JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Agricultural and Environmental Chemistry

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J. Agric. Food Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jafc.9b05185 • Publication Date (Web): 23 Sep 2019

Design, Synthesis, and Structure−Activity Relationship of New Arylpyrazole Pyrimidine Ether Derivatives As Fungicides

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ABSTRACT

To explore a novel fungicide for effectively against cucumber downy mildew (CDM), a series of new arylpyrazole containing pyrimidine ether derivatives were designed and synthesized by employing the intermediate derivatization method (IDM). The structures of synthesized compounds were identified by ^{1}H NMR, ^{13}C NMR, elemental analyses, MS, and X-ray diffraction. Bioassays demonstrated that some of the title compounds exhibited excellent fungicidal activities against CDM. Especially, compound **7** (EC_{50} = 1.22 mg/L) displayed significantly higher bioactivity than commercial fungicides diflumetorim, flumorph, and nearly equal effect to cyazofamid. The relationship between structure and fungicidal activity of the synthesized compounds was discussed as well. The study showed that compound **7** was a promising fungicide candidate for further development.

KEYWORDS: pyrimidine ether derivatives, intermediate derivatization method (IDM), fungicidal activities, structure−activity relationship

INTRODUCTION

Fungicides are one of important parts in agrochemical crops protection products, which are applied widely to major crops, such as rice, fruits, corn and vegetables against diseases.[1] However, the frequent use and misuse of fungicides inevitably lead to developing serious resistance. So it is a huge challenge and an urgent need for discovering and developing new fungicides with innovated structures and improved activities.[2]

Arylpyrazolol is a common chemical intermediate often found not only in medicines field for treatment of diseases such as pain, cancer, inflammation, neurodegenerative, heart failure and renal diseases,[3,4] but also in agrochemical field to protect crops from pests or diseases. Pyraclostrobin, pyrametostrobin and pyraoxystrobin are commercial/in development fungicides containing an arylpyrazole moiety.[5-8] Pyraclostrobin is the most outstanding representative for its advantages of broad spectrum, high efficacy and safety on non-targets, and its big market value with global sales of $920 million in 2017. More importantly, some excellent features in arylpyrazolol itself including ease of synthesis, ease of diverse derivation, low toxicity and low cost, fully meet the requirement of intermediate selection of the intermediate derivatization method (IDM), a highly efficient approach to discover agrochemical candidates.[9-23]

In prior arts, more and more literatures regarding pyrimidine ether compounds with fungicidal activity have been published. For example, Ciba-Geigy

(Syngenta) reported pyrimidine ethers containing pyrazolyl acrylic acid derivatives as effective microbicides for controlling fungal diseases, especially against *cercospora arachidicola* on groundnut plants and *phytophthora infestans* on tomatoes.[24] Imperial Chemical Industriesplc (Syngenta) found a series of pyrimidine diethers for combating fungi, especially fungal infections of plants, such as *pyricularia oryzae* on rice, *puccinia recondita*, *puccinia striiformis* and other rusts on wheat.[25] Bayer reported a pyrimidinyloxyphenyl-2-methoxyiminoacetates with a promising bioactivity against *leptosphaeria nodorum* on wheat, *phytophthora* on tomato, and *venturia* on apple.[26] High & New Tech Research Center of Henan Academy of Sciences found a series of ((6-substituted-pyrimidine-4-oxy)phenyl)-3-methyl methoxyacrylates, which had a excellent control for *fusarium oxysporum*, *cercospora arachidicola hori*, *rhizoctonia cerealis* and more.[27]

In this study, considering the excellent performance of the arylpyrazolol in crop protection field, in order to explore new skeleton fungicidal compounds with improved activity, (4-chlorophenyl)hydrazine hydrochloride was used as starting material by employing the intermediate derivatization method to obtain the key intermediate 1-(4-chlorophenyl)-1*H*-pyrazol-3-ol, followed by replacement with methoxy acrylate group of azoxystrobin to achieve a series of novel pyrimidine ether derivatives containing an arylpyrazole moiety (Figure 1). The detailed synthesis and structure−activity relationships of these compounds were 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. Elemental analyses were determined on a Yanaco MT-3CHN elemental analyzer (Yanaco, Kyoto, Japan). ^{1}H NMR spectra was recorded with a Mercury 300 or 600 MHz spectrometer (Varian, Palo Alto, CA) with deuterochloroform or deuterium dimethyl sulfoxide as the solvent and tetramethylsilane (TMS) as the internal standard. ^{13}C NMR spectra was recorded with a Mercury 151 MHz spectrometer (Varian, Palo Alto, CA) with deuterochloroform or deuterium dimethyl sulfoxide as the solvent and tetramethylsilane (TMS) as the internal standard. Mass spectra were acquired with Agilent 1100 series LC-MSD-Trap-VL (Agilent Technologies, Milford, MA). Melting points were determined on a Büchi M-569 melting point apparatus (Büchi Labortechnik AG, Flawil, Switzerland) and were uncorrected. X-ray structure determination was recorded with XtaLAB mini (Rigaku, The Woodlands, TX). All plant and fungus materials were obtained from the Agrochemical Discovery Department in Shenyang Sinochem Agrochemicals R&D Co. Ltd. (Shenyang, China).

The general synthetic methods for compounds **1−31** were shown in Figures 2, 4 and 5, and their structures were listed in Tables 1−5. The silica gel chromatography was performed with a column of 254 mm × 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 4-((1-(4-chlorophenyl)-1*H*-pyrazol-3-yl)oxy)-6-(4-(trifluoro-methyl)phenoxy)-pyrimidine, 7. (General procedure for compounds **1-26**) (Figure 2)

*Synthesis of 1-(4-chlorophenyl)pyrazolidin-3-one (**A-1**)*

Sodium (10.35 g, 450 mmol) was cut into small pieces and added to 250 mL of ethanol. Then (4-chlorophenyl)hydrazine hydrochloride (26.85 g, 150 mmol) was added at 30-35 ^{o}C after sodium was completely dissolved, followed by addition of acrylamide (14.2 g, 200 mmol) after stirring for 15 min. The reaction mixture was stirred for 6 h when the temperature rised to 80 ^{o}C, until the color of reaction mixture gradually turned from red to light red. After reaction completion, the mixture was concentrated under reduced pressure, the residue was poured into water, and extracted with ethyl acetate. The extract was dried over anhydrous magnesium sulfate, filtered, and evaporated to obtain intermediate **A-1** as a brown solid: 27.2 g (92%).

*Synthesis of 1-(4-chlorophenyl)-1H-pyrazol-3-ol (**B-1**)*

A-1 (27.2 g, 138 mmol) was dissolved in *N*-methylpyrrolidone, following ferric chloride (0.26 g, 1.6 mmol) was added to the flask equipped with a bubbler, and then the reaction mixture was heated to 100 ^{o}C for 6-7 h. After the reaction completion, the mixture was extracted, dried, filtered and concentrated. The residue was purified via silica gel chromatography with ethyl acetate/60−90 ^{o}C

petroleum ether (1:7, v/v) as eluent to obtain intermediate **B-1** as a pale white solid: 25.6 g (94%), m.p. 190.8 °C.

*Synthesis of 4-chloro-6-((1-(4-chlorophenyl)-1H-pyrazol-3-yl)oxy)pyrimidine (**C-1**)*

To a solution of 4,6-dichloropyrimidine (3.83 g, 26 mmol) and potassium carbonate (7.09 g, 51 mmol) in 120 mL of DMF was added **B-1** (5 g, 26 mmol). The reaction mixture was then heated to 90 °C for 4 h, and monitored by TLC until the reaction was completed. The mixture was extracted, dried, filtered, concentrated to obtain intermediate **C-1** as a yellow solid: 7.3 g (92%), m.p. 150.4 °C.

*Synthesis of 4-((1-(4-chlorophenyl)-1H-pyrazol-3-yl)oxy)-6-(4-(trifluoro-methyl)phenoxy)pyrimidine (compound **7**)*

To a solution of **C-1** (0.4 g, 1.3 mmol) and potassium carbonate (0.36 g, 2.6 mmol) in 20 mL of DMF was added 4-(trifluoromethyl)phenol (0.21 g, 1.3 mmol). The reaction mixture was then heated to 100 °C for 2 h, and monitored by TLC until the reaction was completed. 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 (1:5, v/v) as eluent to obtain 0.47 g compound **7** as a light yellow solid: 0.47 g (84%), m.p. 157 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.50 (s, 1H, pyrimidyl-2-H), 7.89 (s, 1H,

pyrazol-5-H), 7.71 (d, *J* = 8.1 Hz, 2H, 4-CF_3-Ph-3,5-2H), 7.60 (d, *J* = 8.4 Hz, 2H, 4-Cl-Ph-2,6-2H), 7.42 (d, *J* = 8.4 Hz, 2H, 4-Cl-Ph-3,5-2H), 7.29 (d, *J* = 8.1 Hz, 2H, 4-CF_3-Ph-2,6-2H), 6.64 (s, 1H, pyrimidyl-5-H), 6.37 (s, 1H, pyrazol-4-H). ^{13}C NMR (151 MHz, $CDCl_3$) *δ* 170.77, 170.31, 158.12, 157.87, 154.89, 138.14, 132.02, 129.51, 128.14, 127.12 (d, *J* = 3.4 Hz), 121.87, 119.72, 99.25, 93.24. Anal. Calcd (%) for $C_{20}H_{12}ClF_3N_4O_2$: C, 55.51; H, 2.79; N, 12.95. Found: C, 55.55; H, 2.74; N, 12.98. MS m/z 433.0 $[M + H]^+$ (calcd $[M + H]^+$ 433.06).

The crystal structure of target compound **7** was also determined by X-ray diffraction analyses. Its crystal structure is shown in Figure 3.

Synthesis of 4-((1-(4-chlorophenyl)-1*H*-pyrazol-4-yl)oxy)-6-(4-(trifluoro-methyl)phenoxy)-pyrimidine, 27. (Figure 4)

*Synthesis of (E)-1-chloro-3-(2-(4-chloro-phenyl)hydrazono)propan-2-one (**D**)*

86 mL of water and 43 mL of 37% concentrated hydrochloric acid were dropped into 4-chloroaniline (11.16 g, 120 mmol), which was stirred in ice bath for 30 min ahead of time. When the reaction solution reached 0 ^{o}C, sodium nitrite (8.69 g, 126 mmol) in aqueous solution was dropwise added within 30 min. Then 4-chloroacetoacetic acid (16.38 g, 120 mmol) and sodium acetate (24.60 g, 300 mmol) in aqueous solution were added to the reaction mixture in turn after it turned the starch potassium iodide paper to blue, and they were stirred at room temperature until a large amount of solid was generated. After

reaction completion, the mixture was filtered, washed and dried to obtain intermediate **D** as a pale orange solid: 18 g (76%).

*Synthesis of 1-(4-chlorophenyl)-1H-pyrazol-4-ol (**E**)*

To a solution of **D** (9.80 g, 50 mmol) and sodium hydroxide (5.00 g, 125 mmol) in 100mL of methanol, stirring at 40 °C for 3-6 h, and the reaction was monitored by TLC until the reaction was completed. Then the mixture was concentrated under reduced pressure, added water, adjusted the pH to neutral, and poured into water and extracted with ethyl acetate. The extract was dried over anhydrous magnesium sulfate and evaporated. The residue was purified via silica gel chromatography with ethyl acetate/60−90 °C petroleum ether (1:8 v/v) as eluent to obtain **E** as a yellow solid: 6 g (75%), m.p. 122.5 °C.

*Synthesis of 4-chloro-6-((1-(4-chlorophenyl)-1H-pyrazol-4-yl)oxy)pyrimidine (**F**)*

To a solution of 4,6-dichloropyrimidine (3.83 g, 26 mmol) and potassium carbonate (7.09 g, 51 mmol) in 120 mL of DMF was added **E** (5 g, 26 mmol). The reaction mixture was then heated to 90 °C for 4 h, and monitored by TLC until the reaction was completed. The mixture was extracted, dried, filtered, concentrated to obtain intermediate **F** as a white solid: 7.1 g (90%).

*Synthesis of 4-((1-(4-chlorophenyl)-1H-pyrazol-4-yl)oxy)-6-(4-(trifluoro-methyl)phenoxy)pyrimidine (compound **27**)*

To a solution of **F** (0.4 g, 1.3 mmol) and potassium carbonate (0.36 g, 2.6

mmol) in 20 mL of DMF was added 4-(trifluoromethyl)phenol (0.21 g, 1.3 mmol). The reaction mixture was then heated to 100 °C for 2 h, and monitored by TLC until the reaction was completed. 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 (1:7 v/v) as eluent to obtain 0.43 g compound **27** as a yellow solid: 0.43 g (77%), m.p. 142 °C. ^{1}H NMR (600 MHz, DMSO) *δ* 8.78 (s, 1H, pyrazol-5-H), 8.54 (s, 1H, pyrimidyl-2-H), 7.92 (s, 1H, pyrazol-3-H), 7.87 (dd, *J* = 15.6, 8.7 Hz, 4H, 4-Cl-Ph-2,6-2H; 4-CF_3-Ph-3,5-2H), 7.59 (d, *J* = 8.7 Hz, 2H, 4-Cl-Ph-3,5-2H), 7.48 (d, *J* = 8.4 Hz, 2H, 4-CF_3-Ph-2,6-2H), 6.90 (s, 1H, pyrimidyl-5-H). Anal. Calcd (%) for $C_{20}H_{12}ClF_3N_4O_2$: C, 55.51; H, 2.79; N, 12.95. Found: C, 55.56; H, 2.76; N, 12.92.

Synthesis of 3-((6-((1-(4-chlorophenyl)-1*H*-pyrazol-3-yl)oxy)pyrimidin-4-yl)oxy)-5-methylisoxazole, 30. (General procedure for compounds **28-31**) (Figure 5)

To a solution of **C-1** (0.4 g, 1.3 mmol) and potassium carbonate (0.36 g, 2.6 mmol) in 20 mL of DMF was added 5-methylisoxazol-3-ol (0.13 g, 1.3 mmol). The reaction mixture was then heated to 100 °C for 2 h, and monitored by TLC until the reaction was completed. 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 (1:8 v/v) as eluent to obtain 0.38 g compound **30** as a white solid: 0.38 g (79%), m.p. 207.1 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.75 (s, 1H, pyrimidyl-2-H), 8.62 (d, J = 2.7 Hz, 1H, pyrazol-5-H), 7.85 (d, J = 9.0 Hz, 2H, 4-Cl-Ph-2,6-2H), 7.69 (s, 1H, oxazolyl-H), 7.58 (d, J = 9.0 Hz, 2H, 4-Cl-Ph-3,5-2H), 6.53 (d, J = 2.7 Hz, 1H, pyrazol-4-H), 6.00 (s, 1H, pyrimidyl-5-H)), 2.41 (s, 3H, CH_3). Anal. Calcd (%) for $C_{17}H_{12}ClN_5O_3$: C, 55.22; H, 3.27; N, 18.94. Found: C, 55.26; H, 3.23; N, 18.99.

Fungicidal Assay.

Each of the test compounds (4 mg) was first dissolved in 5 mL of acetone/methanol (1:1, v/v), and 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, and then the test solution was sprayed on the host plant with a homemade 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 5×10^5 spores/mL using a PS289 Procon Boy WA double action 0.3 mm airbrush (GSI, Tokyo, Japan). The cucumber plants were stored in a humidity chamber (24 ± 1 ^{o}C, RH > 95%, dark) and then transferred into a greenhouse (18−30 ^{o}C, 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 from 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] × 100. The EC_{50} values were calculated by Duncan's new multiple range method using DPS version 14.5. The fungicidal test results of compounds **1−31** against CDM are listed in Tables 1−5.

RESULTS AND DISCUSSION

Synthesis. The synthesized compounds were characterized by ^{1}H NMR and elemental analyses, and some compounds were further characterized by ^{13}C NMR and MS. The chemical structure of lead compound **7** was unequivocally determined by X-ray crystallography (Figure 3). All spectral and analytical data were consistent with the assigned structures.

Structure−Activity Relationships (SAR).

Discovery of Lead Compound 4

Initially, to verify the feasibility of our design concept, the starting material, (4-chlorophenyl)hydrazine hydrochloride, was employed to obtain the key intermediate 1-(4-chlorophenyl)-1*H*-pyrazol-3-ol, the crucial moiety of pyraclostrobin. Then the obtained intermediate was used to replace the methoxy acrylate moiety of azoxystrobin, to achieve primary compound **1** (Table 1). The bioassay results indicated that the synthesized compound only had weak activity (EC_{50} > 400 mg/L) against CDM, and hardly any bioactivity against other tested market targets wheat powdery mildew (WPM), southern rust (SR), and cucumber anthracnose (CA). Next, considering the structure feature, we tried to changing the substituent position of cyanogen by using 3-hydroxybenzonitrile and 4-hydroxybenzonitrile to synthesize compounds **2** and **3**. However, these two compounds both didn't show improved activity (EC_{50} > 400 mg/L) against CDM, so did against the other diseases. Then we selected 4-chlorophenol as the probe to design compound **4** by means of usual optimization strategy around phenyl ring. Then, we replaced the cyanide group with the chlorine atom to get the compound **4**, fortunately, it had better activity (EC_{50} = 167.21 mg/L) against CDM than the first three compounds. So compound **4** (Table 1) was regarded as the lead compound to carry out further optimization on phenyl ring targeting CDM.

Optimization of Lead Compound 4

Compound **4** was conducted optimization from 4 aspects, including the electronic properties, spatial characteristics of the substituent groups, changes of substituent position and numbers of substituents. Firstly, we changed the substituent group of *para*-position according to the the electronic properties. We synthesized six compounds, compounds **5-8** with the electron-withdrawing groups, **9-10** with the electron-donating groups (Table 2**)**. We could find that the compounds **5-8**, which had the electron-withdrawing groups, all showed higher bioactivity than lead compound **4**. Although the improvement in activity of the compounds **5** and **6** (replaced with fluorine and bromine atoms respectively) was not relatively obvious, compounds **7** and **8** (substituted with strong electron-withdrawing groups CF_3 and NO_2 respectively) exhibited a sharply increased efficacy compared to the lead compound **4**, especially compound **7** (EC_{50} = 1.22 mg/L) displayed significantly higher bioactivity than commercial fungicides diflumetorim (EC_{50} = 23.06 mg/L), flumorph (EC_{50} = 7.77 mg/L), and nearly equal effect to cyazofamid (EC_{50} = 1.01 mg/L). The reasons why compound **7** had excellent bioactivity were listed as below: CF_3 group usually provides a very strong positive contribution to biological activities;[28] there existed the unique properties of the fluorine atom, such as high thermal stability and lipophilicity.[29] These indicated that electron-withdrawing groups possibly have positive effect on activity, especially the strong electron-withdrawing substituent. While compound **9** with

Rn of CH_3, a weak electron-donating substituent, had hardly any fungicidal activity (EC_{50} > 400 mg/L), however, compound **10** with Rn of OCH_3, a high electron-donating substituent, showed much better activity (EC_{50} = 57.24 mg/L) versus the lead compound **4** (EC_{50} = 167.21 mg/L). Secondly, compound **11** with Rn of *t*-Bu was synthesized to explore the influence of spatial effect. Surprisingly, the results displayed that the presence of a larger group probably could not produce negative influence as usual rather than positive influence on activity. Thirdly, two compounds were synthesized to evaluate the effect of the substituent position on fungicidal activity using the chlorine atoms as probes. We changed the position of single chlorine atom to *meta*-position and *ortho*-position of the phenyl ring to obtain compounds **12** and **13**, these two compounds both exhibited higher fungicidal activity than the lead compound **4** (29.57 and 26.08 mg/L, respectively, versus 167.21 mg/L), indicating *meta*-position and *ortho*-position are both helpful to enhance activity and could be selected as potential lead compounds for further optimization in future studies. Finally, we introduced one more or two chlorine atoms into other positions of phenyl ring, while keeping the 4 position fixed as chlorine. Compounds **14-16** were prepared. The assay result exhibited that compound **14** with Rn of 2,4-Cl_2 showed almost equal activity (EC_{50} = 174.35 mg/L) to the compound **4**; while the fungicidal activity of compounds **15** and **16** decreased sharply, which suggested that the increase of substituent numbers probably brings negative influence on bioactivity. Consequently, based on the significant

303 contribution of CF_3 group in compound **7** and similar contribution of 2,4-Cl_2
304 groups in compound **14**, compound **17** with Rn of 2-Cl-4-CF_3 was further
305 designed and synthesized, to our disappointment, compound **17** did not
306 express satisfactory control as we anticipated; meanwhile, compound **18**
307 bearing two CF_3 groups didn't work well either, not embodying the synergistic
308 effect of two dominant groups. So far, the result of the optimization of
309 compound **4** was identification of compound **7** with a *para*-Cl group on the left
310 phenyl ring and a *para*-CF_3 group on the right phenyl ring as the optimized
311 structure, the second lead compound with greatly improved fungicidal activity.
312

313 **Effect of Substituents of Pyrimidine Ring and Linker between Pyrimidine**
314 **and Phenyl Groups on Fungicidal Activity**

315 To determine if changing the substituents of pyrimidine and the linker between
316 pyrimidine and phenyl groups would bring enhanced fungicidal activity, we
317 synthesized and screened a series of compounds **19-26** (Table 3). First of all,
318 we used lead compound **7** as template for further optimization, and kept Rn
319 group fixed as 4-CF_3, while changing R_1, R_2, X in turn to synthesize
320 compounds **19-21**. When CH_3 group was introduced into R_1 and R_2 position to
321 get the compounds **19** and **20** respectively, the bioactivity both reduced greatly.
322 So did replacing O with NH for changing linker (compound **21**). When Rn was
323 4-OCH_3, neither replacement of R_2 with CH_3 nor replacement of X with NH
324 resulted in dramatical reduction of activity. However, when Rn was 4-CH_3, the

above mentioned substitution of R_2 and X both result in outstanding activity enhancement. On the basis of the positive information, we further synthesized compound **26** bearing both R_2 of CH_3 and X of NH with hope to find higher activity compound than compound **25**, even than compound **7**, but the results went contrary to our wishes. Generally speaking, the effect of substituent of pyrimidine ring and linker between pyrimidine and phenyl groups on fungicidal activity mostly depends on Rn group of phenyl ring.

Activity of Different Position of Pyrimidine Ether Group Attached to Pyrazole Ring

To investigate the effect of different position of pyrimidine ether group attached to pyrazole ring on the activity of molecules, compound **27** was designed and synthesized (Table 4). The activity decreased obviously (EC_{50} > 400 mg/L) versus compound **7** (EC_{50} = 1.22 mg/L). The poor result showed that when we changed attached position of pyrimidine ether group to pyrazole ring from 3-position to 4-position, the bioactivity diminished dramatically.

Activity of Different Heterocycles Attached to Pyrimidine Ring

To primarily evaluate if replacing the right side phenyl ring with a nitrogen-containing heterocycle would change bioactivity of intact molecules, we synthesized a series of compounds **28-31** (Table 5**)**. The compounds **29** and **31** relating to pyridinyl substituted with CF_3 and pyridazinonyl had hardly

any bioactivity. On the contrary, the compounds **28** and **30**, whose heterocyclic groups were pyridin-2-yl and 5-CH_3-isoxazol-3-yl respectively, both exhibited superior bioactivity, although still less than compound **7**. The result suggested that various kinds of nitrogen-containing heterocycles might have different influence on the biological activity of compounds, further detailed studies are in progress.

On the basis of data presented in Tables 1−5, a clear-cut, well-defined relationship between chemical structure and biological activity had taken shape by examining the effect of the electronic properties, the spatial characteristics, the substituted position and numbers, substituents of pyrimidine ring, linker between pyrimidine and phenyl groups, different position of pyrimidine ether group attached to pyrazole ring, different heterocycles attached to pyrimidine ring. The *para*-position single CF_3-substituted phenyl derivative, compound **7**, exhibited significantly improved bioactivity compared to some commercial varieties. Compound **25** (Rn = 4-CH_3, X = NH, R_1 = R_2 = H) was a good lead compound for further modification. Single electron-withdrawing substituent on the *para*-position of phenyl ring and the 3-position where the pyrimidine ether group was attached to pyrazole ring might improve fungicidal activity effectively.

Above all, we can conclude that compound **7** is the optimal structure with desired activity. It offers a control with an EC_{50} value of 1.22 mg/L against CDM, displaying significantly higher bioactivity than commercial fungicides

diflumetorim, flumorph, and nearly equal effect to cyazofamid, which is a promising candidate for future development. Further synthesis of analogues, structure optimization studies, and field trials of compound **7** are in progress.

SUPPORTING INFORMATION

Characterization for intermediates **B-1**, **C-1**, **C-2**, **C-3**, **E**, **F**, and compounds **1-6**, **8-26**, **28, 29, 31**. This material is available free of charge via the Internet at http://pubs.acs.org. The atomic coordinates for compound **7** have been deposited at the Cambridge Crystallographic Data Centre. CCDC ID: 1946262 contains the supplementary crystallographic data for this Article. 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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FIGURE CAPTIONS

Figure 1. An overview of the design of new arylpyrazole pyrimidine ether derivatives

Figure 2. Synthetic route to compounds **1-26**

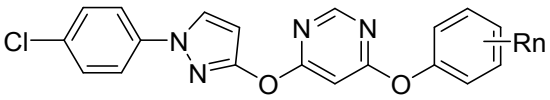
Figure 3. X-ray single-crystal diffraction of compound **7**

Figure 4. Synthetic route to compound **27**

Figure 5. Synthetic route to compounds **28-31**

TABLES

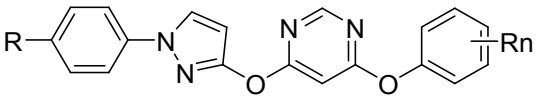
Table 1. Chemical Structures and Fungicidal Activity of Arylpyrazole Pyrimidine Ether Derivatives (compounds **1** to **4**)



compound	Rn	EC_{50} (mg/L)	95% d[a]
1	2-CN	>400	/ [b]
2	3-CN	>400	/ [b]
3	4-CN	>400	/ [b]
4 (lead 1)	4-Cl	167.21	17.52-1595.67
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.

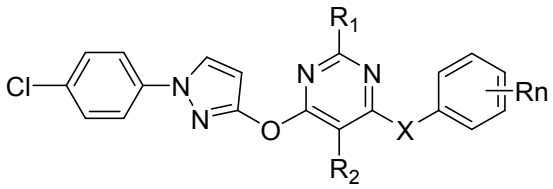
Table 2. Chemical Structures and Fungicidal Activity of Arylpyrazole Pyrimidine Ether Derivatives (compounds **5** to **18**)



compound	R	Rn	EC_{50} (mg/L)	95% d[a]
4 (lead 1)	Cl	4-Cl	167.21	17.52-1595.67
5	Cl	4-F	59.87	49.27-72.76
6	Cl	4-Br	75.32	59.25-95.76
7 (lead 2)	Cl	4-CF_3	1.22	1.04-1.42
8	Cl	4-NO_2	24.45	20.92-28.59
9	Cl	4-CH_3	>400	/ [b]
10	Cl	4-OCH_3	57.24	47.00-69.72
11	Cl	4-t-Bu	54.25	45.11-65.24
12	Cl	3-Cl	29.57	25.90-33.77
13	Cl	2-Cl	26.08	22.91-29.69
14	Cl	2,4-Cl_2	174.35	12.99-2339.93
15	Cl	3,4-Cl_2	>400	/ [b]
16	Cl	2,4,6-Cl_3	>400	/ [b]
17	Cl	2-Cl-4-CF_3	182.78	11.25-2970.68
18	CF_3	4-CF_3	100-400	/ [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.

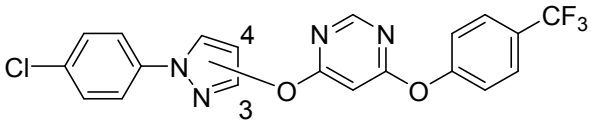
Table 3. Chemical Structures and Fungicidal Activity of Arylpyrazole Pyrimidine Ether Derivatives (compounds **19** to **26**)



compound	R_1	R_2	X	Rn	EC_{50} (mg/L)	95% d[a]
7 (lead 2)	H	H	O	4-CF_3	1.22	1.04-1.42
19	CH_3	H	O	4-CF_3	>400	/ [b]
20	H	CH_3	O	4-CF_3	>400	/ [b]
21	H	H	NH	4-CF_3	25.64	22.23-29.57
10	H	H	O	4-OCH_3	57.24	47.00-69.72
22	H	CH_3	O	4-OCH_3	>400	/ [b]
23	H	H	NH	4-OCH_3	>400	/ [b]
9	H	H	O	4-CH_3	>400	/ [b]
24	H	CH_3	O	4-CH_3	43.97	37.54-51.50
25	H	H	NH	4-CH_3	3.87	0.46-32.49
26	H	CH_3	NH	4-CH_3	85.76	64.05-114.83
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.

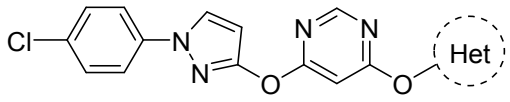
Table 4. Chemical Structures and Fungicidal Activity of Arylpyrazole Pyrimidine Ether Derivatives (compound **27**)



compound	Position of Pyrimidine ether Group Attached to Pyrazole Ring	EC_{50} (mg/L)	95% d[a]
7 (lead 2)	3-position	1.22	1.04-1.42
27	4-position	>400	/ [b]

[a] Confidence limit. [b] The value could not be measured accurately.

Table 5. Chemical Structures and Fungicidal Activity of Arylpyrazole Pyrimidine Ether Derivatives (compounds **28** to **31**)



compound	Het	EC_{50} (mg/L)	95% d[a]
7 (lead 2)	4-CF_3-phenyl	1.22	1.04-1.42
28	pyridin-2-yl	27.99	24.50-31.98
29	5-CF_3- pyridin-2-yl	>400	/ [b]
30	5-CH_3-isoxazol-3-yl	54.25	45.11-65.24
31	2-(*t*-butyl)-4-chloro-pyridazin-3(2*H*)-one-5-yl	>400	/ [b]

[a] Confidence limit. [b] The value could not be measured accurately.

FIGURE GRAPHICS

Figure 1

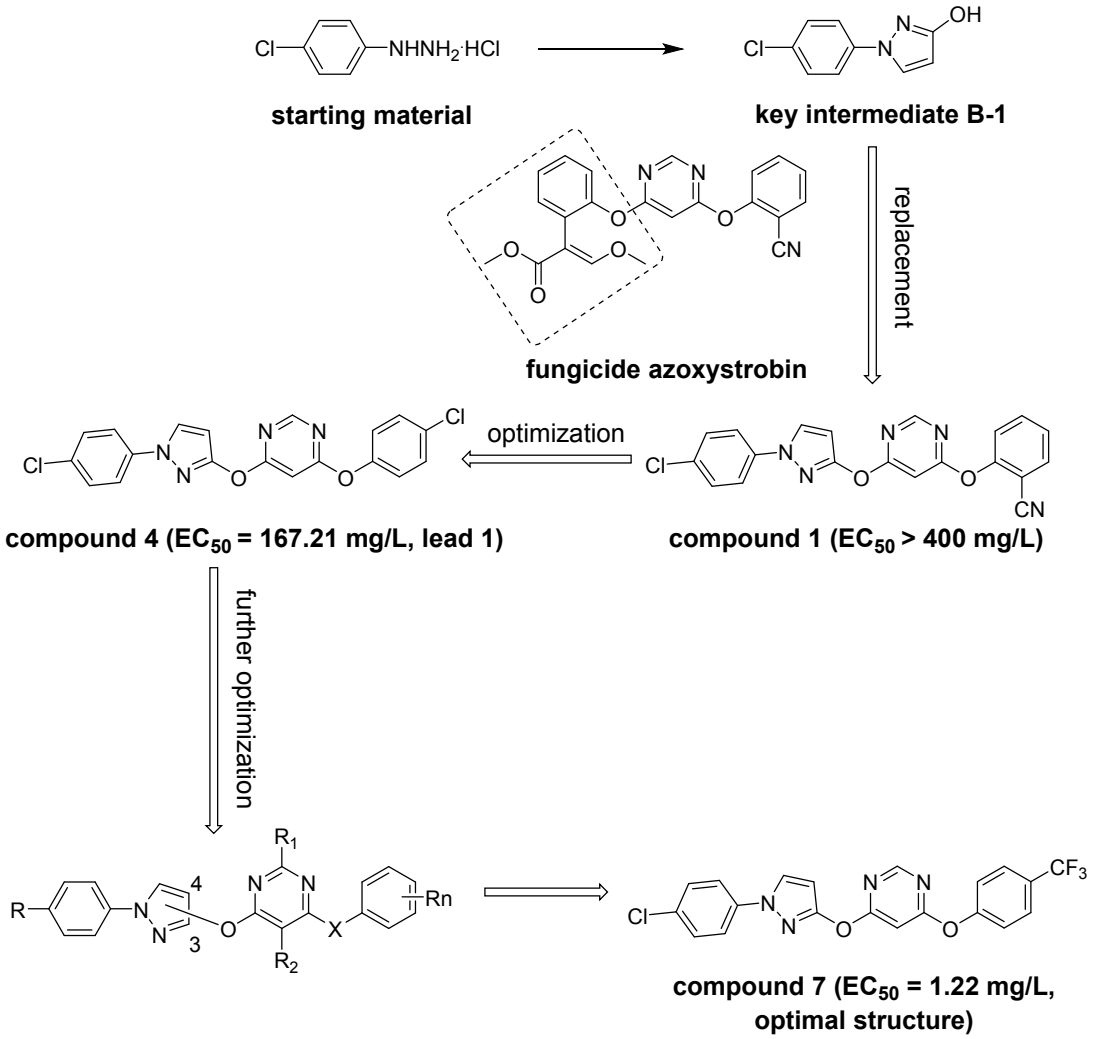


Figure 2

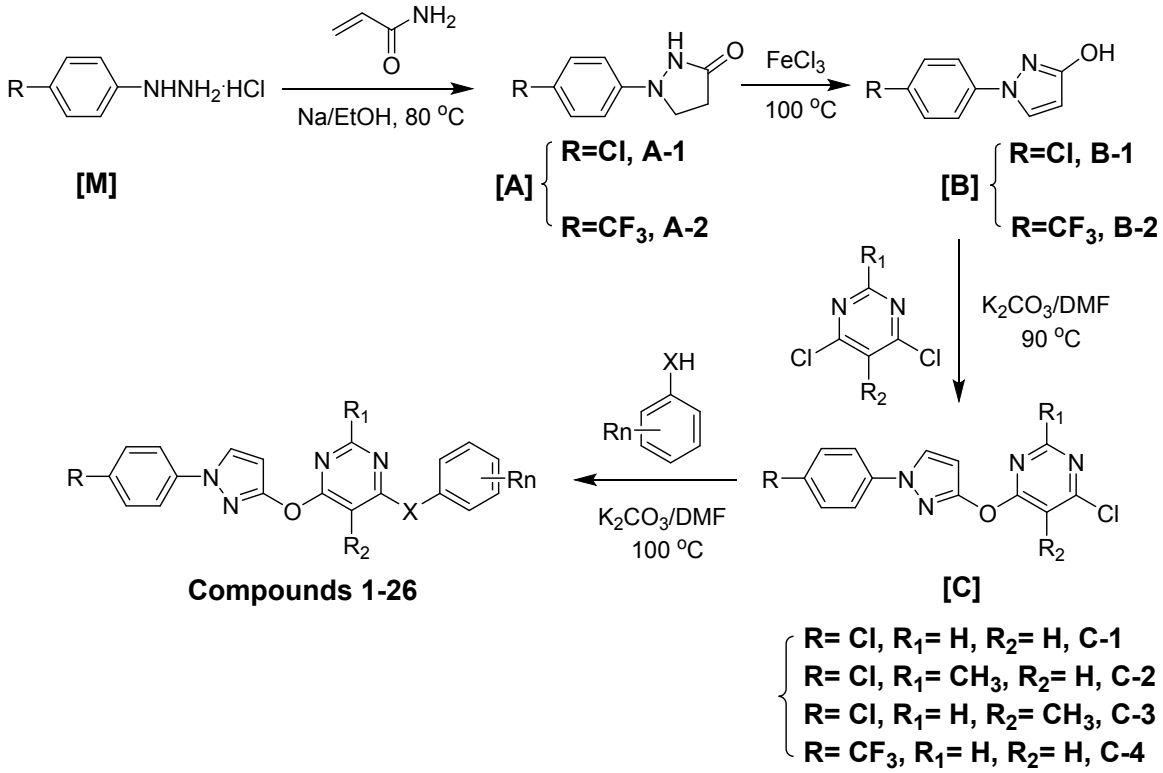

R
NHNH2·HCl
NH2
O
Na/EtOH, 80 °C
[M]
H
N
N
O
[A]
R=Cl, A-1
R=CF3, A-2
FeCl3
100 °C
OH
[B]
R=Cl, B-1
R=CF3, B-2
K2CO3/DMF
90 °C
R1
N
N
Cl
Cl
R2
[C]
R= Cl, R1= H, R2= H, C-1
R= Cl, R1= CH3, R2= H, C-2
R= Cl, R1= H, R2= CH3, C-3
R= CF3, R1= H, R2= H, C-4
XH
Rn
K2CO3/DMF
100 °C
X
Compounds 1-26

Figure 3

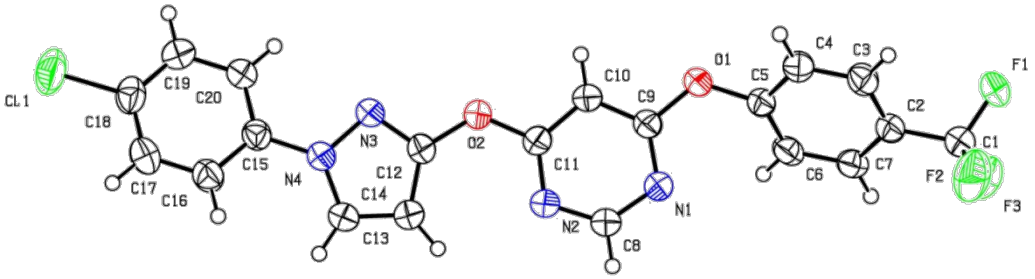

CL1
C18
C19
C20
C15
C17
C16
N4
N3
C12
C14
C13
O2
C11
C10
C9
N2
C8
N1
O1
C5
C4
C3
C2
C1
C7
C6
F1
F2
F3

Figure 4

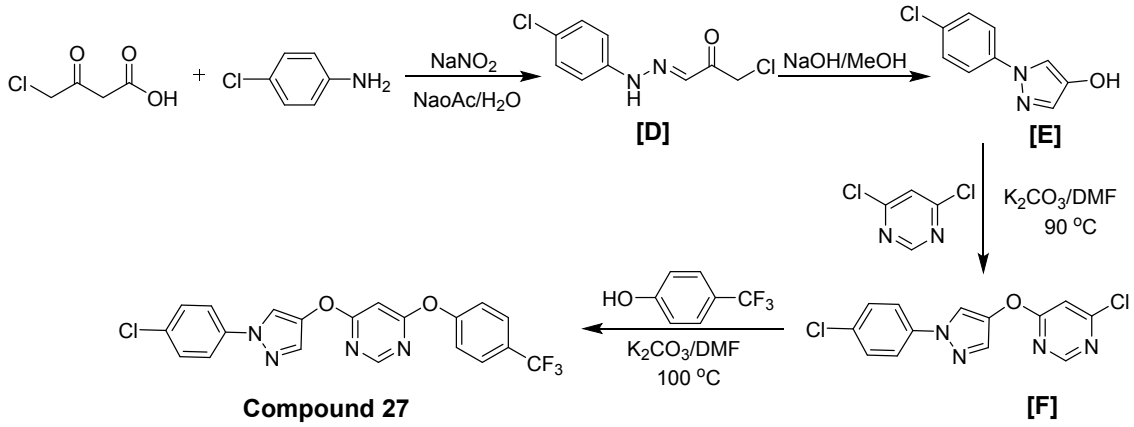
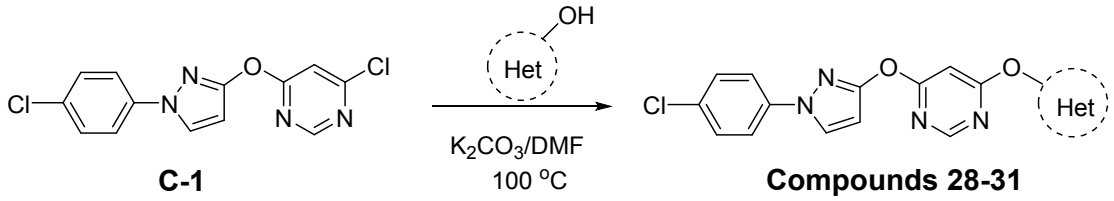
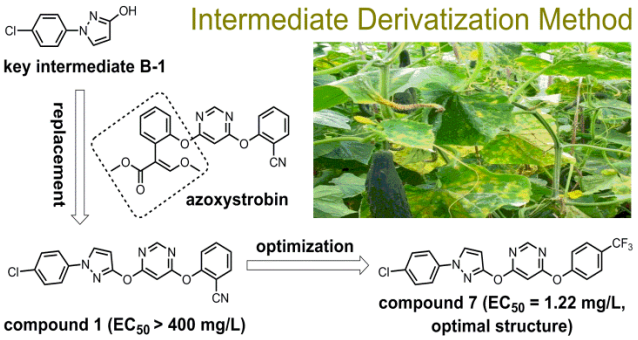

NaNO2
NaoAc/H2O
[D]
NaOH/MeOH
[E]
K2CO3/DMF
90 °C
[F]
HO
CF3
K2CO3/DMF
100 °C
Compound 27

Figure 5



C-1 **Compounds 28-31**

TOC graphic


Intermediate Derivatization Method
key intermediate B-1
replacement
azoxystrobin
optimization
compound 1 (EC_{50} > 400 mg/L)
compound 7 (EC_{50} = 1.22 mg/L, optimal structure)