4d**). White solid; yield 68.7%; m. p. 168–170°C; $R_f = 0.33$ (ethyl acetate: petroleum ether, 1:2); IR (KBr, cm^{-1}): ν : 3,433 (NH), 1749 (COO), 1,687 (CON); ^1H NMR (400 MHz, CDCl_3 , ppm) δ : 12.13 (s, 1H, NH), 8.25 (d, $J = 9.6$ Hz, 1H, Ar-H), 7.61 (t, $J = 8.8$ Hz, 1H, Ar-H), 7.19 (t, $J = 7.6$ Hz, 1H, Ar-H), 7.10 (d, $J = 8.4$ Hz, 1H, Ar-H), 5.30 (t, $J = 9.2$ Hz, 1H, H-3'), 5.18–5.08 (m, 3H, H-1', H-2', H-4'), 4.32–4.22 (m, 2H, H-5', H-6'), 4.12 (s, 3H, OCH₃), 3.86–3.82 (m, 1H, H-6''), 2.16 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.01 (s, 3H, CH_3); ^{13}C NMR (150 MHz, CDCl_3 , ppm) δ : 171.01 (COCH₃), 170.13 (COCH₃), 169.40 (COCH₃), 169.38 (COCH₃), 162.61 (CONH), 161.06 (thiadiazole-C), 158.08 (thiadiazole-C), 155.19 (Ar-C), 135.32 (Ar-C), 132.83 (Ar-C), 121.94 (Ar-C), 118.07 (Ar-C), 111.82 (Ar-C), 84.17 (C-1'), 76.32 (C-5'), 73.63 (C-3'), 69.67 (C-2'), 67.85 (C-4'), 61.83 (C-6'), 56.48 (OCH₃), 21.77 (CH_3), 20.72 (CH_3), 20.68 (CH_3), 20.60 (CH_3); HRMS [$\text{M} + \text{H}$]⁺ calculated for $\text{C}_{24}\text{H}_{27}\text{N}_3\text{O}_{11}\text{S}_2$: m/z 598.1142, found 598.1161.

(2*R*,3*R*,4*S*,5*R*,6*R*)-2-(acetoxymethyl)-6-((5-(3-methoxybenzamido)-1,3,4-thiadiazol-2-yl)thio)tetrahydro-2*H*-pyran-3,4,5-triyltriacetate (**4e**). White solid; yield 73.2%; m. p. 169–171°C; $R_f = 0.41$ (ethyl acetate: petroleum ether, 1:2); IR (KBr, cm^{-1}): ν : 3,450 (NH), 1749 (COO), 1,666 (CON); ^1H NMR (400 MHz, CDCl_3 , ppm) δ : 11.40 (s, 1H, NH), 7.67 (d, $J = 7.7$ Hz, 1H, Ar-H), 7.58 (s, 1H, Ar-H), 7.48 (t, $J = 8.0$ Hz, 1H, Ar-H), 7.23 (d, $J = 10.7$ Hz, 1H, Ar-H), 5.29 (t, $J = 9.2$ Hz, 1H, H-3'), 5.21–5.04 (m, 3H, H-1', H-2', H-4'), 4.34–4.11 (m, 2H, H-5', H-6'), 3.90 (s, 3H, OCH₃), 3.75–3.71 (m, 1H, H-6''), 2.13 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.02 (s, 3H, CH_3); ^{13}C NMR (150 MHz, CDCl_3 , ppm) δ : 170.54 (COCH₃), 170.23 (COCH₃), 169.95 (COCH₃), 169.50 (CONH), 165.04 (thiadiazole-C), 160.01 (thiadiazole-C), 132.19 (Ar-C), 130.08 (Ar-C), 120.61 (Ar-C), 120.05 (Ar-C), 113.18 (Ar-C), 84.44 (C-1'), 75.17 (C-5'), 71.62 (C-3'), 67.16 (C-2'), 66.98 (C-4'), 61.56 (C-6'), 55.60 (OCH₃), 20.74 (CH_3), 20.66 (CH_3), 20.65 (CH_3), 20.56 (CH_3); HRMS [$\text{M} + \text{H}$]⁺ calculated for $\text{C}_{24}\text{H}_{27}\text{N}_3\text{O}_{11}\text{S}_2$: m/z 598.1142, found 598.1162.

(2*R*,3*R*,4*S*,5*R*,6*R*)-2-(acetoxymethyl)-6-((5-(4-methoxybenzamido)-1,3,4-thiadiazol-2-yl)thio)tetrahydro-2*H*-pyran-3,4,5-triyltriacetate (**4f**). White solid; yield 75.0%; m. p. 166–168°C; $R_f = 0.52$ (ethyl acetate: petroleum ether, 1:2); IR (KBr, cm^{-1}): ν : 3,462 (NH), 1747 (COO), 1,664 (CON); ^1H NMR (400 MHz, CDCl_3 , ppm) δ : 8.04 (d, $J = 8.9$ Hz, 2H, Ar-H), 7.04 (d, $J = 8.9$ Hz, 2H, Ar-H), 5.28 (t, $J = 9.2$ Hz, 1H, H-3'), 5.20–5.04 (m, 3H, H-1', H-2', H-4'), 4.32–4.17 (m, 2H, H-5', H-6'), 3.92 (s, 3H, OCH₃), 3.83–3.77 (m, 1H, H-6''), 2.15 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.01 (s, 3H, CH_3); ^{13}C NMR (150 MHz, CDCl_3 , ppm) δ : 170.58 (COCH₃),

170.44 (COCH₃), 169.87(CONH), 169.79(thiadiazole-C), 163.63 (thiadiazole-C), 155.45 (Ar-C), 131.02 (Ar-C), 123.57 (Ar-C), 114.53 (Ar-C), 83.14 (C-1'), 74.69 (C-5'), 71.15 (C-3'), 68.07 (C-2'), 67.31 (C-4'), 62.42 (C-6'), 56.07 (OCH₃), 20.91 (CH₃), 20.88 (CH₃), 20.79 (CH₃), 20.76 (CH₃); HRMS [M + H]⁺ calculated for C₂₄H₂₇N₃O₁₁S₂: m/z 598.1142, found 598.1162.

(2*R*,3*R*,4*S*,5*R*,6*R*)-2-(acetoxymethyl)-6-((5-(2-fluorobenzamido)-1,3,4-thiadiazol-2-yl)thio)tetrahydro-2*H*-pyran-3,4,5-triyltriacetate (**4g**). White solid; yield 55.4%; m. p. 173–175°C; R_f = 0.61 (ethyl acetate: petroleum ether, 1:2); IR (KBr, cm⁻¹) ν: 3,421 (NH), 1749 (COO), 1,676 (CON); ¹H NMR (400 MHz, CDCl₃, ppm) δ: 10.29 (s, 1H, NH), 8.17 (t, J = 7.8 Hz, 1H, Ar-H), 7.66 (d, J = 7.4 Hz, 1H, Ar-H), 7.38 (t, J = 8.0 Hz, 1H, Ar-H), 7.31–7.23 (m, 1H, Ar-H), 5.35–5.23 (m, 1H, H-3'), 5.19–5.08 (m, 3H, H-1', H-2', H-4'), 4.31 (dd, J = 12.5, 5.0 Hz, 1H, H-5'), 4.21 (dd, J = 12.5, 2.0 Hz, 1H, H-6'), 3.85–3.83 (m, 1H, H-6''), 2.16 (s, 3H, CH₃), 2.10 (s, 3H, CH₃), 2.04 (s, 3H, CH₃), 2.01 (s, 3H, CH₃); ¹³C NMR (150 MHz, CDCl₃, ppm) δ: 170.93 (COCH₃), 170.07 (COCH₃), 169.34 (COCH₃), 169.29 (COCH₃), 164.45 (CONH), 163.34 (thiadiazole-C), 155.18 (thiadiazole-C), 132.36 (Ar-C), 130.21 (Ar-C), 129.51 (Ar-C), 128.78 (Ar-C), 83.81 (C-1'), 76.48 (C-5'), 73.57 (C-3'), 69.68 (C-2'), 67.69 (C-4'), 61.69 (C-6'), 20.73 (CH₃), 20.60 (CH₃); HRMS [M + H]⁺ calculated for C₂₃H₂₄FN₃O₁₀S₂: m/z 586.0932, found 586.0964.

(2*R*,3*R*,4*S*,5*R*,6*R*)-2-(acetoxymethyl)-6-((5-(3-fluorobenzamido)-1,3,4-thiadiazol-2-yl)thio)tetrahydro-2*H*-pyran-3,4,5-triyltriacetate (**4h**). White solid; yield 70.2%; m. p. 174–176°C; R_f = 0.63 (ethyl acetate: petroleum ether, 1:2); IR (KBr, cm⁻¹) ν: 3,435 (NH), 1749 (COO), 1,670 (CON); ¹H NMR (400 MHz, CDCl₃, ppm) δ: 12.30 (s, 1H, NH), 8.29 (s, 1H, Ar-H), 7.81 (d, J = 8.7 Hz, 1H, Ar-H), 7.46 (t, J = 7.9 Hz, 1H, Ar-H), 5.32–5.28 (m, 1H, H-3'), 5.20–5.05 (m, 3H, H-1', H-2', H-4'), 4.28 (dd, J = 12.5, 5.0 Hz, 1H, H-5'), 4.24–4.11 (m, 1H, H-6'), 3.78–3.73 (m, 1H, H-6''), 2.14 (s, 3H, CH₃), 2.09 (s, 3H, CH₃), 2.05 (s, 3H, CH₃), 2.02 (s, 3H, CH₃); ¹³C NMR (150 MHz, CDCl₃, ppm) δ: 170.93 (COCH₃), 170.07 (COCH₃), 169.34 (COCH₃), 169.29 (COCH₃), 164.45 (CONH), 163.34 (thiadiazole-C), 155.18 (thiadiazole-C), 132.36 (Ar-C), 130.21 (Ar-C), 129.51 (Ar-C), 128.78 (Ar-C), 83.81 (C-1'), 76.48 (C-5'), 73.57 (C-3'), 69.68 (C-2'), 67.69 (C-4'), 61.69 (C-6'), 20.73 (CH₃), 20.60 (CH₃); HRMS [M + H]⁺ calculated for C₂₃H₂₄FN₃O₁₀S₂: m/z 586.0932, found 586.0963.

(2*R*,3*R*,4*S*,5*R*,6*R*)-2-(acetoxymethyl)-6-((5-(4-fluorobenzamido)-1,3,4-thiadiazol-2-yl)thio)tetrahydro-2*H*-pyran-3,4,5-triyltriacetate (**4i**). White solid; yield 65.8%; m. p. 170–172°C; R_f = 0.64 (ethyl acetate: petroleum ether, 1:2); IR (KBr, cm⁻¹) ν: 3,475 (NH), 1751 (COO), 1,676 (CON); ¹H NMR (400 MHz, CDCl₃, ppm) δ: 12.54 (s, 1H, NH), 8.36 (d, J = 8.2 Hz, 2H, Ar-H), 7.85 (d, J = 8.3 Hz, 2H, Ar-H), 5.28 (t, J = 9.3 Hz, 1H, H-3'), 5.18–5.09 (m, 2H, H-1', H-2'), 5.00 (d, J = 10.0 Hz, 1H, H-4'), 4.33 (dd, J = 12.5, 4.8 Hz, 1H, H-5'), 4.23 (dd, J = 12.5, 2.0 Hz, 1H, H-6'), 3.86–3.82 (m, 1H, H-6''), 2.17 (s, 3H, CH₃), 2.09 (s, 3H, CH₃), 2.04 (s, 3H, CH₃), 2.01 (s, 3H, CH₃); ¹³C NMR (150 MHz, CDCl₃, ppm) δ: 170.31 (COCH₃), 170.21 (COCH₃), 170.00 (COCH₃), 169.58 (COCH₃), 166.22 (CONH), 159.08 (thiadiazole-C), 158.51 (thiadiazole-C), 134.35 (Ar-C), 134.29 (Ar-C), 115.52 (Ar-C), 115.38 (Ar-C), 83.14 (C-1'),

74.72(C-5'), 71.83(C-3'), 67.12(C-2'), 66.83(C-4'), 61.34 (C-6'), 20.73 (CH₃), 20.67 (CH₃), 20.65 (CH₃), 20.59 (CH₃); HRMS [M + H]⁺ calculated for C₂₃H₂₄FN₃O₁₀S₂: m/z 586.0932, found 586.0962.

(2*R*,3*R*,4*S*,5*R*,6*R*)-2-(acetoxymethyl)-6-((5-(2-chlorobenzamido)-1,3,4-thiadiazol-2-yl)thio)tetrahydro-2*H*-pyran-3,4,5-triyltriacetate (**4j**). White solid; yield 70.1%; m. p. 178–180°C; R_f = 0.55 (ethyl acetate: petroleum ether, 1:2); IR (KBr, cm⁻¹) ν: 3,441 (NH), 1747 (COO), 1,668 (CON); ¹H NMR (400 MHz, CDCl₃, ppm) δ: 10.48 (s, 1H, NH), 7.90 (d, J = 8.4 Hz, 1H, Ar-H), 7.57–7.41 (m, 3H, Ar-H), 5.31–5.11 (m, 4H, H-3', H-1', H-2', H-4'), 4.32–4.19 (m, 2H, H-5', H-6'), 3.86–3.81 (m, 1H, H-6''), 2.15 (s, 3H, CH₃), 2.10 (s, 3H, CH₃), 2.04 (s, 3H, CH₃), 2.01 (s, 3H, CH₃); ¹³C NMR (150 MHz, CDCl₃, ppm) δ: 170.93 (COCH₃), 170.12 (COCH₃), 169.37 (COCH₃), 169.33 (COCH₃), 163.64 (CONH), 160.98 (thiadiazole-C), 155.66 (thiadiazole-C), 133.34 (Ar-C), 131.70 (Ar-C), 131.24 (Ar-C), 131.19 (Ar-C), 131.02 (Ar-C), 127.60 (Ar-C), 84.00 (C-1'), 76.39 (C-5'), 73.57 (C-3'), 69.65 (C-2'), 67.80 (C-4'), 61.75 (C-6'), 20.78 (CH₃), 20.68 (CH₃), 20.60 (CH₃); HRMS [M + H]⁺ calculated for C₂₃H₂₄ClN₃O₁₀S₂: m/z 602.0641, found 602.0663.

(2*R*,3*R*,4*S*,5*R*,6*R*)-2-(acetoxymethyl)-6-((5-(3-chlorobenzamido)-1,3,4-thiadiazol-2-yl)thio)tetrahydro-2*H*-pyran-3,4,5-triyltriacetate (**4k**). White solid; yield 65.3%; m. p. 179–180°C; R_f = 0.66 (ethyl acetate: petroleum ether, 1:2); IR (KBr, cm⁻¹) ν: 3,442 (NH), 1749 (COO), 1,674 (CON); ¹H NMR (400 MHz, CDCl₃, ppm) δ: 12.34 (s, 1H, NH), 8.16 (s, 1H, Ar-H), 8.08 (d, J = 7.8 Hz, 1H, Ar-H), 7.66 (d, J = 8.0 Hz, 1H, Ar-H), 7.52 (t, J = 7.9 Hz, 1H, Ar-H), 5.29 (t, J = 9.2 Hz, 1H, H-3'), 5.22–5.06 (m, 3H, H-1', H-2', H-4'), 4.37–4.10 (m, 2H, H-5', H-6'), 3.84–3.81 (m, 1H, H-6''), 2.14 (s, 3H, CH₃), 2.09 (s, 3H, CH₃), 2.05 (s, 3H, CH₃), 2.02 (s, 3H, CH₃); ¹³C NMR (150 MHz, CDCl₃, ppm) δ: 170.89 (COCH₃), 170.12 (COCH₃), 169.35 (COCH₃), 169.33 (COCH₃), 164.26 (CONH), 163.15 (thiadiazole-C), 155.75 (thiadiazole-C), 135.22 (Ar-C), 133.60 (Ar-C), 132.57 (Ar-C), 130.32 (Ar-C), 128.74 (Ar-C), 126.94 (Ar-C), 83.98 (C-1'), 76.37 (C-5'), 73.52 (C-3'), 69.64 (C-2'), 67.79 (C-4'), 61.75 (C-6'), 20.74 (CH₃), 20.66 (CH₃), 20.60 (CH₃); HRMS [M + H]⁺ calculated for C₂₃H₂₄ClN₃O₁₀S₂: m/z 602.0641, found 602.0661.

(2*R*,3*R*,4*S*,5*R*,6*R*)-2-(acetoxymethyl)-6-((5-(4-chlorobenzamido)-1,3,4-thiadiazol-2-yl)thio)tetrahydro-2*H*-pyran-3,4,5-triyltriacetate (**4l**). White solid; yield 78.5%; m. p. 178–180°C; R_f = 0.48 (ethyl acetate: petroleum ether, 1:2); IR (KBr, cm⁻¹) ν: 3,450 (NH), 1751 (COO), 1,672 (CON); ¹H NMR (400 MHz, CDCl₃, ppm) δ: 11.88 (s, 1H, NH), 8.13 (d, J = 8.6 Hz, 2H, Ar-H), 7.55 (d, J = 8.6 Hz, 2H, Ar-H), 5.29 (t, J = 9.2 Hz, 1H, H-3'), 5.21–5.00 (m, 3H, H-1', H-2', H-4'), 4.36–4.25 (m, 1H, H-5'), 4.20 (dd, J = 12.5, 2.0 Hz, 1H, H-6'), 3.84–3.81 (m, 1H, H-6''), 2.15 (s, 3H, CH₃), 2.11 (s, 3H, CH₃), 2.04 (s, 3H, CH₃), 2.01 (s, 3H, CH₃); ¹³C NMR (150 MHz, CDCl₃, ppm) δ: 170.59 (COCH₃), 170.26 (COCH₃), 169.92 (COCH₃), 169.48 (COCH₃), 164.45 (CONH), 163.56 (thiadiazole-C), 155.49 (thiadiazole-C), 139.98 (Ar-C), 130.24 (Ar-C), 129.32 (Ar-C), 129.06 (Ar-C), 84.26 (C-1'), 75.32 (C-5'), 71.62 (C-3'), 67.09 (C-2'), 66.90 (C-4'), 61.66 (C-6'), 20.79 (CH₃), 20.70 (CH₃), 20.68 (CH₃),

20.58 (CH₃); HRMS [M + H]⁺ calculated for C₂₃H₂₄ClN₃O₁₀S₂: m/z 602.0641, found 602.0664.

(2R,3R,4S,5R,6R)-2-(acetoxymethyl)-6-((5-(2-bromobenzamido)-1,3,4-thiadiazol-2-yl)thio)tetrahydro-2H-pyran-3,4,5-triyltriacetate (**4m**). White solid; yield 69.4%; m. p. 190–192°C; R_f = 0.65 (ethyl acetate: petroleum ether, 1:2); IR (KBr, cm⁻¹) ν: 3,473 (NH), 1745 (COO), 1,689 (CON); ¹H NMR (400 MHz, CDCl₃, ppm) δ: 11.35 (s, 1H, NH), 7.76 (d, J = 7.2 Hz, 1H, Ar-H), 7.72 (d, J = 7.4 Hz, 1H, Ar-H), 7.51–7.44 (m, 2H, Ar-H), 5.29 (t, J = 9.2, 1H, H-3'), 5.16–5.08 (m, 3H, H-1', H-2', H-4'), 4.29 (dd, J = 12.5, 5.0 Hz, 1H, H-5'), 4.18 (dd, J = 12.5, 2.0 Hz, 1H, H-6'), 3.82–3.78 (m, 1H, H-6''), 2.14 (s, 3H, CH₃), 2.10 (s, 3H, CH₃), 2.05 (s, 3H, CH₃), 2.02 (s, 3H, CH₃); ¹³C NMR (150 MHz, CDCl₃, ppm) δ: 170.62 (COCH₃), 170.25 (COCH₃), 169.99 (COCH₃), 169.49 (COCH₃), 164.27 (CONH), 163.20 (thiadiazole-C), 155.98 (thiadiazole-C), 135.18 (Ar-C), 133.49 (Ar-C), 132.58 (Ar-C), 130.28 (Ar-C), 128.69 (Ar-C), 126.99 (Ar-C), 84.45 (C-1'), 75.22 (C-5'), 71.60 (C-3'), 67.17 (C-2'), 66.96 (C-4'), 61.64 (C-6'), 20.73 (CH₃), 20.67 (CH₃), 20.57 (CH₃); HRMS [M + H]⁺ calculated for C₂₃H₂₄BrN₃O₁₀S₂: m/z 646.0171, found 646.0161.

(2R,3R,4S,5R,6R)-2-(acetoxymethyl)-6-((5-(3-bromobenzamido)-1,3,4-thiadiazol-2-yl)thio)tetrahydro-2H-pyran-3,4,5-triyltriacetate (**4n**). White solid; yield 60.2%; m. p. 191–193°C; R_f = 0.70 (ethyl acetate: petroleum ether, 1:2); IR (KBr, cm⁻¹) ν: 3,475 (NH), 1753 (COO), 1,676 (CON); ¹H NMR (400 MHz, CDCl₃, ppm) δ: 12.30 (s, 1H, NH), 8.29 (s, 1H, Ar-H), 8.11 (d, J = 9.2 Hz, Ar-H), 7.81 (d, J = 8.7 Hz, 1H, Ar-H), 7.46 (t, J = 7.9 Hz, 1H, Ar-H), 5.29 (t, J = 9.3 Hz, 1H, H-3'), 5.17–5.12 (m, 3H, H-1', H-2', H-4'), 4.28 (dd, J = 12.5, 5.0 Hz, 1H, H-5'), 4.19–4.16 (m, 1H, H-6'), 3.78–3.73 (m, 1H, H-6''), 2.14 (s, 3H, CH₃), 2.09 (s, 3H, CH₃), 2.05 (s, 3H, CH₃), 2.02 (s, 3H, CH₃); ¹³C NMR (150 MHz, CDCl₃, ppm) δ: 170.87 (COCH₃), 170.11 (COCH₃), 169.34 (COCH₃), 169.32 (COCH₃), 164.09 (CONH), 163.06 (thiadiazole-C), 155.82 (thiadiazole-C), 136.53 (Ar-C), 132.78 (Ar-C), 131.53 (Ar-C), 130.56 (Ar-C), 127.36 (Ar-C), 123.19 (Ar-C), 84.00 (C-1'), 76.39 (C-5'), 73.52 (C-3'), 69.67 (C-2'), 67.80 (C-4'), 61.76 (C-6'), 20.74 (CH₃), 20.68 (CH₃), 20.60 (CH₃); HRMS [M + H]⁺ calculated for C₂₃H₂₄BrN₃O₁₀S₂: m/z 646.0171, found 646.0162.

(2R,3R,4S,5R,6R)-2-(acetoxymethyl)-6-((5-(4-bromobenzamido)-1,3,4-thiadiazol-2-yl)thio)tetrahydro-2H-pyran-3,4,5-triyltriacetate (**4o**). White solid; yield 70.3%; m. p. 188–190°C; R_f = 0.75 (ethyl acetate: petroleum ether, 1:2); IR (KBr, cm⁻¹) ν: 3,435 (NH), 1751 (COO), 1,674 (CON); ¹H NMR (400 MHz, CDCl₃, ppm) δ: 12.45 (s, 1H, NH), 8.11 (d, J = 8.5 Hz, 2H, Ar-H), 7.72 (d, J = 8.5 Hz, 2H, Ar-H), 5.30 (t, J = 9.2 Hz, 1H, H-3'), 5.18–5.02 (m, 3H, H-1', H-2', H-4'), 4.32 (dd, J = 12.6, 4.8 Hz, 1H, H-5'), 4.20 (d, J = 12.3 Hz, 1H, H-6'), 3.82–3.79 (m, 1H, H-6''), 2.16 (s, 3H, CH₃), 2.13 (s, 3H, CH₃), 2.04 (s, 3H, CH₃), 2.01 (s, 3H, CH₃); ¹³C NMR (150 MHz, CDCl₃, ppm) δ: 170.93 (COCH₃), 170.07 (COCH₃), 169.34 (COCH₃), 169.29 (COCH₃), 164.45 (CONH), 163.34 (thiadiazole-C), 155.18 (thiadiazole-C), 132.36 (Ar-C), 130.21 (Ar-C), 129.51 (Ar-C), 128.78 (Ar-C), 83.81 (C-1'), 76.48 (C-5'), 73.57 (C-3'), 69.68 (C-2'), 67.69 (C-4'), 61.69 (C-6'), 20.73 (CH₃), 20.60 (CH₃); HRMS [M + H]⁺ calculated for C₂₃H₂₄BrN₃O₁₀S₂: m/z 646.0171, found 646.0161.

(2R,3R,4S,5R,6R)-2-(acetoxymethyl)-6-((5-(2-nitrobenzamido)-1,3,4-thiadiazol-2-yl)thio)tetrahydro-2H-pyran-3,4,5-triyltriacetate (**4p**). Yellow solid; yield 53.4%; m. p. 188–190°C; R_f = 0.42 (ethyl acetate: petroleum ether, 1:2); IR (KBr, cm⁻¹) ν: 3,458 (NH), 1751 (COO), 1,689 (CON); ¹H NMR (400 MHz, CDCl₃, ppm) δ: 13.02 (s, 1H, NH), 8.22 (d, J = 7.9 Hz, 1H, Ar-H), 7.85–7.75 (m, 3H, Ar-H), 5.32 (t, J = 9.0 Hz, 1H, H-3'), 5.15–5.03 (m, 3H, H-1', H-2', H-4'), 4.28 (dd, J = 12.5, 5.1 Hz, 1H, H-5'), 4.18 (dd, J = 12.5, 2.0 Hz, 1H, H-6'), 3.86–3.81 (m, 1H, H-6''), 2.29 (s, 3H, CH₃), 2.12 (s, 3H, CH₃), 2.05 (s, 3H, CH₃), 2.04 (s, 3H, CH₃); ¹³C NMR (150 MHz, CDCl₃, ppm) δ: 170.97 (COCH₃), 170.14 (COCH₃), 169.38 (COCH₃), 169.27 (COCH₃), 164.63 (CONH), 162.12 (thiadiazole-C), 134.30 (thiadiazole-C), 131.80 (Ar-C), 129.81 (Ar-C), 129.50 (Ar-C), 124.88 (Ar-C), 84.14 (C-1'), 76.38 (C-5'), 73.47 (C-3'), 69.76 (C-2'), 67.73 (C-4'), 61.71 (C-6'), 20.72 (CH₃), 20.67 (CH₃), 20.60 (CH₃); HRMS [M + H]⁺ calculated for C₂₃H₂₄N₄O₁₂S₂: m/z 613.0915, found 613.0908.

(2R,3R,4S,5R,6R)-2-(acetoxymethyl)-6-((5-(4-nitrobenzamido)-1,3,4-thiadiazol-2-yl)thio)tetrahydro-2H-pyran-3,4,5-triyltriacetate (**4q**). Yellow solid; yield 55.8%; m. p. 189–191°C; R_f = 0.67 (ethyl acetate: petroleum ether, 1:2); IR (KBr, cm⁻¹) ν: 3,437 (NH), 1751 (COO), 1,678 (CON); ¹H NMR (400 MHz, CDCl₃, ppm) δ: 13.02 (s, 1H, NH), 8.48–8.42 (m, 4H, Ar-H), 5.29 (t, J = 9.3 Hz, 1H, H-3'), 5.19–5.12 (m, 2H, H-1', H-2'), 4.97 (d, J = 10.0 Hz, 1H, H-4'), 4.35 (dd, J = 12.6, 4.6 Hz, 1H, H-5'), 4.26–4.22 (m, 1H, H-6'), 3.88–3.84 (m, 1H, H-6''), 2.19 (s, 3H, CH₃), 2.15 (s, 3H, CH₃), 2.05 (s, 3H, CH₃), 2.01 (s, 3H, CH₃); ¹³C NMR (150 MHz, CDCl₃, ppm) δ: 170.93 (COCH₃), 170.03 (COCH₃), 169.34 (COCH₃), 169.31 (COCH₃), 163.85 (CONH), 163.78 (thiadiazole-C), 155.13 (thiadiazole-C), 150.75 (Ar-C), 135.98 (Ar-C), 130.16 (Ar-C), 124.19 (Ar-C), 83.33 (C-1'), 76.66 (C-5'), 73.49 (C-3'), 69.98 (C-2'), 67.59 (C-4'), 61.62 (C-6'), 20.72 (CH₃), 20.69 (CH₃), 20.60 (CH₃), 20.58 (CH₃), 20.57 (CH₃); HRMS [M + H]⁺ calculated for C₂₃H₂₄N₄O₁₂S₂: m/z 613.0915, found 613.0906.

Antifungal Activity *In Vitro*

The *in vitro* antifungal activities of the target compounds against *G. zeae*, *Botryosphaeria dothidea* (*B. dothidea*), *Phomopsis* sp., *P. infestans*, and *Thanatephorus cucumeris* (*T. cucumeris*) are evaluated by using the poison plate technique. All of the target compounds **4a–4q** were dissolved in 1 ml DMSO before mixing with 90 ml potato dextrose agar (PDA) to prepare concentration of 50 μg/ml. Then, mycelia dishes of approximately 4 mm diameter were cut from the culture medium. A mycelium is obtained using a germ-free inoculation needle and inoculated in the middle of the PDA plate aseptically. The inoculated plates are incubated at 27 ± 1°C for 5 days. DMSO in sterile distilled water served as the negative control and Dimethomorph served as the positive control. Each treatment condition consisted of three replicates (Maddila et al., 2016). The relative inhibition rates *I* (%) were calculated as follows equation, where *C* was the diameter of fungal growth on untreated PDA, *T* was the diameter of fungi on treated PDA.

$$I (\%) = [(C - T)/(C - 0.4)] \times 100\%$$

Antibacterial Activity *In Vitro*

The *in vitro* antibacterial activities of the target compounds **4a–4q** against *Xoo* and *Xcc* were evaluated by using the turbidimeter test, the commercial agricultural antibacterial Thiodiazole-copper used as control. The test compounds were dissolved in 150 μ L of dimethylformamide (DMF) and diluted with 0.1% (v/v) Tween-20 to prepare two concentrations of 200 and 100 μ g/ml. One milliliter of the liquid sample was added to the 40 ml non-toxic nutrient broth medium (NB: 1.5 g of beef extract, 2.5 g of peptone, 0.5 g of yeast powder, 5.0 g of glucose, and 500 ml of distilled water, pH 7.0–7.2). Then, 40 μ L of NB medium containing *Xoo* or *Xcc* was added to 5 ml of solvent NB containing the test compounds or Thiodiazole-copper. The inoculated test tubes were incubated at $30 \pm 1^\circ\text{C}$ under continuous shaking at 180 rpm for 48 h. The culture growth was monitored spectrophotometrically by measuring the optical density at 600 nm (OD_{600}) and expressed as corrected turbidity (Dalgaard et al., 1994). The relative inhibition rates *I* (%) were calculated as follows equation, where C_{tur} was the corrected turbidity value of bacterial growth on untreated NB, T_{tur} was the corrected turbidity value of bacterial growth on treated NB.

$$I (\%) = (C_{\text{tur}} - T_{\text{tur}})/C_{\text{tur}} \times 100\%$$

RESULTS AND DISCUSSION

In this study, the target compounds **4a–4q** were synthesized in five steps, including acetylation, bromination, thioetherification, chlorination, and condensation. Among of them, it was found that 2,3,4,6-tetra-*O*-acetyl- α -*D*-glucopyranosyl bromide **1** reacted with 2-amino-5-mercapto-1,3,4-thiadiazole to obtain (2*R*,3*R*,4*S*,5*R*,6*R*)-2-(acetoxymethyl)-6-((5-amino-1,3,4-thiadiazol-2-yl)thio)-tetrahydro-2*H*-pyran-3,4,5-triyltriacetate **2** of β -configuration with high stereo selectivity in acetone solution of NaOH at room temperature, which indicated that the reaction process was S_N2 and configuration transformation occurred in the reaction process.

All the synthesized compounds were characterized by ^1H NMR, ^{13}C NMR, and HRMS. In the ^1H NMR spectra of the obtained amide, pyran and acetyl proton signals should be distinguished. For example, for compound **4i**, the proton signals of NH group was observed as a singlet at 12.54 ppm, signals of benzene ring protons were registered at 8.36 and 7.85 ppm, respectively, and the proton signal of pyran was registered in the range of 5.18–3.82 ppm. Moreover, four singlets at 2.17, 2.09, 2.04, and 2.01 ppm indicated to CH_3 protons of acetyl.

The *in vitro* antifungal activities of the target compounds were evaluated against five different fungus including *P. infestans*, *G.*

TABLE 1 | The *in vitro* antifungal activities of the target compounds **4a–4q** at 50 μ g/ml.

Compounds	Inhibition rate (%)				
	<i>G. zea</i>	<i>B. dothidea</i>	<i>P. infestans</i>	<i>Phomopsis sp</i>	<i>T. cucumeris</i>
4a	58.6 \pm 2.2	58.1 \pm 1.6	44.4 \pm 1.5	21.0 \pm 2.4	17.1 \pm 1.2
4b	62.2 \pm 1.4	54.8 \pm 0.7	28.5 \pm 2.0	38.7 \pm 1.3	29.0 \pm 1.2
4c	65.7 \pm 1.3	60.1 \pm 1.1	19.8 \pm 0.6	43.0 \pm 2.9	56.9 \pm 2.4
4days	58.9 \pm 1.1	52.0 \pm 1.2	40.9 \pm 1.4	50.0 \pm 1.3	44.5 \pm 1.5
4e	53.6 \pm 0.7	40.7 \pm 1.1	29.4 \pm 0.7	26.7 \pm 0.4	32.0 \pm 1.4
4f	51.7 \pm 1.1	43.3 \pm 0.1	35.0 \pm 1.9	30.8 \pm 2.3	42.2 \pm 2.0
4g	58.4 \pm 1.2	60.7 \pm 1.2	77.3 \pm 2.1	56.7 \pm 2.1	62.0 \pm 1.0
4h	35.6 \pm 0.6	33.5 \pm 0.8	73.0 \pm 1.0	30.8 \pm 1.0	22.2 \pm 2.2
4i	48.9 \pm 1.7	58.1 \pm 1.5	83.5 \pm 0.6	55.2 \pm 2.1	64.3 \pm 1.5
4j	58.3 \pm 1.6	51.1 \pm 0.9	30.1 \pm 2.6	58.4 \pm 1.7	44.7 \pm 1.6
4k	55.2 \pm 2.2	55.2 \pm 1.2	61.9 \pm 2.0	43.7 \pm 2.0	37.0 \pm 1.8
4L	58.0 \pm 2.3	49.2 \pm 1.3	70.0 \pm 1.2	31.5 \pm 0.9	59.8 \pm 0.9
4m	73.1 \pm 1.0	41.0 \pm 1.6	63.6 \pm 1.3	48.4 \pm 1.1	44.3 \pm 1.6
4n	70.3 \pm 1.1	45.6 \pm 1.1	73.1 \pm 1.8	33.7 \pm 0.8	58.5 \pm 1.8
4o	45.0 \pm 2.2	22.1 \pm 0.9	75.9 \pm 1.2	40.0 \pm 2.3	54.3 \pm 1.7
4p	53.4 \pm 1.9	61.3 \pm 1.1	79.0 \pm 1.1	64.0 \pm 1.3	62.8 \pm 0.7
4q	56.8 \pm 1.5	62.0 \pm 2.0	81.1 \pm 0.3	63.1 \pm 1.2	65.1 \pm 1.3
Dimethomorph	74.3 \pm 2.0	72.3 \pm 1.6	78.2 \pm 1.1	69.3 \pm 1.6	68.3 \pm 1.6

TABLE 2 | The EC_{50} values of compounds **4i**, **4p**, and **4q** against *P. infestans*.

Compds	Toxic regression equation	<i>r</i>	EC_{50} (μ g/ml)
4i	$y = 0.85x + 4.53$	0.98	3.43 \pm 1.5
4p	$y = 0.98x + 4.22$	0.98	6.15 \pm 2.1
4q	$y = 1.13x + 4.20$	0.97	5.02 \pm 1.8
Dimethomorph	$y = 0.94x + 4.30$	0.99	5.52 \pm 1.2

zea, *B. dothidea*, *Phomopsis sp.*, and *T. cucumeris*. Bioassay results, as shown in **Table 1**, revealed that the target compounds exhibited moderate to good antifungal activities against *P. infestans*, *G. zea*, *B. dothidea*, *Phomopsis sp.*, and *T. cucumeris*, with the inhibitory rates range of 19.8–83.5%, 35.6–73.1%, 22.1–62.0%, 21.0–64.0%, and 17.1–65.1%, respectively. Meanwhile, it was found that the inhibitory rates of the target compounds against *G. zea* in the range of 35.6–73.1% at the 50 μ g/ml, which was higher than the previously reported

TABLE 3 | The *in vitro* antibacterial activities of the target compounds **4a–4q**.

Compds	Xoo		Xcc	
	200 µg/ml	100 µg/ml	200 µg/ml	100 µg/ml
4a	60.1 ± 1.1	38.1 ± 2.1	64.9 ± 1.2	31.7 ± 2.2
4b	63.5 ± 1.5	37.3 ± 1.3	60.1 ± 2.2	39.2 ± 1.4
4c	54.2 ± 2.0	38.5 ± 1.0	55.4 ± 1.9	34.8 ± 2.1
4days	58.6 ± 1.8	42.3 ± 1.3	66.8 ± 2.1	36.3 ± 2.8
4e	44.0 ± 2.1	35.2 ± 1.5	51.4 ± 1.5	34.9 ± 2.2
4f	43.6 ± 1.9	32.6 ± 1.6	47.3 ± 1.5	25.8 ± 1.7
4g	49.0 ± 1.5	31.7 ± 2.3	33.2 ± 1.9	16.6 ± 1.5
4h	45.2 ± 1.5	33.4 ± 2.1	67.2 ± 2.0	43.3 ± 2.6
4i	59.4 ± 2.2	34.4 ± 1.7	68.6 ± 1.0	39.6 ± 1.4
4j	53.5 ± 1.6	32.8 ± 1.3	61.9 ± 1.3	45.5 ± 2.1
4k	51.0 ± 1.6	31.6 ± 1.1	26.5 ± 1.8	15.6 ± 1.7
4L	71.2 ± 0.9	42.6 ± 1.0	77.5 ± 1.4	45.3 ± 2.6
4m	74.4 ± 1.2	44.8 ± 1.5	77.5 ± 1.6	42.3 ± 1.6
4n	68.4 ± 2.1	42.6 ± 1.1	79.0 ± 2.0	47.2 ± 1.8
4o	74.6 ± 1.6	43.8 ± 1.3	75.8 ± 2.8	45.1 ± 1.3
4p	70.1 ± 2.5	43.1 ± 1.4	76.2 ± 2.0	43.1 ± 1.2
4q	69.7 ± 1.2	42.3 ± 1.4	80.8 ± 2.5	45.0 ± 1.3
Thiadiazole-copper	76.2 ± 1.3	45.2 ± 1.3	86.2 ± 2.1	44.5 ± 1.7

inhibitory activity of *N*-(2-chloro-4-phenyl-5-(trifluoromethyl)cyclopenta-1,4-dien-1-yl)-5-((4-nitrobenzyl)thio)-1,3,4-thiadiazol-2-amine against *G. zeae* (23.9%) at the 50 µg/ml (Xie et al., 2016). Especially, compound **4i** and **4q** showed higher antifungal activity against *P. infestans*, with the inhibition rates of 83.5%, 81.1%, respectively, than that of Dimethomorph (78.2%). Based on the preliminary antifungal bioassays, the EC₅₀ values of partial compounds against *P. infestans* were also tested and presented in **Table 2**. **Table 2** showed that compounds **4i** exhibited good bioactivities against *P. infestans*, with EC₅₀ values of 3.43 µg/ml, which were higher than that of Dimethomorph (5.52 µg/ml). While, the target compounds showed lower antibacterial activities (**Table 3**) against *Xoo* and *Xcc* at 200 and 100 µg/ml than those of Thiodiazole-copper as well as the amide derivatives containing 1,3,4-thiadiazole of the previously reported by Chen (Chen J. et al., 2019).

From the structure-activity relationships (SAR) analysis, it was found that there was clear SAR against *P. infestans*. Inspection of the chemical structures of the target compounds suggests that the group R in the target compounds significantly influence the antifungal activity against *P. infestans*. With a fluorinated or nitrified substituent (4-F and 4-NO₂) on the phenyl ring, the compounds exhibited enhanced bioactivity against *P. infestans* (**4i** and **4q**). Further, the position of substituent groups in the phenyl ring also plays an important role in the antifungal activity against *P. infestans*, with a four substituent (4-F or 4-NO₂) in the

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phenyl ring exhibited higher antifungal activity than other positions.

CONCLUSION

A series of novel 1,3,4-thiadiazole derivatives of glucosides were prepared via acetylation, bromination, thioetherification, chlorination, and condensation. Bioassay results showed that some of the target compounds revealed better inhibitory activity against *P. infestans*. In addition, SAR analysis found that the type and position of substituent groups in the phenyl ring of the target compounds plays an important role in increasing the antifungal activity against *P. infestans*.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**; further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

MC and WW contributed to the synthesis, purification, characterization of all compounds, and prepared the original manuscript. XZ and DL performed the biological activity research. HL and ZZ analyzed the experimental results. GZ and XQ drafted the first and second version of the manuscript. All authors discussed, edited, and approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fchem.2021.645876/full#supplementary-material>.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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