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## DESIGN, SYNTHESIS AND *IN VITRO* ANTIFUNGAL EVALUATION OF NOVEL TRIAZOLE DERIVATIVES

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**Abstract** – As a part of our continuing research on triazole derivatives antifungal agents, 10 novel target compounds containing 1,2,4-triazole moiety were synthesized and characterized by the spectroscopic analysis, and their *in vitro* antifungal activities against four phytopathogenic fungi were assayed systematically. Especially, compound **3c** displayed excellent activity against *F. graminearum* with the median effective concentration value (EC<sub>50</sub>) of 5.03 µg/mL, and the value was extremely close to that of tebuconazole (EC<sub>50</sub> = 3.13 µg/mL). Generally, for carbonyl compounds containing morpholine moiety, introducing 4-F to benzene ring obviously improved activities against most of tested fungi in varying degree.

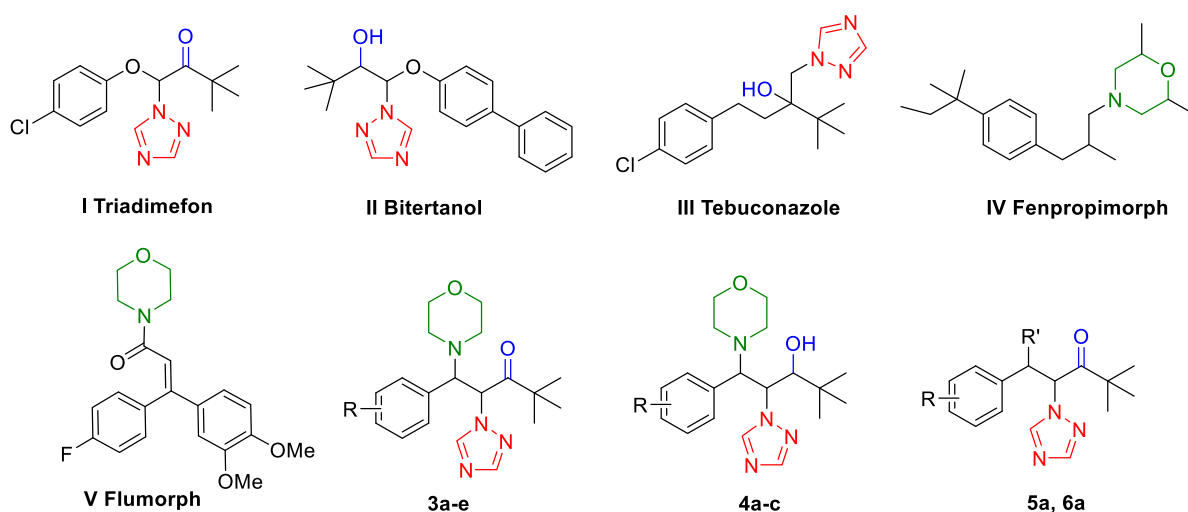
## INTRODUCTION

Various diseases caused by phytopathogenic fungi have been one of the most severe concerns in a wide range of field.<sup>1</sup> More severely, many of the phytopathogenic fungi can produce mycotoxins harmful to animal and human health.<sup>2-4</sup> To date, the application of plant fungicide is considered as an efficient measure for preventing and controlling fungal diseases.<sup>5,6</sup> Nevertheless, long-term use of the available traditional fungicides, such as carbendazim, isothiazolinone, benzimidazoles and dicarboximides, not only leads to increasing resistance, but also results in harm to the environment and plants.<sup>7</sup> Therefore, development of a novel, highly active, environmentally friendly and promising antifungal agent is urgently required.

Agriculture today, various synthesized fungicides have been extensively developed and commercially available.<sup>8</sup> The representatives are triazole fungicides, such as triadimefon, bitertanol, tebuconazole and

so on (**Figure 1, I-III**). As an important class of sterol biosynthesis inhibitors, they are of great significance to protect crops, and have achieved a unique position in the chemical control of fungal diseases since their discovery in the late 1960 s.<sup>8,9</sup>

Another important type is morpholine fungicides, like fenpropimorph, flumorph, and dimethomorph, etc. (**Figure 1, IV, V**), whose action mechanism is thought to be inhibiting biosynthesis of ergosterol or affecting the formation of cell wall of fungi.<sup>10</sup> Because of their features of high efficiency and broad spectrum, these fungicides attracted great attention of many researchers, and have been used for crop protection currently.



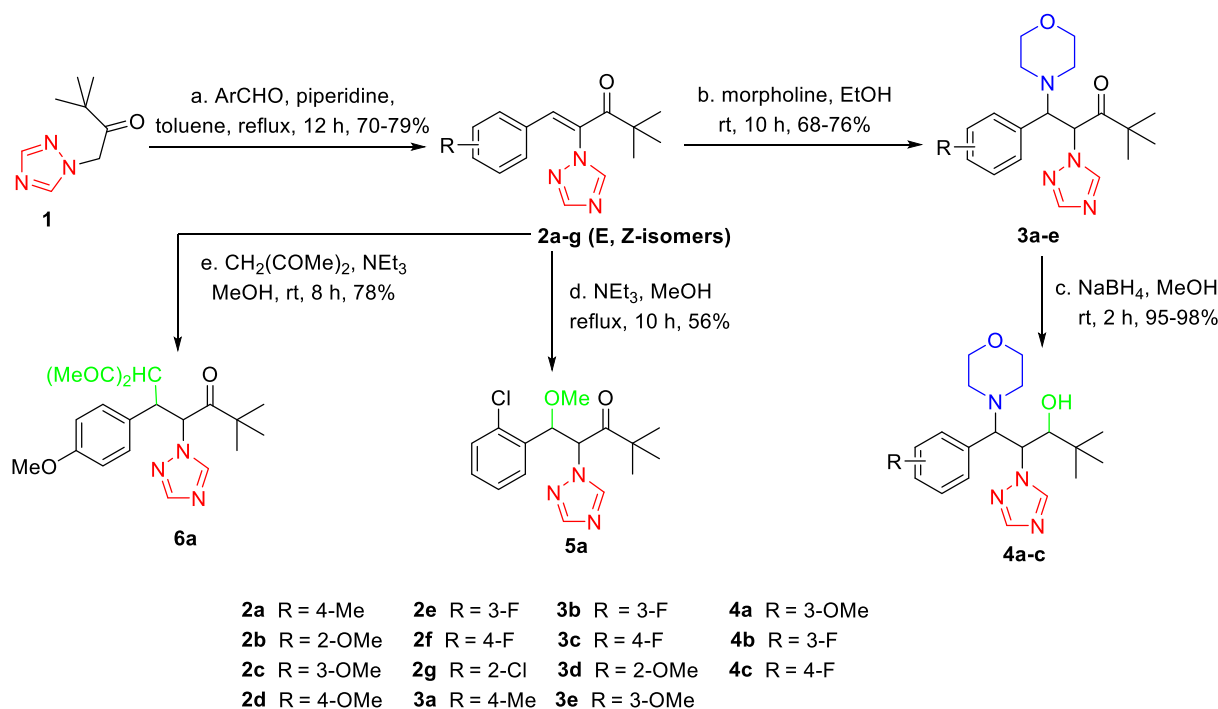
**Figure 1.** Chemical structures of the representative types of triazole fungicides (**I-III**), morpholine fungicides (**IV, V**), and target compounds (**3a-6a**)

Considering the positive effects of the two type of fungicides described above, in this study, we combined the active structure of triazole fungicides with morpholine or other active groups to design and synthesize a series of novel triazole derivatives (**Figure 1, 3a-6a**), aiming for obtaining compounds possessing more potent antifungal activities, especially against resistant fungi, as well as establishing their structure–activity relationships (SAR). Based on this reason, antifungal activity of the derivatives were evaluated systematically. So far, no systematic research on the synthesis and bioactivity of these compounds was found.

## RESULTS AND DISCUSSION

The general synthetic route of target compounds was illustrated in **Scheme 1**. Commercially available 1,2,4-triazol-1-ylpinacolone (**1**) was used as a starting material to react with substituted benzaldehyde, in

the presence of piperidine, and toluene was chosen as the solvent under reflux conditions to prepare compounds **2a-g** in 70-79% yields. Next, compounds **2a-g** were severally reacted with morpholine in ethanol to give desired compounds **3a-e**, which further reacted with sodium borohydride to afford the corresponding secondary alcohols **4a-c**. Meanwhile, compound **2g** reacted with solvent (methanol) under reflux condition in the presence of triethylamine to afford compound **5a**. Finally, compound **6a** was also obtained through the above same type reaction.



**Scheme 1.** Synthetic route of novel triazole derivatives (**3a-6a**)

According to the mycelium linear growth rate method reported by us,<sup>11</sup> all target compounds **3a-e**, **4a-c**, **5a**, **6a** were screened for antifungal activity *in vitro* at 50 µg/mL against four plant pathogenic fungi (*F. solani*, *F. graminearum*, *F. oxysporum*, *C. lunata*). Tebuconazole (≥ 99%), a commercial fungicide, was used as the positive control. The results of preliminary antifungal activity of compounds were listed in **Table 1**, and the results showed all the compounds displayed the activity in varying degrees against each of the tested fungi. In general, compounds **3c** and **5a** exhibited higher activity against almost all tested plant pathogenic fungi than other tested compounds. Especially for *F. graminearum*, compound **3c** exhibited satisfying activity with the inhibition rate of 81.3%, which was closed to that of tebuconazole (97.8%). Moreover, compound **3c** showed inhibitory activities against all tested plant pathogenic fungi with inhibition rates over 50%, which exhibited the broader antifungal spectrum.

**Table 1.** Preliminary antifungal activity of novel triazole derivatives against 4 fungi at 50 µg/mL in 72 h and the corresponding EC<sub>50</sub> values

Compounds		Average inhibition rate ± SD (%) (n=3)				EC <sub>50</sub> <sup>a)</sup>
No.	R	<i>F. solani</i>	<i>F. graminearum</i>	<i>F. oxysporum</i>	<i>C. lunata</i>	µg/mL
<b>3a</b>	4-Me	57.9 ± 4.0	53.5 ± 2.1	43.3 ± 1.3	47.1 ± 2.0	/
<b>3b</b>	3-F	37.1 ± 0.9	36.9 ± 0.9	49.4 ± 1.7	42.2 ± 1.2	/
<b>3c</b>	4-F	68.4 ± 1.9	<b>81.3 ± 2.7</b>	53.3 ± 2.2	54.2 ± 1.9	5.03
<b>3d</b>	2-OMe	44.4 ± 2.1	43.8 ± 1.9	32.1 ± 1.0	41.2 ± 3.1	/
<b>3e</b>	3-OMe	52.8 ± 2.7	30.0 ± 1.1	49.8 ± 1.5	44.5 ± 2.7	/
<b>4a</b>	3-OMe	21.4 ± 0.7	29.9 ± 1.1	27.5 ± 1.5	30.1 ± 2.7	/
<b>4b</b>	3-F	27.7 ± 1.0	13.5 ± 0.6	32.0 ± 1.9	28.6 ± 2.3	/
<b>4c</b>	4-F	19.3 ± 1.5	40.1 ± 1.5	30.6 ± 2.3	32.5 ± 1.6	/
<b>5a</b>	/	52.8 ± 1.8	54.7 ± 2.9	53.3 ± 2.0	47.1 ± 1.0	/
<b>6a</b>	/	21.4 ± 0.9	46.3 ± 2.6	34.8 ± 1.9	15.3 ± 1.3	/
Tebuconazole		95.0 ± 1.2	97.8 ± 0.5	76.9 ± 3.5	85.2 ± 2.0	3.13

<sup>a)</sup> EC<sub>50</sub> values of compound **3c** and tebuconazole against *F. graminearum*.

In order to further explore the antifungal potential and structure–activity relationship, the compound **3c** with inhibition rates >80% at 50 µg/mL was further examined to determine their median effective concentrations (EC<sub>50</sub>) against *F. graminearum*. The results were shown in the right column of **Table 1**. From the right column, it was clearly seen that compound **3c** displayed excellent activity against *F. graminearum* with EC<sub>50</sub> value of 5.03 µg/mL, and the value was extremely close to that of tebuconazole (EC<sub>50</sub> = 3.13 µg/mL), the positive control.

By comparing the inhibition rates of all the target compounds at 50 µg/mL in **Table 1**, it was obviously seen that almost all of the triazole derivatives possessed higher activity against most of the tested fungi. Furthermore, the results described above revealed that introduction of different substituents to benzene ring significantly influenced the antifungal activities. Generally, for carbonyl compounds containing morpholine moiety, introducing 4-F to benzene ring obviously improved activities against most of tested fungi in varying degree.

In conclusion, a series of novel triazole derivatives were synthesized and determined by the spectra analysis, and their antifungal activity was assayed systematically. All the synthesized compounds showed the obvious growth inhibition activity against all the tested fungi. Among them, compound **3c** showed the highest activity against *F. graminearum* with an EC<sub>50</sub> value of 5.03 µg/mL, and the value was extremely close to that of tebuconazole (EC<sub>50</sub> = 3.13 µg/mL). SAR analysis showed that the type as well as position of substituents on benzene ring could make significant effects on the activity as mentioned above.

## EXPERIMENTAL

### General

Tebuconazole ( $\geq 99\%$ ), a commercial fungicide, was purchased from Yi Fang Biotechnology Co. Ltd. (Zhejiang, China); Commercially available 1,2,4-triazol-1-ylpinacolone (**1**) was used as a starting material, was purchased from Jiangsu Yancheng Chemical Factory (Jiangsu, China). Other reagents and solvents were obtained locally and of analytical grade or purified according to standard methods before use. The water used was redistilled and ion-free. The plant pathogenic fungi (*Fusarium. solani*, *Fusarium. graminearum*, *Fusarium. oxysporum*, *Curvularia. lunata*) were provided by the Center of Pesticide Research, Northwest A&F University, China. The silica gel and GF<sub>254</sub> silica gel of analytical thin-layer chromatography (TLC) were produced by the Qingdao Haiyang Chemical Co., Ltd., which we utilized during the experiment procedure. Melting points (mp) were determined on an XT-4 micro-melting point apparatus and uncorrected. Infrared spectra (IR) were performed on a Bruker TENSOR 27 spectrometer with KBr disks. Nuclear magnetic resonance spectra (NMR) were recorded on a Bruker Avance 300 or 400 MHz instrument using tetramethylsilane (TMS) as the internal standard. Chemical shifts ( $\delta$  values) and coupling constants ( $J$  values) are given in parts per million and hertz, respectively. High-resolution mass spectra (HRMS) were carried out with an AB SCIEX Triple TOF 5600<sup>+</sup> spectrometer (AB SCIEX, Boston, MA, USA).

### General procedure for the preparation of compounds **2a-g**

Based on a previously reported method with modification.<sup>12</sup> To a solution of 1,2,4-triazol-1-ylpinacolone **1** (8.36 g, 50.0 mmol) and 4-methylbenzaldehyde (6.60 g, 55.0 mmol) in toluene (50 mL) was slowly added piperidine (0.50 mL, 5.46 mmol) and stirred uniformly in a round-bottomed flask. Then, the mixture was heated to reflux for 12 h until the reaction was completed according to TLC detection. Next, concentrated in vacuo to give the crude product. Afterwards, the crude product was dissolved in EtOAc (30 mL), followed by washing with 5% glacial acetic acid (30 mL), brine (30 mL), and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. After filtration and removal of the solvent, the residue was purified by column chromatography on silica gel to produce the key intermediate compound **2a** (9.43 g, yield 70%). Similarly, compounds **2b-g** were synthesized according to the above procedure.

### General procedure for the preparation of compounds **3a-e**

To a stirred solution of  $\alpha$ ,  $\beta$ -unsaturated ketone **2a** (538 mg, 2.0 mmol) in absolute EtOH (10 mL) was added morpholine (0.26 mL, 3.0 mmol) dropwise, then the resulting mixture was stirred about 10 h at room temperature until the reaction was completed. Next, the solvent was removed under vacuum to obtain the crude product, followed by adding 95% EtOH (5 mL), and then recrystallized to produce the key compound **3a** (541 mg, yield 76%). Similarly, compounds **3b-e** were synthesized according to the above procedure. Exemplary data of compounds **3a-e** were provided in the Characterization.

**General procedure for the preparation of compounds 4a-c**

To a stirred solution of **3e** (372 mg, 1.0 mmol) in MeOH (5 mL) was added slowly sodium borohydride (57 mg, 1.5 mmol), then the reaction was stirred for 2.0 h at room temperature until the reaction was completed. Next, the solvent was removed under vacuum to obtain the crude product, the crude product was dissolved in EtOAc (10 mL), followed by washing with brine (10 mL), and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. After filtration and removal of the solvent, the residue was purified by column chromatography on silica gel to produce the desired compound **4a** (355 mg, yield 95%). Similarly, compounds **4b-c** were synthesized according to the above procedure. Exemplary data of compounds **4a-c** were provided in the Characterization.

**General procedure for the preparation of compounds 5a, 6a**

To a stirred solution of  $\alpha$ ,  $\beta$ -unsaturated ketone **2g** (289 mg, 1.0 mmol) in MeOH (5 mL) was added triethylamine (15  $\mu$ L, 0.1 mmol) dropwise, then the resulting mixture was stirred and heated to reflux about 10 h until the reaction was completed. Next, the solvent was removed under vacuum to obtain the residue, the residue was further purified by column chromatography on silica gel to produce the desired compound **5a** (180 mg, yield 56%). Similarly, compound **6a** was synthesized according to the above procedure. Exemplary data of compounds **5a, 6a** were provided in the Characterization.

**Characterization**

Compound **3a**: Yield: 76%, white crystals, mp 171-173 °C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 8.24 (s, 1H), 7.93 (s, 1H), 7.27-7.15 (m, 2H), 7.10-7.07 (m, 2H), 6.08 (d,  $J$  = 11.6 Hz, 1H), 4.39 (d,  $J$  = 11.6 Hz, 1H), 3.48-3.42 (m, 2H), 3.35-3.30 (m, 2H), 2.53-2.46 (m, 2H), 2.34 (s, 3H), 2.30-2.23 (m, 2H), 0.88 (s, 9H); IR (KBr, cm<sup>-1</sup>): 2956, 1723, 1507, 1268, 1115, 892; HRMS (ESI) calcd for C<sub>20</sub>H<sub>29</sub>N<sub>4</sub>O<sub>2</sub><sup>+</sup> [M+H]<sup>+</sup>: 357.2285, found 357.2281.

Compound **3b**: Yield: 70%, colorless crystals, mp 158-160 °C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 8.23 (s, 1H), 7.96 (s, 1H), 7.39-7.29 (m, 1H), 7.08-7.05 (m, 1H), 7.05-6.99 (m, 1H), 6.96-6.91 (m, 1H), 6.07 (d,  $J$  = 11.5 Hz, 1H), 4.45 (d,  $J$  = 11.5 Hz, 1H), 3.50-3.43 (m, 2H), 3.38-3.32 (m, 2H), 2.56-2.50 (m, 2H), 2.31-2.24 (m, 2H), 0.91 (s, 9H); IR (KBr, cm<sup>-1</sup>): 2969, 1717, 1449, 1258, 1111, 772; HRMS (ESI) calcd for C<sub>19</sub>H<sub>26</sub>FN<sub>4</sub>O<sub>2</sub><sup>+</sup> [M+H]<sup>+</sup>: 361.2034, found 361.2039.

Compound **3c**: Yield: 72%, white crystals, mp 167-169 °C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 8.22 (s, 1H), 7.94 (s, 1H), 7.27-7.18 (m, 2H), 7.10-7.04 (m, 2H), 6.05 (d,  $J$  = 11.6 Hz, 1H), 4.44 (d,  $J$  = 11.6 Hz, 1H), 3.49-3.43 (m, 2H), 3.37-3.31 (m, 2H), 2.53-2.47 (m, 2H), 2.29-2.23 (m, 2H), 0.89 (s, 9H); IR (KBr, cm<sup>-1</sup>): 2976, 1711, 1504, 1224, 1112, 661; HRMS (ESI) calcd for C<sub>19</sub>H<sub>26</sub>FN<sub>4</sub>O<sub>2</sub><sup>+</sup> [M+H]<sup>+</sup>: 361.2034, found 361.2033.

Compound **3d**: Yield: 68%, white crystals, mp 179-181 °C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 8.27 (s, 1H), 7.94 (s, 1H), 7.30-7.25 (m, 1H), 7.15-7.12 (m, 1H), 6.97-6.90 (m, 2H), 6.30-6.26 (m, 1H), 4.94-4.92 (m,

1H), 3.86 (s, 3H), 3.47-3.32 (m, 4H), 2.55-2.49 (m, 2H), 2.28-2.21 (m, 2H), 0.93 (s, 9H); IR (KBr,  $\text{cm}^{-1}$ ): 2969, 1710, 1495, 1248, 1119, 767; HRMS (ESI) calcd for  $\text{C}_{20}\text{H}_{29}\text{N}_4\text{O}_3^+$   $[\text{M}+\text{H}]^+$ : 373.2234, found 373.2229.

Compound **3e**: Yield: 69%, white crystals, mp 153-155 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 8.24 (s, 1H), 7.94 (s, 1H), 7.31-7.25 (m, 1H), 6.88-6.84 (m, 1H), 6.81-6.78 (m, 1H), 6.73-6.72 (m, 1H), 6.08 (d,  $J = 11.6$  Hz, 1H), 4.40 (d,  $J = 11.6$  Hz, 1H), 3.82 (s, 3H), 3.49-3.42 (m, 2H), 3.37-3.31 (m, 2H), 2.56-2.46 (m, 2H), 2.32-2.25 (m, 2H), 0.90 (s, 9H); IR (KBr,  $\text{cm}^{-1}$ ): 2952, 1711, 1459, 1277, 1122, 762; HRMS (ESI) calcd for  $\text{C}_{20}\text{H}_{29}\text{N}_4\text{O}_3^+$   $[\text{M}+\text{H}]^+$ : 373.2234, found 373.2236.

Compound **4a**: Yield: 95%, white crystals, mp 90-92 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 8.24 (s, 1H), 7.97 (s, 1H), 7.37-7.27 (m, 1H), 6.92-6.88 (m, 1H), 6.76-6.73 (m, 1H), 6.70-6.69 (m, 1H), 5.12-5.08 (m, 1H), 4.29-4.25 (m, 1H), 3.84 (s, 3H), 3.43-3.32 (m, 4H), 3.29-3.24 (m, 1H), 2.49-2.42 (m, 2H), 2.16-2.10 (m, 2H), 0.68 (s, 9H); HRMS (ESI) calcd for  $\text{C}_{20}\text{H}_{31}\text{N}_4\text{O}_3^+$   $[\text{M}+\text{H}]^+$ : 375.2391, found 375.2387.

Compound **4b**: Yield: 98%, white crystals, mp 87-89 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 8.26 (s, 1H), 7.96 (s, 1H), 7.28 (s, 1H), 7.12-7.04 (m, 1H), 6.97-6.95 (m, 1H), 6.90-6.85 (m, 1H), 5.11-5.07 (m, 1H), 4.34-4.30 (m, 1H), 3.70 (s, 1H), 3.43-3.29 (m, 4H), 3.21-3.18 (m, 1H), 2.48-2.42 (m, 2H), 2.16-2.09 (m, 2H), 0.67 (s, 9H); HRMS (ESI) calcd for  $\text{C}_{19}\text{H}_{28}\text{FN}_4\text{O}_2^+$   $[\text{M}+\text{H}]^+$ : 363.2191, found 363.2195.

Compound **4c**: Yield: 96%, white crystals, mp 262-263 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 8.24 (s, 1H), 7.97 (s, 1H), 7.27-7.13 (m, 2H), 7.12-7.04 (m, 2H), 5.09-5.05 (m, 1H), 4.33-4.30 (m, 1H), 3.62 (s, 1H), 3.42-3.29 (m, 4H), 3.18-3.15 (m, 1H), 2.45-2.39 (m, 2H), 2.14-2.07 (m, 2H), 0.67 (s, 9H); IR (KBr,  $\text{cm}^{-1}$ ): 3215, 2962, 1509, 1211, 1114, 861; HRMS (ESI) calcd for  $\text{C}_{19}\text{H}_{28}\text{FN}_4\text{O}_2^+$   $[\text{M}+\text{H}]^+$ : 363.2191, found 363.2197.

Compound **5a**: Yield: 56%, white crystals, mp 128-130 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 8.23 (s, 1H), 7.65 (s, 1H), 7.38-7.37 (m, 1H), 7.23-7.19 (m, 1H), 7.13-7.10 (m, 1H), 6.96-6.94 (m, 1H), 5.93 (s, 1H), 5.33 (s, 1H), 3.23 (s, 3H), 1.14 (s, 9H); IR (KBr,  $\text{cm}^{-1}$ ): 2967, 1720, 1485, 1096, 770; HRMS (ESI) calcd for  $\text{C}_{16}\text{H}_{21}\text{ClN}_3\text{O}_2^+$   $[\text{M}+\text{H}]^+$ : 322.1317, found 322.1311.

Compound **6a**: Yield: 78%, white crystals, mp 179-181 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 8.10 (s, 1H), 7.89 (s, 1H), 7.19-7.16 (m, 2H), 6.84-6.81 (m, 2H), 5.64-5.60 (m, 1H), 4.74-4.67 (m, 1H), 4.48-4.45 (m, 1H), 3.77 (s, 3H), 1.90 (s, 3H), 1.71 (s, 3H), 0.67 (s, 9H); IR (KBr,  $\text{cm}^{-1}$ ): 2978, 1693, 1509, 1256, 1182, 1021; HRMS (ESI) calcd for  $\text{C}_{21}\text{H}_{28}\text{N}_3\text{O}_4^+$   $[\text{M}+\text{H}]^+$ : 386.2074, found 386.2070.

### ***In vitro* antifungal assay**

The *in vitro* antifungal activities of compounds against four plant pathogenic fungi (*F. solani*, *F. graminearum*, *F. oxysporum*, *C. lunata*) were investigated using the mycelium linear growth rate method. The test fungi, provided by the Center of Pesticide Research, Northwest A&F University, China,

maintained on potato dextrose agar (PDA) medium slants were subcultured for 48 h in Petri dishes prior to testing and used for inoculation of fungal strains on PDA plates. All the test compounds and the positive control (Tebuconazole) were completely dissolved in 0.5 mL dimethyl sulfoxide (DMSO) and the solution was added to 9.5 mL of sterile water. The resulting solution was added to 90 mL of melted PDA medium at a proper temperature below 50 °C. After quickly and completely mixing, the medium containing the compounds at a concentration of 50 µg/mL was poured into sterilized Petri dishes for screening. The solution of DMSO without any compounds mixed with PDA served as the blank control. When the medium in the plate was partially solidified, a 5 mm thick and 4 mm diameter disc of fungus cut from beforehand subcultured Petri dishes was placed at the centre of semi-solid medium. The dishes were kept in an incubator at 28 °C for 72 h. Three replicates were performed for each experiment. The growth inhibitory rates were calculated according to the following formula and expressed as means: average inhibition rate (%) =  $[(d_c - d_o) - (d_s - d_o)] / (d_c - d_o) \times 100\%$ , where  $d_o$  is the diameter of the fungus cut,  $d_c$  is the diameter of a fungal colony in the blank test, and  $d_s$  is the diameter of a fungal colony in the compound-treated test.

Based on the above results of *in vitro* antifungal activity, the more active compound **3c** (inhibition rate > 80.0% in **Table 1**) was selected to determine their median effective concentration (EC<sub>50</sub>) according to the same method described above. A stock solution was prepared by dissolving the tested compounds in DMSO, and then diluted by DMSO using serial two-fold dilution method to obtain a series of stock solutions. Each stock solution was respectively mixed with the autoclaved PDA medium to prepare a set of mediums containing 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125 µg/mL of the tested compounds. Meanwhile, 0.5% DMSO in culture medium was used as a blank control. Each experiment was performed in triplicate. The concentration (µg/mL) of the compound was transformed to the corresponding logarithm value (lgC). LgC values for each compound and its corresponding probit values were used to establish toxicity regression equation by the linear least-square fitting method. EC<sub>50</sub> values was calculated from the toxicity regression equations by using GraphPad Prism software ver.5.0 (GraphPad Software Inc, San Diego, CA, USA).

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