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Article

Design, Synthesis and Biological Activities of Novel *N*-Aryl-1*H*-pyrazole-5-carboxylate Derivatives

ZHANG Xiulan[1*], ZHANG Huan[1], MA Jinlong[2], YU Shujing[2] and LI Zhengming[2*]

1. College of Science, Tianjin University of Science & Technology, Tianjin 300457, P. R. China;

2. State Key Laboratory of Elemento-Organic Chemistry, Institute of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, P. R. China

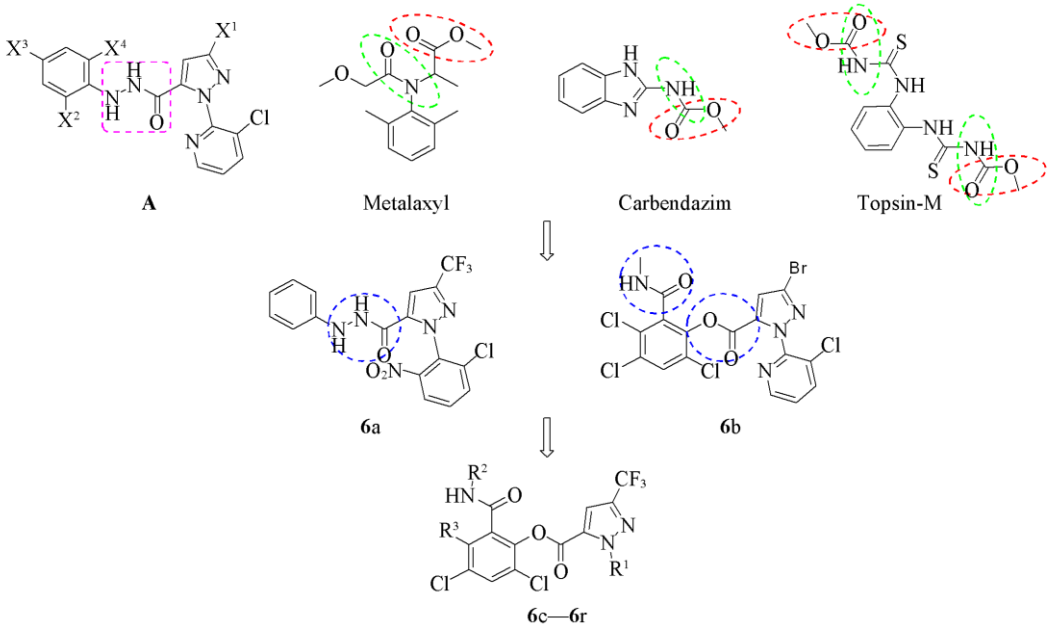
Abstract In an attempt to search for potent fungicide, a series of novel *N*-aryl-1*H*-pyrazole-5-carboxylate derivatives was designed and synthesized. Their chemical structures were characterized by ^{1}H NMR spectra and high resolution mass spectrometry(HRMS). The preliminary bioassay results indicated that some target compounds displayed better fungicidal activities against certain fungi at 50 μg/mL or favorable antitumor activities at 5 μg/mL compared with chlorothalonil and 5-fluorouracil, respectively. The structure-activity relationship demonstrated that the introduction of ester group and amide bond was favorable to the improvement of activities against *Physalospora piricola* and *Phytophthora capsici*.

Keywords *N*-Aryl-1*H*-pyrazole-5-carboxylate; Fungicidal activity; Antitumor activity

1 Introduction

Plant pathogenic fungi are commonly found in the environment. Twenty thousand different fungal species need to live on plant hosts to extend their life cycle, which brings serious harm to agricultural production. Fungicides have the characteristics of fast response, high efficiency, low cost and easy to use, so the main step of preventing and controlling crop diseases is still using fungicides. Today in China, there are some problems existing in this field, such as old fungicides, increasing resistance and high residue, so it needs to constantly develop fungicides with new mechanism and independent intellectual property rights.

Nitrogenous heterocyclic compounds with unique structural characteristic, physiological function and high activity have been widely used in the synthesis of medicine and pesticide. Pyrazole derivatives especially have become a research hot spot in the field of agricultural chemicals due to their good herbicidal activity[1—2], insecticidal activity[3—5], fungicidal activity[6—8], antiviral activity[9—10], antitumor activity[11—13] and so on. It was reported that *N*-pyridyl-1*H*-pyrazole-carbohydrazide derivatives **A**[14](Scheme 1) exhibited good



Scheme 1 Design strategy of target compounds

*Corresponding authors. Email: nkzml@vip.163.com; zxl840327@163.com

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inhibitory activity against some fungi at 50 μg/mL. Further optimization of the structure is expected to obtain potent fungicide. It is an effective method to optimize lead compound according to the strategy of bioisosterism[15]. The high electronegativity of nitrogen atom in pyridine ring in compounds **A** would induce the π electron to move toward N[16]. It was postulated that nitrobenzene and pyridine ring may have similar properties and the introduction of nitrobenzene was likely to provide the molecular diversity while maintaining the biological activity. 2-Chloro-6-nitrophenyl-1*H*-pyrazole-5-carbohydrazide(**6**a) was synthesized firstly by replacing pyridine ring with nitrobenzene. Meanwhile, many fungicides contain both ester group and amide bond, such as metalaxyl, carbendazim and topsin-m(Scheme 1). It was speculated that the introduction of ester group and amide bond to the structure of compound **A** may also help improve the activity. Then, *N*-pyridyl-1*H*-pyrazole-carboxylate(**6**b) was obtained. The preliminary bioassay indicated that they had moderate or good fungicidal activity. Encouraged by this discovery, the group further optimized the structure in the next step. By combining the structures of compounds **6**a and **6**b, *N*-2-chloro-6-nitrophenyl-1*H*-pyrazole-5-carboxylate compounds **6**c—**6**l were designed and synthesized. Then, compounds **6**m—**6**p containing 2-chloro-4-nitrophenyl group were synthesized to identify the effect of the position of nitro in phenylpyrazole moiety on fungicidal activity. To study the effect of chlorine atom on fungicidal activity, compounds **6**q—**6**r containing 2,4-dinitrophenyl group in absence of chlorine atom were synthesized. All the target compounds were evaluated for their fungicidal activities and antitumor activities.

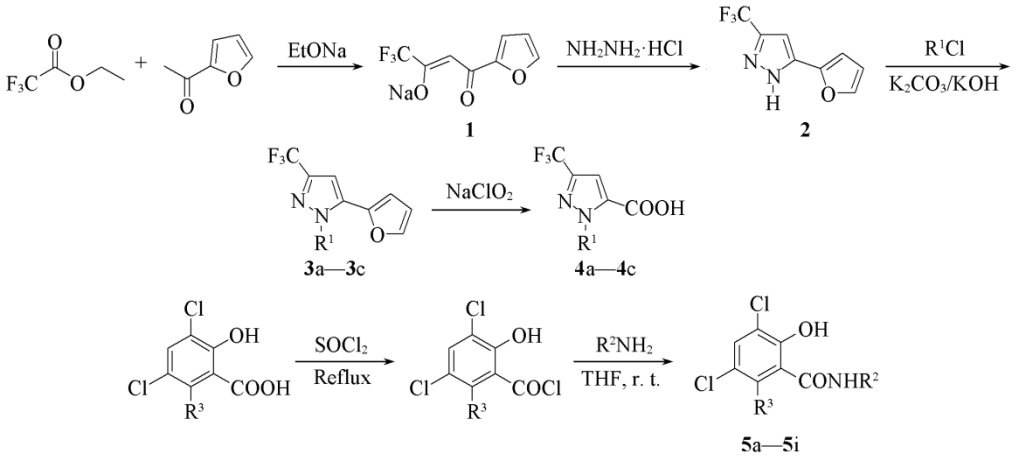
2 Experimental

2.1 Instruments and Materials

^{1}H NMR spectra were recorded on a Bruker 300 or 400 MHz nuclear magnetic resonance spectrometer in $CDCl_3$ or DMSO-d_6 solution with tetramethylsilane(TMS) as internal standard. High resolution mass spectrometry(HRMS) data were obtained on a Varian quantum field theory electrospray ionization(QFT-ESI) instrument. The melting points were determined on an X-4 binocular microscope melting point apparatus and were uncorrected. All the reagents were of analytical grade and obtained from commercial suppliers and treated with conventional methods.

2.2 General Synthetic Procedure for Intermediates 1—5

The synthetic route of intermediates is shown in Scheme **2**. Intermediates **1**—**4** were synthesized according to previous work[17,18] and intermediates **5**a—**5**j were prepared according to the literature[19].



R^1=2-chloro-6-nitrophenyl(A); 2-chloro-4-nitrophenyl(B); 2,4-dinitrophenyl(C). **3**a, **4**a: R^1=A; **3**b, **4**b: R^1=B; **3**c, **4**c: R^1=C; **5**a: R^2=Me, R^3=Cl; **5**b: R^2=*i*-Pr, R^3=Cl; **5**c: R^2=cyclopropyl, R^3=Cl; **5**d: R^2=*t*-Bu, R^3=Cl; **5**e: R^2=cyclohexyl, R^3=Cl; **5**f: R^2=Me, R^3=H; **5**j: R^2=*i*-Pr, R^3=H; **5**h: R^2=cyclopropyl, R^3=H; **5**i: R^2=*t*-Bu, R^3=H; **5**j: R^2=cyclohexyl, R^3=H.

Scheme 2 Synthesis of intermediates 1—5

2,3,5-Trichloro-6-hydroxy-*N*-methylbenzamide(**5**a): yield 87.2%, a white solid, ^{1}H NMR(400 MHz, $CDCl_3$), δ: 13.13(s, 1H, OH), 7.59(s, 1H, Ph-H), 7.46(s, 1H, NH—CO), 3.08(d, J=4.8 Hz, 3H, CH_3).

2,3,5-Trichloro-6-hydroxy-*N*-isopropylbenzamide(**5**b): yield 88.5%, a white solid, ^{1}H NMR(400 MHz, $CDCl_3$), δ: 7.58(s, 1H, Ph-H), 7.19(s, 1H, NH-CO), 4.32[m, 1H, C**H**$(CH_3)_2$], 1.31[d, J=6.6 Hz, 6H, CH(C**H**$_3)_2$].

2,3,5-Trichloro-*N*-cyclopropyl-6-hydroxybenzamide(**5**c): yield 76.8%, a white solid, ^{1}H NMR(400 MHz, $CDCl_3$), δ: 7.58(s, 1H, Ph-H), 7.49(s, 1H, NH-CO), 2.96[m, 1H, C**H**$(CH_2)_2$], 1.00—0.91[m, 2H, CH(C**H**$_2)_2$], 0.73—0.65[m, 2H, CH(C**H**$_2)_2$].

N-(Tert-butyl)-2,3,5-trichloro-6-hydroxybenzamide(**5**d): yield 82.3%, a white solid, ^{1}H NMR(400 MHz, $CDCl_3$), δ: 7.56(s, 1H, Ph-H), 7.09(s, 1H, NH-CO), 1.49[s, 9H,$(CH_3)_3$].

2,3,5-Trichloro-*N*-cyclohexyl-6-hydroxybenzamide(**5**e): yield 77.8%, a white solid, ^{1}H NMR(400 MHz, $CDCl_3$), δ: 12.96(s, 1H, OH), 7.58(s, 1H, Ph-H), 7.28(s, 1H, NH-CO), 4.09—4.00[m, 1H, C**H**$(CH_2)_5$], 2.04—1.99[m, 2H, CH(C**H**$_2)_5$], 1.79—1.71[m, 2H, CH(C**H**$_2)_5$], 1.68—1.60[m, 1H, CH(C**H**$_2)_5$], 1.53—1.20[m, 5H, CH(C**H**$_2)_5$].

3,5-Dichloro-2-hydroxy-*N*-methylbenzamide(**5**f): yield 85.0%, a white solid, ^{1}H NMR(400 MHz, $CDCl_3$), δ: 12.86(s, 1H, OH), 7.49(d, J=2.3 Hz, 1H, Ph-H), 7.27(d, J=2.4 Hz, 1H, Ph-H), 6.37(s, 1H, NH-CO), 3.03(d, J=4.8 Hz, 3H, CH_3).

3,5-Dichloro-2-hydroxy-*N*-isopropylbenzamide(**5**g): yield 98.0%, a white solid, ^{1}H NMR(400 MHz, $CDCl_3$), δ: 13.02(s, 1H, OH), 7.48(d, *J*=2.4 Hz, 1H, Ph-H), 7.24(d, *J*=2.4 Hz, 1H, Ph-H), 6.07(s, 1H, NH-CO), 4.35—4.20[m, 1H, C**H**$(CH_3)_2$], 1.29[d, *J*=6.6 Hz, 6H, CH(C**H**$_3)_2$].

3,5-Dichloro-*N*-cyclopropyl-2-hydroxybenzamide(**5**h): yield 82.6%, a white solid, ^{1}H NMR(400 MHz, $CDCl_3$), δ: 12.84(s, 1H, OH), 7.49(d, *J*=2.3 Hz, 1H, Ph-H), 7.21(d, *J*=2.4 Hz, 1H, Ph-H), 6.43(s, 1H, NH-CO), 2.91—2.86[m, 1H, C**H**$(CH_2)_2$], 0.99—0.88[m, 2H, CH(C**H**$_2)_2$], 0.69—0.64[m, 2H, CH(C**H**$_2)_2$].

N-(Tert-butyl)-3,5-dichloro-2-hydroxybenzamide(**5**i): yield 60.7%, a white solid, ^{1}H NMR(400 MHz, $CDCl_3$), δ: 7.46(d, *J*=2.4 Hz, 1H, Ph-H), 7.19(d, *J*=2.4 Hz, 1H, Ph-H), 6.06(s, 1H, NH-CO), 1.48[s, 9H, $(CH_3)_3$].

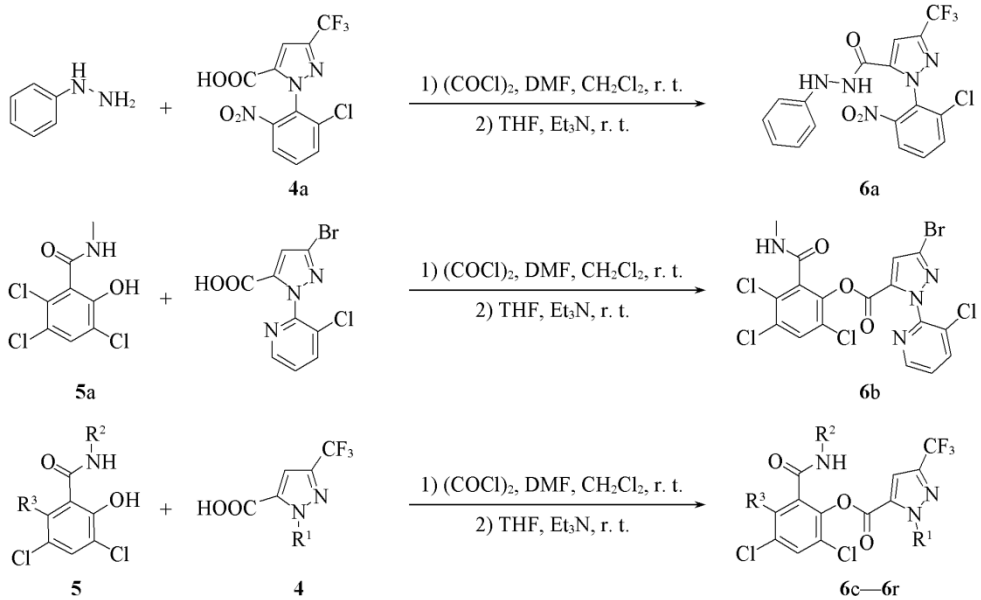
3,5-Dichloro-*N*-cyclohexyl-2-hydroxybenzamide(**5**j): yield 96.7%, a white solid, ^{1}H NMR(400 MHz, $CDCl_3$), δ: 7.48(d, *J*=2.4 Hz, 1H, Ph-H), 7.24(d, *J*=2.4 Hz, 1H, Ph-H), 6.12(s, 1H, NH-CO), 4.00—3.91[m, 1H, C**H**$(CH_2)_5$], 2.04—2.00[m, 2H, CH(C**H**$_2)_5$], 1.82—1.75[m, 2H, CH(C**H**$_2)_5$], 1.70—1.65[m, 1H, CH(C**H**$_2)_5$], 1.48—1.37[m, 2H, CH(C**H**$_2)_5$], 1.31—1.17[m, 3H, CH(C**H**$_2)_5$].

2.3 General Synthetic Procedure for Compounds 6a—6r

To a solution of intermediate **4**[or 3-bromo-1-(3-chloropyridin-2-yl)-1*H*-pyrazole-5-carboxylic acid](1 mmol) in dichloromethane(20 mL) were added oxalyl chloride(1.5 mmol) and dimethylformamide(2 drops). The solution was stirred at room temperature for 3 h, and the progress was monitored by thin layer chromatography(TLC). Then the mixture was concentrated *in vacuo* to obtain the crude acyl chloride.

The solution of crude acyl chloride in tetrahydrofuran(15 mL) was added dropwise slowly to a stirred solution of intermediate **5**(or phenylhydrazine)(1 mmol) in tetrahydrofuran(20 mL) in an ice bath, and triethylamine(1.5 mmol) was added after 20 min. The solution was concentrated *in vacuo* after stirred for 2—3 h at room temperature. The residue was extracted with ethyl acetate and washed with 1 mol/L hydrochloric acid solution, saturated sodium bicarbonate solution and saturated salt water, respectively. The organic layer was dried with anhydrous sodium sulfate, filtered, concentrated and further purified by flash chromatography on silica gel with petroleum ether/ethyl acetate to give compounds **6**a—**6**r(Scheme 3). The yields, melting points, ^{1}H NMR and HRMS of them were described as follows.

1-(2-Chloro-6-nitrophenyl)-*N*'-phenyl-3-(trifluoromethyl)-1*H*-pyrazole-5-carbohydrazide(**6a**): yield 65.2%, a white solid, m. p. 68—70 °C; ^{1}H NMR(400 MHz, $CDCl_3$), δ: 8.08(dd, *J*=8.3, 1.4 Hz, 1H, Ph-H), 7.91(d, *J*=3.5 Hz, 1H, Ph-NH-N**H**), 7.80(dd, *J*=8.1, 1.3 Hz, 1H, Ph-H), 7.62(t, *J*=8.3 Hz, 1H, Ph-H), 7.22(t, *J*=7.8 Hz, 2H, Ph-H), 7.14(s, 1H, pyrazolyl-H), 6.92(t, *J*=7.4 Hz, 1H, Ph-H), 6.80(d, *J*=8.2 Hz, 2H, Ph-H), 6.07(d, *J*=4.4 Hz, 1H, Ph-N**H**-NH). HRMS, *m*/*z* calcd. for $C_{17}H_{12}ClF_3N_5O_3^+$([M+H]$^+$): 426.0575, found: 426.0575.



R^1=2-chloro-6-nitrophenyl(A); 2-chloro-4-nitrophenyl(B); 2,4-dinitrophenyl(C); **6**c: R^1=A, R^2=Me, R^3=Cl; **6**d: R^1=A, R^2=*i*-Pr, R^3=Cl; **6**e: R^1=A, R^2=cyclopropyl, R^3=Cl; **6**f: R^1=A, R^2=*t*-Bu, R^3=Cl; **6**g: R^1=A, R^2=cyclohexyl, R^3=Cl; **6**h: R^1=A, R^2=Me, R^3=H; **6**i: R^1=A, R^2=*i*-Pr, R^3=H; **6**j: R^1=A, R^2=cyclopropyl, R^3=H; **6**k: R^1=A, R^2=*t*-Bu, R^3=H; **6**l: R^1=A, R^2=cyclohexyl, R^3=H; **6**m: R^1=B, R^2=Me, R^3=Cl; **6**n: R^1=B, R^2=*i*-Pr, R^3=Cl; **6**o: R^1=B, R^2=cyclopropyl, R^3=Cl; **6**p: R^1=B, R^2=*t*-Bu, R^3=Cl; **6**q: R^1=C, R^2=Me, R^3=Cl; **6**r: R^1=C, R^2=cyclopropyl, R^3=Cl.

Scheme 3 Synthesis of target compounds 6a—6r

3,4,6-Trichloro-2-(methylcarbamoyl)phenyl-3-bromo-1-(3-chloropyridin-2-yl)-1*H*-pyrazole-5-carboxylate(**6**b): yield 78.5%, a white solid, m. p. 135—137 °C; ^{1}H NMR(400 MHz, DMSO-d_6) δ: 8.59(s, 2H, pyridyl-H), 8.28(d, *J*=8.1 Hz, 1H, pyridyl-H), 8.16(s, 1H, Ph-H), 7.71(s, 1H, NH-CO), 7.64(s, 1H, pyrazolyl-H), 2.70(s, 3H, CH_3). HRMS, *m*/*z* calcd. for $C_{17}H_{10}BrCl_4N_4O_3^+$([M+H]$^+$): 538.8655, found: 538.8655.

3,4,6-Trichloro-2-(methylcarbamoyl)phenyl-1-(2-chloro-6-nitrophenyl)-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxylate (**6**c): yield 78.2%, a white solid, m. p. 150—152 °C; ^{1}H NMR

(400 MHz, $CDCl_3$), δ: 8.14(d, J=8.3 Hz, 1H, Ph-H), 7.85(d, J=8.2 Hz, 1H, Ph-H), 7.68(t, J=8.2 Hz, 1H, Ph-H), 7.59(s, 2H, Ph-H, pyrazolyl-H), 5.61(q, J=5.0 Hz, 1H, CO-NH), 2.90(d, J=4.8 Hz, 3H, CH_3). HRMS, m/z calcd. for $C_{19}H_{10}Cl_4F_3N_4O_5^+$([M+H]$^+$): 572.9322, found: 572.9316.

3,4,6-Trichloro-2-(isopropylcarbamoyl)phenyl-1-(2-chloro-6-nitrophenyl)-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxylate(**6**d): yield 80.3%, a white solid, m. p. 174—176 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ: 8.15(d, J=8.3 Hz, 1H, Ph-H), 7.85(d, J=8.2 Hz, 1H, Ph-H), 7.68(t, J=8.2 Hz, 1H, Ph-H), 7.58(s, 2H, Ph-H, pyrazolyl-H), 5.49(d, J=8.1 Hz, 1H, CO-NH), 4.21—4.14[m, 1H, C**H**$(CH_3)_2$], 1.15[d, J=1.9 Hz, 3H, CH(C**H**$_3)_2$], 1.14[d, J=1.9 Hz, 3H, CH(C**H**$_3)_2$]. HRMS, m/z calcd. for $C_{21}H_{14}Cl_4F_3N_4O_5^+$([M+H]$^+$): 600.9635, found: 600.9637.

3,4,6-Trichloro-2-(cyclopropylcarbamoyl)phenyl-1-(2-chloro-6-nitrophenyl)-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxylate(**6**e): yield 74.1%, a white solid, m. p. 154—156 °C; ^{1}H NMR(400 MHz, $CDCl_3$), δ: 8.15(dd, J=8.3, 1.3 Hz, 1H, Ph-H), 7.85(dd, J=8.2, 1.3 Hz, 1H, Ph-H), 7.68(t, J=8.3 Hz, 1H, Ph-H), 7.60(s, 1H, Ph-H), 7.58(s, 1H, pyrazolyl-H), 5.77(d, J=3.1 Hz, 1H, CO-NH), 2.81—2.76[m, 1H, C**H**$(CH_2)_2$], 0.85—0.81[m, 2H, CH(C**H**$_2)_2$], 0.53—0.49[m, 2H, CH(C**H**$_2)_2$]. HRMS, m/z calcd. for $C_{21}H_{12}Cl_4F_3N_4O_5^+$([M+H]$^+$): 598.9479, found: 598.9474.

2-(Tert-butylcarbamoyl)-3,4,6-trichlorophenyl-1-(2-chloro-6-nitrophenyl)-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxylate(**6**f): yield 78.5%, a white solid, m. p. 151—153 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ: 8.14(d, J=8.3 Hz, 1H, Ph-H), 7.85(d, J=8.2 Hz, 1H, Ph-H), 7.69(t, J=8.2 Hz, 1H, Ph-H), 7.58(s, 1H, Ph-H), 7.56(s, 1H, pyrazolyl-H), 5.45(s, 1H, CO-NH), 1.34[s, 9H, $(CH_3)_3$]. HRMS, m/z calcd. for $C_{22}H_{16}Cl_4F_3N_4O_5^+$([M+H]$^+$): 614.9792, found: 614.9797.

3,4,6-Trichloro-2-(cyclohexylcarbamoyl)phenyl-1-(2-chloro-6-nitrophenyl)-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxylate(**6**g): yield 78.5%, a white solid, m. p. 166—168 °C; ^{1}H NMR(400 MHz, $CDCl_3$), δ: 8.16(d, J=8.3 Hz, 1H, Ph-H), 7.85(d, J=8.2 Hz, 1H, Ph-H), 7.69(t, J=8.2 Hz, 1H, Ph-H), 7.57(s, 2H, Ph-H, pyrazolyl-H), 5.52(d, J=8.4 Hz, 1H, CO-NH), 3.91—3.82[m, 1H, C**H**$(CH_2)_5$], 1.97—1.81[m, 2H, CH(C**H**$_2)_5$], 1.77—1.54[m, 3H, CH(C**H**$_2)_5$], 1.44—1.29[m, 2H, CH(C**H**$_2)_5$], 1.19—1.00[m, 3H, CH(C**H**$_2)_5$]. HRMS, m/z calcd. for $C_{24}H_{18}Cl_4F_3N_4O_5^+$([M+H]$^+$): 640.9948, found: 640.9945.

2,4-Dichloro-6-(methylcarbamoyl)phenyl-1-(2-chloro-6-nitrophenyl)-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxylate (**6**h): yield 51.6%, a white solid, m. p. 176—178 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ: 8.12(dd, J=8.3, 1.4 Hz, 1H, Ph-H), 7.84(dd, J=8.2, 1.4 Hz, 1H, Ph-H), 7.68(t, J=8.3 Hz, 1H, Ph-H), 7.60(s, 1H, pyrazolyl-H), 7.54(d, J=2.5 Hz, 1H, Ph-H), 7.52(d, J=2.5 Hz, 1H, Ph-H), 5.90(s, 1H, CO-NH), 2.89(d, J=4.9 Hz, 3H, CH_3). HRMS, m/z calcd. for $C_{19}H_{11}Cl_3F_3N_4O_5^+$([M+H]$^+$): 536.9742, found: 536.9739.

2,4-Dichloro-6-(isopropylcarbamoyl)phenyl-1-(2-chloro-6-nitrophenyl)-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxylate (**6**i): yield 52.3%, a white solid, m. p. 206—208 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ: 8.13(dd, J=8.3, 1.4Hz, 1H, Ph-H), 7.84(dd, J=8.2, 1.4 Hz, 1H, Ph-H), 7.67(t, J=8.2 Hz, 1H, Ph-H), 7.59(s, 1H, pyrazolyl-H), 7.53(d, J=2.5 Hz, 1H, Ph-H), 7.51(d, J=2.5 Hz, 1H, Ph-H), 5.75(d, J=7.9 Hz, 1H, CO-NH), 4.20—4.07[m, 1H, C**H**$(CH_3)_2$], 1.15[d, J=1.9 Hz, 3H, CH(C**H**$_3)_2$], 1.13[d, J=1.9 Hz, 3H, CH(C**H**$_3)_2$]. HRMS, m/z calcd. for $C_{21}H_{15}Cl_3F_3N_4O_5^+$([M+H]$^+$): 565.0055, found: 565.0050.

2,4-Dichloro-6-(cyclopropylcarbamoyl)phenyl-1-(2-chloro-6-nitrophenyl)-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxylate(**6**j): yield 57.2%, a white solid, m. p. 184—186 °C; ^{1}H NMR(400 MHz, $CDCl_3$), δ: 8.13(dd, J=8.3, 1.4 Hz, 1H, Ph-H), 7.85(dd, J=8.2, 1.4 Hz, 1H, Ph-H), 7.67(t, J=8.3 Hz, 1H, Ph-H), 7.59(s, 1H, pyrazolyl-H), 7.53(d, J=2.5 Hz, 1H, Ph-H), 7.52(d, J=2.5 Hz, 1H, Ph-H), 6.04(s, 1H, CO-NH), 2.76—2.81[m, 1H, C**H**$(CH_2)_2$], 0.79—0.86[m, 2H, CH(C**H**$_2)_2$], 0.49—0.43[m, 2H, CH(C**H**$_2)_2$]. HRMS, m/z calcd. for $C_{21}H_{13}Cl_3F_3N_4O_5^+$([M+H]$^+$): 562.9898, found: 562.9904.

2-(Tert-butylcarbamoyl)-4,6-dichlorophenyl-1-(2-chloro-6-nitrophenyl)-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxylate (**6**k): yield 60.5%, a white solid, m. p. 191—193 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ: 8.14(dd, J=8.3, 1.4 Hz, 1H, Ph-H), 7.84(dd, J=8.2, 1.4 Hz, 1H, Ph-H), 7.67(t, J=8.2 Hz, 1H, Ph-H), 7.58(s, 1H, pyrazolyl-H), 7.52(d, J=2.5 Hz, 1H, Ph-H), 7.49(d, J=2.5 Hz, 1H, Ph-H), 5.78(s, 1H, CO-NH), 1.33[s, 9H, $(CH_3)_3$]. HRMS, m/z calcd. for $C_{22}H_{17}Cl_3F_3N_4O_5^+$([M+H]$^+$): 579.0211, found: 579.0207.

2,4-Dichloro-6-(cyclohexylcarbamoyl)phenyl-1-(2-chloro-6-nitrophenyl)-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxylate (**6**l): yield 46.8%, a white solid, m. p. 185—187 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ: 8.14(dd, J=8.3, 1.4 Hz, 1H, Ph-H), 7.84(dd, J=8.2, 1.4 Hz, 1H, Ph-H), 7.67(t, J=8.3 Hz, 1H, Ph-H), 7.59(s, 1H, pyrazolyl-H), 7.52(d, J=2.5 Hz, 1H, Ph-H), 7.51(d, J=2.5 Hz, 1H, Ph-H), 5.77(d, J=8.2 Hz, 1H, CO-NH), 3.87—3.78[m, 1H, C**H**$(CH_2)_5$], 1.90—1.86[m, 2H, CH(C**H**$_2)_5$], 1.70—1.63[m, 3H, CH(C**H**$_2)_5$], 1.41—1.31[m, 2H, CH(C**H**$_2)_5$], 1.19—1.02[m, 3H, CH(C**H**$_2)_5$]. HRMS, m/z calcd. for $C_{24}H_{19}Cl_3F_3N_4O_5^+$([M+H]$^+$): 605.0368, found: 605.0366.

3,4,6-Trichloro-2-(methylcarbamoyl)phenyl-1-(2-chloro-4-nitrophenyl)-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxylate (**6**m): yield 52.6%, a white solid, m. p. 142—144 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ: 8.41(d, J=2.4 Hz, 1H, Ph-H), 8.30(dd, J=8.7, 2.5 Hz, 1H, Ph-H), 7.73(d, J=8.7 Hz, 1H, Ph-H), 7.62(s, 1H, Ph-H), 7.55(s, 1H, pyrazolyl-H), 5.67(q, J=5.1 Hz, 1H, CO-NH), 2.94(d, J=4.9 Hz, 3H, CH_3). HRMS, m/z calcd. for $C_{19}H_{10}Cl_4F_3N_4O_5^+$([M+H]$^+$): 572.9322, found: 572.9316.

3,4,6-Trichloro-2-(isopropylcarbamoyl)phenyl-1-(2-chloro-4-nitrophenyl)-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxylate(**6**n): yield 49.2%, a white solid, m. p. 225—227 °C; ^{1}H NMR(400 MHz, $CDCl_3$), δ: 8.42(d, J=2.4 Hz, 1H, Ph-H), 8.29(dd, J=8.7, 2.5 Hz, 1H, Ph-H), 7.69(d, J=8.7 Hz, 1H, Ph-H), 7.61(s, 1H, Ph-H), 7.55(s, 1H, pyrazolyl-H), 5.49(d, J=8.2 Hz, 1H, CO-NH), 4.23—4.15[m, 1H, C**H**$(CH_3)_2$], 1.15[d, J=6.6 Hz, 6H, CH(C**H**$_3)_2$]. HRMS, m/z calcd. for $C_{21}H_{14}Cl_4F_3N_4O_5^+$([M+H]$^+$): 600.9635, found: 600.9627.

3,4,6-Trichloro-2-(cyclopropylcarbamoyl)phenyl-1-(2-chloro-4-nitrophenyl)-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxylate(**6**o): yield 47.3%, a white solid, m. p. 209—211 °C; ^{1}H NMR(400 MHz, $CDCl_3$), δ: 8.42(d, J=2.4 Hz, 1H, Ph-H),

8.30(dd, *J*=8.7, 2.4 Hz, 1H, Ph-H), 7.72(d, *J*=8.7 Hz, 1H, Ph-H), 7.61(s, 1H, Ph-H), 7.56(s, 1H, pyrazolyl-H), 5.78(s, 1H, CO-NH), 2.81—2.78[m, 1H, C**H**$(CH_2)_2$], 0.90—0.84[m, 2H, CH(C**H**$_2)_2$], 0.52—0.48[m, 2H, CH(C**H**$_2)_2$]. HRMS, *m*/*z* calcd. for $C_{21}H_{12}Cl_4F_3N_4O_5^+$([M+H]$^+$): 598.9479, found: 598.9484.

2-(Tert-butylcarbamoyl)-3,4,6-trichlorophenyl-1-(2-chloro-4-nitrophenyl)-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxylate(**6**p): yield 60.6%, a white solid, m. p. 98—100 °C; ^{1}H NMR(400 MHz, $CDCl_3$), *δ*: 8.42(d, *J*=2.4 Hz, 1H, Ph-H), 8.29(dd, *J*=8.7, 2.4 Hz, 1H, Ph-H), 7.70(d, *J*=8.7 Hz, 1H, Ph-H), 7.59(s, 1H, Ph-H), 7.55(s, 1H, pyrazolyl-H), 5.46(s, 1H, CO-NH), 1.35[s, 9H,$(CH_3)_3$]. HRMS, *m*/*z* calcd. for $C_{22}H_{16}Cl_4F_3N_4O_5^+$([M+H]$^+$): 614.9792, found: 614.9783.

3,4,6-Trichloro-2-(methylcarbamoyl)phenyl-1-(2,4-dinitrophenyl)-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxylate (**6**q): yield 90.5%, a white solid, m. p. 92—94 °C; ^{1}H NMR (400 MHz, $CDCl_3$), *δ*: 9.05(d, *J*=2.5 Hz, 1H, Ph-H), 8.65(dd, *J*=8.7, 2.5 Hz, 1H, Ph-H), 7.92(d, *J*=8.7 Hz, 1H, Ph-H), 7.60(s, 2H, Ph-H, pyrazolyl-H), 5.66(q, *J*=5.3 Hz, 1H, CO-NH), 2.97(d, *J*=4.9 Hz, 3H, CH_3). HRMS, *m*/*z* calcd. for $C_{19}H_{10}Cl_3F_3N_5O_7^+$ ([M+H]$^+$): 581.9592, found: 581.9592.

3,4,6-Trichloro-2-(cyclopropylcarbamoyl)phenyl-1-(2,4-dinitrophenyl)-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxylate (**6**r): yield 83.6%, a white solid, m. p. 176—178 °C; ^{1}H NMR (400 MHz, $CDCl_3$), *δ*: 9.06(d, *J*=2.5 Hz, 1H, Ph-H), 8.64(dd, *J*=8.7, 2.5 Hz, 1H, Ph-H), 7.91(d, *J*=8.7 Hz, 1H, Ph-H), 7.60(s, 1H, Ph-H), 7.59(s, 1H, pyrazolyl-H), 5.80(d, *J*=2.9 Hz, 1H, CO-NH), 2.84—2.78[m, 1H, C**H**$(CH_2)_2$], 0.89—0.84[m, 2H, CH(C**H**$_2)_2$], 0.57—0.53[m, 2H, CH(C**H**$_2)_2$]. HRMS, *m*/*z* calcd. for $C_{21}H_{12}Cl_3F_3N_5O_7^+$([M+H]$^+$): 607.9749, found: 607.9748.

2.4 Biological Assay

The fungicidal activities of the target compounds against six kinds of fungi were screened and evaluated according to the literature[20]. Chlorothalonil was used as control.

The antitumor activities of the target compounds and 5-fluorouracil against Lung cancer cells(A549), liver cancer cells(Bel7402) and colon cancer cells(HCT-8) were determined by the MTT assay according to the standard procedures[21]. The optical density(OD) was measured at 540 nm and the cell growth inhibition can be defined as

$$\text{Growth inbibition rate(GIR)(\%)}=\frac{(OD_{\text{cell negative control}}-OD_{\text{compound control}})}{(OD_{\text{cell negative control}}-OD_{\text{blank}})}\times 100\%$$

3 Results and Discussion

3.1 Fungicidal Activity

The fungicidal activities of the target compounds *in vitro* are showed in Table 1. Preliminary bioassay proved that the title compounds exhibited moderate to good activity against *Fusarium omysporum*, *Physalospora piricola*, *Alternaria solani*, *Cercospora arachidicola, Gibberella sanbinetti* and *Phytophthora capsici* at a concentration of 50 μg/mL. The overall activities of compounds **6**a, **6**c and **6**q against fungi were good. Compounds **6**a, **6**b and **6**c exhibited 92.9%, 60.0% and 70.0% inhibition rates, respectively, against *Cercospora arachidicola,* higher than that of chlorothalonil(58.8%). Most of the targets expressed potent fungicidal effects against *Physalospora piricola,* especially compounds **6**c, **6**j and **6**k(92.0%, 96.2% and 92.3%, respectively), better than that of chlorothalonil(88.5%). Compounds **6**a, **6**c and **6**q were also superior to chlorothalonil (63.6%) against *Alternaria solani*, the activities were 81.3%, 72.5% and 75.0%, respectively. Besides, compounds **6**a, **6**m and **6**q showed 87.1%, 80.6% and 83.9% fungicidal activities against *Gibberella sanbinetti*, which were more outstanding than the control(73.1%). The structure-activity relationship demonstrated that when R^2 is methyl, the activity was superior to other alkyls, the position of nitro and the existence of

Table 1 Fungicidal activities of compounds 6a—6r

Compd.	Fungicidal activity(%)(50 μg/mL)					
	Fusarium omysporum	*Cercospora arachidicola*	*Physalospora piricola*	*Alternaria solani*	*Gibberella sanbinetti*	*Phytophthora capsici*
6a	81.0	92.9	69.2	81.3	87.1	55.2
6b	46.7	60.0	54.5	41.7	47.8	22.2
6c	63.3	70.0	92.0	72.5	43.5	36.1
6d	30.0	45.0	15.9	20.8	17.4	19.4
6e	40.0	55.0	18.2	25.0	17.4	33.3
6f	36.7	45.0	45.5	25.0	30.4	33.3
6g	36.7	35.0	50.0	25.0	17.4	30.6
6h	23.8	21.4	76.9	31.3	41.9	44.8
6i	47.6	50.0	19.2	25.0	32.3	20.7
6j	38.1	14.3	96.2	31.3	35.5	37.9
6k	38.1	42.9	92.3	31.3	51.6	34.5
6l	47.6	50.0	69.2	18.8	32.3	34.5
6m	47.6	35.7	69.2	43.8	80.6	44.8
6n	23.8	0.0	57.7	12.5	29.0	31.0
6o	23.8	35.7	57.7	37.5	48.4	27.6
6p	14.3	35.7	76.9	43.8	71.0	48.3
6q	33.3	50.0	76.9	75.0	83.9	58.6
6r	47.6	21.4	76.9	25.0	67.7	69.0
Chlorothalonil	84	58.8	88.5	63.6	73.1	82.6

chlorine atom in the benzene ring showed little effect on activity. The introduction of ester group and amide bond facilitated the activities against *Physalospora piricola* and *Phytophthora capsici*. But the activities of carboxylates against other four kinds of fungi did not exceed that of the carbohydrazide compound **6**a.

3.2 Antitumor Activity

The antitumor activities of the title compounds against A549, Bel7402 and HCT-8 are listed in the Table 2. The results indicated that some compounds possessed certain degree of antitumor activities. Specifically, the inhibitory rate of compound **6**c against Bel7402 at 5 μg/mL was 76.96%, exceeded that of the control(50.75%). Compounds **6**c, **6**d and **6**p revealed a comparable activity against A549 to 5-fluorouracil. Compounds **6**a and **6**m had moderate activities against HCT-8.

Table 2 Antitumor activities *in vitro* of compounds 6a—6r

Compd.	Inhibitory rate(%) of cancer cells(5 μg/mL)			Compd.	Inhibitory rate(%) of cancer cells(5 μg/mL)		
	HCT-8	Bel7402	A549		HCT-8	Bel7402	A549
6a	32.31	6.36	15.84	**6**k	–7.71	–37.23	–5.31
6b	–7.94	8.52	–3.52	**6**l	4.31	–4.69	1.36
6c	2.23	76.96	41.48	**6**m	31.62	–5.52	6.54
6d	4.15	27.36	45.49	**6**n	–19.82	–37.69	–3.89
6e	–5.70	14.35	2.92	**6**o	–12.07	–14.25	23.83
6f	4.96	4.34	–21.22	**6**p	0.27	–17.82	49.08
6g	–1.13	6.62	–16.95	**6**q	1.08	–25.43	–2.04
6h	6.05	–24.73	5.19	**6**r	18.97	–16.70	4.81
6i	16.70	–11.31	7.17	5-Fluorouracil	59.12	50.75	65.39
6j	17.00	–19.52	11.04				

4 Conclusions

On the basis of carbohydrazide, a series of novel *N*-aryl-1*H*-pyrazole-5-carboxylate derivatives was designed, synthesized and characterized by ^{1}H NMR and HRMS. Comparing with chlorothalonil, some compounds exhibited better fungicidal activities against certain fungi. It was found that the nitrophenyl was a good substitute for pyridyl group in the structure of lead compound. Besides, some carboxylates presented favorable antitumor activity. The novel structures deserve further exploration.

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