Synthesis and Bioactivities of Novel 1-(3-Chloropyridin-2-yl)-*N*-Substituted-5-(Trifluoromethyl)-Pyrazole Carboxamide Derivatives

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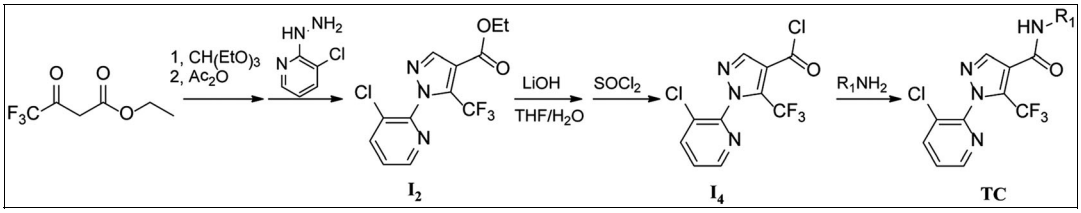

A series of novel 1-(3-chloropyridin-2-yl)-*N*-substituted-5-(trifluoromethyl)-pyrazole carboxamide derivatives **TC$_1$–TC$_{11}$** were synthesized and characterized by IR, ^{1}H NMR, ^{13}C NMR, MS, and elemental analysis. All the target compounds were tested *in vitro* for their antibacterial activities and antifungal activities. The preliminary bioassays indicated that compound **TC$_6$** exhibited excellent activity against *Xanthomonas oryzae* (94.9% and 84.9%) at different concentrations (200 μg/mL and 100 μg/mL), which was higher than that of Bismerthiazol (94.6% and 64.0%), respectively. At the same time, most of the compounds exhibited moderate antifungal activities against four kinds of phytopathogenic fungi

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INTRODUCTION

Heterocyclic compounds have been extensively studied for their powerful applications in many fields. Among them, pyrazole amides have already been used as pharmaceuticals and agrochemicals due to their various bioactivities, including antitumor, anti-inflammatory, antipyretic, herbicidal, antifungal, and insecticidal activities [1–11]. Some compounds have already been commercialized as fungicides, like sedaxane (Syngenta, 2005), isopyrazam (Syngenta, 2006), bixafen (Bayer, 2005), and fluxapyroxad (BASF, 2008). On the other hand, compounds containing pyridine also have been used as pesticides because of their diverse bioactivities [12–14].

In our previous work [15], we have demonstrated that 1-methyl-(phenyl)-pyrazole-carboxamides containing pyridine moiety have moderate fungicidal activities. In order to look for highly active compounds against phytopathogenic microbioorganisms, the methyl or phenyl group at 1-position of the pyrazolering was replaced with 3-chloropyridine moiety, and a series of new 1-(3-chloropyridin-2-yl)-*N*-substituted-5-(trifluoro methyl)-pyrazole carboxamide derivatives were synthesized. Preliminary bioassay showed that title compounds have good inhibitory activity against plant pathogenic bacterial and moderate activities against plant pathogenic fungi.

RESULTS AND DISCUSSION

Chemistry. The synthetic route to target compounds (TCs) is shown in Scheme 1. The intermediate **I$_2$** was synthesized by the cyclization reaction from **I$_1$** in the presence of 3-chloro-2-hydrazinylpyridine [16]. The key intermediate **I$_3$** was obtained from **I$_2$** through hydrolysis process in the presence of a weak base lithium hydroxide [17,18]. Subsequently, intermediate **I$_3$** was refluxed in $SOCl_2$ to give pyrazole acyl chloride (**I$_4$**). TCs were obtained by the reaction of different amines with **I$_4$** with the yields ranging from 42–99% in the presence of NaH (Scheme 1). In order to optimize the synthetic conditions of the key intermediate **I$_3$**, a series of different bases and solvents were evaluated, and the results are listed in Table 1. It can be seen that LiOH is better than NaOH and KOH for the hydrolysis reaction, with the yield up to 85% (Table 1, entries 1–3). Various solvents were further examined, among which, THF/H_2O (1/1) gave the best yield of 98% (Table 1, entries 3–5). Based on the aforementioned results, we chose LiOH as the base and THF/H_2O as the solvent to prepare the intermediate **I$_3$**.

Bioactivities. ***Antibacterial activity.*** Preliminary bioassay of the title compounds against tobacco bacterial wilt and *Xanthomonas oryzae* were conducted by the reported method [19], and Bismerthiazol was used as the control. As shown in Table 2, most of the title compounds exhibited a certain degree of antibacterial activities against *X. oryzae* at

Scheme 1. Synthetic route of the target compounds.

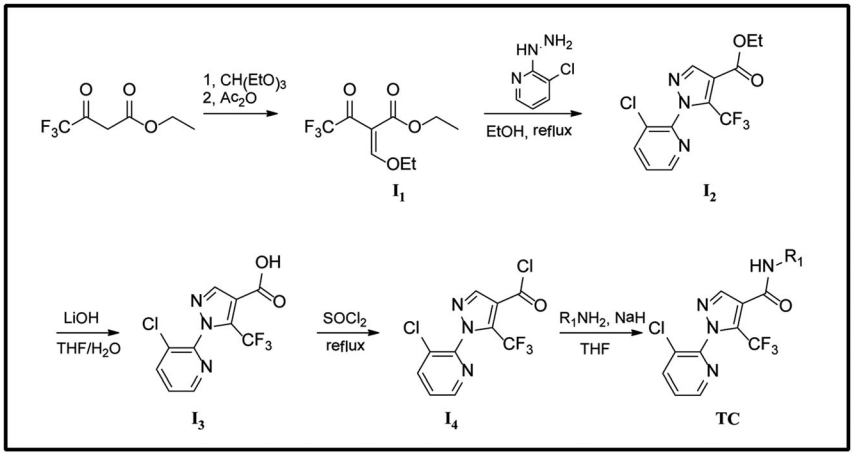


Table 1

Effect of different bases and solvents for synthesis of intermediate $\mathbf{I_3}$.

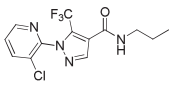
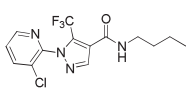
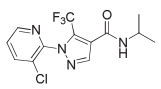
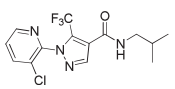
Entry	Base	Solvent	Yield (%)
1[a]	NaOH	H_2O	75
2[a]	KOH	H_2O	70
3[a]	LiOH	H_2O	85
4[b]	LiOH	THF	86
5[b]	LiOH	THF/H_2O	98

[a]Reactions were carried out at room temperatures.
[b]Reactions were carried out at refluxing temperatures.

200 μg/mL and 100 μg/mL, respectively. Among them, compounds ***TC₆***, ***TC₇***, and ***TC₁₁*** showed 84.9%, 83.4%, and 71.8% antibacterial activities against *X. oryzae* at 100 μg/mL, respectively, which was higher than that of the control (64.0%). Notably, compound ***TC₆*** exhibited excellent activity against *X. oryzae* (94.9%, 84.9%, 60.1%, and 37.3%) at different concentrations (200 μg/mL, 100 μg/mL, 50 μg/mL, and 25 μg/mL), which was higher than that of Bismerthiazol (94.6%, 64.0%, 45.3%, and 27.3%), respectively. Compound ***TC₆*** also exhibited better activity against tobacco bacterial wilt (51.1%, 22.1%) at 200 μg/mL and 100 μg/mL than the control (49.4%, 0%).

Table 2

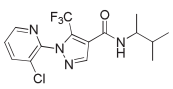
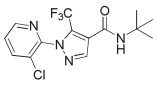
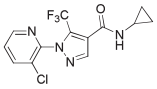
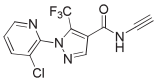
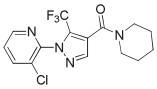
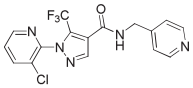
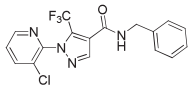
Inhibition effect of title compounds against tobacco bacterial wilt and *Xanthomonas oryzae*.

		Inhibition rate (%)					
		Tobacco bacterial wilt		*Xanthomonas oryzae*			
Compound	Structure	200 μg/mL	100 μg/mL	200 μg/mL	100 μg/mL	50 μg/mL	25 μg/mL
TC_1		24.6 ± 8.6	0	29.8 ± 1.7	9.1 ± 1.4	—	—
TC_2		0	0	62.0 ± 6.6	12.8 ± 2.7	—	—
TC_3		41.8 ± 3.4	41.4 ± 1.6	65.2 ± 6.5	12.8 ± 2.7	—	—
TC_4		—	—	**0**	**0**	—	—

(Continued)

Table 2

(Continued)

		Inhibition rate (%)					
		Tobacco bacterial wilt		*Xanthomonas oryzae*			
Compound	Structure	200 μg/mL	100 μg/mL	200 μg/mL	100 μg/mL	50 μg/mL	25 μg/mL
TC_5		0	0	48.2 ± 1.9	23.8 ± 0.8	—	—
TC_6		51.1 ± 3.6	22.1 ± 6.0	94.9 ± 6.2	84.9 ± 1.7	60.1 ± 5.0	37.3 ± 5.5
TC_7		0	0	89.7 ± 1.6	83.4 ± 1.6	48.9 ± 3.1	31.6 ± 1.2
TC_8		0	0	66.0 ± 7.7	8.1 ± 3.1	—	—
TC_9		35.0 ± 2.7	33.3 ± 2.4	61.7 ± 4.7	57.0 ± 6.0	—	—
TC_{10}		20.3 ± 1.7	12.9 ± 1.3	67.2 ± 3.0	31.4 ± 5.8	—	—
TC_{11}		25.1 ± 10.2	20.1 ± 6.0	83.0 ± 1.5	71.8 ± 1.5	10.9 ± 5.5	0
Bismerthiazol		49.4 ± 11.4	0	94.6 ± 0.2	64.0 ± 1.2	45.3 ± 3.4	27.3 ± 1.7

"—" not test.

Table 3

Inhibition effect of title compounds on phytopathogenic fungi at 100 μg/mL.

	Inhibition rate (%)			
Compound	*F. oxysporum*	*B. cinerea*	*B. cinereapers*	*S. sclerotiorum*
TC_1	7.7 ± 1.5	36.3 ± 3.9	76.6 ± 2.8	31.8 ± 1.9
TC_2	8.4 ± 1.6	14.5 ± 1.4	11.9 ± 1.2	61.3 ± 3.6
TC_3	9.1 ± 1.6	25.4 ± 1.3	33.1 ± 1.3	17.8 ± 1.8
TC_4	10.5 ± 1.7	34.3 ± 1.9	74.4 ± 3.6	31.3 ± 1.6
TC_5	10.9 ± 1.7	0	22.4 ± 1.6	28.1 ± 1.6
TC_6	9.4 ± 1.6	10.4 ± 2.0	0	14.7 ± 1.6
TC_7	12.1 ± 1.7	0	0	13.7 ± 1.8
TC_8	10.4 ± 1.7	0	0	12.3 ± 2.2
TC_9	14.1 ± 1.6	18.4 ± 1.6	0	36.0 ± 1.9
TC_{10}	13.1 ± 1.5	0	0	17.4 ± 1.6
TC_{11}	13.8 ± 1.6	28.0 ± 2.3	0	24.3 ± 2.0
Hymexazol	63.1 ± 2.5	66.8 ± 1.0	67.1 ± 1.2	51.3 ± 1.9

Fungicidal activity. The title compounds were also tested against four kinds of phytopathogenic fungi, which were *Fusarium oxysporum*, *Botrytis cinerea*, *Botrytis cinerea Pers*, and *Sclerotinia sclerotiorum*, through fungi growth inhibition method using potato dextrose agar as the culture medium and hymexazol as the control [20]. The results revealed that some compounds exhibited certain anti-fungicidal activities against the aforementioned fungi at 100 μg/mL. Compounds **TC$_1$** and **TC$_4$** showed inhibition activities of 76.6%, and 74.4% respectively against *Botrytis cinerea Pers*. Compound ***TC$_2$*** had an inhibition activity of 61.3% against *S. sclerotiorum*, which was higher than that of hymexazol (51.3%) (Table 3).

CONCLUSIONS

In summary, a series of novel 1-(3-chloropyridin-2-yl)-*N*-substitutied-5-(trifluoromethyl)-pyrazole carboxamide derivatives were designed and synthesized. Preliminary bioassay results revealed that most of the title compounds exhibited good inhibitory bioactivities against *X. oryzae*, and part of the title compounds is active against four phytopathogenic fungi. Among them, compound ***TC$_6$*** exhibited excellent activity against *X. oryzae* (94.9% and 84.9%) at different concentrations (200 μg/mL and 100 μg/mL), which was higher than that of Bismerthiazol (94.6% and 64.0%), respectively, which is promising for further development. The structure optimization of the title compounds based on this work is currently underway.

EXPERIMENTAL

Instruments. Melting points of the compounds were determined on a XT-4 binocular microscope (Beijing Tech Instrument Co., China). Infrared spectra were recorded on a Bruker VECTOR 22 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on JEOL-ECX-500 spectrometers. Chemical shifts were reported in parts per million (ppm) down field from TMS with the solvent resonance as the internal standard. Coupling constants (*J*) were reported in Hz and referred to apparent peak multiplications. Mass spectral studies were conducted on an Agilent 5973 organic mass spectrometer. Elemental analysis was performed using an Elemental Vario-III CHN analyzer.

General procedure for the preparation of the target compounds *TC$_1$*–*TC$_{11}$*. Intermediate **I$_3$** (0.59 m*M*), NaH (4 m*M*), anhydrous THF (5 mL), and substituted amine (1.18 m*M*) were added into a 25 mL three round bottom flask. The reaction mixture was stirred at room temperature for 12 h. Then, the organic solvent was removed under reduced pressure. After that, 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, filtered, and followed by the removal of the solvent under vacuum. The crude residue was further purified by flash column chromatography on a silica gel to afford the desired product.

1-(3-chloropyridin-2-yl)-*N*-propyl-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide (*TC$_1$*). white solid, yield 96%, mp 135–136°C; IR (KBr cm^{-1}): ν_{max} 3256, 3132, 3081, 2968, 2935, 2877, 1660, 1591, 1593, 1574, 1549, 1481, 1463, 1382, 1374, 1240, 1059, 983, 876, 806, 698, 653 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.63–8.62 (m, 2H, pyridine H and NH), 8.35 (d, J = 8.0 Hz, 1H, pyridine H), 8.27 (s, 1H, pyrazole H), 7.78–7.75 (m, 1H, pyridine H), 3.20 (t, J = 6.3 Hz, 2H, NCH_2), 1.54–1.50 (m, 2H, CH_2), 0.89 (t, J = 7.4 Hz, 3H,CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 160.0, 148.2, 147.9, 140.9, 131.2, 130.9, 128.5, 128.4, 121.7, 120.7, 41.3, 22.8, 11.9; MS (ESI): m/z 333 $[M+H]^+$. *Anal*. Calcd ($C_{13}H_{12}ClF_3N_4O$): C, 46.93; H, 3.64; N, 16.84. Found: C, 46.97; H, 3.98; N, 16.87.

1-(3-chloropyridin-2-yl)-*N*-butyl-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide (*TC$_2$*). white solid, yield 99%, mp 131–132°C; IR (KBr cm^{-1}): ν_{max} 3344, 3255, 3116, 3069, 2933, 2876, 2863, 1659, 1640, 1578, 1531, 1477, 1437, 1379, 1301, 1080, 982, 877, 665 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.63–8.61 (m, 2H, pyridine H and NH), 8.57 (d, J = 8.0 Hz, 1H, pyridine H), 8.27 (s, 1H, pyrazole H), 7.78, 7.76 (dd, J = 8.0, 4.6 Hz, 1H, pyridine H), 3.25–3.21 (m, 2H, NCH_2), 1.52–1.47 (m, 2H, CH_2), 1.37–1.30 (m, 2H, CH_2), 0.90 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 160.0, 148.2, 147.9, 140.9, 130.9, 128.5, 128.4, 121.7, 120.7, 118.56, 39.2, 31.6, 20.1, 14.2; MS (ESI): m/z 347 $[M+H]^+$. *Anal*. Calcd ($C_{14}H_{14}ClF_3N_4O$): C, 48.50; H, 4.07; N, 16.16. Found: C, 48.68; H, 4.42; N, 16.48.

1-(3-chloropyridin-2-yl)-*N*-isopropyl-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide (*TC$_3$*). white solid, yield 96%, mp 153–154°C; IR (KBr cm^{-1}): ν_{max} 3461, 3260, 3068, 2983, 2937, 2884, 2823, 1637, 1586, 1574, 1544, 1482, 1461, 1390,1373, 1060, 1050, 982, 879, 69, 558 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.52 (d, J = 4.6, 1H, pyridine H), 8.04 (s, 1H, NH), 7.95 (d, J = 8.0 Hz, 1H, pyridine H), 7.50, 7.48 (dd, J = 8.0, 4.6 Hz, 1H, pyridine H), 5.76 (s, 1H, pyrazole H), 4.31–4.24 (m, 1H, CH), 1.26 (d, J = 6.5 Hz, 6H, $2CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.8, 148.3, 147.2, 140.9, 139.7, 129.3, 126.7, 121.0, 120.4, 118.3, 42.3, 22.6; MS (ESI): m/z 333 $[M+H]^+$. *Anal*. Calcd ($C_{13}H_{12}ClF_3N_4O$): C, 46.93; H, 3.64; N, 16.84. Found: C, 46.92; H, 4.01; N, 16.89.

1-(3-chloropyridin-2-yl)-*N*-isobutyl-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide (*TC$_4$*). white solid, yield 96%, mp 134–135°C; IR (KBr cm^{-1}): ν_{max} 3283, 3085, 2965,

2931, 2872, 1669, 1645, 1593, 1575, 1549, 1481, 1437, 1373, 1143,1055, 982, 1050, 871, 806, 697 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 8.64–8.62 (m, 2H, NH and pyridine H), 8.37, 8.35 (dd, J = 8.1, 1.8 Hz, 1H, pyridine H), 8.28 (s, 1H, pyrazole H), 7.78. 7.77 (dd, J = 8.6, 5.2 Hz, 1H, pyridine H), 3.03 (t, J = 6.6 Hz, 2H, CH_2), 1.85–1.77 (m, 1H, CH), 0.90 (d, J = 6.9 Hz, 6H, $2CH_3$); ^{13}C NMR (125 MHz, DMSO-d_6): δ 160.2, 148.3, 147.9, 140.9, 130.8, 128.5, 128.4, 121.7, 120.8, 118.6, 47.0, 28.6, 20.7; MS (ESI): m/z 347 $[M+H]^+$. *Anal*. Calcd ($C_{14}H_{14}ClF_3N_4O$): C, 48.50; H, 4.07; N, 16.16. Found: C, 48.47; H, 4.19; N, 16.09.

1-(3-chloropyridin-2-yl)-*N*-(3-methylbutan-2-yl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide (*TC*$_5$). white solid, yield 95%, mp 163–164°C; IR (KBr cm^{-1}): ν_{max} 3274, 3067, 2966, 2935, 2880, 1667, 1641, 1588, 1574, 1543, 1480, 1437, 1394, 1375,1142, 1084, 980, 872, 806, 697 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 8.63 (d, J = 3.5 Hz, 1H, pyridine H), 8.40–8.35 (m, 2H, NH and pyridine H), 8.26 (s, 1H, pyrazole H), 7.78, 7.76 (dd, J = 8.0, 4.6 Hz, 1H, pyridine H), 3.83–3.77 (m, 1H, N-CH), 1.75–1.70 (m, 1H, CH), 1.09 (d, J = 6.9 Hz, 3H, CH_3), 0.90, 0.88 (dd, J = 6.3, 1.7 Hz, 6H, $2CH_3$); ^{13}C NMR (125 MHz, DMSO-d_6): δ 159.6, 148.2, 147.9, 140.9, 128.5, 128.4, 122.0, 119.3, 118.6, 50.6, 33.1, 19.5, 19.2, 17.7; MS (ESI): m/z 361 $[M+H]^+$. *Anal*. Calcd ($C_{15}H_{16}ClF_3N_4O$): C, 49.94; H, 4.47; N, 15.53. Found: C, 49.92; H, 4.44; N, 15.47.

1-(3-chloropyridin-2-yl)-*N*-(tert-butyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide (*TC*$_6$). white solid, yield 89%, mp.183–184°C; IR (KBr cm^{-1}): ν_{max} 3451, 2985, 1646, 1587, 1576, 1539, 1481, 1457, 1390, 1363, 1063, 1054, 984, 866 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.51 (d, J = 5.2 Hz, 1H, pyridine H), 8.01 (s, 1H, pyrazole H), 7.96 (d, J = 8.0 Hz, 1H, pyridine H), 7.50, 7.48 (dd, J = 8.1, 4.6 Hz, 1H, pyridine H), 5.77 (s, 1H, NH), 1.46 (s, 9H, $3CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.0, 148.3, 147.1, 140.9, 139.7, 130.9, 129.28, 126.6, 122.0, 118.3, 52.4, 28.7; MS (ESI): m/z 347 $[M+H]^+$. *Anal*. Calcd ($C_{14}H_{14}ClF_3N_4O$): C, 48.50; H, 4.07; N, 16.16. Found: C, 48.35; H, 4.17; N, 16.30.

1-(3-chloropyridin-2-yl)-*N*-cyclopropyl-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide (*TC*$_7$). white solid, yield 89%, mp 137–138°C; IR (KBr cm^{-1}): ν_{max} 3508, 3288, 3119, 3031, 1681, 1576, 1525, 1481, 1457, 1434, 1352, 1031, 984, 649 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 8.67–8.53 (m, 1H, pyridine H), 8.65 (d, J = 3.5 Hz, 1H, NH), 8.62 (d, J = 6.3 Hz, 1H, pyridine H), 8.36 (d, J = 8.0 Hz, 1H, pyridine H), 8.26 (s, 1H, pyrazole H), 7.78, 7.76 (dd, J = 8.0, 4.6 Hz, 1H, pyridine H), 2.83–2.79 (m, 1H, CH), 0.73–0.69 (m, 1H, CH_2), 0.59–0.53 (m, 1H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6): δ 161.2, 148.3, 147.9, 141.0, 140.9, 130.9, 128.5, 128.5, 121.4, 120.7, 118.6, 23.3; MS (ESI): m/z 331 $[M+H]^+$. *Anal*. Calcd ($C_{13}H_{10}ClF_3N_4O$): C, 47.22; H, 3.05; N, 16.94. Found: C, 47.27; H, 3.33; N, 17.01.

1-(3-chloropyridin-2-yl)-*N*-(prop-2-yn-1-yl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide (*TC*$_8$). white solid, yield 83%, mp 108–109°C; IR (KBr cm^{-1}): ν_{max} 3280, 3128, 3057, 2951, 2816, 2127, 1782, 1548, 1502, 1558, 1513, 1418, 1370, 1348, 1042, 988, 658, 569 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.53 (d, J = 4.6 Hz, 1H, pyridine H), 8.08 (s, 1H, pyrazole H), 7.97 (d, J = 8.0 Hz, 1H, pyridine H), 7.51, 7.50 (dd, J = 7.5, 4.6 Hz, 1H, pyridine H), 6.17 (s, 1H,NH), 4.26, 4.25 (dd, J = 5.2, 2.3 Hz, 2H, CH_2), 2.31 (t, J = 2.3 Hz, 1H, CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.1, 148.2, 147.2, 140.9, 139.7, 129.3, 126.8, 120.0, 119.6, 118.1, 78.8, 72.4, 29.9; MS (ESI): m/z 329 $[M+H]^+$. *Anal*. Calcd ($C_{13}H_8ClF_3N_4O$): C, 47.51; H, 2.45; N, 17.34. Found: C, 47.81; H, 2.77; N, 17.59.

(1-(3-chloropyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazol-4-yl)(piperidin-1-yl)methanone (*TC*$_9$). white solid, yield 89%, mp 137–138°C; IR (KBr cm^{-1}): ν_{max} 3345, 3001, 2953, 2925, 2854, 1704, 1573, 1465, 1458, 1433, 1386, 1280, 1049, 797, 756, 649 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 8.67–8.53 (m, 1H, pyridine H), 8.32 (d, J = 8.0Hz, 1H, pyridine H), 8.22 (s, 1H, pyrazole H), 7.74, 7.73 (dd, J = 8.0, 4.6 Hz, 1H, pyridine H), 2.78 (t, J = 7.7Hz, 4H, $2CH_2$), 0.68–0.41 (m, 6H, $3CH_2$); ^{13}C NMR (125 MHz, DMSO-d_6): δ 161.2, 148.3, 147.9, 141.0, 140.9, 130.9, 128.5, 128.5, 121.4, 120.7, 38.6, 23.3, 20.4; MS (ESI): m/z 359 $[M+H]^+$. *Anal*. Calcd ($C_{15}H_{14}ClF_3N_4O$): C, 50.22; H, 3.93; N, 15.62. Found: C, 49.99; H, 4.22; N, 15.75.

1-(3-chloropyridin-2-yl)-*N*-(pyridin-4-ylmethyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide (*TC*$_{10}$). light yellow solid, yield 42%, mp 194–196°C; IR (KBr cm^{-1}): ν_{max} 3269, 3131, 3068, 2919, 2849, 1734, 1686, 1588, 1539, 1482, 1435, 1377, 1241, 1148, 1058, 990, 795, 634 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.74 (d, J = 8.2 Hz, 1H, pyridine H), 8.74 (d, J = 8.2 Hz, 1H, pyridine H), 8.52 (dd, J = 2.5, 4.9 Hz, 2H, pyridine H), 8.30 (s, 1H, pyrazole H), 8.03 (dd, J = 7.1, 8.3 Hz, 2H, pyridine H), 7.56–7.42 (m, 1H, pyridine H), 4.61 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.9, 150.8, 150.1, 148.1, 147.2, 147.0, 140.8, 139.8, 129.3, 126.8, 122.5, 121.5, 119.8, 42.9; MS (ESI): m/z 382 $[M+H]^+$. *Anal*. Calcd ($C_{16}H_{11}ClF_3N_5O$): C, 50.34; H, 2.90; N, 18.53. Found: C, 50.03; H, 3.20; N, 18.61.

1-(3-chloropyridin-2-yl)-*N*-benzyl-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide (*TC*$_{11}$). white solid, yield 88%, mp 160–161°C; IR (KBr cm^{-1}): ν_{max} 3256, 3079, 2968, 2935, 2877, 1660, 1641, 1593, 1574, 1549, 1459, 1437, 1374, 1040, 983, 876, 743, 698 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 9.22 (s, 1H, NH), 8.64 (s, 1H, pyrazole H), 8.37–8.36 (m, 2H, pyridine H), 7.78, 7.76 (dd, J = 8.0, 4.5 Hz, 1H, pyridine H), 7.32 –7.27 (m, 5H,

benzene H), 4.44 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6): δ 160.1, 148.3, 147.9, 141.0, 140.9, 139.6, 128.9, 128.8, 128.48, 127.8, 127.5, 121.2, 120.7, 118.6, 43.0; MS (ESI): m/z 381 $[M+H]^+$. *Anal*. Calcd ($C_{17}H_{12}ClF_3N_4O$): C, 53.63; H, 3.18; N, 14.71. Found: C, 54.02; H, 3.21; N, 15.06.

Antibacterial activities assay. The antibacterial activities were evaluated *in vitro* against tobacco bacterial wilt and *X. oryzae* by the turbidimeter test according to the reference [19]. The title compounds were dissolved in 80 μL DMSO, and diluted with sterile distilled water containing 0.1% Tween20 (4 mL) to prepare the stock solution. Then, the stock solution (1 mL) was added to 4 mL solvent NB nontoxic nutrient broth liquid medium, 3 g of beef extract, 5 g of peptone, 1 g of yeast powder, 10 g of glucose, and 1 L of distilled water, pH 7.0–7.2 in tubes. Then, 40 μL of NB containing tobacco bacterial wilt or *X. oryzae* was added to 5 mL of solvent NB containing the test compounds and the controls. The inoculated test tubes were incubated with continuous shaking at 180 rpm for 24 h at 30 ± 1°C. DMSO in sterile distilled water served as the blank control, where Bismerthiazol served as the positive control. Each treatment condition consisted of three replicates. Culture growth was monitored with a spectrophotometer by measuring the optical density at 600 nm (OD_{600}) given by corrected turbidity values. The inhibition rate of bacterial growth was calculated by formula I (%) = (C − T)/C × 100%, where C represents the corrected optical density value of bacterial growth on the blank control, T represents the corrected optical density value of bacterial growth on treated NB, and I represents the inhibition rate.

Antifungal activities assay. The antifungal activities were evaluated *in vitro* against *F. oxysporum*, *B. cinerea*, *botrytis cinerea Pers*, and *S. sclerotiorum* by fungi growth inhibition method according to the reference [20]. The title compounds were dissolved in DMSO (1 mL) and then added into 9-mL sterilized water containing Tween80 (1%) before mixing with potato dextrose agar (PDA, 90 mL). The compounds were tested at a concentration of 100 μg/mL. The stock solution was transferred into three 9-cm diameter of Petri dishes evenly. Then, mycelia dishes of approximately 4-mm diameter were cut from the culture medium and inoculated in the middle of the PDA plate aseptically. The inoculated plates were incubated at 27 ± 1 °C for 5 days. DMSO in sterile distilled water was used as the blank control, whereas hymexazol served as the positive control. Each treatment condition consisted of three replicates. Radial growth of the fungal colonies was measured, and the data were statistically analyzed. Inhibitory effects on these fungi were calculated by the formula I (%) = [(C − T)/(C − 0.4)] × 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.

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