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Antiviral and Antibacterial Activities of *N*-(4-Substituted phenyl) Acetamide Derivatives Bearing 1,3,4-Oxadiazole Moiety

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In this paper, a series of *N*-(4-substituted phenyl) acetamide derivatives bearing 1,3,4-oxadiazole moiety were synthesised. Preliminary bioassays revealed that these compounds not only exhibited favourable antiviral activities toward tobacco mosaic virus (TMV) but also demonstrated sustained inhibition activities against plant pathogenic bacteria, including *Xanthomonas oryzae* pv. *oryzae*, *Ralstonia solanacearum*, and *Xanthomonas axonopodis* pv. *citri.* Among the derivatives, **TC_8** and **TC_{20}** exerted the strongest curative activities against TMV, with half-maximal effective concentration (EC_{50}) values of 239.5 and 236.2 μg/mL, respectively, which were comparable to that of ningnanmycin (EC_{50}=273.2 μg/mL). Given their simple synthesis, the target compounds can serve as alternative antiviral candidates.

Keywords 1,3,4-oxadiazole, acetamide, synthesis, antiviral, antibacterial

Introduction

Plant diseases caused by pathogenic viruses and bacteria have attracted extensive attention because of their significant threats towards agricultural products.[1-5] Tobacco mosaic disease exhibits typical symptoms, such as mosaic-like mottling and discoloration on the leaves; this disease is mainly caused by the invasion of tobacco mosaic virus (TMV) and can occur at all growth stages of tobacco, thereby resulting in large economic losses annually.[6-8] Some plant viral inhibitors (ribavirin and ningnanmycin) have been used to combat TMV, but rare chemicals can completely cure the infected plant tissues or fully protect plants from infection under field conditions.[9] Moreover, pathogenic bacteria can lead to various diseases, such as rice bacterial leaf blight, tobacco bacterial wilt and citrus bacterial canker, which are caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), *Ralstonia solanacearum* (*R. solanacearum*), and *Xanthomonas axonopodis* pv. *citri* (*Xac*), respectively.[10-12] Although some commercial bactericides had been employed to address these diseases, their poor efficiency, high phytotoxicity, or bactericide resistance had been paid more and more attention worldwide. Thus, exploring and developing highly efficient antiviral and antibacterial agents to manage plant diseases remain a significant task.

Compounds with 1,3,4-oxadiazole scaffold exhibit a wide range of biological activities, including antibacterial, antifungal, antiviral and antitumour activities.[13-21] Furthermore, our previous work verified that 1,3,4-oxadiazole derivatives displayed potent antibacterial and anti-fungal activities and resulted in two antibacterial candidates for commercialisation.[22-25] With their potent bioactivity toward pathogenic microorganism, considerable efforts have been exerted to explore and develop alternative pesticide agents based on the scaffold of 1,3,4-oxadiazole.

An amide bond can modulate the physical properties of a molecule, and bioactivities are often tuned by introducing an amide bond into target compounds.[26] Recently, some fungicides containing an amide bond have been discovered and successfully commercialised; these fungicides include boscalid, fluopicolide and salicylanilide. Hence, a series of *N*-(4-substituted phenyl) acetamide derivatives bearing 1,3,4-oxadiazole moiety were designed by introducing an amide bond into 1,3,4-oxadiazole derivatives (Figure 1). Within these molecules, an amide bond was placed at the 2-position of 1,3,4-oxadiazole. Moreover, the title compounds were bioassayed against pathogenic virus (TMV) and pathogenic bacteria (*Xoo*, *R. solanacearum* and *Xac*).

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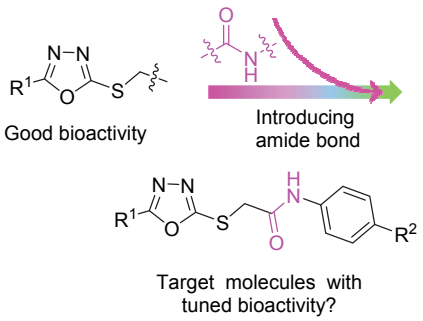

Figure 1 Design strategy of the target compounds.

Experimental

Materials

Melting points were determined using an XT-4 binocular microscope (Beijing Tech Instrument Co., China) and left uncorrected. Elemental analysis was performed using an Elemental Vario-III CHN analyzer. NMR spectra were obtained by using a JEOL-ECX-500 apparatus. Chemical shifts were reported 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. Mass spectral studies were conducted on an Agilent 5973 organic mass spectrometer. The course of the reactions was monitored by thin-layer chromatography analysis on silica gel GF254. All reagents were analytical grade or CP. All anhydrous solvents were dried and purified according to standard techniques before use.

Antivir alactivity against TMV

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 d 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 d after inoculation. Three replicates were used for each compound. The inhibitory rate of the compound was calculated according to the following formula ("av" means average):

Inhibition rate=[(av local lesion no. of control (not treated with compound) – av local lesion no. smeared with drugs)/av local lesion no. of control (not treated with compound)]×100%.

***In vitro* antibacterial bioassay (turbidimeter test)** In our study, all the synthesized target compounds were evaluated for their antibacterial activities against *Xoo*, *R. solanacearum*, 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* (*R. solanacearum* or *Xac*), incubated on the phase of logarithmic growth, was added to 5 mL of solvent NB containing the test compounds and positive control. The inoculated test tubes were incubated at (28±1) ℃ and continuously shaken at 180 r/min for 24—48 h until the bacteria were incubated on 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 $I=(C-T)/C\times100\%$. 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. The experiment was repeated three times.

General synthesis protocols of target compounds (TC_1—TC_{30})

The intermediate 2-bromo-*N*-(4-(substituted)-phenyl)acetamide (**5**, 1.58 mmol) was added into a mixture of 5-substituted-1,3,4-oxadiazole-2-thiol (**3**, 1.58 mmol) and K_2CO_3 (1.83 mmol) in the solvent acetone, then the solution was stirred at room temperature for 6 h. After that, the organic layer was poured into ice-cold water to give precipitates, filtered, and dried. Finally, the pure title compounds **TC_1—TC_{30}** were obtained through recrystallization in methanol.

***N*-(4-Fluorophenyl)-2-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC_1)** A white solid, m.p. 199—200 ℃; yield 71%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.48 (s, 1H), 7.92 (d, J=6.7 Hz, 2H, Ar-H), 7.62 (dd, J=8.5, 1.6 Hz, 2H, Ar-H), 7.56 (d, J=6.5 Hz, 2H, NH-Ar-H), 7.13 (d, J=15.8 Hz, 2H, NH-Ar-H), 4.31 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.3 (C=O), [164.9, 164.2] (oxadiazol), [159.7, 157.8, 137.3, 135.6, 130.1, 128.7, 122.4, 121.5 (d, J=8.0 Hz), 116.0 (d, J=11.5 Hz)] (aromatic), 37.3 (CH_2); IR (KBr) ν_{max}: 3282, 3050, 2930, 1684, 1671, 1546, 1508, 1476, 835, 809 cm^{-1}; MS (ESI) m/z: 364.1 ([M+H]$^+$).

***N*-(4-Chlorophenyl)-2-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC_2)** A white solid, m.p. 197—198 ℃; yield 78%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.57 (s, 1H, NH), 7.92 (d, J=8.6 Hz, 2H, Ar-H), 7.62 (d, J=8.6 Hz, 2H, Ar-H), 7.58 (d, J=8.9

Hz, 2H, NH-Ar-H), 7.35 (d, J=8.9 Hz, 2H, NH-Ar-H), 4.32 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.6 (C=O), [164.9, 164.2] (oxadiazol), [138.1, 137.3, 130.1, 129.3, 128.7, 127.8, 122.4, 121.2] (aromatic), 37.3 (CH_2); IR (KBr) ν_{max}: 3309, 3083, 2929, 1684, 1662, 1607, 1548, 1474, 837, 813 cm^{-1}; MS (ESI) m/z: 380.0 ([M+H]$^+$).

***N*-(4-Bromophenyl)-2-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC_3)** A white solid, m.p. 209−210 ℃; yield 87%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.56 (s, 1H, NH), 7.93 (d, J=8.6 Hz, 2H, Ar-H), 7.62 (d, J=8.6 Hz, 2H, NH-Ar-H), 7.52 (d, J=8.9 Hz, 2H, Ar-H), 7.48 (d, J=9.0 Hz, 2H, NH-Ar-H), 4.32 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.6 (C=O), [164.9, 164.2] (oxadiazol), [138.5, 137.3, 132.3, 130.2, 128.7, 122.4, 121.6, 115.9] (aromatic), 37.5 (CH_2); IR (KBr) ν_{max}: 3240, 3047, 2931, 1675, 1537, 1488, 1474, 1242, 838, 825 cm^{-1}. MS (ESI) m/z: 423.9 ([M+H]$^+$).

***N*-(4-*p*-Tolyl)-2-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC_4)** A light yellow solid, m.p. 207−209 ℃; yield 77%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.37 (s, 1H, NH), 7.98 (d, J=8.6 Hz, 2H, Ar-H), 7.67 (d, J=8.6 Hz, 2H, Ar-H), 7.48 (d, J=8.4 Hz, 2H, NH-Ar-H), 7.14 (d, J=8.3 Hz, 2H, NH-Ar-H), 4.34 (s, 2H, CH_2), 2.25 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.1 (C=O), [164.9, 164.3] (oxadiazol), [137.3, 136.7, 133.2, 130.1, 129.8, 128.7, 122.4, 119.7] (aromatic), 37.4 (CH_2), 21.0 (CH_3); IR (KBr) ν_{max}: 3252, 3048, 2970, 2920, 1673, 1603, 1540, 1511, 1472, 1383, 1245, 844, 814 cm^{-1}; MS (ESI) m/z: 360.3 ([M+H]$^+$).

***N*-(4-Methoxyphenyl)-2-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC_5)** A white solid, m.p. 210−212 ℃; yield 89%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.32 (s, 1H, NH), 7.95 (d, J=8.6 Hz, 2H, Ar-H), 7.64 (d, J=8.6 Hz, 2H, Ar-H), 7.49 (d, J=9.1 Hz, 2H, NH-Ar-H), 6.90 (d, J=9.0 Hz, 2H, NH-Ar-H), 4.33 (s, 2H, CH_2), 3.70 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 164.9 (C=O), [164.8, 164.3] (oxadiazol), [156.0, 137.3, 132.3, 130.1, 128.70, 122.4, 121.2, 114.5] (aromatic), 55.7 (OCH_3), 37.2 (CH_2); IR (KBr) ν_{max}: 3248, 3056, 2932, 2835, 1670, 1602, 1543, 1510, 1474, 1241, 846, 828 cm^{-1}; MS-ESI (+) m/z: 376.1 ([M+K]$^+$).

***N*-(4-Nitrophenyl)-2-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC_6)** A light yellow solid, m.p. 228−229 ℃; yield 58%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.03 (s, 1H, NH), 8.20 (d, J=8.9 Hz, 2H, NH-Ar-H), 7.92 (d, J=8.4 Hz, 2H, NH-Ar-H), 7.80 (d, J=9.0 Hz, 2H, Ar-H), 7.61 (d, J=8.4 Hz, 2H, Ar-H), 4.39 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.5 (C=O), [165.00, 164.1] (oxadiazol), [145.1, 143.0, 137.3, 130.2, 128.7, 125.7, 122.3, 119.5] (aromatic), 37.4 (CH_2); IR (KBr) ν_{max}: 3363, 3111, 2928, 1707, 1604, 1536, 1507, 1470, 1248, 870, 836 cm^{-1}; MS-ESI (+) m/z: 391.2 ([M+H]$^+$).

***N*-(4-Fluorophenyl)-2-((5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC_7)** A white solid, m.p. 215−216 ℃; yield 60%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.56 (s, 1H, NH), 7.91 (d, J=6.2 Hz, 1H, NH-Ar-H), 7.66 (d, J=8.0 Hz, 1H, NH-Ar-H), 7.62−7.58 (m, 1H, Ar-H), 7.52 (t, J=8.4 Hz, 3H, Ar-H), 7.48 (d, J=8.8 Hz, 2H, NH-Ar-H), 4.32 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.5 (C=O), [164.6, 163.7] (oxadiazol), [138.5, 133.9, 132.2 (d, J=8.3 Hz), 131.7, 128.4, 122.6, 121.6, 115.8] (aromatic), 37.3 (CH_2); IR (KBr) ν_{max}: 3309, 3069, 2930, 1717, 1672, 1653, 1598, 1517, 1380, 1255, 829, 741 cm^{-1}; MS-ESI (+) m/z: 364.1 ([M+H]$^+$).

***N*-(4-Chlorophenyl)-2-((5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC_8)** A white solid, m.p. 168−169 ℃; yield 63%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.58 (s, 1H, NH), 7.91 (d, J=7.8 Hz, 1H, NH-Ar-H), 7.66 (d, J=8.0 Hz, 1H, NH-Ar-H), 7.60 (d, J=7.9 Hz, 1H, Ar-H), 7.58 (d, J=8.8 Hz, 2H, Ar-H), 7.50 (t, J=7.7 Hz, 1H, Ar-H), 7.35 (d, J=8.6 Hz, 2H, NH-Ar-H), 4.33 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.5 (C=O) [164.6, 163.8] (oxadiazol), [138.1, 133.9, 132.2, 131.7, 129.3, 128.4, 127.8, 122.6, 121.2] (aromatic), 37.3 (CH_2); IR (KBr) ν_{max}: 3268, 3070, 2979, 1684, 1608, 1546, 1482, 1464, 1280, 1038, 817, 765 cm^{-1}; MS-ESI (+) m/z: 380.0 ([M+H]$^+$).

***N*-(4-Bromophenyl)-2-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC_9)** A white solid, m.p. 180−181 ℃; yield 60%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.55 (s, 1H, NH), 7.91 (d, J=6.3 Hz, 1H, NH-Ar-H), 7.67 (d, J=7.7 Hz, 1H, NH-Ar-H), 7.60 (t, J=7.0 Hz, 1H, Ar-H), 7.52 (t, J=8.6 Hz, 3H, Ar-H), 7.48 (d, J=8.9 Hz, 2H, NH-Ar-H), 4.33 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.5 (C=O); [164.5, 163.8] (oxadiazol), [138.6, 133.9, 132.3, 132.2, 131.7, 128.4, 122.7, 121.6, 115.8] (aromatic), 37.3 (CH_2); IR (KBr) ν_{max}: 3309, 3065, 2978, 1684, 1607, 1545, 1482, 1464, 1247, 1036, 815, 765 cm^{-1}; MS (ESI) m/z: 424.0 ([M+H]$^+$).

***N*-(4-*p*-Tolyl)-2-((5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC_{10})** A white solid, m.p. 122−123 ℃; yield 47%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.33 (s, 1H, NH), 7.91 (d, J=9.3 Hz, 1H, NH-Ar-H), 7.67 (d, J=8.0 Hz, 1H, NH-Ar-H), 7.60 (t, J=7.8 Hz, 1H, Ar-H), 7.50 (t, J=7.5 Hz, 1H, Ar-H), 7.43 (d, J=8.4 Hz, 2H, Ar-H), 7.09 (d, J=8.3 Hz, 2H, NH-Ar-H), 4.31 (s, 2H, CH_2), 2.22 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.0 (C=O), [164.7, 163.7] (oxadiazol), [136.7, 133.8, 133.2, 132.2, 131.7, 130.1, 129.8, 128.4, 122.7, 119.7] (aromatic), 37.3 (CH_2), 21.0 (CH_3); IR (KBr) ν_{max}: 3272, 3132, 2923, 1688, 1653, 1610, 1554, 1507, 1399, 1204, 1171, 1036, 818, 764 cm^{-1}; MS (ESI) m/z: 360.1 ([M+H]$^+$).

***N*-(4-Methoxyphenyl)-2-((5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC_{11})** A white solid, m.p. 150−152 ℃; yield 68%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.27 (s, 1H, NH), 7.92 (d, J=7.8 Hz, 1H, Ar-H), 7.67 (d, J=8.0 Hz, 1H, Ar-H), 7.62−7.58 (m, 1H, Ar-H), 7.50 (t, J=7.5 Hz, 1H, Ar-H), 7.45

(d, J=9.0 Hz, 2H, NH-Ar-H), 6.86 (d, J=9.0 Hz, 2H, NH-Ar-H), 4.29 (s, 2H), 4.29 (s, 2H, CH_2), 3.68 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 164.7 (C=O), [164.6, 163.7] (oxadiazol), [156.0, 133.8, 132.3, 132.2, 131.7, 128.4, 121.2, 114.5] (aromatic), 55.7 (OCH_3), 37.2 (CH_2); IR (KBr) ν_{max}: 3298, 3140, 3087, 1680, 1610, 1559, 1512, 1482, 1244, 1177, 1044, 835, 764 cm^{-1}; MS (ESI) m/z: 376.0 ([M+H]$^+$).

***N*-(4-Nitrophenyl)-2-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC$_{12}$)** A light yellow solid, m.p. 218−220 ℃; yield 70%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.03 (s, 1H, NH), 8.21 (d, J=9.2 Hz, 2H, NH-Ar-H), 7.91 (d, J=6.2 Hz, 1H, Ar-H), 7.80 (d, J=9.2 Hz, 2H, NH-Ar-H), 7.66 (d, J=7.9 Hz, 1H, Ar-H), 7.59 (t, J=6.9 Hz, 1H, Ar-H), 7.50 (t, J=7.5 Hz, 1H, Ar-H), 4.40 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 166.4 (C=O), [164.5, 163.8] (oxadiazol), [145.2, 143.3, 133.9, 132.2, 131.7, 128.4, 125.7, 122.6, 119.5] (aromatic), 37.4 (CH_2); IR (KBr) ν_{max}: 3309, 3087, 2996, 1684, 1615, 1546, 1467, 1408, 1329, 1212, 1166, 1109, 857, 770, 753, 737 cm^{-1}; MS (ESI) m/z: 391.2 ([M+H]$^+$).

***N*-(4-Fluorophenyl)-2-((5-(3-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC$_{13}$)** A white solid, m.p. 136−138 ℃; yield 62%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.54 (s, 1H, NH), 7.95 (s, 1H), 7.93 (d, J=7.8 Hz, 1H, Ar-H), 7.71 (d, J=8.0 Hz, 1H, Ar-H), 7.64 (d, J=3.8 Hz, 1H, Ar-H), 7.62 (d, J=4.6 Hz, 2H, NH-Ar-H), 7.18 (t, J=8.9 Hz, 2H, NH-Ar-H), 4.37 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.4 (C=O), [164.5, 164.5] (oxadiazol), [135.6, 134.6, 132.4, 132.0, 126.4, 125.5 (d, J=21.5 Hz), 121.5 (d, J=8.1 Hz), 116.0 (d, J=22.5 Hz)] (aromatic), 37.2 (CH_2); IR (KBr) ν_{max}: 3285, 3075, 2975, 1658, 1546, 1510, 1478, 1409, 1215, 1169, 1080, 839, 805, 773, 724 cm^{-1}; MS (ESI) m/z: 364.1 ([M+H]$^+$).

***N*-(4-Chlorophenyl)-2-((5-(3-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC$_{14}$)** A white solid, m.p. 163−165 ℃; yield 74%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.03 (s, 1H, NH), 8.21 (d, J=8.6 Hz, 2H, Ar-H), 7.92 (d, J=8.0 Hz, 2HNH-Ar-H), 7.80 (d, J=8.4 Hz, 2H, NH-Ar-H), 7.62 (d, J=8.0 Hz, 2H, Ar-H), 4.39 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 166.5 (C=O), [165.0, 164.1] (oxadiazol), [145.2, 143.0, 137.3, 130.2, 128.7, 125.7, 122.3, 119.5] (aromatic), 37.4 (CH_2); IR (KBr) ν_{max}: 3313, 2974, 2926, 1660, 1594, 1520, 1407, 1202, 1183, 1080, 823, 791, 772, 718 cm^{-1}; MS (ESI) m/z: 380.0 ([M+H]$^+$).

***N*-(4-Bromophenyl)-2-((5-(3-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC$_{15}$)** A white solid, m.p. 179−181 ℃; yield 53%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.58 (s, 1H, NH), 7.92−7.87 (m, 2H, Ar-H), 7.66 (d, J=7.9 Hz, 1H, Ar-H), 7.57 (t, J=7.9 Hz, 1H, Ar-H), 7.53 (d, J=8.9 Hz, 2H, NH-Ar-H), 7.47 (d, J=8.9 Hz, 2H, NH-Ar-H), 4.32 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.7 (C=O), [164.6, 164.4] (oxadiazol), [138.5, 134.6, 132.4, 132.3, 132.0, 126.4, 125.6, 125.4, 121.6, 115.9] (aromatic), 37.3 (CH_2); IR (KBr) ν_{max}: 3312, 3030, 2926, 1659, 1589, 1525, 1488, 1396, 1202, 1183, 1080, 821, 791, 772, 718 cm^{-1}; MS (ESI) m/z: 424.0 ([M+H]$^+$).

***N*-(*o*-Tolyl)-2-((5-(3-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC$_{16}$)** A white solid, m.p. 150 − 152 ℃; yield 54%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.34 (s, 1H, NH), 7.93−7.87 (m, 2H, ArH), 7.66 (d, J=8.0 Hz, 1H, ArH), 7.57 (t, J=7.9 Hz, 1H, ArH), 7.43 (d, J=8.3 Hz, 2H, NH-Ar-H), 7.09 (d, J=8.2 Hz, 2H, NH-Ar-H), 4.31 (s, 2H, CH_2), 2.21 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.2 (C=O), [164.5, 136.7] (oxadiazol), [134.6, 133.2, 132.4, 132.0, 129.8, 126.4, 125.6, 125.4, 119.7] (aromatic), 37.3 (CH_2), 21.0 (CH_3); IR (KBr) ν_{max}: 3264, 3020, 2930, 1688, 1654, 1554, 1463, 1380, 1201, 1170, 1078, 816, 719, 774, 716 cm^{-1}; MS (ESI) m/z: 360.1 ([M+H]$^+$).

***N*-(4-Methoxyphenyl)-2-((5-(3-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC$_{17}$)** A white solid, m.p. 143−144 ℃; yield 48%; ^{1}H NMR (500 MHz, DMSO-d_6) δ:10.31 (s, 1H, NH), 7.96−7.87 (m, 2H, Ar-H), 7.67 (d, J=8.0 Hz, 1H, Ar-H), 7.58 (t, J=7.9 Hz, 1H, Ar-H), 7.46 (d, J=8.9 Hz, 2H, NH-Ar-H), 6.86 (d, J=8.8 Hz, 2H, NH-Ar-H), 4.29 (s, 2H, CH_2), 3.68 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 164.9 (C=O), [164.5, 156.0] (oxadiazol), [134.6, 132.4, 132.3, 132.0, 129.8, 127.6, 126.4, 125.6, 125.4, 121.2, 114.8, 114.6] (aromatic), 55.7 (OCH_3), 37.2 (CH_2); IR (KBr) ν_{max}: 3322, 3065, 2839, 1650, 1550, 1470, 1248, 1169, 1033, 833, 792, 775, 719 cm^{-1}; MS (ESI) m/z: 376.1 ([M+H]$^+$).

***N*-(4-Nitrophenyl)-2-((5-(3-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC$_{18}$)** A light yellow solid, m.p. 206−207 ℃; yield 77 %; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.03 (s, 1H, NH), 8.20 (d, J=9.1 Hz, 2H, NH-Ar-H), 7.87 (d, J=7.5 Hz, 2H, NH-Ar-H), 7.80 (d, J=9.2 Hz, 2H, Ar-H), 7.63 (d, J=8.2 Hz, 1H, Ar-H), 7.57 (t, J=7.9 Hz, 1H, Ar-H), 4.39 (s, 2H CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 166.6 (C=O), [164.6, 164.3] (oxadiazol), [145.2, 143.1, 134.6, 132.4, 132.0, 126.4, 125.7, 125.6, 125.4, 119.5] (aromatic), 37.4 (CH_2); IR (KBr) ν_{max}: 3326, 3074, 2837, 1700, 1570, 1556, 1507, 1332, 1253, 1171, 1109, 856, 792, 773, 721 cm^{-1}; MS (ESI) m/z: 391.2 ([M+H]$^+$).

***N*-(4-Fluorophenyl)-2-((5-(4-chlorobenzyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC$_{19}$)** A white solid, m.p. 129−130 ℃; yield 67%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.41 (s, 1H, NH), 7.53 (dd, J=9.1, 5.0 Hz, 2H, NH-Ar-H), 7.34 (d, J=8.4 Hz, 2H, NH-Ar-H), 7.29 (d, J=8.5 Hz, 2H, Ar-H), 7.13 (t, J=8.9 Hz, 2H, Ar-H), 4.23 (s, 2H, CH_2), 4.20 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 166.9 (C=O), [165.2, 163.9] (oxadiazol), [159.7, 157.7, 135.6, 133.7, 132.5, 131.4, 129.2, 121.4 (d, J=8.0 Hz), 116.0 (d, J=8.0 Hz)] (aromatic), 37.1 (CH_2), 30.5 (CH_2); IR (KBr) ν_{max}: 3309, 3094, 2837, 1676, 1588, 1480, 1410, 1168, 1092, 837, 808, 771, 695 cm^{-1}; MS (ESI) m/z: 378.1([M+H]$^+$).

***N*-(4-Chlorophenyl)-2-((5-(4-chlorobenzyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC$_{20}$)** A white solid,

m.p. 136—137 ℃; yield 80%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.49 (s, 1H, NH), 7.54 (d, J=8.3 Hz, 2H, NH-Ar-H), 7.35 (d, J=2.5 Hz, 2H, NH-Ar-H), 7.33 (d, J=2.0 Hz, 2H, Ar-H), 7.29 (d, J=7.9 Hz, 2H, Ar-H), 4.23 (s, 2H, CH_2), 4.21 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 166.9 (C=O), [165.4, 163.8] (oxadiazol), [138.1, 133.7, 132.5, 131.3, 129.3, 129.3, 127.8, 121.2] (aromatic), 37.1 (CH_2), 30.5 (CH_2); IR (KBr) ν_{max}: 3313, 3131, 2938, 1683, 1554, 1491, 1406, 1176, 1097, 1066, 823, 778 cm^{-1}; MS (ESI) *m*/*z*: 394.1 ([M+H]$^+$).

***N*-(4-Bromophenyl)-2-((5-(4-chlorobenzyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC_{21})** A white solid, m.p. 137—138 ℃; yield 92 %; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.48 (s, 1H, NH), 7.49 (d, J=9.2 Hz, 2H, NH-Ar-H), 7.47 (d, J=9.2 Hz, 2H, NH-Ar-H), 7.34 (d, J=8.4 Hz, 2H, Ar-H), 7.28 (d, J=8.5 Hz, 2H, Ar-H), 4.23 (s, 2H, CH_2), 4.21 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 166.9 (C=O), [165.5, 163.8] (oxadiazol), [138.5, 133.7, 132.5, 132.2, 131.3, 129.2, 121.5, 115.8] (aromatic), 37.1 (CH_2), 30.5 (CH_2); IR (KBr) ν_{max}: 3316, 3126, 2938, 1684, 1550, 1490, 1473, 1179, 820, 778 cm^{-1}; MS (ESI) *m*/*z*: 438.3 ([M+H]$^+$).

***N*-(*p*-Tolyl)-2-((5-(4-chlorobenzyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC_{22})** A white solid, m.p. 156—157 ℃; yield 97%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.26 (s, 1H, NH), 7.40 (d, J=7.4 Hz, 2H, NH-Ar-H), 7.33 (d, J=8.0 Hz, 2H, NH-Ar-H), 7.29 (d, J=8.1 Hz, 2H, Ar-H), 7.08 (d, J=7.9 Hz, 2H, Ar-H), 4.23 (s, 2H, CH_2), 4.20 (s, 2H, CH_2), 2.22 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 166.8 (C=O), [165.0, 163.9] (oxadiazol), [136.7, 133.8, 133.2, 132.5, 131.3, 129.8, 129.2, 119.6] (aromatic), 37.2 (CH_2), 30.5 (CH_2), 21.0 (CH_3); IR (KBr) ν_{max}: 3317, 3133, 2939, 1681, 1554, 1491, 1473, 1380, 1173, 814, 719 cm^{-1}; MS (ESI) *m*/*z*: 374.1 ([M+H]$^+$).

***N*-(4-Methoxyphenyl)-2-((5-(4-chlorobenzyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC_{23})** A white solid, m.p. 138—139 ℃; yield 74%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.19 (s, 1H, NH), 7.40 (d, J=9.0 Hz, 2H, NH-Ar-H), 7.32 (d, J=8.4 Hz, 2H, Ar-H), 7.27 (d, J=8.5 Hz, 2H, Ar-H), 6.84 (d, J=9.0 Hz, 2H, NH-Ar-H), 4.24 (s, 2H, CH_2), 4.16 (s, 2H, CH_2), 3.67 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 166.9 (C=O), [164.8, 155.8] (oxadiazol), [133.8, 133.7, 132.5, 132.3, 131.3, 129.2, 121.2, 114.5] (aromatic), 55.7 (OCH_3), 37.1 (CH_2), 30.5 (CH_2), 166.9, 164.8, 155.8, 133.8, 133.7, 132.5, 132.3, 131.3, 129.2, 121.2, 114.5, 55.7, 37.1, 30.5; IR (KBr) ν_{max}: 3296, 3088, 2834, 1669, 1558, 1512, 1482, 1247, 1166, 823, 775 cm^{-1}; MS (ESI) *m*/*z*: 390.1 ([M+H]$^+$).

***N*-(4-Nitrophenyl)-2-((5-(4-chlorobenzyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC_{24})** A light yellow solid, m.p. 153—155 ℃; yield 61%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.95 (s, 1H, NH), 8.21 (d, J=9.2 Hz, 2H, NH-Ar-H), 7.76 (d, J=9.2 Hz, 2H, NH-Ar-H), 7.33 (d, J=6.4 Hz, 2H, Ar-H), 7.28 (d, J=8.5 Hz, 2H, Ar-H), 4.28 (s, 2H, CH_2), 4.23 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 167.0 (C=O), [166.4, 163.7] (oxadiazol), [145.2, 143.0, 133.7, 132.5, 131.3, 129.2, 125.7, 119.4] (aromatic), 37.2 (CH_2), 30.5 (CH_2); IR (KBr) ν_{max}: 3272, 3092, 2933, 1684, 1570, 1510, 1481, 1337, 1172, 1112, 858, 751 cm^{-1}; MS (ESI) *m*/*z*: 405.3 ([M+H]$^+$.

***N*-(4-(Fluorophenyl)-2-((5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC_{25})** A light yellow solid, m.p. 217—218 ℃; yield 57%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.03 (s, 1H, NH), 8.21 (d, J=9.0 Hz, 2H, Ar-H), 7.92 (d, J=8.5 Hz, 2H, Ar-H), 7.80 (d, J=9.0 Hz, 2H, NH-Ar-H), 7.62 (d, J=8.3 Hz, 2H, NH-Ar-H), 4.32 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.4 (C=O); [164.9, 164.0] (oxadiazol), [160.5, 135.6, 129.6 (d, J=9.2 Hz), 128.6 (d, J=9.1 Hz), 121.5 (d, J=8.1 Hz), 119.2 (d, J=7.7 Hz), 117.2 (dd, J=22.4, 15.9 Hz), 116.4—115.9 (m) (aromatic)], 37.2 (CH_2); IR (KBr) ν_{max}: 3263, 3072, 2890, 1627, 1594, 1500, 1480, 1248, 843, 833, 776, 732 cm^{-1}; MS (ESI) *m*/*z*: 348.2 ([M+H]$^+$).

***N*-(4-Chlorophenyl)-2-((5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC_{26})** A white solid, m.p. 200—202 ℃; yield 61%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.62 (s, 1H, NH), 8.02 (dd, J=8.8, 5.4 Hz, 2H, Ar-H), 7.62 (d, J=8.9 Hz, 2H, Ar-H), 7.44 (t, J=8.8 Hz, 2H, NH-Ar-H), 7.39 (d, J=8.8 Hz, 2H, NH-Ar-H), 4.36 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.6 (C=O), [165.0, 163.9] (oxadiazol), [163.6, 138.1, 134.3, 133.7, 130.6, 129.6 (d, J=8.7 Hz), 129.3, 127.8, 121.2, 120.2, 117.2 (d, J=22.3 Hz)] (aromatic), 37.3 (CH_2); IR (KBr) ν_{max}: 3309, 3077, 2860, 1663, 1605, 1546, 1500, 1489, 1238, 841, 814, 730, 624 cm^{-1}; MS (ESI) *m*/*z*: 364.0 ([M+H]$^+$.

***N*-(4-Bromophenyl)-2-((5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC_{27})** A white solid, m.p. 200—202 ℃; yield 66%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.58 (s, 1H, NH), 7.98 (dd, J=8.8, 5.4 Hz, 2H Ar-H), 7.51 (d, J=8.9 Hz, 2H, Ar-H), 7.45 (d, J=9.0 Hz, 2H, NH-Ar-H), 7.39 (t, J=8.8 Hz, 2H, Ar-H), 4.31 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.7 (C=O), [165.0, 163.9] (oxadiazol), [163.6, 138.5, 132.3, 129.6 (d, J=9.3 Hz), 121.6, 120.2, 117.2 (d, J=22.3 Hz), 115.8] (aromatic), 37.3 (CH_2); IR (KBr) ν_{max}: 3309, 3129, 2890, 1664, 1605, 1546, 1500, 1488, 1380, 1224, 841, 814, 730, 624 cm^{-1}; MS (ESI) *m*/*z*: 407.9 ([M+H]$^+$).

***N*-(*p*-Tolyl)-2-((5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC_{28})** A white solid, m.p. 172—173 ℃; yield 83%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.32 (s, 1H, NH), 7.93 (t, J=10.6 Hz, 2H, Ar-H), 7.62 (d, J=8.5 Hz, 2H, NH-Ar-H), 7.41 (d, J=8.3 Hz, 2H, Ar-H), 7.09 (d, J=8.2 Hz, 2H, Ar-H), 4.30 (s, 2H, CH_2), 2.25 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.1 (C=O), [164.9, 164.3] (oxadiazol), [137.3, 136.7, 133.2, 130.1, 129.8, 128.7, 122.4, 119.7] (aromatic), 37.4 (CH_2), 21.0 (CH_3); IR (KBr) ν_{max}: 3249, 3043, 2925, 1673, 1607, 1540, 1534, 1501, 1380, 1229, 844, 812, 735, 622 cm^{-1}; MS (ESI)

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m/*z*: 344.1 ([M+H]$^+$).

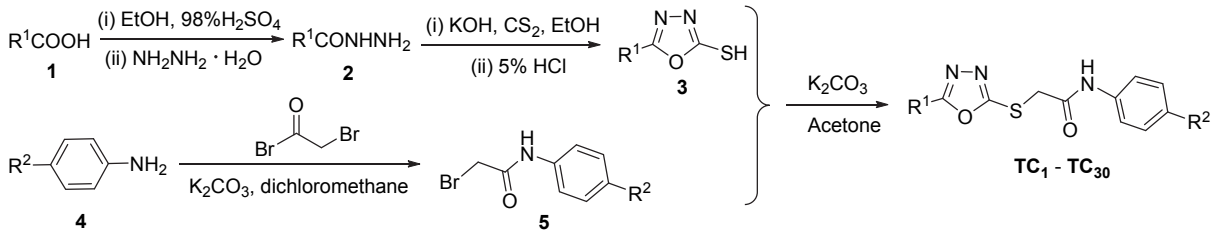
***N*-(4-Methoxyphenyl)-2-((5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC$_{29}$)** A white solid, m.p. 188—189 ℃; yield 63%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.28 (s, 1H, NH), 7.98 (d, J=13.6 Hz, 2H, Ar-H), 7.45 (d, J=8.8 Hz, 2H, Ar-H), 7.39 (t, J=8.7 Hz, 2H, NH-Ar-H), 6.86 (d, J=8.8 Hz, 2H, NH-Ar-H), 4.28 (s, 2H, CH_2), 3.68 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.6 (C=O), [164.9, 164.0] (oxadiazol), [159.7, 156.1, 132.3, 130.5, 129.8, 129.6 (d, J=9.1 Hz), 121.2 117.2 (d, J=22.5 Hz), 114.7 (d, J=46.3 Hz)] (aromatic), 55.7 (OCH_3), 37.3 (CH_2); IR (KBr) ν_{max}: 3248, 3052, 2935, 1670, 1607, 1595, 1509, 1380, 1228, 849, 832, 735, 623 cm^{-1}; MS (ESI) *m*/*z*: 360.1 ([M+H]$^+$).

***N*-(4-Nitrophenyl)-2-((5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamides (TC$_{30}$)** A light yellow solid, m.p. 184—185 ℃; yield 62%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.02 (s, 1H, NH), 8.21 (d, J=8.7 Hz, 2H, NH-Ar-H), 7.98 (dd, J=8.0, 5.7 Hz, 2H, NH-Ar-H), 7.80 (d, J=9.2 Hz, 2H, Ar-H), 7.40 (t, J=8.8 Hz, 2H, Ar-H), 4.38 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 166.6 (C=O), [165.6, 165.0] (oxadiazol), [163.8 (d, J=21.3 Hz), 145.2, 143.1, 129.6 (d, J=9.3 Hz), 125.7, 120.2, 119.5, 117.2 (d, J=21.3 Hz)] (aromatic), 37.4 (CH_2); IR (KBr) ν_{max}: 3264, 3085, 2935, 1690, 1611, 1560, 1509, 1380, 1229, 849, 832, 754, 623 cm^{-1}; MS (ESI) *m*/*z*: 375.1 ([M+H]$^+$).

Results and Discussion

The synthetic route for the title compounds (**TC$_1$—TC$_{30}$**) is illustrated in Scheme 1. The starting material (substituted benzoic or benzyl carboxylic acid (**1**)) was treated via esterification, hydrazidation, cyclisation and acidification to provide an important intermediate, namely, 5-substituted-1,3,4-oxadiazole-2-thiol (**3**).[27,28] Another crucial intermediate (**5**) was synthesized through the substitution reaction between 4-substituted anilines and 2-bromoacetyl bromide under the base of K_2CO_3 in dichloromethane. Finally, the title compounds **TC$_1$—TC$_{30}$** were obtained by incubating **3** and **5** in acetone at room temperature for 6 h. All the target compounds were characterised by IR, ^{1}H NMR, ^{13}C NMR,

Scheme 1 Synthetic route of the title compounds (**TC$_1$—TC$_{30}$**)



1 **2** **3** **4** **5**

TC$_1$: R^1 = 4-Cl-C$_6$H$_4$-	R^2 = F	**TC$_{11}$**: R^1 = 2-Cl-C$_6$H$_4$-	R^2 = OCH_3	**TC$_{21}$**: R^1 = 4-Cl-C$_6$H$_4$-CH$_2$-	R^2 = Br
TC$_2$: R^1 = 4-Cl-C$_6$H$_4$-	R^2 = Cl	**TC$_{12}$**: R^1 = 2-Cl-C$_6$H$_4$-	R^2 = NO_2	**TC$_{22}$**: R^1 = 4-Cl-C$_6$H$_4$-CH$_2$-	R^2 = CH_3
TC$_3$: R^1 = 4-Cl-C$_6$H$_4$-	R^2 = Br	**TC$_{13}$**: R^1 = 3-Cl-C$_6$H$_4$-	R^2 = F	**TC$_{23}$**: R^1 = 4-Cl-C$_6$H$_4$-CH$_2$-	R^2 = OCH_3
TC$_4$: R^1 = 4-Cl-C$_6$H$_4$-	R^2 = CH_3	**TC$_{14}$**: R^1 = 3-Cl-C$_6$H$_4$-	R^2 = Cl	**TC$_{24}$**: R^1 = 4-Cl-C$_6$H$_4$-CH$_2$-	R^2 = NO_2
TC$_5$: R^1 = 4-Cl-C$_6$H$_4$-	R^2 = OCH_3	**TC$_{15}$**: R^1 = 3-Cl-C$_6$H$_4$-	R^2 = Br	**TC$_{25}$**: R^1 = 4-F-C$_6$H$_4$-	R^2 = F
TC$_6$: R^1 = 4-Cl-C$_6$H$_4$-	R^2 = NO_2	**TC$_{16}$**: R^1 = 3-Cl-C$_6$H$_4$-	R^2 = CH_3	**TC$_{26}$**: R^1 = 4-F-C$_6$H$_4$-	R^2 = Cl
TC$_7$: R^1 = 2-Cl-C$_6$H$_4$-	R^2 = F	**TC$_{17}$**: R^1 = 3-Cl-C$_6$H$_4$-	R^2 = OCH_3	**TC$_{27}$**: R^1 = 4-F-C$_6$H$_4$-	R^2 = Br
TC$_8$: R^1 = 2-Cl-C$_6$H$_4$-	R^2 = Cl	**TC$_{18}$**: R^1 = 3-Cl-C$_6$H$_4$-	R^2 = NO_2	**TC$_{28}$**: R^1 = 4-F-C$_6$H$_4$-	R^2 = CH_3
TC$_9$: R^1 = 2-Cl-C$_6$H$_4$-	R^2 = Br	**TC$_{19}$**: R^1 = 4-Cl-C$_6$H$_4$CH$_2$-	R^2 = F	**TC$_{29}$**: R^1 = 4-F-C$_6$H$_4$-	R^2 = OCH_3
TC$_{10}$: R^1 = 2-Cl-C$_6$H$_4$-	R^2 = CH_3	**TC$_{20}$**: R^1 = 4-Cl-C$_6$H$_4$-CH$_2$-	R^2 = Cl	**TC$_{30}$**: R^1 = 4-F-C$_6$H$_4$-	R^2 = NO_2

Table 1 Antiviral activities of the target compounds against tobacco mosaic virus (TMV) *in vivo* at 500 μg/mL

Compd	Curative activity/%	Protective activity/%	Compd	Curative activity/%	Protective activity/%
TC$_1$	40.3±2.1	52.7±6.3	**TC$_{17}$**	49.5±1.4	52.9±1.6
TC$_2$	46.7±1.0	41.2±1.7	**TC$_{18}$**	46.9±7.2	56.7 ±4.9
TC$_3$	45.2±1.3	51.7±4.8	**TC$_{19}$**	30.5±2.5	46.6±3.4
TC$_4$	43.3±3.4	54.7±5.7	**TC$_{20}$**	60.2±3.2	54.1±3.1
TC$_5$	53.8±3.2	59.5±3.0	**TC$_{21}$**	40.6±4.2	51.2±3.4
TC$_6$	32.9±7.0	51.1±2.5	**TC$_{22}$**	36.6±4.0	39.5±4.7
TC$_7$	52.7±4.2	48.9±5.1	**TC$_{23}$**	57.3±3.0	50.0±2.2
TC$_8$	59.8±2.7	46.1±5.3	**TC$_{24}$**	49.2±1.0	61.7±4.4
TC$_9$	44.9±1.9	49.2±5.8	**TC$_{25}$**	37.6±1.9	50.1±2.2
TC$_{10}$	57.5±3.9	56.2±1.1	**TC$_{26}$**	52.3±1.2	49.4±1.8
TC$_{11}$	47.9±8.1	50.9±3.9	**TC$_{27}$**	57.4±1.7	43.5±1.3

Continued

Compd	Curative activity/%	Protective activity/%	Compd	Curative activity/%	Protective activity/%
TC_{12}	57.5±2.4	55.7±7.5	**TC_{28}**	45.3±2.3	45.0±5.0
TC_{13}	51.3±3.5	52.8±3.1	**TC_{29}**	55.3±1.2	47.0±1.3
TC_{14}	48.4±1.6	58.5±5.6	**TC_{30}**	48.4±3.0	46.4±2.8
TC_{15}	55.6±4.3	52.0±1.6	Ningnanmycin	57.9±1.4	65.7±1.2
TC_1	40.1±4.4	47.7±6.0			

Table 2 Half-maximal effective concentration (EC_{50}) values of the curative activities of **TC_8**, **TC_{10}**, **TC_{12}**, **TC_{15}**, **TC_{20}**, **TC_{23}**, **TC_{27}** and **TC_{29}** against TMV

Compd	R^1	R^2	Regression equation	r^2	EC_{50}/(μg•mL^{-1})
TC_8	2-Cl-C_6H_4–	4-Cl	y=0.7154x+3.2979	0.95	239.5±2.5
TC_{10}	2-Cl-C_6H_4–	4-CH_3	y=0.6847x+3.3197	0.97	284.5±3.1
TC_{12}	2-Cl-C_6H_4–	4-NO_2	y=0.6646x+3.3599	0.96	293.6±2.8
TC_{15}	3-Cl-C_6H_4–	4-Br	y=0.6465x+3.4134	0.99	284.3±2.0
TC_{20}	4-Cl-C_6H_4-CH_2–	4-Cl	y=0.7493x+3.2217	0.97	236.2±2.7
TC_{23}	4-Cl-C_6H_4-CH_2–	4-OCH_3	y=0.6559x+3.3971	0.98	278.0±2.5
TC_{27}	4-F-C_6H_4–	4-Br	y=0.6030x+3.5292	0.98	274.9±1.8
TC_{29}	4-F-C_6H_4–	4-OCH_3	y=0.7118x+3.2633	0.96	274.9±2.5
Ningnanmycin	—	—	y=0.7221x+3.2406	0.95	273.2±3.4

and MS; NMR spectra are provided in the supplementary data.

The antiviral activities of the compounds (**TC_1**—**TC_{30}**) toward TMV were investigated *in vivo* as previously described.[29] As shown in Table 1, most of the target compounds showed acceptable curative and protective activities against TMV, with values ranging within 40.1%—60.2% and 41.2%—61.7% at the concentration of 500 μg/mL, respectively. Among these compounds, **TC_8** and **TC_{20}** exhibited favourable curative activities against TMV, with inhibition rates of 59.8% and 60.2%, respectively, which were slightly higher than that of the positive control Ningnanmycin (inhibition ratio, 57.9%). Moreover, the inhibition rates of compounds **TC_{10}**, **TC_{12}**, **TC_{23}** and **TC_{27}** were 57.5%, 57.5%, 57.3% and 57.4%, respectively, which were also comparable with that of ningnanmycin. All the title compounds exhibited weaker protective activities than ningnanmycin (protective activity, 65.7%).

The half-maximal effective concentration (EC_{50}) values of compounds **TC_8**, **TC_{10}**, **TC_{12}**, **TC_{15}**, **TC_{20}**, **TC_{23}**, **TC_{27}** and **TC_{29}** for curative activity against TMV were evaluated and listed in Table 2. Compounds **TC_8** and **TC_{20}** exerted stronger curative activity than ningnanmycin. The EC_{50} values of these compounds were 239.5 and 236.2 μg/mL, respectively, and that of ningnanmycin was 273.2 μg/mL. In addition, compounds **TC_{10}**, **TC_{12}**, **TC_{15}**, **TC_{23}**, **TC_{27}** and **TC_{29}** showed comparable activities with ningnanmycin, and their EC_{50} values were 284.5, 293.6, 284.3, 278.0, 274.9, 274.9 and 273.2 μg/mL, respectively. These results revealed that the kind of compounds could serve as applicable and alternative antiviral candidates.

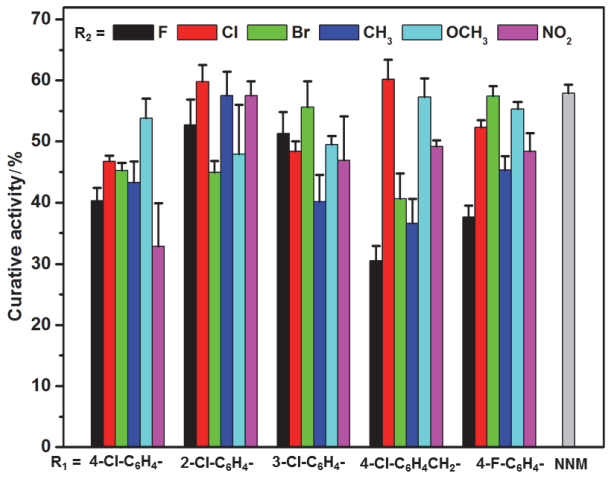


Figure 2 Comparison of curative activity of (**TC_1**—**TC_{30}**) against TMV *in vivo*. NNM is Ningnanmycin.

The preliminary structure–activity relationship (SAR) was elucidated in accordance with the curative effect against TMV. From Figure 2, it was noted that the substituents on 5-position of 1,3,4-oxadiazole moiety or arylamine had a great impact on the anti-TMV activity. The result demonstrated that the electronic effect of substituents on the arylamine did exert the effect toward the bioactivity. For example, when R^1 is 4-chlorophenyl, a better electron-donating group (—OCH_3, R^2, 53.8%) on the arylamine exhibited higher anti-TMV activity than that of other electron-withdrawing groups (—F, 40.3%; —Cl, 46.7%; —Br, 45.2%; —NO_2, 32.9%) or a weaker electron-donating group (—CH_3, 43.3%). Moreover, the position of the substituent also affected

the curative effect. When R^2 is −F, −Cl, $-CH_3$, or $-NO_2$, the order of activities of chloro-substituted compounds followed 2-Cl-C_6H_4−>3-Cl-C_6H_4− (or 4-Cl-C_6H_4−). It was worth mentioning that the antivirus activity was significantly enhanced when a soft methylene group was placed within 4-Cl-C_6H_4− and 1,3,4-oxadiazole moiety [46.7% for **TC_2** (without $-CH_2-$), 60.2% for **TC_{20}** (with $-CH_2-$)]. Additionally, different halogen groups also affected the anti-TMV activity to acertain extent; for example, compared with compounds **TC_{19}** (F, 30.5%), **TC_{20}** (Cl, 60.2%), and **TC_{21}** (Br, 40.6%). In view of the above results, **TC_8** (R^1 = 4-Cl-C_6H_4 −, R^2 = Cl) and **TC_{20}** (R^1 = 4-Cl-$C_6H_4CH_2$−, R^2=Cl) exerted the strongest curative activities against TMV than ningnanmycin, suggesting that the two compounds could serve as potential plant viral inhibitors against TMV.

To determine whether these compounds can maintain their antibacterial activity, Gram-negative pathogenic bacteria *Xoo*, *R. solanacearum* and *Xac* were screened. In the present study, turbidimeter test was employed to examine their antibacterial activities *in vitro*, and positive controls (bismerthiazol and thiodiazole copper) were used for bioactivity comparison under the same conditions.[30-33] As shown in Table 3, all the target compounds exhibited certain inhibition effects against the three bacteria at concentrations of 200 and 100 μg/mL. Among these compounds, **TC_{16}** and **TC_{29}** exhibited favourable bioactivities against *Xoo*, with inhibition rates of 96.2% and 95.9% at the concentration of 200 μg/mL, respectively; these rates were higher than that of the control agent bismerthiazol (56.1%). The SAR on the anti-*Xoo* activity demonstrated that an electron-donating group ($-OCH_3$, **TC_{29}**, 95.9%) on the arylamine exhibited higher antibacterial activity than other electron-withdrawing groups (−F, **TC_{25}**, 68.4%;

Table 3 Inhibition effect of the title compounds against *Xoo*, *R. solanacearum*, and *Xac*

Compd.	Inhibition/%					
	Xoo		*R. solanacearum*		*Xac*	
	200 μg/mL	100 μg/mL	200 μg/mL	100 μg/mL	200 μg/mL	100 μg/mL
TC_1	68.3±9.5	14.5±4.8	39.4±5.9	19.3±5.2	77.5±7.3	18.5±7.2
TC_2	69.1±2.1	27.4±3.9	17.6±4.4	9.5±3.3	35.7±2.3	32.8±7.8
TC_3	62.4±7.7	36.6±6.8	19.8±4.9	14.4±5.1	52.4±9.1	26.2±1.8
TC_4	62.7±9.8	51.8±9.5	22.9±5.0	9.8±7.4	55.3±6.1	40.0±2.1
TC_5	25.3±7.2	17.5±9.0	25.3±7.2	17.3±9.0	38.7±8.3	29.8±1.6
TC_6	46.4±4.8	21.9±4.7	10.1±0.6	4.4±0.4	68.8±5.3	38.3±2.2
TC_7	39.5±8.8	23.0±5.0	31.6±6.2	11.6±2.9	43.4±6.4	35.5±3.4
TC_8	72.6±3.1	38.6±2.8	49.4±6.9	28.5±5.4	0	0
TC_9	34.7±4.6	8.8±1.4	30.7±7.7	4.9± 2.3	60.4±2.7	53.7±5.3
TC_{10}	72.4±9.3	25.4±3.5	57.3±5.1	44.6±2.8	18.3±7.7	0
TC_{11}	13.8±3.1	6.8±4.0	32.3±3.1	23.9±7.8	23.5±5.4	15.9±6.4
TC_{12}	43.5±2.7	19.4±0.8	27.5±3.0	15.2±5.5	65.8±8.1	49.8±2.7
TC_{13}	58.9±4.5	42.7±3.8	47.7±4.2	9.3±5.0	60.5±2.7	53.5±5.3
TC_{14}	53.4±9.1	27.3±9.6	32.4±4.9	28.0±2.5	57.8±2.2	45.8±1.1
TC_{15}	36.5±6.8	16.2±6.3	20.7±6.0	11.1±0.9	55.4±8.1	50.5±1.8
TC_{16}	96.2±4.1	14.3±4.8	46.3±7.6	37.3±7.0	55.6±1.1	54.3±5.9
TC_{17}	66.3±7.6	27.3±3.9	24.1±4.7	21.4±7.3	43.4±8.6	24.5±4.3
TC_{18}	68.5±4.8	36.1±6.8	76.5±5.6	31.8±1.7	62.5±8.3	56.2±3.9
TC_{19}	28.2±4.8	12.3±3.4	29.6±6.0	18.4±4.0	33.2±4.1	30.1±1.8
TC_{20}	62.1±1.5	17.5±9.0	30.7±5.9	10.5±3.6	75.7±2.1	60.9±9.7
TC_{21}	21.4±3.0	12.4±4.7	19.8±2.7	6.2±5.9	51.4±1.8	44.4±3.7
TC_{22}	67.1±3.7	23.4±5.0	45.9±5.0	25.5±6.6	62.3±1.0	48.6±4.6
TC_{23}	−	38.3±2.8	26.6±5.5	11.4±8.6	50.6±4.3	42.8±0.9
TC_{24}	80.3±6.0	8.5±1.4	19.3±7.2	6.4±5.9	70.1±6.4	67.3±0.7
TC_{25}	68.4±8.6	25.1±3.5	37.5±5.3	15.6±6.8	11.8±7.0	6.9±3.4
TC_{26}	59.4±7.1	6.8±4.0	30.6±5.5	18.2±4.9	23.4±2.6	21.3±6.4
TC_{27}	63.5±8.2	19.2±0.8	10.4±6.5	9.3±6.0	30.3±4.6	18.6±5.6
TC_{28}	63.7±6.0	42.4±3.8	26.6±2.4	11.7±5.9	33.5±4.7	12.4±4.4
TC_{29}	95.9±9.0	27.6±9.6	51.2±4.0	30.9±7.4	75.7±4.0	33.8±9.5
TC_{30}	11.2±6.5	0	12.3±2.7	11.5±6.0	63.2±9.6	51.5±4.0
Bismerthiazol	56.1±7.3	49.3±5.4	−	−	−	−
Thiodiazole copper	−	−	92.5±2.7	52.3±4.9	51.4±2.4	22.7±2.6

—Cl, **TC_{26}**, 59.4%; —Br, **TC_{27}**, 63.5%; —NO_2, **TC_{30}**, 11.2%). Additionally, the order of activities followed **TC_{16}** (3-Cl-C_6H_4—, 96.2%) > **TC_{10}** (2-Cl-C_6H_4—, 74.2%) > **TC_4** (4-Cl-C_6H_4—, 62.7%), suggesting that the position of the substituent also affected the anti-*Xoo* activity. Compounds **TC_1**, **TC_{20}**, **TC_{24}** and **TC_{29}** showed strong inhibition effects against *Xac* (70%), which were higher than that of thiodiazole copper (51.4%). These results showed that these compounds exhibited sustained inhibition activities against plant pathogenic bacteria, as indicated in our previous work.

Conclusions

In summary, a series of *N*-(4-substituted phenyl) acetamide derivatives possessing 1,3,4-oxadiazole moiety were synthesised and evaluated for their anti-TMV and antibacterial activities. Preliminary bioassays revealed that these compounds not only exhibited good antiviral activities toward TMV but also demonstrated sustained inhibition activities against plant pathogenic bacteria (*Xoo*, *R. solanacearum* and *Xac*). In particular, **TC_8** and **TC_{20}** exerted the best curative activities against TMV, with EC_{50} values of 239.5 and 236.2 μg/mL, respectively, which were comparable to that of ningnanmycin (273.2 μg/mL), and the related SAR was summarized. Moreover, the kind of compounds also showed broad-spectrum bactericidal activity. For example, compounds **TC_{16}** and **TC_{29}** exhibited favourable bioactivities against *Xoo* with inhibition rates of 96.2% and 95.9% at the concentration of 200 μg/mL, respectively. Given their simple synthesis, the target compounds could be developed as alternative antiviral and antibacterial candidates.

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