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Article

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Design, Synthesis and Structure-activity Relationship of New Pyrimidinamine Derivatives Containing an Aryloxy Pyridine Moiety

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ABSTRACT

The pyrimidinamine diflumetorim is an ideal template for the discovery of agrochemical lead compounds due to its unique mode of action, novel chemical structure and lack of reported resistance. In order to develop a new pyrimidinamine fungicide effective against cucumber downy mildew (CDM), a series of new pyrimidinamine derivatives containing an aryloxy pyridine moiety were designed and synthesized by employing the recently reported intermediate derivatization method (IDM). The structures of all compounds were identified by ^{1}H NMR, elemental analyses, HRMS and X-ray diffraction. Bioassays demonstrated that some of the title compounds exhibited excellent fungicidal activities against CDM. Compound **9** gave the best activity (EC_{50} = 0.19 mg/L), which is significantly better than the commercial fungicides diflumetorim, flumorph and cyazofamid. The relationship between structure and fungicidal activity of the synthesized pyrimidinamines was explored. The study showed that compound **9** is a promising fungicide candidate for further development.

KEYWORDS: intermediate derivatization method (IDM), fungicidal activities, cucumber downy mildew, structure-activity relationship

INTRODUCTION

Agrochemicals play an important role in modern agriculture by increasing both crop quality and yield while improving living standards. Fungicides are a major section of crop protection products, which are applied widely to major crops such as rice, corn, fruits and vegetables. In 2014 the global market for fungicides was valued at $16,365 million, equivalent to 25.9% of the global agrochemical market; this represents an increase of 4.4% in comparison with 2013.[1] However, with the widespread application of fungicides, the development of resistance is inevitable. Based on the report from the Fungicide Resistance Action Committee (FRAC), fungicides with known modes of action are classified according to target sites into more than 50 groups. Among these, it was reported that over 40 target sites have already developed medium to high resistance risk to many familiar classes of fungicides which involved the vast majority of fungicide structures.[2] The discovery of fungicide candidates with innovative structures is urgently needed to address this problem. Pyrimidinamines, which act as mitochondrial complex I electron transport inhibitors (MET I), are one of the few types of fungicides without reported resistance.[2] Diflumetorim is the representative pyrimidinamine fungicide targeting MET I, its structure is significantly different from commercially available fungicides, and also different from MET I inhibitors that are insecticides. More importantly, it has no cross resistance with the existing conventional fungicides, and is safe to non-target organisms.[3a] These

properties suggest that pyrimidinamines may be promising templates for the discovery of new fungicide candidates.

The diaryl ether moiety is commonly found in many molecules of agricultural importance such as pyrethroid insecticides, (aryloxy)phenoxypropionate herbicides and triazole fungicides such as difenoconazole.[3] Additionally, the pyridine moiety is found in several fungicides, insecticides/acaricides and herbicides.[4] The introduction of a pyridine ring in place of a phenyl ring usually leads to pesticides that have lower toxicity and higher systemicity or selectivity.[5] 2-Chloro-5-(chloromethyl)pyridine and substituted nicotinic acids bearing reactive groups such as Cl, COOH and chloromethyl, are promising intermediates of low cost and toxicity, which can be readily modified to give structural diversity.

In this study, we sought to develop new pyrimidinamine derivatives with improved fungicidal activity by employing intermediate derivatization method (IDM), a recently reported highly efficient approach to discover agrochemical candidates.[6-20] 2-Chloro-5-(chloromethyl)pyridine or 6-chloronicotinic acid derivatives were used as starting materials to obtain key intermediates which produced a series of pyrimidinamine derivatives containing an aryloxy pyridine moiety (Figure 1). The detailed synthesis, bioassay results, and structure−activity relationships of these compounds are discussed below.

MATERIALS AND METHODS

All chemicals such as starting materials and reagents were commercially

available (Sinopharm Chemical reagent Co. Ltd., Shanghai, China) and used without further purification except as indicated. Melting points were determined on a Büchi M-569 melting point apparatus (Büchi Labortechnik AG, Flawil, Switzerland) and are uncorrected. ^{1}H NMR spectra were recorded with a Mercury 300 MHz spectrometer (Varian, Palo Alto, CA) with deuterochloroform as the solvent and tetramethylsilane (TMS) as the internal standard. Elemental analyses were determined on a Yanaco MT-3CHN elemental analyzer (Yanaco, Kyoto, Japan). Mass spectra were acquired with an Agilent Accurate-Mass-Q-TOF MS 6520 system (Agilent Technologies, Milford, MA) equipped with an electrospray ionisation (ESI) source. X-ray structure determination was recorded with XtaLAB mini (Rigaku, The Woodlands, TX). All plant and bacteria materials were obtained from the Agrochemical Discovery Department in Shenyang Sinochem Agrochemicals R&D Co. Ltd (Shenyang, China).

The general synthetic methods for compounds **1-37** are shown in Figures 2, 3 and 5-9 and their structures are listed in Tables 1-4. The silica gel chromatography was performed with a column of 254 mm x 26 mm i.d. (Synthware glass Co. Ltd., Beijing, China) using 100-140 mesh silica gel (Sinopharm Chemical reagent Co. Ltd., Shanghai, China).

Synthesis of 5-chloro-*N*-((6-(4-chlorophenoxy)pyridin-3-yl)methyl)-6-ethylpyrimidin-4-amine, 26. (General procedure for compounds **1, 2, 25** and **26**) (Figure 2)

Synthesis of 6-(4-chlorophenoxy)nicotinonitrile (**M-1**, R = Cl)

Sodium hydride (1.03 g, 70%, 30 mmol) was added into a solution of 4-chlorophenol (2.57 g, 20 mmol) in 30 mL of DMF, the reaction mixture was stirred at room temperature for 4 h, followed by addition of 6-chloronicotinonitrile (2.76 g, 20 mmol), and then the reaction mixture was heated to 100 ^{o}C for another 10 h. After reaction completion, the mixture was poured into water and extracted with ethyl acetate. The extract was dried over anhydrous magnesium sulfate and concentrated under reduced pressure to give intermediate **M-1** as a pale brown solid: 4.27 g (93%).

Synthesis of (6-(4-chlorophenoxy)pyridin-3-yl)methanamine (**M-2**, R = Cl)

To a solution of **M-1** (2.31 g, 10 mmol), Raney nickel (1.0 g) and 10 mL of 25% aqueous ammonia in 50 mL ethanol, hydrogen was introduced, then the reaction mixture was stirred at room temperature for 3-15 h and monitored by TLC until the reaction was complete, Raney nickel was filtered, and the solution was concentrated under reduced pressure to give **M-2** as a jade-green sticky liquid: 2.55 g (95%).

To a solution of **M-2** (0.24 g, 1.0 mmol) and potassium carbonate (0.21 g, 1.5 mmol) in 10 mL DMF was added 4,5-dichloro-6-ethylpyrimidine (0.16 g, 1.0 mmol), prepared according to literature methods.[21,22] Then the reaction mixture was heated to 80 ^{o}C for 2 h, and monitored by TLC until the reaction was complete. The reaction mixture was poured into water and extracted with ethyl acetate. The organic phase was washed successively with water and

saturated brine, dried, filtered and evaporated under reduced pressure. The residue was purified via silica gel chromatography with ethyl acetate/60-90 °C petroleum ether (1:3, v/v) as eluent to obtain 0.28 g compound **26** as a white solid: 0.28 g (75%), m.p. 86 ^{o}C. ^{1}H NMR (300MHz, $CDCl_3$) δ 1.31 (t, J=7.5Hz, 3H, CH_2CH_3), 2.80 (q, 2H, CH_2CH_3), 4.68 (d, J=6.0 Hz, 2H, CH_2), 5.71 (bs, 1H, NH), 6.91 (d, J=8.4Hz, 1H, pyridine 3-H), 7.07 (d, J=8.7Hz, 2H, Ph-2,6-2H), 7.34 (d, J=8.7Hz, 2H, Ph-3,5-2H), 7.72 (dd, J=8.4,2.7Hz, 1H, pyridine 4-H), 8.16 (s, 1H, pyridine 6-H), 8.44 (s, 1H, pyrimidine 2-H). Anal. Calcd (%) for $C_{18}H_{16}Cl_2N_4O$: C, 57.61; H, 4.30; N, 14.93. Found: C, 57.53; H, 4.33; N, 14.89. HRMS m/z 374.0698 $[M + H]^+$ (calcd $[M + H]^+$ 374.0701).

Synthesis of 5-chloro-*N*-(2-(6-(4-chlorophenoxy)pyridin-3-yl)ethyl)-6-methylpyrimidin-4-amine, 9. (General procedure for compounds **3-24**) (Figure 3)

Synthesis of methyl 6-(4-chlorophenoxy)nicotinate (**M-3**, R = Cl)

To a solution of 4-chlorophenol (25.6 g, 0.2 mol) in 350 mL DMF was added 70% sodium hydride (103 g, 3.0 mol) in portions. The reaction mixture was stirred for 4 h at room temperature, and then methyl 6-chloronicotinate (34.2 g, 0.2 mol) was added in portions. Next the reaction temperature was raised to 100 ^{o}C and maintained for 10 h, and monitored by TLC until the reaction was complete. The resulting solution was poured into water and extracted with ethyl acetate. The organic phase was successively washed with water and saturated brine, dried, filtered and evaporated under reduced pressure, the

cooled residue was filtered and washed with petroleum ether, to obtain intermediate **M-3** as a brown solid: 42.0 g, m.p. 65 ^{o}C.

Synthesis of (6-(4-chlorophenoxy)pyridin-3-yl)methanol (**M-4**, R = Cl)

To a solution of **M-3** (52.6 g, 0.2 mol) in 500 mL anhydrous ether was added dropwise 65% Red-Al (74.5 g, 0.24 mol) in toluene at 0 ^{o}C. The reaction mixture was stirred for 4 h at room temperature, cooled to 0 ^{o}C, and 10% sodium hydroxide solution prepared fresh was added dropwise until the reaction solution was clarified. Then the reaction temperature was raised to 35 ^{o}C to react for 2 h, and monitored by TLC until the reaction was complete. The solution was poured into water and extracted with ethyl acetate. The organic phase was successively washed with water and saturated brine, dried, filtered and evaporated under reduced pressure. The residue was purified via silica gel chromatography with ethyl acetate/60-90 °C petroleum ether (1:3, v/v) as eluent to obtain **M-4** as a white solid: 42.2 g, m.p. 101 ^{o}C.

Synthesis of 5-(chloromethyl)-2-(4-chlorophenoxy)pyridine (**M-5**, R = Cl)

To a solution of **M-4** (23.5 g, 0.1 mol) in 350 mL dichloromethane was dropwise added thionyl chloride (17.9 g, 0.15 mol) at 0 ^{o}C. The reaction mixture was stirred for 4 h at room temperature, and monitored by TLC until the reaction was complete, excess thionyl chloride was evaporated and the residue was poured into water and extracted with ethyl acetate. The organic phase was successively washed with water, saturated sodium bicarbonate solution and saturated brine, dried, filtered and evaporated under reduced

pressure, to obtain **M-5** as a white solid: 22.8 g, m.p. 79 ^{o}C.

Synthesis of 2-(6-(4-chlorophenoxy)pyridin-3-yl)acetonitrile (**M-6**, R = Cl)

To a solution of sodium cyanide (2.69 g, 55 mmol) dissolved in 300 mL dimethyl sulfoxide was added **M-5** (13.9 g, 50 mmol) and 18-Crown-6 (0.69 g, 2.63 mmol) at 40 °C. The reaction mixture was raised to 80 ^{o}C to react for 2 h, and monitored by TLC until the reaction was complete. The residue was poured into water, extracted with toluene, the organic phase was successively washed with water and saturated brine, dried, filtered and evaporated under reduced pressure. The residue was purified via silica gel chromatography with ethyl acetate/60-90 °C petroleum ether (1:3, v/v) as eluent to obtain **M-6** as a white solid: 11.2 g, m.p. 101 ^{o}C.

Synthesis of 2-(6-(4-chlorophenoxy)pyridin-3-yl)ethanamine (**M-7**, R = Cl)

To a solution of **M-6** (2.44 g, 10 mmol), Raney nickel (1.0 g) and 10 mL of 25% aqueous ammonia in 50 mL ethanol was introduced hydrogen gas, then the reaction mixture was stirred at room temperature for 3-15 h and monitored by TLC until the reaction was complete. Raney nickel was filtered, and the solution was concentrated under reduced pressure to give **M-7** as a jade-green sticky liquid: 2.30 g (95%).

To a solution of **M-7 (**0.25 g, 1.0 mmol) and potassium carbonate (0.21 g, 1.5 mmol) in 10 mL DMF was added 4,5-dichloro-6-methylpyrimidine (0.16 g, 1.0 mmol). Then the reaction temperature was raised to 80 ^{o}C and maintained for 2 h, and monitored by TLC until the reaction was complete. The mixture was

poured into water and extracted with ethyl acetate. The organic phase was successively washed with water and saturated brine, then dried, filtered and evaporated under reduced pressure. The residue was purified via silica gel chromatography with ethyl acetate/60-90 °C petroleum ether (1:4, v/v) as eluent to obtain compound **9** as a colorless oil: 0.28 g (74%). ^{1}H NMR (300MHz, $CDCl_3$) δ 2.46 (s, 3H, CH_3), 2.91 (t, J=6.9Hz, 2H, CH_2CH_2NH), 3.75 (q, 2H, CH_2CH_2NH), 5.43 (bs, 1H, NH), 6.89(d, J=8.4Hz, 1H, pyridine 3-H), 7.07 (d, J=6.6Hz, 2H, Ph-2,6-2H), 7.35 (d, J=6.6Hz, 2H, Ph-3,5-2H), 7.58 (dd, J=8.4,2.7Hz, 1H, pyridine 4-H), 8.03 (s, 1H, pyridine 6-H), 8.39 (s, 1H, pyrimidine 2-H). Anal. Calcd (%) for $C_{18}H_{16}Cl_2N_4O$: C, 57.61; H, 4.30; N, 14.93. Found: C, 57.59; H, 4.26; N, 14.99. HRMS m/z 374.0694 $[M + H]^+$ (calcd $[M + H]^+$ 374.0701).

The crystal structure of target compound **9** was also determined by X-ray diffraction analyses. Its crystal structure is shown in Figure 4.

Synthesis of 5-chloro-4-((6-(4-chlorophenoxy)pyridin-3-yl)methoxy)-6-methylpyrimidine, 27. (General procedure for compounds **27** and **28**) (Figure 5)

To a solution of (6-(4-chlorophenoxy)pyridin-3-yl)methanol (**M-4)** (0.23 g, 1.0 mmol) and potassium carbonate (0.21 g, 1.5 mmol) in 10 mL DMF was added 4,5-dichloro-6-methylpyrimidine (0.16 g, 1.0 mmol). Then the reaction temperature was raised to 80 °C and maintained for 2 h, and monitored by TLC until the reaction was complete. The mixture was poured into water and

extracted with ethyl acetate. The organic phase was successively washed with water and saturated brine, dried, filtered and evaporated under reduced pressure. The residue was purified via silica gel chromatography with ethyl acetate/60-90 °C petroleum ether (1:4, v/v) as eluent to obtain compound **27** as a colorless oil: 0.28 g (77%). ^{1}H NMR (300MHz, $CDCl_3$) δ 2.43 (s, 3H, CH_3) 5.07 (s, 2H, CH_2), 6.92 (d, *J*=8.4Hz, 1H, pyridine 3-H), 7.06 (d, *J*=8.4Hz, 2H, Ph-2,6-2H), 7.35 (d, *J*=8.4Hz, 2H, Ph-3,5-2H), 7.81 (dd, *J*=8.4,2.1Hz, 1H, pyridine 4-H), 8.06 (s, 1H, pyridine 6-H), 8.17 (s, 1H, pyrimidine 2-H). Anal. Calcd (%) for $C_{17}H_{13}Cl_2N_3O_2$: C, 56.37; H, 3.62; N, 11.60. Found: C, 56.42; H, 3.59; N, 11.68. HRMS *m*/*z* 361.0391 $[M + H]^+$ (calcd $[M + H]^+$ 361.0385).

Synthesis of *N*-(1-(6-(4-(*tert*-butyl)phenoxy)pyridin-3-yl)ethyl)-5-chloro-6-ethylpyrimidin-4-amine, 30. (General procedure for compounds **29** and **30**) (Figure 6) Compound **30** was obtained according to a literature method as a colorless oil in 49% yield.[23] ^{1}H NMR (300MHz, $CDCl_3$) δ 1.26 (t, *J*=7.5Hz, 3H, CH_2CH_3), 1.33 (s, 9H, *t*-Bu), 1.61 (d, *J*=6.9Hz, 3H, $CHCH_3$), 2.78 (q, 2H, CH_2CH_3), 5.36 (q, 1H, $CHCH_3$), 5.52 (bs, 1H, NH), 6.87 (d, *J*=8.4Hz, 1H, pyridine 3-H), 7.05 (d, *J*=6.9Hz, 2H, Ph-2,6-2H), 7.39 (d, *J*=6.9Hz, 2H, Ph-3,5-2H), 7.67 (dd, *J*=8.4,2.7Hz, 1H, pyridine 4-H), 8.22 (s, 1H, pyridine 6-H), 8.39 (s, 1H, pyrimidine 2-H). Anal. Calcd (%) for $C_{19}H_{19}ClN_4O$: C, 64.31; H, 5.40; N, 15.79. Found: C, 64.22; H, 5.44; N, 15.80. HRMS *m*/*z* 410.1878 $[M + H]^+$ (calcd $[M + H]^+$ 410.1873).

Synthesis of 1-(5-chloro-6-methylpyrimidin-4-yl)-3-(2-(6-(4-chlorophenoxy)pyridin-3-yl) ethyl)urea, 31. (Figure 7)

Synthesis of 2-(4-chlorophenoxy)-5-(2-isocyanatoethyl)pyridine (**M-10**)

To a 0 ^{o}C solution of triphosgene (3.5 mL) in dichloromethane (25 mL) and saturated $NaHCO_3$ (25 mL) was added 2-(6-(4-chlorophenoxy)-pyridin-3-yl)ethanamine (**M-7)** (0.74 g, 3.0 mmol). The reaction mixture was stirred at room temperature for 2.5 h and then the layers are separated. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to half volume. Dichloromethane (20 mL) was added to obtain **M-10** as a solution in dichloromethane.

To a 0 ^{o}C solution of 5-chloro-6-methylpyrimidin-4-amine (0.28 g, 2.0 mmol) in dichloromethane (5 mL) was added isocyanate **M-10** prepared above and then *N,N*-diisopropylethylamine (DIPEA) (3 mL).[24] The reaction mixture was stirred at room temperature for 1 h and then diluted with ethyl acetate and washed with saturated NH_4Cl and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified via silica gel chromatography with ethyl acetate/60-90 °C petroleum ether (1:2, v/v) as eluent to obtain compound **31** as a white solid: 0.33 g (40%). ^{1}H NMR (300MHz, $CDCl_3$) δ 2.48 (s, 3H, CH_3), 2.73 (t, *J*=6.9Hz, 2H, $NHCH_2\mathit{CH_2}$), 3.46-3.51 (m, 2H, $NH\mathit{CH_2}CH_2$), 4.92(bs, 1H, $\mathit{NH}CH_2CH_2$), 6.85 (d, *J*=8.4Hz, 1H, pyridine 3-H), 7.03 (d, *J*=8.1Hz, 2H, Ph-2,6-2H), 7.33 (d, *J*=8.1Hz,

2H, Ph-3,5-2H), 7.54 (d, *J*=7.2Hz, 1H, pyridine 4-H), 7.96 (s, 1H, pyridine 6-H), 8.32 (s, 1H, pyrimidine 2-H). Anal. Calcd (%) for $C_{19}H_{19}ClN_4O$: C, 64.37; H, 5.30; N, 15.79. Found: C, 64.31; H, 5.40; N, 15.79. HRMS *m*/*z* 417.0755 $[M + H]^+$ (calcd $[M + H]^+$ 417.0759).

Synthesis of 5-Chloro-*N*-(2-(2-(4-chlorophenoxy)pyridin-3-yl)ethyl)-6-ethylpyrimidin-4-amine, 35. (General procedure for compounds **32-35**) (Figure 8)

The procedures for preparing intermediates (**M-11** to **M-15**) and desired compound **35** were analogous to those for the corresponding intermediates (**M-3** to **M-7)** and compound **9**, only replacing the starting materials, methyl 6-chloronicotinate and 4,5-dichloro-6-methylpyrimidine, with methyl 2-chloronicotinate (1.71 g, 10 mmol) and 4,5-dichloro-6-ethylpyrimidine (1.76 g, 10 mmol) respectively.

5-Chloro-*N*-(2-(2-(4-chlorophenoxy)pyridin-4-yl)ethyl)-6-ethylpyrimidin-4-amine, 37. (General procedure for compounds **36** and **37**) (Figure 9)

The procedures for preparing intermediates (**M-16** to **M-20**) and desired compound **37** were analogous to those for the corresponding intermediates (**M-3** to **M-7)** and compound **9**, only replacing the starting materials, methyl 6-chloronicotinate and 4,5-dichloro-6-methylpyrimidine, with ethyl 2-chloroisonicotinate (1.86 g, 10 mmol) and 4,5-dichloro-6-ethylpyrimidine(1.76 g, 10 mmol) respectively.

Fungicidal assay

Each of the test compounds (4 mg) was first dissolved in 5 mL acetone/methanol (1:1, v/v), then 5 mL of water containing 0.1% Tween 80 was added to generate a 10 mL stock solution of concentration 400 mg/L. Serial test solutions were prepared by diluting the above solution (testing range 3.13-400 mg/L).

Evaluations of fungicidal activity of the synthesized compounds against cucumber downy mildew (CDM) was performed as follows: cucumber seeds (*Cucumis sativus* L.) were grown to the one-leaf and one-heart stage, then the test solution was sprayed on the host plant with a home-made sprayer. After 24 h, the leaf of host plant was inoculated with Sporangium suspension of the fungus *Pseudoperonospora cubensis* cultured by Shenyang Sinochem Agrochemicals R&D Co. Ltd. (Shenyang, China) at a concentration of $5x10^5$ spores/mL using PS289 Procon Boy WA double action 0.3 mm airbrush (GSI, Tokyo, Japan). The cucumber plants were stored in a humidity chamber (24±1 ^oC, RH>95%, dark) and then transferred into a greenhouse (18-30 ^oC, RH>50-60%) 24 h after infection. Three replicates were carried out. The activity of each compound was estimated by visual inspection after 7 d and screening results were reported in the range 0% (no control) to 100% (complete control). The inhibitory activity (%) was estimated as: [(viability of the blank control-viability of the treatment) / viability of the blank control] x 100. The ED_{50} values were calculated by Duncan's new multiple range method using DPS version 14.5.

The fungicidal test results of compounds **1-37** against CDM are listed in Tables 1-4.

RESULTS AND DISCUSSION

Synthesis. According to the schemes shown in Figures 2-9, 37 title compounds were synthesized with yields of 40-80%. Chemical structures of compounds 1-37 are shown in Tables 1-4. The synthesized compounds were characterized by ^{1}H NMR and elemental analyses/HRMS. The chemical structure of lead compound **9** was unequivocally determined by x-ray crystallography (Figure 4). All spectral and analytical data were consistent with the assigned structures.

Structure-Activity Relationships (SAR).

Discovery of lead compounds 3 and 4

Initially, in order to verify the feasibility of our design concept, mimicking the skeleton of diflumetorim, the starting materials, phenol and 6-chloronicotinonitrile, were employed to obtain the key intermediate (6-phenoxypyridin-3-yl)methanamine, from which we obtained compounds **1** and **2**. These compounds can be considered as diflumetorim analogs in which the phenyl moiety is replaced with a pyridinyl moiety. The bioassay results indicated that the synthesized compounds had activity against four important fungal diseases, CDM, cucumber grey mold (CGM), wheat powdery mildew (WPM) and southern corn rust (SCR),[25] particularly against CDM. The activity of compound **1** (R_1 = CH_3) against CDM was very weak (EC_{50} > 400 mg/L)

compared with diflumetorim (EC_{50} = 23.06 mg/L), however, compound **2** (R_1 = C_2H_5) displayed activity equal to diflumetorim (EC_{50} = 23.17 mg/L). On the basis of these results, CDM was selected as the test target for optimization. Next, taking inspiration from the structure of insecticide flufenerim, which is similar to diflumetorim,[26] we synthesized compounds **3** and **4** with Alk = CH_2CH_2 (Table 1). These two compounds exhibited higher fungicidal activity than the corresponding analogs with Alk = CH_2 (7.33 mg/L and 7.02 mg/L respectively versus 23.06 mg/L), which encouraged us to synthesize additional analogs.

Optimization of lead compounds 3 and 4

Using compounds **3** and **4** as a starting point for additional analogs, we next turned our attention to modifications in the phenyl ring including changes of substituent position, numbers of substituents, the electronic properties and spatial characteristics of the substituent groups (Table 2). First, six compounds, **5-10**, were synthesized to evaluate the position effect of the substituent on fungicidal activity using the chlorine atoms as probes. When a single chlorine was present in the *ortho*-position of phenyl ring (compounds **5** and **6**), there was no improvement in fungicidal activity. When a single chlorine was present in the *meta*-position of phenyl ring (compounds **7** and **8**), the fungicidal activity decreased significantly. When a single chlorine was present in the *para*-position (compounds **9** and **10**), we observed a significant increase in fungicidal activity; compounds **9** and **10** gave much lower EC_{50} values of 0.19

mg/L and 2.65 mg/L, respectively, versus initial leads **3** and **4** (7.33 mg/L and 7.02 mg/L), especially compound **9** displayed superior control to the commercial fungicides flumorph and cyazofamid, indicating that single substitution at the *para*-position probably has a positive effect on bioactivity. Next, we further selected typical electron-donating groups CH_3 and OCH_3, and electron-withdrawing group CF_3 to carry out some fine modification on *para*-position of phenyl ring to investigate the electronic properties of substituents. We synthesized six compounds, **11** and **12** with the electron donating group (CH_3), **13** and **14** with the electron donating group (OCH_3), **15** and **16** with the electron withdrawing group (CF_3) respectively (Table 2). The bioassay results showed that these six compounds **11** to **16** were less efficacious than compounds **9** and **10** (3.13 - 11.65 mg/L versus 0.19 mg/L, 2.65 mg/L), indicating that higher electron donating or withdrawing group has possibly a negative effect on bioactivity. Then in order to assess the effect of substituent numbers specifically relating to double substituted phenyl on fungicidal activity, we employed some low cost and commonly used phenol intermediates bearing two substituents to prepare compounds **17** to **24** (Table 2). Compound **18** with Rn of 2-CH_3-4-Cl showed low fungicidal activity with 30.25 mg/L EC_{50} and the other compounds of this type **17**, **19** to **23** were also less active, with EC_{50} values ranging from 3.75 mg/L to 10.44 mg/L. However, compound **24** (Table 2) with Rn = 2-Cl-4-CF_3 showed promising activity (EC_{50} of 1.10 mg/L) which is approaching of compound **9** and may be considered for

exploring new compounds with improved activity. So far, the result of the optimization of compounds **3** and **4** is identification of compound **9** with *para*-Cl group on phenyl ring as the optimized structure, the third lead compound with greatly improved fungicidal activity.

Effect of linker between pyrimidine and pyridine groups on fungicidal activity

To determine if changing the linker between pyrimidine and pyridine groups (X-Alk) would bring enhanced fungicidal activity, we synthesized and screened a series of compounds **25** to **31** (Table 3). When CH_2CH_2 was replaced with CH_2 on Alk and keeping the X group fixed as NH, both compounds **25** which gave EC_{50} of 7.91 mg/L and **26** which showed EC_{50} of 5.79 mg/L were much less efficacious than compound **9** which gave EC_{50} of 0.19 mg/L. These results were consistent with the conclusion obtained above. When introducing O-CH_2 into X-Alk instead of NH-CH_2CH_2, both compounds **27** and **28** exhibited a sharply reduced efficacy compared with the lead compound **9** (more than 400 mg/L for compound **27**, 137.8 mg/L for compound **28** versus 0.19 mg/L for compound **9**). The branched Alk in diflumetorim was employed in compounds **29** and **30** with Alk = $CH(CH_3)$. The replacement of CH_2 with $CH(CH_3)$ did not enhance fungicidal activity. The introduction of *t*-Bu into the *para*-position on phenyl suggests that the presence of a group with large spatial effect probably leads to considerable reduction in activity. A possible explanation for the lower activity associated with large substituents is that such substituents would block

the interaction of target enzyme with these bulky compounds.[10] Additionally, we considered a urea bridge, an extensively utilized moiety in herbicides.[3c] We designed NH(CO)NH substructure for X group based on compound **9** to achieve compound **31**. However, disappointedly, compound **31** had hardly any fungicidal activity with a very high EC_{50} value of more than 400 mg/L.

Activity of different position of pyrimidinamine group attached to pyridine ring

To investigate the effect of different position of pyrimidinamine group attached to pyridine ring on the activity of intact molecules, six positional isomers were designed and synthesized (Table 4). Generally speaking, when pyrimidinamine group was attached to the 3-position of the pyridine ring (compounds **32** to **35**), the fungicidal activity indicated the lowest, followed by 4-position analogues **36** and **37**, showing obviously higher activity especially compound **36** with EC_{50} of 6.49 mg/L, but still much lower than compound **9**. In addition, we noted that compounds with CH_3 on R_1 were much more active in contrast with their counterparts of C_2H_5 in this series. The poor results of compounds **32**-**37** in Table 4 suggested that when the pyrimidinamine group was attached to the pyridine ring at 3- or 4-position, the fungicidal activity diminished dramatically in this class of compounds.

Based on data presented in Tables 1-4, a clear-cut, well-defined relationship between chemical structure and biological activity has taken shape by examining the effect of the substituted position, the numbers of substituents,

411 the electronic properties and spatial characteristics of the substituent groups
412 on phenyl ring, linker between pyrimidine and pyridine groups, and the
413 different position of pyrimidinamine group attached to pyridine ring. The
414 *para*-position single chlorine substituted phenyl derivative, compound **9**,
415 showed significantly improved fungicidal activity compared to the
416 commercialized controls. Compound **24** with Rn of 2-Cl-4-CF_3 is a good
417 candidate for further exploration of new compounds with improved activity.
418 Variation in the linker between pyrimidine and pyridine groups did not enhance
419 fungicidal activity, while the 5-position where pyrimidinamine group is attached
420 to pyridine ring provided optimal activity.
421 From the above we can conclude that compound **9** is the optimal structure with
422 desired activity. It offers a control with EC_{50} value of 0.19 mg/L against CDM,
423 significantly higher than commercial fungicides diflumetorim, flumorph and
424 cyazofamid. Compound **9**, which has also shown activities against CSR, WPM
425 and RB in addition to CDM,[25] is a promising candidate for further development.
426 This study demonstrates the effectiveness of our design concept by using
427 intermediate derivatization method to discover bioactive compounds. The
428 discovery of new pyrimidinamine fungicides may delay the development of
429 resistance and contribute to resistance management. Further synthesis of
430 analogs, structure optimization studies and field trials of compound **9** are in
431 progress.
432

ACKNOWLEDGEMENT

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SUPPORTING INFORMATION

Characterization for intermediates **M-1**, **3-7**, **11-20**, and compounds **1**-**8**, **10**-**25**, **28**, **29**, **32**-**37**. This material is available free of charge via the Internet at http://pubs.acs.org. The atomic coordinates for compound **9** have been deposited at the Cambridge Crystallographic Data Centre. CCDC ID: 1501756 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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REFERENCES

(1) Mcdougall, P. *Phillips McDougall AgriService*; Phillips McDougall AgriService: Midlothian, UK, May 2016; p 219.

(2) FRAC (Fungicide Resistance Action Committee) Code List ©*2016, fungicides sorted by mode of action (including FRAC code numbering), www.frac.info (accessed Aug 10, 2016).

(3) (a) Liu, C. L. *World Compendium of Fungicides*; Chemical Industry Press: Beijing, China, 2006. (b) *World Compendium of Insecticides*; Chemical Industry Press: Beijing, China, 2012. (c) *World Compendium of Herbicides*; Chemical Industry Press: Beijing, China, 2002.

(4) Guan, A. Y.; Liu, C. L.; Sun, X. F.; Xie, Y.; Wang, M. A. Discovery of pyridine-based agrochemicals by using intermediate derivatization methods. *Bioorg. Med. Chem.* **2016**, *24*, 342–353.

(5) Liu, C. L. *Proceedings of Research and Development of New Agrochemicals;* Chemical Industry Press Publishers: Beijing, 2002; pp 93-94.

(6) Liu, C. L.; Guan, A. Y.; Yang, J, D.; Chai, B. S.; Li, M.; Li, H. C.; Yang, J. C.; Xie. Y. Efficient approach to discover novel agrochemical candidates: intermediate derivatization method. *J. Agric. Food Chem.* **2016**, *64*, 45-51.

(7) Guan, A. Y.; Liu, C. L.; Yang, X. P.; Dekeyser, M. Application of the intermediate derivatization approach in agrochemical discovery. *Chem. Rev.* **2014**, *114*, 7079-7107.

(8) Liu, C. L. Approach and application of novel agrochemical innovation. *Chin.*

J. Pestic. **2011**, *50*, 20-23.

(9) Liu, C. L. New agrochemical candidates from intermediate derivatization method based on bioisosteric replacement. In *Frontiers of Modern Chemical Engineering, Metallurgy, and Material Technologies, 7th Academic Conference of Chemical, Metallurgy and Material Engineering Department*; Wang, J. K., Ed.; Chemical Industry Press Publishers: Beijing, China, 2009; pp 86-94.

(10) Guan, A. Y.; Liu, C. L.; Huang, G.; Li, H. C.; Hao, S. L.; Xu, Y.; Li, Z. N. Design, synthesis, and structure-activity relationship of novel aniline derivatives of chlorothalonil. *J. Agric. Food Chem.* **2013**, *61*, 11929−11936.

(11) Xie, Y.; Chi, H. W.; Guan, A. Y.; Liu, C. L.; Ma, H. J.; Cui, D. L. Design, synthesis, and herbicidal activity of novel substituted 3-(pyridin-2-yl)benzenesulfonamide derivatives. *J. Agric. Food Chem.* **2014**, *62*, 12491-12496.

(12) Li, M.; Liu, C. L.; Yang, J. C.; Zhang, J. B.; Li, Z. N.; Zhang, H.; Li, Z. M. Synthesis and biological activity of new (*E*)-α-(methoxyimino)benzeneacetate derivatives containing a substituted pyrazole ring. *J. Agric. Food Chem.* **2010**, *58*, 2664-2667.

(13) Yang, J. C.; Li, M.; Wu, Q.; Liu, C. L.; Chang, X. H. Design, synthesis and insecticidal evaluation of aryloxy dihalopropene derivatives. *Bioorg. Med. Chem.* **2016**, *24*, 383-390.

(14) Xie, Y.; Chi, H. W.; Guan, A. Y.; Liu, C. L.; Ma, H. J.; Cui, D. L. Synthesis

496 and evaluation of substituted 3-(pyridin-2-yl)benzenesulfonamide derivatives
497 as potent herbicidal agents. *Bioorg. Med. Chem.* **2016**, *24*, 428-434.
498 (15) Li, H. C.; Guan, A. Y.; Huang, G.; Liu, C. L.; Li, Z. N.; Xie, Y.; Lan, J.
499 Design, synthesis and structure-activity relationship of novel diphenylamine
500 derivatives. *Bioorg. Med. Chem.* **2016**, *24*, 453-461.
501 (16) Guan, A.Y.; Liu, C. L.; Li, M.; Zhang, H.; Li, Z. N.; Li, Z. M. Design,
502 synthesis and structure-activity relationship of novel coumarin derivatives. *Pest*
503 *Manag. Sci.* **2011**, *67*, 647-655.
504 (17) Chai, B. S.; Liu, C. L.; Li, H. C.; He, X. M.; Luo, Y. M.; Huang, G.; Zhang,
505 H.; Chang, J. B. Design, synthesis and acaricidal activity of novel strobilurin
506 derivatives containing pyrimidine moieties. *Pest Manag. Sci.* **2010**, *66*,
507 1208-1214.
508 (18) Chai, B. S.; Liu, C. L.; Li, H. C.; Zhang, H.; Liu, S. W.; Huang, G.; Chang, J.
509 B. The discovery of SYP-10913 and SYP-11277: novel strobilurin acaricides.
510 *Pest Manag. Sci.* **2011**, *67*, 1141-1146.
511 (19) Li, M.; Liu, C. L.; Li, L.; Yang, H.; Li, Z. N.; Zhang, H.; Li, Z. M. Design,
512 synthesis and biological activities of new strobilurin derivatives containing
513 substituted pyrazoles. *Pest Manag. Sci.* **2010**, *66*, 107-112.
514 (20) Li, M.; Liu, C. L.; Zhang, J.; Wu, Q.; Hao, S. L.; Song, Y. Q. Design,
515 synthesis and structure-activity relationship of novel insecticidal
516 dichloro-allyloxy-phenol derivatives containing substituted pyrazol-3-ols. *Pest*
517 *Manag. Sci.* **2013**, *69*, 635-641.

(21) Liu, C. L.; Sun, X. F.; Zhao, J.; Wang, L. Z.; Li, K. K.; Zhang, J. B.; Chen, X. M.; Guan, A. Y.; Song, Y. Q.; Lan, J.; Ma, S.; Ru, L. J. Pyrazolyl pyrimidinamine compound and application thereof. PCT Int. Appl. WO 2015085935, June 18, 2015.

(22) Wang, L. Z.; Sun, X. F.; Lan, J.; Zhang, J. B.; Wang, B.; Guan, A. Y.; Zhou, J. Z.; Yang, F.; Song, Y. Q.; Liu, C. L. Substituted pyrimidinamine compound and uses thereof. CN Patent 104292169, Jan 21, 2015.

(23) Drumm, J. E. III.; Lett, R. M.; Stevenson, T. M. Preparation of fungicidal and miticidal and arthropodicidal aminopyrimidine agrochemicals. PCT Int. Appl. WO 9507278, Mar 16, 1995.

(24) Barlaam, B.; Fennell, M.; Germain, H.; Green, T.; Hennequin, L.; Morgentin, R.; Olivier, A.; Ple, P.; Vautier, M.; Costello, G. New heterocyclic analogues of 4-(2-chloro-5-methoxyanilino)quinazolines as potent and selective c-Src kinase inhibitors. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5446-5449.

(25) Guan, A. Y.; Yang, F.; Wang, J. F.; Chen, W.; Fan, X. X.; Wang, X. L.; Chen, X, M.; Li, K. K.; Li, Z. N.; Liu, C. L. Substituted aryloxyl pyridine compound and uses thereof. CN Patent 105732585, July 6, 2016.

(26) Ghanim, M.; Lebedev, G.; Kontsedalov, S.; Ishaaya, I. Flufenerim, a novel insecticide acting on diverse insect pests: biological mode of action and biochemical aspects. *J. Agric. Food Chem.* **2011**, *59*, 2839-2844.

FIGURE CAPTIONS

Figure 1. An overview of the design of new pyrimidinamine derivatives containing an aryloxy pyridine moiety

Figure 2. Synthetic route to compounds **1, 2, 25** and **26**

Figure 3. Synthetic route to compounds **3-24**

Figure 4. X-ray single-crystal diffraction of compound **9**.

Figure 5. Synthetic route to compounds **27** and **28**

Figure 6. Synthetic route to compounds **29** and **30**

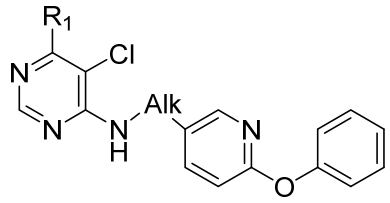
Figure 7. Synthetic route to compound **31**

Figure 8. Synthetic route to compounds **32-35**

Figure 9. Synthetic route to compounds **36** and **37**

TABLES

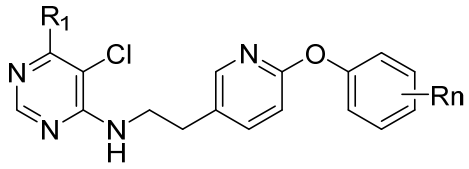
Table 1. Chemical Structures and Fungicidal Activity of Pyrimidinamine Derivatives Containing Aryloxy Pyridine Moiety (compounds **1** to **4**)



compound	R_1	Alk	EC_{50} (mg/L)	95% d[a]
1	CH_3	CH_2	>400	/ [b]
2	C_2H_5	CH_2	23.17	15.86-33.86
3	CH_3	CH_2CH_2	7.33	3.33-16.12
4	C_2H_5	CH_2CH_2	7.02	3.12-15.80
diflumetorim			23.06	16.13-32.95
flumorph			7.77	6.48-9.32
cyazofamid			1.01	0.62-1.63

[a] confidence limit. [b] the value could not be measured accurately.

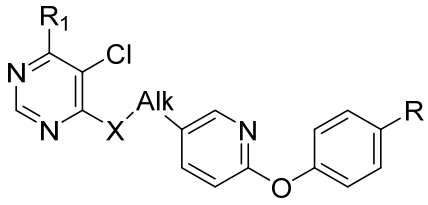
Table 2. Chemical Structures and Fungicidal Activity of Pyrimidinamine Derivatives Containing Aryloxy Pyridine Moiety (compounds **5** to **24**)



compound	R_1	Rn	EC_{50} (mg/L)	95% d[a]
3 (Lead 1)	CH_3	H	7.33	3.33-16.12
4 (Lead 2)	C_2H_5	H	7.02	3.12-15.80
5	CH_3	2-Cl	13.10	3.27-52.39
6	C_2H_5	2-Cl	26.40	17.75-39.26
7	CH_3	3-Cl	>400	/ [b]
8	C_2H_5	3-Cl	>400	/ [b]
9 (Lead 3)	CH_3	4-Cl	0.19	0.14-0.26
10	C_2H_5	4-Cl	2.65	1.61-4.35
11	CH_3	4-CH_3	3.13-6.25	/ [b]
12	C_2H_5	4-CH_3	5.50	3.57-8.50
13	CH_3	4-OCH_3	5.54	3.75-8.16
14	C_2H_5	4-OCH_3	11.65	9.26-14.66
15	CH_3	4-CF_3	3.13-6.25	/ [b]
16	C_2H_5	4-CF_3	3.13-6.25	/ [b]
17	CH_3	2-CH_3-4-Cl	9.44	3.34-26.65
18	C_2H_5	2-CH_3-4-Cl	30.25	19.62-46.65
19	CH_3	2,4-Cl_2	9.11	3.15-26.35
20	C_2H_5	2,4-Cl_2	10.44	3.93-27.72
21	CH_3	2,5-Cl_2	6.61	4.67-9.37
22	C_2H_5	2,5-Cl_2	3.75	2.33-6.04
23	CH_3	2-Cl-4-CF_3	6.47	4.57-9.16
24	C_2H_5	2-Cl-4-CF_3	1.10	0.80-1.52
diflumetorim			23.06	16.13-32.96
flumorph			7.77	6.48-9.32
cyazofamid			1.01	0.62-1.63

[a] confidence limit. [b] the value could not be measured accurately.

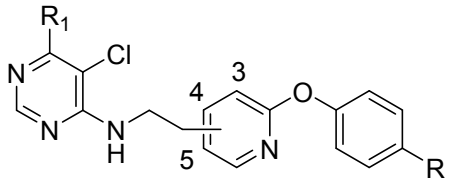
Table 3. Chemical Structures and Fungicidal Activity of Pyrimidinamine Derivatives containing Aryloxy Pyridine Moiety (compounds **25** to **31**)



compound	R_1	X	Alk	R	EC_{50} (mg/L)	95% d[a]
9 (Lead 3)	CH_3	NH	CH_2CH_2	Cl	0.19	0.14-0.26
25	CH_3	NH	CH_2	Cl	7.91	3.97-15.77
26	C_2H_5	NH	CH_2	Cl	5.79	4.02-8.36
27	CH_3	O	CH_2	Cl	>400	/ [b]
28	C_2H_5	O	CH_2	Cl	137.84	51.51-368.85
29	C_2H_5	NH	$CH(CH_3)$	H	18.76	11.87-29.66
30	C_2H_5	NH	$CH(CH_3)$	*t*-Bu	69.54	40.44-119.59
31	CH_3	NH(CO)NH	CH_2CH_2	Cl	>400	/ [b]
diflumetorim					23.06	16.13-32.96
flumorph					7.77	6.48-9.32
cyazofamid					1.01	0.62-1.63

[a] confidence limit. [b] the value could not be measured accurately.

Table 4. Chemical Structures and Fungicidal Activity of Pyrimidinamine Derivatives containing Aryloxy Pyridine Moiety (compounds **32** to **37**)



compound	R_1	position of pyrimidinamine group attached to pyridine ring	R	EC_{50} (mg/L)	95% d[a]
9 (Lead 3)	CH_3	5-position	Cl	0.19	0.14-0.26
10	C_2H_5	5-position	Cl	2.65	1.61-4.35
32	CH_3	3-position	H	60.84	44.32-83.53
33	C_2H_5	3-position	H	326.58	179.20-595.16
34	CH_3	3-position	Cl	232.59	177.24-305.23
35	C_2H_5	3-position	Cl	>400	/ [b]
36	CH_3	4-position	Cl	6.49	2.45-17.19
37	C_2H_5	4-position	Cl	92.14	71.00-119.57
diflumetorim				23.06	16.13-32.96
flumorph				7.77	6.48-9.32
cyazofamid				1.01	0.62-1.63

[a] confidence limit. [b] the value could not be measured accurately.

FIGURE GRAPHICS

Figure 1

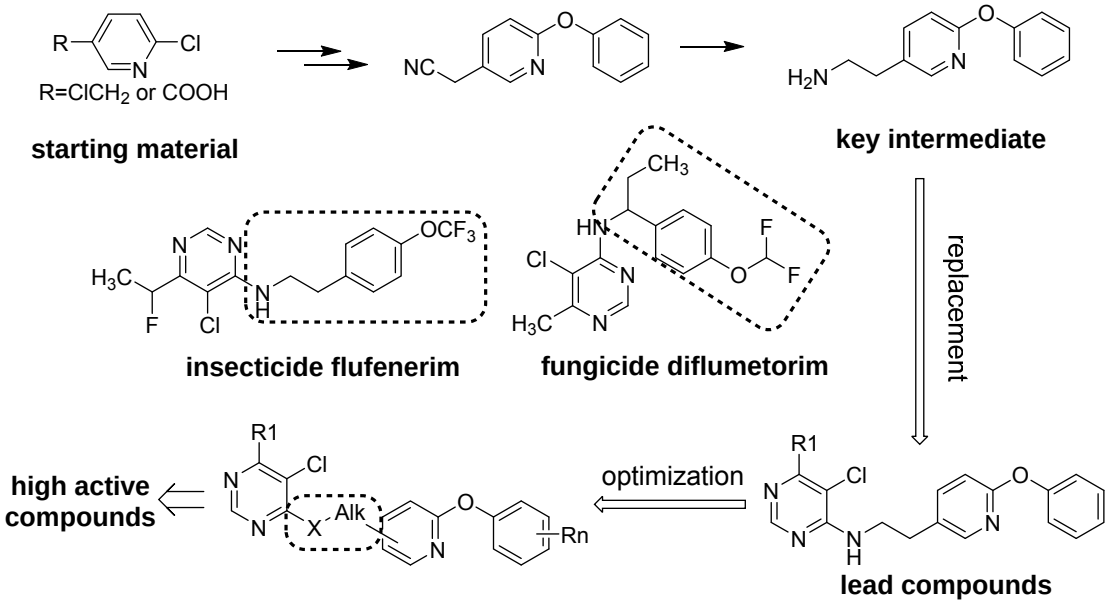
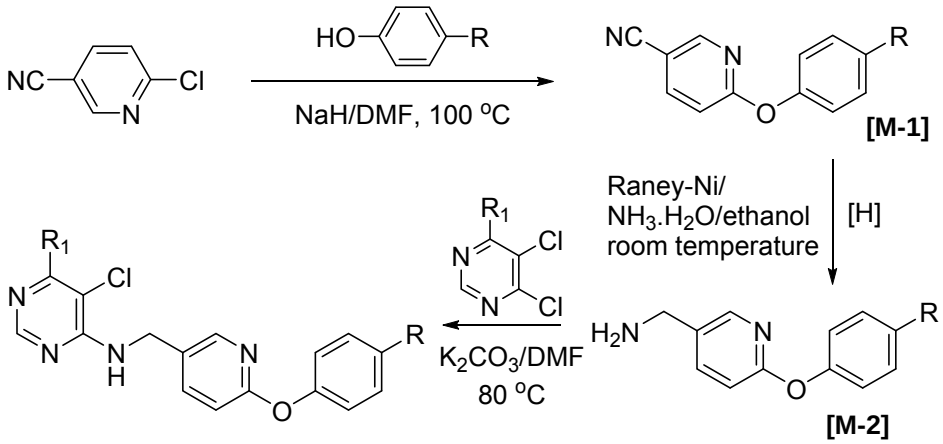
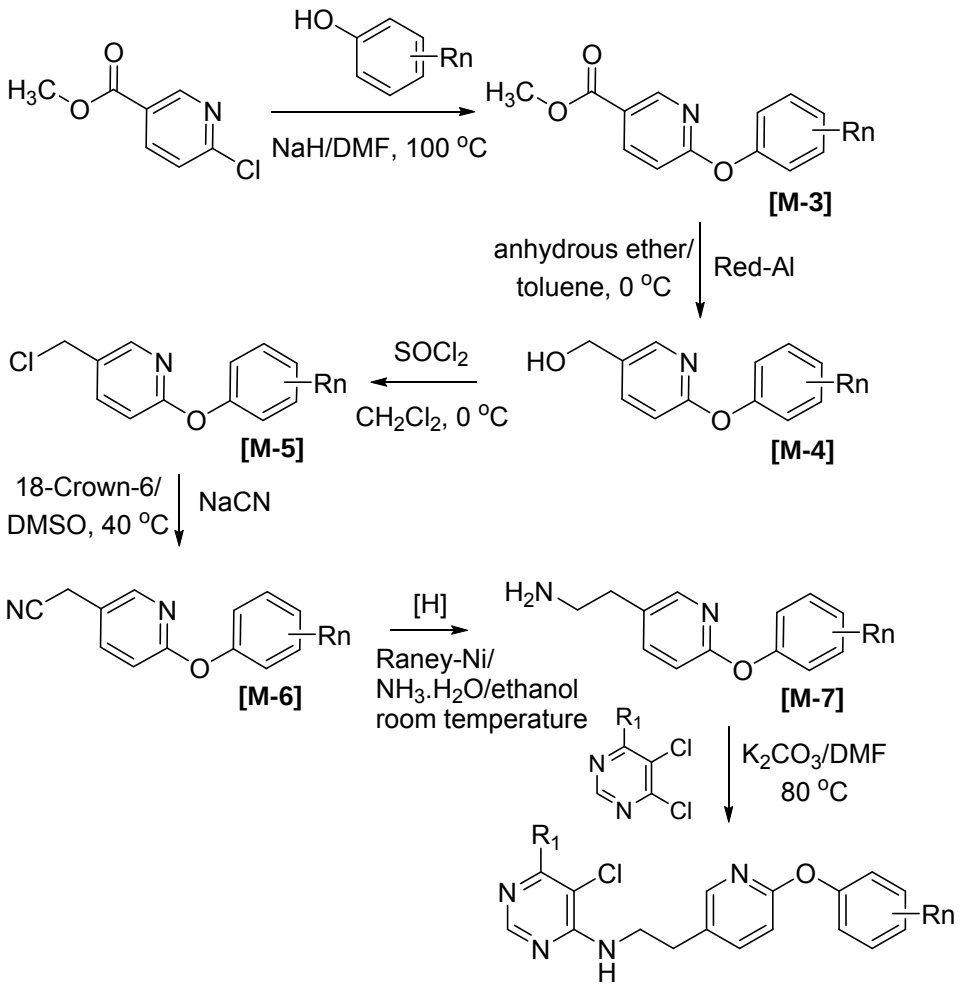


Figure 2



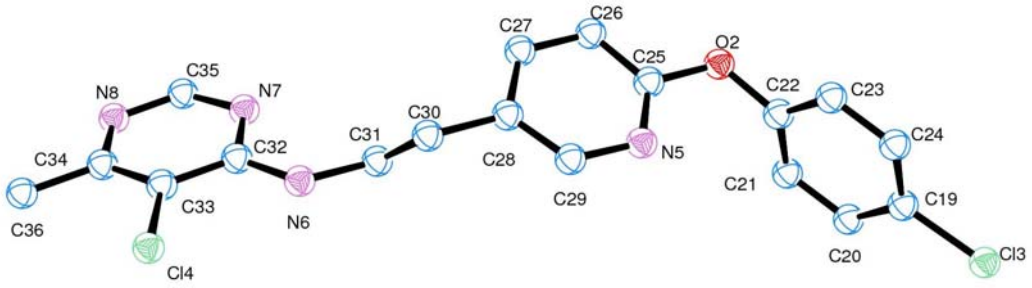
Compounds 1, 2, 25 and **26**

Figure 3



Compounds 3-24

Figure 4


C27
C26
C25
O2
C22
C23
C24
C19
Cl3
C20
C21
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C32
C33
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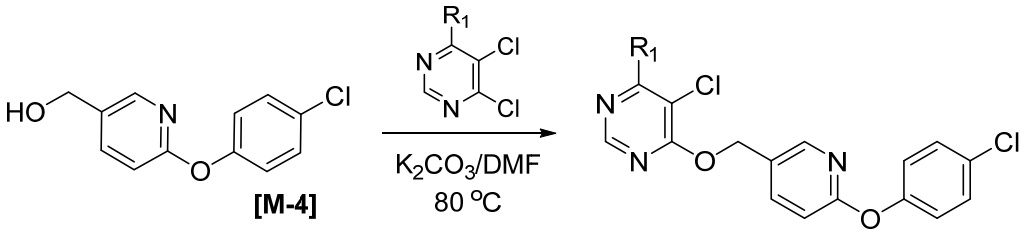
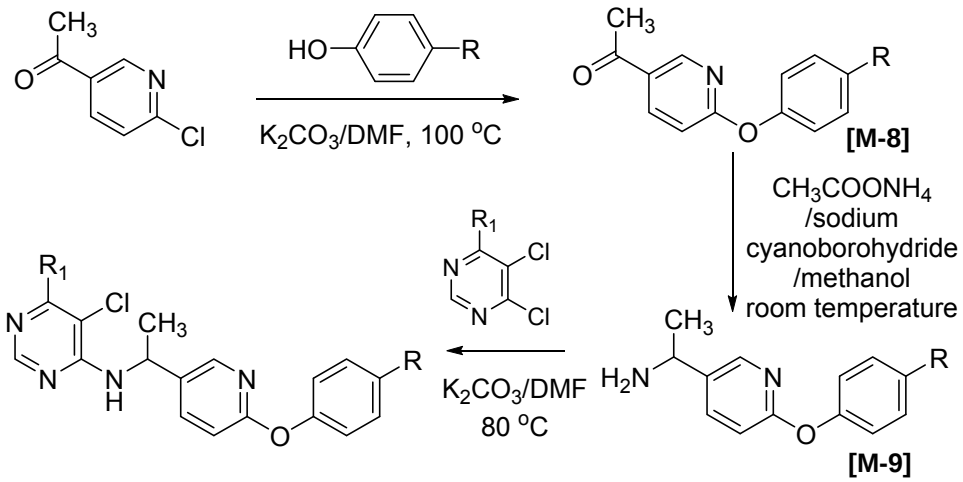
C34
C36
N8
C35
N7

Figure 5



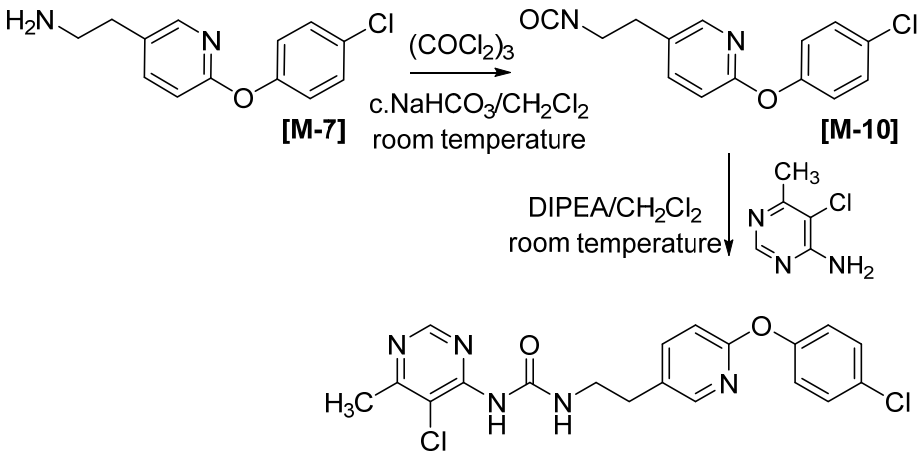
Compounds 27 and **28**

Figure 6



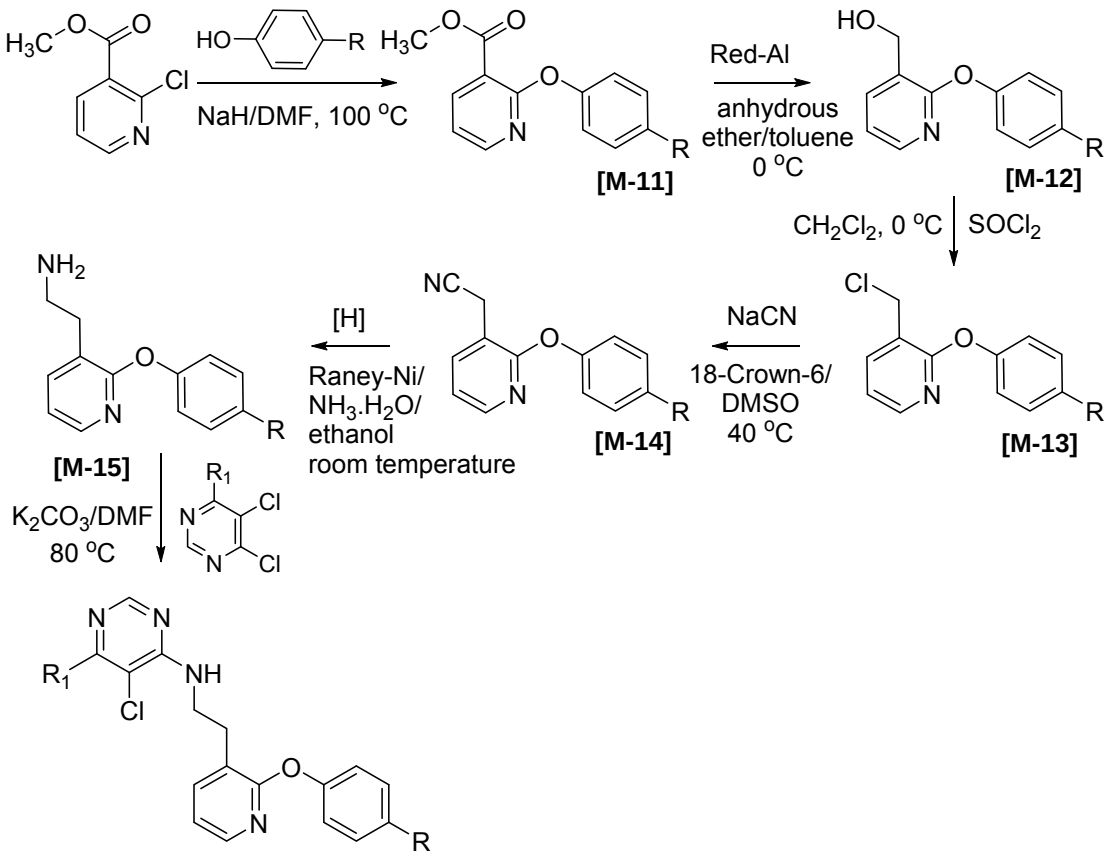
Compounds 29 and **30**

Figure 7



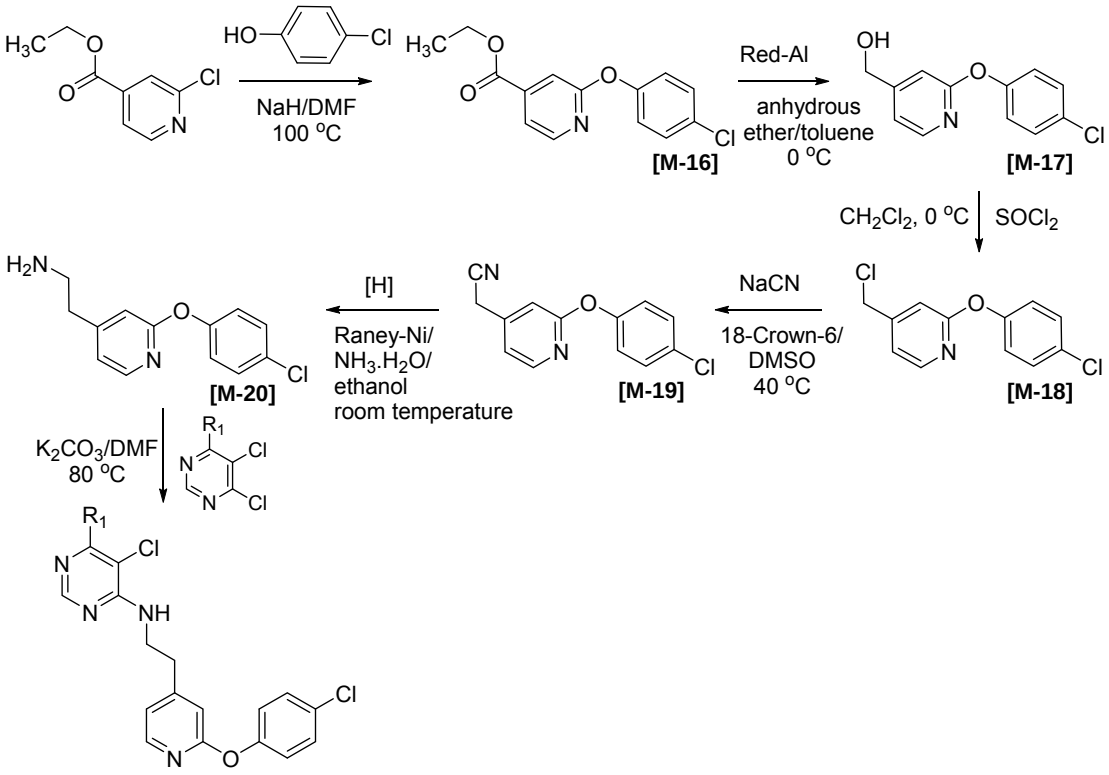
Compound 31

Figure 8



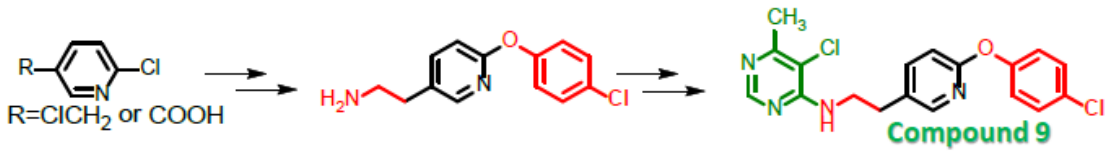
Compounds 32-35

Figure 9



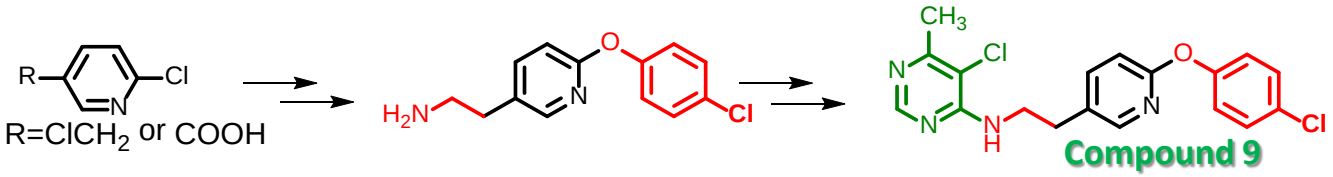
Compounds 36 and **37**

GRAPHIC FOR TABLE OF CONTENTS



Intermediate Derivatization Method

COMPOUND	EC_{50} (mg/L) against CDM
Compound 9	0.19
Diflumetorim	23.06
Flumorph	7.77
Cyazofamid	1.01



Intermediate Derivatization Method

COMPOUND	EC_{50} (mg/L) against CDM
Compound 9	0.19
Diflumetorim	23.06
Flumorph	7.77
Cyazofamid	1.01