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Design, Synthesis, and Structure–Activity Relationship of Novel Aniline Derivatives of Chlorothalonil

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Supporting Information

ABSTRACT: Chlorothalonil with both low cost and low toxicity is a popularly used fungicide in the agrochemical field. The presence of nucleophilic groups on this compound allows further chemical modifications to obtain novel chlorothalonil derivatives. Fluazinam, another commercially available agent with a broad fungicidal spectrum, has a scaffold of diaryl amine structure. To mimic this backbone structure, a variety of (un)substituted phenyl amines was used as nucleophilic agents to react with chlorothalonil to obtain compounds with a diphenyl amine structure. Via an elegant design, two leads, 2,4,5-trichloro-6-(2,4-dichlorophenylamino)isophthalonitrile (**7**) and 2,4,5-trichloro-6-(2,4,6-trichlorophenylamino)isophthalonitrile (**11**), with potential fungicidal activity were discovered after a preliminary bioassay screen. These two leads were further modified to obtain final products by replacing the chlorine groups in the phenyl ring in phenyl amine with other functional groups. These functional groups with various electronic properties and spatial characteristics were considered to explore the relationship between structure and fungicidal activity. The results indicate that the electron-withdrawing group NO_2 on the 4 position on the right phenyl ring plays a unique role on enhancing the fungicidal activity. The compounds were identified by proton nuclear magnetic resonance and elemental analysis. Bioassays demonstrated that some of the title compounds exhibited excellent fungicidal activities against cucumber downy mildew at 25 mg/L. Compound **20** has been shown as the optimal structure with 85% control against cucumber downy mildew at 6.25 mg/L concentration. The relationship between structure and fungicidal activity is reported. The present work demonstrates that chlorothalonil derivatives can be used as possible lead compounds for developing novel fungicides.

KEYWORDS: *Chlorothalonil derivatives, intermediate derivatization methods, fungicidal activities, structure–activity relationship*

INTRODUCTION

Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) (Bravo) is a well-established broad spectrum fungicide with $310 M sales in 2011 in the agrochemical field. It is effective against fungal diseases, such as gray mold, early and late blights, leaf spots, anthracnose, fruit rots, rusts, and downy mildews, that threaten numerous vegetable, small fruit, stone fruit, ornamental, turf, and other agricultural crops with both lower manufacturing cost and relatively low toxicity of LD_{50} > 10 000 mg/kg orally of rats.[1] Because of its success in crop protection, a great deal of synthetic work has been performed, aimed at creating various analogues of chlorothalonil.[2−7] Our interest in chlorothalonil analogues was to apply our new agrochemical discovery approach, which we call intermediate derivatization methods, to try to obtain novel fungicidally active compounds.

Intermediate derivatization methods use a three-pronged approach to agrochemical discovery: common intermediate method, terminal group replacement method, and active compound derivatization method.[8−10] Among these three approaches, the terminal group replacement method has been proven as an effective and practical method.[11−16] However, the active compound derivatization method is another strategy with big potential to discover novel biological active compounds. This approach requires further optimization based on existing compounds. Usually, these existing compounds are selected from small molecules available in nature with particularly potential biological properties or those used in known agrochemical and/or pharmaceutical products. Another key feature of these starting compounds is that these small molecules possess chemically active functional groups amenable to derivatization and usually very low cost to cut down the manufacturing cost of the final product. Further, at the very beginning of a project, three major factors, including low toxicity of environmentally friendly intermediates, simple process of preparation, and novel mechanism of action with innovated structure, need to be systematically considered.

Chlorothalonil possesses reactive groups, Cl and CN, which can easily be modified. Thus, chlorothalonil is able to be derivatized by typical organic chemical reactions, such as nucleophile substitution, hydrolysis, and addition reactions. On the basis of these considerations mentioned above, we launched a comprehensive project to contribute our efforts to modify chlorothalonil. As a part of this project, in this paper, we report an interesting result based on modification of one of the Cl atoms on chlorothalonil to mimic the diaryl amine backbone structure of fluazinam, another commercially available agent with broad spectrum fungicidal activity. A variety of aryl amines was used as nucleophilic agents to react with chlorothalonil to obtain compounds with a diaryl amine structure. The detailed

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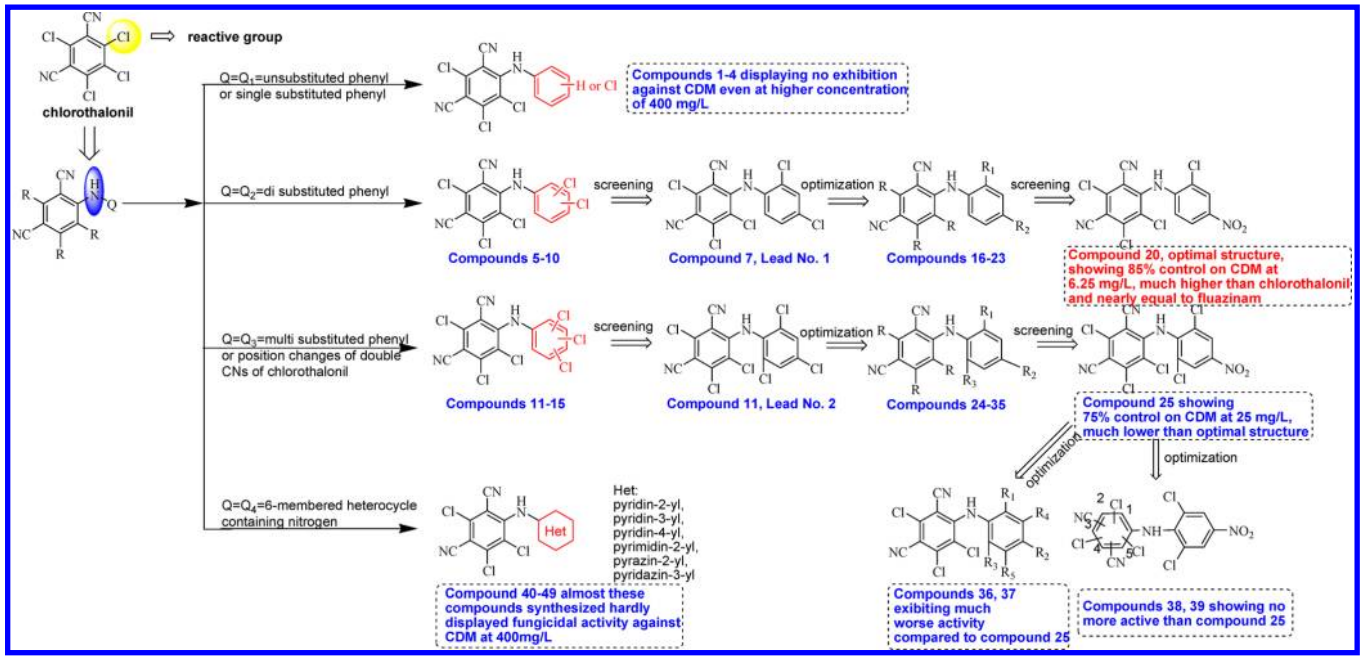

Figure 1. Overview of structure−activity relationships in aniline derivatives of chlorothalonil.

syntheses, bioassays, and structure−activity relationships of these compounds are discussed below.

MATERIALS AND METHODS

All starting materials and reagents were commercially available and used without further purification, except as indicated. Melting points were determined on a Büchi melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded with a Mercury 300 (Varian, 300 MHz) spectrometer with deuterochloroform as the solvent and tetramethylsilane (TMS) as the internal standard. Elemental analyses were determined on a Yanaco MT-3CHN elemental analyzer. All plant and bacteria materials were obtained from the Agrochemical Discovery Department at the Shenyang Research Institute of Chemical Industry.

An overview of the synthesis of aniline derivatives of chlorothalonil and their structure−activity relationships is presented in Figure 1.

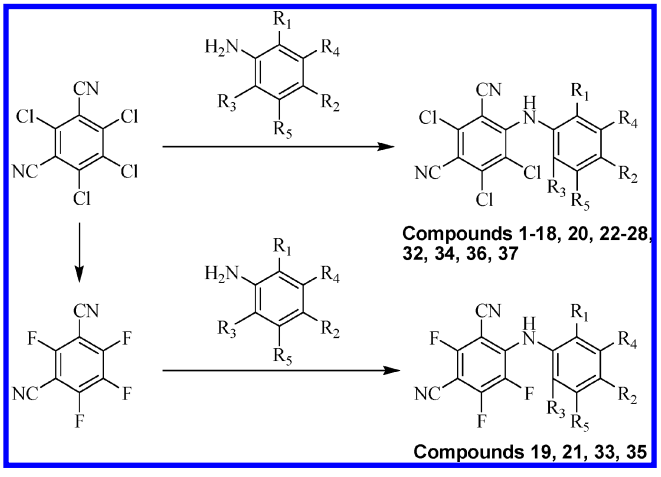

Figure 2. Synthesis of compounds **1**−**28** and **32**−**37**.

The general synthetic methods possessing simple reaction and operation, high yields, and more importantly, very low synthesis cost for compounds **1**−**49** are shown in Figures 2−6. Representative procedures are given below. The yields were not optimized, and each target compound was identified and verified by ^{1}H NMR and elemental analyses.

Synthesis of Target Compounds (1−28 and 32−49).[17−21] *Synthesis of 2,4,5-Trichloro-6-(2-chloro-4-nitrophenylamino)-isophthalonitrile (**20**, the Optimal Compound; Figures 2, 5, and 6) and General Procedure for Compounds **1**−**18**, **22**−**28**, **32**, **34**, and **36**−**49**.* 2-Chloro-4-nitroaniline (1.30 g, 7.5 mmol) was dissolved in 40 mL of *N*,*N*-dimethylformamide (DMF), and sodium hydroxide (0.60 g, 15.0 mmol) was added to the solution. The solution was stirred for 10 min, and 2,4,5,6-tetrachloroisophthalonitrile (chlorothalonil, 1.99 g, 7.5 mmol) was then added. The reaction mixture was stirred at room temperature and monitored by thin-layer chromatography (TLC). After completion of the reaction (5 h), the mixture was added to 100 mL of water and extracted with ethyl acetate (3 × 200 mL). The combined extracts were washed with brine, dried (anhydrous magnesium sulfate), and filtered. The filtrate was evaporated, and the crude product was purified via silica gel column chromatography, using a 1:4 (v/v) mixture of ethyl acetate and petroleum ether (boiling point range of 60−90 °C) as the eluting solvent to obtain compound **20** as a yellow solid: 2.60 g (86%), mp 220−222 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.42 (d, 1H, Ph-3-H, J = 2.7 Hz), 8.20 (dd, 1H, Ph-5-H, ^3J = 9.0 Hz, ^4J = 2.7 Hz), 7.07 (s, 1H, NH), 7.04 (d, 1H, Ph-6-H, J = 8.7 Hz). Anal. Calcd (%) for $C_{14}H_4Cl_4N_4O_2$: C, 41.83; H, 1.00; N, 13.94. Found: C, 41.71; H, 1.05; N, 14.01.

*Synthesis of 4-(2-Chloro-4-(trifluoromethyl)phenylamino)-2,5,6-trifluoroisophthalonitrile (**19**; Figure 2) and General Procedure for Compounds **21**, **33**, and **35**.* Compound **19** was prepared from 2-chloro-4-(trifluoromethyl)aniline and 2,4,5,6-tetrafluoroisophthalonitrile[22,23] using the same procedure as compound **20** as a yellow solid with a yield of 88%, mp 122−124 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.78 (s, 1H, Ph-3-1H), 7.59 (d, J = 9.0 Hz, 1H, Ph-5-1H), 7.24 (d, J = 9.0 Hz, 1H, Ph-6-1H, J = 8.4 Hz), 6.82 (br, 1H, NH). Anal. Calcd (%) for $C_{15}H_4ClF_6N_3$: C, 47.96; H, 1.07; N, 11.19. Found: C, 47.89; H, 1.12; N, 11.22.

Synthesis of 3,5-Dichloro-*N*-(4-chlorophenyl)-4-(2,3,5-trichloro-4,6-dicyanophenylamino)benzamide (30; Figure 3) and General Procedure for Compound 29.[17,21] *Synthesis of 3,5-Dichloro-4-(2,3,5-trichloro-4,6-dicyanophenylamino)benzoic Acid (Intermediate **1**).* Compound **28** (13.31 g, 31.0 mmol) was dissolved in 120 mL of mixture solution of tetrahydrofuran (THF) and water (1:4, v/v), and sodium hydroxide (2.45 g, 61.0 mmol) was added to the solution. The solution was stirred in an oil bath at 50 °C and monitored by TLC. After completion of the reaction (after 5 h),

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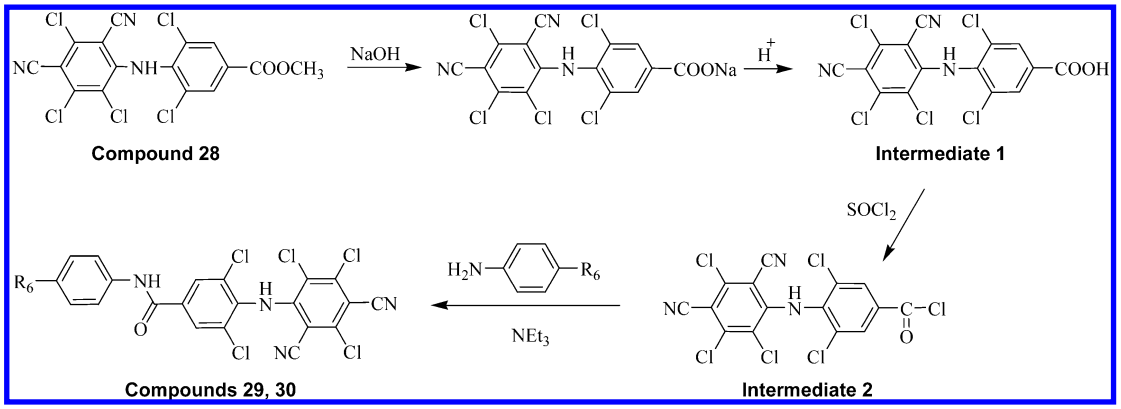

Figure 3. Synthesis of compounds **29** and **30**.

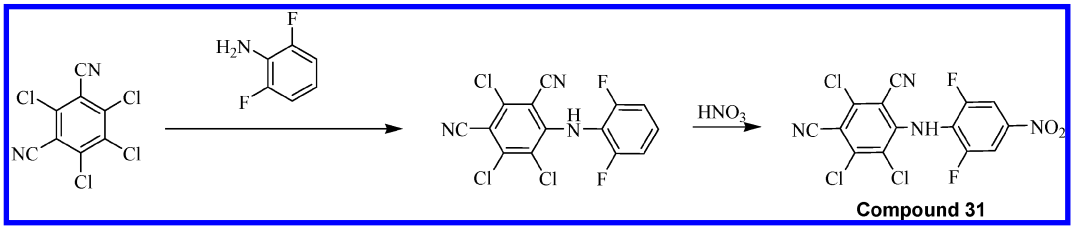

Figure 4. Synthesis of compound **31**.

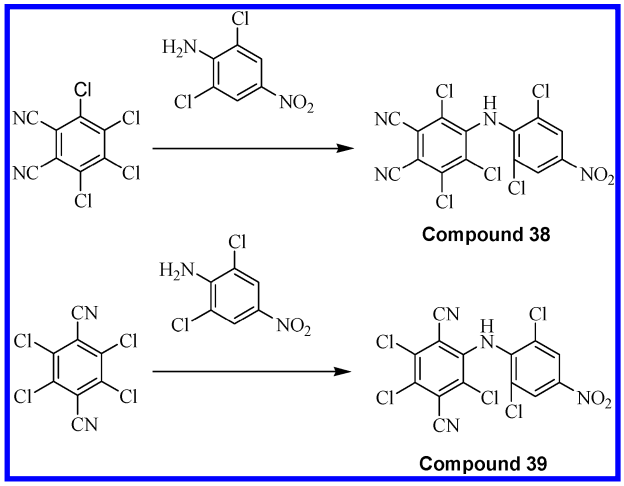

Figure 5. Synthesis of compounds **38** and **39**.

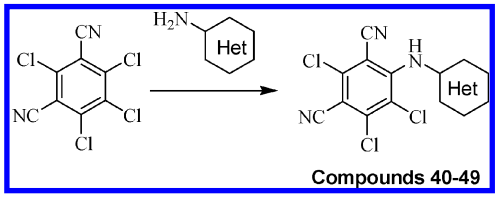

Figure 6. Synthesis of compounds **40**–**49**.

the mixture was added to 500 mL of water and extracted with ethyl acetate (3 × 500 mL). The pH of the aqueous phase was adjusted to 5–6 with dilute hydrochloric acid, and the solid was filtered (intermediate **1**) and used without further purification.

*Synthesis of 3,5-Dichloro-4-(2,3,5-trichloro-4,6-dicyanophenylamino)-benzoyl Chloride (Intermediate **2**).* Intermediate **1** (5.54 g, 12.72 mmol) was dissolved in 100 mL of petroleum ether followed by 2 drops of DMF, and then sulfurous dichloride (2.27 g, 19.08 mmol) was added to the solution. The solution was refluxed in an oil bath at 85 °C and monitored by TLC. After completion of the reaction (2 h), the mixture was evaporated under reduced pressure to obtain intermediate **2**, which was used without further purification.

*Synthesis of 3,5-Dichloro-N-(4-chlorophenyl)-4-(2,3,5-trichloro-4,6-dicyanophenylamino)benzamide (**30**).* Intermediate **2** (0.40 g, 0.91 mmol) was added to a solution of 4-chloroaniline (0.12 g, 0.909 mmol) and triethylamine (0.10 g, 1.00 mmol) in 50 mL of THF, and the reaction mixture was stirred in an oil bath at 45 °C for 5 h. After reaction completion (TLC), the reaction mixture was poured into 30 mL of saturated brine and extracted with ethyl acetate, and the extract was dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified on a silica gel column [ethyl acetate/petroleum ether (boiling point range of 60–90 °C) = 1:3, as an eluent] to give compound **30** as a white solid: 0.46 g (92% based on intermediate **2**), mp 275–276 °C.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 10.50 (d, 1H, CONH, J = 12.9 Hz), 8.13 (dd, 2H, Ph-2,6-2H, ^3J = 15.7 Hz, ^4J = 1.2 Hz), 7.81 (d, 2H, 4-Cl-Ph-3,5-2H, J = 9.0 Hz), 7.31–7.35 (m, 2H, 4-Cl-Ph-2,6-2H). Anal. Calcd (%) for $C_{21}H_8Cl_6N_4O$: C, 46.28; H, 1.48; N, 10.28. Found: C, 46.33; H, 1.41; N, 10.26.

Synthesis of 2,4,5-Trichloro-6-(2,6-difluoro-4-nitrophenylamino)isophthalonitrile (31; Figure 4).[17,21] Intermediate 2,4,5-trichloro-6-(2,6-difluorophenylamino)isophthalonitrile was prepared from 2,6-difluoroaniline and 2,4,5,6-tetrachloroisophthalonitrile using the same procedure as compound **20** as a yellow solid with a yield of 83%.

To the mixture of 2,4,5-trichloro-6-(2,6-difluorophenylamino)isophthalonitrile (0.68 g, 2.0 mmol) in concentrated sulfuric acid (20 mL) was added fuming nitric acid (d = 1.52, 10 mL) dropwise for 20 min with sufficient stirring. After stirring at room temperature for 1 h, the reaction mixture was poured into ice water, and the resulting precipitate was collected by filtration and washed with water to obtain compound **31** as a pale white solid, mp 204–206 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.97–8.01 (dd, 2H, Ph-3,5-2H, ^3J = 10.8 Hz, ^4J = 3.0 Hz), 6.70 (s, 1H, NH). Anal. Calcd (%) for $C_{14}H_3Cl_3F_2N_4O_2$: C, 41.67; H, 0.75; N, 13.88. Found: C, 41.71; H, 0.81; N, 13.81.

Fungicidal Assay. Each of the test compounds (4 mg) was first dissolved in 5 mL of mixture of acetone and methanol (1:1 by volume), and then 5 mL of water containing 0.1% Tween 80 was added to generate a 10 mL stock solution of 400 mg/L concentration. Serial test solutions were prepared by diluting the above solution (testing range of 6.25–400 mg/L).

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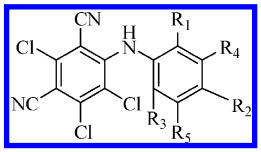
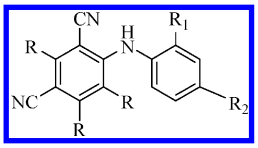
Table 1. Chemical Structures and Fungicidal Activity of Aniline Derivatives of Chlorothalonil (Compounds 1−15)



						biological activity against CDM (% control at the given concentration in mg/L)					
compound	R_1	R_2	R_3	R_4	R_5	400	100	50	25	12.5	6.25
1	H	H	H	H	H	0	*a*	*a*	*a*	*a*	*a*
2	Cl	H	H	H	H	0	*a*	*a*	*a*	*a*	*a*
3	H	H	H	Cl	H	0	*a*	*a*	*a*	*a*	*a*
4	H	Cl	H	H	H	0	*a*	*a*	*a*	*a*	*a*
5	Cl	H	H	Cl	H	100	35	0	*a*	*a*	*a*
6	Cl	H	Cl	H	H	80	*a*	*a*	*a*	*a*	*a*
7 lead number 1	Cl	Cl	H	H	H	100	100	20	*a*	*a*	*a*
8	H	H	H	Cl	Cl	60	*a*	*a*	*a*	*a*	*a*
9	Cl	H	H	H	Cl	100	0	*a*	*a*	*a*	*a*
10	H	Cl	H	Cl	H	85	*a*	*a*	*a*	*a*	*a*
11 lead number 2	Cl	Cl	Cl	H	H	98	95	75	15	*a*	
12	Cl	Cl	H	Cl	H	80	*a*	*a*	*a*	*a*	
13	CH_3	CH_3	CH_3	H	H	80	*a*	*a*	*a*	*a*	
14	Cl	Cl	H	H	Cl	100	0	*a*	*a*	*a*	
15	H	Cl	H	Cl	Cl	85	*a*	*a*	*a*	*a*	
chlorothalonil						100	100	80	30	*a*	*a*
fluazinam						100	100	100	100	100	90

[a]No data.

Table 2. Chemical Structures and Fungicidal Activity of Aniline Derivatives of Chlorothalonil (Compounds 16−23)



				biological activity against CDM (% control at the given concentration in mg/L)					
compound	R	R_1	R_2	400	100	50	25	12.5	6.25
7 lead number 1	Cl	Cl	Cl	100	100	20	*a*	*a*	*a*
16	Cl	CH_3	Cl	100	0	*a*	*a*	*a*	*a*
17	Cl	NO_2	Cl	100	70	30	10	*a*	*a*
18	Cl	Cl	CF_3	100	100	98	80	20	*a*
19	F	Cl	CF_3	100	100	98	80	*a*	*a*
20 optimal structure	Cl	Cl	NO_2	100	100	98	98	98	85
21	F	Cl	NO_2	100	95	80	40	*a*	*a*
22	Cl	NO_2	NO_2	100	95	80	60	*a*	*a*
23	Cl	CN	NO_2	100	*a*	*a*	*a*	*a*	*a*
chlorothalonil				100	100	80	30	*a*	
fluazinam				100	100	100	100	100	90

[a]No data.

Evaluations of fungicidal activities of the synthesized compounds against cucumber downy mildew (CDM) were performed as follows: Briefly, a whole plant is used in this test, and the testing solution is sprayed to the host plant by a special plant sprayer. The plant is inoculated with fungus after 24 h. According to the infecting characteristics of fungus, the plant is stored in a humidity chamber and then transferred into a greenhouse after infection is finished. The other plants are placed in a greenhouse directly. The activity of each compound was estimated by visual inspection after 7 days, and screening results were reported as a range from 0% (no control) to 100% (complete control).

The test results of the fungicidal activities of compounds **1**−**49** against CDM are listed in Tables 1−5.

RESULTS AND DISCUSSION

Synthesis. According to the schemes shown in Figures 2−6, 49 title compounds were synthesized with yields of 50−95%, as shown in Tables 1−5. The synthesized compounds were characterized by ^{1}H NMR and elemental analyses. All spectral and analytical data were consistent with the assigned structures.

Structure−Activity Relationship (SAR). *Discovery of Lead Compounds.* Initially, chlorothalonil was reacted with

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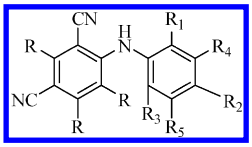
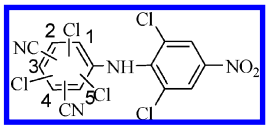
Table 3. Chemical Structures and Fungicidal Activity of Aniline Derivatives of Chlorothalonil (Compounds 24−37)



compound	R	R_1	R_2	R_3	R_4	R_5	biological activity against CDM (% control at the given concentration in mg/L) 400	100	50	25	12.5	6.25
11 lead number 2	Cl	Cl	Cl	Cl	H	H	98	95	75	15	*a*	*a*
24	Cl	Cl	Br	Cl	H	H	100	70	40	20	*a*	*a*
25	Cl	Cl	NO_2	Cl	H	H	100	100	100	75	*a*	*a*
26	Cl	Cl	CF_3	Cl	H	H	50	*a*	*a*	*a*	*a*	*a*
27	Cl	Br	OCF_3	Br	H	H	85	*a*	*a*	*a*	*a*	
28	Cl	Cl	CO_2CH_3	Cl	H	H	20	*a*	*a*	*a*	*a*	
29	Cl	Cl	CONHPh	Cl	H	H	98	0	*a*	*a*	*a*	
30	Cl	Cl	CONH(4-Cl-Ph)	Cl	H	H	55	*a*	*a*	*a*	*a*	
31	Cl	F	NO_2	F	H	H	100	100	100	98	70	40
32	Cl	Cl	NO_2	F	H	H	100	100	98	95	50	40
33	F	Cl	NO_2	F	H	H	100	95	70	30	*a*	*a*
34	Cl	Br	NO_2	Br	H	H	100	100	100	100	30	*a*
35	F	Br	NO_2	Br	H	H	100	98	90	85	*a*	*a*
36	Cl	CH_3	NO_2	NO_2	Cl	H	100	100	0	*a*	*a*	*a*
37	Cl	Cl	CN	CN	Cl	Cl	100	0	*a*	*a*	*a*	*a*
20 optimal structure	Cl	Cl	NO_2	H	H	H	100	100	98	98	98	85

[a]No data.

Table 4. Chemical Structures, Physical Properties. and Fungicidal Activity of Cyano Isomers of Compound 25 (Compounds 38 and 39)



compound	position of double CNs	biological activity against CDM (% control at the given concentration in mg/L) 400	100	50	25	12.5
38	2,3 position	100	100	95	90	0
39	1,4 position	100	100	100	100	20
25	1,3 position	100	100	100	75	*a*
20 optimal structure		100	100	98	98	98

[a]No data.

aniline to give compound **1**, which did not show any control of CDM (Table 1). Then, 9 compounds (compounds **2**−**10**) were synthesized to evaluate the effect of the substituent position of R_1, R_2, R_3, R_4, and R_5 on fungicidal activity using the chlorine atoms as probes. When a single chlorine was introduced into any position of the right phenyl ring (compounds **2**−**4**), there was no improvement in fungicidal activity. Next, we synthesized dichloro (compounds **5**−**10**) and trichloro (compounds **11**−**15**) analogues. Fungicidal assays identified two lead compounds, compound **7** with 2,4-Cl_2 substituents (R_1 = R_2 = Cl, and R_3 = R_4 = R_5 = H) and compound **11** with 2,4,6-Cl_3 substituents on the right phenyl ring, which displayed 100 and 95% control, respectively, against CDM at 100 mg/L. Although when screened at the lower concentration of 50 mg/L, compounds **7** and **11** showed lower fungicidal activity than the commercial fungicide chlorothalonil (20 and 75%, respectively, versus 80% for chlorothalonil), we were encouraged to further modify these two leads to obtain compounds with better fungicidal activity. More importantly, we obtained very useful structure−activity information based on the preliminary results; namely, substitution at the 2,4 and 2,4,6 positions of the right phenyl ring may be key to improving fungicidal activity of the whole molecule compared to other substituted positions. We next considered varying the electronic properties and spatial characteristics of the substituent groups at these positions. These changes are described below, and data are presented in Tables 2 and 3.

Optimization of Compound 7. Using compound **7** as a starting point for additional analogues, we turned our attention to replacing the Cl atoms on the 2 and 4 positions of the right side phenyl ring with other electron-donating groups, such as CH_3, and/or electron-withdrawing groups, such as CO_2CH_3, CF_3, NO_2, and CN (Table 2). First, we varied substituents on the 2 position and kept the 4 position fixed as Cl. We synthesized two compounds **16** with an electron-donating group (CH_3) and **17** with an electron-withdrawing group (NO_2), respectively. The bioassay results showed that compound **16** was less efficacious than lead compound **7** (0 versus 100% at 100 mg/L), indicating that the electron-donating group has a negative effect on bioactivity. In contrast, the fungicidal activity of compound **17** increased moderately compared to the lead compound **7** (30 versus 20% at 50 mg/L), implying that the electron-withdrawing group is helpful for enhancing activity. Then, we varied the 4 position and kept the 2 position fixes as Cl. We synthesized two compounds with electron-withdrawing groups at the 4 position, compound **18** with a CF_3 group and compound **20** with a NO_2 group (Table 2). To our surprise, compound **18** exhibited 80% control at 25 mg/L, and compound **20** gave 98% control at 25 mg/L and 85% control at 6.25 mg/L. Both of these compounds were more efficacious than chlorothalonil, which only showed 30% control of CDM at 25 mg/L. Furthermore, to investigate if the fungicidal activity

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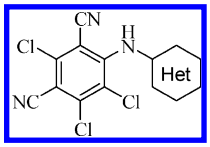
Table 5. Chemical Structures and Fungicidal Activity of Heterocyclic Amine Derivatives of Chlorothalonil (Compounds 40−49)



		biological activity against CDM (% control at the given concentration in mg/L)					
compound	Het	400	100	50	25	12.5	6.25
40	pyridin-2-yl	0	*a*	*a*	*a*	*a*	*a*
41	5-Br-pyridin-2-yl	30	*a*	*a*	*a*	*a*	*a*
42	pyridin-3-yl	0	*a*	*a*	*a*	*a*	*a*
43	6-Br-pyridin-3-yl	80	*a*	*a*	*a*	*a*	*a*
44	2-Cl-pyridin-4-yl	90	80	30	10	*a*	*a*
45	pyrimidin-2-yl	0	*a*	*a*	*a*	*a*	*a*
46	4,6-2CH_3-pyrimidin-2-yl	0	*a*	*a*	*a*	*a*	*a*
47	4,6-2OCH_3-pyrimidin-2-yl	0	*a*	*a*	*a*	*a*	*a*
48	6-Cl-pyrazin-2-yl	98	0	*a*	*a*	*a*	*a*
49	6-Cl-pyridazin-3-yl	100	0	*a*	*a*	*a*	*a*

[a]No data.

could be improved further when both 2- and 4-Cl atoms were substituted by electron-withdrawing groups, compounds (compound **22** with 2-NO_2 and 4-NO_2 and compound **23** with 2-CN and 4-NO_2) were designed and synthesized. However, the fungicidal activity results showed that neither compound was as effective as the lead compound **20**. The result of the optimization of compound **7** is identification of compound **20** with a 2-Cl-4-NO_2 group as the optimized structure with greatly improved fungicidal activity.

Optimization of Compound 11. Using compound **11** as a starting point for additional analogues, we turned our attention to replacing the Cl atoms on the 2, 4, and 6 positions of the right side phenyl ring (Table 3). First, we varied substituents on the 4 position while maintaining the 2 and 6 positions as Cl or Br. These substituents are typical groups with both electronic effect and spatial effect simultaneously. While the 4-Br analogue (compound **24**) showed lower fungicidal activity than lead compound **11** (40 versus 75%, respectively, at 50 mg/L), the NO_2 analogue (compound **25**) gave improved activity (100 versus 75%, respectively, at 50 mg/L). At a 25 mg/L dose, a larger difference in activity between compounds **25** and **11** was observed (75 versus 15%, respectively). However, when the 4-Cl atom of compound **11** was replaced with CF_3 (compound **26**), OCF_3 (compound **27**), or CO_2CH_3 (compound **28**), weaker fungicidal activity resulted. On the basis of these observations, the electron-withdrawing group NO_2 rather than CF_3, OCF_3, and CO_2CH_3 plays an important role in enhancing the fungicidal activity. It was a surprising result that CF_3 and OCF_3 at the 4 position did not exhibit excellent bioactivity because it has been shown that CF_3 usually provides a very strong positive contribution to biological activities.[24] In our case, this may be because the 4 postion is not an optimum location for these fluorine-containing groups. Furthermore, when large spatial groups, such as CONHPh and CONH(4-Cl-Ph), were induced into the 4 position of lead compound **11**, compounds **29** and **30** exhibited reduced effect compared to the lead compound **11** as well. A possible explanation for the lower activity associated with large subsitutuents is that large substituents would block the interaction of the target enzyme and these bulky compounds.

Considering that many fluorine-containing compounds exhibit significant agricultural bioactivities, owing to the unique properties of the fluorine atom, such as high thermal stability and lipophilicity,[25] further optimization was conducted by introducing fluorine into the 2 and/or 6 position with the 4 position fixed as NO_2 because the NO_2 group was demonstrated to be beneficial in compound **25**. To our excitement, as we expected, these two compounds (**31** with 2,6-F_2 and **32** with 2-Cl-6-F) had much higher activity than compound **25** (2,6-Cl_2). Compound **31** gave 98% control at 25 mg/L compared to 75% control shown by compound **25**, while compound **32** gave 95% control. When the 2,6-Cl_2 atoms in compound **11** were replaced by Br atoms (compound **34**), fungicidal activity was similar to compounds **31** and **32**. With continuing interest to induce fluorine into the lead compound, we turned our attention to replacing all Cl atoms of the left side ring with F atoms, resulting in compounds **33** and **35** (Table 3). However, these two compounds showed lower fungicidal activity than their corresponding Cl analogues. The results suggest that it is not always true that the more fluorine atoms, the better bioactivity. Further, the position of the fluorine atom in the intact molecule may also play a crucial role in its bioactivity.

Finally, two compounds **36** and **37** were prepared to evaluate the effect of including additional substituents in the right side ring on fungicidal activity (Table 3). The results show that neither compound **36** nor compound **37** was as efficacious as lead compound **25**. The result of the optimization of compound **11** is identification of compound **31** with a 2,6-F_2-4-NO_2 group as the optimized structure with greatly improved fungicidal activity.

Activity of Cyano Isomers of Compound 25. To determine if the cyano isomers of compound **25** would show improved fungicidal activity, we synthesized and screened compounds **38** and **39** (Table 4). Both compounds **38**, which gave 90% control at 25 mg/L, and **39**, which showed 100% control at 25 mg/L, were more efficacious than compound **25**, which gave 75% control at 25 mg/L.

Activity of Heterocyclic Amino Analogues of Chlorothalonil. To determine if replacing the right side phenyl ring with a nitrogen heterocycle would enhance fungicidal activity in this class of compounds, we prepared a series of 10 heterocyclic amino analogues of chlorothalonil, which were derived from pyridine, pyrimidine, and pyrazine heterocycles (Table 5).

The only analogue that showed any appreciable activity was compound **44**, in which the heterocyclic group was 2-Cl-pyridin-4-yl. The poor results from these analogues suggest that introduction of nitrogen in the aromatic ring was detrimental to the fungicidal activity in this class of compounds.

On the basis of data presented in Tables 1−5, a clear-cut, well-defined relationship between the chemical structure and biological activity has taken shape by examining the effect of different kinds of electron-withdrawing, electron-donating, and spatially demanding groups on the fungicidal activity of aniline derivatives of chlorothalonil. 2,4-Disubstituted aniline derivatives, especially compound **20**, which possesses a 2-chloro-4-nitro substitution in the right side phenyl ring, showed improved fungicidal activity compared to chlorothalonil. 2,4,6-Trisubstituted aniline derivatives, especially compound **25**, which possesses a 2,6-Cl_2-4-NO_2 substitution in the right side phenyl ring, were more efficacious than chlorothalonil. Cyano isomers of compound **25**, namely, compounds **38** and **39**, were slightly more efficacious than compound **25**, while nitrogen heterocyclic amine derivatives were very weak or inactive.

From what has been discussed above, we can draw the conclusion that compound **20** derived from chlorothalonil with a simple process and relative low cost, is the optimal structure with desired activity. It offers a control of 85% against CDM at 6.25 mg/L concentration, much higher than chlorothalonil and nearly equal to fluazinam. Compound **20**, which has also shown activity against rice blast and gray mold, besides CDM,[21] is a promising candidate for further development. This study demonstrates the effectiveness of our intermediate derivatization method approach to the discovery of bioactive compounds. Further synthesis of analogues, structure optimization studies, and field trials of compound **20** are in progress.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H NMR and melting point data for compounds **2**, **3**, **5**−**18**, **21**−**29**, and **32**−**49**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ REFERENCES

(1) British Columbia Ministry of Agriculture, Food and Fisheries. *Pesticide Info: Chlorothalonil*; British Columbia Ministry of Agriculture, Food and Fisheries: Vancouver, British Columbia, Canada, 2004; http://www.agf.gov.bc.ca/pesticides/infosheets/chlorothalonil.pdf.

(2) Yan, S. J; Huang, C.; Su, C. X.; Ni, Y. F.; Lin, J. Facile route to 1,3-diazaheterocycle-fused [1,2*b*]isoquinolin-1(2*H*)-one derivatives via substitution−cyclization reactions. *J. Comb. Chem.* **2010**, *12*, 91−94.

(3) Yan, S. J; Huang, C.; Zeng, X. H.; Huang, R.; Lin, J. Solvent-free, microwave assisted synthesis of polyhalo heterocyclic ketene aminals as novel anti-cancer agents. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 48−51.

(4) Yan, S. J; Zheng, H.; Huang, C.; Yan, Y. Y.; Lin, J. Synthesis of highly functionalized 2,4-diaminoquinazolines as anticancer and anti-HIV agents. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4432−4435.

(5) Hrenn, H.; Schwack, W.; Seilmeier, W.; Wieser, H. Synthesis of a chlorothalonil peptide conjugate mimicking protein-bound pesticide residues. *Tetrahedron Lett.* **2003**, *44*, 1911−1913.

(6) Kontrec, D.; Vinkovic, V.; Sunjic, V. Preparation and evaluation of chiral stationary phases based on *N*,*N*-2,4-(or 4,6)-disubstituted 4,5-(or 2,5)-dichloro-1,3-dicyanobenzene. *Chirality* **2000**, *12*, 63−70.

(7) Shi, L. P.; Jiang, K. M.; Jiang, J. J.; Jin, Y.; Tao, Y. H.; Li, K.; Wang, X. H.; Lin, J. Synthesis and antimicrobial activity of polyhalobenzonitrile quinazolin-4(3*H*)-one derivatives. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 5958−5963.

(8) 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.

(9) Liu, C. L. Innovation of pesticide discovery methods and candidate compounds. *High-technol. Ind.* **2008**, *9*, 79−81.

(10) Liu, C. L. Approach and application of novel agrochemical innovation. *Chin. J. Pestic.* **2011**, *50*, 20−23.

(11) Guan, A. Y.; Liu, C. L.; Li, M.; Zhang, H.; Li, Z. N.; Li, Z. M. Design, synthesis and structure−activity relationship of novel coumarin derivatives. *Pest Manage. Sci.* **2011**, *67*, 647−655.

(12) Chai, B. S.; Liu, C. L.; Li, H. C.; He, X. M.; Luo, Y. M.; Huang, G.; Zhang, H.; Chang, J. B. Design, synthesis and acaricidal activity of novel strobilurin derivatives containing pyrimidine moieties. *Pest Manage. Sci.* **2010**, *66*, 1208−1214.

(13) Chai, B. S.; Liu, C. L.; Li, H. C.; Zhang, H.; Liu, S. W.; Huang, G.; Chang, J. B. The discovery of SYP-10913 and SYP-11277: Novel strobilurin acaricides. *Pest Manage. Sci.* **2011**, *67*, 1141−1146.

(14) Li, M.; Liu, C. L.; Li, L.; Yang, H.; Li, Z. N.; Zhang, H.; Li, Z. M. Design, synthesis and biological activities of new strobilurin derivatives containing substituted pyrazoles. *Pest Manage. Sci.* **2010**, *66*, 107−112.

(15) Li, M.; Liu, C. L.; Zhang, J.; Wu, Q.; Hao, S. L.; Song, Y. Q. Design, synthesis and structure−activity relationship of novel insecticidal dichloro-allyloxy-phenol derivatives containing substituted pyrazol-3-ols. *Pest Manage. Sci.* **2013**, *69*, 635−641.

(16) Guan, A. Y.; Liu, C. L.; Li, Z. N.; Zhang, M. X. Synthesis and bioactivity of novel coumarin derivatives containing (*E*)-methyl 2-(methoxyimino)-2-phenylacetates. *Chin. Chem. Lett.* **2011**, *22*, 663−666.

(17) Liu, C. L.; Huang, G.; Lan, J.; Li, H. C.; Li, Z. N.; Hao, S. L.; Guan, A. Y.; Cui, D. L. Cyano-diphenylamine-containing compounds and applications thereof. CN 102827032 A, 2012.

(18) Li, H. C.; Huang, G.; Li, Z. N.; Guan, A. Y.; Lan, J.; Hao, S. L.; Liu, C. L. Compound containing aminobenzonitrile and application thereof. CN 102827071 A, 2012.

(19) Hao, S. L.; Jiang, A. R.; Li, Z. N.; Li, H. C.; Wang, J. K.; Guan, A. Y.; Liang, B.; Song, Y. Q.; Liu, C. L. Compound containing *o*-dicyanoaniline and application thereof. CN 102827033 A, 2012.

(20) Hao, S. L.; Guan, A. Y.; Lan, J.; Jiang, A. R.; Li, Z. N.; Li, H. C.; Liang, B.; Cui, D. L.; Song, Y. Q.; Liu, C. L. *p*-Dicyanoaniline-containing compounds and applications thereof. CN 102827034 A, 2012.

(21) Liu, C. L.; Huang, G.; Lan, J.; Hao, S. L.; Li, Z. N.; Li, H. C.; Guan, A. Y.; Jiang, A. R.; Xu, Y. Substituted cyanoaniline compounds, preparation and use thereof. WO 2012171484 A1, 2012.

(22) Lin, J.; Xu, R.; Mao, D. S.; Yan, S. J.; Yang, L. J.; Liu, F. C. Novel benzoylurea compound and use thereof. CN 1465563 A, 2004.

(23) Lin, J.; Yan, S. J.; Mao, D. S.; Xu, R.; Yang, L. J.; Liu, F. C. Synthesis of novel benzoylphenylurea chitin inhibitors from chlorothalonil. *Chin. Chem. Lett.* **2003**, *14*, 1219−1222.

(24) Filler, R.; Kobayashi, Y. *Biomedical Aspects of Fluorine Chemistry*; Elsevier Biomedical Press: Amsterdam, Netherlands, 1986.
(25) Hudlicky, M. *Chemistry of Organic Fluorine Compounds II*; American Chemical Society (ACS): Washington, D.C., 1995; pp 979, 1145.