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Design, synthesis and insecticidal activities of N-(4-cyano-1-phenyl-1H-pyrazol-5-yl)-1,3-diphenyl-1H-pyrazole-4-carboxamide derivatives

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Experimental

General

All of the synthesized compounds were chemically characterized by thin layer chromatography (TLC), proton nuclear magnetic resonance (^{1}H NMR) and elemental microanalyses (CHN). ^{1}H NMR spectra were measured on a Bruker AV-300 spectrometer at 25 ℃ and referenced to Me4Si. Chemical shifts were reported in ppm (δ) using the residual solvent line as internal standard. Splitting patterns were designed as s, singlet; d, doublet; t, triplet; m, multiplet. ESI-MS spectra were recorded on a Mariner System 5304 Mass spectrometer. Elemental analyses were performed on a CHN-O-Rapid instrument and were within ±0.4% of the theoretical values. Melting points were determined on a XT4 MP apparatus (Taike Corp., Beijing, China) and were as read. Analytic thin-layer chromatography was performed on the glass backed silica gel sheets (silica gel 60A GF254). All compounds were detected using UV light (254 nm or 365 nm).

Chemical synthesis

General procedure for synthesis of 1,3-diphenyl-1H-pyrazole-4-carboxylic acid (3a-3d)

The intermediates 1,3-diphenyl-1H-pyrazole-4-carboxylic acid (**3a-3d**) were synthesized as follows: para-substituted acetophenone (20mmol) interact with phenylhydrazine hydrochloride (20mmol) couple with sodium acetate (40mmol) in anhydrous ethanol to form 1-phenyl-2-(1-phenylethylidene) hydrazine (**1a-1d**), which was then dissolved in a cold mixed solution of DMF (20 mL) and $POCl_3$ (16 mL),stirred at 50-60℃ for 5h. The resulting mixture was poured into ice-cold water, a saturated solution of sodium hydroxide was added to neutralize the mixture, and the solid precipitate was filtered, washed with water, dried and recrystallized from ethanol to give the compounds **2a-2d**. Then the product (7mmol) which was dissolved in acetone was added into the mixture solution of $NaClO_2$ (20mmol) and NH_2SO_3H (20mmol), the mixture was poured into ice-cold water, stirred for 2h, then at room temperature for 2h. After completion of the reaction, the solvent was concentrated under reduced pressure to remove acetone, then it is dissolved in ethyl acetate. The mixture was extracted from ethyl acetate with thiosulfate solution and saturated sodium chloride successively, then dried concentrated gave the compounds **3a-3d**.

General procedure for synthesis of 5-amino-1-aryl-1H-pyrazole-4-carbonitriles (4a-4e)

A stirred mixture of para-substituted phenyl hydrazine hydrochloride(0.025 mol) was dissolved in H_2O (30 mL), then the mixture was basified to pH 7-8 by the dropwise addition of 10％ NaOH solution to form para-substituted phenyl hydrazine. Then, which with Ethoxymethylenemalononitrile in ethanol medium was refluxed for 3h. After completion of the reaction, the reaction mixture was allowed to cool at room temperature, the solid **4a-4d** was filtered under vacuum. The crude product obtained was recrystallized from DMF to afford the pure product.

General procedure for Synthesis of N-(4-cyano-1-phenyl-1H-pyrazol-5-yl)-1,3-diphenyl-1H-pyrazole-4-carboxamide (5a-5s)

To a stirred solution of the intermediates compound **3a-3d** (1mmol) with triethylamine (2 mmol) into DMF (12 mL) medium, then a mixture of EDCI (1 mmol) and HOBt (1 mmol) was placed in the reaction system, stirred at room temperature for 30 min, the mixture of compound **4a-4d** (1mmol) and DMF (5mL) was added in the reaction system, the reaction mixture was monitored by TLC. After completion of the

reaction, the product was extracted from chloroform with water, 0.2 mol/L hydrochloric acid, water, 2 mol/L sodium hydroxide, saturated sodium chloride successively, and then dried, concentrated, and purified by preparative thin layer chromatography (PE : EA = 8 : 1) followed by recrystallization from ethanol.

N-(4-cyano-1-phenyl-1H-pyrazol-5-yl)-1,3-diphenyl-1H-pyrazole-4-carboxamide (5a).

Pale yellow crystal, yield 66%, mp: 175-176℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.93 (s, 1H), 8.09 (d, J = 8.5 Hz, 1H), 7.95-7.92 (m, 2H), 7.89-7.86 (m, 2H), 7.60-7.40 (m, 11H). MS (ESI): 431.2 ($C_{26}H_{19}N_6O$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{18}N_6O$: C, 72.55; H, 4.21; N, 19.52; Found: C, 72.62; H, 4.22; N, 19.58.

N-(4-cyano-1-(4-fluorophenyl)-1H-pyrazol-5-yl)-1,3-diphenyl-1H-pyrazole-4-carboxamide (5b).

Pale yellow crystal, yield 64%, mp: 179-181℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.93 (s, 1H), 8.09 (d, J = 8.4 Hz, 1H), 7.95– 7.91 (m, 2H), 7.90–7.85 (m, 2H), 7.60–7.42 (m, 10H), ^{13}C NMR (151 MHz, Chloroform-*d*) δ 158.56, 155.73, 143.49, 138.73, 133.34, 130.63, 129.81, 129.52, 129.15, 128.93, 128.72, 128.43, 128.23, 124.77, 120.52, 119.92, 108.38, 106.78, 77.2. MS (ESI): 449.1 ($C_{26}H_{17}N_6O$, $[M+ H]^+$). Anal. Calcd for$C_{26}H_{17}N_6O$: C, 69.63; H, 3.82; N, 18.74; Found: C, 69.71; H, 3.92; N, 18.82.

N-(1-(4-chlorophenyl)-4-cyano-1H-pyrazol-5-yl)-1,3-diphenyl-1H-pyrazole-4-carboxamide (5c).

Pale yellow crystal, yield 62%, mp: 182-184℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.93 (s, 1H), 8.08 (dt, J = 8.4, 0.9 Hz, 1H), 7.95-7.91 (m, 2H), 7.89-7.85 (m, 2H), 7.60-7.42 (m, 10H), ^{13}C NMR (151 MHz, Chloroform-*d*) δ 158.57, 155.72, 143.47, 134.77, 133.36, 130.62, 130.13, 129.81, 129.52, 129.15, 128.92, 128.73, 128.43, 128.23, 124.80, 120.50, 119.92, 108.38, 106.76, 77.2. MS (ESI): 465.1 ($C_{26}H_{18}ClN_6O$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{17}ClN_6O$: C, 67.17; H, 3.69; N, 18.08; Found: C, 67.25; H, 3.86; N, 18.12.

N-(4-cyano-1-(p-tolyl)-1H-pyrazol-5-yl)-1,3-diphenyl-1H-pyrazole-4-carboxamide (5d).

Pale yellow crystal, yield 63%, mp: 183-185℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.93 (s, 1H), 8.09 (d, J = 8.4 Hz, 1H), 7.96-7.91 (m, 2H), 9.90-7.84 (m, 2H), 7.60-7.39 (m, 10H), 2.42 (m, 3H), ^{13}C NMR (151 MHz, Chloroform-*d*) δ 158.56, 155.73, 143.49,

138.73, 133.34, 130.63, 130.49, 129.81, 129.52, 129.15, 128.93, 128.72, 128.42, 128.23, 124.78, 120.52, 119.92, 108.38, 106.78, 77.2, 21.15. MS (ESI): 445.2 ($C_{27}H_{21}N_6O$, $[M+H]^+$). Anal. Calcd for $C_{27}H_{20}N_6O$: C, 72.96; H, 4.54; N, 18.91; Found: C, 73.03; H, 4.62; N, 18.98.

N-(4-cyano-1-(4-nitrophenyl)-1H-pyrazol-5-yl)-1,3-diphenyl-1H-pyrazole-4-carboxamide (5e).

Pale yellow crystal, yield 67%, mp: 181-183℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (s, 1H), 8.08 (dd, *J* = 8.4, 0.8 Hz, 1H), 7.96-7.84 (m, 4H), 7.61-7.50 (m, 4H), 7.50-7.40 (m, 6H). MS (ESI): 476.1 ($C_{26}H_{18}N_7O_3$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{17}N_7O_3$: C, 65.68; H, 3.60; N, 20.62; Found: C, 65.84; H, 3.68; N, 20.71.

N-(4-cyano-1-phenyl-1H-pyrazol-5-yl)-1-(4-fluorophenyl)-3-phenyl-1H-pyrazole-4-carboxamide (5f).

Pale yellow crystal, yield 61%, mp: 208-210℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (s, 1H), 8.09 (d, *J* = 8.4 Hz, 1H), 7.96-7.89 (m, 2H), 7.88–7.82 (m, 2H), 7.59-7.38 (m, 7H), 7.31-7.26 (m, 2H), 7.25-7.18 (m, 1H). MS (ESI): 449.1 ($C_{26}H_{17}FN_6O$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{17}FN_6O$: C, 69.63; H, 3.82; N, 18.74; Found: C, 69.71; H, 3.86; N, 18.83.

N-(4-cyano-1-(4-fluorophenyl)-1H-pyrazol-5-yl)-1-(4-fluorophenyl)-3-phenyl-1H-pyrazole-4-carboxamide (5g).

Pale yellow crystal, yield 61%, mp: 162-163℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.05 (s, 1H), 8.49 (s, 1H), 7.83-7.75 (m, 5H), 7.57-7.44 (m, 5H), 7.25-7.18 (m, 3H). MS (ESI): 467.1 ($C_{26}H_{16}F_2N_6O$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{16}F_2N_6O$: C, 66.95; H, 3.46; N, 18.02; Found: C, 67.03; H, 3.52; N, 18.10.

N-(1-(4-chlorophenyl)-4-cyano-1H-pyrazol-5-yl)-1-(4-fluorophenyl)-3-phenyl-1H-pyrazole-4-carboxamide (5h).

Pale yellow crystal, yield 63%, mp: 167-169℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.05 (s, 1H), 8.49 (s, 1H), 7.85-7.75 (m, 4H), 7.54-7.45 (m, 6H), 7.26-7.14 (m, 3H). MS (ESI): 483.1 ($C_{26}H_{16}ClFN_6O$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{16}ClFN_6O$: C, 64.67; H, 3.34; N, 17.40; Found: C, 64.77; H, 3.42; N, 17.43.

N-(4-cyano-1-(p-tolyl)-1H-pyrazol-5-yl)-1-(4-fluorophenyl)-3-phenyl-1H-pyrazole-4-carboxa

mide (5i).

Pale yellow crystal, yield 65%, mp: 167-169℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.05 (s, 1H), 8.49 (s, 1H), 7.82-7.76 (m, 4H), 7.58-7.42 (m, 4H), 7.41-7.27 (m, 2H), 7.25-7.16 (m, 3H), 2.42 (s, 3H). MS (ESI): 463.2 ($C_{27}H_{19}FN_6O$, $[M+H]^+$). Anal. Calcd for $C_{27}H_{19}FN_6O$: C, 70.12; H, 4.14; N, 18.17; Found: C, 70.22; H, 4.33; N, 18.35.

N-(4-cyano-1-(4-nitrophenyl)-1H-pyrazol-5-yl)-1-(4-fluorophenyl)-3-phenyl-1H-pyrazole-4-carboxamide (5j).

Pale yellow crystal, yield 65%, mp:191-193℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.05 (s, 1H), 8.49 (s, 1H), 7.82-7.75 (m, 4H), 7.59-7.38 (m, 7H), 7.25-7.16 (m, 3H). MS (ESI): 494.1 ($C_{26}H_{16}FN_7O_4$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{16}FN_7O_4$: C, 63.28; H, 3.27; N, 19.87; Found: C, 63.36; H, 3.35; N, 20.07.

1-(4-chlorophenyl)-N-(4-cyano-1-phenyl-1H-pyrazol-5-yl)-3-phenyl-1H-pyrazole-4-carboxamide (5k).

Pale yellow crystal, yield 61%, mp: 230-231℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (s, 1H), 8.09 (d, J = 8.4 Hz, 1H), 7.94-7.89 (m, 2H), 7.85-7.81 (m, 2H), 7.60-7.39 (m, 10H). MS (ESI): 465.1 ($C_{26}H_{17}ClN_6O$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{17}ClN_6O$: C, 67.17; H, 3.69; N, 18.08; Found: C, 67.35; H, 3.75; N, 18.12.

1-(4-chlorophenyl)-N-(4-cyano-1-(4-fluorophenyl)-1H-pyrazol-5-yl)-3-phenyl-1H-pyrazole-4-carboxamide (5l).

Pale yellow crystal, yield 61%, mp: 245-247℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 8.09 (dt, J = 8.4, 0.9 Hz, 1H), 7.94-7.90 (m, 2H), 7.85-7.81 (m, 2H), 7.56-7.43 (m, 9H). MS (ESI): 483.1 ($C_{26}H_{16}ClFN_6O$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{16}ClFN_6O$: C, 64.67; H, 3.34; N, 17.40; Found: C, 64.83; H, 3.56; N, 17.46.

1-(4-chlorophenyl)-N-(1-(4-chlorophenyl)-4-cyano-1H-pyrazol-5-yl)-3-phenyl-1H-pyrazole-4-carboxamide (5m).

Pale yellow crystal, yield 63%, mp: 228-230℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (s, 1H), 8.09 (dt, J = 8.4, 0.9 Hz, 1H), 7.95-7.88 (m, 2H), 7.85-7.80 (m, 2H), 7.57-7.40 (m, 9H). MS (ESI): 499.1 ($C_{26}H_{16}Cl_2N_6O$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{16}Cl_2N_6O$: C, 62.54; H, 3.23; N, 16.83; Found: C, 62.62; H, 3.41; N, 17.05.

1-(4-chlorophenyl)-N-(4-cyano-1-(p-tolyl)-1H-pyrazol-5-yl)-3-phenyl-1H-pyrazole-4-carboxa

mide (5n).

Pale yellow crystal, yield 64%, mp: 232-233℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 8.09 (d, J = 8.5 Hz, 1H), 7.97-7.88 (m, 2H), 7.86-7.77 (m, 2H), 7.57-7.52 (m, 2H), 7.46-7.42 (m, 3H), 7.39–7.30 (m, 4H), 2.42 (s, 3H). MS (ESI): 479.1 ($C_{27}H_{29}ClN_6O$, $[M+H]^+$). Anal. Calcd for $C_{27}H_{19}ClN_6O$: C, 67.71; H, 4.00; N, 17.55; Found: C, 67.91; H, 4.09; N, 17.68.

1-(4-chlorophenyl)-N-(4-cyano-1-(4-nitrophenyl)-1H-pyrazol-5-yl)-3-phenyl-1H-pyrazole-4-carboxamide (5o).

Pale yellow crystal, yield 65%, mp: 228-230℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 8.09 (d, J = 8.4 Hz, 1H), 7.94 – 7.89 (m, 2H), 7.85-7.80 (m, 2H), 7.56-7.41 (m, 9H). MS (ESI):510.1 ($C_{26}H_{16}ClN_7O_3$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{16}ClN_7O_3$: C, 61.24; H, 3.16; N, 19.23; Found: C, 61.44; H, 3.32; N, 19.32.

N-(4-cyano-1-phenyl-1H-pyrazol-5-yl)-3-phenyl-1-(p-tolyl)-1H-pyrazole-4-carboxamide (5p).

Pale yellow crystal, yield 66%, mp: 242-244℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.05 (s, 1H), 8.50 (s, 1H), 7.82 (d, J = 7.7, 2H), 7.67 (d, J = 8.4 Hz, 2H), 7.58–7.27 (m, 10H), 2.42 (s, 3H). MS (ESI): 445.1 ($C_{27}H_{20}N_6O$, [M+H]+). Anal. Calcd for $C_{27}H_{20}N_6O$: C, 72.96; H, 4.54; N, 18.91; Found: C, 73.04; H, 4.61; N, 19.08.

N-(4-cyano-1-(4-fluorophenyl)-1H-pyrazol-5-yl)-3-phenyl-1-(p-tolyl)-1H-pyrazole-4-carboxamide (5q).

Pale yellow crystal, yield 63%, mp: 138-140℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.05 (s, 1H), 8.50 (s, 1H), 7.83-7.78 (m, 2H), 7.68-7.66 (m, 2H), 7.54-7.42 (m, 6H), 7.35 – 7.26 (m, 3H), 1.58 (s, 3H). MS (ESI): 463.1 ($C_{27}H_{19}FN_6O$, $[M+H]^+$). Anal. Calcd for $C_{27}H_{19}FN_6O$: C, 70.12; H, 4.14; N, 18.17; Found: C, 70.20; H, 4.26; N, 18.45.

N-(1-(4-chlorophenyl)-4-cyano-1H-pyrazol-5-yl)-3-phenyl-1-(p-tolyl)-1H-pyrazole-4-carboxamide (5r).

Pale yellow crystal, yield 63%, mp: 172-174℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.04 (s, 1H), 8.50 (s, 1H), 7.83-7.78 (m, 2H), 7.68-7.66 (m, 2H), 7.54-7.42 (m, 6H), 7.35-7.26 (m, 3H), 2.42 (s, 3H), ^{13}C NMR (151 MHz, Chloroform-*d*) δ158.56, 154.64, 149.74, 141.48, 138.02, 136.76, 135.47, 134.76, 131.40, 130.78, 130.14, 129.21, 128.95, 128.72, 126.77, 125.38, 122.31, 119.67, 113.69, 77.2, 21.01. MS (ESI): 479.1

($C_{27}H_{19}ClN_6O$, $[M+H]^+$). Anal. Calcd for $C_{27}H_{19}ClN_6O$: C, 67.71; H, 4.00; N, 17.55; Found: C, 67.94; H, 4.18; N, 17.57.

N-(4-cyano-1-(p-tolyl)-1H-pyrazol-5-yl)-3-phenyl-1-(p-tolyl)-1H-pyrazole-4-carboxamide (5s).

Pale yellow crystal, yield 61%, mp: 135-137℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.04 (s, 1H), 8.50 (s, 1H), 7.85-7.78 (m, 2H), 7.69-7.65 (m, 2H), 7.52-7.46 (m, 3H), 7.36-7.29 (m, 6H), 2.42 (s, 3H), 2.42 (d, J = 1.5 Hz, 6H). MS (ESI): 459.2 ($C_{28}H_{22}N_6O$, $[M+H]^+$). Anal. Calcd for $C_{28}H_{22}N_6O$: C, 73.35; H, 4.84; N, 18.33; Found: C, 73.43; H, 5.02; N, 18.39.

Biological assay

The larvicidal activity of the title compounds and contrast compound ethiprole and chlorantraniliprole against diamondback moth was tested by the leaf-dip method using the reported procedure. The bioassay was replicated at 25 ± 1℃ according to statistical requirements. Assessments were made on a dead/alive basis, and mortality rates were corrected applying Abbott's formula.

Fresh cabbage discs were dipped into the test solutions containing title compounds and the control reagent of for 10s, after air-drying, the treated leaf disks were placed individually in a petri dish lined with filter paper. Thirty larvae of second-instar P. xylostella were carefully transferred to the dried treated leaf disk. Percentage mortalities were evaluated 3 days after treatment. Each treatment was performed three times. The insecticidal activity is summarized in Table 1.

Homology modeling

Plutella xylostella ryanodine receptor (PxRyR) sequences were obtained from the NCBI database (http://www.ncbi.nlm.nih.gov/protein/AEI91094.1). Due to exploring the binding mode bettwen small molecules and protein receptor, we choosed the main domain containing binding sites (LEU3771-GLU5164, 1454 residues) as the major functional domain in the RIP1 protein. In the next step, we submitted this segment sequence into the I-TASSER server maintained by Zhang group (http://zhanglab.ccmb.med.umich.edu/I-TASSER/).

Molecular docking

Docking of compound **5g**, chlorantraniliprole, and fipronil were performed using GLIDE (2012, Schrödinger). A 10-Å search grid was used using the center of mass of active residues. GLIDE docking was carried out in standard precision (SP) mode, and at least 10 poses were requested with a docking score cutoff of 7.0 (anything lower than7.0 was treated as a hit). The poses were inspected in Maestro 9.3 and selected for further analysis.