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Synthesis of novel 1,3,4-oxadiazole derivatives containing diamides as promising antibacterial and antiviral agents

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Abstract In this paper, a variety of novel 1,3,4-oxadiazole derivatives possessing diamides were synthesized and tested for their antibacterial and antiviral activity. Preliminary antibacterial assays indicated that some intermediates and title compounds displayed excellent inhibition effects against plant pathogens *Xanthomonas oryzae pv. oryzae* (*Xoo*) and *Xanthomonas axonopodis pv. citri* (*Xac*). Further studies revealed that compound **H15** exhibited the strongest activities against *Xoo* and *Xac* with minimal EC_{50} values of 0.7 and 5.9 μg/mL, respectively. Antiviral bioassays suggested that some of these structures displayed appreciable curative activities and moderate protective effects against tobacco mosaic virus (TMV) in vivo. Among them, compound **H8** exerted the best chemotherapeutic effect against TMV with the curative rate of 60.0% at 500 μg/mL, which was comparable with those of commercial agricultural antiviral agent ningnanmycin (54.2%). Given their significant biological activities, this kind of compound could serve as new leading compounds in the study of antibacterial and antiviral chemotherapy.

Keywords 1,3,4-Oxadiazole · Diamides · Synthesis · Antibacterial · Antiviral

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Introduction

Agricultural disease-causing bacteria as "plant killers" can cause necrotic lesions on leaves, stems, and/or fruits, which consequently result in a significant loss in agricultural output [1–3]. *Xanthomonas oryzae pv. oryzae* (*Xoo*) is a gram-negative bacteria causing bacterial leaf blight of rice which is one of the serious worldwide diseases of rice in most of the rice growing countries [4–6]. As another important gram-negative plant-pathogenic bacteria, *Xanthomonas axonopodis pv. citri* (*Xac*), can cause an extremely persistent disease-citrus bacterial canker through entering the natural openings (stomata) or wounds of plants in all producing countries [7–9]. Therefore, management of crops against spoilage by invading pathogenetic bacteria is a necessity. Using chemicals to address these bacterial diseases has become one of the effective measures [10, 11]. To date, great progress has been made in the exploration and development of antibacterial agents, and has led to a few of them being commercialized, such as bismerthiazol (BT) and thiodiazole copper (TC). However, these agents cannot meet the desired requirements due to their poor efficiency and/or bacterial resistance [12, 13]. Thus, a search for new types of inhibitors with excellent bioactivities is urgently needed in pesticide science.

Tobacco mosaic virus (TMV), as a classical pathogenic virus, can infect nine plant families, and at least 125 individual species including tobacco, tomato, pepper, potato, and cucumber [14]. Infected plants by TMV can give characteristic patterns with "mosaic"-like mottling and discoloration on the leaves, which consequently affect the quality and output of agricultural products every year [15, 16]. In the past decades, a few of the TMV-inhibitors have been commercialized, such as ningnanmycin (NNM) and ribavirin. They gave curative rates of 56.0 and 38.5% at a high dosage of 500 μg/mL, respectively. Moreover, their photosensitivity and/or water stickiness have become a major limitation for their field application [17, 18]. Obviously, exploring new high-efficient agents is required in antiviral drug discovery.

Natural substructures possess advantages over other general building blocks in the construction of bioactive molecules for several reasons, such as low mammalian toxicity, impressive biodegradability, the specific ability to the target species, desirable biological activities, as well as good biocompatibility [19, 20]. Oxadiazole, a natural skeleton, is usually utilized as the bioisosteric replacement of the amide bonds [21–24]. This scaffold was commonly observed with increased hydrolytic and metabolic stability, and improved pharmacokinetic performance in vivo, which endows it an important structural motif for the pharmaceutical industry [25, 26]. From the literature review, compounds bearing oxadiazole have displayed an impressive array of biological activities including antitumor, herbicidal, insecticidal, and antifungal activities [24, 27–30]. Particularly, their antibacterial and antiviral activities have attracted the interests of chemists [31–34]. For example, Khalilullah and co-workers had evaluated the antimicrobial activity of a series of 1,3,4-oxadizole derivatives containing 1,4-benzodioxane ring, and found that some of the tested compounds showed better antibacterial and antifungal activities than those of chloramphenicol and fluconazole [35]. Barral and co-workers reported a series of 5-phenyl-2-[2-(2-thienyl)ethenyl]-1,3,4-oxadiazole

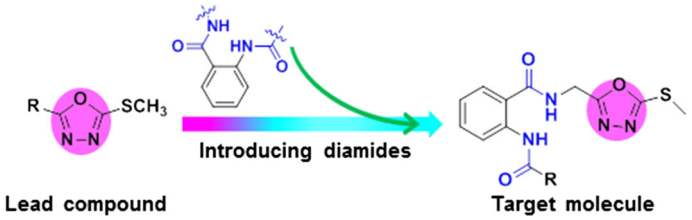


Fig. 1 Design strategy of the target compound

derivatives exhibiting submicromolar activity on the four dengue virus serotypes [36]. Neda and co-workers had synthesized a series of novel 1,2,4-oxadiazoles, and found that this kind of structure exhibited antitumor activities towards 12 cancer cell lines [37]. Meanwhile, it is noted that the synthesis of oxadiazole motif is economical and facile, which has made it become the promising pharmacophore for the construction of bioactive molecules [38]. As another crucial building block, diamide bonds, existing in many natural products, have been proved to possess diverse chemotherapeutic potentials of their derivatives [39–46]. Moreover, many compounds containing diamide bonds have been commercialized and used as pesticides, such as chlorantraniliprole, flubendiamide (insecticides), and fenpyrazamine (fungicide). In view of the aforementioned facts, the design of compounds possessing both oxadiazole pharmacophore and diamide bonds might result in bioactive molecules with better efficacy owing to the synergistic effect of these valuable moieties. Herein, a series of 1,3,4-oxadiazole derivatives bearing diamides were designed and synthesized, in which the diamides were placed at the 5-postion of 1,3,4-oxadiazole. All the title compounds were examined for their antibacterial and antiviral activities. To the best of our knowledge, no studies have so far reported the usage of this kind of compound against pathogenic *Xoo*, *Xac*, and TMV (Fig. 1).

Methods and materials

Chemistry

All the chemicals were purchased from Aladdin, and used as received. The organic solvents were distilled before used. Melting points of the compounds were determined on a XT-4 binocular microscope (Beijing Tech Instrument Co., China). NMR spectra were obtained by using a JEOL-ECX-500 apparatus. Chemical shifts were reported in parts per million (ppm) down field from TMS with the solvent resonance as the internal standard. Coupling constants (J) were reported in Hz and referred to apparent peak multiplications.

General synthetic procedures for the intermediates and target compounds

*N-(2-hydrazinyl-2-oxoethyl)-2-nitrobenzamide (**C**)* 2-Nitrobenzoic acid (3.59 g, 21.5 mmol) and $SOCl_2$ (15 mL) were refluxed for 5 h. The solvent was removed

under reduced pressure, and re-dissolved by dry tetrahydrofuran (THF, 8 mL). It was added dropwise into a solution of glycine ethyl ester hydrochloride (3.00 g, 21.5 mmol) and triethylamine (TEA, 7.45 mL, 53.7 mmol) in dry THF (30 mL) on ice bath, and then the mixture was stirred at room temperature for 8 h. After that, the solvent was removed under reduced pressure, and re-dissolved by ethyl acetate. The organic layer was washed by water, brine, dried with sodium sulfate, filtered, and followed by the removal of the solvent under vacuum, and re-dissolved in 20 mL ethanol containing 3 mL 80% hydrazine hydrate. The mixture was refluxed for 12 h. The solvent was removed under reduced pressure, and the precipitate was washed with water to give a light yellow solid (**C**), yield 56.8%, m. p. 206–207 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.04 (s, 1H, NHNH$_2$), 8.91 (t, J = 5.8 Hz, 1H, NHCH$_2$), 7.99 (dd, J = 7.9, 1.4 Hz, 1H, phenyl-H), 7.76 (td, J = 7.5, 1.2 Hz, 1H, phenyl-H), 7.68–7.64 (m, 2H, phenyl-H), 4.23 (s, 2H, NH$_2$), 3.79 (d, J = 5.8 Hz, 2H, NHCH$_2$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.7, 165.7, 147.1, 133.4, 131.9, 130.9, 129.3, 124.0, 41.2; MS (ESI): m/z = 239 [M + H$^+$].

*N-(2-hydrazinyl-2-oxoethyl)-3-nitrobenzamide (**C′**)* The synthesis of **C′** was carried out by the same method of **C**, and the difference is that 3-nitrobenzoic acid was used to replace 2-nitrobenzoic acid, a light yellow solid, yield 90.7%, m. p. 160–161 °C. ^{1}H NMR (500 MHz, DMSO-D6) δ 9.23 (s, 1H, NHNH$_2$), 9.16 (t, J = 5.8 Hz, 1H, NHCH$_2$), 8.71 (s, 1H, phenyl-H), 8.37 (dd, J = 8.2, 2.2 Hz, 1H, phenyl-H), 8.29 (d, J = 7.7 Hz, 1H, phenyl-H), 7.77 (t, J = 8.0 Hz, 1H, phenyl-H), 4.26 (s, 2H, NH$_2$), 3.88 (d, J = 5.8 Hz, 2H, NHCH$_2$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.1, 164.6, 147.7, 135.6, 133.9, 130.1, 126.0, 122.3; MS (ESI): m/z = 239 [M + H$^+$].

The synthesis of **D**, **D′**, **E**, and **E′** were carried out by our previous methods [47].

*N-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)-2-nitrobenzamide (**D**)* A yellow solid, yield 54.2%, m. p. >230 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.52 (s, 1H, SH), 9.44 (t, J = 5.6 Hz, 1H, NH), 8.07 (dd, J = 8.1, 0.8 Hz, 1H, phenyl-H), 7.83 (td, J = 7.5, 1.0 Hz, 1H, phenyl-H), 7.73 (td, J = 8.0, 1.3 Hz, 1H, phenyl-H), 7.63 (dd, J = 7.6, 1.2 Hz, 1H, phenyl-H), 4.54 (d, J = 5.7 Hz, 2H, N–CH$_2$); ^{13}C NMR (126 MHz, DMSO-d_6) 178.0, 165.9, 160.8, 146.9, 133.8, 131.4, 131.3, 129.1, 124.3, 34.4; MS (ESI): m/z = 281 [M + H$^+$].

*N-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)-3-nitrobenzamide (**D′**)* A yellow solid, yield 75.2%, m. p. 144–145 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.50 (s, 1H, SH), 9.60 (t, J = 5.4 Hz, 1H, NHCH$_2$), 8.77–8.64 (m, 1H, phenyl-H), 8.42 (ddd, J = 8.2, 2.3, 1.0 Hz, 1H, phenyl-H), 8.35–8.26 (m, 1H, phenyl-H), 7.81 (t, J = 8.0 Hz, 1H, phenyl-H), 4.60 (d, J = 5.5 Hz, 2H, NHCH$_2$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 177.9, 164.5, 161.1, 147.9, 134.5, 133.8, 130.4, 126.5, 122.1, 34.8; MS (ESI): m/z = 281 [M + H$^+$].

*N-((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)-2-nitrobenzamide (**E**)* A yellow solid, yield 31.4%, m. p. 108–109 °C; ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.92 (dd, J = 8.1, 0.8 Hz, 1H, phenyl-H), 7.76 (t, J = 5.7 Hz, 1H, NH), 7.59 (td, J = 7.5, 0.9 Hz, 1H, phenyl-H), 7.52–7.47 (m, 2H, phenyl-H), 4.68 (d, J = 5.9 Hz, 2H),

2.58 (s, 3H, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.0, 166.2, 164.2, 146.3, 133.9, 131.8, 130.9, 129.0, 124.5, 34.8, 14.6; MS (ESI): m/z = 295 [M + H^+].

*N-((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)-3-nitrobenzamide (**E′**)* A yellow solid, yield 58.1%, m. p. 129–130 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 8.66 (t, *J* = 1.9 Hz, 1H, phenyl-H), 8.34 (ddd, *J* = 8.2, 2.2, 1.0 Hz, 1H, phenyl-H), 8.20–8.16 (m, 1H, phenyl-H), 7.91 (t, *J* = 5.5 Hz, 1H, NH), 7.61 (t, *J* = 8.0 Hz, 1H, phenyl-H), 4.88 (d, *J* = 5.8 Hz, 2H, N–CH_2), 2.69 (s, 3H, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.7, 165.2, 164.7, 148.3, 134.8, 133.5, 130.0, 126.6, 122.4, 35.2, 14.7; MS (ESI): m/z = 295 [M + H^+].

*2-Amino-N-((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)benzamide (**F**)* Intermediate **E** (0.50 g, 1.7 mmol) was added into an ethanol solution (20 mL) containing zinc powder (1.11 g, 17.0 mmol) and NH_4Cl (0.91 g, 17.0 mmol), then the mixture was refluxed for 10 h. After that, the mixture was filtrated, and solvent was removed under reduced pressure, the pure compound (**F**) could be obtained by column chromatography using (CH_2Cl_2:EtOAc = 4:1) as the eluent, a yellow solid, yield 47.3%, m. p. 120–121 °C. ^1H NMR (500 MHz, $CDCl_3$) δ 7.39 (dd, *J* = 7.9, 1.3 Hz, 1H, phenyl-H), 7.26–7.22 (m, 1H, phenyl-H), 6.70–6.65 (m, 3H, phenyl-H&NH), 5.55 (s, 2H, NH_2), 4.81 (d, *J* = 5.7 Hz, 2H, N–CH_2), 2.72 (s, 3H, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.2, 166.3, 164.7, 149.2, 133.2, 127.5, 117.6, 116.9, 114.6, 34.7, 14.8; MS (ESI): m/z = 265 [M + H^+].

*3-Amino-N-((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)benzamide (**F′**)* The synthesis of **F′** was carried out as synthesis of **F**, a white solid, yield 39.0%, m. p. 128–129 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 7.19 (t, *J* = 7.8 Hz, 1H, phenyl-H), 7.15 (t, *J* = 2.0 Hz, 1H, phenyl-H), 7.13–7.09 (m, 1H, phenyl-H), 7.00 (t, *J* = 5.1 Hz, 1H, NH), 6.80 (ddd, *J* = 7.9, 2.4, 0.9 Hz, 1H, phenyl-H), 4.82 (d, *J* = 5.7 Hz, 2H, N–CH_2), 2.69 (s, 3H, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.9, 166.3, 164.8, 147.0, 134.5, 129.7, 118.6, 116.8, 114.0, 35.0, 14.7; MS (ESI): m/z = 265 [M + H^+].

*2-Acetamido-N-((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)benzamide (**G1**)* Acetylchloride (0.09 g, 1.14 mmol) was added into a solution of **F** (0.20 g, 0.76 mmol) and TEA (0.2 mL) in dry THF (8 mL), then the mixture was stirred at room temperature for 12 h. After that, the solvent was removed under reduced pressure, and re-dissolved by ethyl acetate. The organic layer was washed by water, brine, dried with sodium sulfate, filtered, and followed by the removal of the solvent under vacuum. The pure compound (**G1**) could be obtained by column chromatography using (CH_2Cl_2:CH_3OH = 20:1) as the eluent, a white solid, yield 11.4%, m. p. 143–144 °C. ^1H NMR (500 MHz, $CDCl_3$) δ 10.84 (s, 1H, NHCOCH$_3$), 8.52 (d, *J* = 7.8 Hz, 1H, phenyl-H), 7.55 (d, *J* = 7.4 Hz, 1H, phenyl-H), 7.46 (t, *J* = 7.5 Hz, 1H, phenyl-H), 7.35 (s, 1H, NH–CH_2), 7.07–7.04 (m, 1H, phenyl-H), 4.79 (d, *J* = 5.5 Hz, 2H, NCH_2), 2.71 (s, 3H, SCH_3), 2.18 (s, 3H, $COCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.3, 169.2, 166.5, 164.3, 139.7, 133.3, 127.1, 123.0, 121.7, 119.3, 34.9, 25.5, 14.7; MS (ESI): m/z = 307 [M + H^+].

The synthesis of **G2**, **G2′** and **G3** were carried out by synthetic protocols of **G1**.

*N-((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)-2-propionamidobenzamide (**G2**)* A white solid, yield 60.9%, m. p. 121–122 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.87 (s, 1H, NHCOCH$_2$), 8.59 (d, J = 8.3 Hz, 1H, phenyl-H), 7.55 (d, J = 7.9 Hz, 1H, phenyl-H), 7.48 (t, J = 7.9 Hz, 1H, phenyl-H), 7.19 (t, J = 5.0 Hz, 1H, NHCH$_2$), 7.06 (t, J = 7.7 Hz, 1H, phenyl-H), 4.81 (d, J = 5.7 Hz, 2H, N–CH$_2$), 2.71 (s, 3H, SCH_3), 2.43 (q, J = 7.6 Hz, 2H, COCH$_2$), 1.24 (t, J = 7.6 Hz, 3H, COCH$_2$CH$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.0, 169.2, 166.5, 164.2, 139.9, 133.3, 127.0, 122.8, 121.7, 119.2, 34.9, 31.6, 14.7, 9.7; MS (ESI): m/z = 321 [M + H$^+$].

*N-((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)-3-propionamidobenzamide (**G2′**)* A light yellow solid, yield 72.3%, m. p. 128–129 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (s, 1H, NHCOCH$_2$), 8.16 (t, J = 5.7 Hz, 1H, NHCH$_2$), 7.79 (s, 1H, phenyl-H), 7.72 (d, J = 8.1 Hz, 1H, phenyl-H), 7.44 (d, J = 7.7 Hz, 1H, phenyl-H), 7.19 (t, J = 7.9 Hz, 1H, phenyl-H), 4.73 (d, J = 5.8 Hz, 2H, N–CH$_2$), 2.62 (s, 3H, SCH_3), 2.33 (q, J = 7.5 Hz, 2H, COCH$_2$), 1.13 (t, J = 7.5 Hz, 3H, COCH$_2$CH$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.3, 167.9, 166.2, 165.1, 138.7, 133.7, 129.2, 123.6, 122.8, 118.6, 35.0, 30.5, 14.6, 9.7; MS (ESI): m/z = 321 [M + H$^+$].

*2-benzamido-N-((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)benzamide (**G3**)* A white solid, yield 27.4%, m. p. 216–217 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.19 (s, 1H, NHCO), 9.57 (t, J = 5.3 Hz, 1H, NHCH$_2$), 8.63 (d, J = 8.5 Hz, 1H, phenyl-H), 7.92–7.56 (m, 7H, phenyl-H), 7.24 (t, J = 7.8 Hz, 1H, phenyl-H), 4.73 (d, J = 5.4 Hz, 2H, CH$_2$), 2.66 (s, 1H, SCH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.0, 164.8, 164.7, 164.5, 139.4, 134.5, 132.8, 132.1, 129.0, 128.3, 126.9, 123.1, 120.6, 119.8, 34.4, 14.3; MS (ESI): m/z = 369 [M + H$^+$].

*1-(5-((2-(((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)carbamoyl)phenyl)amino)-5-oxopentyl)pyridin-1-ium bromide (**H4**)* 5-bromopentanoyl chloride (0.45 mmol) was added into a mixture of intermediate **F** (0.12 g, 0.45 mmol) and TEA (0.91 mmol) in 10 mL CH_2Cl_2. Then it was stirred at room temperature for 5 h. After that, the solvent was removed under reduced pressure, and re-dissolved by ethyl acetate. The organic layer was washed by water, brine, dried with sodium sulfate, filtered, and followed by the removal of the solvent under vacuum. The crude product was incubated in 5 mL pyridine at 50 °C for 12 h. After that, the extra pyridine was removed under reduced pressure. The crude residue was further purified by flash column chromatography on a silica gel using CH_2Cl_2 and CH_3OH (6:1) as the eluent to afford the desired product (**H4**), a yellow liquid, yield 41.2%. ^{1}H NMR (500 MHz, CD_3OD) δ 9.05 (d, J = 5.6 Hz, 2H, pyridine-H), 8.59 (t, J = 7.9 Hz, 1H, pyridine-H), 8.18 (d, J = 8.2 Hz, 1H, phenyl-H), 8.12 (t, J = 7.0 Hz, 2H, pyridine-H), 7.68 (d, J = 9.3 Hz, 1H, phenyl-H), 7.48 (t, J = 7.9 Hz, 1H, phenyl-H), 7.19 (t, J = 8.1 Hz, 1H, phenyl-H), 4.72 (s, 2H, NH–CH$_2$), 4.69 (t, J = 7.5 Hz, 2H, N–CH$_2$), 2.70 (s, 3H, SCH_3), 2.48 (t, J = 7.1 Hz, 2H, COCH$_2$), 2.13–2.05 (m, 2H, NCH$_2$CH$_2$), 1.79–1.70 (m, 2H, CH$_2$); ^{13}C NMR (126 MHz, CD_3OD) δ 173.2, 171.2, 167.7, 166.5, 146.9, 146.0, 139.2, 133.3, 129.6, 129.1, 125.0, 123.8, 123.2, 62.7, 37.4, 35.6, 31.7, 22.9, 14.7; MS (ESI): m/z = 426 [M − Br].

The synthesis of **Hn** (n = 5, 6, 7, 8, 9, 10, 15) and **Hn′** (n = 10, 15) were carried out by synthetic protocols of **H4**.

*1-(6-((2-(((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)carbamoyl)phenyl)amino)-6-oxohexyl)pyridin-1-ium bromide (**H5**)* A yellow liquid, yield 42.0%; ^{1}H NMR (500 MHz, CD_3OD) δ 9.04 (d, J = 6.2 Hz, 2H, pyridine-H), 8.58 (t, J = 7.8 Hz, 1H, pyridine-H), 8.26 (d, J = 8.3 Hz, 1H, phenyl-H), 8.10 (t, J = 7.0 Hz, 2H, pyridine-H), 7.73 (d, J = 9.3 Hz, 1H, phenyl-H), 7.51 (t, J = 8.6 Hz, 1H, phenyl-H), 7.21 (t, J = 7.9 Hz, 1H, phenyl-H), 4.76 (s, 2H, NH$\underline{CH_2}$), 4.68 (t, J = 7.5 Hz, 2H, N–CH_2), 2.71 (s, 3H, SCH_3), 2.43 (t, J = 7.2 Hz, 2H, $COCH_2$), 2.12–2.04 (m, 2H, $NCH_2\underline{CH_2}$), 1.81–1.73 (m, 2H, CH_2), 1.49–1.42 (m, 2H, CH_2); ^{13}C NMR (126 MHz, CD_3OD) δ 173.8, 171.2, 167.6, 166.6, 146.8, 146.0, 139.5, 133.4, 129.5, 129.1, 124.8, 123.3, 122.9, 62.8, 38.1, 35.6, 31.9, 26.3, 25.7, 14.7; MS (ESI): m/z = 440 [M − Br].

*1-(7-((2-(((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)carbamoyl)phenyl)amino)-7-oxoheptyl)pyridin-1-ium bromide (**H6**)* A yellow liquid, yield 62.2%; ^{1}H NMR (500 MHz, CD_3OD) δ 9.04 (d, J = 5.6 Hz, 2H, pyridine-H), 8.58 (t, J = 8.4 Hz, 1H, pyridine-H), 8.29 (d, J = 8.3 Hz, 1H, phenyl-H), 8.11 (t, J = 7.0 Hz, 2H, pyridine-H), 7.73 (d, J = 9.2 Hz, 1H, phenyl-H), 7.50 (t, J = 8.5 Hz, 1H, phenyl-H), 7.20 (t, J = 7.6 Hz, 1H, phenyl-H), 4.76 (s, 2H, NH$\underline{CH_2}$), 4.67 (t, J = 7.5 Hz, 2H, N–CH_2), 2.71 (s, 3H, SCH_3), 2.40 (t, J = 7.3 Hz, 2H, $COCH_2$), 2.08–2.01 (m, 2H, $NCH_2\underline{CH_2}$), 1.75–1.67 (m, 2H, CH_2), 1.49–1.40 (m, 4H, $2CH_2$); ^{13}C NMR (126 MHz, CD_3OD) δ 174.1, 171.1, 167.6, 166.6, 146.8, 145.9, 139.6, 133.4, 129.5, 129.1, 124.7, 123.1, 122.9, 62.9, 38.4, 35.6, 32.1, 29.3, 26.7, 26.1, 14.7; MS (ESI): m/z = 454 [M − Br].

*1-(8-((2-(((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)carbamoyl)phenyl)amino)-8-oxooctyl)pyridin-1-ium bromide (**H7**)* A yellow liquid, yield 19.1%; ^{1}H NMR (500 MHz, CD_3OD) δ 9.02 (d, J = 6.3 Hz, 2H, pyridine-H), 8.60 (t, J = 8.4 Hz, 1H, pyridine-H), 8.32 (d, J = 8.5 Hz, 1H, phenyl-H), 8.12 (t, J = 6.9 Hz, 2H, pyridine-H), 7.72 (d, J = 7.8 Hz, 1H, phenyl-H), 7.51 (t, J = 9.2 Hz, 1H, phenyl-H), 7.20 (t, J = 7.6 Hz, 1H, phenyl-H), 4.75 (s, 2H, NH$\underline{CH_2}$), 4.65 (t, J = 7.6 Hz, 2H, N–CH_2), 2.72 (d, J = 1.2 Hz, 3H, SCH_3), 2.40 (t, J = 7.2 Hz, 2H, $COCH_2$), 2.13–1.97 (m, 2H, $NCH_2\underline{CH_2}$), 1.78–1.60 (m, 2H, CH_2), 1.45–1.40 (m, 6H, $3CH_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.7, 169.5, 165.7, 165.0, 145.0, 144.9, 144.8. 139.8, 132.8, 128.5, 123.1, 121.5, 119.7, 62.3, 38.4, 35.1, 31.8, 29.8, 28.1, 25.6, 25.1, 14.8; MS (ESI): m/z = 468 [M − Br].

*1-(9-((2-(((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)carbamoyl)phenyl)amino)-9-oxononyl)pyridin-1-ium bromide (**H8**)* A yellow liquid, yield 28.6%; ^{1}H NMR (500 MHz, CD_3OD) δ 9.03 (d, J = 5.6 Hz, 2H, pyridine-H), 8.59 (t, J = 7.9 Hz, 1H, pyridine-H), 8.31 (d, J = 8.3 Hz, 1H, phenyl-H), 8.12 (t, J = 6.9 Hz, 2H, pyridine-H), 7.73 (d, J = 7.9 Hz, 1H, phenyl-H), 7.50 (t, J = 7.8 Hz, 1H, phenyl-H), 7.19 (t, J = 8.0 Hz, 1H, phenyl-H), 4.76 (s, 2H, NH$\underline{CH_2}$), 4.65 (t, J = 7.6 Hz, 2H, N–CH_2), 2.71 (s, 3H, SCH_3), 2.38 (t, J = 7.4 Hz, 2H, $COCH_2$), 2.04 (m, 2H, $NCH_2\underline{CH_2}$), 1.76–1.60 (m, 2H, CH_2), 1.38 (m, 8H, $4CH_2$); ^{13}C NMR (126 MHz,

CD_3OD) δ 174.3, 171.1, 167.6, 166.5, 146.8, 145.9, 139.7, 133.4, 129.5, 129.1, 124.7, 122.9, 122.8, 63.1, 38.7, 35.6, 32.4, 29.9, 29.8, 29.7, 26.9, 26.5, 14.7; MS (ESI): m/z = 482 [M − Br].

*1-(10-((2-(((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)carbamoyl)phenyl)amino)-10-oxodecyl)pyridin-1-ium bromide (**H9**)* A yellow liquid, yield 36.4%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.17 (s, 1H, NHCO), 9.17 (d, J = 5.9 Hz, 2H, pyridine-H), 8.78 (t, J = 5.6 Hz, 1H, $NHCH_2$), 8.50 (d, J = 8.3 Hz, 1H, phenyl-H), 8.39 (t, J = 7.4 Hz, 1H, pyridine-H), 8.03 (t, J = 7.0 Hz, 2H, pyridine-H), 7.97 (d, J = 7.4 Hz, 1H, phenyl-H), 7.41 (t, J = 7.9 Hz, 1H, phenyl-H), 7.06 (t, J = 7.7 Hz, 1H, phenyl-H), 4.83 (d, J = 5.7 Hz, 2H, $NHCH_2$), 4.71 (t, J = 7.6 Hz, 2H, NCH_2), 2.69 (s, 3H, SCH_3), 2.39 (t, J = 6.9 Hz, 2H, $COCH_2$), 1.89 (dt, J = 14.6, 7.2 Hz, 2H, NCH_2CH_2), 1.70 (dt, J = 14.0, 7.0 Hz, 2H, $COCH_2CH_2$), 1.27–1.23 (m, 10H, $5CH_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.7, 169.5, 165.7, 164.9, 145.0, 144.9, 139.8, 132.8, 128.5, 123.1, 121.5, 119.7, 62.3, 38.4, 35.1, 31.8, 29.8, 28.3, 28.1, 28.0, 25.6, 25.1, 14.8; MS (ESI): m/z = 496 [M − Br].

*1-(11-((2-(((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)carbamoyl)phenyl)amino)-11-oxoundecyl)pyridin-1-ium bromide (**H10**)* A light yellow solid, yield 38.7%, m. p. 105–106 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 9.04 (d, J = 5.3 Hz, 2H, pyridine-H), 8.60 (t, J = 7.8 Hz, 1H, pyridine-H), 8.31 (d, J = 8.3 Hz, 2H, pyridine-H), 8.12 (t, J = 7.1 Hz, 1H, phenyl-H), 7.73 (d, J = 7.9 Hz, 1H, phenyl-H), 7.50 (t, J = 8.6 Hz, 1H, phenyl-H), 7.19 (t, J = 7.6 Hz, 1H, phenyl-H), 4.76 (s, 2H, $NHCH_2$), 4.65 (t, J = 7.5 Hz, 2H, N–CH_2), 2.71 (s, 3H, SCH_3), 2.38 (t, J = 7.5 Hz, 2H, $COCH_2$), 2.10–1.91 (m, 2H, NCH_2CH_2), 1.75–1.59 (m, 2H, $COCH_2CH_2$), 1.37–1.31 (m, 12H, $6CH_2$); ^{13}C NMR (126 MHz, CD_3OD) δ 174.4, 171.1, 167.5, 166.5, 146.8, 145.9, 139.7, 133.4, 129.5, 129.1, 124.7, 122.9, 122.8, 63.1, 38.8, 35.6, 32.4, 30.3, 30.2, 30.1, 29.9, 27.1, 26.6, 14.7; MS (ESI): m/z = 510 [M − Br].

*1-(11-((3-(((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)carbamoyl)phenyl)amino)-11-oxoundecyl)pyridin-1-ium bromide (**H10′**)* A white solid, yield 57.6%, m. p. 163–164 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 9.04 (d, J = 5.8 Hz, 2H, pyridine-H), 8.59 (t, J = 7.8 Hz, 1H, pyridine-H), 8.12 (t, J = 7.0 Hz, 3H, pyridine-H & phenyl-H), 7.73 (d, J = 8.0 Hz, 1H, phenyl-H), 7.57 (d, J = 7.8 Hz, 1H, phenyl-H), 7.41 (t, J = 7.9 Hz, 1H, phenyl-H), 4.77 (s, 2H, $NHCH_2$), 4.65 (t, J = 7.5 Hz, 2H, NCH_2), 2.70 (s, 3H, SCH_3), 2.40 (t, J = 7.4 Hz, 2H, $COCH_2$), 2.07–1.91 (m, 2H, NCH_2CH_2), 1.75–1.60 (m, 2H, $COCH_2CH_2$), 1.36–1.31 (m, 12H, $6CH_2$); ^{13}C NMR (126 MHz, CD_3OD) δ 174.8, 169.9, 167.5, 166.6, 146.8, 145.9, 140.4, 135.4, 130.1, 129.5, 124.6, 123.7, 120.3, 63.1, 37.9, 35.7, 32.4, 30.2, 30.1, 29.9, 27.1, 26.7, 14.7; MS (ESI): m/z = 510 [M − Br].

*1-(16-((2-(((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)carbamoyl)phenyl)amino)-16-oxohexadecyl)pyridin-1-ium bromide (**H15**)* A colourless liquid, yield 44.0%; ^{1}H NMR (500 MHz, CD_3OD) δ 9.01 (d, J = 5.7 Hz, 2H, pyridine-H), 8.60 (t, J = 7.8 Hz, 1H, pyridine-H), 8.32 (d, J = 8.3 Hz, 1H, phenyl-H), 8.12

(t, $J = 7.0$ Hz, 2H, pyridine-H), 7.71 (d, $J = 7.9$ Hz, 1H, phenyl-H), 7.50 (t, $J = 8.0$ Hz, 1H, phenyl-H), 7.19 (t, $J = 7.5$ Hz, 1H, phenyl-H), 4.76 (s, 2H, $NHCH_2$), 4.64 (t, $J = 7.5$ Hz, 2H, NCH_2), 2.72 (s, 3H, SCH_3), 2.39 (t, $J = 7.6$ Hz, 2H, $COCH_2$), 2.06–1.98 (m, 2H, $NCH_2\underline{CH_2}$), 1.73–1.65 (m, 2H, $COCH_2\underline{CH_2}$), 1.43–1.24 (m, 22H, $11CH_2$); ^{13}C NMR (126 MHz, CD_3OD) δ 166.6, 146.9, 145.9, 133.4, 129.5, 129.0, 124.7, 123.0, 122.9, 63.1, 35.5, 32.5, 30.7, 30.6, 30.5, 30.4, 30.3, 30.2, 30.1, 27.2, 26.7 (s), 14.7; MS (ESI): m/z = 580 [M − Br].

*1-(16-((3-(((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)carbamoyl)phenyl)amino)-16-oxohexadecyl)pyridin-1-ium bromide (**H15′**)* A white solid, yield 45.8%, m. p. 123–124 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 9.03 (d, $J = 6.0$ Hz, 2H, pyridine-H), 8.60 (t, $J = 7.8$ Hz, 1H, pyridine-H), 8.14–8.10 (m, 3H, pyridine-H & phenyl-H), 7.73 (d, $J = 9.0$ Hz, 1H, phenyl-H), 7.57 (d, $J = 7.7$ Hz, 1H, phenyl-H), 7.42 (t, $J = 7.9$ Hz, 1H, phenyl-H), 4.77 (s, 2H, $NHCH_2$), 4.65 (t, $J = 7.4$ Hz, 2H, $N–CH_2$), 2.71 (s, 3H, SCH_3), 2.39 (t, $J = 7.4$ Hz, 2H, $COCH_2$), 2.10–1.93 (m, 2H, $NCH_2\underline{CH_2}$), 1.77–1.58 (m, 2H, $COCH_2\underline{CH_2}$), 1.37–1.27 (m, 22H, $11CH_2$); ^{13}C NMR (126 MHz, CD_3OD) δ 174.9, 170.0, 167.6, 166.6, 146.8, 145.9, 140.4, 135.4, 130.1, 129.5, 124.6, 123.7, 120.3, 63.1, 38.0, 35.7, 32.5, 30.6, 30.5, 30.4, 30.3, 30.2, 30.1, 27.1, 26.8, 14.7; MS (ESI): m/z = 580 [M − Br].

In vitro antibacterial bioassay

In our study, all the synthesized target compounds were evaluated for their antibacterial activities against *Xoo* and *Xac* by the turbidimeter test in vitro. Dimethylsulfoxide in sterile distilled water served as a blank control. Bismerthiazol and Thiodiazole Copper served as positive controls. Approximately 40 μL of solvent NB (1.5 g beef extract, 2.5 g peptone, 0.5 g yeast powder, 5.0 g glucose, and 500 mL distilled water; pH 7.0–7.2) containing *Xoo* (or *Xac*), incubated in the phase of logarithmic growth, was added to 5 mL of solvent NB containing different concentrations of the test compounds and positive control, such as 100, 50 μg/mL (for preliminary bioassay), 80, 40, 20, 10, 5 μg/mL, 20, 10, 5, 2.5, 1.25 μg/mL, or 1.25, 0.625, 0.3125, 0.156, 0.078 μg/mL (for EC_{50} detection, depend on the bioactivity of different compounds, the concentration was chosen as two times the decline trend). The inoculated test tubes were incubated at 28 ± 1 °C and continuously shaken at 180 rpm for 24–48 h until the bacteria were incubated in the logarithmic growth phase. The growth of the cultures was monitored on a microplate reader by measuring the optical density at 595 nm (OD_{595}) given by 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 values of bacterial growth on untreated NB (blank control), and T is the corrected turbidity values of bacterial growth on treated NB. By using the 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.

In vivo antiviral bioassay

Curative activity of the target compounds against TMV in vivo

Growing *Nicotiana tabacum* L. leaves of the same age were selected. The leaves were inoculated with TMV (concentration of 6×10^{-3} mg/mL) by dipping and brushing the whole leaves, which had previously been scattered with silicon carbide. The leaves were then washed with water after inoculation for 0.5 h. The compound solution was smeared on the left side of the leaves, and the solvent was smeared on the right side as the control. The number of local lesions was counted and recorded 3–4 days after inoculation. Three replicates were set up for each.

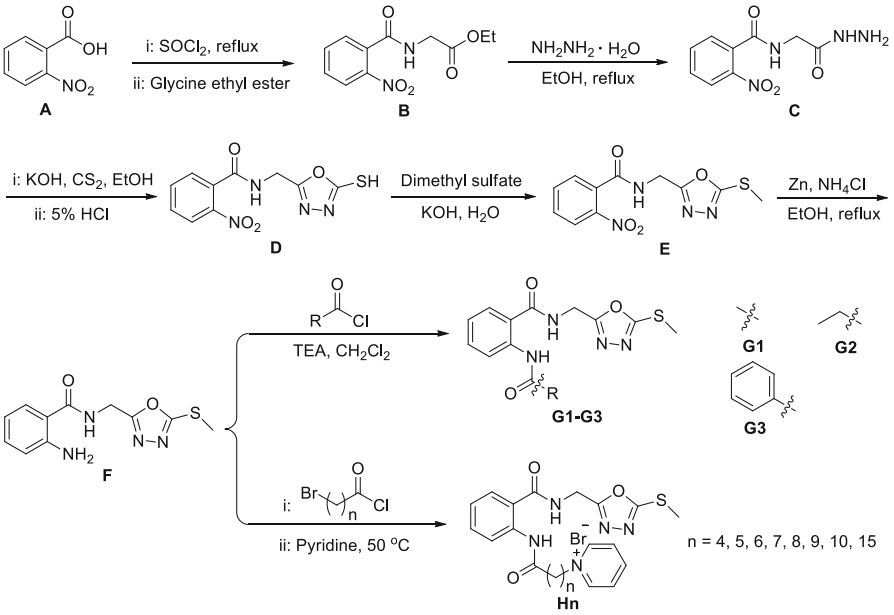
Protection activity of the target compounds against TMV in vivo

The compound solutions were smeared on the left side of the *N. tabacum* L. leaves, and the solvents were smeared on the right side as the control sample for growing *N. tabacum* L. leaves. After 12 h, crude TMV (concentration of 6×10^{-3} mg/mL) was inoculated on whole leaves at the same concentration on each side of the leaves, which were previously scattered with silicon carbide. After 0.5 h, the leaves were washed with water and then dried. The number of local lesions was recorded 3–4 days after inoculation. Three replicates were used for each compound. The inhibitory rate of the compound was calculated according to the following formula:

$$\begin{aligned}\text{Inhibition rate} = [&(\text{average local lesion number of control}(\text{not treated with compound})\\ &-\text{average local lesion number smeared with drugs})/\\ &\text{average local lesion number of control}\\ &(\text{not treated with compound})] \times 100\%.\end{aligned}$$

Results and discussion

The structure and synthesis of title compounds (**G1–G3** and **Hn**) are illustrated in Scheme 1. Ethyl 2-(2-nitrobenzamido)acetate (**B**) was synthesized from 2-nitrobenzoic acid (**A**) by chlorination, followed by treatment with glycine ethyl ester hydrochloride. The reaction of **B** and hydrazine hydrate in ethanol gave the desired intermediate *N*-(2-hydrazinyl-2-oxoethyl)-2-nitrobenzamide (**C**) that was then treated with carbon disulfide in the presence of potassium hydroxide to form *N*-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)-2-nitrobenzamide (**D**). Subsequently, a substitution reaction with dimethyl sulfate to give *N*-((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)-2-nitrobenzamide (**E**) that was dealt with by zinc and ammonium chloride to give an important immediate (**F**) bearing an amino group. The target molecules (**G1–G3**) were obtained via treating **F** with substituted acyl chloride. Intermediate (**F**) was reacted by two-step consecutive reactions with bromide-tailored acyl chloride and pyridine to provide title compounds (**Hn**, n = 4, 5, 6, 7, 8,



Scheme 1 Synthetic route of the target compounds (**G1–G3** and **Hn**)

9, 10, 15). All the structures were confirmed by ^{1}H NMR, ^{13}C NMR, and MS (detailed NMR spectra see supplementary data).

Turbidimeter tests [48, 49] was carried out to evaluate their antibacterial activities toward pathogenic bacteria *Xoo* and *Xac* in vitro, and the commercial antibacterial agents (**BT** and **TC**) were used as positive controls under the same conditions. As shown in Table 1, some intermediates and most of the target compounds displayed certain growth suppression effects against *Xoo* and *Xac* at 100 or 50 μg/mL. Among them, compounds **D**, **F**, **H10**, and **H15** exerted the strongest antibacterial effect toward *Xoo* with inhibition rates of 99.3, 86.5, 90.5, and 99.7% at 100 μg/mL, respectively, which were better than those of **BT** (56.1%) or **TC** (43.1%). Moreover, the intermediate **D** and compound **H15** remained the best antibacterial functions against *Xoo* with the rate above 98% as the concentrate decreased two fold. Further studies indicated that compounds **D** and **H15** showed admirable inhibition effects against *Xoo* with EC_{50} values of 10.1 and 0.7 μg/mL (Table 2), respectively, which were quite superior to those of **BT** (EC_{50} = 92.6 μg/mL) or **TC** (EC_{50} = 121.8 μg/mL). Compounds **G2**, **H4**, **H6**, and **H15** gave the inhibition rates of 98.0, 67.6, 70.0, and 99.8% against *Xac* at 100 μg/mL, respectively, which were better than that of **TC** (56.0%) at 100 μg/mL. It was noted that only compound **H15** still maintained the potent inhibition effect against *Xac* with the rate of 99.8% at 50 μg/mL. EC_{50} value for **H15** against *Xac* was determined as 5.9 μg/mL. Based on the above bioassays, this kind of compound could be further studied as alternative antibacterial agents.

Preliminary structure-antibacterial activity relationship analysis toward *Xoo* and *Xac* was elucidated according to the bioassay results. It is worth mentioning that the

Table 1 Inhibition rates (%) of compounds (**D**, **D′**, **E**, **E′**, **F**, **F′**, **G1–G3**, **G2′**, **Hn**, **H10′**, and **H15′**) against *Xoo* and *Xac* in vitro

Compounds	*Xoo*		*Xac*	
	100 μg/mL	50 μg/mL	100 μg/mL	50 μg/mL
D	99.3 ± 0.7	98.5 ± 1.1	3.9 ± 5.5	0
E	34.7 ± 3.9	26.2 ± 4.1	13.8 ± 2.9	12.6 ± 5.6
F	86.5 ± 4.4	33.7 ± 7.2	34.1 ± 3.4	14.0 ± 4.7
G1	25.8 ± 1.8	20.2 ± 9.0	14.5 ± 7.2	0
G2	37.8 ± 2.9	26.0 ± 2.9	98.0 ± 2.5	20.1 ± 1.7
G3	68.3 ± 5.3	19.2 ± 6.7	13.8 ± 2.9	12.6 ± 5.6
H4	39.6 ± 4.6	34.8 ± 4.3	67.6 ± 2.3	32.5 ± 4.8
H5	19.2 ± 6.7	18.5 ± 6.5	32.0 ± 3.3	20.5 ± 0.7
H6	30.7 ± 7.2	21.4 ± 4.8	70.0 ± 1.5	29.3 ± 1.9
H7	26.9 ± 3.1	25.1 ± 4.3	33.8 ± 1.7	22.6 ± 6.9
H8	38.4 ± 3.0	34.2 ± 1.8	53.3 ± 4.5	5.7 ± 3.2
H9	39.3 ± 6.4	32.8 ± 2.0	0	0
H10	90.5 ± 4.6	45.5 ± 4.9	29.9 ± 5.1	4.3 ± 2.1
H15	99.7 ± 1.7	98.3 ± 2.7	99.8 ± 0.3	99.8 ± 0.3
D′	99.2 ± 0.2	99.0 ± 0.2	28.5 ± 5.0	21.1 ± 0.8
E′	88.8 ± 2.9	55.5 ± 5.4	28.8 ± 6.9	18.4 ± 5.3
F′	63.0 ± 4.0	33.1 ± 1.9	17.6 ± 1.5	10.9 ± 3.8
G2′	61.2 ± 1.9	39.3 ± 4.8	5.0 ± 1.7	4.1 ± 0.5
H10′	74.0 ± 0.8	42.1 ± 3.8	71.5 ± 1.0	44.5 ± 3.2
H15′	86.8 ± 3.9	56.8 ± 5.2	11.6 ± 1.4	9.4 ± 0.7
BT	53.7 ± 1.2	35.3 ± 3.4	–	–
TC	43.1 ± 3.2	28.5 ± 1.7	56.0 ± 1.2	37.6 ± 2.3

Table 2 EC_{50} values of target compounds against pathogenic bacteria *Xoo* and *Xac*

Compd.	*Xoo*			*Xac*		
	Regression equation	r	EC_{50} (μg/mL)	Regression equation	r	EC_{50} (μg/mL)
D	$y = 1.578x + 4.175$	0.96	10.1 ± 4.6	–	–	–
H15	$y = 1.282x + 5.197$	0.97	0.7 ± 0.5	$y = 1.617x + 3.752$	0.95	5.9 ± 0.1
D′	$y = 0.861x + 4.063$	0.99	12.2 ± 1.1	–	–	–
H15′	$y = 1.166x + 3.032$	0.99	48.7 ± 1.7	–	–	–
BT	$y = 1.499x + 2.052$	0.98	92.6 ± 2.1	–	–	–
TC	$y = 1.540x + 1.788$	0.98	121.8 ± 3.6	$y = 2.153x + 0.938$	0.98	77.0 ± 2.0

substituents had a great impact on bioactivity. The bioactivity against *Xoo* was dramatically decreased by introducing an aliphatic group (–CH_3, **E**, 34.7%) on the mercapto group (–SH, **D**, 99.3%), indicating that the sulfhydryl group might own the specific ability to bind the target species of bacteria. An increased bioactivity was observed when the nitro-group (–NO_2, **E**, 34.7%) was reduced into an amino group (–NH_2, **F**, 86.5%), suggesting a hydrogen-bond donor is favourable for the

bioactivity. The anti-*Xoo* activity was improved about two fold when the aromatic ring (phenyl group, **G3**, 68.3%) replaced the aliphatic substituents (–CH_3, **G1**, 25.8%; –CH_2CH_3, **G2**, 37.8%) in the molecules, probably attributed to the molecular lipid solubility. It was noted that the inhibition effect was enhanced with increasing the alkyl length ($n = 15$, **H15**, 99.7%; $n = 10$, **H10**, 90.5%; $n = 9$, **H9**, 39.3%; $n = 8$, **H8**, 38.4%; $n = 7$, **H7**, 26.9%), might be ascribed to the enhanced molecular hydrophobicity. In contrast, a suitable aliphatic substituent (–CH_2CH_3, **G2**, 98.0%) showed better anti-*Xac* activity than those of other aliphatic (–CH_3, **G1**, 14.5%) or aromatic (phenyl group, **G3**, 13.8%) substituents. The anti-*Xac* effect reached the maximum when n was 15, indicating that a suitable hydrophobicity/hydrophilicity balance was gained for **H15** against *Xac* (Fig. 2).

In order to optimize the molecular structure and investigate the position of the amide bond toward bioactivity, intermediates (**D′**, **E′**, **F′**) and target compounds (**G2′**, **H10′**, **H15′**) were selectively synthesized by using 3-nitrobenzoic acid as the starting material in instead of 2-nitrobenzoic acid. All the compounds were tested for their antibacterial activities under the same conditions. As indicated in Tables 1 and 2, intermediate **D′** almost maintained the same anti-*Xoo* effect with EC_{50} value of 12.2 μg/mL (**D**, 10.1 μg/mL), while intermediate **E′** gave improved anti-*Xoo* activity with an inhibition rate of 88.8% at 100 μg/mL (**E**, 34.7%). A decreased bioactivity against *Xoo* was observed for compounds **H10** (from 90.5 to 74.0%, 100 μg/mL) and **H15** (EC_{50} value from 0.7 to 28.9 μg/mL). Meanwhile, it was disadvantageous to anti-*Xac* activity when the substituent at the meta-position of the benzene ring, as indicated by the comparison of compounds **H15′** (11.6%, 100 μg/mL) and **H15** (99.8%, 100 μg/mL). The above fact revealed that the position of the amide bond on the benzene ring did have a significant impact on the bioactivity.

The anti-TMV activity of the intermediates and all title compounds were tested in vivo as previously described [50, 51], and the commercial agent **NNM** was chosen as the positive control. As shown in Table 3, most of the tested compounds displayed certain curative and protective activities against TMV at 500 μg/mL. Among them, compounds **H6**, **H8**, and **H15** displayed the strongest curative activity with the

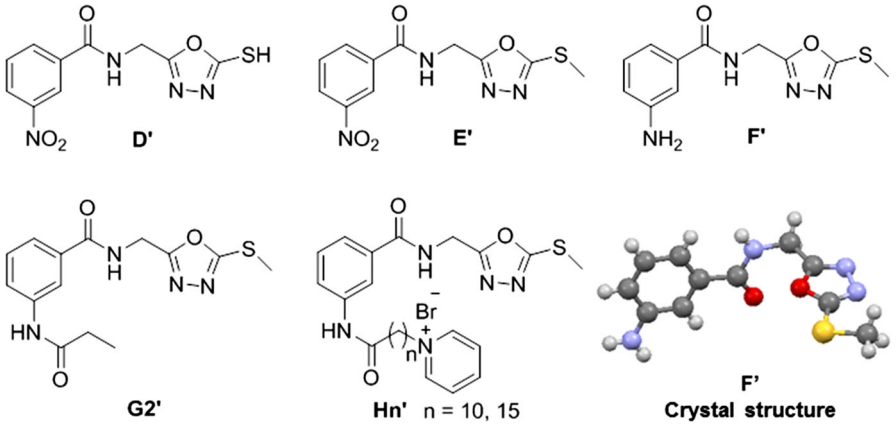


Fig. 2 Structures of compounds **D′**, **E′**, **F′**, **G2′**, **H10′**, **H15′** with crystal structure of **F′**

Table 3 Anti-TMV activities of compounds (**D**, **D′**, **E**, **E′**, **F**, **F′**, **G1**–**G3**, **G2′**, **Hn**, **H10′**, and **H15′**) at 500 μg/mL in vivo

Compounds	Rate (%)		Compounds	Rate (%)	
	Curative activity	Protective activity		Curative activity	Protective activity
D	38.5 ± 1.2	35.2 ± 3.1	**H8**	60.0 ± 5.6	36.4 ± 1.0
E	36.9 ± 5.1	14.4 ± 2.9	**H9**	26.9 ± 2.9	43.3 ± 3.0
F	26.8 ± 5.2	54.5 ± 2.9	**H10**	48.7 ± 5.1	25.2 ± 2.9
G1	22.3 ± 6.4	54.6 ± 5.2	**H15**	51.9 ± 3.0	45.6 ± 4.2
G2	47.2 ± 2.8	38.8 ± 4.5	**D′**	41.8 ± 1.0	41.7 ± 1.7
G3	44.8 ± 9.5	36.8 ± 0.8	**E′**	17.5 ± 1.2	32.2 ± 1.6
H4	7.1 ± 1.7	51.2 ± 7.6	**F′**	17.7 ± 1.2	42.6 ± 2.2
H5	37.4 ± 3.5	27.8 ± 5.5	**G2′**	49.3 ± 2.0	19.6 ± 2.4
H6	50.6 ± 4.7	42.9 ± 2.5	**H10′**	33.9 ± 1.3	20.2 ± 1.0
H7	37.1 ± 3.3	23.5 ± 1.1	**H15′**	35.3 ± 2.3	19.3 ± 0.8
NNM	54.2 ± 2.9	65.7 ± 2.2		–	

therapeutic rates of 50.6, 60.0, and 51.9%, respectively, which were comparable with that of **NNM** (54.2%). Meanwhile, compounds **F**, **G1** and **H4** displayed moderate protective effects with values of 54.5, 54.6, and 51.2%, respectively, which were lower than that of **NNM** (65.7%). The structure–activity relationship analysis was elucidated based on the preliminary bioassays. For compounds **H4**, **H6**, **H8**, and **H10**, the curative activity reached the maximum with $n = 6$, suggesting that the appropriate hydrophobicity/hydrophility ratio of a molecule was advantageous to the bioactivity. The position of substituents also affected the anti-TMV activity, as indicated by the comparison of curative activity of compounds **H10** (48.7%) and **H10′** (33.9%), **H15** (51.9%) and **H15′** (35.3%), revealing that the amide bond at the ortho-position of the benzene ring might be beneficial for anti-TMV activity.

Conclusion

In summary, two series of novel 1,3,4-oxadiazole derivatives bearing diamides were fabricated and evaluated their antibacterial and antiviral activity. Antibacterial assays indicated that intermediates **D** and **D′** showed excellent inhibition effects against *Xoo* with EC_{50} values of 10.1 and 12.2 μg/mL, respectively, which were quite superior to those of commercial antibacterial agents **BT** ($EC_{50} = 92.6$ μg/mL) or **TC** ($EC_{50} = 121.8$ μg/mL). Title compound **H15** exhibited the best activities against *Xoo* and *Xac* with minimal EC_{50} values of 0.7 and 5.9 μg/mL, respectively. Meanwhile, antiviral bioassays suggested compound **H8** exerted the best curative activity against TMV with a rate of 60.0% at 500 μg/mL, which was comparable with that of commercial agent **NNM** (54.2%). Given their potent bioactivities, these kinds of compounds could be further studied as promising antibacterial and antiviral candidates.

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