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Synthesis, Structure and Biological Activity of Novel 1,2,4-Triazole Mannich Bases Containing a Substituted Benzylpiperazine Moiety

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A series of novel Mannich bases with trifluoromethyl-1,2,4-triazole and substituted benzylpiperazine moieties were synthesized. Their structures were confirmed by IR, ^{1}H NMR and elemental analysis. The single crystal structure of compound 4r was also determined. The preliminary bioassays showed that most of the lead compounds had low herbicidal activity against *Brassica campestris*, *Echinochloa crusgalli*, and KARI enzyme. However, most of them exhibited significant fungicidal activity at the dosage of 50 μg/mL toward five test fungi. Among the 18 novel compounds, several showed superiority over the commercial fungicide Triadimefon against *Cercospora arachidicola* and *Fusarium oxysporum* f. sp. *cucumerinum* during this study. Meanwhile, some compounds displayed plant growth regulatory activity at the dosage of 10 μg/mL.

Key words: 1,2,4-triazole, benzylpiperazine, fungicidal activity, herbicidal activity, Mannich base, plant growth regulatory activity

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Sulfur- and nitrogen-linked heterocyclic compounds have received considerable attention in recent times because of their medicinal and pesticidal importance (1–4). Triazoles, like many other five-membered heterocyclic compounds are used very often in pharmacological, medicinal, and agricultural applications. The compounds containing a triazole ring, such as 1-(substituted phenyl)-3-(1-alkoxy carboxyl alkoxy)-1,2,4-1*H*-triazoles have shown a versatility and useful biological properties, and they have been developed as fungicides, herbicides, or plant growth regulators (PGRS) (5). The incorporation of different active functional groups into triazole ring was proved to be a good way to produce novel active pesticides (6).

In recent years, many 1,2,4-triazole derivatives such as Diniconazole, Triadimefon, Triadimenol, Flusilazole, Fluconazole, Itraconazole, and so on (Figure 1) have been found and developed as fungicides. These compounds represent the most important category of fungicides to date and have excellent protective, curative, and eradicant power toward a wide spectrum of crop diseases (7–9). Meanwhile some structures with a 1,2,4-triazole ring also exhibited outstanding herbicidal activity (10,11). Because of their diverse properties, 1,2,4-triazole pesticides may become one of the focuses in the research and development of agrochemicals. Thus, the synthesis of broader spectrum and highly bioactive 1,2,4-triazole compounds has become an active research in agricultural chemistry. Further, piperazine derivatives were also found to possess various biological activities (12–14). Many literature references have shown that Mannich bases possess potent biological activity such as antibacterial, antifungal, anti-inflammatory, antimalarial, and pesticide properties (15–18).

In our previous work, we reported some interesting Mannich bases derived from 1,2,4-triazole Schiff bases (19), and benzotriazoles containing benzylpiperzine ring, which are associated with various useful herbicidal activity (12). In view of all these facts and as continuation of our research on pesticidal important heterocycles, hereby a series of novel Mannich bases with trifluoromethyl-1,2,4-triazole and substituted benzylpiperazine moieties were synthesized, and their herbicidal, fungicidal, and PGR activity were investigated. It was worthy of note that the structures we synthesized here are similar to those of benzotriazoles Mannich bases we reported earlier, during our search for novel inhibitors of KARI (one of the key herbicidal target enzymes involved in the biosynthesis of chain amino acids) (12). So the *in vitro* and *in vivo* herbicidal activity of lead compounds were both investigated. The preliminary biological tests showed that some compounds exhibit significant fungicidal activity and PGR activity.

Experimental

Instruments and materials

The melting points were determined on an X-4 binocular microscope melting point apparatus (Beijing Tech Instrument Co., Beijing, China) and are uncorrected. Infrared spectra were recorded on a Nicolet MAGNA-560 spectrophotometer (Nicolet Instrument Corp., Madison, WI, USA) as KBr tablets. ^{1}H NMR spectra were measured on a Bruker AC-P500 instrument (400 MHz) (Bruker, Fallanden, Switzerland) using TMS as an internal standard and DMSO-d_6 or $CDCl_3$ as solvent. Elemental analysis was performed on a Vario EL elemental analyzer

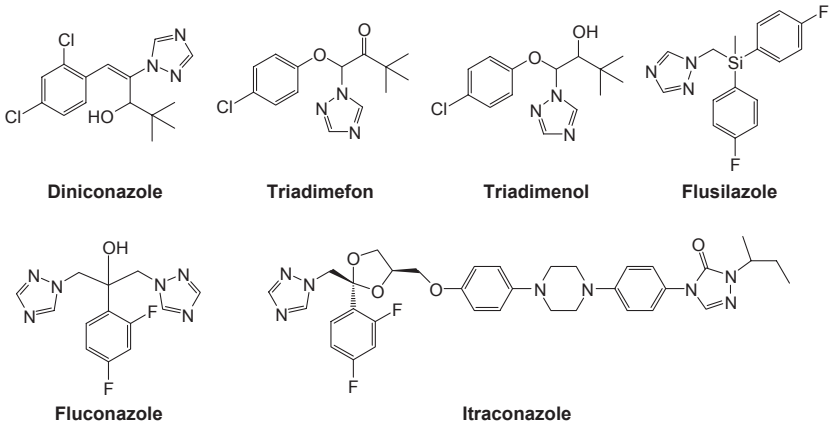


Figure 1: Some representative structures of triazole fungicides.

(Elementar, Hanau, Germany). Crystallographic data of the compound were collected on a Rigaku MM-07 Saturn 724 CCD diffractometer (Rigaku International Corp., Tokyo, Japan). All of the solvents and materials are of analytical grade.

Synthesis

The lead compounds were synthesized according to the route shown in Scheme 1.

Synthesis of compound triazolthiol (2)

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

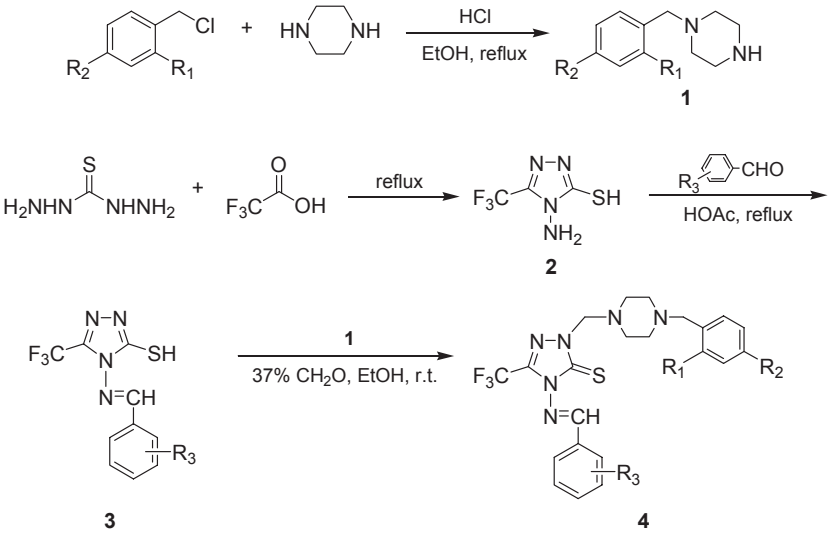
Preparation of 4-(substituted)benzylpiperazine (1)

The procedures are similar to that described previously (21). To a solution of anhydrous piperazine (50 mmol) in 20 mL 96% of ethanol was added conc. HCl (25 mmol). The mixture was stirred under reflux, and substituted benzyl chlorine (25 mmol) was added dropwise for over 5 min. The mixture was refluxed for 4–8 h with TLC monitoring, then left to stand overnight at room temperature. The solid precipitated was filtered and washed with ethanol, the filtrate was evaporated in vacuum, and the residue was dissolved in 30 mL of saturated K_2CO_3 aq., extracted with chloroform (8 mL × 5). The chloroform solution was dried with anhydrous Na_2SO_4 and evaporated in vacuum. The residue was then distilled under reduced pressure to give compound **1** as a colorless liquid.

1a (R_1=R_2=H): yield 58%, bp 131–134 °C/10 mmHg [Lit. bp 154–160 °C/18 mmHg (22)]; **1b** (R_1=H, R_2=Cl): yield 41%, bp 138–141 °C/10 mmHg [Lit. bp 92–96 °C/0.1 mmHg (23)]; **1c** (R_1=R_2=Cl): yield 36%, bp 147–150 °C/6 mmHg [Lit. bp 106–108 °C/0.05 mmHg (23)].

General synthetic procedures for 4-(substituted)benzylideneamino-5-trifluoromethyl-4*H*-1,2,4-triazole-3-thiol (3)

Compound **2** (10 mmol) and aromatic aldehyde (10.5 mmol) were mixed in acetic acid (15 mL). After having been stirred and refluxed



Scheme 1: Synthetic route of title compounds.

for 15 min, the reaction mixture was cooled to room temperature. The resulting crystals were filtered and washed with ethanol to give Schiff base **3**.

3a (R_3=H): white crystal, yield 74%, mp 198–199 °C [Lit. mp 200–201 °C (19)]; ^{1}H 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).

3b (R_3=2-F): white crystals, yield 82%, mp 192–193 °C; ^{1}H 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).

3c (R_3=4-MeO): white crystals, yield 71%, mp 195–196 °C; ^{1}H 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).

3d (R_3=3,4-Me_2): white crystals, yield 73%, mp 205–206 °C; ^{1}H 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 (ds, 6H, CH_3).

3e (R_3=4-Cl): white crystals, yield 72%, mp 208–209 °C; ^{1}H 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).

3f (R_3=2-NO_2): yellow crystals, yield 89%, mp 201–202 °C; ^{1}H 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-substituted-benzylpiperazin-1-yl)methyl]-4-(substituted)benzylideneamino-3-trifluoromethyl-1*H*-1,2,4-triazole-5(4*H*)-thione (4)

Schiff base **3** (1 mmol) and 40% formalin (1.2 mmol) were dissolved in 15 mL of ethanol, and the mixture was stirred at room temperature for 10 min. A solution of substituted benzylpiperazine **1** (1 mmol) in 2 mL ethanol was slowly added to. Then, the mixture was stirred for 2–3 h and placed in a refrigerator overnight. The resulting precipitate was filtered and recrystallized from ethanol to give 1,2,4-triazole Mannich base **4**.

Crystal structure determination

Compound **4r** was dissolved in hot ethyl alcohol, and the resulting solution was allowed to stand in air at room temperature to give single crystal of **4r**. A yellow crystal of **4r** suitable for X-ray diffraction with dimensions of 0.30 × 0.28 × 0.16 mm was mounted on a Rigaku MM-07 Saturn 724 CCD diffractometer with *Mo-K*α radiation (λ = 0.71073 Å) for data collection. A total of 13449 reflections were collected in the range of $2.01 < \theta < 25.02$ by using a phi and scan modes at 113(2) K, of which 4345 were independent with R_{int} = 0.0277. 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 $P2_1/n$ of the monoclinic system with cell parameters: a = 11.884(2) Å, b = 8.6972(17) Å, c = 24.051(5) Å, α = 90°, β = 96.33(3)°, γ = 90°, V = 2470.7(9) Å^3, Z = 4, D_c = 1.544 mg/m^3, μ = 0.406 per mm, and F(000) = 1176. The final refinement converged at R = 0.0315, wR = 0.0814 for 3852 observed reflections with $I > 2\sigma(I)$, where $W = 1/[\sigma^2(F_O^2) + (0.0554P)^2 + 0.2627P]$ with $P = (F_O^2 + 2F_c^2)/3$, S = 1.046, $(\Delta/\sigma)_{max} < 0.0001$, $(\Delta\rho)_{max}$ = 0.252 e/Å^3 and $(\Delta\rho)_{min}$ = −0.312 e/Å^3.

The herbicidal activity

In vivo herbicidal activity of compounds **4a-r** was determined by rape root and barnyardgrass cup tests according to the reported method (24).

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 only in distilled water. The inhibitive rates were calculated from the root length using the following equation:

$$\text{Relative inhibition rate}\,(\%) = [(\text{CK} - \text{PT})/\text{CK}] \times 100\%$$

where CK is the average root length during the blank assay and PT is the average root length after treatment during testing.

Inhibition of the seedling growth of Barnyardgrass (*Echinochloa crusgalli*)

The evaluated compounds were dissolved in water and emulsified if necessary. Ten 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 the above-ground plant parts from each cup were measured and the means were calculated. The check test was carried out only in distilled water. The inhibitive rates were calculated from the plant heights using the following equation:

$$\text{Relative inhibition rate}\,(\%) = [(\text{CK} - \text{PT})/\text{CK}] \times 100\%$$

where CK is the average plant height during the blank assay and PT is the average plant height after treatment during testing.

KARI activity test

The cloning of rice KARI has been described previously (25), and enzyme expression and purification followed that protocol. Protein concentrations were estimated *via* the bicinchoninic acid method

and protein purity was assessed by SDS-PAGE (26,27). KARI activity was measured with a continuous assay method (25), following the consumption of NADPH at 340 nm and 30 °C. Assay solutions contained 0.2 mM NADPH, 1 mM $MgCl_2$, 0.1 mM substrate (2-acetolactate), and inhibitors (**4a-r** and IpOHA), in 0.1 M phosphate buffer (pH 8.0). Inhibitors were preincubated with the enzyme, NADPH, and $MgCl_2$ in phosphate buffer at 30 °C for 10 min. The reaction was then started by adding the substrate. The percentage of the inhibition was calculated using the following equation:

$$\text{Relative inhibition rate}\,(\%) = [(\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}})/\text{Abs}_{\text{blank}}] \times 100\%$$

where $\text{Abs}_{\text{blank}}$ and $\text{Abs}_{\text{sample}}$ are the absorption curve slopes of NADPH at 340 nm with blank and inhibitor in testing solution, respectively.

The fungicidal activity

Fungicidal activity of **4a-r** against *G. zeae* Petch, *Phytophthora infestans* (Mont.) de Bary, *Cercospora arachidicola*, *Botryosphaeria berengeriana* f. sp. *piricola* (Nose) koganezawa et Sakuma, and *Fusarium oxysporum* f.sp. *cucumerinum* were evaluated using the mycelium growth rate test (28).

The method for testing the primary biological activity was performed in an isolated culture. Under a sterile condition, 1 mL of sample was added to the culture plates, followed by the addition of 9 mL of culture medium. The final mass concentration was 50 μg/mL. The blank assay was performed with 1 mL of sterile water. Circle mycelium with a diameter of 4 mm was cut using a drill. The culture plates were cultivated at 24 ± 1 °C. The extended diameters of the circle mycelium were measured after 72 h. The relative inhibition rate of the circle mycelium compared to blank assay was calculated *via* the following equation:

$$\text{Relative inhibition rate}\,(\%) = [(d_{\text{ex}} - d_{\text{ex}})/d'_{\text{ex}}] \times 100\%$$

where d_{ex} is the extended diameter of the circle mycelium during the blank assay; and d'_{ex} is the extended diameter of the circle mycelium during testing.

The blank test was made using acetone. Three replicates were performed. The fungicidal tests results were given in Table 1.

The plant growth regulatory activity

The PGR activity of compounds **4a**-**r** was evaluated by means of cucumber cotyledon test according to a reported procedure (29). The cucumber seeds (JINKE, No. 4) were supplied by the Biological Assay Center, Nankai University, China. These seeds were incubated at 24 °C in a dark room for 3 days and 10 pieces of cotyledons of the same size were selected. The test samples were dissolved in *N,N*-dimethylformamide (DMF) at a concentration of 10 μg/mL. A sample solution (0.3 mL) was sprayed over a filter paper (6 cm diameter), and solvent was volatilized to dryness on air. The filter paper thus prepared was placed into an incubation vessel (6 cm diameter) and soaked with distilled water (3 mL). Finally, 10 pieces of cotyledons were added. These cotyledons were incubated at 24 °C in a dark room for 3 days. The rhizogenesis numbers of every 10 pieces of hypocotyls were measured. Each treatment was performed three times. In contrast, the distilled water was used as a control. The relative ratios of cucumber cotyledon rhizogenesis were calculated according to the following formula:

$$\text{Relative ratio}\,\% = (N_S - N_C)/N_C \times 100\%$$

where N_S and N_C are the numbers of cucumber cotyledon rhizogenesis of tested compound and control experiment, respectively.

Results and Discussion

Synthesis

The synthesis procedures for compound **4** were shown in Scheme 1. Based on an acetic acid–solvent method reported by Wu *et al.* for the condensation of amine and aldehyde (30), Schiff base **3**, namely 4-amino-5-trifluoromethyl-4*H*-1,2,4-trizole-3-thiol, was prepared successfully with high yield and short reaction time. The Mannich reaction of **3** with formaldehyde and substituted benzylpiperazine **1** in ethanol at room temperature led to novel trifluoromethyl-substituted 1,2,4-triazole Mannich base **4** in 44–83% yield. Compounds **4** were identified by melting point, ^{1}H NMR and IR spectra. The measured elemental analyses were also consistent with the corresponding calculated ones (see Supporting Information).

Proton magnetic resonance spectra

The proton magnetic resonance spectra of the Mannich bases have been recorded in $CDCl_3$ and those of the Schiff bases in DMSO-d_6. A comparison of the spectra of the products with the intermediates leads to the following conclusions: The –CH=N proton appeared at δ 9.73–10.88 as a singlet in the Schiff base **3**, which shifted downfield to δ 10.07–11.21 in Mannich bases **4**. The Schiff bases **3** can exist either as a thione or the thiol tautomeric forms or as an equilibrium mixture of both forms, because they have a thioamide, -NH-C(=S) function. The chemical shift at δ 14.84–14.96 as a singlet in **3** may be because of SH proton, indicating that **3** existed not as thione but as the thiol tautomeric forms in solution (31). In the Mannich bases **4**, neither a NH signal nor a thiol SH signal is visible. The signal of CH_2 protons neighboring to the triazole ring was observed at δ approximately 5.22 as a singlet, and the substituted benzyl CH_2 proton appeared at δ 3.45–3.56 as a singlet in Mannich bases **4**. The chemical shifts at 2.86–2.88 ppm and 2.46–2.54 ppm may be because of piperazine ring protons, which were appeared as two broad singlets, respectively. The signal because of benzene ring protons appears in the region δ 6.98–8.23 in the spectra of all products and intermediates.

Infrared spectra

The spectra of Mannich bases **4a**, **4g**, and **4m** showed bands at 1601–1600 per cm for C=N stretching. The characteristic stretching vibrations ν (C-F) and ν (C=S) appears at 1320–1318 per cm, 1197–1194 per cm and 1166–1154 per cm, respectively.

Table 1: The fungicidal and plant growth regulatory activity of compounds **4a-r**

	Growth inhibition (%, 50 μg/mL)					
Compound	*G. zeae* Petch	Phytophthora infestans *(Mont.) de Bary*	Cercospora arachidicola	*Botryosphaeria berengeriana* f. sp. *piricola* (Nose) koganezawa et Sakuma	*Fusarium oxysporum* f.sp. *cucumerinum*	Rhizogenesis (%, 10 μg/mL)
4a	33.2 ± 2.7	21.0 ± 1.8	70.3 ± 1.9	10.8 ± 2.4	10.1 ± 1.1	4.6 ± 1.8
4b	50.1 ± 2.1	60.4 ± 1.2	70.4 ± 2.4	70.1 ± 1.4	80.4 ± 2.8	68.6 ± 3.2
4c	0	0	0	0	18.7 ± 2.2	10.4 ± 2.0
4d	0	0	19.5 ± 1.3	0	0	109.3 ± 3.4
4e	0	10.3 ± 0.8	50.3 ± 2.7	0	0	97.6 ± 1.7
4f	50.2 ± 4.1	53.1 ± 3.5	72.3 ± 3.2	20.1 ± 3.3	40.2 ± 1.6	62.7 ± 2.8
4g	0	24.3 ± 2.0	50.4 ± 2.3	19.6 ± 1.4	50.9 ± 3.2	-12.7 ± 1.1
4h	50.3 ± 2.9	51.7 ± 2.1	70.6 ± 3.4	52.6 ± 4.0	71.9 ± 3.1	51.1 ± 2.3
4i	11.2 ± 2.0	19.7 ± 2.3	50.7 ± 2.9	10.0 ± 0.9	60.4 ± 3.4	16.2 ± 1.7
4j	0	19.6 ± 2.0	44.5 ± 3.3	0	40.1 ± 3.1	27.9 ± 2.8
4k	30.6 ± 2.5	30.1 ± 3.2	60.7 ± 3.0	0	30.3 ± 1.8	39.5 ± 2.6
4l	18.8 ± 2.3	19.8 ± 2.1	70.2 ± 3.4	30.0 ± 1.9	50.2 ± 2.4	16.4 ± 1.3
4m	50.3 ± 2.6	51.8 ± 4.5	70.4 ± 4.1	30.5 ± 2.2	70.8 ± 3.1	39.5 ± 2.3
4n	50.0 ± 2.7	50.4 ± 2.3	60.2 ± 2.9	41.5 ± 1.2	82.6 ± 1.8	45.3 ± 1.6
4o	51.8 ± 2.4	36.0 ± 1.3	50.2 ± 3.3	0	50.4 ± 3.0	22.0 ± 1.8
4p	50.7 ± 3.6	50.9 ± 3.9	80.8 ± 2.0	70.3 ± 3.2	70.5 ± 2.8	62.9 ± 3.0
4q	30.0 ± 2.1	50.5 ± 2.8	70.4 ± 3.4	70.6 ± 3.7	60.7 ± 2.9	10.2 ± 0.7
4r	30.5 ± 1.8	30.2 ± 2.4	63.6 ± 3.8	41.6 ± 2.8	60.8 ± 3.1	45.3 ± 3.3
Triadimefon	72.6 ± 3.6	66.1 ± 2.7	73.4 ± 2.3	86.7 ± 3.2	81.5 ± 2.6	50.2 ± 3.4

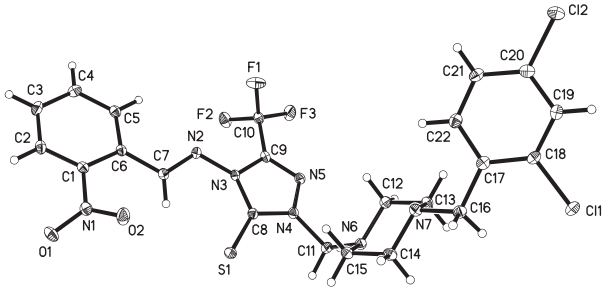


Figure 2: Molecular structure of compound **4r**.

Crystal structure

The structure of compound **4r** was further confirmed by single-crystal X-ray diffraction analysis (Figure 2). From the molecular structure, it can be seen that both groups on the N atoms of piperazine ring (triazole-CH_2 and 2,4-dichlorophenyl-CH_2) are in the e-bond positions of chair conformation in the six-member ring. The dihedral angel between 2-nitrobenzene ring and triazole ring is 2.6°, which indicates the two rings are almost coplanar in the molecular structure. The X-ray analysis also reveals that in this typical compounds **4r**, the substituted benzene ring and triazole ring are on the opposite sides of the C=N double bond (Figure 2). The torsion angle of C(6)-C(7)-N(2)-N(3) is 177.33°, which indicates that the C=N double bond is in the (*E*)-configuration.

Herbicidal activity

To investigate the KARI inhibitory activity and herbicidal activity of the compounds referring to those of benzotriazoles Mannich bases, we reported before during our search for novel KARI inhibitors (12), *N*-hydroxy-*N*-isopropyloxamate (IpOHA) (32), a potent inhibitor of KARI *in vitro*, was used as a control. As shown in Table 2, compounds **4m** and **4r** showed higher inhibition abilities of rape root at a concentration of 100 μg/mL. Compound **4a** showed obvious inhibitory activity to the rice KARI enzyme with an inhibition of 76.6%. Except that, most of compounds exhibited weak herbicidal activity *in vivo* and *in vitro* against rape and barnyardgrass and KARI enzyme.

Fungicidal activity

The *in vitro* fungicidal results of the Mannich bases **4a-r** in inhibiting the mycelial growth of five test fungi were listed in Table 1. The results of preliminary bioassays were compared with that of a commercial fungicide Triadimefon. As indicated in Table 1, some compounds exhibited potential fungicidal activity. For example, compounds **4b**, **4f**, **4h**, and **4m**-**4p** possess approximately 50% inhibitory rates against *G. zeae* Petch. Also compound **4b** held a 60.4% inhibitory rate against *Phytophthora infestans* (Mont.) de Bary. It was observed that most of the compounds exhibited significant inhibition effect against *Cercospora arachidicola* (inhibition rates of 50.2–80.8%) and the inhibitory effect of **4p** (80.8%) on *Cercospora arachidicola* was better than that of the control Triadimefon (73.4%). For *Botryosphaeria berengeriana* f. sp. *piricola* (Nose) koganezawa et Sakuma, compounds **4b**, **4h**, **4p,** and **4q** showed favorable activity and held 70.1, 52.6, 70.3, and 70.6% inhibitory rates, respectively. In addition, almost half of the compounds exhibited good inhibition effect against *Fusarium oxysporum* f. sp. *cucumerinum* (inhibition rates of 60.4–82.6%). Especially, compound **4b** and **4n** showed 80.4 and 82.6% inhibi-

Table 2: Herbicidal activities data of title compounds (% inhibition)

Compound	R_1	R_2	R_3	Rape root test (*Brassica campestris*) 100 μg/mL (10 μg/mL)	Barnyardgrass cup test (*Echinochloa crusgalli*) 100 μg/mL (10 μg/mL)	KARI test 100 μg/mL
4a	H	H	H	27.8	10.4	76.6
4b	H	H	2-F	10.7	15.2	21.3
4c	H	H	4-MeO	17.0	5.3	9.7
4d	H	H	3,4-Me_2	0	5.0	0
4e	H	H	4-Cl	2.2	30.2	1.1
4f	H	H	2-NO_2	17.4	10.1	1.9
4g	H	Cl	H	31.4	15.2 (5.1)	0
4h	H	Cl	2-F	30.1 (10.3)	20.4	0
4i	H	Cl	4-MeO	0.9	30.2	–
4j	H	Cl	3,4-Me_2	7.6	10.1 (5.0)	21.6
4k	H	Cl	4-Cl	39.3	15.3 (5.2)	15.9
4l	H	Cl	2-NO_2	27.8	15.4	17.5
4m	Cl	Cl	H	50.2	5.1	22.8
4n	Cl	Cl	2-F	38.1	15.4	12.1
4o	Cl	Cl	4-MeO	33.4	20.4 (10.2)	9.57
4p	Cl	Cl	3,4-Me_2	13.9	25.4 (5.3)	28.8
4q	Cl	Cl	4-Cl	7.2	15.1	28.0
4r	Cl	Cl	2-NO_2	48.0	20.4 (5.2)	30.0
IpOHA				71.6 (50.2)	14.4	99.5

–, Indicates the compound cannot be dissolved in our test system, so no data obtained.

tory activity against *Fusarium oxysporum* f.sp. *cucumerinum*, respectively, and the effects of which were comparable to that of the control (81.5%). It was worthy of note that in these Mannich bases, compounds with two chlorine atoms on benzene ring of benzyl group (at 4-position of piperazine ring), exhibited more obvious activity than others.

Plant growth regulatory activity

Plant growth regulatory (PGR) activities are defined here as those effects produced by the compound that leaves the numbers of cucumber cotyledon rhizogenesis indistinguishable from the untreated control in the same testing condition. The positive value of activity data means the compound exhibits potential promoting activity and can be an activator for the plant growth, while the negative value means the compound exhibits potential inhibitory activity and can be an inhibitor for the plant growth. The PGR activity of the lead compounds **4a-r** was screened by cucumber cotyledon test at a concentration of 10 μg/mL. As shown in Table 1, some compounds, such as **4b**, **4d**, **4e**, **4f**, **4h**, and **4p**, exhibited better promoting activity than Triadimefon, with values of 68.6, 109.3, 97.6, 62.7, 51.1, and 62.9%, respectively. These results were consistent with those of their low *in vivo* herbicidal activity based on *Brassica campestris* and *Echinochloa crusgalli* tests at a dose of 10 μg/mL (almost no activity), referring to the herbicidal activity data listed in Table 2. Compound **4g** displayed weak inhibitory activity for the plant growth, with values of −12.7%, while the same effect could also be observed in its herbicidal activity assays.

It was found that compounds without substituent in the benzene ring of the benzyl group (R_1=R_2=H) almost have a higher level of promoting activity for plant growth than others (R_1=H, R_2=Cl and R_1=R_2=Cl). For herbicidal activity of the lead compound, compounds with one or two chloro group(s) in the benzene ring of the benzyl group showed relatively favorable activity than others; however, the extent of the herbicidal effect was varying with concentration. On the whole, the promoting activity of the lead compounds is mainly for plant growth. Among these compounds, 1-[(4-benzylpiperazin-1-yl)methyl]-4-(3,4-dimethyl)benzylideneamino-3-trifluoromethyl-1*H*-1,2,4-triazole-5(4*H*)-thione (**4d**) was the most effective and least inhibitory to plant and could be a favorable activator of plants growth at a low dose of concentration.

Based on these preliminary studies, the fungicidal activity was outstanding for this series of compounds. It appeared prior to the synthesis and testing of substituted derivatives that the fungicidal activity might be influenced by steric factors. Other novel Mannich bases derive from Schiff bases containing various alkyl substituents, or other aryl groups, such as heterocycles, may result in novel fungicides. Anyhow, it is important for their chemical structure that both two chloro groups are in the benzene ring of the benzyl group of such kind of compounds.

In summary, we have conveniently synthesized a series of trifluoromethyl-substituted 1,2,4-triazole Mannich bases containing substituted benzylpiperazine ring *via* Mannich reaction in good yields. The preliminary bioassays showed that most of the compounds had low herbicidal activity against *Brassica campestris*, *Echinochloa crusgalli*,

and KARI enzyme. However, most of them exhibited significant fungicidal activity at the dosage of 50 μg/mL toward five test fungi. Among the 18 novel compounds, several showed superiority over the commercial fungicide Triadimefon against *Cercospora arachidicola* and *Fusarium oxysporum* f.sp. *cucumerinum* during this study and could be further developed as fungicides. Meanwhile, some compounds displayed PGR activity at the dosage of 10 μg/mL in the preliminary studies.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Analytical data for compounds **4**.

Table S2. ^{1}H NMR spectral data of compounds **4**.

Table S3. IR spectral data of compounds **4**.

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