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Synthesis of 4-Methyl-1,2,3-thiadiazole Derivatives via Ugi Reaction and Their Biological Activities†

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The Ugi reaction is a green and rapid one-pot reaction for lead derivation. To develop novel candidate pesticides with diverse biological activities, two series of 4-methyl-1,2,3-thiadiazole derivatives containing active substructures of 3-chloro-4-methylphenyl or 3-fluoro-4-methylphenyl, respectively, were rationally designed and synthesized via Ugi reaction according to the principle of combinations of bioactive substructures. All of the structures of newly synthesized compounds were confirmed by proton nuclear magnetic resonance and high-resolution mass spectrometry. Biological activities of the target compounds including fungicide activity, antivirus activity in vitro and in vivo, and systemic acquired resistance were evaluated systematically. The results indicated that derivatives containing 3-(trifluoromethyl)phenyl and 2-methylphenyl possessed a potential wide spectrum of fungicidal activity. Derivatives containing 3-(trifluoromethyl)phenyl and 4-hydroxyphenyl possessed good potential direct antivirus activities against tobacco mosaic virus (TMV) in vitro, and the replacement of Cl atom by F atom improved their direct inhibition activities against TMV in vitro. Derivatives containing phenyl, 2-(trifluoromethyl)phenyl, 3-(trifluoromethyl)phenyl, 3-nitrophenyl, 4-nitrophenyl, 2-methylphenyl, and 4-hydroxyphenyl possessed good potential bioactivities in vivo including protection, inactivation, curative, and induction activities against TMV. These studies indicate that the newly synthesized 4-methyl-1,2,3-thiadiazole derivatives possessed good potential bioactivities, and a combination of bioactive substructures via Ugi reaction was an effective way to find bioactive compounds for novel pesticide development.

KEYWORDS: 4-Methyl-1,2,3-thiadiazole; systemic acquired resistance; Ugi reaction; fungicide

INTRODUCTION

Since thiabendazole was discovered and developed as a fungicide by Merck & Co. Inc. in the 1960s, many thiazoles including a number of widely used fungicides (e.g., tricyclazole, probenazole, and trifluzamide) and the best plant elicitor [i.e., acibenzolar-*S*-methyl (BTH)] have been introduced into the pesticide market (*1*, *2*). As a class of compounds with various interesting properties, 1,2,3-thiadiazoles are becoming a rapidly growing and independent branch of the chemistry of thiazoles. Many 1,2,3-thiadiazoles have shown biological activities such as antiviral (*3*,*4*), systemic acquired resistance (*5*, *6*), fungicidal (*5*, *7*−*10*), and insecticidal activities (*11*). Moreover, because of their various biological activities, 1,2,3-thiadiazoles have attracted considerable attention, and 1,2,3-thiadizoles have also been introduced into other active compounds as an active moiety for lead discovery (*12*). Meanwhile, the property of easy breakdown of the 1,2,3-thiadiazole ring into low molecular weight compounds favors the use of its derivatives as pesticides with low toxicity (*13*). Tiadinil (TDL), a 4-methly-1,2,3-thiadiazole-5-carboxamide derivative containing a 3-chloro-4-methylphenyl moiety, has been recognized as a well-known commercialized fungicide as well as a plant elicitor (*14*). However, studies on the wide biological spectrum of 1,2,3-thiadiazoles are seldom reported. An elicitor is any compound with low or no direct activity against a pathogen, but acts by stimulating the immunosystem of the host plants to produce systemic acquired resistance against the succeeding pathogen attack (*15*, *16*). Unluckily, very limited documents report the development of novel elicitors, too.

Combinatorial chemistry has attracted much attention as an efficient tool for drug discovery. The use of combinatorial chemistry or parallel synthesis for the optimization of highly promising lead compounds arising from the drug-discovery processes around privileged structures and their combinations has been successful in many discovery and development efforts (*17*). Our studies noted that the Ugi four-component condensation reaction (U-4CR) could be an efficient and convenient green method to combine several active moieties into one molecule, and it could be convenient to implement structural modification via alternation of diverse starting materials. Generally, U-4CR refers to the reaction between an amine, a carbonyl compound (aldehyde or ketone), an isocyanide, and a carboxylic

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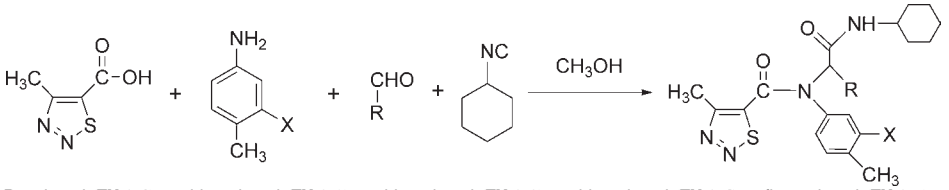
Table 1. Yields and Physical Properties of the Target Compounds

compd	yield (%)	appearance, mp (°C)	compd	yield (%)	appearance, mp (°C)
ZX-3-1	50	white solid, 175−180	**ZX-4-1**	52	white solid, 183−185
ZX-3-2	52	white needle crystal, 176−180	**ZX-4-2**	22	white needle crystal, 171−174
ZX-3-3	76	white solid, 198−203	**ZX-4-3**	81	white needle crystal, 200−201
ZX-3-4	78	white solid, 215−218	**ZX-4-4**	78	white needle crystal, 223−225
ZX-3-5	55	white solid, 132−135	**ZX-4-5**	62	white solid, 139−141
ZX-3-6	72	white solid, 206−208	**ZX-4-6**	75	white needle crystal, 223−225
ZX-3-7	89	white solid, 213−217	**ZX-4-7**	78	white solid, 204−206
ZX-3-8	8	yellow solid, 152−158	**ZX-4-8**	52	white solid, 167−169
ZX-3-9	48	white solid, 205−207	**ZX-4-9**	46	white solid, 168−170
ZX-3-10	81	white solid, 214−216	**ZX-4-10**	74	white needle crystal, 224−226
ZX-3-11	45	white solid, 171−173	**ZX-4-11**	68	white solid, 187−189
ZX-3-12	71	white solid, 204−208	**ZX-4-12**	90	white solid, 217−219
ZX-3-13	75	white solid, 113−115	**ZX-4-13**	71	white solid, 231−233
ZX-3-14	47	white solid, 131−134	**ZX-4-14**	62	white needle crystal, 170−172
ZX-3-15	46	white solid, 172−173	**ZX-4-15**	84	white needle crystal, 209−211
ZX-3-16	60	white solid, 209−211	**ZX-4-16**	79	white solid, 178−180
ZX-3-17	3	white solid, 167−172	**ZX-4-17**	22	white needle crystal, 171−174
ZX-3-18	40	white needle crystal, 135−139			

Scheme 1. Molecule Structures and Synthesis of the Target Compounds[a]



[a] X = Cl (**series ZX-3**), R = phenyl (**ZX-3-1**); 2-chlorophenyl (**ZX-3-2**); 3-chlorophenyl (**ZX-3-3**); 4-chlorophenyl (**ZX-3-4**); 2-fluorophenyl (**ZX-3-5**); 3-fluorophenyl (**ZX-3-6**); 4-fluorophenyl (**ZX-3-7**); 2-(trifluoromethyl)phenyl (**ZX-3-8**); 3-(trifluoromethyl)phenyl (**ZX-3-9**); 4-(trifluoromethyl)phenyl (**ZX-3-10**); 2-nitrophenyl (**ZX-3-11**); 3-nitrophenyl (**ZX-3-12**); 4-nitrophenyl (**ZX-3-13**); 2-methylphenyl (**ZX-3-14**); 3-methylphenyl (**ZX-3-15**); 4-methylphenyl (**ZX-3-16**); 3-hydroxyphenyl (**ZX-3-17**); 4-hydroxyphenyl (**ZX-3-18**); X = F (**series ZX-4**), R = phenyl (**ZX-4-1**); 2-chlorophenyl (**ZX-4-2**); 3-chlorophenyl (**ZX-4-3**); 4-chlorophenyl (**ZX-4-5**); 2-fluorophenyl (**ZX-4-5**); 3-fluorophenyl (**ZX-4-6**); 4-fluorophenyl (**ZX-4-7**); 2-(trifluoromethyl)phenyl (**ZX-4-8**); 3-(trifluoromethyl)phenyl (**ZX-4-9**); 4-(trifluoromethyl)phenyl (**ZX-4-10**); 2-nitrophenyl (**ZX-4-11**); 3-nitrophenyl (**ZX-4-12**); 4-nitrophenyl (**ZX-4-13**); 2-methylphenyl (**ZX-4-14**); 3-methylphenyl (**ZX-4-15**); 4-methylphenyl (**ZX-4-16**); 4-hydroxyphenyl (**ZX-4-17**).

acid, which is a convenient rapid one-pot reaction with the process itself and the disposal of its succedent being very simple (*18*−*20*).

Our previous studies suggested that some 4-methyl-1,2,3-thiadiazole-5-carboxamide derivatives designed and synthesized showed good biological activities (*5*, *21*−*23*), fluorinated 4-methyl-1,2,3-thiadiazoles had good systemic acquired resistance, and 4-methyl-1,2,3-thiadiazole was an important active substructure (*5*, *21*, *23*). These studies inspired our current hypothesis that the introduction of phenyl moieties into the target compounds may favor the biological activities of 1,2,3-thiadiazoles, and here we designed and synthesized two series of 4-methyl-1,2,3-thiadiazole-5-carboxamide derivatives via U-4CR for the fungicidal, antiviral, and systemic acquired resistance activity determination because 1,2,3-thiadiazoles could be decomposed by releasing N_2 and regarded as environmentally friendly molecules (*24*). One series contained a 3-chloro-4-methylphenyl moiety and the other one contained a 3-fluoro-4-methylphenyl moiety (**Scheme 1**). In U-4CR, 4-methyl-1,2,3-thiadiazole-5-carboxylic acid was chosen as the acid component and cyclohexyl isocyanide was chosen as the isocyanide component; the amine components were 3-chloro-4-methylbenzenamine and 3-fluoro-4-methylbenzenamine, respectively, as described in the above two series. Because aromatic rings play important roles in chemical and biological recognition (*25*), various substituted benzaldehyde derivatives were used as the aldehyde component. The most important difference between this study and our former study is in the substructures coupled with 4-methyl-1,2,3-thiadiazole. Here, these were all aromatic amines, and there was no H atom combined in the carboxylic amine N nearest the 4-methyl-1,2,3-thiadiazole ring in the products, and some of them also showed biological activities. Our results indicated that 4-methyl-1,2,3-thiadiazole derivatives had good potential bioactivities, and combination of bioactive substructures via Ugi reaction was an effective measure for structure−activity relationship studies and novel pesticide development.

EXPERIMENTAL METHODS

Equipment for Structural Characterization. Melting points of all compounds were determined on an X-4 binocular microscope (Gongyi Technical Instrument Co., Henan, China), and the thermometer was not corrected. Proton NMR spectra were obtained using a Bruker AVANCE-400 MHz spectrometer, and chemical shift values (δ) were reported as parts per million (δ) with deutero dimethyl sulfoxide (DMSO-d_6) as the solvent and tetramethylsilane (TMS) as the internal standard. High-resolution mass spectrometry (HRMS) data were obtained on an FTICR-MS Varian 7.0T FTICR-MS instrument. 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 by using silica gel.

Preparation of Cyclohexyl Isocyanide (3). Ethyl formate (1.0 mol) was added dropwise to cyclohexylamine (1.2 mol) in an ice−water bath with stirring. After the exothermic reaction ceased, the solution was refluxed for 5 h. Ethanol and excess cyclohexylamine were distilled off under reduced pressure, and the residue *N*-cyclohexylformamide was obtained as a white solid, with a yield of 85%. A solution including 1.0 mol of *N*-cyclohexylformamide, 500 mL of pyridine, and 250 mL of petroleum ether (bp 60−90 °C) was mixed in a three-neck flask, round-bottom flask. Phosphorus oxychloride (0.6 mol) was added dropwise to the solution in an ice−water bath with stirring. After all phosphorus oxychloride was added, the mixture was refluxed for 5 h, and a solid appeared in the mixture. Ice water was added gradually with stirring until

Table 2. ^{1}H NMR and HRMS Data of the Target Compounds

compd	^{1}H NMR (solvent: $CDCl_3$)	HRMS (*m*/*z*)
ZX-3-1	δ 7.29−6.94 (m, 8H), 6.17 (s, 1H), 5.39 (d, *J* = 7.6 Hz, 1H), 3.90−3.82 (m, 1H), 2.87 (s, 3H), 2.25 (s, 3H), 1.99−1.00 (m, 10H)	[M + Na^+]: 505.1435 found: 505.1433
ZX-3-2	δ 7.39−6.89 (m, 7H), 6.57 (s, 1H), 5.50 (d, *J* = 7.8 Hz, 1H), 3.92−3.85 (m, 1H), 2.86 (s, 3H), 2.21 (s, 3H), 2.05−1.0 (m, 10H)	[M + Na^+]: 539.1046 found: 539.1047
ZX-3-3	δ 7.28−7.01 (m, 7H), 6.08 (s, 1H), 5.45 (d, *J* = 7.6 Hz, 1H), 3.89−3.81 (m, 1H), 2.87 (s, 3H), 2.28 (s, 3H), 2.00−1.03 (m, 10H)	[M + Na^+]: 539.1046 found: 539.1041
ZX-3-4	δ 7.24−6.97 (m, 7H), 6.11 (s, 1H), 5.40 (d, *J* = 8.0 Hz, 1H), 3.88−3.81 (m, 1H), 2.87 (s, 3H), 2.28 (s, 3H), 2.00−1.02 (m, 10H)	[M + Na^+]: 539.1046 found: 539.1038
ZX-3-5	δ 7.29−6.93 (m, 7H), 6.44 (s, 1H), 5.43 (d, *J* = 7.8 Hz, 1H), 3.93−3.84 (m, 1H), 2.86 (s, 3H), 2.23 (s, 3H), 2.03−1.01 (m, 10H)	[M + Na^+]: 523.1341 found: 523.1338
ZX-3-6	δ 7.25−6.91 (m, 7H), 6.10 (s, 1H), 5.44 (d,*J* = 7.8 Hz, 1H), 3.89−3.82 (m, 1H), 2.87 (s, 3H), 2.27 (s, 3H), 2.01−1.03 (m, 10H)	[M + Na^+]: 523.1341 found: 523.1348
ZX-3-7	δ 7.16−6.92 (m, 7H), 6.14 (s, 1H), 5.40 (d, *J* = 7.8 Hz, 1H), 3.89−3.81 (m, 1H), 2.87 (s, 3H), 2.27 (s, 3H), 2.01−1.02 (m, 10H)	[M + Na^+]: 523.1341 found: 523.1348
ZX-3-8	δ 7.72−7.05 (m, 7H), 6.58 (s, 1H), 5.29 (d, *J* = 7.8 Hz, 1H), 3.88−3.80 (m, 1H), 2.86 (s, 3H), 2.23 (s, 3H), 2.04−1.09 (m, 10H)	[M + Na^+]: 573.1309 found: 573.1304
ZX-3-9	δ 7.54−6.95 (m, 7H), 6.20 (s, 1H), 5.49 (d, *J* = 8.0 Hz, 1H), 3.90−3.83 (m, 1H), 2.88 (s, 3H), 2.26 (s, 3H), 2.02−1.02 (m, 10H)	[M + Na^+]: 573.1309 found: 573.1308
ZX-3-10	δ 7.54−6.98 (m, 7H), 6.16 (s, 1H), 5.44 (d, *J* = 8.0 Hz, 1H), 3.88−3.84 (m, 1H), 2.88 (s, 3H), 2.28 (s, 3H), 1.98−1.02 (m, 10H)	[M + Na^+]: 573.1309 found: 573.1304
ZX-3-11	δ 7.93−6.91 (m, 7H), 6.63 (s, 1H), 5.66 (d, *J* = 7.0 Hz, 1H), 3.88−3.81 (m, 1H), 2.86 (s, 3H), 2.21 (s, 3H), 2.03−1.01 (m, 10H)	[M + Na^+]: 550.1286 found: 550.1288
ZX-3-12	δ 8.16−7.00 (m, 7H), 6.21 (s, 1H), 5.59 (d, *J* = 7.5 Hz, 1H), 3.90−3.84 (m, 1H), 2.89 (s, 3H), 2.27 (s, 3H), 2.03−1.0 (m, 10H)	[M + Na^+]: 550.1286 found: 550.1285
ZX-3-13	δ 8.12−7.00 (m, 7H), 6.18 (s, 1H), 5.55 (d, *J* = 7.8 Hz, 1H), 3.90−3.83 (m, 1H), 2.88 (s, 3H), 2.28 (s, 3H), 2.02−1.04 (m, 10H)	[M + Na^+]: 550.1286 found: 550.1287
ZX-3-14	δ 7.20−6.81 (m, 7H), 6.51 (s, 1H), 5.29 (d, *J* = 7.8 Hz, 1H), 3.92−3.84 (m, 1H), 2.88 (s, 3H), 2.43 (s, 3H), 2.22 (s, 3H), 1.98−1.00 (m, 10H)	[M + Na^+]: 519.1592 found: 519.1591
ZX-3-15	δ 7.14−6.89 (m, 7H), 6.13 (s, 1H), 5.38 (d, *J* = 8.3 Hz, 1H), 3.89−3.82 (m, 1H), 2.87 (s, 3H), 2.26 (s, 6H), 1.99−1.01 (m, 10H)	[M + Na^+]: 519.1592 found: 519.1598
ZX-3-16	δ 7.06−6.94 (m, 7H), 6.13 (s, 1H), 5.36 (d, *J* = 8.0 Hz, 1H), 3.88−3.81 (m, 1H), 2.87 (s, 3H), 2.30 (s, 3H), 2.26 (s, 3H), 1.99−1.00 (m, 10H)	[M + Na^+]: 519.1592 found: 519.1593
ZX-3-17	δ 6.99−6.69 (m, 7H), 6.17−6.07 (m, 2H), 5.49 (d, *J* = 8.3 Hz, 1H), 3.87−3.80 (m, 1H), 2.84 (s, 3H), 2.26 (s, 3H), 1.95−0.98 (m, 10H)	[M + Na^+]: 521.1385 found: 519.1383
ZX-3-18	δ 6.97−6.69 (m, 8H), 6.05 (s, 1H), 5.57 (d, *J* = 8.0 Hz, 1H), 3.86−3.78 (m, 1H), 2.82 (s, 3H), 2.26 (s, 3H), 1.95−1.01 (m, 10H)	[M + Na^+]: 521.1385 found: 519.1387
ZX-4-1	δ 7.28−6.87 (m, 8H), 6.19 (s, 1H), 5.38 (d, *J* = 8.0 Hz, 1H), 3.93−3.84 (m, 1H), 2.86 (s, 3H), 2.15 (d, *J* = 1.8 Hz, 1H), 1.99−1.00 (m, 10H)	[M + Na^+]: 489.1731 found: 489.1740
ZX-4-2	δ 7.37−6.85 (m, 7H), 6.58 (s, 1H), 5.51 (d, *J* = 6.2 Hz, 1H), 3.93−3.84 (m, 1H), 2.86 (s, 3H), 2.11 (d, *J* = 1.8 Hz, 1H), 2.04−1.03 (m, 10H)	[M + Na^+]: 523.1341 found: 523.1344
ZX-4-3	δ 7.21−6.93 (m, 7H), 6.11 (s, 1H), 5.43 (d, *J* = 8.0 Hz, 1H), 3.88−3.83 (m, 1H), 2.88 (s, 3H), 2.18 (d, *J* = 1.8 Hz, 1H), 2.00−1.03 (m, 10H)	[M + Na^+]: 523.1341 found: 523.1347
ZX-4-4	δ 7.23−6.91 (m, 7H), 6.13 (s, 1H), 5.39 (d, *J* = 8.0 Hz, 1H), 3.88−3.81 (m, 1H), 2.87 (s, 3H), 2.18 (d, *J* = 1.8 Hz, 1H), 2.00−1.01 (m, 10H)	[M + Na^+]: 523.1341 found: 523.1341
ZX-4-5	δ 7.28−6.89 (m, 7H), 6.45 (s, 1H), 5.54 (d, *J* = 8.0 Hz, 1H), 3.93−3.83 (m, 1H), 2.86 (s, 3H), 2.14 (d, *J* = 1.8 Hz, 1H), 2.03−1.04 (m, 10H)	[M + Na^+]: 507.1637 found: 507.1642

Table 2. Continued

compd	^{1}H NMR (solvent: $CDCl_3$)	HRMS (*m*/*z*)
ZX-4-6	δ 7.24−6.90 (m, 7H), 6.12 (s, 1H), 5.43 (d, *J* = 7.8 Hz, 1H), 3.89−3.82 (m, 1H), 2.88 (s, 3H), 2.18 (d, *J* = 2.0 Hz, 1H), 2.01−1.03 (m, 10H)	[M + Na^+]: 507.1637 found: 507.1639
ZX-4-7	δ 7.16−6.91 (m, 7H), 6.16 (s, 1H), 5.40 (d, *J* = 8.0 Hz, 1H), 3.88−3.83 (m, 1H), 2.87 (s, 3H), 2.18 (d, *J* = 1.8 Hz, 1H), 2.00−1.01 (m, 10H)	[M + Na^+]: 507.1637 found: 507.1640
ZX-4-8	δ 7.70−6.87 (m, 7H), 6.58 (s, 1H), 5.28 (d, *J* = 7.5 Hz, 1H), 3.87−3.80 (m, 1H), 2.85 (s, 3H), 2.13 (d, *J* = 1.5 Hz, 1H), 2.04−0.96 (m, 10H)	[M + Na^+]: 557.1605 found: 557.1606
ZX-4-9	δ 7.54−6.93 (m, 7H), 6.22 (s, 1H), 5.47 (d, *J* = 8.0 Hz, 1H), 3.90−3.83 (m, 1H), 2.89 (s, 3H), 2.16 (d, *J* = 1.8 Hz, 1H), 2.01−1.02 (m, 10H)	[M + Na^+]: 557.1605 found: 557.1612
ZX-4-10	δ 7.53−6.93 (m, 7H), 6.18 (s, 1H), 5.44 (d, *J* = 8 Hz, 1H), 3.89−3.82 (m, 1H), 2.88 (s, 3H), 2.18 (d, *J* = 1.8 Hz, 1H), 2.01−1.02 (m,10H)	[M + Na^+]: 557.1605 found: 557.1597
ZX-4-11	δ 7.93−6.87 (m, 7H), 6.63 (s, 1H), 5.59 (d, *J* = 8.0 Hz, 1H), 3.89−3.83 (m, 1H), 2.88 (s, 3H), 2.12 (d, *J* = 1.8 Hz, 1H), 2.04−1.02 (m, 10H)	[M + Na^+]: 534.1582 found: 534.1577
ZX-4-12	δ 8.15−6.96 (m, 7H), 6.23 (s, 1H), 5.60 (d, *J* = 8.0 Hz, 1H), 3.91−3.83 (m, 1H), 2.89 (s, 3H), 2.17 (d, *J* = 1.8 Hz, 1H), 2.04−1.05 (m, 10H)	[M + Na^+] :534.1582 found: 534.1586
ZX-4-13	δ 8.12−6.97 (m, 7H), 6.21 (s, 1H), 5.54 (d, *J* = 7.5 Hz, 1H), 3.91−3.84 (m, 1H), 2.89 (s, 3H), 2.20 (d,*J* = 1.8 Hz, 1H), 2.04−1.05 (m, 10H)	[M + Na^+]: 534.1582 found: 534.1586
ZX-4-14	δ 7.19−6.81 (m, 7H), 6.52 (s, 1H), 5.31 (d, *J* = 7.5 Hz, 1H), 3.92−3.84 (m, 1H), 2.88 (s, 3H), 2.43 (s, 3H), 2.12 (d, *J* = 1.3 Hz, 1H), 2.00−1.00 (m, 10H)	[M + Na^+]: 503.1888 found: 503.1882
ZX-4-15	δ 7.13−6.89 (m, 7H), 6.15 (s, 1H), 5.38 (d, *J* = 7.8 Hz, 1H), 3.89−3.82 (m, 1H), 2.88 (s, 3H), 2.25 (s, 3H), 2.16 (d, *J* = 1.8 Hz, 1H), 1.98−1.00 (m, 10H)	[M + Na^+]: 503.1888 found: 503.1881
ZX-4-16	δ 7.02−6.88 (m, 7H), 6.15 (s, 1H), 5.35 (d, *J* = 8.3 Hz, 1H), 3.88−3.81 (m, 1H), 2.87 (s, 3H), 2.30 (s, 3H), 2.16 (d, *J* = 1.8 Hz, 1H), 1.97−0.99 (m, 10H)	[M + Na^+]: 503.1888 found: 503.1887
ZX-4-17	δ 7.02−6.67 (m, 7H), 6.13 (s, 1H), 5.38 (d, *J* = 8.0 Hz, 1H), 5.04 (s, 1H), 3.88−3.81 (m, 1H), 2.87 (s, 3H), 2.17 (d, *J* = 1.8 Hz, 1H), 1.98−1.01 (m, 10H)	[M + Na^+]: 505.1680 found: 505.1672

all solid dissolved, the organic phase was separated, the aqueous phase was extracted with petroleum ether, and the extracts were combined with the organic phase. The resulting organic phase was washed with water and dried over magnesium sulfate. Pyridine and petroleum ether were distilled off, and the residue was obtained under reduced pressure; cyclohexyl isocyanide, a colorless foul-smelling liquid, was obtained with a yield of 73% (*26*).

Preparation of 4-Methyl-1,2,3-thiadiazolyl-5-carboxylic Acid. 4-Methyl-1,2,3-thiadiazole-5-carboxylic acid was prepared according to the description in ref *5*.

General Synthetic Procedure for 4-Methyl-1,2,3-thiadiazole-5-carboxamide Derivatives via U-4CR. Benzaldehyde (0.30 g, 2.8 mmol) was added to a solution of 3-chloro-4-methylbenzenamine (0.42 g, 3.0 mmol) in methanol in an ice−water bath, and then the reaction mixture was stirred for 6 h at room temperature. A solution of 4-methyl-1,2,3-thiadiazole-5-carboxylic acid (0.47 g, 3.6 mmol) in methanol was added to the mixture. Five minutes later, cyclohexyl isocyanide (0.28 g, 2.6 mmol) was added to the mixture in an ice−water bath. The product precipitated as a white solid within several minutes, and after stirring overnight, the crude product was collected by filtration. All of the crude products were obtained in this way but purified by different procedures. Most of the title compounds were purified by washing and recrystallization in methanol, whereas compounds **ZX-3-8**, **ZX-3-14**, **ZX-3-17**, **ZX-3-18**, **ZX-4-5**, and **ZX-4-9** were purified by column chromatography on silica gel using ethyl acetate and petroleum ether (60−90 °C) with 1:2−1:4 (v/v) as an eluent (*21*−*23*). The yields and physical properties of the target compounds are listed in **Table 1**, and their proton nuclear magnetic resonance (^{1}H NMR) and HRMS data are listed in **Table 2**.

Biological Screening. Biological activities of the target compounds including fungicide and direct antivirus activities such as protection, inactivation, and curative effects and systemic acquired resistance were evaluated systematically according to the standard operation practice (SOP) as described in ref *5* and the following procedures.

Fungicide Screening. Preliminary screening was conducted by fungus growth inhibition method according to reference using potato dextrose agar (PDA) as cultivation medium (*5*). Fungi used in this studies included *Alternaria solani* (AS), *Cercospora arachidicola* (CA), *Cercospora beticola* (CB), *Colletotrichum lagenarium* (CL), *Fusarium oxysporum* (FO), *Gibberella zeae* (GZ), *Phyricularia grisea* (PG), *Phytophthora infestans* (Mont) de Bary (PI), *Physalospora piricola* (PP), *Pellicularia sasakii* (PS), and *Rhizoctonia solani* (RS).

Systemic Acquired Resistance Screening. Systemic acquired resistance of the target compounds was detected using tobacco against the tobacco mosaic virus (TMV) system as described in ref *5*. The induction activity was evaluated using the antivirus inhibition ratio, which was calculated by the average number of viral inflammations on the inoculated leaves with the corresponding control accordingly. TDL and Ribavirin were chosen as positive control and negative control, respectively, and all compounds were tested at concentrations of 100 and 50 μg/mL, respectively.

Protective Effects of the Target Compounds against TMV in Vivo. Healthy fresh tobacco plants at six-leaf stage were selected for the tests. The compound solution was smeared on the whole leaves, and then the leaves were dried in the greenhouse. After 12 h, TMV at a concentration of 5.88×10^{-2} μg/mL was inoculated on the upper three leaves using the conventional juice robbing method,

Table 3. Fungicide Activity Determination of Target Compounds[a]

compd	CB	FO	CA	AS	GZ	PP	PG	PS	CL	RS	PI
ZX-3-1	36	25	61	32	nd	22	40	17	9	63	32
ZX-3-2	27	0	48	26	nd	13	40	12	5	67	26
ZX-3-3	18	11	39	21	nd	26	20	12	9	65	21
ZX-3-4	0	nd	29	38	35	27	29	50	32	40	41
ZX-3-5	16	nd	43	38	46	30	14	33	47	49	35
ZX-3-6	27	21	39	21	nd	22	15	17	9	69	21
ZX-3-7	27	0	26	37	nd	26	25	12	5	67	35
ZX-3-8	0	nd	29	38	12	46	43	33	53	60	41
ZX-3-9	22	31	21	32	nd	31	24	30	20	20	20
ZX-3-10	0	nd	57	31	19	19	14	22	16	37	29
ZX-3-11	11	22	16	35	nd	41	24	22	32	29	0
ZX-3-12	0	nd	57	38	12	19	0	20	26	40	41
ZX-3-13	11	22	16	35	nd	38	18	25	24	29	12
ZX-3-14	0	nd	14	56	23	35	0	43	37	43	35
ZX-3-15	27	18	17	37	nd	35	10	24	14	63	35
ZX-3-16	27	18	35	42	nd	26	50	24	18	69	26
ZX-3-17	44	34	47	56	nd	48	29	22	44	43	24
ZX-3-18	33	38	53	50	nd	31	41	37	8	46	32
ZX-4-1	33	nd	21	0	45	0	14	0	14	28	5
ZX-4-2	0	nd	29	19	23	31	14	22	26	37	47
ZX-4-3	13	nd	14	25	27	8	29	28	16	34	29
ZX-4-4	0	nd	0	38	12	23	43	22	37	43	24
ZX-4-5	0	nd	29	38	0	31	43	33	42	49	41
ZX-4-6	17	nd	21	0	38	0	0	0	5	42	5
ZX-4-7	0	nd	43	44	8	19	14	17	26	40	29
ZX-4-8	75	nd	29	44	15	23	29	11	47	37	53
ZX-4-9	0	nd	71	100	62	85	14	74	95	97	88
ZX-4-10	50	nd	57	0	24	0	14	6	5	35	0
ZX-4-11	67	nd	21	0	41	0	0	0	5	37	5
ZX-4-12	33	nd	57	0	28	32	14	45	10	30	11
ZX-4-13	17	nd	14	0	34	0	0	0	0	40	16
ZX-4-14	67	nd	79	44	34	53	57	16	43	51	47
ZX-4-15	33	nd	21	0	34	0	0	0	0	28	0
ZX-4-16	17	nd	14	0	28	0	29	0	0	33	5
ZX-4-17	17	nd	36	0	24	0	14	14	5	40	0

[a] AS, *Alternaria solani*; CA, *Cercospora arachidicola*; CB, *Cercospora beticola*; CL, *Colletotrichum lagenarium*; FO, *Fusarium oxysporum*; GZ, *Gibberella zeae*; PG, *Phyricularia grisea*; PI, *Phytophthora infestans* (Mont) de Bary; PP, *Physalospora piricola*; PS, *Pellicularia sasakii* (Shirai); PT, *Puccinia triticina* Eriks; RS, *Rhizoctonia solani* Kuhn; nd, not detected.

and the solvent was smeared on the lower three leaves as a control. The local lesion numbers were then recorded 2−3 days after inoculation. For each compound, three repetitions were conducted. All compounds were tested at concentrations of 500 and 100 μg/mL, respectively.

Inactivation Effect of the Target Compounds against TMV in Vivo. Healthy fresh tobacco plants at six-leaf stage were selected for the tests. The TMV virus at a concentration of 5.88×10^{-2} μg/mL was inhibited by mixing with the target compound solution at the same volume for 30 min. Then the mixture was inoculated on the upper three leaves using the conventional juice robbing method, and the solvent was smeared on the lower three leaves as a control. The local lesion numbers were then recorded 2−3 days after inoculation. For each compound, three repetitions were conducted. All compounds were tested at concentrations of 500 and 100 μg/mL, respectively.

Curative Effect of Target Compounds on TMV in Vivo. Healthy fresh tobacco plants at six-leaf stage were selected for the tests. TMV at a concentration of 5.88×10^{-2} μg/mL was inoculated on the whole leaves using the conventional juice robbing method. After the leaves were dried in the greenhouse, the compound solution was smeared on the upper three leaves, and the solvent was smeared on the lower three leaves as control. The local lesion numbers were then recorded 2−3 days after inoculation. For each compound, three repetitions were conducted. All compounds were tested at concentrations of 500 and 100 μg/mL, respectively.

The activities of protection, inactivation, and curative effects against TMV were calculated by the average number of 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 (protection, inactivation, and curative effects in vivo) (%), CK is the average number of viral inflammations on the control leaves in vivo, and A is the average number of viral inflammations on the target compound treated leaves in vivo.

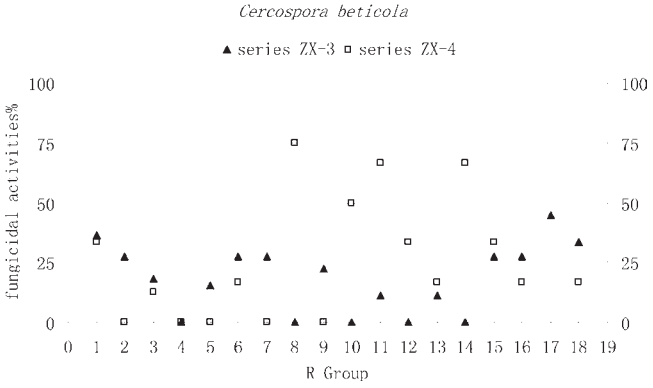


Figure 1. Parallel activity contrasts between series **ZX-3** and series **ZX-4** against CB. The fungicidal activities were tested at 50 μg/mL. The numbers on the *x*-axis represent the R group in **Scheme 1**: 1 = phenyl; 2 = 2-chlorophenyl; 3 = 3-chlorophenyl; 4 = 4-chlorophenyl; 5 = 2-fluorophenyl; 6 = 3-fluorophenyl; 7 = 4-fluorophenyl; 8 = 2-(trifluoromethyl)phenyl; 9 = 3-(trifluoromethyl)phenyl; 10 = 4-(trifluoromethyl)phenyl; 11 = 2-nitrophenyl; 12 = 3-nitrophenyl; 13 = 4-nitrophenyl; 14 = 2-methylphenyl; 15 = 3-methylphenyl; 16 = 4-methylphenyl; 17 = 3-hydroxyphenyl; 18 = 4-hydroxyphenyl.

RESULTS AND DISCUSSION

Preparation of the Novel 4-Methyl-1,2,3-thiadiazole-5-carboxyamide Derivatives via U-4CR. Optimization of a lead structure can be conducted via diverse methods. This study used U-4CR as the synthesis method via parallel synthesis, and the target compounds were proved to be easily synthesized and purified. In U-4CR, the order of adding the reactants is very important; usually, isocyanides in acidic medium are unstable, so it is necessary to add isocyanides at the last step. Moreover, it is well-known that the preformation of the imine favors the reaction (*21*, *23*). For these reasons, our experiments mixed an amine component and an aldehyde component first to get an intermediate imine, and then a carbonyl component was added in the mixture for certain time intervals before an isocyanide component was added. Because the Ugi reaction is favored by high concentrations of the reactants, the amount of methanol added to the reaction was as small as possible, just to make the agitation effective. After all components were mixed, products usually precipitated in solvent within 1 h. However, some products containing strong polar group such as nitro or hydroxy were not easily precipitated at room temperature; that process took several days, or precipitation occurred only after removal of most of the solvent. Our studies did not optimize the reaction conditions, and the mother liquor was not recycled; therefore, some yields of title compounds were low. All structures of the title compounds synthesized were confirmed by ^{1}H NMR and HRMS.

Biological Activity. *Fungicidal Activity*. Eleven fungi representing typical fungi often occurring in the Chinese agro-ecosystem, including AS, CA, CB, CL, FO, GZ, PG, PI, PP, PS, and RS, were chosen for fungicide screening, and the results are shown in **Table 3**. All compounds showed a certain degree of fungicidal

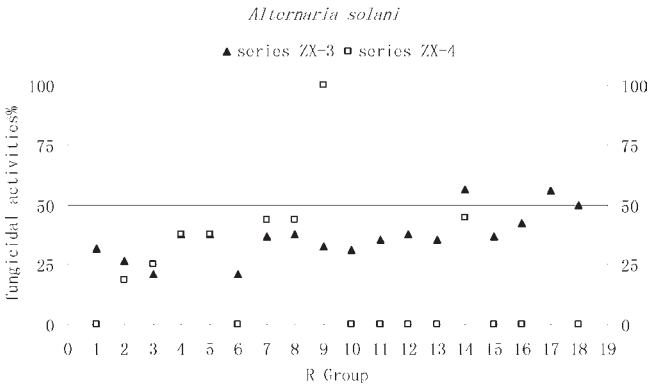


Figure 2. Parallel activity contrasts between series **ZX-3** and series **ZX-4** against AS. The fungicidal activities were tested at 50 μg/mL. The numbers on the *x*-axis represent the R group in **Scheme 1**: 1 = phenyl; 2 = 2-chlorophenyl; 3 = 3-chlorophenyl; 4 = 4-chlorophenyl; 5 = 2-fluorophenyl; 6 = 3-fluorophenyl; 7 = 4-fluorophenyl; 8 = 2-(trifluoromethyl)phenyl; 9 = 3-(trifluoromethyl)phenyl; 10 = 4-(trifluoromethyl)phenyl; 11 = 2-nitrophenyl; 12 = 3-nitrophenyl; 13 = 4-nitrophenyl; 14 = 2-methylphenyl; 15 = 3-methylphenyl; 16 = 4-methylphenyl; 17 = 3-hydroxyphenyl; 18 = 4-hydroxyphenyl.

activity at 50 μg/mL; the results indicate that compounds **ZX-4-8**, **ZX-4-10**, **ZX-4-11**, and **ZX-4-14** exhibited 75, 50, 67, and 67% fungicidal activity against CB, respectively; compounds **ZX-3-1**, **ZX-3-10**, **ZX-3-12**, **ZX-3-18**, **ZX-4-9**, **ZX-4-10**, **ZX-4-12**, and **ZX-4-14** showed 61, 57, 57, 52.63, 71, 57, 57, and 79% fungicidal activities against CA, respectively; compounds **ZX-3-18**, **ZX-3-14**, **ZX-3-17**, and **ZX-4-9** showed 50, 56, 56, and 100% fungicidal activities against AS, respectively; compound **ZX-4-9** showed 62% fungicidal activity against GZ; compounds **ZX-4-9** and ZX-4-14 showed 85 and 53% fungicidal activities against PP, respectively; compounds **ZX-3-16** and **ZX-4-14** showed 50 and 57% fungicidal activities against PG, respectively; compounds **ZX-3-4** and **ZX-4-9** showed 50 and 74% fungicidal activities against PS, respectively; compounds **ZX-3-8** and **ZX-4-9** showed 63 and 95% fungicidal activities against CL, respectively; compounds **ZX-3-1**, ZX-3-2, **ZX-3-3**, **ZX-3-6**, **ZX-3-7**, **ZX-3-8**, **ZX-3-15**, **ZX-3-16**, ZX-4-9, and **ZX-4-14** exhibited 63, 67, 65, 69, 67, 60, 63, 69, 97, and 51% fungicidal activities against RS, respectively; and compounds **ZX-4-8** and **ZX-4-9** showed 53 and 88% fungicidal activities against PI, respectively. As the results described above, most of the target compounds showed a very narrow spectrum against the fungi tested; however, two of them showed broad-spectrum activities against several fungi. For example, compound ZX-4-9 had good activities against AS (100%), CA (71%), CL (95%), GZ (62%), PI (88%), PP (85%), PS (74%), and RS (97%), and **ZX-4-14** also had good fungicide activities against CA (79%), CB (67%), PG (57%), PP (53%), and RS (51%).

As mentioned above, the differences between two series of target compounds were that they contained different moieties of 3-chloro-4-methylphenyl and 3-fluoro-4-methylphenyl, respectively. According to the results, the structure−activity relationship via parallel activity contrasting between the two series of compounds against fungi at the same concentration (50 μg/mL) can be evaluated. Most of the contrasting results between the chlorine and fluorine in the two series had almost equal fungicidal activities as a whole. However, series **ZX-4** showed better activity against CB but worse activity against AS than series **ZX-3** as a whole (**Figures 1** and **2**). Moreover, when the R group was 2-substituted with strong electron-withdrawing groups in the benzene ring such as trifluoro and nitro groups, the fungicidal activities were usually raised. This coincided with our former studies that strongly electroattractive groups favored the fungicide activity of 1,2,3-thiadiazole-containing molecules (*15*)

Table 4. Inhibition Activities against TMV in Vitro

	concentration			concentration	
compd	500 μg/mL	100 μg/mL	compd	500 μg/mL	100 μg/mL
ZX-3-1	24 ± 4	19	**ZX-4-1**	19 ± 1	19 ± 5
ZX-3-2	20 ± 2	19 ± 3	**ZX-4-2**	23 ± 2	21 ± 4
ZX-3-3	29 ± 2	21 ± 2	**ZX-4-3**	39 ± 7	15 ± 2
ZX-3-4	29 ± 4	38 ± 8	**ZX-4-4**	30 ± 1	28 ± 1
ZX-3-5	24 ± 3	18	**ZX-4-5**	23 ± 4	22 ± 1
ZX-3-6	18 ± 7	9 ± 3	**ZX-4-6**	29 ± 3	9 ± 1
ZX-3-7	16 ± 1	15 ± 3	**ZX-4-7**	36 ± 5	40 ± 15
ZX-3-8	38 ± 2	18 ± 5	**ZX-4-8**	48 ± 5	33
ZX-3-9	19 ± 4	22 ± 5	**ZX-4-9**	67 ± 5	29 ± 9
ZX-3-10	34 ± 1	11 ± 1	**ZX-4-10**	25 ± 3	10 ± 3
ZX-3-11	47 ± 2	15 ± 7	**ZX-4-11**	29 ± 9	17 ± 3
ZX-3-12	9	11 ± 2	**ZX-4-12**	58 ± 1	44 ± 2
ZX-3-13	32	26 ± 2	**ZX-4-13**	39	26 ± 2
ZX-3-14	42 ± 5	38 ± 11	**ZX-4-14**	52 ± 2	23 ± 5
ZX-3-15	19	11 ± 3	**ZX-4-15**	29 ± 2	18
ZX-3-16	40 ± 5	25 ± 13	**ZX-4-16**	36 ± 5	52 ± 8
ZX-3-17	35 ± 4	24 ± 3	**ZX-4-17**	70 ± 4	20 ± 4
ZX-3-18	17 ± 1	14 ± 2			
Tiadinil	51 ± 4	37 ± 5	Ribavirin	38 ± 5	28 ± 2

Anti-TMV Activity and Systemic Acquired Resistance Activity. TDL and Ribavirin were used as two positive controls. The results of activity screening by half leaf juicy method against TMV indicated that (**Table 4**) most compounds showed a certain degree of direct inhibition activities against TMV. The activity of any of the compounds including **ZX-4-8**, **ZX-4-9**, **ZX-4-12**, **ZX-4-14**, and **ZX-4-17** was equal to or higher than that of TDL at 500 μg/mL. Compounds **ZX-3-4**, **ZX-3-14**, **ZX-4-7**, **ZX-4-8**, **ZX-4-12**, and **ZX-4-16** had good activities of 38, 38, 40, 33, 44, and 52%, respectively, equal to or higher than that of TDL (37%) at 100 μg/mL. Moreover, compounds **ZX-3-8**, **ZX-3-11**, **ZX-3-14**, **ZX-3-16**, **ZX-4-3**, **ZX-4-8**, **ZX-4-9**, **ZX-4-12**, **ZX-4-13**, **ZX-4-14**, and **ZX-4-17** had inhibition activities of 38, 47, 42, 40, 48, 67, 58, 38, 52, and 70%, respectively, which was equal to or higher than that of Ribavirin (38%) at 500 μg/mL, and compounds **ZX-3-4**, ZX-3-14, **ZX-4-4**, **ZX-4-7**, **ZX-4-8**, **ZX-4-9**, **ZX-4-12**, **ZX-4-13**, and **ZX-4-16** had activities of 38, 38, 28, 40, 33, 29, 26, 44, and 52%, respectively, which was equal to or higher than that of Ribavirin (28%) at 100 μg/mL. All of these results indicated that more compounds belonging to series **ZX-4** had better activities than members of series **ZX-3**; the replacement of Cl by F might improve their direct inhibition activities against TMV in vitro.

On the basis of the results of screening in vitro, some compounds with good direct anti-TMV activity in vitro were chosen for further evaluation of protection, inactivation, and curative effect and induction activities against TMV in vivo (**Table 5**). To study the structure−activity relationship conveniently, seven compounds with various phenyl moieties from one series including **ZX-4-1**, **ZX-4-8**, **ZX-4-9**, **ZX-4-12**, **ZX-4-13**, **ZX-4-14**, and **ZX-4-17** were chosen for comparative studies against TMV in vivo. As shown in **Table 5**, all seven compounds had a certain degree of activity against TMV in vivo. It was noteworthy that compounds containing phenyl, trifluoromethylphenyl, and hydroxyphenyl such as **ZX-4-1**, **ZX-4-8**, and **ZX-4-17** had equal protection effects with corresponding control at 100 and 500 μg/mL. Moreover, compounds **ZX-4-1**, **ZX-4-9**, **ZX-4-12**, **ZX-4-13**, **ZX-4-14**, and **ZX-4-17** had curative effects equal to or higher than controls at 500 and 100 μg/mL, respectively; that is to say, trifluoromethyl-, nitro-, and hydroxyl-substituted phenyls were beneficial to the curative effect. Among these compounds,

Table 5. Antivirus in Vivo and Systemic Acquired Resistance Activity of the Title Compounds

	protective effect (%)		curative effect (%)		inactivation effect (%)		induction effect (%)	
compd	500 μg/mL	100 μg/mL	500 μg/mL	100 μg/mL	500 μg/mL	100 μg/mL	100 μg/mL	50 μg/mL
ZX-4-1	49	41	81	47	29	11	54	69
ZX-4-8	51	49	30	13	39	26	45	57
ZX-4-9	54	19	42	28	31	21	38	68
ZX-4-12	55	27	56	39	62	48	68	50
ZX-4-13	38	18	59	36	57	48	68	36
ZX-4-14	35	23	47	18	60	27	58	63
ZX-4-17	55	40	43	34	68	65	65	44
Tiadinil	48	35	44	12	70	70	49	47
Ribavirin	44	40	46	14	71	57	10	40

compound **ZX-4-1** stood out, with curative effects of 81 and 47% at 100 and 500 μg/mL, respectively. As for activity evaluation of the inactivation effect, only compound **ZX-4-17** had an equal effect with control at 500 and 100 μg/mL, respectively. The evaluation of systemic acquired resistance can be described by the induction activity. All seven compounds had induction activities equal to or higher than control agent TDL at 500 and 100 μg/mL, respectively.

Our studies on the synthesis of 4-methyl-1,2,3-thiadiazole derivatives and biological evaluation indicated that the combination of bioactive substructures via Ugi reaction was an effective way to find potential bioactive compounds.

ABBREVIATIONS USED

AS, *Alternaria solani*; BTH, acibenzolar-*S*-methyl; CA, *Cercospora arachidicola*; CB, *Cercospora beticola*; CL, *Colletotrichum lagenarium*; DMSO-d_6, deutero-, dimethyl sulfoxide; FO, *Fusarium oxysporum*; GZ, *Gibberella zeae*; ^{1}H NMR, hydrogen nuclear magnetic resonance; HRMS, high-resolution mass spectrometry; nd, not detected; PDA, potato dextrose agar; PG, *Phyricularia grisea*; PI, *Phytophthora infestans* (Mont) de Bary; PP, *Physalospora piricola*; PS, *Pellicularia sasakii*; RS, *Rhizoctonia solani*; SOP, standard operation practice; TDL, Tiadinil; TMS, tetramethylsilane; TMV, tobacco mosaic virus; U-4CR, Ugi four-component condensation reaction.

NOTE ADDED AFTER ASAP PUBLICATION

There was an error in the page numbers of reference 22 in the version of this paper published ASAP December 14, 2009; the corrected version published ASAP February 3, 2010.

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