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Synthesis and Biological Activity of Some Novel Trifluoromethyl-Substituted 1,2,4-Triazole and Bis(1,2,4-Triazole) Mannich Bases Containing Piperazine Rings

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A series of trifluoromethyl-substituted 1,2,4-triazole Mannich base **6** and bis(1,2,4-triazole) Mannich base **7** containing pyrimidinylpiperazine rings via the Mannich reaction were synthesized and characterized by infrared (IR), ^{1}H nuclear magnetic resonance (NMR), and elemental analysis. The fungicidal tests indicated that most of compounds **6** and **7** possessed excellent fungicidal activity. Among 19 novel compounds, some showed superiority over commercial fungicides Dimethomorph, Thiophanate-methyl, Iprodione, and Zhongshengmycin. Some compounds also exhibited favorable herbicidal activity in the preliminary studies. On the basis of the comparative molecular field analysis (CoMFA), five novel compounds were subsequently synthesized, their activities were estimated fairly accurately, and compounds **6-A1** and **7-A2** displayed good fungicidal activity against *Pseudoperonospora cubensis* (96.9 and 84.9%) as **6h** and **7c**, respectively.

KEYWORDS: 1,2,4-Triazole; pyrimidinylpiperazine; Mannich base; fungicidal activity; herbicidal activity

INTRODUCTION

The application of agrochemicals to protect vegetable and cereal crops is an established part of conventional agriculture. This has provided healthy crops and increased yields as well as economic benefits for over many years. The main purpose of the research for agrochemicals is to develop novel active compounds with lower application doses and high selectivity and that are environmentally friendly (*1*, *2*). Compounds containing a 1,2,4-triazole ring are often associated with various useful pesticidal activity (*3*−*6*). In the early 1970s, the azole fungicides were synthesized and used for the protection of various crops. Since the discovery of these fungicides, a variety of other 1,2,4-triazole compounds have been discovered (*7*−*9*). The introduction of these 1,2,4-triazole sterol biosynthesis inhibitors represented significant progress in the chemical control of fungal diseases. This class of pesticides includes several excellent systemic fungicides with long, protective, and curative activity against a broad spectrum of foliar, root, and seedling diseases caused by many ascomycetes, basidiomycetes, and imperfect fungi (*10*, *11*). On the other hand, some structures with a 1,2,4-triazole ring also exhibited outstanding herbicidal activity. For example, 3-amino-1,2,4-triazole was introduced as a selective herbicide and has been used successfully in the control of several woody species during the mid-1950s (*12*). Also, in the early 1990s, 3-trifluoromethyl-4-aryl-1,2,4-triazole-5(4*H*)-thiones were reported to possess good herbicidal activity (*13*). Furthermore, bioactive compounds possess a trifluoromethyl moiety that might be expected to enhance their biological activity (*14*−*17*), which is often considered an important point by researchers for designing various biological molecules. All of these results encouraged us to synthesize some other novel compounds containing such structural moiety.

In our previous work, we reported some interesting Mannich bases derived from 1,2,4-triazole Schiff base (*18*) and benzotriazoles containing a pyrimidinylpiperzine ring, which are associated with various useful biological activities (*19*). As a continuation of our work, a series of novel Mannich base and bis-Mannich base with trifluoromethyl-1,2,4-triazole and pyrimidinylpiperazine moiety were synthesized and their fungicidal and herbicidal activities were investigated in this paper.

MATERIALS AND METHODS

Instruments and Materials. The melting points were determined on a X-4 binocular microscope melting point apparatus (Beijing Tech Instrument Co., Beijing, China) and were uncorrected. Infrared spectra were recorded on a Nicolet MAGNA-560 spectrophotometer as KBr tablets. ^{1}H nuclear magnetic resonance (NMR) spectra were measured on a Bruker AC-P500 instrument (400 MHz) using tetramethylsilane (TMS) as the internal standard and dimethylsulfoxide (DMSO)-d_6 or $CDCl_3$ as the solvent. Elemental analyses were performed on a Vario EL elemental

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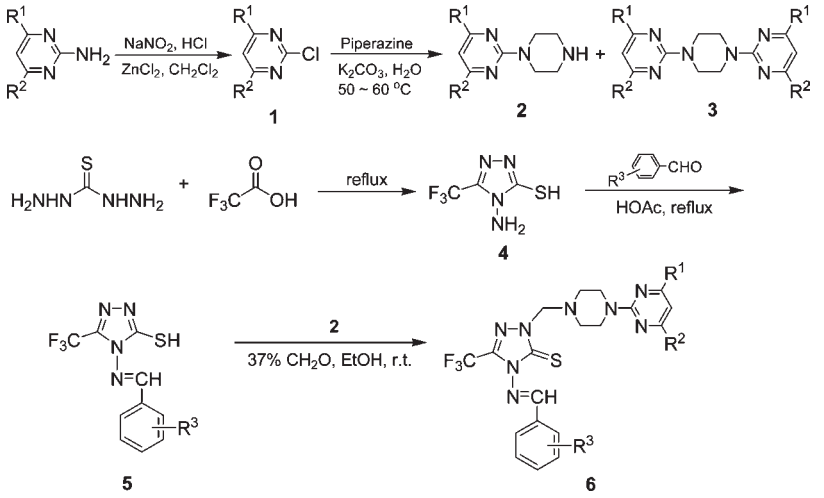
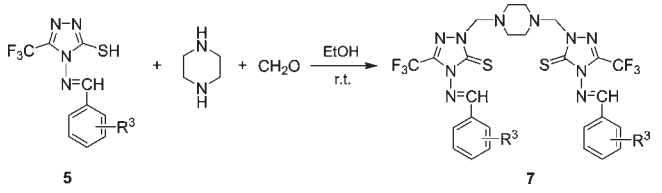
Scheme 1



Scheme 2



analyzer. Crystallographic data of the compound were collected on a Rigaku MM-07 Saturn 724 charge coupled device (CCD) diffractometer. All of the solvents and materials were analytical-grade.

Synthetic Procedures. 4-Amino-5-trifluoromethyl-4*H*-1,2,4-triazole-3-thiol **4** was prepared according to the literature (*20*).

General Synthetic Procedures for 4-(4,6-Disubstituted-pyrimidin-2-yl)piperazine 2. 2-Chloro-4,6-disubstituted-pyrimidines **1** were prepared by the reaction of the diazonium salts of 4,6-disubstituted-pyrimidin-2-amines with concentrated HCl and $ZnCl_2$ (*21*). Compound **2** was prepared according to ref *22*, and the method was improved. To a stirred solution of piperazine (45 mmol) and K_2CO_3 (16.5 mmol) in water (20 mL) was added chloropyrimidine **1** (18 mmol) in small portions at 50−65 °C. The mixture was stirred for 1 h at 60−65 °C and cooled to 35 °C. The yellow solid, 1,4-bis(4,6-disubstituted-pyrimidin-2-yl)piperazine **3**, was filtered off, and the filtrate was then extracted 3 times with chloroform, dried over Na_2SO_4, and evaporated in vacuum to give compound **2**, which was used for the following reactions without further purification.

2a ($R^1 = R^2 = H$): yellow oil, yield 88%. **2b** ($R^1 = H$; $R^2 = Me$): yellow solid, yield 81%, mp 45−48 °C. **2c** ($R^1 = R^2 = Me$): yellow solid, yield 79%, mp 82−84 °C.

3a ($R^1 = R^2 = H$): yellow solid, yield 4%, mp 273−274 °C [literature mp 275−278 °C (*23*)]. **3b** ($R^1 = H$, $R^2 = Me$): yellow crystal, yield 14%, mp 180−182 °C. **3c** ($R^1 = R^2 = Me$): yellow crystal, yield 19%, mp 216−218 °C.

General Synthetic Procedures for 4-(Substituted)benzylideneamino-5-trifluoromethyl-4*H*-1,2,4-triazole-3-thiol 5. Compound **4** (10 mmol) and aromatic aldehyde (10.5 mmol) were mixed in acetic acid (15 mL). After the reaction mixture was stirred and refluxed for 15 min, it was cooled to room temperature. The resulting crystals were filtered and washed with ethanol to give Schiff base **5**.

5a ($R^3 = H$): white crystal, yield 74%, mp 198−199 °C [literature mp 200−201 °C (*18*)]. ^1H NMR (DMSO-d_6, 400 MHz) δ: 14.90 (s, 1H, SH), 9.97 (s, 1H, CH), 7.58−7.92 (m, 5H, Ph-H).

5b (R^3 = 2-F): white crystals, yield 82%, mp 192−193 °C. ^1H NMR (DMSO-d_6, 400 MHz) δ: 14.93 (s, 1H, SH), 10.44 (s, 1H, CH), 7.41−8.03 (m, 4H, Ph-H).

5c (R^3 = 4-MeO): white crystals, yield 71%, mp 195−196 °C. ^1H NMR (DMSO-d_6, 400 MHz) δ: 14.84 (s, 1H, SH), 9.73 (s, 1H, CH), 7.86 (d, J = 8.8 Hz, 2H, Ph-H), 7.13 (d, J = 8.8 Hz, 2H, Ph-H), 3.86 (s, 3H, OCH_3).

5d (R^3 = 3,4-Me_2): white crystals, yield 73%, mp 205−206 °C. ^1H NMR (DMSO-d_6, 400 MHz) δ: 14.86 (s, 1H, SH), 9.79 (s, 1H, CH), 7.66 (s, 1H, Ph-H), 7.62 (d, J = 8.0 Hz, 1H, Ph-H), 7.35 (d, J = 8.0 Hz, 1H, Ph-H), 2.31 (s, 6H, CH_3).

5e (R^3 = 4-Cl): white crystals, yield 72%, mp 208−209 °C. ^1H NMR (DMSO-d_6, 400 MHz) δ: 14.92 (s, 1H, SH), 10.05 (s, 1H, CH), 7.93 (d, J = 8.4 Hz, 2H, Ph-H), 7.67 (d, J = 8.4 Hz, 2H, Ph-H).

5f (R^3 = 2-NO_2): yellow crystals, yield 89%, mp 201−202 °C. ^1H NMR (DMSO-d_6, 400 MHz) δ: 14.96 (s, 1H, SH), 10.88 (s, 1H, CH), 7.87−8.23 (m, 4H, Ph-H).

General Synthetic Procedures for 1-[4-(4,6-Disubstituted-pyrimidin-2-yl)piperazin-1-yl)methyl]-4-(substituted)benzylideneamino-3-trifluoromethyl-1*H*-1,2,4-triazole-5(4*H*)-thione 6. Schiff base **5** (1 mmmol) and 40% formalin (1.2 mmol) were dissolved in ethanol (15 mL), and the mixture was stirred at room temperature for 10 min. A solution of pyrimidylpiperazine **2** (1 mmol) in ethanol (2 mL) was slowly added dropwise. Then, the reaction mixture was stirred for 2−3 h and placed in a refrigerator overnight. The resulting precipitate was filtered and recrystallized from ethanol to give Mannich base **6**.

General Synthetic Procedures for 1,1′-[Piperazin-1,4-diylbis(methylene)]bis[4-(substituted)benzylideneamino-3-trifluoromethyl-1*H*-1,2,4-triazole-5(4*H*)-thione] 7. Schiff base **5** (1.8 mmmol) and 40% formalin (2 mmol) were dissolved in ethanol (30 mL), and the mixture was stirred at room temperature for 10 min. A solution of piperazine (0.9 mmol) in ethanol (2 mL) was slowly added dropwise. Then, the reaction mixture was stirred for 2−3 h and placed in a refrigerator overnight. The resulting precipitate was filtered and recrystallized from ethanol to give bis-Mannich base **7**.

Fungicidal Activity Tests. Fungicidal activity of compounds **6** and **7** against *Corynespora cassiicola*, *Pseudomonas syringae* pv. *lachrymans*, *Ascochyta citrallina* Smith, *Pseudoperonospora cubensis*, and *Sclerotinia sclerotiorum* were evaluated according to ref *24*, and a potted plant test method was adopted. Four commericial fungicides, Dimethomorph, Thiophanate-methyl, Iprodione, and Zhongshengmycin, were evaluated as controls at the same condition. Germination was conducted by soaking cucumber seeds in water for 2 h at 50 °C and then keeping the seeds moist for 24 h at 28 °C in an incubator. When the radicles were 0.5 cm, the seeds were grown in plastic pots containing a 1:1 (v/v) mixture of vermiculite and peat. Cucumber plants used for inoculations were at the stage of two seed leaves. Tested compounds and commercial fungicides were sprayed with a hand spray on the surface of the seed leaves on a fine morning, at the standard concentration of 500 μg/mL. After 2 h, inoculations of *C. cassiicola*, *A. citrullina* Smith, and *P. cubensis* were carried out by spraying a conidial suspension, inoculation of *P. syringae* pv. *lachrymans* was carried out by spraying a suspension, and inoculation of *S. sclerotiorum* was carried out by spraying a mycelial suspension. The experiment was repeated 4 times. After inoculation, the plants were maintained at 18−30 °C [mean temperature of 24 °C and above 80% relative humidity (RH)]. The fungicidal activity were evaluated when the nontreated cucumber plant (blank) fully developed symptoms. The area of inoculated treated leaves covered by disease symptoms was assessed and compared to that of nontreated ones to determine the average disease index (*24*). The relative

Table 1. Analytical Data for Compounds **6** and **7**

compound	R^1	R^2	R^3	yield (%)	mp (°C)	appearance	formula	elemental analysis (calculated %) C	H	N
6a	H	H	H	48	156−158	white crystal	$C_{19}H_{19}F_3N_8S$	50.68 (50.89)	4.53 (4.27)	25.10 (24.99)
6b	H	H	2-F	65	150−152	white crystal	$C_{19}H_{18}F_4N_8S$	48.64 (48.92)	4.21 (3.89)	23.88 (24.02)
6c	H	H	4-MeO	64	154−156	white crystal	$C_{20}H_{21}F_3N_8OS$	49.96 (50.20)	4.65 (4.42)	23.27 (23.42)
6d	H	Me	H	44	138−140	white crystal	$C_{20}H_{21}F_3N_8S$	51.67 (51.94)	4.94 (4.58)	24.38 (24.23)
6e	H	Me	2-F	42	113−115	white crystal	$C_{20}H_{20}F_4N_8S$	49.96 (49.99)	4.49 (4.20)	23.56 (23.32)
6f	H	Me	4-MeO	72	163−164	white crystal	$C_{21}H_{23}F_3N_8OS$	50.97 (51.21)	4.98 (4.71)	23.02 (22.75)
6g	H	Me	3,4-Me_2	58	158−159	white crystal	$C_{22}H_{25}F_3N_8S$	53.87 (53.87)	5.28 (5.14)	23.24 (22.84)
6h	H	Me	4-Cl	48	160−162	white crystal	$C_{20}H_{20}ClF_3N_8S$	48.05 (48.34)	4.29 (4.06)	22.69 (22.55)
6i	H	Me	2-NO_2	58	88−89	yellow crystal	$C_{20}H_{20}F_3N_9O_2S$	47.08 (47.33)	3.81 (3.97)	24.53 (24.84)
6j	Me	Me	H	54	154−156	white crystal	$C_{21}H_{23}F_3N_8S$	52.91 (52.93)	4.65 (4.86)	23.54 (23.51)
6k	Me	Me	2-F	46	153−154	white crystal	$C_{21}H_{22}F_4N_8S$	50.26 (51.00)	4.31 (4.48)	22.71 (22.66)
6l	Me	Me	4-MeO	52	160−161	white crystal	$C_{22}H_{25}F_3N_8OS$	52.12 (52.16)	5.02 (4.97)	22.23 (22.12)
6m	Me	Me	3,4-Me_2	47	162−164	white crystal	$C_{23}H_{27}F_3N_8S$	54.33 (54.75)	5.46 (5.39)	21.81 (22.21)
6n	Me	Me	4-Cl	63	171−173	white crystal	$C_{21}H_{22}ClF_3N_8S$	48.95 (49.36)	4.72 (4.34)	21.61 (21.93)
6o	Me	Me	2-NO_2	48	143−145	yellow crystal	$C_{21}H_{22}F_3N_9O_2S$	48.43 (48.36)	4.32 (4.25)	23.87 (24.17)
7a			H	60	198−199[a]	white crystal	$C_{26}H_{24}F_6N_{10}S_2$	47.64 (47.70)	4.01 (3.70)	20.96 (21.40)
7b			2-F	63	201−202[a]	white crystal	$C_{26}H_{22}F_8N_{10}S_2$	45.14 (45.22)	3.50 (3.21)	20.16 (20.28)
7c			4-MeO	75	200−201[a]	white crystal	$C_{28}H_{28}F_6N_{10}O_2S_2$	47.13 (47.05)	4.08 (3.95)	19.45 (19.60)
7d			3,4-Me_2	80	204−206[a]	white crystal	$C_{30}H_{32}F_6N_{10}S_2$	50.42 (50.69)	4.38 (4.54)	19.38 (19.71)
6-A1	H	H	3,4-Me_2	52	166−168	white crystal	$C_{21}H_{23}F_3N_8S$	52.90 (52.93)	4.78 (4.86)	23.67 (23.51)
6-A2	H	H	4-Cl	72	191−192	white crystal	$C_{19}H_{18}ClF_3N_8S$	46.92 (47.26)	3.84 (3.76)	22.88 (23.20)
6-A3	H	H	2-NO_2	63	153−155	yellow crystal	$C_{19}H_{18}F_3N_9O_2S$	45.98 (46.25)	3.74 (3.68)	25.26 (25.55)
7-A1			4-Cl	88	214−215[a]	white crystal	$C_{26}H_{22}Cl_2F_6N_{10}S_2$	42.91 (43.16)	3.30 (3.06)	18.80 (19.36)
7-A2			2-NO_2	72	196−197[a]	yellow crystal	$C_{26}H_{22}F_6N_{12}O_4S_2$	41.92 (41.94)	3.20 (2.98)	22.23 (22.57)

[a] Decomposed.

control efficacy of compounds compared to the blank assay was calculated via the following equation:

$$\text{relative control efficacy (\%)} = [(\text{CK} - \text{PT})/\text{CK}] \times 100\%$$

where CK is the average disease index during the blank assay and PT is the average disease index after treatment during testing.

Herbicidal Activity Tests. *Inhibition of the Root Growth of Rape* (*Brassica campestris*). The evaluated compounds were dissolved in water and emulsified if necessary. Rape seeds were soaked in distilled water for 4 h before being placed on a filter paper in a 6 cm Petri plate, to which 2 mL of inhibitor solution had been added in advance. Usually, 15 seeds were used on each plate. The plate was placed in a dark room and allowed to germinate for 65 h at 28 ± 1 °C. The lengths of 10 rape roots selected from each plate were measured, and the means were calculated. The check test was carried out in distilled water only. The percentage of the inhibition was calculated.

Inhibition of the Seedling Growth of Barnyardgrass (*Echinochloa crusgalli*). The evaluated compounds were dissolved in water and emulsified if necessary. A total of 10 barnyardgrass seeds were placed into a 50 mL cup covered with a layer of glass beads and a piece of filter paper at the bottom, to which 5 mL of inhibitor solution had been added in advance. The cup was placed in a bright room and allowed to germinate for 65 h at 28 ± 1 °C. The heights of seedlings of above-ground plant parts from each cup were measured, and the means were calculated. The check test was carried out in distilled water only. The percentage of the inhibition was calculated.

Crystal Structure Determination. Compound **6i** was dissolved in hot alcohol, and the resulting solution was allowed to stand in air at room temperature to give a single crystal of compound **6i**. A yellow crystal of compound **6i** suitable for X-ray diffraction with dimensions of 0.16 × 0.14 × 0.10 mm was mounted on a Rigaku MM-07 Saturn 724 CCD diffractometer with Mo Kα radiation ($\lambda = 0.710\,73$ Å) for data collection. A total of 17 003 reflections were collected in the range of $2.01 < \theta < 25.02$ using phi and scan modes at 113(2) K, of which 4363 were independent with $R_{int} = 0.0614$. All calculations were refined anisotropically (SHELXS-97). All hydrogen atoms were located from a difference Fourier map, placed at calculated positions, and included in the refinements in the riding mode with isotropic thermal parameters. The compound crystallizes in space group $Pca2_1$ of the orthorhombic system with cell parameters: $a = 20.312(4)$ Å, $b = 14.395(3)$ Å, $c = 8.8173(18)$ Å, $\alpha = 90°$, $\beta = 90°$, $\gamma = 90°$, $V = 2578.2(9)$ Å^3, $Z = 4$, $D_c = 1.367$ mg/m^3, $\mu = 0.186$ mm^{-1}, and $F(000) = 1100$. The final refinement converged at $R = 0.0603$ and $wR = 0.1494$ for 3552 observed reflections with $I > 2\sigma(I)$, where $W = 1/[\sigma^2(F_o^2) + (0.0980P)^2 + 0.0000P]$ with $P = (F_o^2 + 2F_c^2)/3$, $S = 1.073$, $(\Delta/\sigma)_{max} < 0.0001$, $(\Delta\rho)_{max} = 0.434$ e/Å^3, and $(\Delta\rho)_{min} = -0.265$ e/Å^3.

Three-Dimensional Quantitative Structure−Activity Relationship (3D QSAR) Analysis. Molecular modeling was performed using SYBYL 6.91 software, and the comparative molecular field analysis (CoMFA) method has been performed according to our previous papers (*25*, *26*). The fungicidal activities of 19 compounds (**6a**−**6o** and **7a**−**7d**, for training sets compounds) against *P. cubensis* (% *I*) at 500 μg/mL used to derive the CoMFA analysis model were listed in **Table 7**. The activity was expressed in terms of *D* by the formula $D = \log\{[I/(100 - I)] \times \text{MW}\}$, where *I* is the percent control efficacy and MW is the molecular weight of the tested compounds. The compound **6i**, owing to the determination of the crystal structure, was used as a template to build the other molecular structures. Each structure was fully geometry-optimized using a conjugate gradient procedure based on the TRIPOS force field and Gasteiger and Hückel charges. Because these compounds share a common skeleton, 19 atoms marked with an asterisk were used for root-mean-square (rms) fitting onto the corresponding atoms of the template structure. 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. The CoMFA steric and electrostatic fields generated were scaled by the CoMFA−STD method in SYBYL. The electrostatic fields were ignored at the lattice points with maximal steric interactions. A partial least-squares approach was used to derive the 3D QSAR, in which the CoMFA descriptors were used as independent variables and ED values were used as dependent variables. The cross-validation with the leave-one-out option and the SAMPLS program (*27*) rather than column filtering was carried out to obtain the optimal number of components to be used in the final analysis. After the optimal number of components was determined, a non-cross-validated analysis was performed without column filtering. The modeling capability

Table 2. ^{1}H NMR Spectral Data of Compounds **6** and **7**

compound	δ ($CDCl_3$, 400 MHz)
6a	10.34 (s, 1H, CH), 6.46−8.29 (m, 8H, Ar−H), 5.28 (s, 2H, CH_2), 3.87 (t, J = 4.8 Hz, 4H, piperazine-H), 2.90 (t, J = 4.8 Hz, 4H, piperazine-H)
6b	10.70 (s, 1H, CH), 6.45−8.29 (m, 7H, Ar−H), 5.28 (s, 2H, CH_2), 3.86 (t, J = 4.8 Hz, 4H, piperazine-H), 2.90 (t, J = 4.8 Hz, 4H, piperazine-H)
6c	10.07 (s, 1H, CH), 8.28 (d, J = 4.8 Hz, 2H, pyrimidine-H), 7.82 (d, J = 8.8 Hz, 2H, Ph-H), 6.98 (d, J = 8.8 Hz, 2H, Ph-H), 6.47 (t, J = 4.8 Hz, 1H, pyrimidine-H), 5.28 (s, 2H, CH_2), 3.88 (s, 3H, OCH_3), 3.86 (t, J = 4.8 Hz, 4H, piperazine-H), 2.90 (t, J = 4.8 Hz, 4H, piperazine-H)
6d	10.35 (s, 1H, CH), 8.14 (d, J = 4.4 Hz, 1H, pyrimidine-H), 7.47−7.88 (m, 5H, Ph-H), 6.35 (d, J = 4.4 Hz, 1H, pyrimidine-H), 5.29 (s, 2H, CH_2), 3.87 (t, J = 4.0 Hz, 4H, piperazine-H), 2.89 (t, J = 4.0 Hz, 4H, piperazine-H), 2.31 (s, 3H, CH_3)
6e	10.70 (s, 1H, CH), 8.13 (d, J = 4.4 Hz, 1H, pyrimidine-H), 7.15−8.09 (m, 4H, Ph-H), 6.35 (d, J = 4.4 Hz, 1H, pyrimidine-H), 5.28 (s, 2H, CH_2), 3.88 (bs, 4H, piperazine-H), 2.89 (bs, 4H, piperazine-H), 2.31 (s, 3H, CH_3)
6f	10.08 (s, 1H, CH), 8.13 (d, J = 4.8 Hz, 1H, pyrimidine-H), 7.82 (d, J = 8.0 Hz, 2H, Ph-H), 6.99 (d, J = 8.0 Hz, 2H, Ph-H), 6.35 (d, J = 4.8 Hz, 1H, pyrimidine-H), 5.28 (s, 2H, CH_2), 3.88 (bs, 7H, CH_3O + piperazine-H), 2.89 (bs, 4H, piperazine-H), 2.31 (s, 3H, CH_3)
6g	10.11 (s, 1H, CH), 8.13 (d, J = 4.8 Hz, 1H, pyrimidine-H), 7.63 (s, 1H, Ph-H), 7.60 (d, J = 8.0 Hz, 1H, Ph-H), 7.24 (d, J = 8.0 Hz, 1H, Ph-H), 6.35 (d, J = 4.8 Hz, 1H, pyrimidine-H), 5.28 (s, 2H, CH_2), 3.87 (bs, 4H, piperazine-H), 2.89 (bs, 4H, piperazine-H), 2.33 (s, 3H, Ph−CH_3), 2.32 (s, 3H, Ph−CH_3), 2.31 (s, 3H, pyrimidine-CH_3)
6h	10.44 (s, 1H, CH), 8.13 (d, J = 4.8 Hz, 1H, pyrimidine-H), 7.80 (d, J = 8.0 Hz, 2H, Ph-H), 7.47 (d, J = 8.0 Hz, 2H, Ph-H), 6.35 (d, J = 4.8 Hz, 1H, pyrimidine-H), 5.28 (s, 2H, CH_2), 3.87 (bs, 4H, piperazine-H), 2.89 (bs, 4H, piperazine-H), 2.31 (s, 3H, CH_3)
6i	11.21 (s, 1H, CH), 7.70−8.18 (m, 5H, Ph-H + pyrimidine-H), 6.36 (d, J = 4.8 Hz, 1H, pyrimidine-H), 5.28 (s, 2H, CH_2), 3.88 (bs, 4H, piperazine-H), 2.90 (bs, 4H, piperazine-H), 2.31 (s, 3H, CH_3)
6j	10.35 (s, 1H, CH), 7.47−7.88 (m, 5H, Ph-H), 6.25 (s, 1H, pyrimidine-H), 5.29 (s, 2H, CH_2), 3.89 (t, J = 4.4 Hz, 4H, piperazine-H), 2.89 (t, J = 4.4 Hz, 4H, piperazine-H), 2.26 (s, 6H, CH_3)
6k	10.70 (s, 1H, CH), 7.15−8.09 (m, 4H, Ph-H), 6.25 (s, 1H, pyrimidine-H), 5.29 (s, 2H, CH_2), 3.88 (t, J = 4.0 Hz, 4H, piperazine-H), 2.89 (t, J = 4.0 Hz, 4H, piperazine-H), 2.26 (s, 6H, CH_3)
6l	10.08 (s, 1H, CH), 7.83 (d, J = 8.0 Hz, 2H, Ph-H), 6.99 (d, J = 8.0 Hz, 2H, Ph-H), 6.25 (s, 1H, pyrimidine-H), 5.29 (s, 2H, CH_2), 3.89 (bs, 7H, CH_3O + piperazine-H), 2.88 (bs, 4H, piperazine-H), 2.26 (s, 6H, CH_3)
6m	10.11 (s, 1H, CH), 7.63 (s, 1H, Ph-H), 7.60 (d, J = 8.0 Hz, 1H, Ph-H), 7.25 (d, J = 8.0 Hz, 1H, Ph-H), 6.25 (s, 1H, pyrimidine-H), 5.28 (s, 2H, CH_2), 3.88 (t, J = 4.4 Hz, 4H, piperazine-H), 2.88 (t, J = 4.4 Hz, 4H, piperazine-H), 2.34 (s, 3H, Ph−CH_3), 2.33 (s, 3H, Ph−CH_3), 2.26 (s, 6H, pyrimidine-CH_3)
6n	10.44 (s, 1H, CH), 7.80 (d, J = 8.0 Hz, 2H, Ph-H), 7.47 (d, J = 8.0 Hz, 2H, Ph-H), 6.25 (s, 1H, pyrimidine-H), 5.28 (s, 2H, CH_2), 3.88 (bs, 4H, piperazine-H), 2.88 (bs, 4H, piperazine-H), 2.26 (s, 6H, CH_3)
6o	11.21 (s, 1H, CH), 7.70−8.18 (m, 4H, Ph-H), 6.25 (s, 1H, pyrimidine-H), 5.29 (s, 2H, CH_2), 3.89 (bs, 4H, piperazine-H), 2.89 (bs, 4H, piperazine-H), 2.26 (s, 6H, CH_3)
7a	10.35 (s, 2H, CH), 7.48−7.59 (m, 10H, Ph-H), 5.19 (s, 4H, CH_2), 2.88 (s, 8H, piperazine-H)
7b	10.71 (s, 2H, CH), 7.15−8.10 (m, 8H, Ph-H), 5.18 (s, 4H, CH_2), 2.88 (s, 8H, piperazine-H)
7c	10.08 (s, 2H, CH), 7.83 (d, J = 8.8 Hz, 4H, Ph-H), 6.99 (d, J = 8.8 Hz, 4H, Ph-H), 5.18 (s, 4H, CH_2), 3.89 (s, 6H, OCH_3), 2.87 (s, 8H, piperazine-H)
7d	10.12 (s, 2H, CH), 7.24−7.64 (m, 6H, Ph-H), 5.18 (s, 4H, CH_2), 2.88 (s, 8H, piperazine-H), 2.34 (s, 6H, CH_3), 2.33 (s, 6H, CH_3)
6-A1	10.11 (s, 1H, CH), 8.28 (d, J = 4.8 Hz, 2H, pyrimidine-H), 7.63 (s, 1H, Ph-H), 7.60 (d, J = 8.0 Hz, 1H, Ph-H), 7.24 (d, J = 8.0 Hz, 1H, Ph-H), 6.47 (t, J = 4.8 Hz, 1H, pyrimidine-H), 5.28 (s, 2H, CH_2), 3.87 (t, J = 4.8 Hz, 4H, piperazine-H), 2.90 (t, J = 4.8 Hz, 4H, piperazine-H), 2.33 (s, 3H, CH_3), 2.32 (s, 3H, CH_3)
6-A2	10.44 (s, 1H, CH), 8.28 (d, J = 4.8 Hz, 2H, pyrimidine-H), 7.80 (d, J = 8.8 Hz, 2H, Ph-H), 7.46 (d, J = 8.8 Hz, 2H, Ph-H), 6.47 (t, J = 4.8 Hz, 1H, pyrimidine-H), 5.28 (s, 2H, CH_2), 3.87 (t, J = 4.4 Hz, 4H, piperazine-H), 2.90 (t, J = 4.4 Hz, 4H, piperazine-H)
6-A3	11.22 (s, 1H, CH), 8.29 (d, J = 4.8 Hz, 2H, pyrimidine-H), 7.70−8.19 (m, 4H, Ph-H), 6.47 (t, J = 4.8 Hz, 1H, pyrimidine-H), 5.28 (s, 2H, CH_2), 3.87 (t, J = 4.4 Hz, 4H, piperazine-H), 2.91 (t, J = 4.4 Hz, 4H, piperazine-H)
7-A1	10.45 (s, 2H, CH), 7.81 (d, J = 8.4 Hz, 4H, Ph-H), 7.47 (d, J = 8.4 Hz, 4H, Ph-H), 5.17 (s, 4H, CH_2), 2.87 (s, 8H, piperazine-H)
7-A2	11.21 (s, 2H, CH), 7.71−8.19 (m, 8H, Ph-H), 5.18 (s, 4H, CH_2), 2.89 (s, 8H, piperazine-H)

(goodness of fit) was judged by the correlation coefficient squared, r^2, and the prediction capability (goodness of prediction) was indicated by the cross-validated r^2 (q^2).

RESULTS AND DISCUSSION

Synthesis. The synthesis procedures for compounds **6** were shown in **Scheme 1**, and the synthesis procedures for compounds **7** were shown in **Scheme 2**. According to the method described in ref *22* for the synthesis of required 4-(4,6-disubstituted-pyrimidin-2-yl)piperazine **2**, a small amount of 1,4-bis(4,6-disubstituted-pyrimidin-2-yl) piperazine **3** was also obtained, which was not mentioned in the literature. Compound **3** was further confirmed by ^{1}H NMR spectra. In reference to an acetic acid solvent method reported by Wu et al. for the condensation of amine and aldehyde (*28*), Schiff base **5**, namely, 4-amino-5-trifluoromethyl-4*H*-1,2,4-trizole-3-thiol, was prepared successfully. The Mannich reaction of compound **5** with formaldehyde and pyrimidyl-piperazine **2** in ethanol at room temperature led to novel trifluoromethyl-substituted 1,2,4-triazole Mannich base **6**. In a 2:1 molar ratio of Schiff base **5** and piperazine, novel trifluoromethyl-substituted bis(1,2,4-triazole) Mannich base **7** was prepared conveniently in 60−88% yield using the same procedures for compound **6**. Compounds **6** and **7** were identified by ^{1}H NMR and infrared (IR) spectra (Tables **2** and **3**). The measured elemental analyses were also consistent with the corresponding calculated ones (**Table 1**).

Table 3. IR Spectral Data of Compounds **6** and **7**

compound	ν (cm^{-1}) (KBr)
6a	2860, 2825 (C−H), 1587 (C═N), 1550, 1506, 1483, 1465 (Ar), 1310, 1205 (C−F), 1161 (C═S)
6d	2980, 2830 (C−H), 1578 (C═N), 1566, 1489, 1467, 1449 (Ar), 1316, 1194 (C−F), 1161 (C═S)
6j	2941, 2853 (C−H), 1577 (C═N), 1504, 1467, 1449 (Ar), 1311, 1194 (C−F), 1156 (C═S)
7a	2935, 2822 (C−H), 1599 (C═N), 1583, 1463, 1452 (Ar), 1317, 1193 (C−F), 1156 (C═S)

Crystal Structure. The structure of compound **6i** was further confirmed by single-crystal X-ray diffraction analysis (**Figure 1**). From the molecular structure, it can be seen that both groups on the N atoms of the piperazine ring (triazole-CH_2 and pyrimidine) are in the e-bond positions of chair conformation in the six-membered ring. The dihedral angle between the 2-nitrobenzene ring and the triazole ring is 32°, which indicates that the two rings are not coplanar in the molecular structure. The X-ray analysis also reveals that, in this typical compound **6i**, the substituted benzene ring and the triazole ring are on the opposite sides of the C═N double bond (**Figure 1**). The torsion angle of

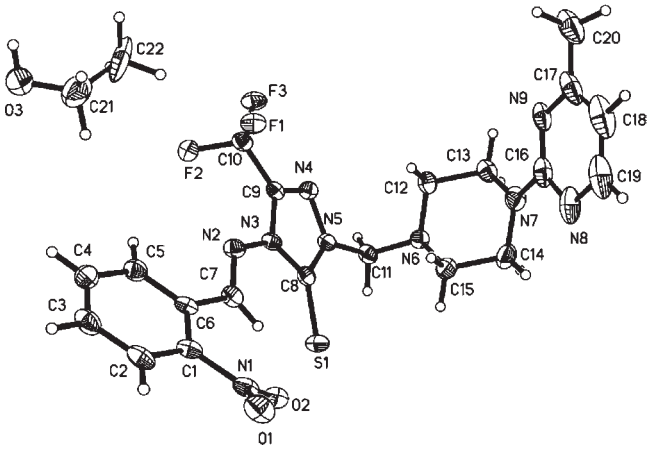


Figure 1. Molecular structure of compound **6i**.

Table 4. Fungicidal Activity of Compounds (Percent Relative Control Efficacy)

compound	*C. cassiicola*	*P. syringae* pv *lachrymans*	*A. citrullina* Smith	*P. cubensis*	*S. sclerotiorum*
6a	56.2	55.8	37.1	66.3	41.7
6b	40.3	45.4	61.3	64.9	47.3
6c	73.4	68.1	73.4	79.5	75.3
6d	49.2	20.4	62.4	78.6	36.8
6e	−5.3	62.3	57.8	75.5	52.2
6f	52.8	30.6	67.7	72.5	50.9
6g	38.7	22.0	70.0	69.5	51.9
6h	71.1	21.1	61.3	91.2	70.3
6i	73.8	44.5	44.3	76.8	32.8
6j	22.1	55.0	61.5	74.1	48.0
6k	78.7	56.1	83.5	79.2	72.0
6l	83.7	50.3	87.6	87.3	62.6
6m	85.9	59.5	82.9	67.8	48.9
6n	94.4	61.5	62.8	91.6	38.0
6o	87.8	46.5	88.4	77.2	39.3
7a	70.9	56.4	25.6	78.9	67.6
7b	84.7	6.8	86.5	79.9	25.9
7c	44.1	55.6	89.7	84.5	62.0
7d	67.4	36.7	26.0	78.7	32.7
Dimethomorph	95.3	14.0	98.4	83.8	98.4
Thiophanate-methyl	78.8	54.2	100	68.7	83.4
Iprodione	53.5	38.5	98.4	55.9	97.8
Zhongshengmycin	90.2	69.7	100	62.6	84.4

C(6)−C(7)−N(2)−N(3) is 176.52°, which indicates that the C═N double bond is in the *E* configuration.

Fungicidal Activity. The *in vivo* fungicidal results of the Mannich base **6a**−**6o** and bis-Mannich base **7a**−**7d** against *C. cassiicola*, *P. syringae* pv. *lachrymans*, *A. citrallina* Smith, *P. cubensis*, and *S. sclerotiorum* were listed in **Table 4**. Most of the compounds showed promising results in inhibiting the mycelial growth of all of the test fungi at a concentration of 500 μg/mL. Meanwhile, all of these tested compounds were found safe for the cucumber plants. The comparison of the fungicidal activity of compounds **6** and **7** for five test fungi to those of commercial fungicides leads to the following conclusions: (a) Compounds **6l**, **6m**, **6n**, **6o**, and **7b** exhibited a significant inhibition effect against *C. cassiicola*, and the fungicidal activities (control efficacy of 83.7−94.4%) were higher than those of Thiophanate-methyl and Iprodione. Especially, compound **6n** showed a control efficacy of 94.4%, which was similar to that of the most active fungicide Dimethomorph (95.3%). It can be seen that variances among R^1, R^2, and R^3 can greatly effect the fungicidal activity of compounds against *C. cassiicola*. When $R^1 = R^2 =$ Me and a bulky group at the 4 position of the benzene ring in Mannich base **6** (e.g., compound **6n**, $R^3 =$ Cl), there is an apparent increase of fungicidal activity, while the introduction of the electron-withdrawing group in the benzene ring of bis-Mannich base **7** is favorable to the improvement of activity. (b) Compounds **6c**, **6e**, and **6n** possessed efficacy rates 68.1, 62.3, and 61.5% against *P. syringae* pv. *lachrymans*, respectively. All of the three compounds were more effective than Dimethomorph, Thiophanate-methyl, and Iprodione. Among them, compound **6c** had almost the same activity as that of Zhongshengmycin against *P. syringae* pv. *lachrymans*. For this fungi, when $R^1 =$ H and $R^2 =$ Me (Mannich base **6**), compounds with an electron-donating group in the benzene ring will affect lower fungicidal activities than those of others. However, the small and electron-donating group in the benzene ring of bis-Mannich base **7** is favorable. (c) Compounds **6l**, **6o**, **7b**, and **7c** held 86.5−89.7% efficacy rates against *A. citrallina* Smith, while all of them were less effective than all of the contrasts. It was found that compounds with two methyl groupds in the pyrimidine ring ($R^1 = R^2 =$ Me) have a higher level of fungicidal activity than others ($R^1 = R^2 =$ H and $R^1 =$ H and $R^2 =$ Me), which indicates that the introduction of hydrophobic groups at 4 and 6 positions of the pyrimidine ring could be an activation process for this group of compounds. (d) All of the compounds exhibited good control efficacy against *P. cubensis* (exceeding 64% efficacy rate), and most of them had higher activity than Thiophanate-methyl, Iprodione, and Zhongshengmycin. It was worthy to note that compounds **6h**, **6l**, **6n**, and **7c**, whose efficacy rates were 91.2, 87.3, 91.6, and 84.5%, respectively, were found to be more effective compared to the most active fungicide Dimethomorph (83.8%) against *P. cubensis*. To further explore the comprehensive structure−activity relationship on the basis of these data, CoMFA was subsequently performed. (e) For *S. sclerotiorum*, compounds **6c**, **6h**, and **6k** exhibited favorable fungicidal activity and held 75.3, 70.3, and 72.0% efficacy rates, respectively. However, all of them were less effective than all of the contrasts.

Table 5. Herbicidal Activity of Compounds (Percent Inhibition)

	rape (*B. campestris*) root test		barnyardgrass (*E. crusgalli*) cup test	
compound	100 μg/mL	10 μg/mL	100 μg/mL	10 μg/mL
6a	66.8	49.2	0	0
6b	74.5	70.8	15.3	9.9
6c	78.8	53.5	18.6	0
6d	12.5	0	15.3	8.7
6e	24.4	0	8.0	0
6f	30.3	0	34.3	15.3
6g	11.2	0	27.8	0
6h	68.6	0	96.7	10.2
6i	0	0	33.9	11.5
6j	71.1	36.9	9.8	0
6k	72.4	67.1	33.2	17.1
6l	67.7	30.6	29.5	0
6m	20.5	6.0	8.7	0
6n	88.5	9.5	36.6	12.3
6o	11.2	0	10.2	0
7a	35.1	0	25.1	16.2
7b	4.3	0	26.6	10.7
7c	29.5	0	25.5	5.5
7d	27.4	0	28	1.5
Chlorsulfuron	80.4	76.0	29.9	8.2

Herbicidal Activity. As shown in **Table 5**, most compounds **6** and **7** displayed herbicidal activity based on the rape (*B. campestris*) root and barnyardgrass (*E. crusgalli*) cup tests at a concentration of 100 μg/mL. Compounds **6a**−**6c**, **6h**, **6j**−**6l**, and **6n** showed inhibition rates of 66.8−88.5% to the root growth of dicotyledonous rape at 100 μg/mL. Especially, compounds **6b**

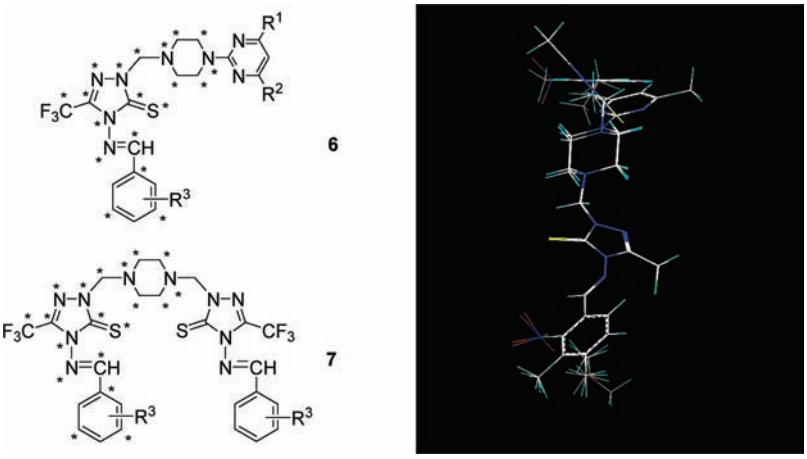


Figure 2. Superimposition of compounds **6** and **7** for 3D QSAR studies.

Table 6. Summary of CoMFA Analysis

						contribution (%)	
	q^2	r^2	S	F	compound	steric	electrostatic
CoMFA	0.526	0.913	0.085	24.448	**6h**	74.6	25.4

and **6k** held by inhibition rates 70.8 and 67.1% at the concentration of 10 μg/mL, respectively, which were similar to that of contrast Chlorsulfuron. Against the seedling growth of monocotyledonous barnyardgrass, all of the compounds showed little inhibitory activitity at 10 μg/mL, but compounds **6h** exhibited 96.7% inhibition at 100 μg/mL. On the whole, these compounds might exhibit less of an effect in comparison to the commercial sulfonylureas against rape root at 1 μg/mL [e.g., Chlorsulfuron, 64.2% inhibition; Metsulfuron-methyl, 81.0% inhibition (*29*)]; however, some showed favorable herbicidal activity in these preliminary studies and might be further optimized to enhance their activity.

CoMFA Analysis. The CoMFA method is widely used in drug design, because it allows for rapid generation of predictive information of QSAR from the biological activity of newly designed molecules (*30*). Starting from the structural alignment of **Figure 2**, comprehensive CoMFA analyses were performed. The results of these computations were summarized in **Table 6**. A comparison of experimental and predicted activity by CoMFA for all of the compounds in this study was presented in **Table 7**. The cross-validated results were assessed by their q^2 value (see the Materials and Methods), where a value above 0.3 indicates that the probability of chance correlation is less than 5% and a value over 0.5 is highly significant. As listed in **Table 6**, a predictive CoMFA model was established with the cross-validated coefficient $q^2 = 0.526$ and the conventional correlation coefficient $r^2 = 0.913$. The contributions of steric and electrostatic fields are 74.6 and 25.4% as shown in panels **a** and **b** of **Figure 3**, respectively.

In **Figure 3**, the isocontour diagrams of the steric and electrostatic field contributions ("standard deviation × coefficient") obtained from the CoMFA analysis are illustrated. The steric field contour map is plotted in **Figure 3a**. The green displays 2 positions, where a bulky group would be favorable for higher fungicidal activity. In contrast, yellow indicates positions where a decrease in the bulk of the target molecules is favored. As shown in **Figure 3a**, the CoMFA steric contour plots obviously indicated that a yellow region is located around the 2 and 3 position of the benzene ring. This means that the bulky substituents at the 2 and 3 position will decrease the fungicidal activity. For example, some compounds bearing substituents at the 2 position of the benzene ring, such as **6b**, **6e**, **6i**, **6j**, and **6o**, displayed lower fungicidal activity. The electrostatic contour plot is shown in **Figure 3b**. The blue contour defines a region where an increase in the positive charge will result in an increase in the activity, whereas the red contour defines a region of space where increasing electron density is favorable. As shown in **Figure 3b**, the target compounds bearing an electron-donating group at the 4 or 6 position of the pyrimidine ring or an electron-withdrawing group at the 3 or 4 position of the benzene ring, such as **6h**, **6n**, and **7c**, displayed higher activity. According to the 3D QSAR model obtained, five novel Mannich bases (**6-A1**−**6-A3**, **7-A1**, and **7-A2**) as test set compounds were subsequently synthesized and bioassayed. As shown in **Table 7**, their activities were estimated fairly accurately from the analysis, indicating that the alignment of the molecules was valid. For example, compounds **6-A1** and 7-A2 were found to display good fungicidal activity against

Table 7. Experimental and Predicted Fungicidal Activity (*P. cubensis*) of 3D QSAR

compound	MW	I	ED[a]	PD[b]	residual
6a	448	66.3	2.945	3.002	−0.057
6b	466	64.9	2.935	3.003	−0.068
6c	478	79.5	3.268	3.162	0.106
6d	462	78.6	3.230	3.249	−0.019
6e	480	75.5	3.170	3.139	0.031
6f	492	72.5	3.113	3.272	−0.159
6g	490	69.5	3.048	3.072	−0.024
6h	496	91.2	3.711	3.701	0.010
6i	507	76.8	3.225	3.265	−0.040
6j	476	74.1	3.134	3.250	−0.116
6k	494	79.2	3.274	3.250	0.024
6l	506	87.3	3.541	3.605	−0.064
6m	504	67.8	3.026	3.028	−0.002
6n	510	91.6	3.745	3.599	0.146
6o	521	77.2	3.247	3.259	−0.012
7a	654	78.9	3.388	3.296	0.092
7b	690	79.9	3.438	3.394	0.044
7c	714	84.5	3.590	3.606	−0.016
7d	710	78.7	3.419	3.458	−0.039
6-A1[c]	476	96.9	4.173	4.002	0.171
6-A2[c]	482	74.2	3.142	3.341	−0.199
6-A3[c]	493	76.2	3.198	2.903	0.295
7-A1[c]	722	81.0	3.488	3.568	−0.080
7-A2[c]	744	84.9	3.622	3.677	−0.055

[a] ED = experimental D value. [b] PD = predicted D value. [c] Test set compounds.

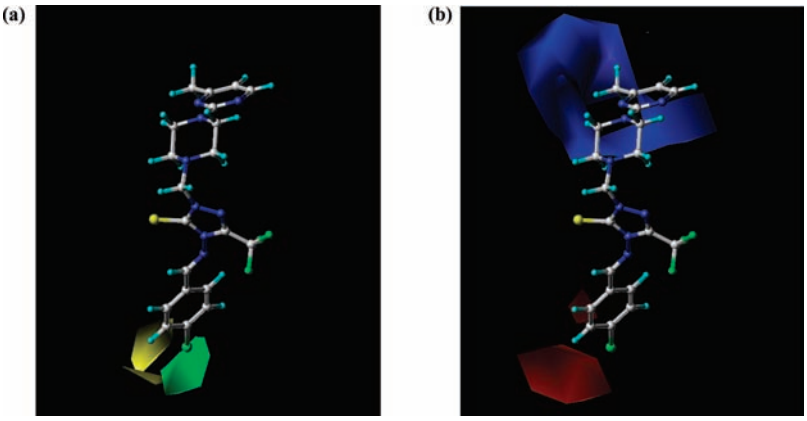


Figure 3. (a) Steric map from the CoMFA model. (b) Electrostatic map from the CoMFA model.

P. cubensis (96.9 and 84.9%) as compounds **6h** and **7c**, respectively. These results provide useful information for further optimization of the compounds.

In summary, we have conveniently synthesized a series of trifluoromethyl-substituted 1,2,4-triazole Mannich base **6** and bis(1,2,4-triazole) Mannich base **7** containing pyrimidinylpiperazine rings via the Mannich reaction in good yields. The fungicidal tests indicated that most compounds **6** and **7** possessed excellent fungicidal activity. Among 19 novel compounds, some showed superiority over the commercial fungicides Dimethomorph, Thiophanate-methyl, Iprodione, and Zhongshengmycin during the present studies and could be further developed as fungicides. Some compounds also exhibited favorable herbicidal activity in the preliminary studies. In addition, the 3D QSAR results provide useful information for guiding optimization of such structures to accelerate the discovery of compounds with high fungicidal activity.

LITERATURE CITED

(1) Kramer, W.; Schirmer, U. *Modern Crop Protection*; Wiley-VCH: Weinheim, Germany, 2007.

(2) Tanaka, K.; Tsukamoto, Y.; Sawada, Y.; Kasuya, A.; Hotta, H.; Ichinose, R.; Watanabe, T.; Toya, T.; Yokoi, S.; Kawagishi, A.; Ando, M.; Sadakane, S.; Katsumi, S.; Masui, A. Chromafenozide: A novel lepidopteran insect control agent. *Annu. Rep. Sankyo Res. Lab.* **2001**, *53*, 1–49.

(3) Bucchel, K. H.; Draber, W. Ger. Offen. 1940628, 1971. *Chem. Abstr.* **1971**, *74*, No. 125698t.

(4) Greenfield, S. A.; Seldel, M. C.; Von Meyer, W. C. Ger. Offen. 1943915, 1970. *Chem. Abstr.* **1970**, *72*, No. 100713q.

(5) Okano, S.; Yasujaga, K. Jpn. Pat. 7017191, 1970. *Chem. Abstr.* **1970**, *73*, No. 77251x.

(6) Reisser, F. Ger. Offen. 1918795, 1969. *Chem. Abstr.* **1970**, *72* No. 43692f.

(7) Bianchi, D.; Cesti, P.; Spezia, S.; Garavaglia, C.; Mirenna, L. Chemoenzymic synthesis and biological activity of both enantiomeric forms of tetraconazole, a new antifungal triazole. *J. Agric. Food Chem.* **1991**, *39*, 197–201.

(8) Chen, T.; Dwyre-Gygax, C.; Hadfield, S. T.; Willetts, C.; Breuil, C. Development of an enzyme-linked immunosorbent assay for a broad spectrum triazole fungicide: Hexaconazole. *J. Agric. Food Chem.* **1996**, *44*, 1352–1356.

(9) Garca Ruano, J. L.; Cifuentes Garca, M.; Martn Castro, A. M.; Rodrguez Ramos, J. H. First highly stereoselective synthesis of fungicide Systhane. *Org. Lett.* **2002**, *4*, 55–57.

(10) Song, Z.; Nes, W. D. Sterol biosynthesis inhibitors: Potential for transition state analogs and mechanism-based inactivators targeted at sterol methyltransferase. *Lipids* **2007**, *42*, 15–33.

(11) Cao, X.; Li, F.; Hu, M.; Lu, W.; Yu, G.-A.; Liu, S. H. Chiral γ-aryl-1*H*-1,2,4-triazole derivatives as highly potential antifungal agents: Design, synthesis, structure, and in vitro fungicidal activities. *J. Agric. Food Chem.* **2008**, *56*, 11367–11375.

(12) Naylor, A. W. Metabolism of herbicides, complexes of 3-amino-1,2,4-triazole in plant metabolism. *J. Agric. Food Chem.* **1964**, *12*, 21–25.

(13) Simmons, K. A.; Dixson, J. A.; Halling, B. P.; Plummer, E. L.; Plummer, M. J.; Tymonko, J. M.; Schmidt, R. J.; Wyle, M. J.; Webster, C. A.; Baver, W. A.; Witkowski, D. A.; Peters, G. R.; Gravelle, W. D. Synthesis and activity optimization of herbicidal substituted 4-aryl-1,2,4-triazole-5(1*H*)-thiones. *J. Agric. Food Chem.* **1992**, *40*, 297–305.

(14) Filler, R.; Kobayashi, Y. *Biomedicinal Aspects of Fluorine Chemistry*; Kodansha and Elsevier Biomedical: Amsterdam, The Netherlands, 1983.

(15) Clark, R. D. Synthesis and QSAR of herbicidal 3-pyrazolyl α,α,α-trifluorotolyl ethers. *J. Agric. Food Chem.* **1996**, *44*, 3643–3652.

(16) Liu, A.; Wang, X.; Ou, X.; Huang, M.; Chen, C.; Liu, S.; Huang, L.; Liu, X.; Zhang, C.; Zheng, Y.; Ren, Y.; He, L.; Yao, J. Synthesis and fungicidal activities of novel bis(trifluoromethyl)phenyl-based strobilurins. *J. Agric. Food Chem.* **2008**, *56*, 6562–6566.

(17) Liu, A.; Wang, X.; Chen, C.; Pei, H.; Mao, C.; Wang, Y.; He, H.; Huang, L.; Liu, X.; Hu, Z.; Ou, X.; Huang, M.; Yao, J. The discovery of HNPC-A3066: A novel strobilurin acaricide. *Pest Manage. Sci.* **2009**, *65*, 229–234.

(18) Liu, F. M.; Wang, B. L.; Lu, W. J. Synthesis of trifluoromethyl-substituted 1,2,4-triazole mannich bases. *Chin. J. Org. Chem.* **2000**, *20*, 738–742.

(19) He, F. Q.; Liu, X. H.; Wang, B. L.; Li, Z. M. Synthesis and biological activity of new 1-[4-(substituted)-piperazin-1-ylmethyl]-1*H*-benzotriazole. *J. Chem. Res.* **2006**, *12*, 809–811.

(20) George, T.; Mehta, D. V.; Tahilramani, R.; David, J.; Talwalker, P. K. Synthesis of some s-triazoles with potential analgesic and antiinflammatory activities. *J. Med. Chem.* **1971**, *14*, 335–338.

(21) Adams, R. R.; Whitmore, F. C. Heterocyclic basic compounds. IV. 2-Aminoalkylamino-pyrimidines. *J. Am. Chem. Soc.* **1945**, *67*, 735–738.

(22) Zlatoidský, P.; Maliar, T. Synthesis of 1-(4-acyloxybenzoyloxyacetyl)-4-alkylpiperazines and 1-(4-acyloxybenzoyl)-4-alkylpiperazines as inhibitors of chymotrypsin. *Eur. J. Med. Chem.* **1996**, *31*, 669–673.

(23) Matsumoto, K.; Hashimoto, S.; Minatogawa, K.; Munakata, M.; Otani, S. Synthesis of linked heterocycles mediated by high pressure SNAr reactions. *Chem. Express* **1990**, *5*, 473–476.

(24) Shi, Y. X.; Yuan, L. P.; Zhang, Y. B.; Li, B. J. Fungicidal activity of novel fungicide chlorphenomizole and efficacy in controlling main diseases on vegetable in field. *Chin. J. Pestic. Sci.* **2007**, *9*, 126–130.

(25) Zhao, W. G.; Wang, J. G.; Li, Z. M.; Yang, Z. Synthesis and antiviral activity against tobacco mosaic virus and 3D-QSAR of α-substituted-1,2,3-thiadiazoleacetamides. *Bioorg. Med. Chem.* **2006**, *16*, 6107–6111.

(26) Liu, X.-H.; Shi, Y.-X.; Ma, Y.; He, G.-R.; Dong, W.-L.; Zhang, C.-Y.; Wang, B.-L.; Wang, S.-H.; Li, B.-J.; Li, Z.-M. Synthesis of

some *N*,*N*′-diacylhydrazine derivatives with radical scavenging and antifungal activity. *Chem. Biol. Drug Des.* **2009**, *73*, 320–327.

(27) Bush, B. L.; Nachbar, R. B. Sample-distance partial least squares PLS optimized for many variables, with application to CoMFA. *J. Comput.-Aided Mol. Des.* **1993**, *7*, 587–619.

(28) Wu, T. X.; Li, Z. J.; Zhao, J. C. A facile method for the synthesis of 4-amino-5-hydrocarbon-2,4-dihydro-3*H*-1,2,4-triazole-3-thione schiff bases. *Chem. J. Chin. Univ.* **1998**, *19*, 1617–1619.

(29) Wang, B.-L.; Duggleby, R. G.; Li, Z.-M.; Wang, J.-G.; Li, Y.-H.; Wang, S.-H.; Song, H.-B. Synthesis, crystal structure and hebricidal activity of mimics of intermediates of the KARI reaction. *Pest Manage. Sci.* **2005**, *61*, 407–412.

(30) Chen, Q.; Zhu, X. L.; Jiang, L. L.; Liu, Z. M.; Yang, G. F. Synthesis antifungal activity and CoMFA analysis of novel l,2,4-triazolo[1,5-*a*]pyrimidine derivatives. *Eur. J. Med. Chem.* **2008**, *43*, 595–603.

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