Article

JOURNAL OF THE CHINESE CHEMICAL SOCIETY

Synthesis and Antifungal Activity Evaluation of Novel Substituted Pyrimidine-5-Carboxamides Bearing the Pyridine Moiety

Shi-Chun Wang, Fu-Xian Wan, Si Liu, Shuai Zhang and Lin Jiang *

College of Chemistry and Material Science, Shandong Agricultural University, Tai'an, 271018, China

(Received: September 7, 2017; Accepted: November 13, 2017; DOI: 10.1002/jccs.201700310)

A series of novel *N*-(substituted phenyl/benzyl)-2-methylthio-4-((pyridin-3-ylmethyl)amino)pyrimidine-5-carboxamides were synthesized by multistep reactions. The structures of the target compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, and elemental analysis. Their *in vitro* antifungal activities against two kinds of plant pathogenic fungi were evaluated by the mycelial growth rate method. The result showed that at the dosage of 100 μg/mL, several of these compounds exhibited moderate activity against *Botrytis cinerea* with inhibition rates of ~70%, and most compounds (e.g., **5a**, **5c**, **5e**, **5f,** and **5h**) possessed excellent activity against *Sclerotinia Sclerotiorum* with more than 90% inhibition rate.

Keywords: Pyrimidine; Pyridine; Carboxamide; Synthesis; Antifungal activity.

INTRODUCTION

Botrytis cinerea and *Sclerotinia sclerotiorum* are two critical plant pathogenic fungi infecting many economic crops such as fruits and vegetables. The diseases caused by the two fungi can seriously affect the yields of crops: for example, *B. cinerea* reduces tomato yield by 30–50%,[1] and *S. sclerotiorum* reduces rape yield by 20–40%.[2] The main method to control such diseases is by employing chemical fungicides such as carbendazim, boscalid, prochloraz, and so on. In recent years, however, the wide and indiscriminate use of fungicides has led to the development of serious resistance.[3] Therefore, there is an urgent need to develop new and effective antifungal agents with novel structures.

Pyrimidine is an important six-membered heterocyclic nucleus containing two nitrogen atoms. The diversity of the types and positions at pyrimidine ring results in an abundance of its derivatives. A literature survey shows that pyrimidine derivatives possess a broad spectrum of biological properties, such as antifungal,[4] antibacterial,[5] anticancer,[6] anti-inflammatory,[7] antidiabetic,[8] insecticidal,[9] and herbicidal[10] activities. Many pyrimidine-based compounds play important roles in the field of pesticide due to their high activity, low toxicity, and unique action mechanism. For example, mepanipyrim, cyprodinil, diflumetorim, and azoxystrobin are all excellent agricultural fungicides. In addition, among the pyrimidine derivatives, pyrimidinecarboxamides represent a promising class of bioactive substances and have received much attention in the field of medicine. For example, 2-thioxo-1,2-dihydro pyrimidine-5-carboxamides (I) have moderate antibacterial activity against *Bacillus subtilis*[11]; *N*-(piperidin-3-yl) pyrimidine-5-carboxamides (II) can be used as renin inhibitor[12]; 1,2-dihydropyrimidine-5-carboxamide containing morpholine moiety (III) exhibits good antihypertensive activity[13]; and di-substituted amino pyrimidine-5-carboxamides (IV) synthesized by Liang behave as excellent BMX kinase inhibitors.[14] Their structures are shown in Figure 1. However, this series of compounds have rarely been studied in the form of pesticides.

On the other hand, pyridine is a significant nitrogen-containing heterocyclic compound, and its derivatives also show a wide spectrum of biological properties, including antifungal,[15] antimicrobial,[16] antitumor,[17] antiviral,[18] herbicidal,[19] and insecticidal[20] activities. Many pyridine derivatives play crucial roles as agrochemicals due to their high activity, low toxicity, and excellent selectivity. The well-known fungicides boscalid, fluopicolide, and pyribencarb are examples of pyridine derivatives.[21]

In view of the above-mentioned findings, in this study we have linked the (pyridin-3-ylmethyl)amino

*Corresponding author. Email: jiangl@sdau.edu.cn

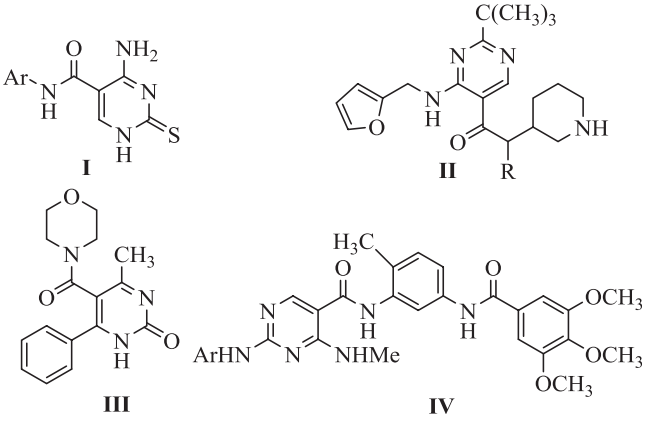


Fig. 1. Structures of some pyrimidine-5-carboxamides.

moiety to the pyrimidine ring bearing a carboxamide group. Thus, we have designed and synthesized a series of novel pyrimidine-5-carboxamides containing the pyridine moiety and evaluated their antifungal activity against *B. cinerea* and *S. sclerotiorum.*

RESULTS AND DISCUSSION

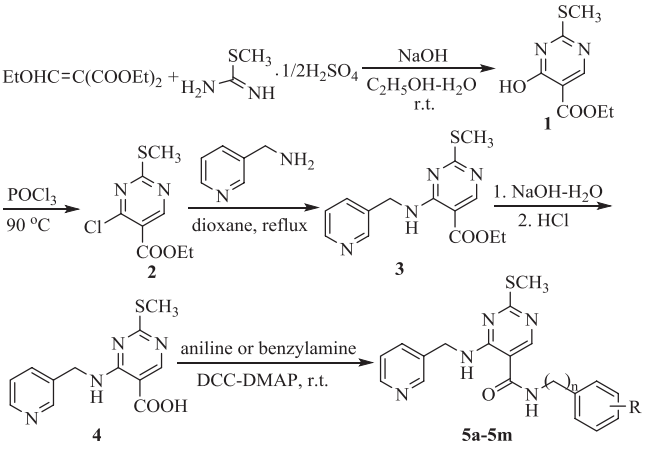
Chemistry

The synthesis of the target compounds **5a**–**5m** is outlined in Scheme 1. Ethyl 4-hydroxy-2-(methylthio) pyrimidine-5-carboxylate (**1**) was prepared by the reaction of diethyl ethoxymethylenemalonate with *S*-methyl isothiourea sulfate in the presence of NaOH,[22] followed by halogenation with $POCl_3$ to convert to its 4-chloro derivative (**2**).[14] Subsequently, **2** was reacted with 3-picolyl amine to yield the key intermediate ethyl 2-methyl thio-4-((pyridin-3-ylmethyl)amino)pyrimidine-5-carboxylate (**3**), which was then hydrolyzed and acidified to give the substituted pyrimidine-5-carboxylic acid (**4**). Finally, **4** was reacted with different substituent amines or benzylamines to afford the target compounds **5**.[23]

The structures of the target compounds were confirmed by ^{1}H NMR, ^{13}C NMR, IR, and elemental analysis. In the ^{1}HNMR spectra, the singlet signals at 2.43–2.54 ppm were assigned to SCH_3 protons, and the signals at 4.73–4.77 ppm were due to the CH_2 protons attached to pyridine ring. The chemical shifts at 7.65–7.73 ppm were attributed to the proton of the pyrimidine ring. The broad signals of the NH protons on the pyrimidine ring were observed at 9.03–9.23 ppm. In the ^{13}C NMR spectra, the chemical shifts of the SCH_3 carbon appeared at 13.4–14.2 ppm and those of CH_2 attached to the pyridine ring at 41.1–42.0 ppm. The signals of carbaxamide carbon were observed at 172.9–175.0 ppm. The signals at 103.7–166.7 ppm were assigned to the aromatic carbons. In their IR spectra, the broad bands at 3225–3276 cm^{-1} were assigned to the N–H stretching vibration, and those at 1633–1660 cm^{-1} were attributed to the C=O of carbaxamide group. The weak or moderate bands of the C–S–C bond could be observed at 710–715 cm^{-1}.

Antifungal activity

The *in vivo* antifungal activities of the target compounds against *B. cinerea* and *S. Sclerotiorum* are listed in Table 1. As can be seen from the table, some target compounds exhibit moderate antifungal activities against *B. cinerea* at 50 μg/mL with inhibition rates of 60.6–67.3%. Some compounds such as **5a**, **5b**, **5e**, **5g**, and **5f** show notable activity with more than 70% inhibition rates at 100 μg/mL but they are lower than that of chlorothalonil (inhibition rate, 87.3%). On the other hand, many compounds show inhibition rates of 61.0–74.8% against *S. Sclerotiorum* at 50 μg/mL, suggesting that they have moderate antifungal activities; except **5b**, **5g,** and **5i**, the other 10 compounds display excellent antifungal activities with more than 90% inhibition rates at 100 μg/mL, and their activities are higher than that of chlorothalonil (inhibition rate, 87.6%). In general, the target compounds possess better inhibition efficacy toward *S. Sclerotiorum* than toward *B. cinerea.* From Table 1, it is also seen that the two kinds of synthesized compounds (**5a**–**5i**) and their *N*-(substituted benzyl)analogs (**5j**–**5m**) showed no obvious difference in their antifungal activity.



Scheme 1. Synthetic route of the title compound **5**.

Table 1. Antifungal activity as inhibition rate for the target compounds **5a**–**5m** (%)

Compd.	R	*n*	*B. cinerea*		*S. sclerotiorum*	
			50 u[b]	100 u	50 u	100 u
5a	H	0	61.2	70.5	66.7	96.2
5b	4-CH_3	0	67.3	73.0	57.8	80.5
5c	4-$CH(CH_3)_2$	0	51.5	64.2	67.0	96.6
5d	4-CH_3O	0	60.6	70.4	58.3	93.2
5e	4-F	0	65.0	71.6	74.8	95.2
5f	3-Cl	0	57.6	66.5	56.9	96.1
5g	4-Cl	0	62.6	71.2	57.4	86.6
5h	4-Br	0	56.5	71.1	61.9	96.7
5i	2-OH-5-Cl	0	58.7	67.5	53.9	79.5
5j	H	1	49.6	69.6	67.4	90.0
5k	4-CH_3O	1	54.8	68.3	64.8	93.1
5l	4-F	1	61.9	68.5	56.1	96.7
5m	4-Cl	1	50.3	68.7	61.0	93.1
CK[a]			82.7	87.3	79.3	87.6

[a] CK, the control, chlorothalonil.
[b] u, unit of the tested concentration (μg/mL).

CONCLUSIONS

In summary, 13 novel *N*-(substituted phenyl)-2-methylthio-4-((pyridin-3-ylmethyl)amino)pyrimidine-5-carboxamides and their *N*-(substituted benzyl) analogs were synthesized from diethyl ethoxymethylene-malonate, *S*-methylisothiourea sulfate, 3-picolylamine, and the substituent amines as starting materials. *In vitro* bioassay showed that most target compounds (e.g. **5a**, **5c**, **5e**, **5f**, and **5h**) exhibited excellent antifungal activities against *S. sclerotiorum* at 100 μg/mL. This study provides a useful reference in the search for novel pyrimidine-based antifungal agents.

EXPERIMENTAL

General

Melting points were determined using a Beijing Tech X-5 microscope melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu IR-440 infrared spectrophotometer using KBr disks. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer using TMS as internal standard (^{1}H NMR at 400 MHz, ^{13}C NMR at 100 MHz). Elemental analyses were carried out on an Elementary Vario EL III analyzer.

All reagents were obtained from commercial sources and used without further purification.

Synthesis of ethyl 2-(methylthio)-4-((pyridin-3-ylmethyl) amino)pyrimidine-5-carboxylate (3)

3-Picolylamine 1.08 g (10 mmol) was added to a mixture of 1.86 g (8 mmol) ethyl 4-chloro-2-(methylthio) pyrimidine-5-carboxylate in dioxane (20 mL). The reaction mixture was refluxed for 2 h, and then concentrated under reduced pressure. The residue was purified by silica gel chromatography with petroleum ether/acetone (4:1 v/v) as eluent to give compound **3**. Yield 91%, white solid, m.p. 166–167°C; IR (KBr) ν cm^{-1}: 3354 (NH), 1681 (C=O), 1572, 1484 (C=C), 714 (C–S–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.67 (s, 2H, Pyrim-NH + Py-H), 8.61 (s, 1H, Py-H), 8.53 (d, 1H, J = 4.0 Hz, Py-H), 7.65 (d, 1H, J = 7.6 Hz, Pyrim-H), 7.26 (t, 1H, J = 5.6 Hz, Py-H), 4.78 (d, 2H, J = 5.6 Hz, Py-CH_2), 4.32 (q, 2H, J = 7.2 Hz, $OC\mathbf{H}_2CH_3$), 2.46 (s, 3H, SCH_3), 1.37 (t, 3H, J = 7.2 Hz, $OCH_2C\mathbf{H}_3$); Anal. Calc. for $C_{14}H_{16}N_4O_2S$: C, 55.25; H, 5.30; N, 18.41%; Found: C, 55.42; H, 5.49; N, 18.20%.

Synthesis of 2-(methylthio)-4-((pyridin-3-ylmethyl)amino) pyrimidine-5-carboxylic acid (4)

The substituted pyrimidine-5-carboxylate (**3**) 3.04 g (10 mmol) in 1 mol/L NaOH solution (30 mL) was refluxed for 20 min. The reaction solution was then adjusted to pH ~ 5 with dilute hydrochloric acid,

whereupon a large amount of solid precipitated. The precipitate was filtered off, washed with water, and dried to afford **4**. Yield 81%, pale yellow solid, m.p. 222–223°C; IR (KBr) ν cm^{-1}: 3322 (NH), 1680 (C=O), 1573, 1475 (C=C), 707 (C–S–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.95 (brs, 1H, Pyrim-NH), 8.57 (s, 1H, Py-H), 8.54 (s, 1H, Py-H), 8.45 (d, 1H, J = 3.2 Hz, Py-H), 7.72 (d, 1H, J = 7.6 Hz, Pyrim-H), 7.24 (q, 1H, J = 2.4 Hz, Py-H), 4.76 (d, 2H, J = 6.0 Hz, Py-CH_2), 2.40 (s, 3H, SCH_3); Anal. Calc. for $C_{12}H_{12}N_4O_2S$: C, 52.16; H, 4.38; N, 20.28%; found C, 52.13; H, 4.60; N, 20.27%.

General procedure for synthesis of *N*-(substituented phenyl/benzyl-4-((pyridin-3-ylmethyl)amino)pyrimidine-5-carboxamides (5)

The appropriate aniline/benzylamine (10 mmol) was added slowly to a mixture of substituted pyrimidine-5-carboxylic acid (**4**) 2.76 g (10 mmol), DCC 2.16 g (10.5 mmol), and DMAP 0.06 g (0.5 mmol) in dry dichloromethane (30 mL) and stirred overnight at room temperature. The progress of the reaction was monitored by TLC with petroleum ether/acetone (2:1, v/v) as a developing solvent. When the reaction was completed, the reaction mixture was filtered off, and the filtrate was evaporated in vacuum. The crude product was recrystallized from DMF–H_2O to afford target compounds **5a**–**5m**.

*N-Phenyl-2-methylthio-4-((pyridin-3-ylmethyl)amino) pyrimidine-5-carboxamide (**5a**)*: Yield 65%, white solid, m.p. 184–185°C; IR (KBr) ν cm^{-1}: 3271 (NH), 1649 (C=O), 1575, 1489 (C=C), 712 (C–S–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.07 (brs, 1H, Pyrim-NH), 8.61 (s, 1H, Py-H), 8.52 (d, 1H, J = 4.4 Hz, Py-H), 8.41 (s, 1H, Py-H), 7.80 (brs, 1H, –C(=O)–NH), 7.65 (d, 1H, J = 8.0 Hz, Pyrim-H), 7.16–7.51 (m, 6H, Py-H + Ph-H), 4.76 (d, 2H, J = 6.0 Hz, Py-CH_2), 2.47 (s, 3H, SCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.9, 165.5, 160.1, 154.0, 149.1, 148.7, 137.4, 135.3, 134.1, 129.0, 124.9, 123.7, 121.2, 104.3, 42.0, 14.2; Anal. Calc. for $C_{18}H_{17}N_5OS$: C, 61.52; H, 4.88; N, 19.93%; found: C, 61.57; H, 5.16; N, 19.74%.

*N-(4-Methylphenyl)-2-methylthio-4-((pyridin-3-yl methyl)amino)-pyrimidine-5-carboxamide (**5b**)*: Yield 70%, white solid, m.p. 226–228°C; IR (KBr) ν cm^{-1}: 3251 (NH), 1645 (C=O), 1574, 1493 (C=C), 715 (C–S–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.08 (brs, 1H, Pyrim-NH), 8.61 (s, 1H, Py-H), 8.51 (d, 1H, J = 4.0 Hz, Py-H), 8.39 (s, 1H, Py-H), 7.89 (brs, 1H, –C(=O)–NH), 7.66 (d, 1H, J = 8.0 Hz, Pyrim-H), 7.16–7.38 (m, 5H, Py-H + Ph-H), 4.75 (d, 2H, J = 5.6 Hz, Py-CH_2), 2.47 (s, 3H, SCH_3), 2.33 (s, 3H, Ph–CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 175.0, 165.4, 160.1, 153.7, 149.2, 148.7, 135.2, 134.9, 134.4, 134.0, 129.6, 123.6, 121.3, 104.4, 42.0, 20.9, 14.2; Anal. Calc. for $C_{19}H_{19}N_5OS$: C, 62.45; H, 5.24; N, 19.16%; found: C, 62.68; H, 5.54; N, 18.88%.

*N-(4-Isopropylphenyl)-2-methylthio-4-((pyridin-3-yl methyl)amino)pyrimidine-5-carboxamide (**5c**)*: Yield 66%, white solid, m.p. 201–202°C; IR (KBr) ν cm^{-1}: 3254 (NH), 1643 (C=O), 1574, 1493 (C=C), 714 (C–S–C); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.08 (brs, 1H, –C(=O)–NH), 9.19 (t, 1H, J = 6 Hz, Pyrim-NH), 8.67 (s, 1H, Py–H), 8.59 (s, 1H, Py–H), 8.45 (d, 1H, J = 3.6 Hz, Py–H), 7.73 (d, 1H, J = 8.0 Hz, Pyrim-H), 7.16–7.59 (m, 5H, Py-H + Ph-H), 4.73 (d, 2H, J = 6.0 Hz, Py-CH_2), 2.87 (m, 1H, CH), 2.53 (s, 3H, SCH_3), 1.22 (d, 6H, J = 6.8 Hz, –CH(C**H**$_3$)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.7, 165.5, 160.2, 153.9, 149.1, 148.7, 145.7, 135.3, 135.0, 134.1, 126.9, 123.6, 121.4, 104.4, 42.0, 33.6, 24.0, 14.1; Anal. Calc. for $C_{21}H_{23}N_5OS$: C, 64.10; H, 5.89; N, 17.80%; found: C, 64.42; H, 6.11; N, 17.52%.

*N-(4-Methoxyphenyl)-2-methylthio-4-((pyridin-3-yl methyl)amino)pyrimidine-5-carboxamide (**5d**)*: Yield 65%, white solid, m.p. 187–188°C; IR (KBr) ν cm^{-1}: 3275 (NH), 1640 (C=O), 1575, 1510 (C=C), 712 (C–S–C); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.04 (brs, 1H, –C(=O)–NH), 9.21 (t, 1H, J = 6.0 Hz, Pyrim–NH), 8.66 (s, 1H, Py–H), 8.58 (s, 1H, Py–H), 8.45 (d, J = 6.0 Hz, 1H, Py–H), 7.73 (d, 1H, J = 8.0 Hz, Pyrim–H), 6.85–7.56 (m, 5H, Py–H + Ph–H), 4.73 (d, 2H, J = 6.0 Hz, Py–CH_2), 3.77 (s, 3H, OCH_3), 2.54 (s, 3H, SCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 173.0, 164.7, 159.4, 155.6, 154.6, 148.7, 147.8, 134.8, 134.3, 131.1, 123.1, 122.3, 113.2, 104.5, 54.8, 41.1, 13.5; Anal. Calc. for $C_{19}H_{19}N_5O_2S$: C, 59.83; H, 5.02; N, 18.36%; found: C, 59.87; H, 5.31; N, 18.25%.

*N-(4-Fluorophenyl)-2-methylthio-4-((pyridin-3-yl methyl)amino)pyrimidine-5-carboxamide (**5e**)*: Yield 65%, white solid, m.p. 230–231°C; IR (KBr) ν cm^{-1}: 3248 (NH), 1654 (C=O), 1578, 1492 (C=C), 716 (C–S–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.05 (brs, 1H, Pyrim-NH), 8.62 (s, 1H, Py-H), 8.53

(d, 1H, J = 3.2 Hz, Py-H), 8.39 (s, 1H, Py-H), 7.67 (brs, 1H, –C(=O)–NH), 7.65 (s, 1H, Pyrim-H), 7.05–7.48 (m, 5H, Py-H + Ph-H), 4.76 (d, 2H, J = 6.0 Hz, Py-CH_2), 2.48 (s, 3H, SCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 173.2, 164.9, 159.3, 157.1, 154.9, 148.8, 147.9, 134.6, 134.4, 123.2, 122.4, 122.3, 114.9, 114.7, 104.5, 41.2, 13.5; Anal. Calc. for $C_{18}H_{16}FN_5OS$: C, 58.82; H, 4.37; N, 18.96%; found: C, 58.42; H, 4.67; N, 18.74%.

*N-(3-Chlorophenyl)-2-methylthio-4-((pyridin-3-ylmethyl)amino)pyrimidine-5-carboxamide (**5f**)*: Yield 62%, white solid, m.p. 175–176°C; IR (KBr) ν cm^{-1}: 3250 (NH), 1660 (C=O), 1576, 1479 (C=C), 717 (C–S–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.04 (brs, 1H, Pyrim-NH), 8.62 (s, 1H, Py-H), 8.53 (d, 1H, J = 4.0 Hz, Py-H), 8.40 (s, 1H, Py-H), 7.80 (brs, 1H, –C(=O)–NH), 7.67 (s, 1H, Pyrim-H), 7.14–7.65 (m, 5H, Py-H + Ph-H), 4.77 (d, 2H, J = 6.0 Hz, Py-CH_2), 2.48 (s, 3H, SCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 175.1, 165.4, 160.1, 153.9, 149.1, 148.8, 138.7, 135.3, 134.6, 133.9, 129.9, 124.8, 123.7, 121.0, 118.8, 103.9, 42.1, 14.2; Anal. Calc. for $C_{18}H_{16}ClN_5OS$: C, 56.03; H, 4.18; N, 18.15%; found: C, 55.61; H, 4.47; N, 17.89%.

*N-(4-Chlorophenyl)-2-methylthio-4-((pyridin-3-ylmethyl)amino)pyrimidine-5-carboxamide (**5g**)*: Yield 68%, white solid, m.p. 245–246°C; IR (KBr) ν cm^{-1}: 3263 (NH), 1655 (C=O), 1575, 1494 (C=C), 716 (C–S–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.03 (brs, 1H, Pyrim-NH), 8.62 (s, 1H, Py-H), 8.53 (d, J = 4.8 Hz, 1H, Py-H), 8.39 (s, 1H, Py-H), 7.72 (brs, 1H, –C(=O)–NH), 7.66 (d, 1H, J = 8.0 Hz, Pyrim-H), 7.26–7.48 (m, 5H, Py-H + Ph-H), 4.77 (d, 2H, J = 6.0 Hz, Py-CH_2), 2.48 (s, 3H, SCH_3); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6) δ (ppm): 173.3, 165.0, 159.3, 155.0, 148.7, 147.9, 137.2, 134.9, 134.4, 128.1, 127.6, 123.1, 121.9, 104.4, 41.2, 13.5; Anal. Calc. for $C_{18}H_{16}ClN_5OS$: C, 56.03; H, 4.18; N, 18.15%; found: C, 55.01; H, 4.41; N, 18.00%.

*N-(4-Bromophenyl)-2-methylthio-4-((pyridin-3-ylmethyl)amino)pyrimidine-5-carboxamide (**5h**)*: Yield 67%, white solid, m.p. 245–246°C; IR (KBr) ν cm^{-1}: 3266 (NH), 1655 (C=O), 1574, 1489 (C=C), 715 (C–S–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 10.23 (brs, 1H, –C(=O)–NH), 9.14 (t, 1H, J = 6.0 Hz, Pyrim-NH), 8.67 (s, 1H, Py-H), 8.58 (s, 1H, Py-H), 8.45 (s, 1H, J = 4.8 Hz, Py-H), 7.73 (d, 1H, J = 8.0 Hz, Pyrim-H), 7.29–7.67 (m, 5H, Py-H + Ph-H), 4.74 (d, 2H, J = 5.6 Hz, Py-CH_2), 2.43 (s, 3H, SCH_3); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6) δ (ppm): 173.4, 165.1, 159.3, 154.9, 148.7, 147.9, 137.6, 134.8, 134.2, 130.9, 123.0, 122.2, 115.6, 104.2, 41.2, 13.5; Anal. Calc. for $C_{18}H_{16}BrN_5OS$: C, 50.24; H, 3.75; N, 16.27%; found: C, 49.92; H, 3.88; N, 15.95%.

*N-(5-Chloro-2-hydroxyphenyl)-2-methylthio-4-((pyridin-3-ylmethyl)amino)pyrimidine-5-carboxamide (**5i**)*: Yield 79%, white solid, m.p. 239–240°C; IR (KBr) ν cm^{-1}: 3276 (NH), 1649 (C=O), 1575, 1492 (C=C), 710 (C–S–C); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.84 (brs, 1H, OH), 9.48 (brs, 1H, –C(=O)–NH), 9.19 (brs, 1H, Pyrim-NH), 8.65 (s, 1H, Py-H), 8.58 (s, 1H, Py-H), 8.45 (s, J = 4.0 Hz, 1H, Py-H), 6.88–7.74 (m, 5H, Py-H + Pyrim-H + Ph-H), 4.74 (s, 2H, Py-CH_2), 2.43 (s, 3H, SCH_3); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6) δ (ppm): 173.3, 165.0, 159.3, 155.0, 148.8, 148.2, 147.9, 134.9, 134.5, 126.2, 124.9, 123.8, 123.2, 122.2, 116.6, 104.2, 41.2, 13.5; Anal. Calc. for $C_{18}H_{16}ClN_5O_2S$: C, 53.80; H, 4.01; N, 17.43%; found: C, 53.62; H, 4.24; N, 17.18%.

*N-Benzyl-2-methylthio-4-((pyridin-3-ylmethyl)amino)pyrimidine-5-carboxamide (**5j**)*: Yeild 59%, white solid, m.p. 152–153°C; IR (KBr) ν cm^{-1}: 3228 (NH), 1634 (C=O), 1577, 1494 (C=C), 710 (C–S–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.22 (brs, 1H, Pyrim-NH), 8.60 (s, 1H, Py-H), 8.51 (d, 1H, J = 3.6 Hz, Py-H), 8.22 (s, 1H, Py-H), 7.66 (d, 1H, J = 8.0 Hz, Pyrim-H), 7.24–7.37 (m, 6H, Py-H + Ph-H), 6.55 (brs, 1H, –C(=O)–NH), 4.74 (d, 2H, J = 6.0 Hz, Py-CH_2), 4.57 (d, J = 5.6 Hz, 2H, Ph-CH_2), 2.44 (s, 3H, SCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.2, 166.8, 160.0, 153.7, 148.9, 148.4, 138.0, 135.1, 134.2, 128.5, 127.6, 127.4, 123.5, 103.8, 43.4, 41.7, 14.1; Anal. Calc. for $C_{19}H_{19}N_5OS$: C, 62.45; H, 5.24%; N, 19.16; found: C, 62.28; H, 5.57; N, 18.88%.

*N-(4-Methoxybenzyl)-2-methylthio-4-((pyridin-3-ylmethyl)amino)pyrimidine-5-carboxamide (**5k**)*: Yield 57%, white solid, m.p. 188–189°C; IR (KBr) ν cm^{-1}: 3234 (NH), 1633 (C=O), 1579, 1494 (C=C), 710 (C–S–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.23 (brs, 1H, Pyrim-NH), 8.59 (s, 1H, Py-H), 8.50 (d, J = 3.6 Hz, 1H, Py-H), 8.21 (s, 1H, Py-H), 7.66 (d, 1H, J = 8.0 Hz, Pyrim-H), 6.86–7.26 (m, 5H, Py-H + Ph-H), 6.54 (brs, 1H, –C(=O)–NH), 4.74 (d, 2H, J = 5.6 Hz, Py-CH_2), 4.57 (d, J = 6.0 Hz, 2H, Ph-CH_2), 3.80 (s, 3H, Ph-CH_3),

2.43 (s, 3H, SCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.6, 166.7, 160.1, 159.1, 153.5, 149.1, 148.6, 135.2, 134.2, 129.8, 129.2, 123.5, 114.1, 103.9, 55.3, 43.2, 41.9, 14.2; Anal. Calc. for $C_{20}H_{21}N_5O_2S$: C, 60.74; H, 5.35; N, 17.71%; found: C, 60.39; H, 5.69; N, 17.50%.

*N-(4-Fluorobenzyl)-2-methylthio-4-((pyridin-3-ylmethyl) amino)pyrimidine-5-carboxamide (**5l**)*: Yield 52%, white solid, m.p. 196–197°C; IR (KBr) ν cm^{-1}: 3232 (NH), 1635 (C=O), 1578, 1495 (C=C), 710 (C–S–C); ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.19 (brs, 1H, Pyrim-NH), 8.61 (s, 1H, Py-H), 8.52 (d, 1H, J = 4.0 Hz, Py-H), 8.22 (s, 1H, Py-H), 7.66 (d, 1H, J = 8.0 Hz, Pyrim-H), 7.02–7.30 (m, 5H, Py-H + Ph-H), 6.40 (brs, 1H, –C(=O)–NH), 4.75 (d, 2H, J = 6.0 Hz, Py-CH_2), 4.54 (d, 2H, J = 6.0 Hz, Ph-CH_2), 2.44 (s, 3H, SCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 172.9, 166.2, 162.3, 159.9, 159.5, 154.3, 148.6, 147.8, 134.8, 134.3, 128.9, 123.1, 114.7, 103.7, 41.6, 41.1, 13.4; Anal. Calc. for $C_{19}H_{18}FN_5OS$: C, 59.52; H, 4.73; N, 18.26%; found: C, 58.96; H, 5.04; N, 17.96%.

*N-(4-Chlorobenzyl)-2-methylthio-4-((pyridin-3-ylmethyl)amino)pyrimidine-5-carboxamide (**5m**)*: Yield 53%, white solid, m.p. 189–190°C; IR (KBr) ν cm^{-1}: 3225 (NH), 1635 (C=O), 1580, 1493 (C=C), 710 (C–S–C); ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.19 (brs, 1H, Pyrim-NH), 8.60 (s, 1H, Py-H), 8.51 (d, 1H, J = 4.0 Hz, Py-H), 8.24 (s, 1H, Py-H), 7.65 (d, 1H, J = 8.0 Hz, Pyrim-H), 7.24–7. 33 (m, 5H, Py-H + Ph-H), 6.58 (brs, 1H, –C(=O)–NH), 4.74 (d, 2H, J = 5.6 Hz, Py-CH_2), 4.54 (d, 2H, J = 5.6 Hz, Ph-CH_2), 2.44 (s, 3H, SCH_3); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6) δ (ppm): 173.0, 166.2, 159.5, 154.4, 148.7, 147.8, 137.8, 134.8, 134.4, 131.6, 128.7, 127.9, 123.1, 103.7, 41.7, 41.1, 13.4; Anal. Calc. for $C_{19}H_{18}ClN_5OS$: C, 57.07; H, 4.54; N, 17.51%; found: C, 57.03; H, 4.84; N, 17.38%.

Bioactivity assay

The *in vitro* fungicidal activities of the target compounds **5a**–**5m** against *B. cinerea* and *S. sclerotiorum* were evaluated by the mycelium growth rate method according to the literature.[24] The concentrations of the tested compounds were 50 and 100 μg/mL, respectively. The commercial fungicide chlorothalonil was used as positive controls, and sterile water was used as blank. Three replicates were performed in the bioassay. The inhibition rate was calculated by the following formula:

$$\text{Inhibition rate } (\%) = [(D_0 - D_1)/D_0] \times 100$$

where D_0 is the expansion diameter of the mycelia in the blank test, and D_1 is the expansion diameter of mycelia in the presence of the tested compound.

ACKNOWLEDGMENT

This work was supported by the Natural Science Foundation of Shandong Province, China (No. ZR2014BM030).

SUPPORTING INFORMATION

Additional supporting information is available in the online version of this article.

REFERENCES

1. C. Y. Zhang, Z. X. Zhang, G. R. Cui, *Plant Protection* **2006**, *32*, 32.
2. H. Hu, Q. Dong, J. Wu, D. Huo, Y. Wang, H. Yang, *J. Anhui Agri. Sci.* **2013**, *41*, 5283.
3. G. A. Bardas, T. Veloukas, O. Koutita, G. S. Karaoglanidis, *Pest Manag. Sci.* **2010**, *66*, 967.
4. D. M. Neumann, A. Cammarata, G. Backes, G. E. Palmer, B. S. Jursic, *Bioorg. Med. Chem.* **2014**, *22*, 813.
5. Y. P. Yi, X. M. Xu, Y. Liu, S. J. Xu, X. Huang, J. P. Liang, R. F. Shang, *Eur. J. Med. Chem.* **2017**, *126*, 687.
6. A. A. Abu-Hashem, F. A. Badria, *J. Chin. Chem. Soc.* **2015**, *62*, 506.
7. A. Orjales, R. Mosquera, B. Lopez, R. Olivera, R. L. Labeaga, M. T. Nunez, *Bioorg. Med. Chem.* **2008**, *16*, 2183.
8. A. Khurshid, A. Saeed, *J. Chin. Chem. Soc.* **2017**, *64*, 224.
9. H. Dai, L. Y. Ye, Y. Li, Y. Fang, Y. J. Shi, Z. P. Liang, J. Shi, *Chin. J. Org. Chem.* **2017**, *37*, 1020.
10. W. Wei, S. Zhou, D. D. Cheng, Y. X. Li, J. B. Liu, Y. T. Xie, Y. H. Li, Z. M. Li, *Bioorg. Med. Chem. Lett.* **2017**, *27*, 3365.
11. A. A. Fadda, R. Rabie, H. A. Etman, A. S. Fouda, *Res. Chem. Intermed.* **2015**, *41*, 740.
12. H. M. Marvaniya, P. K. Parikh, D. J. Sen, *J. Appl. Pharm. Sci.* **2011**, *1*, 109.
13. Y. Imaeda, M. Tawada, S. Suzuki, M. Tomimoto, M. Kondo, N. Tarui, T. Sanada, R. Kanagawa, G. Snell, C. A. Behnke, K. Kubo, T. Kuroita, *Bioorg. Med. Chem.* **2016**, *24*, 5771.

14. X. Liang, F. Lv, B. Wang, K. Yu, H. Wu, Z. Qi, Z. Jiang, C. Chen, A. Wang, W. Miao, W. Wang, Z. Hu, J. Liu, X. Liu, Z. Zhao, L. Wang, S. Zhang, Z. Ye, C. Wang, T. Ren, Y. Wang, Q. Liu, J. Liu, *J. Med. Chem.* **2017**, *60*, 1793.
15. Y. Liu, Z. M. Liu, X. F. Cao, X. Liu, H. L. He, Y. S. Yang, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4779.
16. K. Singaram, D. Marimuthu, S. Baskaran, S. Chinaga, *J. Chin. Chem. Soc.* **2016**, *63*, 758.
17. X. H. Liu, H. F. Liu, X. Shen, B. A. Song, P. S. Bhadury, H. L. Zhu, J. X. Liu, X. B. Qi, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4163.
18. T. X. Li, J. Zhang, J. K. Pan, Z. X. Wu, D. Y. Hu, B. A. Song, *Eur. J. Med. Chem.* **2017**, *125*, 657.
19. X. B. Chen, D. Q. Shi, *Phosphor. Sulfur Silicon Relat. Elem.* **2008**, *183*, 1134.
20. Y. Xu, D. Y. Yang, X. G. Zou, C. H. Rui, Z. Y. Zhou, Y. Q. Ma, Z. H. Qin, *Pest Manag. Sci.* **2017**, *73*, 1927.
21. A. Y. Guan, C. L. Liu, X. F. Sun, Y. Xie, M. A. Wang, *Bioorg. Med. Chem.* **2016**, *24*, 342.
22. T. W. Hai, Y. W. Hong, R. W. Liang, M. Pu, B. Q. Ling, *Chin. J. Appl. Chem.* **2015**, *32*, 412.
23. M. Jadhav, R. S. Kalhapure, S. Rambharose, *J. Ind. Eng. Chem.* **2017**, *47*, 405.
24. J. L. Sun, W. Mu, *Pesticide Science Experimental Techniques and Guidance*, Chemical Industry Press, Beijing, **2009**, p. 228.

