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Synthesis and Biological Activity Evaluation of 1,2,3-Thiadiazole Derivatives as Potential Elicitors with Highly Systemic Acquired Resistance

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Elicitors provide a broad spectrum of systemic acquired resistance by altering the physical and physiological status of the host plants and, therefore, are among the most successful directions in modern pesticide development for plant protection. To develop a novel elicitor with highly systemic acquired resistance, two series of thiazole- and oxadiazole-containing thiadiazole derivatives were rationally designed and synthesized according to the principle of combination of bioactive substructures in this work. Their structures were characterized by ^{1}H nuclear magnetic resonance (NMR), infrared (IR), high-resolution mass spectrometry (HRMS), or elemental analysis. Their potential systemic acquired resistance as an elicitor was also evaluated; bioassay results indicated that, among the 23 compounds synthesized, three compounds, **10a**, **10d**, and **12b**, displayed better systemic acquired resistance than the positive control, tiadinil, a commercialized 1,2,3-thiadiazole-based elicitor. In addition, three other compounds, **10f**, **12c**, and **12j**, exhibited a certain degree of fungus growth inhibition *in vitro* or *in vivo*. Our results demonstrated that, in combination of bioactive substructures is an interesting exploration for novel pesticide development, thiazole- and oxadiazole-containing thiadiazole derivatives are potential elicitors with good systemic acquired resistance.

KEYWORDS: Fungicide; 1,2,3-thiadizole; plant elicitor; 1,3,4-oxadiazole; TMV; thiazole; systemic acquired resistance

1. INTRODUCTION

Fungi and viruses cause dramatic losses in agriculture and horticulture all over the world (*1*). Traditional fungicides play a critical role in the integrated disease management by killing or controlling target fungi directly, sometimes caused adverse effects to the environment, and often led to the fungicide resistance too; it is always of interest to seek out new environment-benign methods for plant protections. Because elicitors themselves and their metabolites had no direct fungicide activity, they induce the immunological system of the plant to produce a broad spectrum of systemic acquired resistance by altering the physical and physiological status of the host plants (*2*). The application of elicitors has been one of the most successful directions in modern pesticide development and environment protection. However, only a limited number of commercial elicitors are available; the most successful elicitor commercialized is acibenzolar-*S*-methyl (BTH) (*3*). Tiadinil (TDL) is another novel fungicide commercialized for disease control in rice fields and has also provided good induction activity of tobacco against tobacco mosaic virus (TMV) and rice blast (*4*). Both products are derivatives of 1,2,3-thiadiazole. While 1,2,3-thiadiazoles have various biological activities, four of them had already been commercialized: BTH and TDL are commercialized as elicitors, thidiazuron is used asa cotton defoliate agrochemical, and cefuzonam is a commercialized medicine (*4*, *5*). In addition, other heterocyclic compounds also have good biological activity; for example, oxadiazoles have good fungicide activity (*6*, *7*), thiazoles also have good fungicide activity, and in fact, thiabendazole, ethaboxam, thifuzamide, and metsulfovax are already commercialized as fungicides (*8*, *9*).

Documents seldom reported the synthesis and biological activity of the heterocyclic compounds containing both thiadiazole and other heterocyclic moieties, such as thiazoles or oxadiazoles in one molecule. Our motivation in this work is to combine different heterocyclic moieties with biological activity with thiadiazole in one molecule and evaluate their biological activity, especially systemic acquired resistance. On this basis, the system for elicitor screening was validated and two series of compounds containing both thiadiazole and thiazole (I) and thiadiazole and

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oxadiazole (II) moieties were designed and synthesized (**Figure 1**). On the basis of the historical effectiveness of thiazole, thiadiazole, and oxadiazole moieties against fungi and viruses, the systemic acquired resistance and fungicidal activity of I and II were evaluated.

2. EXPERIMENTAL PROCEDURES

2.1. Equipments for Structural Characterization. Melting points of all compounds were determined on an X-4 binocular microscope (Gongyi Tech. Instrument Co., Henan, China), and the thermometer was not corrected. Proton nuclear magnetic resonance (NMR) spectra were obtained at 300 MHz using a Bruker AC-P 300 spectrometer; chemical-shift values (δ) were reported as parts per million (ppm); and tetramethylsilane was used as the internal standard. Elemental analyses were determined on a Yanaca CHN Corder MT-3 elemental analyzer. Infrared (IR) was recorded on a Bruker Vector 22 Fourier transform infrared (FTIR) spectrometer using a KBr pellet press. Mass spectra were recorded using a high-resolution mass spectrometry (HRMS) spectrometer. All solvents and liquid reagents were of analytical reagent grade and were dried in advance and distilled before use. Column chromatography purification was carried out using silica gel.

2.2. Preparation of Hydrazine Carboxylic Acid Methyl Ester (2). A mixture of carbonic acid dimethyl ester (**1**, 9.0 g, 0.1 mol) and hydrazine hydrate (5.4 mL, 0.095 mol) was added into a 50 mL round-bottom flask equipped with a condenser. The reaction mixture was heated to 50 °C, stirred for 20 min, and then stirred at room temperature for 20 h. Water, methanol, and excess carbonic acid dimethyl ester (**1**) were distilled off under reduced pressure. After dryness, a white crystal (**2**) (8.1 g) was obtained with a yield of 95% (see ref *10* and **Scheme 1**).

2.3. Preparation of 3-Methoxy Carbonyl Hydrazonoacetic Acid Ethyl Ester (3). A solution containing the compound **2** (5.02 g, 0.06 mol) in ethanol (16.7 mL) was dropwise-added to a solution of ethyl acetoacetate (7.28 g, 0.06 mol) in ethanol (3.7 mL) slowly. After stirring for 6 h at room temperature, water and ethanol were distilled off under reduced pressure and a white product (**3**) (11.26 g) was obtained with a yield of 98% (see ref *11* and **Scheme 1**).

2.4. Preparation of Ethyl 4-Methyl-1,2,3-thiadiazole-5-carboxylate (4). To a solution of 3-methoxy carbonyl hydrazonoacetic acid ethyl ester (**3**, 40.57 g, 0.20 mol) in dichloromethane (45 mL), thionyl chloride (44 mL) was dropwise-added in batches below 20 °C. After stirring in an ice−water bath for 1 h, the reaction mixture was permitted to stand for 20 h at room temperature. The excess thionyl chloride and dichloromethane were distilled off, and the remaining residue was subject to fractional distillation under reduced pressure. A slight yellowish oil (**4**) (25.94 g) was obtained with a yield of 75% (see ref *12* and **Scheme 1**).

2.5. Preparation of 4-Methyl-1,2,3-thiadiazole-5-carboxylic Acid (5). To a solution of sodium hydroxide in methanol (3 M, 80 mL), an intermediate (**4**, 33.4 g, 0.19 mol) was added batchwise. The resulting mixture was then stirred for 16 h at room temperature. The methanol was then distilled off via vacuum, and the remaining sodium salt was washed with ethyl ether, then dissolved into water (200 mL), and acidified with dilute hydrochloric acid. The acid was precipitated, filtered off, and subsequently washed with pentane. A colorless crystal (**5**, 25.9 g) was obtained with a yield of 96%.

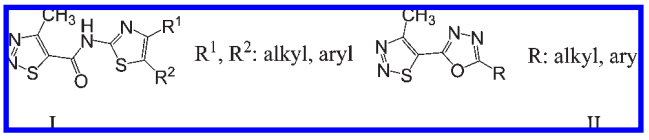


Figure 1. Molecules of the target compounds designed and synthesized.

2.6. Preparation of 4-Methyl-1,2,3-thiadiazole-5-carbonyl Chloride (6). A mixture containing 4-methyl-1,2,3-thiadiazole-5-carboxylic acid (**5**, 9.66 g, 0.067 mol) and thionyl chloride (29 mL) was added into a 100 mL flask and refluxed for 6 h. Excess thionyl chloride was removed under vacuum, and a light yellow oil (**6**) (9.25 g) was obtained by fractional distillation under reduced pressure with a yield of 85% (see ref *13* and **Scheme 1**).

2.7. Preparation of 2-Amino-4-methylthiazole (9a). A mixture of thiourea (7.6 g) in acetone (10 mL) was added into a 100 mL flask equipped with a condenser; I_2 (12.7 g) was added to the slurry. The mixture was poured into ice water (50 mL) after refluxing for 8.5 h, and then the mixture was neutralized by NaOH, followed by extraction with ethyl ether (5 × 20 mL). The ethyl ether layer was dried with anhydrous Na_2SO_4, and after removal of the organic solvent under reduced pressure, fuscous oil (**9a**) (2.8 g) was obtained with a yield of 20.4% (see ref *14* and **Scheme 2**).

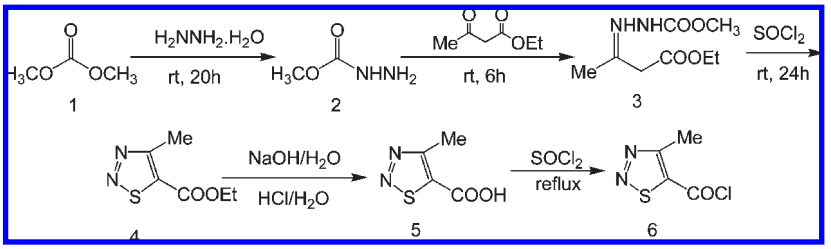
2.8. General Synthetic Procedures for Intermediates 9b and 9c. Thiourea (0.30 mol) and aldehyde (0.15 mol) were mixed in chloroform (75 mL) and cooled in an ice−water bath. Thionyl chloride was added over 8 min, and the exothermic reaction was maintained between 15 and 25 °C. After the chloroform was distilled off, ethanol (200 mL) was added and the reaction mixture was refluxed overnight for about 16 h. Then, the solvent was removed under reduced pressure, and the residue was poured into 200 mL of hot water. After filtration, the filtrate was neutralized to basic with ammonia and extracted with ethyl ether. The ethyl ether layer was dried with anhydrous Na_2SO_4, and then the solvent was removed under reduced pressure to give a light yellow oil: **9b**, 12.0 g, yield 63%; **9c**, 19.4 g, yield 70% (see ref *15* and **Scheme 2**).

2.9. General Synthetic Procedure for Intermediates 9d and 9e. To a mixture of the starting material (**7d**−**7e**, 0.05 mol) and thiourea (0.10 mol), bromine (0.05 mol) was added batchwise to the slurry. The mixture was heated to 80 °C for 16 h, and then it was poured into 100 mL of warm water and stirred until most of the solid was dissolved. After filtration, the solution was cooled and adjusted to pH 8 with concentrated ammonia. The solid collected after filtration was recrystallized from ethanol to give crystals: **9d**, 3.61 g, yield 41%; **9e**, 4.76 g, yield 46% (see ref *16* and **Scheme 2**).

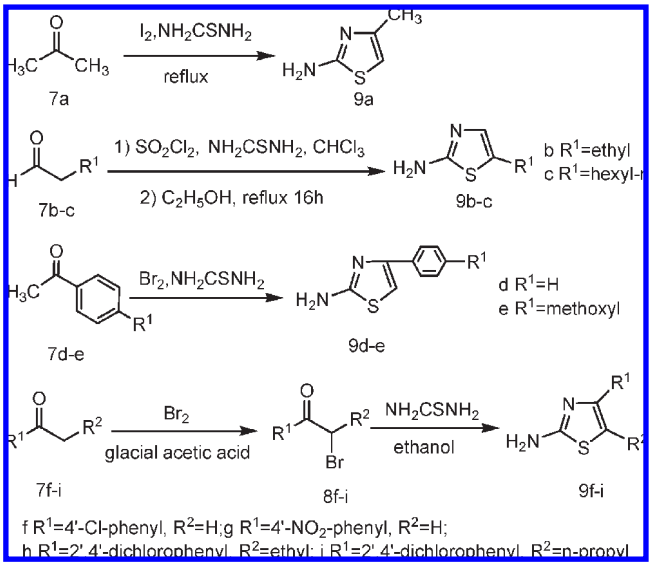
2.10. General Synthetic Procedures for Intermediates 8f and 8g. A mixture of **7f** and **7g** (60 mmol) and glacial acetic acid (40 mL) was cooled, and then bromine was added batchwise. The reaction mixture was kept below 20 °C and stirred overnight. Then, the mixture was poured into 150 mL of ice water, and after filtration and drying, white crystals (*17*) were obtained: **8f**, 13.64 g, yield 97%; **8g**, 9.96 g, yield 75% (see **Scheme 2**).

2.11. General Synthetic Procedures for Intermediates 8h and 8i. A mixture of **7h** and **7i** (30 mmol) and glacial acetic acid (20 mL) was cooled, and then bromine was added batchwise under an ice−water bath. The reaction mixture was kept below 20 °C and stirred overnight. The mixture was then poured into 150 mL of ice water and extracted with ethyl acetate (3 × 20 mL). The ethyl acetate phase was washed with a saturated sodium chloride solution (2 × 40 mL) and dried over

Scheme 1



Scheme 2



anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give a crude of intermediates **8h** and **8i**. The products were purified by column chromatography on silica gel using ethyl acetate and petroleum ether (60−90 °C) with 1:40 as an eluent (see **Scheme 2**).

8h. 8.3 g colorless oil. Yield, 94%. ^{1}H NMR ($CDCl_3$): 1.117 (3H, t, $^3J_{HH}$ = 7.3 Hz, CH_3), 2.035−2.315 (2H, m, CH_2), 5.027 (1H, t, $^3J_{HH}$ = 7.9 Hz, CH), 7.347 (1H, dd, $^3J_{1HH}$ = 6.3 Hz, $^3J_{2HH}$ = 1.8 Hz, benzene−H), 7.444−7.506 (2H, m, benzene−H).

8i. 8.8 g colorless oil. Yield, 95%. ^{1}H NMR ($CDCl_3$): 0.995 (3H, t, $^3J_{HH}$ = 7.5 Hz, CH_3), 1.469−1.571 (2H, m, CH_2), 2.042−2.177 (2H, m, CH_2), 5.093 (1H, t, $^3J_{HH}$ = 5.9 Hz), 7.347 (1H, dd, $^3J_{1HH}$ = 6.3 Hz, $^3J_{2HH}$ = 1.8 Hz, benzene−H), 7.443−7.508 (2H, m, benzene−H).

2.12. Preparation of 2-Amino-4-(4′-chlorophenyl) Thiazole (9f). A mixture of α-bromo-4′-chloroacetophenone (4.6 g, 20 mmol), thiourea (1.52 g, 20 mmol), and ethanol (100 mL) was refluxed for 8 h. After filtration, the precipitate was washed by water and then recrystallized from ethanol to give a white crystal (**9f**) (2.8 g) with a yield of 70% (see ref *18* and **Scheme 2**).

2.13. Preparation of 2-Amino-4′-nitro Phenyl Thiazole (9g). Thiourea (0.80 g, 10 mmol) was added to a solution of α-bromo-4′-nitroacetophenone (2.56 g, 10 mmol) in warmed ethanol (40 mL). The mixture was then refluxed for 1 h. After filtration, washing with ethyl ether, and drying, a yellow crystal (**9g**) (1.95 g) was obtained with a yield of 88% (see ref *19* and **Scheme 2**).

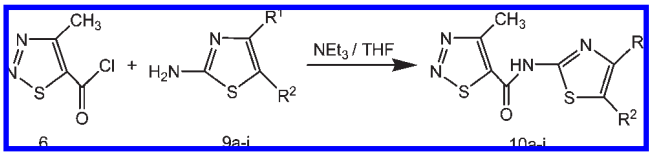
2.14. General Synthetic Procedures for Intermediates 9h and 9i. A solution containing intermediate **8h** or **8i** (14.6 mmol), thiourea (14.6 mmol), and ethanol (15 mL) was refluxed for 5 h. After cooling, the solvent was removed under reduced pressure to give a yellow solid. This solid was dissolved in warm water (15 mL) with stirring and then cooled. Its pH was adjusted to basic with concentrated ammonia, filtered, washed, and dried to give a white crystal (**9h** or **9i**) (see ref *20* and **Scheme 2**).

9h. 3.78 g white crystal. Yield, 95%. mp 238−239 °C. ^{1}H NMR ($CDCl_3$): 1.195 (3H, t, $^3J_{HH}$ = 7.5 Hz, CH_3), 2.544 (2H, q, $^3J_{HH}$ = 7.5 Hz, CH_2), 7.357 (2H, t, $^3J_{HH}$ = 8.2 Hz, benzene−H), 7.529 (1H, d, $^3J_{HH}$ = 1.7 Hz, benzene−H), 8.493 (2H, s, $-NH_2$).

9i. 3.98 g white crystal. Yield, 95%. mp 257−258 °C. ^{1}H NMR ($CDCl_3$): 0.889 (3H, t, $^3J_{HH}$ = 7.3 Hz, CH_3), 1.580 (2H, q, CH_2), 2.455 (2H, t, $^3J_{HH}$ = 7.5 Hz, CH_2), 7.307−7.387 (2H, m, $^3J_{HH}$ = 8.2 Hz, benzene−H), 7.523 (1H, d, $^3J_{HH}$ = 1.8 Hz, benzene−H), 8.675 (2H, s, $-NH_2$).

2.15. General Preparation for 4-Methyl-1,2,3-thiadiazole-5-formylamine (10a−10i). The intermediates **9a−9i** were reacted with intermediate **6** in anhydrous tetrahydrofuran (THF) for 4 h at room temperature with $N(Et)_3$ as an acid acceptor, respectively. Then, the solvent was removed under reduced pressure, and the residue was washed with water to give crude products (**10a−10i**). The products were purified by column chromatography on a silica gel using ethyl acetate and petroleum ether (60−90 °C) at a ratio of 1:2−1:6 as an eluent (see **Scheme 3**).

Scheme 3



*Data for **10a***. Light yellow crystal. Yield, 65%. mp 217−220 °C. ^{1}H NMR (DMSO-d_6): 2.248 (3H, s, CH_3−thiazole), 2.912 (3H, s, CH_3−thiadiazole), 6.755 (1H, s, thiazole−H). HRMS $(M - H)^-$ for $C_8H_8N_4OS_2$, 239.0067; found, 239.0059. IR (KBr pellet press): 3134 (NH, st), 3071, 2969, 2876, 2768, 2674, 1660 (C═O, st), 1615, 1577, 1499, 1449, 1334, 1287, 1208, 1118, 989, 871, 820, 767.

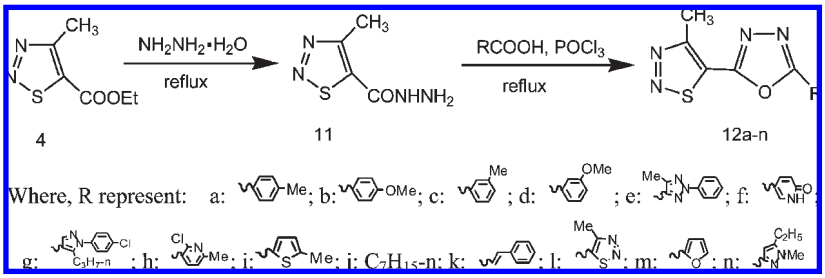
*Data for **10b***. Light yellow crystal. Yield, 63%. mp 168−169 °C. ^{1}H NMR ($CDCl_3$): 1.237 (3H, t, $^3J_{HH}$ = 7.5, CH_3), 2.710 (2H, q, $^3J_{HH}$ = 7.5, CH_2), 2.880 (1H, s, thiadiazole−CH_3), 6.591 (1H, s, thiazole−H). Anal. Calcd for $C_9H_{10}N_4OS_2$: C, 42.50; H, 3.96; N, 22.03. Found: C, 42.60; H, 4.27; N, 21.67. IR (KBr pellet press): 3173 (NH, st), 3085, 2977, 2932, 2875, 1737, 1674, 1606, 1573 (C═O, st), 1496, 1408, 1345, 1224, 1139, 1101, 1027, 687, 815, 766, 695.

*Data for **10c***. Light yellow crystal. Yield, 44%. mp 106−107 °C. ^{1}H NMR ($CDCl_3$): 0.825 (3H, t, $^3J_{HH}$ = 6.5 Hz, CH_3), 1.184−1.251 (6H, m, $-CH_2CH_2CH_2-$), 1.583 (2H, m, $^3J_{HH}$ = 7.0 Hz, $-CH_2$), 2.654 (2H, t, $^3J_{HH}$ = 7.5 Hz, $-CH_2$), 2.902 (3H, s, thiadiazole−CH_3), 6.658 (1H, s, thiazole−H). Anal. Calcd for $C_{13}H_{18}N_4OS_2$: C, 50.30; H, 5.84; N, 18.05. Found: C, 50.18; H, 5.89; N, 18.03. IR (KBr pellet press): 3158 (NH, st), 3101, 2999, 2948, 2929, 2856, 1705, 1617, 1592 (C═O, st), 1548, 1433, 1334, 1205, 873, 821, 763.

*Data for **10d***. Light yellow crystal. Yield, 62%. mp 206−207 °C. ^{1}H NMR ($CDCl_3$): 2.818 (3H, s, thiadiazole−CH_3), 7.123 (1H, s, thiazole−H), 7.270 (3H, t, $^3J_{HH}$ = 1.2 Hz, benzene−H), 7.543−7.574 (2H, m, $^3J_{HH}$ = 7.5 Hz, benzene−H). Anal. Calcd for $C_{13}H_{10}N_4OS_2$: C, 51.64; H, 3.33; N, 18.53. Found: C, 51.60; H, 2.97; N, 18.85. IR (KBr pellet press): 3189 (NH, st), 3059, 2926, 1735, 1671, 1589 (C═O, st), 1567, 1501, 1411, 1337, 1197, 1112, 1027, 870, 818, 722.

*Data for **10e***. Light yellow crystal. Yield, 57%. mp 239−240 °C. ^{1}H NMR ($CDCl_3$): 2.916 (3H, s, thiadiazole−CH_3), 3.845 (3H, s, $-OCH_3$), 6.869 (2H, d, $^3J_{HH}$ = 8.8 Hz, benzene−H), 7.040 (1H, s, thiazole−H), 7.555 (2H, d, $^3J_{HH}$ = 8.8 Hz). Anal. Calcd for $C_{14}H_{12}N_4O_2S_2$: C, 50.55; H, 3.64; N, 16.86. Found: C, 50.59; H, 3.59; N, 16.41. IR (KBr pellet press): 3182 (NH, st), 3109, 3056, 2837, 1737, 1661 (C═O, st), 1605, 1555, 1482, 1371, 1320, 1246, 1178, 1027, 876, 837, 752, 717.

Scheme 4



Data for ***10f***. Light yellow crystal. Yield, 90%. mp 271−272 °C. ^{1}H NMR ($CDCl_3$): 3.108 (3H, s, thiadiazole−CH_3), 7.247 (1H, s, thiazole−H), 7.489 (2H, d, $^3J_{HH}$ = 8.6 Hz, benzene−H), 7.740 (2H, d, $^3J_{HH}$ = 8.6 Hz, benzene−H). Anal. Calcd for $C_{13}H_9ClN_4OS_2$: C, 46.36; H, 2.69; N, 16.63. Found: C, 46.51; H, 2.89; N, 16.63. IR (KBr pellet press): 3200 (NH, st), 3106, 3065, 3977, 3927, 1666 (C═O, st), 1553, 1471, 1318, 1287, 1087, 1062, 878, 837, 763, 711.

Data for ***10g***. Light yellow crystal. Yield, 61%. mp 262−263 °C. ^{1}H NMR ($CDCl_3$): 3.082 (3H, s, thiadiazole−CH_3), 7.471 (1H, s, thiazole−H), 7.989 (2H, d, $^3J_{HH}$ = 8.9 Hz, benzene−H), 8.286 (2H, d, $^3J_{HH}$ = 8.9 Hz, benzene−H). Anal. Calcd for $C_{13}H_9N_5O_3S_2$: C, 44.95; H, 2.61; N, 20.16. Found: C, 44.83; H, 2.51; N, 20.45. IR (KBr pellet press): 3254 (NH, st), 3116, 2927, 1669 (C═O, st), 1597, 1547, 1508, 1343, 1278, 1205, 1107, 1053, 879, 857, 809, 733.

Data for ***10h***. White crystal. Yield, 68%. mp 147−148 °C. ^{1}H NMR ($CDCl_3$): 1.250 (3H, t, $^3J_{HH}$ = 7.5 Hz, CH_3), 2.634 (2H, q, $^3J_{HH}$ = 7.5 Hz, CH_2), 2.865 (3H, s, thiadiazole−CH_3), 6.978 (1H, q, $^3J_{HH}$ = 1.8 Hz, benzene−H), 7.124 (1H, d, $^3J_{HH}$ = 8.2 Hz, CH_2), 7.348 (1H, s, benzene−H), 12.369 (1H, br, N−H). Anal. Calcd for $C_{15}H_{12}Cl_2N_4OS_2$: C, 45.12; H, 3.03; N, 14.03. Found: C, 45.58; H, 3.25; N, 13.83. IR (KBr pellet press): 3153 (NH, st), 3068, 2972, 2930, 2875, 1673 (C═O, st), 1545, 1472, 1379, 1323, 1291, 1213, 1103, 1029, 825.

Data for ***10i***. White crystal. Yield, 65%. mp 181−182 °C. ^{1}H NMR ($CDCl_3$): 0.886 (3H, t, $^3J_{HH}$ = 7.3 Hz, CH_3), 1.617 (2H, m, CH_2), 2.556 (2H, t, $^3J_{HH}$ = 7.5 Hz, CH_2), 2.862 (3H, s, thiadiazole−CH_3), 6.950 (1H, d, $^3J_{HH}$ = 8.2 Hz, benzene−H), 7.112 (1H, q, $^3J_{HH}$ = 1.8 Hz, benzene−H), 7.343 (1H, d, $^3J_{HH}$ = 2.1 Hz, benzene−H), 12.447 (1H, br, N−H). Anal. Calcd for $C_{16}H_{14}Cl_2N_4OS_2$: C, 46.49; H, 3.41; N, 13.55. Found: C, 46.11; H, 3.34; N, 13.29. IR (KBr pellet press): 3137 (NH, st), 3063, 2960, 2932, 2870, 2759, 1735, 1671 (C═O, st), 1630, 1546, 1382, 1319, 1290, 1215, 1102, 1024, 907, 781.

2.16. Preparation of 4-Methyl-1,2,3-thiadiazol-5-carbonyl Hydrazine (11). A mixture of ethyl 4-methyl-1,2,3-thiadiazol-5-carboxylate (**4**, 6.9 g, 40 mmol) and hydrazine hydrate (44 mmol) were refluxed for 10 h. The mixture was then stirred overnight at room temperature. After evaporation under reduced pressure, the residue was washed with 40 mL of petroleum ether and then filtered to give 4.9 g of a light yellow solid (**11**) with a yield of 78% (see ref *21* and **Scheme 4**).

2.17. General Synthetic Procedures for 2-(4′-Methyl-1′,2′,3′-thiadiazol)-5-substituted-1,3,4-oxadiazole Derivatives (12a−12n). A mixture of 4-methyl-1,2,3-thiadiazol-5-carbonyl hydrazine (**11**, 5 mmol), aromatic acids or fatty acids (5 mmol), and $POCl_3$ (7.5 mL) was heated at 120 °C for 6 h. Ice water (80 mL) was added, and then the mixture was neutralized with concentrated ammonia and extracted with dichloromethane (3 × 20 mL). After drying with anhydrous Na_2SO_4, the solvent was evaporated off under reduced pressure to give crude products. These products were purified by column chromatography with silica gel using ethyl acetate and petroleum ether at a ratio of 1:4 as an eluent (see **Scheme 4**).

Data for ***12a***. White crystal. Yield, 25%. mp 135−136 °C. ^{1}H NMR ($CDCl_3$): 2.400 (3H, s, CH_3), 3.100 (3H, s, thiadiazole−CH_3), 7.290 (2H, d, $^3J_{HH}$ = 8.0 Hz, benzene−H), 7.935 (2H, d, $^3J_{HH}$ = 8.3 Hz, benzene−H). Anal. Calcd for $C_{12}H_{10}N_4OS$: C, 55.80; H, 3.90; N, 21.69. Found: C, 55.71; H, 4.00; N, 21.62. IR (KBr pellet press): 2922 (CH, st), 1661 (C═N, st), 1548, 1496, 1441 (vibration of oxadiazole ring), 1178 (vibration of C−O−C), 1178, 1060, 950, 821, 730.

Data for ***12b***. White crystal. Yield, 49%. mp 126−127 °C. ^{1}H NMR ($CDCl_3$): 3.161 (3H, s, thiadiazole−CH_3), 3.913 (3H, s, −OCH_3), 7.047 (2H, d, $^3J_{HH}$ = 8.9 Hz, CH═CH), 8.056 (2H, d, $^3J_{HH}$ = 8.9 Hz, CH═CH). Anal. Calcd for $C_{12}H_{10}N_4O_2S$: C, 52.51; H, 3.52; N, 20.45. Found: C, 52.54; H, 3.67; N, 20.43. IR (KBr pellet press): 3115 (CH, st), 1605, 1496, 1402 (vibration of oxadiazole ring), 1169 (vibration of C−O−C), 1024, 835.

Data for ***12c***. White crystal. Yield, 17%. mp 131−132 °C. ^{1}H NMR ($CDCl_3$): 2.451 (3H, s, CH_3), 3.076 (3H, s, thiadaizole−CH_3), 7.381 (2H, t, $^3J_{HH}$ = 3.6 Hz, benzene−H), 7.947 (2H, d, $^3J_{HH}$ = 2.4 Hz, benzene−H). Anal. Calcd for $C_{12}H_{10}N_4OS$: C, 55.80; H, 3.90; N, 21.69. Found: C, 55.98; H, 4.06; N, 21.23. IR (KBr pellet press): 3485 (st), 3412 (st), 1641, 1547, 1394 (vibration of oxadiazole ring), 1053 (vibration of C−O−C).

Data for ***12d***. White crystal. Yield, 44%. mp 156−157 °C. ^{1}H NMR ($CDCl_3$): 3.161 (3H, s, thiadiazole−CH_3), 4.008 (3H, s, −OCH_3), 7.093−7.130 (2H, m, benzene−H), 7.550−7.608 (1H, m, benzene−H), 8.062 (1H, q, $^3J_{HH}$ = 7.7 Hz, benzene−H). Anal. Calcd for $C_{12}H_{10}N_4O_2S$: C, 52.54; H, 3.67; N, 20.43. Found: C, 52.07; H, 3.89; N, 19.95. IR (KBr pellet press): 3528 (st), 3448 (st), 3469 (st), 2839 (OCH_3, st), 1605, 1525, 1489 (vibration of oxadiazole ring), 1017 (vibration of C−O−C), 1278, 1249, 748.

Data for ***12e***. White crystal. Yield, 42%. mp 159−160 °C. ^{1}H NMR ($CDCl_3$): 2.795 (3H, s, CH_3), 3.202 (3H, s, thiadiazole−CH_3), 7.411−7.460 (1H, m, benzene−H), 7.516−7.569 (2H, m, benzene−H), 8.138 (1H, d, $^3J_{HH}$ = 8.3 Hz). Anal. Calcd for $C_{14}H_{11}N_7OS$: C, 51.68; H, 3.41; N, 30.14. Found: C, 51.60; H, 3.40; N, 29.97. IR (KBr pellet press): 3855, 3746, 3673, 3115 (CH, st), 1648 (C═N, st), 1540, 1510, 1394 (vibration of oxadiazole ring), 1075 (vibration of C−O−C).

Data for ***12f***. White crystal. Yield, 19%. mp 146−147 °C. ^{1}H NMR ($CDCl_3$): 3.177 (3H, s, thiadiazole−CH_3), 7.557 (1H, d, $^3J_{HH}$ = 8.4 Hz, pyridinone−H), 8.381 (1H, dd, $^3J_{1HH}$ = 8.4 Hz, $^3J_{2HH}$ = 2.4 Hz, pyridinone−H), 9.122 (1H, d, $^3J_{HH}$ = 2.4 Hz, pyridinone−H). Anal. Calcd for $C_{10}H_7N_5O_2S$: C, 42.94; H, 2.16; N, 25.04. Found: C, 42.74; H, 2.05; N, 25.33. IR (KBr pellet press): 3035 (CH, st), 1634, 1394 (vibration of oxadiazole ring), 1104 (vibration of C−O−C).

Data for ***12g***. White crystal. Yield, 40%. mp 98−100 °C. ^{1}H NMR ($CDCl_3$): 0.926 (3H, t, $^3J_{HH}$ = 7.3 Hz, CH_3), 1.668 (2H, q, $^3J_{HH}$ = 7.5 Hz, CH_2), 3.078 (2H, t, $^3J_{HH}$ = 7.8 Hz, CH_2), 3.163 (3H, s, thiadiazole−CH_3), 7.423 (2H, dd, $^3J_{1HH}$ = 8.7 Hz, $^3J_{2HH}$ = 1.8 Hz, benzene−H), 7.519 (2H, d, $^3J_{HH}$ = 8.5 Hz, benzene−H), 8.154 (1H, s, pyrazole−H). Anal. Calcd for $C_{17}H_{15}ClN_6OS$: C, 52.78; H, 3.91; N, 21.72. Found: C, 52.50; H, 3.87; N, 21.94. IR (KBr pellet press): 3514, 3441, 3405, 1612, 1394 (vibration of oxadiazole ring), 1060 (vibration of C−O−C).

Data for ***12h***. White crystal. Yield, 48%. mp 119 °C. ^{1}H NMR ($CDCl_3$): 2.675 (3H, s, CH_3), 3.176 (3H, s, thiadiazole−CH_3), 7.315 (1H, d, $^3J_{HH}$ = 7.9 Hz, benzene−H), 8.361 (1H, d, $^3J_{HH}$ = 7.9 Hz, benzene−H). Anal. Calcd for $C_{11}H_8ClN_5OS$: C, 44.72; H, 2.88; N, 23.80. Found: C, 44.98; H, 2.75; N, 23.84. IR (KBr pellet press): 3056 (CH), 2960, 2924, 1603, 1583 (C═N, st), 1546, 1433, 1402, 1350, 1252, 1216, 1153, 1123, 1087, 1043, 964, 846, 821, 747, 681.

Data for ***12i***. White crystal. Yield, 27%. mp 139−140 °C. ^{1}H NMR ($CDCl_3$): 2.601 (3H, s, CH_3), 3.137 (3H, s, CH_3), 6.886 (1H, dd, $^3J_{1HH}$ = 3.9 Hz, $^3J_{2HH}$ = 0.9 Hz, H−thiophene), 7.667 (1H, d, $^3J_{HH}$ = 3.9 Hz, thiophene−H). Anal. Calcd for $C_{10}H_8N_4OS_2$: C, 45.44; H, 3.05; N, 21.20. Found: C, 45.23; H, 2.96; N, 21.25. IR (KBr pellet press):

3535, 3434, 3369 (st), 1641, 1394 (vibration of oxadiazole ring), 1060 (vibration of C−O−C).

Data for ***12j***. White crystal. Yield, 49%. mp liquid at room temperature. ^{1}H NMR ($CDCl_3$): 0.891 (3H, t, $^3J_{HH}$ = 6.7 Hz, CH_3), 1.316−1.432 [8H, m, $-(CH_2)_4-$], 1.832−1.831 (2H, m, CH_2), 2.959 (2H, t, $^3J_{HH}$ = 7.5 Hz), 3.091 (3H, s, CH_3). Anal. Calcd for $C_{12}H_{18}N_4OS$: C, 54.11; H, 6.81; N, 21.03. Found: C, 54.23; H, 6.74; N, 21.25. IR (KBr pellet press): 2931, 2857 (CH, st), 1562 (C=N, st), 1459, 1215 (vibration of oxadiazole ring), 1080 (vibration of C−O−C).

Data for ***12k***. White crystal. Yield, 63%. mp 187−188 °C. ^{1}H NMR ($CDCl_3$): 3.159 (3H, s, CH_3), 7.081 (1H, d, $^3J_{HH}$ = 16.4 Hz, CH=CH), 7.439−7.475 (3H, m, benzene−H), 7.595−7.627 (2H, m, benzene−H), 7.705 (1H, d, $^3J_{HH}$ = 16.4 Hz, CH=CH). Anal. Calcd for $C_{13}H_{10}N_4OS$: C, 57.76; H, 3.73; N, 20.73. Found: C, 57.54; H, 3.48; N, 20.97. IR (KBr pellet press): 3187, 3158, 3035, 1634, 1394 (vibration of oxadiazole ring), 1104 (vibration of C−O−C).

Data for ***12l***. White crystal. Yield, 40%. mp 134−135 °C. ^{1}H NMR ($CDCl_3$): 3.171 (6H, s, thiadiazole−CH_3). Anal. Calcd for $C_8H_6N_6OS_2$: C, 36.08; H, 2.27; N, 31.56. Found: C, 36.09; H, 2.32; N, 31.507. IR (KBr pellet press): 3059, 2989, 2932 (CH, st), 2858, 1564 (C=N, st), 1564, 1504, 1434, 1382, 1343, 1306, 1254, 1209, 1058, 935, 818, 727, 492.

Data for ***12m***. White crystal. Yield, 37%. mp 106−108 °C. ^{1}H NMR ($CDCl_3$): 3.143 (3H, s, CH_3), 6.667 (1H, q, $^3J_{HH}$ = 1.8 Hz, H−thiophene), 7.313 (1H, d, $^3J_{HH}$ = 3.5 Hz, H−thiophene), 7.717 (1H, d, $^3J_{HH}$ = 1.6 Hz, H−thiophene). Anal. Calcd for $C_9H_6N_4O_2S$: C, 46.15; H, 2.58; N, 23.92. Found: C, 46.35; H, 2.45; N, 24.13. IR (KBr pellet press): 3506, 3448, 3390, 1641 (st), 1627, 1394 (vibration of oxadiazole ring), 1060, 1111, 1162 (vibration of C−O−C).

Data for ***12n***. White crystal. Yield, 40%. mp 129−130 °C. ^{1}H NMR ($CDCl_3$): 1.357 (3H, t, $^3J_{HH}$ = 7.5 Hz, CH_3), 2.692 (2H, q, $^3J_{HH}$ = 7.5 Hz, CH_2), 3.161 (3H, s, thiadiazole−CH_3), 3.926 (3H, s, −N−CH_3), 6.786 (1H, s, H−pyrazole). Anal. Calcd for $C_{11}H_{12}N_6OS$: C, 47.81; H, 4.38; N, 30.41. Found: C, 47.62; H, 4.38; N, 30.39. IR (KBr pellet press): 2969 (CH, st), 1649, 1532, 1394 (vibration of oxadiazole ring), 1010 (vibration of C−O−C), 1206.

2.18. Biological Screening. The fungicide activity and systemic acquired resistance activity of the target compounds were evaluated according to the following procedures.

Fungicide Screening. A stock solution of each compound was prepared at 500 μg/mL using *N*,*N*-dimethylformamide (DMF) as a solvent. A working solution (50 μg/mL) was then prepared by diluting the stock solution (0.1 mL) with sterilized water (0.9 mL) in a 10 cm diameter Petri dish. Potato dextrose agar (PDA, 9 mL) was then added to prepare the plate. Before the plate solidification, the PDA was thoroughly mixed by turning around the Petri dish in the sterilized operation desk 5 times to scatter the compounds in PDA evenly. Then, 4 mm of diameter of fungi cake was inoculated on the plate and cultured in the culture tank at 24−26 °C. The diameter of fungi spread was measured 2 days later. Growth inhibition was then calculated using the corresponding control. Fungi used in this study included *Gibberella fujikuroi* (GF), *Fusarium oxysporum* (FO), *Cercospora arachidicola* (CA), *Phoma asparagi* (PA), *Alternaria solani* (AS), *Gibberella zeae* (GZ), *Ustilaginoidea virens* (UV), *Botrytis cinerea* (BC), *Sclerotinia sclerotiorum* (SC), *Alternaria mali* (AM), *Puccinia triticina* Eriks (PT), *Physalospora piricola* (PP), *Valsa mali* (VM), *Glomerella cingulata* (GC), *Colletotrichum lagenarium* (CL), *Alternaria kikuchiana* (AK), *Fusarium vasinfectum* (FV), *Rhizoctonia cerealis* (RC), *Phytophthora infestans* (Mont) de Bary (PI), and *Rhizoctonia solani* Kuhn (RS). For *Puccinia triticina* Eriks screening, identified healthy wheat plants with 3−5 leaves were used and each compound (500 μg/mL) was spread on the plants before fungi spore inoculation. Inhibition percentages were calculated using a corresponding spore stack.

Systemic Acquired Resistance Screening. In this assay, we used the tobacco against the TMV system. Direct antivirus activity was detected before its systemic acquired resistance determination. The *in vitro* activity of each compound against TMV was conducted using the conventional half-leaf juice robbing method: a fresh leaf of 5−6 leaves age of healthy tobacco that had been inoculated with TMV virus by the juice-leaf rubbing method was cut into two halves along the main vein. The concentration of TMV inoculation was 5.88×10^{-2} μg/mL. The two halves were immersed into a solution of the test compound (500 μg/mL) and double-distilled water for 20 min, separately. The half-leaves were then cultured at 25 °C for 72 h under the humidity of 100%, and the viral inflammations on the inoculated leaves were recorded. 2,4-Dioxohexahydro-1,3,5-triazine (DHT) was used as a positive control. Three replicates were performed for each compound. The *in vitro* inhibition ratio was calculated by comparing the average number of the viral inflammations on the two half-leaves according to eq 1. Determination of systemic acquired resistance activity of the tested compound against TMV was conducted according to the following procedures. Chose five pots of 3−5 leaves age of healthy whole tobacco plant for the screening of one compound. All fresh leaves were treated with 20 mL of a target compound (500 μg/mL) by spraying (leaf spray) or irrigation (soil treatment). The plants were then cultured in the green house for about 7 days, after another new leaf was grown large enough for experiment in each pot; each newly grown leaf was inoculated by TMV (5.88×10^{-2} μg/mL) using the juice-leaf rubbing method. Double-distilled water, tiadinil, and BTH were sprayed (leaf spray) or irrigated (soil treatment) as CK and positive controls. Each inoculated tobacco plant was then placed at 25 °C for 72 h of further cultivation; the viral inflammations on the inoculated leaves were recorded. The induction activity was evaluated using the antivirus inhibition ratio, which was calculated by the average number of the viral inflammations on the inoculated leaves with the corresponding control, according to eq 1

$$Y = \frac{\mathrm{CK} - A}{\mathrm{CK}} \times 100 \quad (1)$$

where Y is the antivirus inhibition ratio (*in vitro* or induced *in vivo*) (%), CK is the average number of viral inflammations on the control half-leaf *in vitro* or each induced leave *in vivo*, and A is the average number of viral inflammations on the treatment half-leaf *in vitro* or each leaf induced *in vivo*.

3. RESULTS AND DISCUSSION

3.1. Preparation and Characterization of the Novel 1,2,3-Thiadiazole Derivatives. 1,2,3-Thiadiazoles can be synthesized via different methods (*2*, *22*). The key intermediate, 4-methyl-1,2,3-thiadiazole carboxylic acid in our study, was prepared by the Hurd−Mori reaction with good yield (**Scheme 1**). The target compounds containing 4-methyl-1,2,3-thiadiazole-5-formylamide (**10**) were synthesized using 4-methyl-1,2,3-thiadiazole-5-carbonyl chloride with the corresponding amines in THF with $N(Et)_3$ as an acid acceptor; 4-methyl-1,2,3-thiadiazole carboxylic acid was found to be the main byproduct when the reaction mixture contained high moisture. Our studies did not optimize the reaction conditions, and the reaction system did not conduct in absolute anhydrous conditions; therefore, some yields for synthesis of compounds **10** in our studies were low, and another byproduct $N(Et)_3 \cdot HCl$ was easy to be removed by washing with water. The target compounds containing 2-(4′-methyl-1′,2′,3′-thiadiazol)-5-substituted-1,3,4-oxadiazole (**12**) were synthesized by the reaction of 4-methyl-1,2,3-thiadiazol-5-carbonyl hydrazine (**11**) with different aromatic, herteroaromatic acid or aliphatic acid in the presence of $POCl_3$ under the conditions of refluxing. Low yields of products **12** were due to the formation of acid anhydride by self-condensation of the acid substrates and the oxidation of compounds **11** by $POCl_3$. Higher yields of compounds **12** can be obtained by the reaction of diacylhydrazines with $POCl_3$, while the diacylhydrazines can be prepared by the reaction of intermediates **11** with substituted acid chlorides. All structures of the compounds synthesized were confirmed by ^{1}H NMR, IR, and elemental analysis or HRMS determination.

3.2. Biological Activity. Elicitor is a novel technique for plant protection; however, only limited products are commercialized. The main difficulties in the novel elicitor development were the

Table 1. Validation Data of the Screening System for Plant Activator against TMV[a]

		antivirus activity (%)		induction of systemic acquired resistance (%)	
compound[b]	concentration (μg/mL)	*in vitro*	*in vivo*	leaf spray	soil treatment
BTH	1000	8 ± 2	phototoxicity	97 ± 5	98 ± 3
	500	4 ± 2	8 ± 6	95 ± 7	95 ± 4
	100	2 ± 2	10 ± 6	95 ± 4	96 ± 8
	50	0	15 ± 3	90 ± 6	92 ± 8
	10	0	6 ± 5	50 ± 8	63 ± 7
BABA	2000	5 ± 3	8 ± 7	65 ± 11	80 ± 4
	1000	0	10 ± 6	55 ± 5	80 ± 6
	500	7 ± 4	5 ± 3	50 ± 6	72 ± 7
	100	4 ± 3	9 ± 5	30 ± 4	54 ± 5
	50	0	0	0	46 ± 6
TDL	500	0	0	40 ± 6	60 ± 11
1,2,3-benzothia-diazole	2000	8 ± 4	phototoxicity	12 ± 6	2 ± 3
-7-carboxylic acid	1000	6 ± 2	18 ± 2	8 ± 2	8 ± 4
	500	3 ± 2	5 ± 4	5 ± 1	5 ± 3
	100	5 ± 3	8 ± 6	0	0
	50	7 ± 5	0	3 ± 4	10 ± 2
extractions of *Cynanchum*	100	92 ± 6	76 ± 8	0	7 ± 7
komarovii	50	95 ± 4	60 ± 5	0	7 ± 5
	20	90 ± 5	40 ± 6	5 ± 3	3 ± 1
	10	81 ± 7	18 ± 7	0	0
	5	73 ± 10	0	ND[c]	5 ± 2
	1	35 ± 12	0	ND[c]	2 ± 2
DHT	500	49 ± 8	42 ± 5	5 ± 2	8 ± 4

[a] Data were the average of 20 repeats. [b] BTH, acibenzolar-*S*-methyl; BABA, β-aminobutyric acid; TDL, tiadinil; DHT, 2,4-dioxohexahydro-1,3,5-triazine. [c] ND = not detected.

lack of convenient biological screening methods and an activity evaluation technique. In our previous studies, a systemic screening system was established using cucumber against *Colletotrichum lagenarium* (*23*, *24*). The corresponding standard operation practice (SOP) for tobacco against TMV was also established by a comparison to the corresponding standard elicitors BTH, TDL, and β-aminobutyric acid (BABA), which were also chosen as a positive control, and metabolite of BTH, 1,2,3-benzothiadiazole-7-carboxylic acid, antivirus product 2,4-dioxohexahydro-1,3,5-triazine (DHT), and product of extraction of *Cynanchum komarovii* (the most effective anti-TMV product), which were chosen as a negative control to validate the screening system for the elicitor. The results were shown in **Table 1**.

The data in **Table 1** indicated that TDL, BTH, and BABA had almost no direct antivirus activity *in vitro* or *in vivo*, with the inhibition percentage no more than 20%; however, they all had good induction activity of tobacco against TMV by induction with leaf spraying and soil treatment, while the metabolite of BTH, 1,2,3-benzothiadiazole-7-carboxylic acid, had no activity including direct antivirus activity or systemic acquired resistance against TMV when applied by foliate spraying or via soil treatment. In recent years, our laboratory used DHT and extractions of *Cynanchum komarovii* as positive controls for direct antivirus activity screening, The studies showed that these two products had good antivirus activity *in vitro* and *in vivo*; however, they exhibited no induction of systemic acquired resistance against TMV. These results validated the credibility of the screening system used for activity evaluation of the plant activators. The induction activity of the novel compounds synthesized here was evaluated accordingly. The results in **Table 2** indicated that TDL could stimulate the induction activity of tobacco against TMV, but the induction activity was lower than that of BTH. All of the compounds synthesized had no direct antivirus activity against TMV (data not shown). However, 4-methyl-1,2,3-thiadiazole-5-formyl-(4′-(4″-chlorophenyl)-1′,3′-thiazole)-2′-amine (**10f**) had 40% induction activity, which was similar to that of TDL.

Table 2. Induction Activity of Novel 1,2,3-Thiadiazoles to Tobacco against TMV

compound[a]	concentration (μg/mL)	induction activity (%)	compound	concentration (μg/mL)	induction activity (%)
BTH	500	95 ± 7	**12a**	500	0
BTH	50	92 ± 8	**12b**	500	80 ± 9
TDL	500	40 ± 6	**12b**	50	78 ± 7
TDL	50	63 ± 5	**12c**	500	ND[b]
10a	500	70 ± 4	**12d**	500	0
10a	50	81 ± 8	**12e**	500	10 ± 5
10b	500	30 ± 4	**12f**	500	ND
10c	500	0	**12g**	500	0
10d	500	70 ± 9	**12h**	500	30 ± 8
10d	50	74 ± 9	**12i**	500	ND
10e	500	35 ± 7	**12j**	500	0
10f	500	40 ± 9	**12k**	500	0
10g	500	0	**12l**	500	0
10i	500	20 ± 6	**12m**	500	0
10h	500	0	**12n**	500	20 ± 8

[a] BTH, acibenzolar-*S*-methyl; TDL, tiadinil. [b] ND = not detected.

4-Methyl-1,2,3-thiadiazole-5-formyl-(4′-methyl)-1′,3′-thiazole-2′-amine (**10a**), 4-methyl-1,2,3-thiadiazole-5-formyl-(4′-phenyl)-1′,3′-thiazole-2′-amine (**10d**), and 2-(4′-methyl-1′,2′,3′-thiadizole-5′-)-5-(4″-methoxylphenyl)-1,3,4-oxadiazole (**12b**) showed excellent induction activity of 70%, 70%, and 80%, respectively, and this was validated by the results of screening at lower level concentrations. As can be seen from **Table 2**, thiazole containing 4-methyl-1,2,3-thiadiazole derivatives had good induction activity. A simple substituted thiazole moiety, such as methyl or phenyl substitution, will improve their systemic acquired resistance. For 1,3,4-oxadiazole containing 4-methyl-1,2,3-thiadiazoles, only methoxyl at 4 position of the phenyl ring was observed good systemic acquired resistance. Our studies only synthesized limited derivatives. To conclude the structure and activity relationship, it deserves further synthetic studies and mode of action determination at the molecular level. In addition, another new derivative of

Table 3. Fungicide Activity of 4-Methyl-1,2,3-thiadiazole-5-formylamines (**10**)

	fungi[a]														
compound[b]	GF	FO	CA	PA	AS	GZ	UV	BC	SC	AM	VM	CL	GC	AK	FV
TDL	0	12.5	13.6	**60**	0	25.0	12.0	23.9	34.4	32.1	3.6	22.8	0	0	5.3
10a	**64.3**	15.0	29.4	ND[c]	35.7	7.1	ND	ND	ND	50.0	ND	17.6	ND	17.6	ND
10b	0	53.3	35.7	31.3	21.0	50.0	50.0	0	3.3	25.0	61.1	26.7	36.8	43.8	5.0
10c	**64.3**	ND	29.4	ND	21.0	0	ND	25.0	71.4	ND	31.0	26.7	ND	0	ND
10d	**64.3**	13.3	14.3	6.3	15.8	18.8	55.6	8.3	47.3	10.0	22.2	6.7	15.8	37.5	40.0
10e	10.5	3.3	14.3	ND	4.0	25.0	ND	ND	5.6	15.5	8.7	5.9	4.0	ND	0
10f	**64.3**	46.7	29.4	37.5	0	18.8	11.1	12.0	61.5	15.0	88.9	13.3	68.4	0	40.0
10g	5.3	13.3	35.7	ND	20.0	37.5	ND	ND	11.1	24.1	26.1	17.6	8.0	0	14.3
10h	**64.3**	13.3	14.3	12.5	33.3	25.0	33.3	40	61.5	35.0	44.4	26.7	20.1	37.5	20.0
10i	21.1	10.0	35.7	ND	4.0	25.0	ND	ND	22.2	6.9	4.3	11.8	12.0	ND	3.6

[a] GF, *Gibberella fujikuroi*; FO, *Fusarium oxysporum*; CA, *Cercospora arachidicola*; PA, *Phoma asparagi*; AS, *Alternaria solani*; GZ, *Gibberella zeae*; UV, *Ustilaginoidea virens*; BC, *Botrytis cinerea*; SC, *Sclerotinia sclerotiorum*; AM, *Alternaria mali*; VM, *Valsa mali*; GC, *Glomerella cingulata*; CL, *Colletotrichum lagenarium*; AK, *Alternaria kikuchiana*; FV, *Fusarium vasinfectum*. [b] TDL = tiadinil. [c] ND = not detected.

Table 4. Fungicide Activity of 1,2,3-Thiadiazolo-1,3,4-oxadiazole Derivatives (**12**)

	fungi[a]															
compound[b]	RS	FV	PI	GF	FO	CA	AS	GZ	PP	UV	BC	SC	AM	VM	GC	RC
TDL	ND[c]	5.3	ND	0	12.5	13.6	0	25.0	ND	12	23.9	34.4	32.1	3.6	51.6	ND
12a	67.9	ND	0	ND	0	ND	18.4	ND	ND	ND	2.1	56.0	ND	ND	ND	ND
12b	0	0	ND	0	0	17.6	17.6	37.9	33.3	29.4	31	23.5	37.9	47.2	29.4	31.0
12c	0	0	17.1	24.1	0	23.5	0	24.1	47.2	0	37.9	23.5	41.4	47.2	0	37.9
12d	0	0	20.0	17.2	0	17.6	11.7	31	33.3	0	13.8	23.5	27.6	41.2	0	13.8
12e	0	0	ND	0	0	17.6	17.6	31	44.4	0	41.4	23.5	31	22.2	0	37.9
12f	44.4	24.1	31.0	17.6	33.3	0	31.0	13.8	23.5	22.2	21.1	53.6	27.3	47.2	13.8	13.8
12g	0	0	ND	31.0	0	41.2	17.6	48.3	44.4	0	31.0	23.5	27.6	19.4	0	31.0
12h	22.2	0	30.0	17.2	0	23.5	0	31.0	38.9	17.6	17.2	0	24.1	22.2	0	13.8
12i	0	0	ND	21.1	31.3	9.0	36.8	21.1	39.7	0	0	40.5	38.9	0	13.8	0
12j	26.6	0	**100**	21.1	12.5	0	31.6	34.2	25.4	0	0	32.1	38.9	0	42.1	0
12k	0	23.7	20.0	21.1	36.3	27.3	31.6	36.8	46.0	0	0	0	0	20.3	13.8	0
12l	0	0	ND	21.1	31.3	13.6	44.7	31.6	38.1	0	0	32.1	0	0	13.8	0
12m	ND	ND	0	ND	19.1	87.5	53.6	22.2	38.9	ND	ND	0	ND	ND	42.1	ND
12n	ND	ND	17.1	ND	26.5	33.3	17.9	27.8	16.7	ND	ND	0	ND	ND	42.1	ND

[a] GF, *Gibberella fujikuroi*; FO, *Fusarium oxysporum*; CA, *Cercospora arachidicola*; AS, *Alternaria solani*; GZ, *Gibberella zeae*; PP, *Physalospora piricola*; UV, *Ustilaginoidea virens*; BC, *Botrytis cinerea*; SC, *Sclerotinia sclerotiorum*; AM, *Alternaria mali*; VM, *Valsa mali*; GC, *Glomerella cingulata*; FV, *Fusarium vasinfectum*; RC, *Rhizoctonia cerealis*; PI, *Phytophthora infestans* (Mont) de Bary; RS, *Rhizoctonia solani* Kuhn. [b] TDL = tiadinil. [c] ND = not detected.

1,2,3-thiadiazole synthesized in our group with good activity of systemic acquired resistance is now under development. The results of this study indicated that our idea of combination of thiadiazole with thiazole or oxadiazole moiety is an interesting exploration for novel pesticide development.

According to the current understanding of the mechanism of action of plant elicitors, they act as inducers of the host immune system with no direct activity (*2*). Fungicide activity determination was completed on the following fungi, which represented the typical diseases occurring in the Chinese agricultural ecosystem: GF, FO, CA, PA, AS, GZ, UV, BC, SC, AM, PT, PP, VM, GC, CL, AK, FV, RC, PI, and RS. Results indicated that some compounds had good fungicide activity at 50 μg/mL (**Table 3**). Compounds containing 4-methyl-1,2,3-thiadiazole-5-formylamine (**10a**, **10c**, **10d**, **10f**, and **10h**) inhibited more than 60% of the growth of GF. The percentage of inhibition of **10f** to VM was about 89% (**Table 3**). Compounds containing 4-methyl-1,2,3-thiadiazol-5-1,3,4-oxadiazole (**12a**) had good activity against RS, with growth inhibition of 67.9%. Compound **12j** had excellent activity against PI, with 100% of growth inhibition at 50 μg/mL concentration (**Table 4**).

In vivo studies indicated that two novel compounds, **12c** and **12j**, had a certain extent of efficacy against PT at 500 μg/mL, with 98 and 83% of growth inhibition, respectively. Their activities were lower than that of the positive control chlorothalonil, which had 100% of efficacy against PT at 500 μg/mL. This was the first discovery of derivatives containing 1,2,3-thiadiazole and 1,3,4-oxadiazole that had activity against PT (**Table 5**).

Table 5. Fungicide Activity of 1,2,3-Thiadiazole Derivatives (**10** and **12**) against *Puccinia triticina* Eriks (PT)

compound[a]	concentration (μg/mL)	inhibition (%)	compound	concentration (μg/mL)	inhibition (%)
chlorothalonil	100	100	**12b**	500	0
BTH	500	0	**12c**	500	98
TDL	500	40	**12d**	500	39
10a	500	ND[b]	**12e**	500	0
10c	500	0	**12f**	500	ND
10b	500	40	**12g**	500	0
10d	500	0	**12h**	500	17
10e	500	45	**12i**	500	0
10f	500	0	**12j**	500	83
10g	500	ND	**12k**	500	20
10i	500	30	**12l**	500	28
10h	500	0	**12m**	500	0
12a	500	11	**12n**	500	20

[a] BTH, acibenzolar-*S*-methyl; TDL, tiadinil. [b] ND = not detected.

ABRREVIATIONS USED

TMV, tobacco mosaic virus; PDA, potato dextrose agar; GF, *Gibberella fujikuroi*; FO, *Fusarium oxysporum*; CA, *Cercospora arachidicola*; PA, *Phoma asparagi*; AS, *Alternaria solani*; GZ, *Gibberella zeae*; UV, *Ustilaginoidea virens*; BC, *Botrytis cinerea*; SC, *Sclerotinia sclerotiorum*; AM, *Alternaria mali*; PT, *Puccinia triticina* Eriks; PP, *Physalospora piricola*; VM, *Valsa mali*; GC, *Glomerella cingulata*; CL, *Colletotrichum lagenarium*; AK, *Alternaria kikuchiana*; FV, *Fusarium vasinfectum*; RC, *Rhizoctonia cerealis*; PI, *Phytophthora infestans* (Mont) de Bary; RS, *Rhizoctonia solani* Kuhn; SOP, standard operation practice; BTH, acibenzolar-*S*-methyl; BABA, β-aminobutyric acid; TDL, tiadinil; DHT, 2,4-dioxohexahydro-1,3,5-triazine; ND, not detected.

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LITERATURE CITED

(1) Bos, L. Crop losses caused by viruses. *Crop Prot*. **1982**, *1* (3), 263–282.
(2) Fan, Z. J.; Liu, X. F.; Liu, F. L.; Bao, L. L.; Zhang, Y. G. Progress of researches on induced resistance of plant activator. *Acta Phytophylacica Sin*. **2005**, *32* (1), 87–92.
(3) Gozzo, F. Systemic acquired resistance in crop protection: From nature to a chemical approach. *J. Agric. Food Chem*. **2003**, *51* (16), 4487–4503.
(4) Michiko, Y.; Hideo, N.; Shigeo, Y. Tiadinil, a noval class of activator of systemic acquired resistance, induces defense gene expression and disease resistance in tobacco. *J. Pestic. Sci*. **2004**, *29*, 46–49.
(5) Bakulev, V. A.; Dehaem, W. *The Chemistry of 1,2,3-Thiadiazole*; John Wiley and Sons, Inc.: New York, 2004; pp 229−230.
(6) Pachhamia, V. L.; Parikh, A. R. Studies on 2,5-disubstitued-1,3,4-oxadiazole. Part II. *J. Indian Chem. Soc*. **1988**, *65* (5), 357–361.
(7) Fan, Z. J.; Liu, B.; Liu, X. F.; Zhong, B.; Liu, C. L.; Li, Z. M. Synthesis and bioactivity of pyridine containing 1,3,4-oxadiazole derivatives. *Chem. J. Chin. Univ*. **2004**, *25* (4), 663–666.
(8) Hu, D. Y.; Song, B. A.; He, W. Progresses in the synthesis and biological activity of thiazole derivatives. *Chin. J. Synth. Chem*. **2006**, *14* (4), 319–328.
(9) Xia, Y. Synthesis and fungicidal activity of ethaboxam against oomycete. *World Pestic*. **2005**, *27* (3), 13–17.
(10) Paul, H.; Lange, J.; New, H. Process for making carbohydrazide. U. S. Patent 4,496,761, Jan 29, 1985.
(11) Yang, Z. G.; Yang, G. J.; Ba, Z. H. Thidiazuron analogue and its preparation method. CN1305998, Aug 1, 2001.
(12) Friedrich, A.; Hans, R. K.; Reinhart, R. 1,2,3-Thiadiazole-5-carboxylic acid derivatives, herbicidal and growth regulating compositions containing the same and process for making same. U.S. Patent 4,177,054, Dec 4, 1979.
(13) Shafiee, A.; Mohamadpour, M.; Abtahi, F.; Khoyi, A. Synthesis and antihistaminic activity of 1-[(4-substituted-1,2,3-thiadiazole-5-yl) arymethyl]-4-methyl piperazines. *J. Pharm. Sci*. **1981**, *70* (5), 510–513.
(14) Li, Z. G.; Wang, Q. M.; Huang, J. M. Preparation method of organic intermediate. Chemical Industry Press: Beijing, China, 2000; p 138.
(15) Saul, B. K. Anticancer thiazole-2-ylcarbamoylcarboxylic acids, esters and amides. U.S. 4,321,372, March 23, 1982.
(16) Dodson, R. M.; Carroll, K. L. The reaction of ketones with halogens and thiourea. *J. Am. Chem. Soc*. **1945**, *67* (12), 2242–2243.
(17) Munish, K.; Naveen, A.; Khursheed, A.; et al. Synthesis of β-adrenergic blockers (*R*)-(−)-nifenalol and (*S*)-(+)-sotalol via a highly efficient resolution of a bromohydrin precursor. *Tetrahedron: Asymmetry* **2005**, *16* (3), 717–725.
(18) Singh, S. K.; Naim, S. S.; Sharma, S. Antiparasitic agents. Part 13. Synthesis of 4-aryl-2-substituted aminothiazoles as potential anthelmintics. *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem*. **1989**, *28* (9), 786–789.
(19) Gilles, Q.; Stephane, B.; Cedrik, G. Simple coupling reaction between amino acid and weakly nucleophilic heteroaromatic amines. *J. Comb. Chem*. **2004**, *6*, 695–698.
(20) Nakazato, A.; Kumagai, T. Thiazole derivatives. EP0816362, Jan 7, 1998.
(21) Jalilian, A. R.; Sattari, S.; Bineshmarvasti, M.; Shafiee, A.; Daneshtalab, M. Synthesis and *in vitro* antifungal and cytotoxicity evaluation of thiazolo-4*H*-1,2,3-triazoles and 1,2,3-thiadiazolo-4*H*-1,2,4-triazoles. *Arch. Pharm. Med. Chem*. **2000**, *333*, 347–354.
(22) Kondratieva, M. L.; Pepeleva, A. V.; Belskaia, N. P.; Koksharov, A. V.; Groundwater, P. V.; Robeyns, K.; Meervelt, L. V.; Dehaen, W.; Fan, Z. J.; Bakulev, V. A. A new synthetic method for the 2*H*-[1,2,3] thiadiazolo[5,4-*b*]indoles. *Tetrahedron* **2007**, *63* (14), 3042–3048.
(23) Zhang, Y. G.; Fan, Z. J.; Liu, F. L..; et al. Establishment of screening system for induction of cucumber against *Colletotrichum lagenarium* by β-aminobutyric acid. *Agrochemicals* **2006**, *45* (4), 239–242.
(24) Zhang, Y. G.; Fan, Z. J.; Liu, X. F.; et al. Screening system for elicitation of cucumber against *Colletotrichum lagenarium* induced by BTH. Proceedings of the 3rd International Symposium on Pesticide and Environmental Safety and 7th International Workshop on Crop Protection Chemistry and Regulatory Harmonization, Beijing Pesticide Society (BPS), International Union of Pure and Applied Chemistry (IUPAC), China Agricultural University (CAU), Beijing, People's Republic of China, Oct 9−13, 2007; pp 131−137.

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