Design, Synthesis and Antifungal Evaluation of *N*-Substituted-1-(3-chloropyridin-2-yl)-*N*-(pyridin-4-yl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide Derivatives

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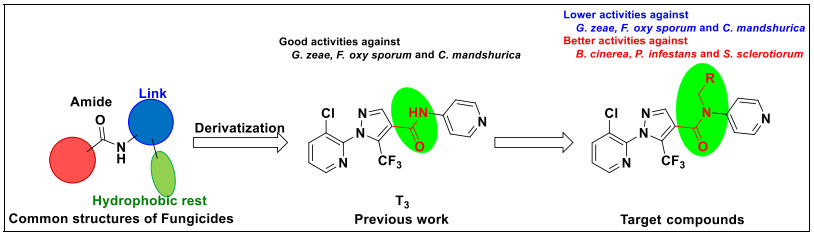

A series of 1-(3-chloropyridin-2-yl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide derivatives which have di-substituents on nitrogen were designed and synthesized. Bioassay results showed that all the synthetic compounds exhibited lower antifungal activities against *Gibberella zeae*, *Cytospora mandshurica*, and *Fusarium oxysporum* than **T_3** (14.7, 21.1, and 32.7 μg/mL), but some of them exhibited better activities against *Botrytis cinerea*, *Phytophthora infestans*, and *Sclerotinia sclerotiorum* than **T_3** (>200, >200, and >200 μg/mL); the EC_{50} values of **7d** and **7c** against *B. cinerea* were 94.9 and 56.2 μg/mL, respectively. The EC_{50} values of **7a**, **7d**, and **7c** against *S. sclerotiorum* were 73.5, 78.7, and 68.5 μg/mL, respectively.

INTRODUCTION

Pyrazole carboxamide compounds have been used as fungicides in agro-chemistry for years and exhibited good activity against many important diseases, such as leaf spots, mildews, molds, and rusts [1–6]. As reported, most pyrazole carboxamide fungicides inhibit fungal respiration by binding to the ubiquinone-binding site (Q-site) of the mitochondrial succinate dehydrogenase complex II in the electron transport chain, which is a functional part of the tricarboxylic acid cycle [7–9]. The structural analysis revealed that amide linker (CONH), which is between pyazole ring and amino part, is very important for the activity. Most substituents groups which replaced amide linker did not keep the activity except thioamide (CSNH) [10].

In our previous work [11,12], a series of *N*-pyridinyl-5-(trifluoromethyl)-pyrazole-4-carboxamide derivatives were designed and synthesized. Among of them, compound 1-(3-chloropyridin-2-yl)-*N*-(pyridin-4-yl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (**T_3**) exhibited good activities against plant pathogenic fungi *Gibberella zeae*, *Cytospora mandshurica*, and *Fusarium oxysporum*, and the EC_{50} values were 14.7, 21.1, and 32.7 μg/mL, respectively. For further study on the role of amide bond played in *N*-pyridinyl-5-trifluoromethyl pyrazole-4-carboxamide derivatives, a series of 1-(3-chloropyridin-2-yl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide derivatives which have di-substituents on nitrogen were designed and synthesized (Fig. 1). Bioassay results showed that all the title compounds exhibited lower antifungal activities against *G. zeae*, *C. mandshurica*, and *F. oxysporum*, but some of them exhibited better activities against *Botrytis cinerea*, *Phytophthora infestans*, and *Sclerotinia sclerotiorum* than **T_3**.

RESULTS AND DISCUSSION

Chemistry. Title compounds **7a–7e** were synthesized in existing literature [13–15] as shown in Scheme 1. Intermediates **2a–2e** were synthesized by pyridin-4-amine and corresponding aldehydes under nitrogen and hydrogenated by $NaBH_4$. Intermediate **4** was synthesized by cyclization reaction from **1** with 3-chloro-2-hydrazinylpyridine (**3**) in the ethanol. Intermediate **5** was obtained from **4** through hydrolysis in the presence of a weak-base lithium hydroxide. Intermediate **5** was then refluxed in $SOCl_2$ to obtain pyrazole acyl chloride (**6**). Target compounds **7a–7e** were obtained by the reaction of **6** with secondary amines **2a–2e** with yields ranging from 43–58% in anhydrous tetrahydrofuran by NaH.

All title compounds were identified by ^{1}H-NMR, ^{13}C-NMR, MS, and IR spectra. Elemental analyses were also

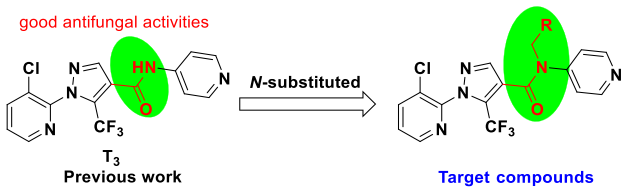

Figure 1. Design of the title compounds. [Color figure can be viewed at wileyonlinelibrary.com]

consistent with the corresponding calculated values. In ^{1}H-NMR, -NH- proton of the secondary amine **2a–2e** showed up at δ 3.62–5.73, and the pyrazole ring proton showed up at δ 7.31–7.90 for title compounds. In the IR spectra of the compounds, the stretching vibration absorption peaks of C=O appeared at 1655–1705 cm^{-1}.

Bioactivities. Preliminary antifungal activity of the title compounds against six kinds of plant phytogenic fungi (*G. zeae*, *C. mandshurica*, *F. oxysporum*, *B. cinerea*, *P. infestans*, and *S. sclerotiorum*) is shown in Table 1, and previous active compound **T$_3$** was used as the control. For title compounds **7a**, **7b**, **7d**, and **7e**, the antifungal activities against *G. zeae*, *C. mandshurica*, and *F. oxysporum* reduced obviously at 100 μg/mL. Even so, the antifungal activities against *B. cinerea*, *P. infestans*, and *S. sclerotiorum* were enhanced effectively, such as **7b** against *S. sclerotiorum*, **7c** against *P. infestans*, and **7d** and **7e** against *B. cinereal* and *S. sclerotiorum*. The EC_{50} values of **7b** against *S. sclerotiorum* and **7c** against *P. infestans* were 73.5 and 41.5 μg/mL, respectively. And the EC_{50} values of **7d** and **7e** against *B. cinereal* and *S. sclerotiorum* were 94.9, 78.7, and 56.2, 68.5 μg/mL, respectively, better than **T$_3$** (>200, >200, and >200 μg/mL).

Analysis of preliminary structure–activity relationships of title compounds **7a–7e** showed that antifungal activity against *G. zeae*, *C. mandshurica*, and *F. oxysporum* obviously reduced when hydrogen of amide bond was substituted by alkyl and pyridinyl (Table 2). The title compounds exhibited better activity against *B. cinerea* and *S. sclerotiorum* than **T$_3$** when isoamyl and benzyl were introduced on -NH- position of amide bond, such as **7d** and **7e**, the steric effect group which introduced in the -NH- bond may enhanced the activity against *B. cinerea* and *S. sclerotiorum* but reduced the activity against *G. zeae*, *C. mandshurica*, and *F. oxysporum*.

Scheme 1. Synthetic route of the target compounds.

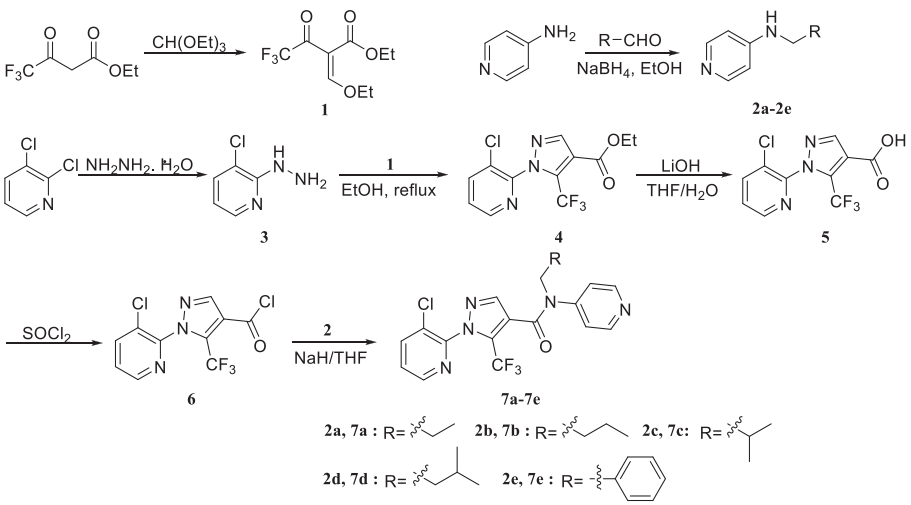

Table 1

Inhibition effect of title compounds against six plant phytopathogenic fungi at 100 μg/mL.

No.	Inhibition (%)					
	G. zeae	*C. mandshurica*	*F. oxysporum*	*B. cinerea*	*P. infestans*	*S. sclerotiorum*
7a	12.7 ± 1.6	14.5 ± 1.8	24.2 ± 3.0	11.5 ± 1.2	14.2 ± 2.9	28.7 ± 2.5
7b	12.0 ± 1.5	11.1 ± 1.8	16.1 ± 1.7	23.0 ± 1.2	16.1 ± 2.8	63.2 ± 1.4
7c	85.7 ± 5.3	67.6 ± 3.4	66.5 ± 2.8	9.7 ± 1.9	78.7 ± 0.9	20.2 ± 2.7
7d	15.3 ± 1.7	6.5 ± 1.9	20.0 ± 1.6	53.0 ± 1.7	22.3 ± 1.2	62.6 ± 1.4
7e	32.2 ± 1.4	12.0 ± 1.6	18.2 ± 1.8	70.3 ± 2.9	19.6 ± 1.5	59.1 ± 2.2
T$_3$	88.0 ± 0.6	77.5 ± 0.8	80.0 ± 0.9	12.0 ± 1.2	12.6 ± 3.0	12.6 ± 3.0

Table 2

The EC_{50} values of compounds **7a–7e** against six kinds of pathogenic fungi.

Compound no.	EC_{50} (μg/mL)					
	G. zeae	*C. mandshurica*	*F. oxysporum*	*B. cinerea*	*P. infestans*	*S. sclerotiorum*
7a	>200	>200	>200	>200	>200	>200
7b	>200	>200	>200	>200	>200	73.5 ± 1.6
7c	45.7 ± 1.9	73.5 ± 1.6	69.2 ± 1.3	>200	41.5 ± 3.1	>200
7d	>200	>200	>200	94.9 ± 3.2	>200	78.7 ± 1.7
7e	163.5 ± 3.4	>200	>200	56.2 ± 2.2	>200	68.5 ± 1.1
T_3	14.7 ± 1.4	21.1 ± 2.7	32.7 ± 3.1	>200	>200	>200

CONCLUSIONS

A series of *N*-substituted-1-(3-chloropyridin-2-yl)-*N*-(pyridin-4-yl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide derivatives were designed and synthesized. Bioassay results showed that when hydrogen of amide bond was substituted by alkyl or benzyl, all the synthetic compounds exhibited lower antifungal activities against *G. zeae*, *C. mandshurica*, and *F. oxysporum* than **T_3** (14.7, 21.1, and 32.7 μg/mL), but some of them exhibited better activities against *B. cinerea*, *P. infestans*, and *S. sclerotiorum* than **T_3** (>200, >200, and 200 μg/mL). The EC_{50} values of **7d** and **7c** against *B. cinerea* were 94.9 and 56.2 μg/mL, respectively. The EC_{50} values of **7a**, **7d**, and **7c** against *S. sclerotiorum* were 73.5, 78.7, and 68.5 μg/mL, respectively.

EXPERIMENTAL

Instruments. Melting points of the compounds were determined on an XT-4 binocular microscope (Beijing Tech Instrument Co., China) and were not corrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a JEOL ECX 500 NMR spectrometer operated at 500 and 125 MHz, respectively, using DMSO-d_6 or CD_3Cl as solvent and tetramethylsilane as an internal standard. Chemical shifts were reported in parts per million (ppm) down field from tetramethylsilane with the solvent resonance as internal standard. Coupling constants (*J*) were reported in Hz and referred to apparent peak multiplications. Mass spectral studies were conducted on an Agilent 5973 mass spectrometer. Elemental analysis was performed using Vario-III CHN analyzer.

General procedure for the preparation of intermediate 2a–2e. Pyridin-4-amine (1 g, 10.6 mmo1), corresponding aldehyde (10.6 mmo1), and ethanol (15 mL) were added into a 25-mL three-round bottom flask. The reaction mixture was refluxed for 6 h under nitrogen. Then $NaBH_4$ (482.4 mg,12.8 mmol) was added at ice-bath slowly, and the mixture was stirred at room temperature for 12 h. Afterward, the mixture was added to ice water (30 mL) and extracted by ethyl acetate (20 mL); the organic layer was dried with anhydrous sodium sulfate and filtered; the solvent was then removed under vacuum. The crude residue was further purified by flash column chromatography (DCM/MeOH = 30/1) on a silica gel to obtain the secondary amine **2a–2e**.

N-propylpyridin-4-amine (**2a**): colorless oil, yield 59%. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.95 (d, *J* = 6.3 Hz, 2H, pyridine H), 6.41 (d, *J* = 6.3 Hz, 2H, pyridine H), 4.12 (s, 1H, NH), 2.95 (t, *J* = 9.8 Hz, 2H, CH_2), 1.58–1.41 (m, 2H, CH_2), 0.88 (t, *J* = 6.5 Hz, 3H, CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 154.17, 149.85, 107.50, 43.94, 22.18, 12.05.

N-butylpyridin-4-amine (**2b**): colorless oil, yield 41%. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.95 (d, *J* = 5.1 Hz, 1H, pyridine H), 6.41 (d, *J* = 5.1 Hz, 1H, pyridine H), 3.70 (s, 1H, NH), 2.98 (t, *J* = 9.3 Hz, 2H, CH_2), 1.51–1.42 (m, 2H, CH_2), 1.39–1.24 (m, 2H, CH_2), 0.87 (t, *J* = 6.8 Hz, 3H, CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 154.18, 149.77, 107.47, 41.82, 31.02, 20.23, 14.27.

N-isobutylpyridin-4-amine (**2c**): colorless oil, yield 50%. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.95 (d, *J* = 5.7 Hz, 2H, pyridine H), 6.44 (d, *J* = 5.7 Hz, 2H, pyridine H), 3.62 (s, 1H, NH), 2.82 (t, *J* = 6.3 Hz, 2H, CH_2), 1.80–1.70 (m, 1H, CH), 0.87 (d, *J* = 6.7 Hz, 6H, CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 154.5, 149.4, 107.5, 49.88, 27.9, 20.8.

N-isopentylpyridin-4-amine (**2d**): colorless oil, yield 63%. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.95 (d, *J* = 4.5 Hz, 2H, pyridine H), 6.41 (d, *J* = 4.5 Hz, 2H, pyridine H), 3.60 (s, 1H, NH), 3.0 (dd, *J* = 12.0, 5.6 Hz, 2H, CH_2), 1.70–1.53 (m, 2H, CH_2), 1.43–1.30 (m, 1H, CH), 0.86 (dd, *J* = 6.6, 2.2 Hz, 6H, CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 154.1, 149.8, 107.5, 39.9, 37.8, 25.8, 22.9.

N-benzylpyridin-4-amine (**2e**): colorless oil, yield 79%. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.96 (d, *J* = 3.6 Hz, 2H, pyridine H), 7.48–6.93 (m, 5H, benzene H), 6.46

(d, J = 3.6 Hz, 2H, pyridine H), 5.73 (s, 1H, NH), 4.28 (s, 2H, CH_2). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 154.0, 149.9, 139.7, 128.9, 127.7, 127.4, 108.0, 45.8.

General procedure for the preparation of intermediate 3. 2, 3-Dichloropyridine (20.0 g, 135.1 μmol) and 80% hydrazine hydrate (67.7 g, 1.4 mmol) were added into a 250-mL three-round bottom flask. The reaction mixture was refluxed for 3 h, cooled to room temperature, and filtered; the residue was washed by ethanol, dried, and obtained **3**, white solid, yield 93.0%, m.p. 161–162°C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.04 (d, J = 5.1 Hz, 1H, pyridine H), 7.61 (s, 1H, NH), 7.57 (d, J = 7.6 Hz, pyridine H), 6.61 (dd, J = 5.1 Hz, J = 7.6 Hz, 1H, pyridine H), 4.21 (s, 2H, NH_2); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 156.3, 146.2, 136.7, 114.1, 113.7.

General procedure for the preparation of the target compounds 7a–7e. Intermediates **2** (586.4 μmol), **6** (645.0 μmol), NaH (2.4 mmol), and anhydrous tetrahydrofuran (5 mL) were added into a 25-mL three-round bottom flask. The reaction mixture was stirred at room temperature for 10 h. The organic solvent was removed under reduced pressure. Afterward, the residue was re-dissolved by ethyl acetate (20 mL). Finally, the organic layer was washed by saturated salt water, dried with anhydrous sodium sulfate, and filtered; the solvent was then removed under vacuum. The crude residue was further purified by flash column chromatography (PE/EA = 2:1) on a silica gel to obtain the desired product **7a–7e**.

N-*Propyl-1-(3-chloropyridin-2-yl)*-N-*(pyridin-4-yl)-5-(trifluoromethyl)-1*H-*pyrazole-carboxamide (7a). White solid, yield 43%, m.p. 122–123°C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.52 (d, J = 9.2 Hz, 1H, pyridine H), 7.90 (d, J = 7.8 Hz, 2H, pyridine H), 7.45 (dd, J = 7.0, 4.6 Hz, 1H, pyridine H), 7.39 (s, 1H, pyrazole H), 7.06 (d, J = 2.8 Hz, 2H, pyridine H), 3.90 (t, J = 7.2 Hz, 2H, CH_2), 1.64 (m, 2H, CH_2), 0.94 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 161.4, 151.3, 149.8, 148.0, 147.12, 139.8, 139.6, 131.5, 129.5, 126.8, 121.9, 120.1, 119.9, 118.0, 51.2, 21.2, 11.2. IR (KBr cm^{-1}): ν_{max} 3446, 3052, 29483, 2917, 2871, 1659, 1585, 1559, 1469, 1440, 1417, 1381, 1286, 1234, 1058, 1051, 968 cm^{-1}. MS (ESI): m/z 410 [M + H]$^+$. *Anal*. Calcd for $C_{18}H_{15}ClF_3N_5O$: C, 52.76; H, 3.69; N, 17.09; Found: C, 52.25; H, 4.03; N, 16.93.

N-*Butyl-1-(3-chloropyridin-2-yl)*-N-*(pyridin-4-yl)-5-(trifluoromethyl)-1*H-*pyrazole-4-carboxamide (7b). White solid, yield 49%, m.p. 147–148°C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.55 (d, J = 4.7 Hz, 1H, pyridine H), 8.47 (d, J = 4.6 Hz, 2H, pyridine H), 7.90 (d, J = 8.0 Hz, 1H, pyridine H), 7.44 (dd, J = 8.0 Hz, 4.7 Hz, 1H, pyridine H), 7.31 (s, 1H, pyrazole H), 7.05 (d, J = 4.8 Hz, 2H, pyridine H), 3.92 (dd, J = 15.1, 8.3 Hz, 2H, CH_2), 2.07–1.06 (m, 4H, CH_2CH_2), 0.90 (dd, J = 5.9, 7.5 Hz, 3H, CH_3). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 161.4, 151.5, 151.2, 149.8, 147.7, 147.1, 139.8, 139.6, 129.5, 126.8, 121.9, 120.1, 119.9, 49.5, 29.9, 20.1, 13.8. IR (KBr cm^{-1}): ν_{max} 3398, 3065, 2948, 2913, 2872, 1655, 1584, 1559, 1481, 1438, 1420, 1394, 1381, 1290, 1234, 1064, 1050, 986, 694 cm^{-1}. MS (ESI): m/z 424 [M + H]$^+$. *Anal*. Calcd for $C_{19}H_{17}ClF_3N_5O$: C, 53.85; H, 4.04; N, 16.52; Found: C, 54.14; H, 4.36; N, 16.71.

N-*Isobutyl-1-(3-chloropyridin-2-yl)*-N-*(pyridin-4-yl)-5-(trifluoromethyl)-1*H-*pyrazole-4-carboxamide (7c). White solid, yield 57%, m.p. 202–203°C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.72 (d, J = 4.7 Hz, 1H, pyridine H), 8.50 (d, J = 4.6 Hz, 2H, pyridine H), 8.16 (d, J = 8.0 Hz, 1H, pyridine H), 7.96 (s, 1H, pyrazole H), 7.54 (dd, J = 7.0, 4.6 Hz, 1H, pyridine H), 7.26 (d, J = 4.8 Hz, 2H, pyridine H), 3.54 (d, J = 13.9 Hz, 2H, CH_2), 2.27–1.80 (m, 1H, CH), 1.25 (d, J = 14.6 Hz, 6H, $2CH_3$). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 159.3, 150.7, 148.0, 147.3, 145.0, 140.5, 139.8, 137.8, 129.3, 127.0, 123.5 120.0, 114.1, 57.6, 29.8, 21.5. IR (KBr cm^{-1}): ν_{max} 3479, 2945, 2919, 2830, 1696, 1627, 1528, 1480, 1419, 1383, 1363, 1330, 1300, 1246, 1061, 1035, 999, 880751 cm^{-1}. MS (ESI): m/z 424 [M + H]$^+$. *Anal*. Calcd for $C_{19}H_{17}ClF_3N_5O$: C, 53.85; H, 4.04; N, 16.52; Found: C, 53.45; H, 4.46; N, 16.76.

N-*Isopentyl-1-(3-chloropyridin-2-yl)*-N-*(pyridin-4-yl)-5-(trifluoromethyl)-1*H-*pyrazole-4-carboxamide (7d). White solid, yield 49%, m.p. 132–133°C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.55 (d, J = 8.2 Hz, 1H, pyridine H), 8.47 (d, J = 4.6 Hz, 2H, pyridine H), 7.90 (s, 1H, pyrazole H), 7.45 (dd, J = 7.8, 5.0 Hz, 1H, pyridine H), 7.39 (d, J = 8.1, 1H, pyridine H), 7.04 (d, J = 7.2 Hz, 2H, pyridine H), 3.95 (m, 2H, CH_2) 2.18–1.11 (m, 3H, CH_2 and CH), 0.90 (d, J = 12.8 Hz, 6H, $2CH_3$). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 161.3, 151.2, 149.8, 147.7, 147.2, 139.8, 139.6, 130.6, 129.5, 126.8, 121.9, 120.1, 120.0, 119.6, 118.0, 48.3, 36.5, 26.1, 22.5. IR (KBr cm^{-1}): ν_{max} 3451, 3068, 2953, 2914, 2870, 1658, 1587, 1562, 1483, 1437, 1417, 1400, 1382, 1287, 1239, 1063, 1052, 988, 692, 596 cm^{-1}. MS (ESI): m/z 438 [M + H]$^+$. *Anal*. Calcd for $C_{20}H_{19}ClF_3N_5O$: C, 54.86; H, 4.37; N, 16.00; Found: C, 55.13; H, 4.78; N, 16.38.

N-*Benzyl-1-(3-chloropyridin-2-yl)*-N-*(pyridin-4-yl)-5-(trifluoromethyl)-1*H-*pyrazole-4-carboxamide (7e). White solid, yield 58%, m.p. 139–140°C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.57 (dd, J = 4.7, 1.3 Hz, 2H, pyridine H), 8.46–8.42 (m, 1H, pyridine H), 8.28 (dd, J = 8.2, 1.2 Hz, 1H, pyridine H), 7.90 (s, 1H, pyrazole H), 7.72 (dd, J = 8.2, 4.7 Hz, 1H pyridine H), 7.33–7.18 (m, 5H, benzene H), 7.14 (d, J = 6.0 Hz, 2H, pyridine H), 5.15 (s, 2H, CH_2). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 161.7, 151.247, 149.1, 148.3, 147.0, 140.9, 140.9, 136.8, 129.1, 129.0, 128.7, 128.3, 128.1, 122.7, 120.4, 51.9. IR (KBr cm^{-1}): ν_{max} 3248, 3180, 3122, 3065, 3033, 2968, 1705, 1602, 1576, 1548, 1482, 1456, 1385, 1033, 980,

697 cm^{-1}. MS (ESI): m/z 458 $[M + H]^+$. *Anal*. Calcd for $C_{22}H_{15}ClF_3N_5O$: C, 57.71; H, 3.30; N, 15.30; Found: C, 57.69; H, 3.24; N, 15.04.

Antifungal activities assay. The antifungal activity of the synthetic compounds was tested *in vitro* against six plant pathogenic fungi (*G. zeae*, *C. mandshurica*, *F. oxysporium*, *B. cinerea*, *P. infestans*, and *S. sclerotiorum*) using mycelia growth inhibition method [16–18]. The title compounds were dissolved in DMSO to prepare the 10 mg/mL stock solution before mixing with potato dextrose agar (PDA 90 mL). The compounds were tested at a concentration of 100 μg/mL. All fungi were cultivated in PDA at 27 ± 1°C for 4 days to make new mycelium for the identification of antifungal activity. Mycelia dishes of approximately 4-mm diameter were then cut from the culture medium. A mycelium was obtained using a germ-free inoculation needle and aseptically inoculated in the middle of the PDA plate. The inoculated plates were incubated at 27 ± 1°C for 5 days. DMSO in sterile distilled water served as the negative control, whereas hymexazol, carboxin, and boscalid served as the positive control. Each treatment condition consisted of three replicates. The radial growth of the fungal colonies was measured, and the data were statistically analyzed. The *in vitro* inhibitory effects of the test compounds on these fungi were calculated by formula $I\ (\%) = [(C - T)/(C - 0.4)] \times 100$, where C represents the diameter of fungal growth on untreated PDA, T represents the diameter of fungi on treated PDA, and I represents the inhibition rate. EC_{50} was defined as the concentration for 50% of maximal effect of mycelia growth and was calculated with SPSS 17.0 software.

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