



# Synthesis and Antifungal Activity of Pyrimidine Derivatives Containing an Amide Moiety

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In this study, 17 novel pyrimidine derivatives containing an amide moiety were synthesized. Then their *in vitro* antifungal activities against *Botryosphaeria dothidea* (*B. dothidea*), *Phomopsis* sp., and *Botrytis cinerea* (*B. cinerea*) were determined. A preliminary biological test showed that compounds 5-bromo-2-fluoro-*N*-(2-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)benzamide (**5f**) and 5-bromo-2-fluoro-*N*-(3-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)benzamide (**5o**) exhibited higher antifungal activity against *Phomopsis* sp., with an inhibition rate of 100% compared to that of Pyrimethanil at 85.1%. In particular, compound **5o** exhibited excellent antifungal activity against *Phomopsis* sp., with the EC<sub>50</sub> value of 10.5 μg/ml, which was even better than that of Pyrimethanil (32.1 μg/ml). As far as we know, this is the first report on the antifungal activities against *B. dothidea*, *Phomopsis* sp., and *B. cinerea* of this series of pyrimidine derivatives containing an amide moiety.

**Keywords:** pyrimidine, amide, synthesize, antifungal activity, kiwifruit soft rot disease

## INTRODUCTION

Plant fungal diseases pose serious threats to crop production and caused huge economic losses throughout the world (Strange and Scott, 2005). In recent years, crop cultivators continually battle with plant fungal diseases affecting crops. The available traditional fungicides used for plant fungal diseases control represent a danger to the living system by killing not only the target fungi but also affecting beneficial living systems (Patel et al., 2014). To protect crops from fungal diseases, commercial agriculture relies heavily on the inputs of chemical pesticides. The resistance of plant fungal diseases against fungicides is rapidly becoming a serious problem. Therefore, the development of novel and promising fungicides is urgently required.

Pyrimidines are important substances in the synthesis of various active molecules that are extensively used in the intermediate skeleton of agrochemicals (Chen et al., 2015; Wu et al., 2015) and have attracted more and more attention due to their extensive biological activities (Sun et al., 2006), including antiviral (Wu et al., 2016a), antibacterial (Wu et al., 2016b), antifungal (Wu et al., 2019, 2020), and insecticidal (Liu et al., 2017; Wu et al., 2020) activities. For example, Zan et al. reported pyrimidine derivatives bearing a dithioacetal moiety as effective antiviral agents for controlling the tomato chlorosis virus (ToCV) (Zan et al., 2021). Zhang et al. found a series of arylpyrazole pyrimidine ether derivatives with promising bioactivity for combating cucumber downy mildew (Zhang et al., 2019). Li and coworkers showed that pyrimidine thiourea derivatives had good herbicidal activities against *Digitaria adscendens* and *Amaranthus retroflexus* (Li et al., 2021a). In the past few years, several pyrimidine compounds have been commercialized as fungicides (such as Pyrimethanil, Fenarimol, Diflufenorim, and Mepanoyrim) for controlling plant fungal diseases,

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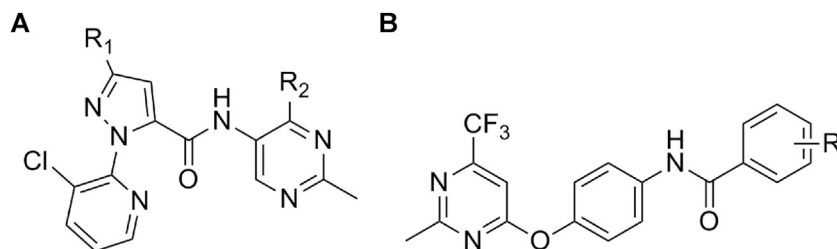
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**FIGURE 1** | Structures of pyrimidine derivatives containing an amide moiety reported in our previous works.



**FIGURE 2** |  $EC_{50}$  values of the target compounds **5f**, **5o**, and **5p** against *Phomopsis* sp.

such as cucumber gray mold (Wang et al., 2021), grape downy mildew (Huang et al., 2018), kiwifruit leaf spot (Shi et al., 2021), and so on. Due to the excellent features of low toxicity, the fact that it is easily synthesized and derived, and considering pyrimidine as a parent compound, the development of promising agrochemical candidates will soon become a reality. Meanwhile, amine, a key moiety in heterocyclic chemistry, play a leading role in pesticide chemistry due to their potent bioactivities including antifungal (Cheng et al., 2020; Chen et al., 2021a), antibacterial (Chen et al., 2021a), antiviral (Tang et al., 2020), herbicidal (Sun et al., 2020), and insecticidal (Chen et al., 2021b; Li et al., 2021b) activities. In our previous work, we reported a series of novel pyrimidine derivatives containing an amine moiety (Figure 1) and found that the target compounds revealed certain antifungal, insecticidal, and antiviral activities (Wu et al., 2016a; Wu et al., 2020).

This study aimed to design and synthesize a series of novel pyrimidine derivatives containing an amide moiety, and investigate their *in vitro* antifungal activities against *Botryosphaeria dothidea* (*B. dothidea*), *Phomopsis* sp., and *Botrytis cinerea* (*B. cinerea*). A biological test showed that compound 5-bromo-2-fluoro-*N*-(3-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)benzamide (**5o**) exhibited excellent antifungal activity against *Phomopsis* sp., with an  $EC_{50}$  value of 10.5  $\mu\text{g/ml}$ , which is even better than Pyrimethanil (32.1  $\mu\text{g/ml}$ ).

## MATERIALS AND METHODS

### General Information

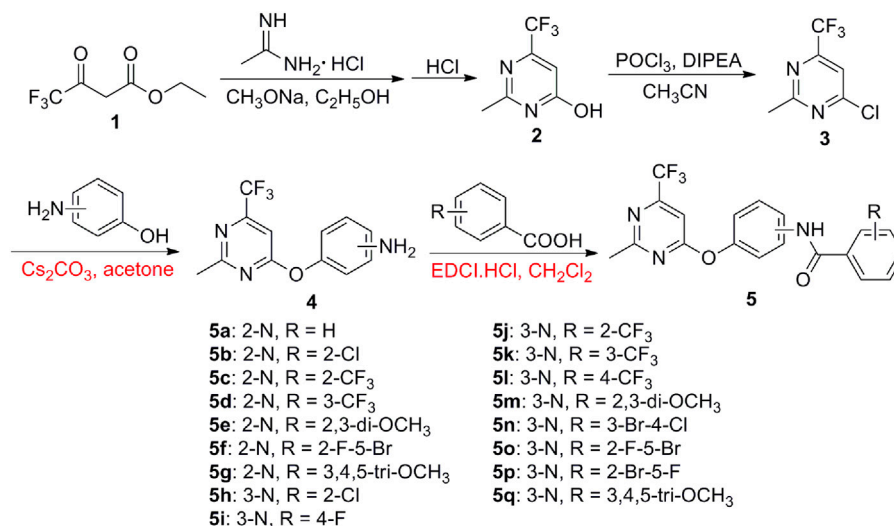
The melting points of the products were determined on an XT-4 binocular microscope (Beijing Tech Instrument Co., China) and

were not corrected.  $^1\text{H}$  and  $^{13}\text{C}$  NMR (solvent  $\text{DMSO}-d_6$ ) spectra were recorded on a Bruker AVANCE HD 600 MHz Digital NMR Spectrometer (Bruker Company, Billerica, MA, United States) at room temperature using TMS as an internal standard. High-resolution mass spectrometry (HRMS) was carried out on an Agilent Technologies 6540 UHD Accurate-Mass Q-TOF LC/MS (Agilent Technologies, Palo Alto, CA, United States). All anhydrous solvents were dried and purified according to standard techniques before use. Unless otherwise noted, all common reagents and solvents were used as obtained from commercial supplies without further purifications.

### Synthesis

#### General Procedure for the Preparation of the Intermediates 2–4

As shown in Scheme 1, to a 250 ml round bottom flask, trifluoroacetoacetate (**1**, 0.05 mol), acetamidine hydrochloride (0.05 mol), sodium methoxide (0.075 mol), and ethanol (100 ml) were added and refluxed for 10 h. After that, the mixture was acidified with dilute HCl to pH 7. The crude products were extracted using ethyl acetate to produce intermediate **2**. Then, intermediate **2** (0.05 mol),  $\text{POCl}_3$  (0.1 mol), and  $\text{CH}_3\text{CN}$  (120 ml) were added to a 250 ml round bottom flask to react for 0.5 h at a reflux temperature and then diisopropylethylamine (0.06 mol) was added dropwise. After continuously refluxing for 8 h, excess  $\text{POCl}_3$  and  $\text{CH}_3\text{CN}$  were distilled and then ice water (60 ml) was added. Finally, the mixture was alkalified with dilute NaOH to pH 9 and extracted using  $\text{CH}_2\text{Cl}_2$  to give intermediate **3**.



**SCHEME 1** | Synthetic routes of the target compounds 5a–5q.

To a 50 ml three-necked round-bottomed flask equipped with a magnetic stirrer, the key intermediate **3** (0.01 mol) was dissolved in acetone (50 ml), Cs<sub>2</sub>CO<sub>3</sub> (0.012 mol), and 3-aminophenol or 2-aminophenol (0.01 mol) were added. The reactions reacted for 5–6 h at room temperature, and the solvent was removed. The residue was added with water, the precipitate formed was filtered off and recrystallized from ethanol to give the intermediate **4**.

### General Procedure for the Preparation of the Target Compounds 5a–5q

To a 50 ml three-necked round-bottomed flask equipped with a magnetic stirrer, the key intermediate **4** (0.02 mol), aromatic acid (0.02 mol), and dimethylaminopyridine (0.0002 mol) dissolved in dichloromethane (10 ml), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.03 mol) were added. The reactions were reacted for 8–10 h at room temperature. The solvent was then dried under vacuum and recrystallized from ethanol to give the pure target compounds **5a–5q**.

The structures were confirmed by <sup>1</sup>H NMR, <sup>13</sup>C NMR, and HRMS. <sup>1</sup>H NMR, <sup>13</sup>C NMR, and HRMS spectral data for the target compounds **5a–5q** are reported in the **Supplementary Material**. In the <sup>1</sup>H NMR spectra of **5a–5q**, one singlet at δ 9.70–10.74 ppm were attributed to the -CONH-. A singlet at δ 6.90–7.54 ppm demonstrated the presence of the proton of the pyrimidine fragment. A singlet at δ 2.31–2.54 ppm integrating for three protons was assigned to Pyrimidine-CH<sub>3</sub> protons. The structure of **5a–5q** was also confirmed by its HRMS spectral data. In all HRMS spectrum, the molecular ion peak was noticed m/z for ([M + H]<sup>+</sup>) corresponding to all of the target molecular weight.

**N-(2-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)benzamide (5a)**. White solid; yield 75%; m.p. 173–175°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 600 MHz, ppm) δ: 9.72 (s, 1H), 7.96 (d, 2H, *J* = 7.8 Hz), 7.76 (d, 1H, *J* = 7.2 Hz), 7.71 (t, 1H, *J* = 8.4 Hz), 7.53 (t,

2H, *J* = 9.0 Hz), 7.41–7.31 (m, 3H, Ph-H), 6.90 (s, 1H), 2.32 (s, 3H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 150 MHz, ppm) δ: 168.66, 164.39, 162.11, 152.99 (q, *J* = 33.6 Hz), 144.29, 134.43, 130.75, 130.08, 129.35, 129.22, 126.84, 126.40, 125.99, 124.06, 122.24 (q, *J* = 272.85 Hz), 100.17, 25.75; HRMS (calcd.) for C<sub>19</sub>H<sub>14</sub>F<sub>3</sub>N<sub>3</sub>O<sub>2</sub> (M + H)<sup>+</sup> 374.1107, found 374.1107.

**2-Chloro-N-(2-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)benzamide (5b)**. White solid; yield 49%; m.p. 157–158°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 600 MHz, ppm) δ: 9.73 (s, 1H), 7.93 (d, 1H, *J* = 7.8 Hz), 7.82 (d, 1H, *J* = 6.0 Hz), 7.66–7.63 (m, 2H), 7.49–7.46 (m, 1H), 7.43 (d, 1H, *J* = 7.8 Hz), 7.40–7.33 (m, 2H), 6.91 (s, 1H), 2.38 (s, 3H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 150 MHz, ppm) δ: 168.75, 162.96, 162.24, 153.25 (q, *J* = 33.6 Hz), 144.14, 134.62, 133.51, 132.34, 131.70, 130.75, 128.70, 127.80, 127.14, 126.60, 123.97, 122.28 (q, *J* = 272.55 Hz), 120.46, 100.24, 25.86; HRMS (calcd.) for C<sub>19</sub>H<sub>13</sub>ClF<sub>3</sub>N<sub>3</sub>O<sub>2</sub> (M + H)<sup>+</sup> 408.0721, found 408.0720.

**N-(2-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)-2-(trifluoromethyl)benzamide (5c)**. White solid; yield 46%; m.p. 148–150°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 600 MHz, ppm) δ: 9.77 (s, 1H), 7.94 (t, 2H, *J* = 6.0 Hz), 7.86–7.81 (m, 3H), 7.41–7.36 (m, 3H), 6.92 (s, 1H), 2.39 (s, 3H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 150 MHz, ppm) δ: 168.75, 163.97, 162.25, 153.52 (q, *J* = 33.6 Hz), 144.03, 133.33, 133.21, 131.22, 130.77, 129.50, 128.33, 128.11 (q, *J* = 31.95 Hz), 127.52, 127.36, 126.57, 124.65 (q, *J* = 272.1 Hz), 123.45, 122.25 (q, *J* = 272.7 Hz), 120.45, 100.23, 25.85; HRMS (calcd.) for C<sub>20</sub>H<sub>13</sub>F<sub>6</sub>N<sub>3</sub>O<sub>2</sub> (M + H)<sup>+</sup> 442.0985, found 442.0979.

**N-(2-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)-3-(trifluoromethyl)benzamide (5d)**. White solid; yield 68%; m.p. 138–140°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 600 MHz, ppm) δ: 9.81 (s, 1H), 8.30 (d, 1H, *J* = 7.8 Hz), 8.14 (s, 1H), 8.09 (d, 1H, *J* = 7.8 Hz), 7.82 (t, 1H, *J* = 7.8 Hz), 7.78 (d, 1H, *J* = 7.2 Hz), 7.48 (d, 1H, *J* = 8.4 Hz), 7.41–7.35 (m, 2H), 6.91 (s, 1H), 2.30 (s, 3H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 150 MHz, ppm) δ: 168.66, 163.13, 162.04,

153.33 (q,  $J = 34.2$  Hz), 144.11, 134.06, 130.82, 130.77, 130.60, 130.55, 130.16 (q,  $J = 31.65$  Hz), 127.09, 126.44, 126.08, 126.00, 124.92 (q,  $J = 270.9$  Hz), 124.18, 123.10 (q,  $J = 270.6$  Hz), 100.10, 25.60; HRMS (calcd.) for  $C_{20}H_{13}F_6N_3O_2$  ( $M + H$ )<sup>+</sup> 442.0985, found 442.0979.

**2,3-Dimethoxy-N-(2-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)benzamide (5e).** White solid; yield 50%; m.p. 130–131°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 600 MHz, ppm)  $\delta$ : 7.63 (s, 1H), 7.54 (d, 1H,  $J = 7.6$  Hz), 7.46 (t, 1H,  $J = 7.4$  Hz), 7.41 (d, 1H,  $J = 8.1$  Hz), 7.35–7.33 (m, 2H), 7.06 (d, 1H,  $J = 8.1$  Hz), 6.97 (t, 1H,  $J = 7.9$  Hz), 6.92 (d, 1H,  $J = 7.6$  Hz), 3.76 (s, 3H), 3.69 (s, 3H), 2.40 (s, 3H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 150 MHz, ppm)  $\delta$ : 169.52, 168.91, 162.84, 156.21 (q,  $J = 35.25$  Hz), 152.54, 147.60, 145.80, 132.19, 131.54, 130.54, 127.24, 124.32, 124.10, 121.66 (q,  $J = 273.45$  Hz), 115.48, 108.51, 103.05, 61.44, 56.28, 25.56; HRMS (calcd.) for  $C_{21}H_{18}F_3N_3O_4$  ( $M + H$ )<sup>+</sup> 434.1322, found 434.1318.

**3,4,5-trimethoxy-N-(2-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)benzamide (5g).** White solid; yield 58%; m.p. 127–128°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 600 MHz, ppm)  $\delta$ : 9.74 (s, 1H), 7.72 (d, 1H,  $J = 7.8$  Hz), 7.40–7.35 (m, 3H), 7.21 (s, 2H, Ph-H), 6.89 (s, 1H), 3.78 (s, 6H), 3.75 (s, 3H), 2.33 (s, 3H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 150 MHz, ppm)  $\delta$ : 168.77, 163.92, 153.21 (q,  $J = 34.6$  Hz), 153.18, 142.77, 130.66, 126.93, 124.22, 120.40 (q,  $J = 272.3$  Hz), 107.36, 60.67, 56.37, 25.76; HRMS (calcd.) for  $C_{22}H_{20}F_3N_3O_5$  ( $M + H$ )<sup>+</sup> 464.1428, found 464.1420.

**2-Chloro-N-(3-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)benzamide (5h).** White solid; yield 62%; m.p. 136–139°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 600 MHz, ppm)  $\delta$ : 10.74 (s, 1H), 7.75 (s, 1H), 7.62–7.58 (m, 3H), 7.54–7.52 (m, 2H), 7.49–7.45 (m, 2H), 7.04 (dd, 1H,  $J_1 = 1.3$  Hz,  $J_2 = 8.0$  Hz), 2.54 (s, 3H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 150 MHz, ppm)  $\delta$ : 170.52, 169.76, 165.62, 156.54 (q,  $J = 34.65$  Hz), 155.83, 152.36, 140.85, 137.16, 131.74, 130.56, 130.40, 130.18, 129.43, 127.78, 123.67 (q,  $J = 273.45$  Hz), 117.52, 117.46, 112.91, 103.46, 25.94; HRMS (calcd.) for  $C_{19}H_{13}ClF_3N_3O_2$  ( $M + H$ )<sup>+</sup> 408.0721, found 408.0716.

**4-Fluoro-N-(3-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)benzamide (5i).** White solid; yield 85%; m.p. 136–139°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 600 MHz, ppm)  $\delta$ : 9.72 (s, 1H), 8.06–8.04 (dd, 2H,  $J_1 = 5.4$  Hz,  $J_2 = 8.4$  Hz), 7.79 (d, 1H,  $J = 7.2$  Hz), 7.41–7.35 (m, 5H), 7.31 (t, 1H,  $J = 7.8$  Hz), 6.91 (s, 1H), 2.37 (s, 3H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 150 MHz, ppm)  $\delta$ : 168.67, 167.65, 165.07, 163.46, 162.07, 153.20 (q,  $J = 33.75$  Hz), 144.08, 133.11, 130.74, 126.87, 126.30, 125.99, 125.89, 124.18, 122.23 (q,  $J = 272.85$  Hz), 116.48, 100.25, 25.75; HRMS (calcd.) for  $C_{19}H_{13}F_4N_3O_2$  ( $M + H$ )<sup>+</sup> 392.1017, found 392.1013.

**N-(3-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)-2-(trifluoromethyl)benzamide (5j).** White solid; yield 73%; m.p. 140–142°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 600 MHz, ppm)  $\delta$ : 10.80 (s, 1H), 7.87 (d, 1H,  $J = 7.7$  Hz), 7.82 (t, 1H,  $J = 7.3$  Hz), 7.75–7.72 (m, 3H), 7.58 (d, 1H,  $J = 7.9$  Hz), 7.52 (s, 1H), 7.47 (t, 1H,  $J = 8.2$  Hz), 7.05 (d, 1H,  $J = 7.7$  Hz), 2.54 (s, 3H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 150 MHz, ppm)  $\delta$ : 170.48, 169.75, 166.26, 156.33 (q,  $J = 33.6$  Hz), 152.36, 140.81, 136.36, 133.14, 130.55, 129.02, 126.84, 126.46 (q,  $J = 31.05$  Hz), 123.32 (q,  $J = 271.8$  Hz), 121.84 (q,  $J = 272.85$  Hz), 117.50, 112.97, 103.46, 25.91; HRMS (calcd.) for  $C_{20}H_{13}F_6N_3O_2$  ( $M + H$ )<sup>+</sup> 442.0985, found 442.0982.

**N-(3-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)-3-(trifluoromethyl)benzamide (5k).** White solid; yield 73%; m.p. 101–103°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 600 MHz, ppm)  $\delta$ : 10.65 (s, 1H), 8.30 (s, 1H), 8.27 (d, 1H,  $J = 7.8$  Hz), 7.97 (d, 1H,  $J = 7.7$  Hz), 7.81–7.79 (m, 2H), 7.71 (d, 1H,  $J = 8.1$  Hz), 7.50 (s, 1H), 7.48 (d, 1H,  $J = 8.2$  Hz), 7.05 (d, 1H,  $J = 8.0$  Hz), 2.53 (s, 3H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 150 MHz, ppm)  $\delta$ : 170.56, 169.79, 164.71, 156.34 (q,  $J = 33.6$  Hz), 152.29, 140.81, 136.01, 132.35, 130.22, 130.02, 129.81 (q,  $J = 31.8$  Hz), 128.74, 125.31 (q,  $J = 271.8$  Hz), 124.75, 123.52, 121.82 (q,  $J = 273.0$  Hz), 118.39, 117.52, 113.84, 103.36, 25.91; HRMS (calcd.) for  $C_{20}H_{13}F_6N_3O_2$  ( $M + H$ )<sup>+</sup> 442.0985, found 442.0983.

**N-(3-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)-4-(trifluoromethyl)benzamide (5l).** White solid; yield 80%; m.p. 94–96°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 600 MHz, ppm)  $\delta$ : 10.66 (s, 1H), 8.15 (d, 2H,  $J = 8.4$  Hz), 7.94 (d, 2H,  $J = 7.8$  Hz), 7.79 (t, 1H,  $J = 1.8$  Hz), 7.69 (d, 1H,  $J = 9.6$  Hz), 7.52 (s, 1H), 7.49 (t, 1H,  $J = 8.4$  Hz), 7.06 (d, 2H,  $J = 7.8$  Hz), 2.53 (s, 3H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 150 MHz, ppm)  $\delta$ : 170.59, 169.80, 165.13, 156.34 (q,  $J = 34.6$  Hz), 152.31, 140.83, 138.99, 132.11 (q,  $J = 31.62$  Hz), 130.52, 129.16, 125.98, 125.31 (q,  $J = 273.25$  Hz), 120.05 (q,  $J = 272.51$  Hz), 118.35, 117.61, 103.48, 25.98; HRMS (calcd.) for  $C_{20}H_{13}F_6N_3O_2$  ( $M + H$ )<sup>+</sup> 442.0985, found 442.0978.

**2,3-Dimethoxy-N-(3-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)benzamide (5m).** White solid; yield 52%; m.p. 108–109°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 600 MHz, ppm)  $\delta$ : 10.47 (s, 1H), 7.78 (s, 1H), 7.62 (d, 1H,  $J = 7.9$  Hz), 7.51 (s, 1H), 7.45 (t, 1H,  $J = 8.2$  Hz), 7.22–7.17 (m, 2H), 7.13 (dd, 1H,  $J_1 = 1.4$  Hz,  $J_2 = 7.3$  Hz), 7.02 (dd, 1H,  $J_1 = 1.3$  Hz,  $J_2 = 8.0$  Hz), 3.87 (s, 3H), 3.82 (s, 3H), 2.54 (s, 3H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 150 MHz, ppm)  $\delta$ : 170.55, 169.79, 165.59, 156.31 (q,  $J = 34.8$  Hz), 153.05, 152.35, 146.32, 141.05, 131.70, 130.46, 124.72, 121.85 (q,  $J = 273.45$  Hz), 120.45, 117.54, 117.10, 115.23, 112.91, 103.40, 61.57, 56.42, 25.92; HRMS (calcd.) for  $C_{21}H_{18}F_3N_3O_4$  ( $M + H$ )<sup>+</sup> 434.1322, found 434.1318.

**3-Bromo-4-chloro-N-(3-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)benzamide (5n).** White solid; yield 68%; m.p. 143–145°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 600 MHz, ppm)  $\delta$ : 10.56 (s, 1H), 8.35 (d, 1H,  $J = 1.9$  Hz), 7.97 (dd, 1H,  $J_1 = 2.1$  Hz,  $J_2 = 8.3$  Hz), 7.80 (d, 1H,  $J = 8.4$  Hz), 7.76 (s, 1H), 7.68 (d, 1H,  $J = 8.1$  Hz), 7.54–7.52 (m, 2H, Ph-H), 7.49–7.46 (m, 2H), 7.04 (dd, 1H,  $J_1 = 1.4$  Hz,  $J_2 = 8.0$  Hz), 2.53 (s, 3H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 150 MHz, ppm)  $\delta$ : 170.54, 169.79, 163.71, 156.33 (q,  $J = 34.8$  Hz), 152.27, 140.74, 137.07, 135.34, 133.25, 131.05, 130.42, 129.12, 122.11, 121.81 (q,  $J = 273.3$  Hz), 118.31, 117.51, 113.76, 103.37, 25.92; HRMS (calcd.) for  $C_{19}H_{12}BrClF_3N_3O_2$  ( $M + H$ )<sup>+</sup> 485.9826, found 485.9822.

**5-Bromo-2-fluoro-N-(3-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)benzamide (5o).** White solid; yield 52%; m.p. 147–149°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 600 MHz, ppm)  $\delta$ : 10.74 (s, 1H), 7.89–7.87 (m, 1H), 7.79–7.77 (m, 1H, Ph-H), 7.73 (s, 1H), 7.60 (d, 1H,  $J = 7.9$  Hz), 7.50 (s, 1H), 7.47 (t, 1H,  $J = 8.2$  Hz), 7.38 (t, 1H,  $J = 9.3$  Hz), 7.05 (dd, 1H,  $J_1 = 1.4$  Hz,  $J_2 = 8.0$  Hz), 2.53 (s, 3H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 150 MHz, ppm)  $\delta$ : 170.52, 169.76, 161.91, 159.46, 157.80, 156.32 (q,  $J = 34.65$  Hz), 152.34, 140.55, 135.67, 132.64, 130.58, 127.15, 121.83 (q,  $J = 273.15$  Hz), 119.24, 117.66, 116.63, 113.19, 103.42, 25.92;

**TABLE 1** | The antifungal activities of the title compounds against *B. dothidea*, *Phomopsis* sp., and *B. cinerea* at 50 µg/ml.

Compounds	Inhibition rate (%)		
	<i>B. dothidea</i>	<i>Phomopsis</i> sp	<i>B. cinerea</i>
5a	75.6 ± 2.1	73.6 ± 2.2	60.3 ± 1.8
5b	76.2 ± 1.3	81.0 ± 1.3	73.1 ± 1.3
5c	62.5 ± 1.1	75.5 ± 1.8	80.0 ± 2.5
5d	70.5 ± 1.6	80.2 ± 2.2	70.6 ± 1.2
5e	54.6 ± 1.5	76.0 ± 1.4	73.3 ± 1.9
5f	72.3 ± 1.9	100.0 ± 2.1	59.7 ± 2.4
5g	46.8 ± 1.0	60.1 ± 2.0	73.8 ± 2.0
5h	78.5 ± 2.6	86.1 ± 1.9	75.8 ± 2.6
5i	82.1 ± 3.0	84.4 ± 2.1	79.6 ± 1.4
5j	72.4 ± 1.9	78.0 ± 2.2	72.1 ± 3.2
5k	76.9 ± 1.0	81.2 ± 1.8	77.5 ± 1.8
5l	81.1 ± 1.2	84.5 ± 1.7	80.4 ± 1.2
5m	48.9 ± 1.7	57.8 ± 1.3	83.6 ± 1.2
5n	84.1 ± 2.3	91.8 ± 1.4	79.7 ± 2.4
5o	88.5 ± 3.3	100.0 ± 1.0	84.7 ± 2.6
5p	79.9 ± 1.8	93.4 ± 1.5	75.2 ± 1.2
5q	54.5 ± 1.6	62.1 ± 1.4	80.5 ± 0.9
Pyrimethanil	84.4 ± 2.1	85.1 ± 1.4	82.8 ± 1.4

**TABLE 2** | The EC<sub>50</sub> values of the target compounds against *Phomopsis* sp.

Compounds	Toxic regression equation	r	EC <sub>50</sub> (µg/mL)
5f	y = 2.42x + 3.62	0.99	15.1 ± 2.0
5o	y = 2.38x + 4.20	0.99	10.5 ± 1.4
5p	y = 3.45x + 2.65	0.99	19.6 ± 2.5
Pyrimethanil	y = 2.18x + 8.25	0.99	32.1 ± 2.0

HRMS(calcd.) for C<sub>19</sub>H<sub>12</sub>BrF<sub>4</sub>N<sub>3</sub>O<sub>2</sub> (M + H)<sup>+</sup> 470.0122, found 470.0117.

**2-Bromo-5-fluoro-N-(3-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)benzamide (5p)**. White solid; yield 60%; m.p.152–153 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 600 MHz, ppm) δ: 10.78 (s, 1H), 7.78 (q, 1H, *J* = 5.0 Hz), 7.72 (s, 1H), 7.59–7.58 (m, 2H), 7.52 (s, 1H), 7.48 (t, 1H, *J* = 8.2 Hz), 7.34 (dd, 1H, *J*<sub>1</sub> = 3.0 Hz, *J*<sub>2</sub> = 8.6 Hz, Ph-H), 7.06 (dd, 1H, *J*<sub>1</sub> = 1.4 Hz, *J*<sub>2</sub> = 8.0 Hz), 2.54 (s, 3H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 150 MHz, ppm) δ: 170.49, 169.75, 165.15, 162.38, 160.74, 156.31 (q, *J* = 34.65 Hz), 152.36, 140.82, 140.64, 135.16, 130.60, 121.84 (q, *J* = 273.15 Hz), 118.87, 117.59, 116.63, 114.21, 112.98, 103.49, 25.94; HRMS(calcd.) for C<sub>19</sub>H<sub>12</sub>BrF<sub>4</sub>N<sub>3</sub>O<sub>2</sub> (M + H)<sup>+</sup> 470.0122, found 470.0118.

**3,4,5-trimethoxy-N-(3-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)benzamide (5q)**. White solid; yield 78%; m.p.116–117 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 600 MHz, ppm) δ: 10.31 (s, 1H), 7.73 (s, 1H), 7.69 (d, 1H, *J* = 7.8 Hz), 7.51 (s, 1H), 7.48 (t, 1H, *J* = 7.8 Hz), 7.28 (s, 2H), 7.03 (d, 1H, *J* = 7.8 Hz), 3.87 (s, 3H), 3.74 (s, 3H), 2.51 (s, 3H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 150 MHz, ppm) δ: 170.59, 169.79, 165.58, 156.31 (q, *J* = 34.65 Hz), 153.11, 152.27, 141.07, 140.90, 130.37, 130.23, 120.02 (q, *J* = 272.85 Hz), 118.41, 117.19, 113.82, 105.78, 103.35, 60.58, 56.55, 25.92; HRMS(calcd.) for C<sub>22</sub>H<sub>20</sub>F<sub>3</sub>N<sub>3</sub>O<sub>2</sub> (M + H)<sup>+</sup> 464.1428, found 464.1425.

## In Vitro Antifungal Activity Test

The antifungal activities of all synthesized compounds at the concentration of 50 µg/ml were evaluated for their *in vitro* antifungal activities against the pathogenic fungi, including *B. dothidea*, *Phomopsis* sp., and *B. cinerea* by the poison plate technique (Min et al., 2016). All the compounds were dissolved in 1 ml dimethyl sulfoxide (DMSO) before mixing with 90 ml potato dextrose agar (PDA). Mycelia dishes of approximately 5 mm diameter were cut from the culture medium and then picked up with a germfree inoculation needle and inoculated in the middle of the PDA plate aseptically. The inoculated plates were fostered at 27 ± 1°C for 3–4 days. DMSO in sterile distilled water served as a negative control, while Pyrimethanil acted as a positive control. For each treatment, three replicates were conducted. The inhibition rate *I* (%) was calculated by the following formula, where *C* (cm) represents the diameter of fungi growth on untreated PDA, and *T* (cm) represents the diameter of fungi on treated PDA.

$$I(\%) = [(C - T) / (C - 0.4)] \times 100$$

## RESULTS AND DISCUSSION

### Antifungal Activity Test *in vitro*

The *in vitro* antifungal activities of the title compounds against the pathogenic fungi, including *B. dothidea*, *Phomopsis* sp., and *B. cinerea* at 50 µg/ml were tested and the results are shown in **Table 1**. Bioassay results showed that compounds **5i**, **5l**, **5n**, and **5o** had good inhibition rates on *B. dothidea*, with the inhibition rates of 82.1, 81.1, 84.1, and 88.5%, respectively, which were similar to Pyrimethanil (84.4%). Meanwhile, the inhibition rates of compounds **5f**, **5n**, **5o**, and **5p** against *Phomopsis* sp. were 100.0, 91.8, 100.0, and 93.4%, which were even better than Pyrimethanil (85.1%). In addition, some of the target compounds, for example compounds **5c** (80.0%), **5i** (79.6%), **5l** (80.4%), **5m** (83.6%), **5n** (79.7%), **5o** (84.7%), and **5q** (80.5%), showed equally antifungal activity against *B. cinerea* to Pyrimethanil (82.8%).

The EC<sub>50</sub> values and antifungal diagram of the target compounds **5f**, **5o**, and **5p** were also tested and are presented in **Table 2** and **Figure 2** respectively. **Table 2** shows that compounds **5f**, **5o**, and **5p** exhibited excellent antifungal activity against *Phomopsis* sp., with the EC<sub>50</sub> values of 15.1, 10.5, and 19.6 µg/ml, which were superior to that of Pyrimethanil (32.1 µg/ml).

### Structure-Function Relationship Analysis

In order to design novel and more promising active small molecules of pyrimidine derivatives, SAR analysis was also performed. The chemical structure of the target compounds indicated that the position of the amine group and the position and size of the substituent group R of the target compounds significantly influence the antifungal activities against *B. dothidea*, *Phomopsis* sp. and *B. cinerea*. With the amine group at 3-position of the benzene ring and F and Br atoms at 2- and 5-position, respectively, compound **5o** exhibited excellent antifungal activities against *B. dothidea*, *Phomopsis* sp. and *B. cinerea*, which were even better than those of Pyrimethanil. Meanwhile, in general terms, the

antifungal activities of the target compounds with the amine group at 3-position of benzene ring were better than those of the corresponding target compounds with the amine group at 2-position of the benzene ring, for example, **5h** > **5b** and **5k** > **5d**.

## CONCLUSION

In conclusion, a total of 17 pyrimidine derivatives containing an amide moiety were synthesized and evaluated for their *in vitro* fungicidal activities against *B. dothidea*, *Phomopsis* sp., and *B. cinerea* by the poison plate technique. Bioassay results demonstrated that compound 5-bromo-2-fluoro-*N*-(3-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)benzamide (**5o**) exhibited excellent antifungal activity against *Phomopsis* sp., with the EC<sub>50</sub> value of 10.5 µg/ml, which were even better than that of Pyrimethanil. This study provided a practical tool for guiding the design and synthesis of novel and more promising active small molecules of pyrimidine derivatives for controlling *Phomopsis* sp.. This study also demonstrated that this series of pyrimidine derivatives containing an amide moiety can be used to develop potential agrochemicals. In accordance with the pesticide registration requirements in China, further field and toxicity studies of compound **5o** will be undertaken in a future study.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

## REFERENCES

- Chen, M., Lu, D., Zhang, X., Chen, M., Dong, C., Wang, X., et al. (2021a). Synthesis and Biological Activities of Novel S-β-D-Glucopyranoside Derivatives of 1,2,4-triazole. *Phosphorus, Sulfur, Silicon Relat. Elem.*, 1–6. doi:10.1080/10426507.2021.1901704
- Chen, S., Zhang, Y., Liu, Y., and Wang, Q. (2021b). Highly Efficient Synthesis and Acaricidal and Insecticidal Activities of Novel Oxazolines with *N*-Heterocyclic Substituents. *J. Agric. Food Chem.* 69, 3601–3606. doi:10.1021/acs.jafc.0c05558
- Chen, X. M., Wang, S. H., Cui, D. L., and Li, B. (2015). The Synthesis and Herbicidal Activity of 5-(substituted-Phenyl)-4,6-Dioxo-4,5,6,7-Tetrahydropyrazolo[3,4-D]pyrimidines. *J. Heterocyclic Chem.* 52, 607–610. doi:10.1002/jhet.2047
- Cheng, Y.-N., Jiang, Z.-H., Sun, L.-S., Su, Z.-Y., Zhang, M.-M., and Li, H.-L. (2020). Synthesis of 1, 2, 4-triazole Benzoyl Arylamine Derivatives and Their High Antifungal Activities. *Eur. J. Med. Chem.* 200, 112463. doi:10.1016/j.ejmech.2020.112463
- Huang, Y. X., Li, M., Pan, X. J., Wu, X. M., Xiang, X. L., Li, W. Z., et al. (2018). Control Effect of Several Fungicides on Grape Downy Mildew. *Agrochemicals* 57, 836–839. doi:10.16820/j.cnki.1006-0413.2018.11.017
- Li, H., Zhao, Y., Sun, P., Gao, L., Li, Y., Xiong, L., et al. (2021a). Synthesis and Insecticidal Evaluation of Novel Anthranilic Diamides Derivatives Containing 4-Chlorine Substituted *N*-Pyridylpyrazole. *Chin. J. Chem.* 39, 75–80. doi:10.1002/cjoc.202000013
- Li, J.-h., Wang, Y., Wu, Y.-p., Li, R.-h., Liang, S., Zhang, J., et al. (2021b). Synthesis, Herbicidal Activity Study and Molecular Docking of Novel Pyrimidine Thiourea. *Pestic. Biochem. Physiol.* 172, 104766. doi:10.1016/j.pestbp.2020.104766

## AUTHOR CONTRIBUTIONS

WW and QF contributed to the synthesis, purification, characterization of all compounds, and prepared the original manuscript. LW and CW performed the activity research. QF and LW perfected the language and assisted with the structure elucidation and manuscript revision. WW designed and supervised the research and revised the manuscript. All authors discussed, edited, and approved the final version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fchem.2021.695628/full#supplementary-material>.

- Liu, X.-H., Wang, Q., Sun, Z.-H., Wedge, D. E., Becnel, J. J., Estep, A. S., et al. (2017). Synthesis and Insecticidal Activity of Novel Pyrimidine Derivatives Containing Urea Pharmacophore against *Aedes Aegypti*. *Pest Manag. Sci.* 73, 953–959. doi:10.1002/ps.4370
- Min, L.-J., Shi, Y.-X., Wu, H.-K., Sun, Z.-H., Liu, X.-H., Li, B.-J., et al. (2016). Microwave-assisted Synthesis and Antifungal Activity of Some Novel Thioethers Containing 1,2,4-triazole Moiety. *Appl. Sci.* 5, 1211–1220. doi:10.3390/app5041211
- Patel, N., Desai, P., Patel, N., Jha, A., and Gautam, H. K. (2014). Agronanotechnology for Plant Fungal Disease Management: A Review. *Int. J. Curr. Microbiol. App. Sci.* 3, 71–84. doi:10.5455/ijmsph.2014.110320141
- Shi, J. Q., Zhang, R. Q., He, L. N., Chen, J., Hu, A. L., Yin, X. H., et al. (2021). Screening of Fungicide for Controlling Kiwifruit Leaf Spot. *Agrochemicals* 60, 294–296. doi:10.16820/j.cnki.1006-0413.2021.04.016
- Strange, R. N., and Scott, P. R. (2005). Plant Disease: a Threat to Global Food Security. *Annu. Rev. Phytopathol.* 43, 83–116. doi:10.1146/annurev.phyto.43.113004.133839
- Sun, F. F., Ma, N., and Li, Z. M. (2006). 2-Amino-4-(2,2,2-trifluoroethoxy) Pyrimidine. *Acta Cryst.* 62, 3864–3865. doi:10.1107/s1600536806029564
- Sun, X., Ji, Z., Wei, S., and Ji, Z. (2020). Design, Synthesis and Herbicidal Activity of 5-Cyclopropyl-*N*-Phenylisoxazole-4-Carboxamides. *J. Mol. Struct.* 1220, 128628. doi:10.1016/j.molstruc.2020.128628
- Tang, X., Zhang, C., Chen, M., Xue, Y., Liu, T., and Xue, W. (2020). Synthesis and Antiviral Activity of Novel Myricetin Derivatives Containing Ferulic Acid Amide Scaffolds. *New J. Chem.* 44, 2374–2379. doi:10.1039/C9NJ05867B
- Wang, Y., Li, J., Zhang, X. Y., Li, Y. L., Hu, B., Sun, H., et al. (2021). Controlling Test of High Efficiency and Low Toxicity Fungicides to *Botrytis Cinerea* on Cucumber. *Vegetables* 1, 38–41.

- Wu, W.-N., Gao, M.-N., Tu, H., and Ouyang, G.-P. (2016a). Synthesis and Antibacterial Activity of Novel Substituted Purine Derivatives. *J. Heterocyclic Chem.* 53, 2042–2048. doi:10.1002/jhet.2527
- Wu, W.-N., Jiang, Y.-M., Fei, Q., and Du, H.-T. (2019). Synthesis and Fungicidal Activity of Novel 1,2,4-triazole Derivatives Containing a Pyrimidine Moiety. *Phosphorus, Sulfur, Silicon Relat. Elem.* 194, 1171–1175. doi:10.1080/10426507.2019.1633321
- Wu, W.-N., Tai, A.-Q., Chen, Q., and Ouyang, G.-P. (2016b). Synthesis and Antiviral Bioactivity of Novel 2-substituted Methylthio-5-(4-Amino-2-Methylpyrimidin-5-Yl)-1,3,4-Thiadiazole Derivatives. *J. Heterocyclic Chem.* 53, 626–632. doi:10.1002/jhet.2435
- Wu, W., Chen, M., Fei, Q., Ge, Y., Zhu, Y., Chen, H., et al. (2020). Synthesis and Bioactivities Study of Novel Pyridylpyrazol Amide Derivatives Containing Pyrimidine Motifs. *Front. Chem.* 8, 522. doi:10.3389/fchem.2020.00522
- Wu, W., Chen, Q., Tai, A., Jiang, G., and Ouyang, G. (2015). Synthesis and Antiviral Activity of 2-substituted Methylthio-5-(4-Amino-2-Methylpyrimidin-5-Yl)-1,3,4-Oxadiazole Derivatives. *Bioorg. Med. Chem. Lett.* 25, 2243–2246. doi:10.1016/j.bmcl.2015.02.069
- Zan, N., Xie, D., Li, M., Jiang, D., and Song, B. (2020). Design, Synthesis, and Anti-ToCV Activity of Novel Pyrimidine Derivatives Bearing a Dithioacetal Moiety that Targets ToCV Coat Protein. *J. Agric. Food Chem.* 68, 6280–6285. doi:10.1021/acs.jafc.0c00987
- Zhang, P., Guan, A., Xia, X., Sun, X., Wei, S., Yang, J., et al. (2019). Design, Synthesis, and Structure-Activity Relationship of New Arylpyrazole Pyrimidine Ether Derivatives as Fungicides. *J. Agric. Food Chem.* 67, 11893–11900. doi:10.1021/acs.jafc.9b05185

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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