DOI: 10.1002/cjoc.201200268

Synthesis, Bioactivities and Structure Activity Relationship of *N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N*′-phenyl Ureas

Guo, Dandan[a](国丹丹) Wang, Zhiwen[a](王治文) Fan, Zhijin*,[a](范志金)
Zhao, Hui[a](赵晖) Zhang, Wei[b](张薇) Cheng, Jiagao[b](程家高) Yang, Jiaqiang[c](杨家强)
Wu, Qingjun[c](吴青君) Zhang, Youjun[c](张友军) Fan, Qian[c](范谦)

[a] *State Key Laboratory of Elemento-Organic Chemistry*, *Nankai University*, *Tianjin 300071*, *China*
[b] *Shanghai Key Laboratory of Chemical Biology*, *School of Pharmacy*, *East China University of Science and Technology*, *Shanghai 200237*, *China*
[c] *Institute of Vegetables and Flowers*, *Chinese Academy of Agricultural Sciences*, *Beijing 100081*, *China*
[d] *Sichuan Functional Heterocyclic Compounds Engineering Technology Research Center*, *Mianyang*, *Sichuan 621000*, *China*

Twenty nine novel *N*-4-methyl-1,2,3-thiadiazole-5-carbonyl-*N*′-phenyl ureas were designed and synthesized, and their structures were confirmed by proton nuclear magnetic resonance (^{1}H NMR), infra red spectroscopy (IR) and high-resolution mass spectroscopy (HRMS). Compounds **V-9**, **V-11**, **V-12**, **V-15**, **V-19**, **V-21**, **V-22** and **V-24** exhibit excellent activity against *Culex pipiens pallens*. Compounds **V-12** and **V-22** present good insecticidal activity against *Plutella xylostella* L. Their median lethal concentrations (LC_{50}) are 164.15 and 89.69 mg•L^{-1}, respectively. Compound **V-11** also has potential wide spectrum of fungicide activity. Its median effective concentrations (EC_{50}) detected from 3.82 μg•mL^{-1} against *Physalospora piricola* to 31.60 μg•mL^{-1} against *Cercospora arachidicola*. Compounds **V-15** and **V-24** show outstanding induction activities as same as positive controls TDL and ningnanmycin, furthermore **V-24** has the highest induction activity of 41.85%±4.43%. To elucidate the structure activity relationship in these compounds, a 3D-QSAR model has been built. The established model showed a reliable predicting ability with q^2 values of 0.643 and r^2 values of 0.982.

Keywords 4-methyl-1,2,3-thiadiazole derivatives, benzoylurea pesticides, insecticidal activity, 3D QSAR model

Introduction

Most of viral diseases of plants are transmitted through the infection of insects.[1] Insect-plant disease associations, which connect insect, pathogen and plant inseparably, lead to greater risk of economic loss.[2] This requires designation of compounds which can not only control pests but also enhance the defense level of plants. Therefore, these compounds can protect plants from the injury caused by pests, succeeding fungi and virus simultaneously, which means they can show insecticidal activities as well as systemic acquired disease resistance. Systemic acquired resistance (SAR) refers to a distinct signal transduction pathway that plays an important role in the ability of plants to defend themselves against pathogens.[3]

Derivatives of 1,2,3-thiadiazole have versatile biological activities such as anti-Human Immunodeficiency Virus (HIV),[4] antitumor,[5] antibacterial,[6] and antiallergic[7] activities. One of the most significant properties is that SAR can be induced by some 1,2,3-thiadiazole derivatives.[8] Tiadinil (TDL, *N*-(3-chloro-4-methylphenyl)-4-methyl-1,2,3-thiadiazole-5-carboxamide) has been commercialized as an important elicitor for SAR, and has been applied in rice field.[9] Its metabolite 4-methyl-1,2,3-thiadiazole-5-carboxylic acid shows a good SAR-inducing activity as well.[10] Considering insecticidal activity of these compounds, we focused our studies on the environment benign benzoylureas pesticides (BUPs) lead. During the last three decades, BUPs played an important role in agricultural pest control with its unique mode of action on a target site which is absent in plants and mammals,[11] therefore, their insecticidal activity was highly selective, and non-toxic to ecological environment. In order to design compounds with both insecticidal activity and SAR, 1,2,3-thiadiazole group has been introduced into the lead of BUPs.

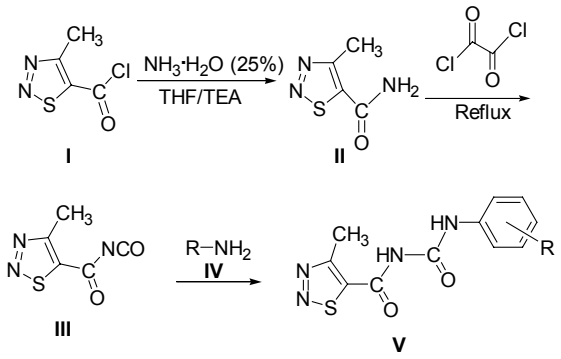
A 3D-QSAR model has been established by CoMFA so as to interpret the relationship between their structures and activities. Comparative molecular field analysis (CoMFA) is one of the most popular QSAR methods,

* E-mail: fanzj@nankai.edu.cn; Tel.: 0086-022-23499464
Received March 19, 2012; accepted September 4, 2012.
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cjoc. 201200268 or from the author.

CHINESE JOURNAL OF CHEMISTRY

which use interactive graphics and statistical techniques for correlating biological properties. In CoMFA, the bioactivities of molecules are correlated with steric and electrostatic potential energies in terms of Lennare-Jones and Coulombic potentials, respectively. The performance of the standard CoMFA procedure requires the specification of both conformation and alignments of molecules.[12] The synthetic routes were shown in Scheme 1. All the structures of the target compounds have been confirmed by ^{1}H NMR, and high-resolution mass spectroscopy. Their bioactivities have been evaluated systemically.

Scheme 1 General synthetic route of the title compounds **V-1**—**V-29**



R=4-nitro, 2-nitro, 3-nitro, 2,4-dinitro, 2-chloro, 4-chloro, 3-chloro, 2,5-dichloro, 3,5-dichloro, 3,4-dichloro, 2,4,5-trichloro, 2,4-difluoro-3,5-dichloro, 4-bromo, 2-fluoro, 2-fluoro-4-hydroxyl, 3-fluoro-4-methyl, 2-methyl-4-chloro, 3-chloro-4-methyl, 2-trifluoromethyl-5-chloro, 2-carboxy, 4-trifluoromethyl, 2-chloro-3,5-ditrifluoromethyl, 4-trifluoromethoxyl, 2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxyl), 2,4-dimethyl, 4-ethyl, 4-carbonyl, 2-hydroxyl, 3,5-dichloro-4-hydroxyl

Experimental

Equipments and materials

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 NMR spectra were obtained using a Bruker AVANCE-400 MHZ spectrometer and chemical shift values (δ) were reported with deutero-dimethyl sulfoxide (DMSO-d_6) as solvent and tetramethylsilane (TMS) as the internal standard. High resolution mass spectrometry (HRMS) data were obtained on a Varian 7.0T FTICR-MS instrument. IR was recorded on a Bruker Vector 22 FTIR spectrometer using a KBr pellet press. All solvents were analytical reagent grade and were dried in advance and distilled before use. Column chromatography purification was carried out using silica gel.

General synthesis

4-Methyl-1,2,3-thiadiazole-5-carboxylic acid was synthesized according to the reference [13].

Synthesis of 4-methyl-1,2,3-thiadiazole-5-carboxylic acid chloride (Compound I)

A mixture of 4-methyl-1,2,3-thiadiazole-5-carboxylic acid (10 g 0.067 mol) in thionyl dichloride (25 mL) was refluxed at 80 ℃ for 6 h. After cooled to room temperature, the thionyl dichloride was removed under reduced pressure. The 4-methyl-1,2,3-thiadiazole-5-carboxylic acid chloride was obtained with the yield of 85% as a light-yellowish oil and used without purification.

Synthesis of 4-methyl-1,2,3-thiadiazole-5-formamide (Compound II)

The mixture of aqueous solution of ammonia (25%, 30 mL), triethylamine (5 mL) and THF (50 mL) was cooled with an ice-water bath, 4-methyl-1,2,3-thiadiazole-5-carboxylic acid chloride (10 g 0.062 mol) was slowly added drop-wise to the cooled mixture. Afterwards, the mixture was stirred in an ice-water bath for another 1 h, and then the mixture was stirred at room temperature overnight. After that, the mixture was poured into the separating funnel, organic layer was collected and the aqueous phase was extracted by THF (15 mL×3). The combined organic layers were washed with saturated brine, and then dried over anhydrous sodium sulfate. After filtration, the organic layer was concentrated under reduced pressure to give a white solid 4-methyl-1,2,3-thiadiazole-5-carboxamide with a yield of 90%, which was used without purification.

Synthesis of 4-methyl-1,2,3-thiadiazole-5-carbonyl isocyanate (Compound III)

A mixture of 4-methyl-1,2,3-thiadiazole-5-carboxamide (2 g 0.014 mol) and oxalyl chloride (3.55 g 0.028 mol) in 1,2-dichloroethane (20 mL) was stirred at room temperature for 30 min, subsequently, the mixture was heated at 80 ℃ for 10 h. After cooling to room temperature, the mixture was concentrated under reduced pressure to give a brown oil as 4-methyl-1,2,3-thiadiazole-5-carbonyl isocyanate with a yield of 80% for further reaction without purification.

Synthesis of target compound *N*-4-methyl-1,2,3-thiadiazole-5-carbonyl-*N*′-phenyl ureas V (1—29)

A solution of 4-methyl-1,2,3-thiadiazole-5-carbonyl isocyanate (0.59 g 3.5 mmol) in 1,2-dichloroethane (5 mL) was added drop-wise to a solution of aromatic amine (3.5 mmol) in 1,2-dichloroethane (20 mL) at room temperature, and the mixture was allowed to stand for 8 h. Then part of the solvent was removed under reduced pressure, and the precipitated solid was filtered to give a solid as crude product. After recrystallization from DMF/ethanol, a pure solid was obtained.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N*′-(4-nitrophenyl) urea (V-1)** White solid, yield 58%, m.p. 178—180 ℃; ^{1}HNMR (DMSO-d_6, 400 MHz) δ: 2.89 (s, 3H, CH_3), 7.92 (d, J=12.4 Hz, 2H, Ar-H), 8.32 (d, J=12.4 Hz, 2H, Ar-H), 10.71 (s, 1H, NH), 11.68 (s, 1H,

NH); IR (KBr) *v*: 3350, 3131, 2945, 1705, 1598, 1513 cm^{-1}; HRMS calcd for $C_{11}H_9N_5O_4S$ ($M^+ + Na$): 330.0267, found 330.0268.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N'*-(2-nitrophenyl) urea (V-2)** White solid, yield, 39%, m.p. 158—160 ℃; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.84 (s, 3H, CH_3), 7.41 (t, J=15.6 Hz, 1H, Ar-H), 7.80 (t, J=15.6 Hz, 1H, Ar-H), 8.18 (d, J=8.0 Hz, 1H, Ar-H), 8.44 (d, J=8.4 Hz, 1H, Ar-H), 11.77 (s, 1H, NH), 11.84 (s, 1H, NH); IR (KBr) *v*: 3556, 3413, 3127, 1697, 1551, 1492 cm^{-1}; HRMS calcd for $C_{11}H_9N_5O_4S$ (M^-−H): 306.0302, found 306.0304.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N'*-(3-nitrophenyl) urea (V-3)** White solid, yield 65%, m.p. 171—173 ℃; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.84 (s, 3H, CH_3), 7.66(t, J=8.2 Hz, 1H, Ar-H), 7.96 (m, 2H, Ar-H), 8.64 (s, 1H, Ar-H), 10.54 (s, 1H, NH), 11.60 (s, 1H, NH); IR (KBr) *v*: 3261, 3104, 2961, 1699, 1597, 1545 cm^{-1}; HRMS calcd for $C_{11}H_9N_5O_4S$ (M^-−H): 306.0302, found 306.0296.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N'*-(2,4-dinitrophenyl) urea (V-4)** Yellow solid, yield 81%, m.p. 164—166 ℃; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.84 (s, 3H, CH_3), 8.61 (d, J=9.2 Hz, 1H, Ar-H), 8.79 (d, J=9.2 Hz, 1H, Ar-H), 8.90 (s, 1H, Ar-H), 12.02 (s, 1H, NH), 12.30 (s, 1H, NH); IR (KBr) *v*: 3138, 3077, 2975, 1698, 1599, 1494 cm^{-1}; HRMS calcd for $C_{11}H_8N_6O_6S$ (M^-−H) 351.0153, found 351.0157.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N'*-(2-chlorophenyl) urea (V-5)** White solid, yield 70%, m.p. 171—173 ℃; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.88 (s, 3H, CH_3), 7.23 (t, J=7.6 Hz, 1H, Ar-H), 7.45 (t, J=8.0 Hz, 1H, Ar-H), 7.62 (d, J=8.0 Hz, 1H, Ar-H), 8.33 (d, J=8.4 Hz, Ar-H), 10.90 (s, 1H, NH), 11.79 (s, 1H, NH); IR (KBr) *v*: 3230, 3126, 2952, 1699, 1589, 767 cm^{-1}; HRMS calcd for $C_{11}H_9ClN_4O_2S$ (M^-−H): 295.0062, found 295.0054.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N'*-(4-chlorophenyl) urea (V-6)** White solid, yield 73%, m.p. 173—175 ℃; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.83 (s, 3H, CH_3), 7.42 (d, J=8.0 Hz, 1H, Ar-H), 7.62 (d, J=7.6 Hz, 1H, Ar-H), 10.28 (s, 1H, NH), 11.48 (s, 1H, NH); IR (KBr) *v*: 3220, 3123, 2946, 1699, 1594, 757 cm^{-1}; HRMS calcd for $C_{11}H_9ClN_4O_2S$ (M^-−H): 295.0062, found 295.0054.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N'*-(3-chlorophenyl) urea (V-7)** White solid, yield 72%, m.p. 166—168 ℃; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.83 (s, 3H, CH_3), 7.19 (d, J=8.0 Hz, 1H, Ar-H), 7.43 (m, 2H, Ar-H), 7.81 (s, 1H, Ar-H), 10.32 (s, 1H, NH), 11.52 (s, 1H, NH); IR (KBr) *v*: 3391, 3128, 1702, 1595, 753 cm^{-1}; HRMS calcd for $C_{11}H_9ClN_4O_2S$ (M^-−H): 295.0062, found 295.0056.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N'*-(2,5-dichlorophenyl) urea (V-8)** White solid, yield 26%, m.p. 186—190 ℃; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.82 (s, 3H, CH_3), 7.24—7.25 (m, 1H, Ar-H), 7.62 (d, J=8.6 Hz, 1H, Ar-H), 8.37 (d, J=2.4 Hz, 1H, Ar-H), 10.97 (s, 1H, NH), 11.86 (s, 1H, NH); IR (KBr) *v*: 3221, 3124, 1709, 1592, 814 cm^{-1}; HRMS calcd for $C_{11}H_8Cl_2N_4O_2S$ (M^-−H): 328.9672, found 328.9684.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N'*-(3,5-dichlorophenyl) urea (V-9)** White solid, yield 72%, m.p. 194—197 ℃; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.82 (s, 3H, CH_3), 7.34 (s, 1H, Ar-H), 7.71 (s, 2H, Ar-H), 10.43 (s, 1H, NH), 11.60 (s, 1H, NH); IR (KBr) *v*: 3219, 3124, 1703, 1590, 757 cm^{-1}; HRMS calcd for $C_{11}H_8Cl_2N_4O_2S$ (M^-−H): 328.9672, found 328.9680.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N'*-(3,4-dichlorophenyl) urea (V-10)** White solid, yield 84%, m.p. 155—157 ℃; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.82 (s, 3H, CH_3), 7.54 (d, J=8.8 Hz, Ar-H), 7.64 (d, J=8.8 Hz, 2H, Ar-H), 7.98 (s, 1H, Ar, 1H -H), 10.41 (s, 1H, NH), 11.56 (s, 1H, NH); IR (KBr) *v*: 3223, 3128, 1696, 1583, 759 cm^{-1}; HRMS calcd for $C_{11}H_8Cl_2N_4O_2S$ (M^-−H): 328.9672, found328.9666.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N'*-(2,4,5-trichlorophenyl) urea (V-11)** White solid, yield 23%, m.p. 122—124 ℃; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.88 (s, 3H, CH_3), 8.05 (s, 1H, Ar-H), 8.59 (s, 1H, Ar-H), 11.03 (s, 1H, NH), 11.98 (s, 1H, NH); IR (KBr) *v*: 3220, 3131, 1692, 1574, 689 cm^{-1}; HRMS calcd for $C_{11}H_7Cl_3N_4O_2S$ (M^-−H): 362.9283, found 362.9286.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N'*-(2,4-difluoro-3,5-dichlorophenyl) urea (V-12)** White solid, yield 92%, m.p. 187—189 ℃; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.82 (s, 3H, CH_3), 8.25 (s, 1H, Ar-H), 10.56 (s, 1H, NH), 11.92 (s, 1H, NH); IR (KBr) *v*: 3232, 3129, 1696, 1603, 1232, 757 cm^{-1}; HRMS calcd for $C_{11}H_6Cl_2F_2N_4O_2S$ (M^-−H): 364.9484, found 364.9480.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N'*-(4-bromophenyl) urea (V-13)** White solid, yield 78%, m.p. 195—197 ℃; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.82 (s, 3H, CH_3), 7.55—7.56 (m, 4H, Ar-H), 10.29 (s, 1H, NH), 11.49 (s, 1H, NH); IR (KBr) *v*: 3216, 3117, 2943, 1590, 1679, 500 cm^{-1}; HRMS calcd for $C_{11}H_9BrN_4O_2S$ (M^-−H): 338.9557, found 338.9551.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N'*-(2-fluorophenyl) urea (V-14)** White solid, yield 86%, m.p. 229—231 ℃; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.84 (s, 3H, CH_3), 7.73 (s, 2H, Ar-H), 7.92 (d, 2H, J=7.2 Hz, Ar-H), 11.58 (s, 1H, NH), 11.90 (s, 1H, NH); IR (KBr) *v*: 3206, 3120, 2947, 1716, 1620, 1229 cm^{-1}; HRMS calcd for $C_{11}H_9FN_4O_2S$ (M^-−H): 279.0357, found 279.0353.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N'*-(2-fluoro-4-hydroxylphenyl) urea (V-15)** Light yellow solid, yield 83%, m.p. 234—236 ℃; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.81 (s, 3H, CH_3), 6.72—6.62 (m, 2H, Ar-H), 7.74 (s, 1H, Ar-H), 9.88 (s, 1H, OH), 10.13 (s, 1H, NH), 11.56 (s, 1H, NH); IR (KBr) *v*: 3212, 3120, 1701, 1613, 1236, 1204 cm^{-1}; HRMS calcd for $C_{11}H_9FN_4O_3S$ (M^-−H): 295.0307, found 295.0303.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N'*-(3-fluoro-4-methylphenyl) urea (V-16)** White solid,

yield 85%, m.p. 191—195 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.82 (s, 3H, CH_3), 7.23—7.26 (m, 2H, Ar-H), 7.50—7.53 (m, 1H, Ar-H), 10.28 (s, 1H, NH), 11.48 (s, 1H, NH); IR (KBr) v: 3222, 3103, 2952, 1704, 1602, 1229, 1674, 1415, 1311 cm^{-1}; HRMS calcd for $C_{12}H_{11}FN_4O_2S$ (M^-—H): 293.0514, found 293.0515.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N′*-(2-methyl-4-chlorophenyl) urea (V-17)** White solid, yield 43%, m.p. 194—196 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.20 (s, 3H, Ar-CH_3), 2.83 (s, 1H, CH_3), 7.29 (d, J=8.7 Hz, 1H, Ar-H), 7.38 (s, 1H, Ar-H), 7.96 (d, J=8.9 Hz, 1H, Ar-H), 10.23 (s, 1H, NH), 11.65 (s, 1H, NH); IR (KBr) v: 3221, 3130, 2937, 1699, 1586, 757 cm^{-1}; HRMS calcd for $C_{12}H_{11}ClN_4O_2S$ (M^-—H): 309.0218, found 309.0215.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N′*-(3-chloro-4-methylphenyl) urea (V-18)** White solid, yield 70%, m.p. 166—168 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.29 (s, 3H, CH_3), 2.82 (s, 3H, CH_3), 7.31—7.38 (m, 2H, Ar-H), 7.79 (s, 1H, Ar-H), 10.26 (s, 1H, NH), 11.51 (s, 1H, NH); IR (KBr) v: 3209, 3118, 2951, 1713, 1603, 752 cm^{-1}; HRMS calcd for $C_{12}H_{11}ClN_4O_2S$ (M^-—H): 309.0218, found 309.0217.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N′*-(2-trifluoromethyl-5-chlorophenyl) urea (V-19)** White solid, yield 50%, m.p. 190—192 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.83 (s, 3H, CH_3), 7.54 (d, J=8.3 Hz, 1H, Ar-H), 7.84 (d, J=8.2 Hz, 1H, Ar-H), 8.66 (s, 1H, Ar-H), 11.09 (s, 1H, NH), 11.92 (s, 1H, NH); IR (KBr) v: 3202, 3137, 2940, 1708, 1607 cm^{-1}; HRMS calcd for $C_{12}H_8ClF_3N_4O_2S$ (M^-—H): 362.9936, found 362.9941.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N*'-(2-carboxyphenyl) urea (V-20)** White solid, yield 91%, m.p. 197—199 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.82 (s, 3H, CH_3), 7.25—7.19 (m, 1H, Ar-H), 7.67—7.59 (m, 1H, Ar-H), 7.98 (d, J=8.0 Hz, 1H, Ar-H), 8.46 (d, J=8.0 Hz, 1H, Ar-H), 11.48 (s, 1H, NH), 12.16 (s, 1H, NH); IR (KBr) v: 3222, 3125, 1681, 1584 cm^{-1}; HRMS calcd for $C_{12}H_{10}N_4O_4S$ (M^-—H): 305.0350, found 305.0349.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N*'-(4-trifluoromethylphenyl) urea (V-21)** White solid, yield 92%, m.p. 168—170 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.83 (s, 3H, CH_3), 7.73 (s, 2H, Ar-H), 7.79 (s, 2H, Ar-H), 10.52 (s, 1H, NH), 11.58 (s, 1H, NH); IR (KBr) v: 3227, 3132, 2944, 1680, 1606, 1318 cm^{-1}; HRMS calcd for $C_{12}H_9F_3N_4O_2S$ (M^-—H): 329.0326, found 329.0326.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N′*-(2-chloro-3,5-ditrifluoromethylphenyl) urea (V-22)** White solid, yield 70%, m.p. 162—164 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.84 (s, 3H, CH_3), 7.95 (d, J=1.6 Hz, 2H, Ar-H), 8.93 (d, J=1.6 Hz, 2H, Ar-H), 11.26 (s, 1H, NH), 12.00 (s, 1H, NH); IR (KBr) v: 3245, 3142, 3113, 1709, 1602, 1223, 759 cm^{-1}; HRMS calcd for $C_{13}H_7ClF_6N_4O_2S$ (M^-—H): 430.9810, found 430.9816.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N′*-(4-trifluoromethoxylphenyl) urea (V-23)**: White solid, yield 75%, m.p. 144—146 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.83 (s, 3H, CH_3), 7.38 (d, J=8.4 Hz, 2H, Ar-H), 7.71 (d, J=8.4 Hz, 2H, Ar-H), 10.33 (s, 1H, NH), 11.50 (s, 1H, NH); IR (KBr) v: 3230, 3124, 2952, 1715, 1611, 1280, 1221 cm^{-1}; HRMS calcd for $C_{12}H_9F_3N_4O_3S$ (M^-—H): 345.0275, found 345.0270.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N′*-[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxyl)phenyl] urea (V-24)** White solid, yield 49%, m.p. 155—157 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.83 (s, 3H, CH_3), 6.61—6.45 (m, 1H, CHF), 7.80 (s, 1H, Ar-H), 8.55 (s, 1H, Ar-H), 11.00 (s, 1H, NH), 11.92 (s, 1H, NH); IR (KBr) v: 3422, 1701, 1588, 1222, 1211, 760 cm^{-1}; HRMS calcd for $C_{14}H_8Cl_2F_6N_4O_3S$ (M^-—H): 494.9526, found 494.9526.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N′*-(2,4-dimethylphenyl) urea (V-25)** White solid, yield 57%, m.p. 176—178 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.26 (s, 6H, CH_3), 2.83 (s, 3H, CH_3), 7.03 (d, J=8.4 Hz, 1H, Ar-H), 7.08 (s, 1H, Ar-H), 7.78 (d, J=8.4 Hz, 1H, Ar-H), 10.14 (s, 1H, NH), 11.54 (s, 1H, NH); IR (KBr) v: 3218, 3131, 2935, 1700, 1557 cm^{-1}; HRMS calcd for $C_{13}H_{14}N_4O_2S$ (M^-—H): 289.0765, found 289.0768.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N′*-(4-ethylphenyl) urea (V-26)** White solid, yield 68%, m.p. 169—161 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.18 (t, J=14.8 Hz, 3H, CH_3), 2.59 (q, J=6.8, 7.6 Hz, 2H, CH_2), 2.83 (s, 3H, CH_3), 7.20 (d, J=7.6 Hz, 2H, Ar-H), 7.47 (d, J=8.0 Hz, 2H, Ar-H), 10.18 (s, 1H, NH), 11.41 (s, 1H, NH); IR (KBr) v: 3224, 3111, 2965, 1711, 1599 cm^{-1}; HRMS calcd for $C_{13}H_{14}N_4O_2S$ (M^-—H): 289.0765, found 289.0763.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N′*-(4-formylphenyl) urea (V-27)** Red solid, yield 95%, m.p. over 240 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.83 (s, 3H, CH_3), 7.80 (d, J=8.4 Hz, 2H, Ar-H), 7.92 (d, J=8.8 Hz, 2H, Ar-H), 9.92 (s, 1H, CHO), 10.57 (s, 1H, NH), 11.58 (s, 1H, NH); IR (KBr) v: 3222, 3114, 1709, 1688, 1547 cm^{-1}; HRMS calcd for $C_{12}H_{10}N_4O_3S$ (M^-—H): 289.0401, found 289.0395.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N′*-(2-hydroxylphenyl) urea (V-28)** White solid, yield 93%, m.p. 186—188 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.88 (s, 3H, CH_3), 6.99—6.83 (m, 3H, Ar-H), 8.16 (d, J=8.0 Hz, 1H, Ar-H), 10.27 (s, 1H, OH), 10.67 (s, 1H, NH), 11.54 (s, 1H, NH); IR (KBr) v: 3278, 3131, 1688, 1547, 1212 cm^{-1}; HRMS calcd for $C_{11}H_{10}N_4O_3S$ (M^-—H): 277.0401, found 277.0397.

***N*-4-Methyl-1,2,3-thiadiazole-5-carbonyl-*N′*-(3,5-dichloro-4-hydroxylphenyl) urea (V-29)** White solid, yield 77%, m.p. 237—239 ℃; ^{1}HNMR (DMSO-d_6, 400 MHz) δ: 2.82 (s, 3H, CH_3), 7.64 (s, 2H, Ar-H), 10.00 (s, 1H, OH), 10.16 (s, 1H, NH), 11.50 (s, 1H, NH); IR (KBr) v: 3234, 1695, 1551, 1215, 754 cm^{-1}; HRMS calcd for $C_{11}H_8Cl_2N_4O_3S$ (M^-—H): 344.9621, found 344.9615.

Biological assay

Biological activities of the target compounds including fungicide and insecticidal activities were evaluated systematically according to the standard operation practice (SOP) as described in Ref. [8] and the following procedures.

Larvicidal activity against *C. pipiens pallens*

Each compound of 2 mg was weighed into a penicillin bottle; 10 mL of acetone was added to dissolve the compound to prepare 200 μg/mL of mother solution. The working solution of 5 μg/mL was prepared by diluting 1 mL of mother solution with 29 mL of water and 10 mL of feeding solution into a 100 mL beaker. The working solutions of 2 or 1 μg/mL were prepared by diluting the solution of 5 μg/mL successively. Ten 4th instar larvae of *C. pipiens pallens* were transferred into the beaker by double distilled water. Thereafter, the beakers with mosquito larvae were put into the standard conditioned rooms for further cultivation with temperature of 25 ℃ and humidity of 80%. After 24 h or 96 h of cultivation, the mortalities of the larvae were calculated relative to the corresponding CK (control check), which was prepared by 1 mL of acetone for substituting 1 mL of mother solution, the observation was conducted until all the larvae metamorphosized into pupation or died. Chlorfluazuron was used as a positive control.

Toxicity against *P. xylostella*

The toxicities of the target compounds against *P. xylostella* were tested by the leaf dip method using the reported procedure.[14] Fresh cabbage leaves were dipped into the 200 μg/mL test water solution for 10 s which was prepared with a 5% of acetone to help the compounds to dissolve. After air-drying for evaporating off the acetone and water, the treated leaves were cut into small pieces and placed in the Petri dishes with 9 cm diameter. Ten individuals of 2nd instars larvae of *P. xylostella* L. were then transferred into the 10 cm diameter of Petri dish. The Petri dishes were finally fastened with rubbers and placed in the standard cultivation room for 96 or 120 h at 25 ℃ with 80% of humidity. The percentage of mortalities was evaluated relative to the corresponding CK which uses water only. The insects which had no reaction when touched by brush pen were regarded as dead insect.

Fungicide screening

Preliminary screening was conducted by fungus growth inhibition method according to Ref. [8]. Fungi used in this studies included *Alternaria solani* (AS), *Botrytis cinerea* (BC), *Cercospora. arachidicola* (CA), *Gibberella zeae* (GZ), *Phytophthora infestans (Mont) de Bary* (PI), *Physalospora piricola* (PP), *Pellicularia-sasakii (Shirai)* (PS), *Sclerotinia sclerotiorum* (SS), and *Rhizoctonia cerealis* (RC). Precision toxicity studies were conducted according to the above methods by determination of growth inhibition of specific fungi using the same compound by five to seven different concentrations; the median effective concentration (EC_{50}) was calculated by the linear regression of logarithm of the concentration with probability of the corresponding growth inhibition by Excel.[15]

Systemic acquired resistance screening

Protective effects of the target compounds against tobacco mosaic virus (TMV) *in vivo* The healthy fresh tobacco leaves growing at 6 leaves age were selected for the tests. The compound solution was smeared on the whole leaves, and then the leaves were dried in the green house. After 12 h, the TMV at a concentration of 5.88×10^{-2} μg/mL 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 tested were conducted at concentrations 100 μg/mL respectively. For the inactivation effect of the target compounds against TMV *in vivo*, the TMV virus with 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. For the curative effect of target compounds on TMV *in vivo*, 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 green house, the compound solution was smeared on the upper three leaves, and the solvent was smeared on the lower three leaves as control. Systemic acquired resistance of the target compounds was detected using tobacco against TMV system as described in reference.[8,16] After 7 days of induction of the target compounds to tobacco plant, TMV at a concentration of 5.88×10^{-2} μg/mL was inoculated on the newly grown leaves using the conventional juice robbing method. 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 accordingly. TDL and ningnanmycin were chosen as positive control and negative control respectively, and all compounds tested were conducted at concentrations of 100 μg/mL respectively. The activity of protection, inactivation, curative effects and induction activity against TMV were calculated by the average number of the viral inflammations on the inoculated leaves with the corresponding control according to Equation 1:

$$Y=\frac{\mathrm{CK}-A}{\mathrm{CK}}\times100 \quad (1)$$

where Y is the antivirus inhibition ratio (protection, inactivation, curative effects and induction activity *in vivo*) (%), CK is the average number of viral inflammations

CHINESE JOURNAL OF CHEMISTRY

on the control leaves *in vivo*, *A* is the average number of viral inflammations on the target compound treated leaves *in vivo*.

Extraction and activity determination of phenylalanine ammonialyase (PAL)

The PAL extraction and assay protocols were conducted according to the description of Zhang *et al.*[17] 5 g of tobacco leaves were cut and homogenized in 10 mL of 25 mmol•L^{-1} of boric acid buffer solutions (pH＝8.0 containing 5 mmol•L^{-1} of mercaptoethanol and 1 mmol•L^{-1} of EDTA) with 0.5 g of PVP and Quartz sand washed and clinched (purity more than 99.8%). The homogenate was filtered through 8 layers of cheesecloth and centrifuged at 10000 g for 15 min at 0—4 ℃, the supernatant fluid was used as crude enzyme of PAL for immediate specific activity determination. Adding 1 mL of PAL crude enzyme and 2 mL of double distilled water to 1 mL solution of 20 mmol•L^{-1} of phenylalanine, the mixture was then heated to (30±1) ℃ for 30 min in the dark, after cooling to room temperature by water, the absorbance (OD) of the mixture was measured at 290 nm. One enzyme unit (U) was calculated by increasing of 0.01 of OD value, specific activity was calculated according to the corresponding content of proteins and was demonstrated as U•[(mg of protein)$^{-1}$•min^{-1}]. Protein had been measured by the method of Coomassie blue according to the description of Bradford *et al.*[18]

3D-QSAR models

In this study, a 3D-QSAR analysis was performed by using CoMFA method within SYBYL 7.0 (Tripos, St. Louis, MO, USA) molecular modeling software. Total 17 compounds including **V-2**, **V-4**, **V-5**, **V-6**, **V-7**, **V-8**, **V-10**, **V-13**, **V-14**, **V-16**, **V-17**, **V-18**, **V-20**, **V-23**, **V-27**, **V-28** and **V-29** were chosen for CoMFA analysis, which showed insecticidal activities against *Culex pipiens pallens* and their activities were within 100%. The activity data were transformed and expressed in terms of *A* by the formula $A=\lg\{a/[100-a]M_w\}$, where *a* is the percentage of inhibition and M_w is the molecular weight of the tested compounds.[12,19,20] The compounds for CoMFA studies were listed in Table 1. Each structure was optimized by molecular mechanics method based on the TRIPOS force field and Gasteiger and Hückel charges. The CoMFA steric and electrostatic interaction fields were calculated at each lattice intersection on a regularly spaced grid of 2.0 Å. The grid pattern was generated automatically by the SYBYL/CoMFA routine, and an sp^3 carbon atom with a van der Waals radius of 1.52 Å and $a+1.0$ charge was used as the probe to calculate the steric (Lennard-Jones 6—12 potential) field energies and electrostatic (Coulombic potential) fields with a distance-dependent dielectric at each lattice point. Values of the steric and electrostatic fields were truncated at ±30.0 kcal/mol. In our study, with standard options for scaling of variables, a standard Leave-One-Out (LOO) cross-validation was finally carried out with the optimal number of components 4 (noncross-validated conventional analysis), then models with the highest r^2 and lowest PRESS was obtained.[12,19,20]

Results and Discussion

Synthesis

As shown in Scheme 1, 4-methyl-1,2,3-thiadiazole-5-carboxylic acid chloride was prepared from 4-methyl-1,2,3-thiadiazole-5-carboxylic acid. Then 4-methyl-1,2,3-thiadiazole-5-carboxylic acid chloride was reacted with ammonia to provide 4-methyl-1,2,3-thiadiazole-5-carboxamide. In the next step, 4-methyl-1,2,3-thiadiazole-5-carbonyl isocyanate was synthesized by the reaction of 4-methyl-1,2,3-thiadia-zole-5-carboxamide and oxalyl chloride with good yield. Finally, the target compounds **V-1—V-29** were produced by the combination of 4-methyl-1,2,3-thiadiazole 5-carbonyl isocyanate and various kinds of aromatic amine successively. Our group found some interesting phenomenon during these processes. First, controlling of temperature of oxalyl chloride and 4-methyl-1,2,3-thiadiazole-5-carboxamide during the refluxing was critical, when the temperature exceeded 120 ℃, 4-methyl-1,2,3-thia-

Table 1 Activities [*A*] of molecules used for 3D-QSAR models

No	V-no	Activity/%	lg *A*			No	V-no	Activity/%	lg *A*		
			Exp	Pre CoMFA	Res				Exp	Pre CoMFA	Res
1	**V-2**	30	−2.86	−2.86	0.00	10	**V-16**	60	−2.29	−2.41	0.12
2	**V-4**	40	−2.72	−2.70	−0.02	11	**V-17**	60	−2.32	−2.31	0.01
3	**V-5**	30	−2.84	−2.85	0.01	12	**V-18**	70	−2.12	−1.99	−0.13
4	**V-6**	40	−2.65	−2.65	0.00	13	**V-20**	20	−3.10	−3.09	0.01
5	**V-7**	30	−2.84	−2.91	0.07	14	**V-23**	10	−3.49	−3.49	0.00
6	**V-8**	20	−3.12	−3.15	0.03	15	**V-27**	20	−3.07	−3.05	−0.02
7	**V-10**	60	−2.34	−2.39	0.05	16	**V-28**	10	−3.40	−3.33	−0.07
8	**V-13**	40	−2.71	−2.75	0.04	17	**V-29**	20	−3.14	−3.11	−003
9	**V-14**	10	−3.40	−3.37	−0.02						

diazole-5-carbonyl isocyanate could be formed in much lower yield. Because the carbonyl isocyanate is unstable, it was important to control the temperature of the reaction and use fresh material.[21] Second, we observed that the reaction occurred as expected even when the aromatic ring contained a hydroxyl group, as in **V-15**, **V-20**, **V-28** and **V-29**. Third, it was found that the reaction would not proceed at or below room temperature when the aromatic ring contained a nitro group, as in **V-1** to **V-4**, but proceeded to completion by refluxing in 1,2-dicholroethane.

Biological activity

Insecticidal Activity against *Culex pipiens pallens*

Different types of substitution in aromatic amine moiety were chosen for structure-activity relationship studies. The results of insecticidal activities of target compounds against *C. pipiens pallens* are listed in Tables 2 and 3. Preliminary screenings were tested at the concentration of 5 μg•mL^{-1} for insecticidal activity against *C. pipiens pallens*. As the results show, the compounds **V-9**, **V-11**, **V-12**, **V-15**, **V-19**, **V-21**, **V-22** and **V-24** exhibited excellent activity, which suggests that introducing halogen atoms and CF_3, OCF_2CHFCF_3, OCF_2CHF_2 groups into the target molecules had improved the insecticidal activity against *C. pipiens pallens*. At a lower test concentration, the mortality of *C. pipiens pallens* also decreased accordingly, with the best active compound, **V-24**, giving 50% control at 0.1 μg•mL^{-1}. None of the test compounds reached the activity of the commercial

Table 2 Insecticidal activities of the compounds **V-1**—**V-29**

Compd.	R	*Plutella xylostella* L.		*Culex pipiens pallens*	
		Concentration/(μg•mL^{-1})	Insecticidal activity/%	Concentration/(μg•mL^{-1})	Insecticidal activity/%
V-1	4-nitro	200	0	5	0
V-2	2-nitro	200	0	5	30
V-3	3-nitro	400	10.3	5	40
V-4	2,4-dinitro	200	0	5	40
V-5	2-chloro	400	5.0	5	30
V-6	4-chloro	400	7.7	5	40
V-7	3-chloro	400	38.5	5	30
V-8	2,5-dichloro	200	0	5	20
V-9	3,5-dichloro	200	5.0	5	100
V-10	3,4-dichloro	400	7.7	5	60
V-11	2,4,5-trichloro	200	3.5	5	100
V-12	2,4-difluoro-3,5-dichloro	400	97.5	5	100
V-13	4-bromo	200	0	5	40
V-14	2-fluoro	400	2.6	5	10
V-15	2-fluoro -4-hydroxyl	400	5.0	5	100
V-16	3-fluoro -4-methyl	200	5.0	5	60
V-17	2-methyl-4-chloro	200	2.5	5	60
V-18	3-chloro-4-methyl	200	0	5	70
V-19	2-trifluoromethyl-5-chloro	400	10.3	5	100
V-20	2-carboxy	400	5.0	5	20
V-21	4-trifluoromethyl	400	27.5	5	100
V-22	2-chloro-3,5-ditrifluoromethyl	400	100	5	100
V-23	4-trifluoromethoxyl	400	59.4	5	10
V-24	2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxyl)	400	75	5	100
V-25	2,4-dimethyl	400	10.3	5	20
V-26	4-ethyl	400	17.9	5	20
V-27	4-formyl	400	12.8	5	20
V-28	2-hydroxyl	400	15	5	10
V-29	3,5-dichloro-4-hydroxyl	400	19.0	5	20
Positive control	Chlorfluazuron	200 400	72.4 100	5	100

CHINESE JOURNAL OF CHEMISTRY

Table 3 Insecticidal active against *Culex pipiens pallens* at lower concentration of compounds **V-9**, **V-11**, **V-12**, **V-15**, **V-19**, **V-22** and **V-24** (Those compounds exhibit 100% at 5 μg•mL^{-1})

Compd.	Concentration/(μg•mL^{-1})	Death rate/%
V-9	2	40
V-11	2	100
	1	40
V-12	2	100
	1	40
V-15	2	60
V-19	2	50
V-22	1	100
	0.5	20
V-24	0.25	100
	0.1	50
Chlorfluazuron	2	100
	1	100
	0.5	100
	0.25	100
	0.1	100
	0.05	100
	0.025	100

standard, chlorfluazuron, which gave 100% control at 0.025 μg•mL^{-1} (Table 3).

Insecticidal activity against *Plutella xylostella*

The results of insecticidal against larval *P. xylostella* were listed in Table 2. We have tested some of these compounds at 200 μg•mL^{-1} at first, however, the insecticidal activities against *Plutella xylostella* of these compounds are unfavorable and hard to compare with each other. Thereafter, we tested the rest of the compounds at 400 μg•mL^{-1}. On the basis of these results, to examine the electronic effect of substitute on the phenyl ring, the electron-donating substitutes CH_3, C_2H_5 and electron-withdrawing substitutes Cl, F, Br, CF_3, CHO, OCF_3, NO_2 were introduced into the target compounds. The bioassay results demonstrated that, compounds with CF_3, OCF_3 presented optimum insecticidal activity against *P. xylostella*, while the rest of compounds with electron-withdrawing substitutes showed lower insecticidal activity than those with electron-donating substitutes. Further results of compounds **V-1**, **V-2**, **V-3** and **V-5**, **V-6**, **V-7** indicated that, the introduction of Cl and NO_2 into *meta*-position of the aromatic ring had acceptable biological activity, however, *para*-substituted and *ortho*-substituted derivatives showed no insecticidal activity. Moreover, as can be seen from Scheme 1 and Table 2, **V-12**, **V-22** and **V-24** had more than 2 substitutes in the phenyl ring; they were the most active compounds with insecticidal activity over 75% against *P. xylostella* at 400 μg•mL^{-1}. Further insecticidal precision toxicological determination results of compounds **V-12** and **V-22** were shown in Table 4 with the LC_{50} value of 164.15 and 89.69 μg•mL^{-1}, respectively. Therefore, substitutes on the phenyl ring played a vital role of the insecticidal activity of the target compounds against *P. xylostella*.

Table 4 Precision toxicological determination of compounds **V-12** and **V-22** against *Plutella xylostella L.* (96 h, %)

Compd.	LC_{50} (95% confidence limits)/(μg•mL^{-1})	Slope±SD	Chi-square
V-12	164.15 (127.10—211.99)	2.94±0.48	7.23
V-22	89.69 (52.50—153.24)	3.95±0.86	0.16
Chlorfluazuron	1.38 (0.14—13.43)	0.76±0.24	0.19

Fungicide activity

Though the main structure of target compounds was the derivatives of a insecticide lead, derivatives of *N*-4-methyl-1,2,3-thiadiazole-5-carbonyl-*N*′-aromatic ureas also showed excellent fungicide activity. The fungicide activities of the title compounds against the representative typical fungi often occurring in the Chinese agro-ecosystem such as AS, BC, CA, GZ, PI, PP, PS, SS and RC detected at 50 μg•mL^{-1} were shown in Table 5. The results indicated that some compounds had good fungicide activity, compounds **V-2**, **V-16** inhibited more than 60% of the growth against AS; compounds **V-1**, **V-2**, **V-9**, **V-11**, **V-13**, **V-16**, **V-22** and **V-25** inhibited more than 50% of the growth to CA; compounds **V-9**, **V-11**, **V-13**, **V-16** and **V-22** inhibited more than 50% of the growth against GZ; compounds **V-2**, **V-11**, **V-13**, **V-14**, **V-22** and **V-25** inhibited more than 50% of the growth against PP; compounds **V-2**, **V-4, V-11**, **V-16**, **V-20**, **V-23**, **V-25**, and **V-28** inhibited more than 50% of the growth against BC; compounds **V-7**, **V-11**, **V-13**, **V-14**, **V-17**, **V-19**, **V-20**, **V-22**, **V-24**, **V-27** and **V-28** inhibited more than 50% of the growth against RC; compounds **V-1**, **V-11** and **V-20** inhibited more than 50% of the growth against PS; compounds **V-9**, **V-11**, **V-13**, **V-25**, **V-28** and **V-29** inhibited more than 70% of the growth against SS; compounds **V-13** inhibited more than 80% of the growth against PI. Compound **V-11** was a broad-spectrum of fungicide activity against all of the fungi tested with growth inhibition from 50% to 100%. Precision toxicity of compound **V-11** was studied further, the EC_{50} values were detected as from 3.82 μg•mL^{-1} against *Physalospora piricola* to 31.60 μg•mL^{-1} against *Cercospora arachidicola*, and these were equal to 14.31 μmol•L^{-1} against *P. piricola* to 86.43 μmol•L^{-1} against *C. arachidicola* among the four tested fungi (Table 6). These results indicated that compound **V-11** was a fungicide lead with good selectivity and deserved for further modification. Halogen-substitution in aromatic derivatives improved the fungicide activity of the title compounds.

Table 5 The antifungal activities of compounds **V-1**—**V-29** *in vitro* at 50 μg•mL^{-1} [a]

Compd.	AS	CA	GZ	PP	BC	RC	PS	SS	PI
V-1	33	77	26	42	27	37	61	32	14
V-2	61	62	33	54	65	48	48	0	14
V-3	0	14	0	32	10	37	0	0	28
V-4	10	38	0	0	56	11	0	14	16
V-5	0	7	0	32	10	27	3	0	24
V-6	0	14	9	29	29	45	44	29	16
V-7	0	21	0	11	19	53	0	50	12
V-8	28	31	13	8	23	31	21	0	14
V-9	39	62	54	4	15	21	33	100	0
V-10	15	26	13	35	23	45	0	3	4
V-11	50	77	72	83	100	96	90	100	9
V-12	49	23	13	10	24	33	2	60	18
V-13	33	54	97	100	23	94	30	100	82
V-14	23	31	27	50	47	57	39	50	14
V-15	8	14	9	8	16	25	4	57	3
V-16	67	62	54	42	58	46	34	32	9
V-17	20	12	38	34	33	70	27	34	7
V-18	10	44	0	0	22	0	0	14	13
V-19	26	7	16	29	41	50	23	30	17
V-20	17	17	22	31	50	65	67	57	14
V-21	17	27	41	27	6	47	21	6	8
V-22	17	50	68	67	29	76	43	57	11
V-23	0	7	0	45	5	33	28	0	16
V-24	10	26	19	30	42	50	5	10	7
V-25	5	57	0	77	59	49	14	73	24
V-26	0	14	0	29	0	37	12	0	16
V-27	10	16	0	42	50	55	33	35	15
V-28	35	37	6	45	54	55	11	71	11
V-29	10	26	3	45	38	40	5	81	22
Chlorfluazuron (Negative control)	5	7	0	4	11	22	3	14	8
Azoxystrobin (Positive control)	60	78	86	100	100	92	77	95	90

[a] AS, *Alternaria solani*; BC, *Botrytis cinerea*; CA, *Cercospora arachidicola*; GZ, *Gibberellazeae*; PI, *Phytophthora infestans* (*Mont*) *de Bary*; PP, *Physalospora piricola*; PS, *Pellicularia sasakii* (*Shirai*) ; SS, *Sclerotinia sclerotiorum*; RC, *Rhizoctonia cerealis*.

Systemic acquired resistance for tobacco against tobacco mosaic virus

The results of anti-TMV activity determination were listed in Table 7. Bioassay indicated that ningnanmycin presented good anti-TMV activity including all test modes, TDL only had good inactivation and induction activities, these results accorded with the reported results.[10,22] While, the negative control chlorfluazuron only had good inactivation activity and certain degree of protective activity which was lower than that of ningnanmycin, among the compounds with best insecticide activity, compound **V-24** had good half leaf activity as that of ningnanmycin did; the five compounds had lower protective activity, curative activity and inactivation activity, but three compounds including **V-15** and **V-24** had good induction activity as that of both TDL and ningnanmycin did, compound **V-24** had highest induction activity of 41.85%±4.43%. All these were validated by PAL activity determination, all these three compounds with good induction acidity improved their PAL activity as compared with TDL did. These results suggested that *N*-4-methyl-1,2,3-thiadiazole-5-carbonyl-*N*′-aromatic ureas possessed good insecticide activity and also showed good systemic acquired resistance, it is valuable for both disease control caused by fungi or virus and insects damage which can also spread the fungi invasion and virus attacking.

3D-QSAR model analysis

To obtain more information about relationship between structure and insecticidal activities against *Culex pipiens pallens*, we need to choose some compounds to do 3D-QSAR analysis. Because these 17 compounds (**V-2**, **V-4**, **V-5**, **V-6**, **V-7**, **V-8**, **V-10**, **V-13**, **V-14**, **V-16**, **V-17**, **V-18**, **V-20**, **V-23**, **V-27**, **V-28**, **V-29**) present a good statistical correlation of their data of predicted activities (red line) and experimental activities (black dots) in Figure 1, we choose them. Under Tripos force field, the best CoMFA model was achieved with optimal components 3, which showed a good q^2 (cross-validate r^2)=0.643, r^2=0.982 and F (variance ratio)=71.771. The proportion of steric and electrostatic contribution was 60.8% and 39.2% respectively. Furthermore, the CoMFA steric and electrostatic fields were plotted as 3D colored contour maps, which were helpful to identify important regions changing in steric and electrostatic fields. In order to explain the field contributions of different properties, *N*-4-methyl-1,2,3-thiadiazole-5-carbonyl-*N*′-[3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxyl)phenyl] urea whose value of insecticidal activity against *Culex pipiens pallens* is 100%, and crystal structure (CCDC 888301) has been obtained displays as a template molecule. The steric and electrostatic contour map from CoMFA analysis was presented in Figure 2. The yellow regions indicated that increasing the steric bulk would decrease the activity. The green regions illustrated that increasing the steric bulk might enhance the activity. Similarly, the red regions indicated where increasing electronegative substitutions could enhance the activity, while the blue regions showed where increasing electropositive substitutions might enhance the activity.

Figure 2 depicted a green region on the side of benzene ring, indicating that a larger bulky group in this region favored the activity of the compound. In contrast, a smaller steric group in the lower left yellow region might decrease activity of the compound. A blue region and a red region were shown on the two sides of ben-

CHINESE JOURNAL OF CHEMISTRY

Table 6 EC_{50} determination of compound **V-11** against four fungi

Fungus	Regression equation	R^2	EC_{50}/(μg•mL^{-1})	EC_{50}/(μg•mL^{-1})
Cercospora arachidicola	$y=0.7548x+3.8680$	0.9496	31.60	86.43
Physalospora piricola	$y=1.2042x+4.2986$	0.9374	3.82	14.31
Rhizoctonia cerealis	$y=1.8112x+3.1698$	0.9829	10.24	27.90
Pellicularia sasakii	$y=2.2147x+1.9125$	0.9481	24.78	67.83

Table 7 Activity against TMV of the target compounds[a]

Compd.	Conc./(μg•mL^{-1})	Half leaf±SD/%	Protection±SD/%	Curative±SD/%	Inactivation±SD/%	Induction±SD/%	PAL specific Activity/U•[(mg of protein)$^{-1}$•min^{-1}]
V-15	100	24.23±3.89	16.23±4.23	27.11±3.36	21.09±2.57	32.62±3.25	2.05
V-22	100	20.87±2.33	28.07±2.01	24.45±2.04	24.15±3.28	19.86±3.25	1.72
V-23	100	26.19±2.06	14.91±5.47	10.22±2.77	27.55±2.70	21.99±3.25	1.89
V-24	100	32.95±3.61	26.75±4.02	20.00±2.67	24.83±3.28	41.85±4.43	2.73
Chlorfluazuron	100	15.15±2.63	38.16±2.63	11.11±2.04	40.48±3.59	23.40±2.13	1.10
TDL	100	15.95±2.01	14.04±3.31	17.78±2.04	45.24±4.82	38.30±2.13	2.21
Ningnanmycin	100	33.50±4.08	48.41±3.77	46.99±2.11	41.32±1.94	39.80±7.42	ND
CK	—	—	—	—	—	—	1.95

[a] ND: not detected; positive control: TDL and Ningnanmycin; negative control: Chlorfluazuron

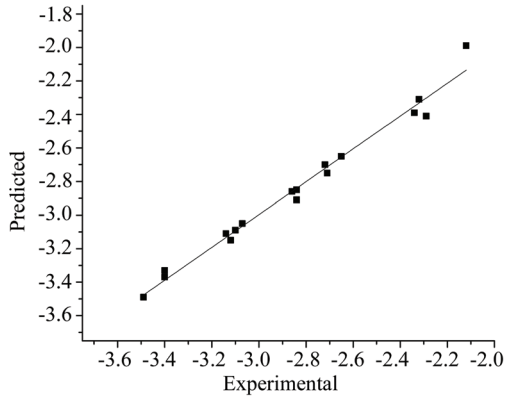


Figure 1 Experimental versus predicted [*A*] for CoMFA model.

zene ring, respectively. The red region of benzene indicated increasing the electronegative substitutions might promote the activity. For example, compounds **V-16** and **V-18**, containing 3-F and 3-Cl substitution respectively, presented an increasing activity in turn. As a result, we designed compounds **V-19**, **V-22** and **V-24**. We use Cl, CF_3 *etc*. to increase the electronegativity of the benzene red region, and use bigger substitutions in the green region, like **V-24**. As can be seen in Table 2, these three compounds exhibit excellent insecticidal activity against *Culex pipiens pallens* as we predicted based on CoMFA analysis.

Conclusions

In summary, 29 novel *N*-4-methyl-1,2,3-thiadiazole-5-carbonyl-*N'*-phenyl ureas were designed and synthesized. Target compounds exhibit insecticidal activities against *C. pipiens pallens* and *P. xylostella* L. Bioassays indicated compounds **V-24** and **V-22** exhibit out-

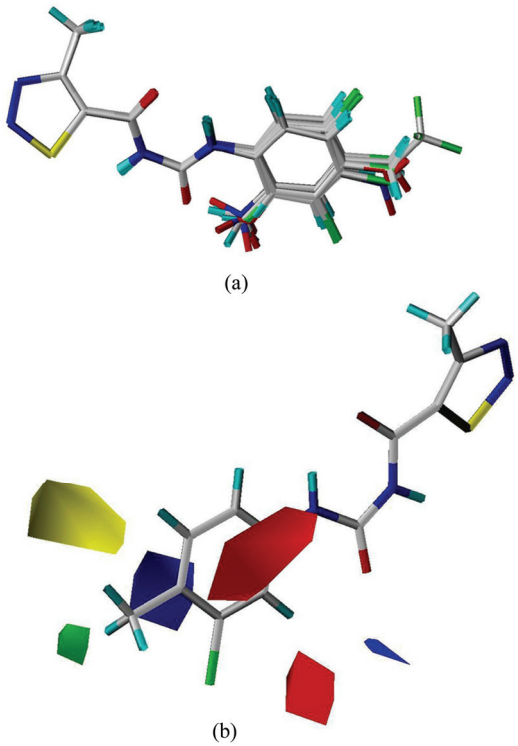


Figure 2 (a) Alignment of the 17 compounds; (b) contour maps for CoMFA model in steric and electrostatic fields. The sterically favored areas are in green; sterically unflavored areas are in yellow. Positive-charge favored areas are in blue; negative-charge favored areas are in red.

standing insecticidal activity against *Culex pipiens pallens* and *Plutella xylostella*, respectively. Compound **V-11** also had a broad-spectrum of fungicide against all of the fungi. Five compounds show good SAR-inducing activity as well. These compounds establish basis for both disease and insect control in agricultural practice and novel pesticide development. Structure-activity relationship studies reveal that, improvement of insecticidal activity requires a reasonable combination of substituent on the phenyl ring, and the type and position of substituent on the phenyl ring are critical. With the contour maps based on steric and electrostatic CoMFA coefficients, we can conclude the relationship between the structures and bioactivity and continue the further design of highly active compounds.

Abrreviations Used

DMF: *N*,*N*-dimethylformamide; DMSO-d_6: deuterodimethyl sulfoxide; EC_{50}: median effective concentrations; HIV: Human Immunodeficiency Virus; ^{1}H NMR: Hydrogen Nuclear Magnetic Resonance; HRMS: High-Resolution Mass Spectrometry; IR: infra red spectroscopy; LC_{50}: median lethal concentrations; PAL: phenylalanine ammonialyase; PXL: *Plutella xylostella* Linnaeu; SOP: standard operation practice; TDL: Tiadinil; TMS: tetramethylsilane; TMV: tobacco mosaic virus; CoMFA: comparative molecular field analysis.

Acknowledgement

This study was funded in part by grants from the National Natural Science Foundation of China (No. 20872071), the Tianjin Natural Science Foundation (No. 10JCZDJC17500), the National Key Project for Basic Research (No. 2010CB126105), the National Key Technology Research and Development Program (No. 2011BAE06B02) and the Foundation of Achievements Transformation and Spreading of Tianjin Agricultural Science and Technology (No. 201002250), Tianjin Key Technology Research and Development Program (No. 11ZCGYNC00100).

References

[1] Brunt, A. A.; Crabtree, K.; Dallwitz, M.; Gibbs, A.; Watson, L.; Zurcher, E. *Viruses of Plants*, CAB International, Cambridge, United Kingdom, **1996**.

[2] Gray, S. M.; Banerjee, N. *Microbiol. Mol. Biol. Reviews* **1999**, *63*, 128.

[3] Ryals, J. A.; Neuenschwander, U. H.; Willits, M. G.; Molina, A.; Stelner, H.; Hunt, M. D. *The Plant Cell* **1996**, *8*, 1809.

[4] Zhan, P.; Liu, X. Y.; Cao, Y.; Wang, Y.; Pannecouque, C.; Erik, D. C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5368.

[5] Wu, M. J.; Sun, Q. M.; Yang, C. H.; Chen, D. D.; Chen, Y. *Bioorg. Med. Chem. Lett*. **2007**, *17*, 869.

[6] Fan, Z. J.; Yang, Z. K.; Zhang, H. K.; Mi, N.; Wang, H.; Cai, F.; Zuo, X.; Zheng, Q. X.; Song, H. B. *J. Agric. Food Chem.* **2010**, *58*, 2630.

[7] Pawar, M. J.; Burungale, A. B.; Karale, B. K. *ARKIVOC* **2009**, (xiii), 97.

[8] Fan, Z. J.; Shi, Z. G.; Zhang, H. K.; Liu, X. F.; Bao, L. L.; Ma, L.; Zuo, X.; Zheng, Q. X.; Mi, N. *J. Agric. Food Chem.* **2009**, *57*, 4279.

[9] Michiko, Y.; Hideo, N.; Shigeo, Y. *J. Pestic. Sci.* **2004**, *29*, 46.

[10] Yasuda, M.; Kusajima, M.; Nakajima, M.; Akutsu, K.; Kudo, T.; Yoshida, S.; Nakashita, H. *J. Pestic. Sci*. **2006**, *31*, 329.

[11] Kramer, W.; Schirmer, U. *Modern Crop Protection Compounds*, Wiley-VCH, Hong Kong, **2007**, p. 819.

[12] Lv, Y.; Yin, C.; Liu, H.; Yi, Z.; Wang, Y. *J. Environ. Sci.-China* **2008**, *20*, 1433.

[13] Larsen, B. D.; Eggert, H.; Harrit, N.; Holm, A. *Acta Chem. Scand.* **1992**, *46*, 482.

[14] Wang, H.; Yang, Z. K.; Fan, Z. J.; Wu, Q. J.; Zhang, Y. J.; Mi, N.; Wang, S. X.; Zhang, Z. C.; Song, H. B.; Liu, F. *J. Agric. Food Chem.* **2011**, *59*, 628.

[15] Wang, Z. H.; Guo, Y. Z.; Zhang, J.; Ma, L.; Song, H. B.; Fan, Z. J. *J. Agric. Food Chem.* **2010**, *58*, 2715.

[16] Zuo, X.; Mi, N.; Fan, Z. J.; Zheng, Q. X.; Zhang, H. K.; Wang, H.; Yang, Z. K. *J. Agric. Food Chem.* **2010**, *58*, 2755.

[17] Zhang, Y. G.; Fan, Z. J.; Liu, X. F.; Jia, J. C.; Ma, L.; Zhong, Z. M. *Proceedings of 3rd International Symposium on Pesticide and Environmental Safety & 7th International Workshop on Crop Protection Chemistry and Regulatory Harmonization*, Editor-in-chief: Jiang Shuren and Racke, K. D., China Agricultural University Press, Beijing, **2007**, pp. 131—137.

[18] Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.

[19] Cui, Z. N.; Zhang, L.; Huang, J.; Ling, Y.; Yang, X. L. *Chin. J. Org. Chem.* **2010**, *30*, 1482.

[20] Liu, X. H.; Shi, Y. X.; Ma, Y.; Zhang, C. Y.; Dong, W. L.; Pan, L.; Wang, B. L.; Li, B. J.; Li, Z. M. *Eur. J. Med. Chem.* **2009**, *44*, 2782.

[21] Liu, Y. H.; Zhao, H. ; Li, G. P. *Isocyanate*, Chemical Industry Press, Beijing, **2004**, p. 92.

[22] Wang, S. X.; Mi, N.; Fan, Z. J.; Fu, Y. F.; Huang, Y.; Wang, H.; Zhang, Z. C.; Zhang, J. G.; Song, H. B.; Belskaya, N. P.; Bakulev, V. A. *Chin. J. Pestic. Sci.* **2010**, *12*, 255.

(Zhao, X.)

