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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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Insect ryanodine receptor is one of the promising targets for the development of novel insecticides. In order to search for potent insecticides targeting the ryanodine receptor (RyR), a series of novel diphenyl-1H-pyrazole derivatives with cyano substituent were designed and synthesized. Their insecticidal activities against diamondback moth (*Plutella xylostella*) indicated that most of the compounds appeared moderate to high activities at the four concentrations. Among these compounds, compound of *N*-(4-cyano-1-(4-fluorophenyl)-*1H*-pyrazol-5-yl)-1-(4-fluorophenyl)-3-phenyl-*1H*-pyrazole-4-carboxamide (**5g**) appeared 84% larvicidal activities against *Plutella xylostella* at the concentration of 0.1 mg/L. Molecular docking showed the predicted binding mode between **5g** and protein receptor, which could be inferred that the title compounds were the possible activators of insect RyR.

Introduction

Highly efficient and broad-spectra insecticidal pyrazole derivatives have been reported in recent years. As shown in Figure 1, those products of pyrazole derivatives have been widely reported[1-6]. Especially, the pyrazole amide compounds combine outstanding potency on many major agricultural pests with low mammalian toxicity and broad-spectrum[7-9]. However, the frequent and widespread use of traditional insecticides could potentially lead to the development of resistance and the implementation of anti-resistance management strategies. For instance, the *Plutellaxylostella* are kinds of the regularly harmful pests of crops in the world[10], and so far this sort of pests have become a tricky thing in pest control because of their resistance to various types of traditional insecticides[11]. Therefore, to cope with the issue of pest resistance, the discovery of new insecticides which act on new biochemical targets is an urgent need for effective pest control.

Figure 1. The products of insecticidal pyrazole derivatives

Recently, two classes of synthetic chemicals, the phthalic diamides[12-13] and the anthranilic diamides[14-15], both of which could evoke the uncontrolled release of calcium from calcium stores by disturbing the insect RyR, have been widely applied in lepidopteran pest control[16-17]. Chlorantraniliprole, as one representative of anthranilic diamide insecticide registered for the control of lepidopteran pests with high selectivity, has exceptional insecticidal activities on diamondback moth[1]. As shown in Figure 2, it can selectively bind to ryanodine receptors (RyR) in insect muscle cells[11, 18], resulting in an uncontrolled release of calcium from internal stores in the sarcoplasmic reticulum, causing unregulated release of internal calcium in the cell and leading to feeding cessation, lethargy, muscle paralysis and ultimately death of target organisms[16, 19, 20]. Moreover, its binding mode is different from that of all other insecticides[21]. According to J. E. Casida's report[22], there are three distinct binding sites in the ryanodine receptors, one for the anthranilic diamides, another for the phthalic diamide, and a third for the classical Ry. Therefore, the ryanodine receptor has been regarded as one of the targets for novel insecticide discovery.

Figure 2. Schematic representation of the ryanodine receptor and the important associated proteins.

Since the discovery of chlorantraniliprole, much of structural modification focusing on the phenyl and N-pyridylpyrazole moieties has been reported, but the employment of cyano moiety has not been fully reported[23, 24]. The discovery of cyantraniliprole, which was obtained by the replacement of the chlorine moiety in chlorantraniliprole[25] (Figure. 3), could lead to significant increases in the potency and physicochemical properties. In addition, several important modifications over chlorantraniliprole scaffold gave rise to the discovery of novel insecticides and high insecticidal activities[23, 26], and those studies indicated that amide group was a highly efficient and key pharmacophore used widely in insecticides design. Based on this, we envisaged that the incorporation of the Nitrile-containing pyrazole pharmacophores into chlorantraniliprole would provide a series of novel activators that exerted their activity.

Figure 3. Chemical structures of chlorantraniliprole and cyantraniliprole.

Herein, we describe the design and synthesis of a series of Nitrile-containing double pyrazole amide derivatives by integrating the pharmacophore structure of chlorantraniliprole and fipronil which was also pyrazole derivatives insecticide registered for the control of lepidopteran pests with high selectivity[26] (Figure. 4). The insecticidal activities of these compounds were evaluated, and subsequently molecular docking was performed for exploring the binding mode bettween small molecules and the ryanodine receptor.

Figure 4. Design of target compounds.

Result and discussion

Chemistry

A series of novel N-(4-cyano-1-phenyl-1H-pyrazol-5-yl)-1,3-diphenyl-1H-pyrazole-4-carboxamide derivatives were synthesized and the general pathway outlined in **Scheme 1-2**[27, 28]. The requisite intermediates pyrazole carbaldehydes (**2**) were prepared by the reaction of the phenyl hydrazones and Vilsmeier – Haack reagent ($DMF/POCl_3$) respectively[29], then oxidation of the structure **2** with $NaOCl_2$ at 0℃, in the presence of sulfamic acid as scavenger, furnished quantitatively the corresponding acids **3**[27, 28]. The available methods for the synthesis of then target compounds are based on the synthesis of 5-amino-1-aryl-1H-pyrazole-4-carbonitrile **4** followed by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) and N-hydroxybenzotriazole (HOBt) in DMF medium in presence of triethylamine as catalyst at room temperature.

Scheme 1. General synthesis of compounds **5a-5s**.

The intermediate structure **4** were obtained from substituted phenyl hydrazine hydrochloride with 2-(ethoxymethylene) malononitrile in ethanol medium, which was refluxed 3h to gain 5-amino-1-aryl-1H-pyrazole-4-carbonitrile **4**[30] (**Scheme 2**).

Scheme 2. General synthesis of compounds **4a-4e**.

The structures of the newly synthesized compounds were confirmed by ^{1}H NMR, mass spectroscopy and element analysis. In the ^{1}H-NMR ($CDCl_3$) spectra of the compounds, the N-H proton was hard to distinguish in the spectra. To prove its existence, the compounds of **5b, 5c, 5d, 5r** were then recorded the ^{13}C-NMR spectrum taken in DMSO.

Bioactivity

The insecticidal activities for the synthesised compounds (**5a-5s**) against *Plutella xylostella* were evaluated using previously reported procedures[31, 32]. In addition, the commercially available fipronil and chlorantraniliprole were used as the control.

Table 1 showed the insecticidal activities of the target compounds **5a-5s**, the contrast fipronil, and chlorantraniliprole against *Plutella xylostella*. The results indicated that almost all the compounds exhibited to some extent insecticidal activities at the drug concentration of 10 or 5 mg/L. Moreover, it was worth noting that several compounds such as compounds **5g**, **5j** and **5m**, had potent activities when compared with the two positive control fipronil and chlorantraniliprole. Especially, compound **5g** showed the most potent insecticidal activity against *Plutella xylostella*, which could be comparable with chlorantraniliprole. Structure–activity relationships showed that the repalcement of the substituent on the benzene ring could

lead to a remarkable change in bioactivity. For instance, the moiety methyl and no substituent on benzene (**5a** and **5s**) exhibited practically negligible activities. As a whole, the insecticidal activities of the target compounds would be increased when the R_1 and R_2 was replaced by some halogen group. Further to say, as to the R_1, R_2 place, the optimal substituent could be concluded as follows: F > Cl > NO_2 > CH_3, according to their insecticidal activities. Therefore, the compounds with R_1 replaced by F would display much higher activities than others.

Tables 1 Insecticidal activity of synthesised compounds against *Plutella xylostella*

Compound	R_1	R_2	Larvicidal activity (%) at a concentration of (mg/L)				
			10	5	1	0.5	0.1
5a	H	H	63	41	12	0	0
5b	H	F	100	100	58	40	32
5c	H	Cl	100	100	64	44	31
5d	H	CH_3	92	63	21	16	0
5e	H	NO_2	100	100	56	33	22
5f	F	H	100	83	47	31	18
5g	F	F	100	100	100	100	84
5h	F	Cl	100	100	100	88	73
5i	F	CH_3	100	100	89	79	51
5j	F	NO_2	100	100	100	96	79
5k	Cl	H	100	86	42	27	19
5l	Cl	F	100	100	100	89	77
5m	Cl	Cl	100	100	100	100	82
5n	Cl	CH_3	100	95	46	22	0
5o	Cl	NO_2	100	100	100	83	62
5p	CH_3	H	95	62	24	0	0
5q	CH_3	F	100	100	53	27	0
5r	CH_3	Cl	100	100	57	32	16
5s	CH_3	CH_3	54	21	0	0	0
fipronil			100	100	87	63	54
chlorantraniliprole			100	100	100	100	89

Molecular docking

In order to explore the binding mode bettween ligands and target protein, we sellected the segment sequence of the cDNA of *Plutella xylostella* ryanodine receptor (LEU3771-GLU5164, 1454 residues) for homology modeling, which contained six transmembrance domains (TM1-6), the putative pore-forming residues, and three possible binding sites (Site 1-3)[34]. The optimal model was generated by the online I-TASSER Server[35], and presented clearly in Figure 5. The three possible binding sites would be considered as the main docking sites in the subsequent docking study. Docking study of these three compounds (compound **5g**, chlorantraniliprole, and fipronil) on the ryanodine receptor models were performed by the GLIDE algorithm developed on the OPLS2005 force field[36]. At the Site 1 position (Figure 6a-c), compound **5g** seemed to be inserted better into the active pocket than the other compounds, and could take full advantage of the hydrophobic interaction formed by GLU326, ASN355, and LEU356; At the Site 2 position (Figure 6d-f), the binding mode of compound **5g** differed form the others, and from the view of binding mode analysis, compound **5g** seemed to interact more tightly with target protein; At the Site 3 position (Figure 6g-i), obviously, the binding mode of compound **5g** was similar to chlorantraniliprole, and different from fipronil. Additionally, the docking energy of compound **5g** at the three sites (Site 1, 2 and 3) was -5.25, -2.45, and -5.19 kcal/mol, respectively, which was much lower than the corresponding fipronil, and could get close to chlorantraniliprole. Therefore, from the above, we could infer the ryanodine receptor would be one potential target protein of this kind of compounds.

Figure 5. Molecular modelling of *Plutella xylostella* ryanodine receptors (PxRyR, LEU3771-GLU5164). This segment contained six transmembrance domains (TM1-6), conserved residues for the putative pore-forming, and three possible binding sites (Site 1-3)[34]. Homology modeling was built by I-TASSER server[35] and the figure was depicted by pymol 1.7.

Figure 6. Molecular docking of compound **5g**, chlorantraniliprole, and fipronil over three possible binding site (Site 1: GLY322-GLY327, Fig 6a-c; Site 2: GLY1036-SER1042, Fig 6d-f; Site 3: TYR1419-GLN1422, Fig 6g-i). Small molecules: yellow stick; Surrounding residues: green stick.

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 (20 mmol) interact with phenylhydrazine hydrochloride (20 mmol) coupled with sodium acetate (40 mmol) 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 (7 mmol) which was dissolved in acetone was added into the mixture solution of $NaClO_2$ (20 mmol) 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 spin dry 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** (1 mmol) 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 (5 mL) 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, Rf value is 0.32) 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-carboxamide (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-carboxamide (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 fipronil and chlorantraniliprole against diamondback

moth was tested by the leaf-dip method using the reported procedure[33]. 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, and 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 between 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)[36]. 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.

Conclusions

In order to search for potent insecticides targeting the ryanodine receptor (RyR), a series of novel diphenyl-1H-pyrazole derivatives with cyano substituent were designed and synthesized. Their insecticidal activities against diamondback moth (Plutella xylostella) indicated that most of the compounds appeared moderate to high activities at the four concentrations. Among these compounds, compound **5g** showed 84% larvicidal activities against Plutella xylostella at the concentration of 0.1 mg/L. Molecular docking showed the predicted binding mode between **5g** and protein receptor, which could be inferred that the title compounds were the possible activators of insect RyR.

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Notes and references

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

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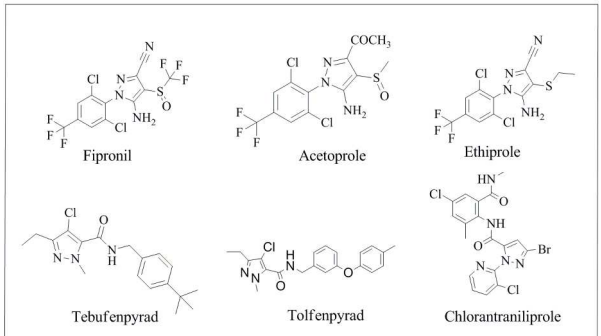

Fig 1.The products of insecticidal pyrazole derivatives

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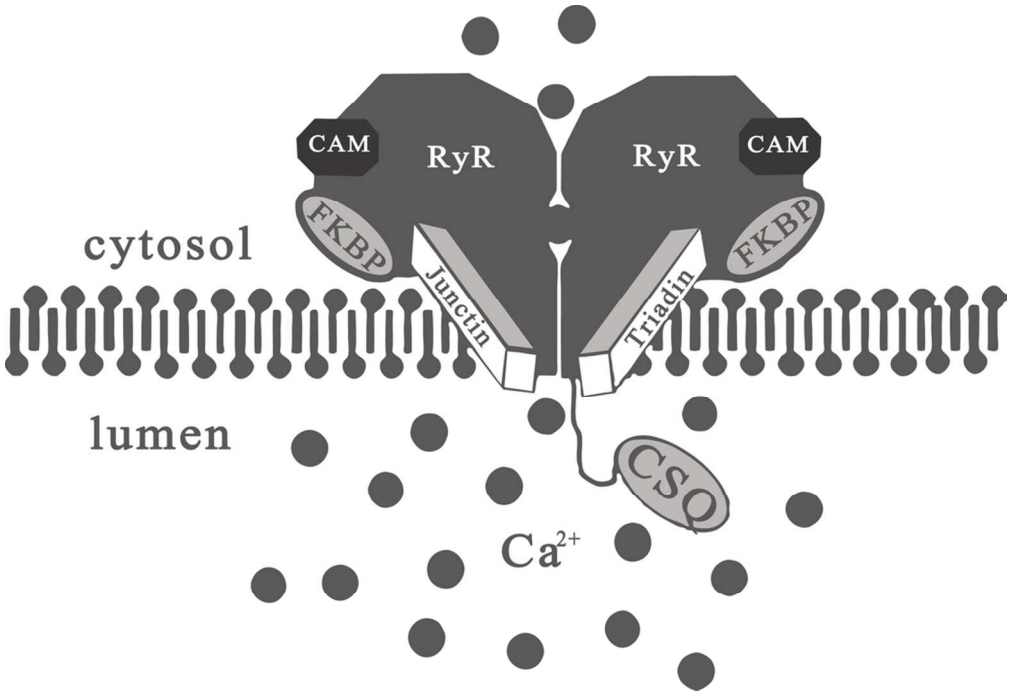

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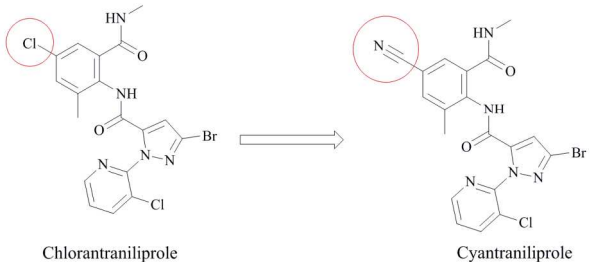

Chlorantraniliprole Cyantraniliprole

Fig 3. Chemical structures of chlorantraniliprole and cyantraniliprole.

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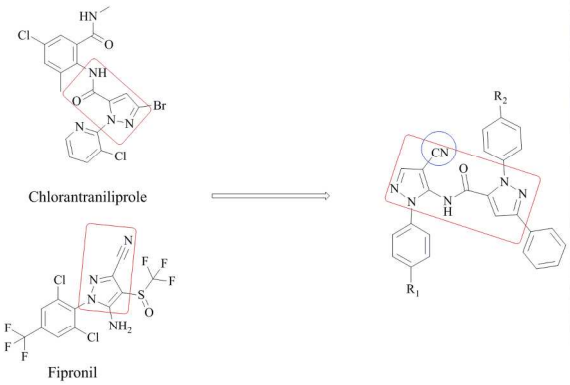

Fig 4. Design of target compounds.

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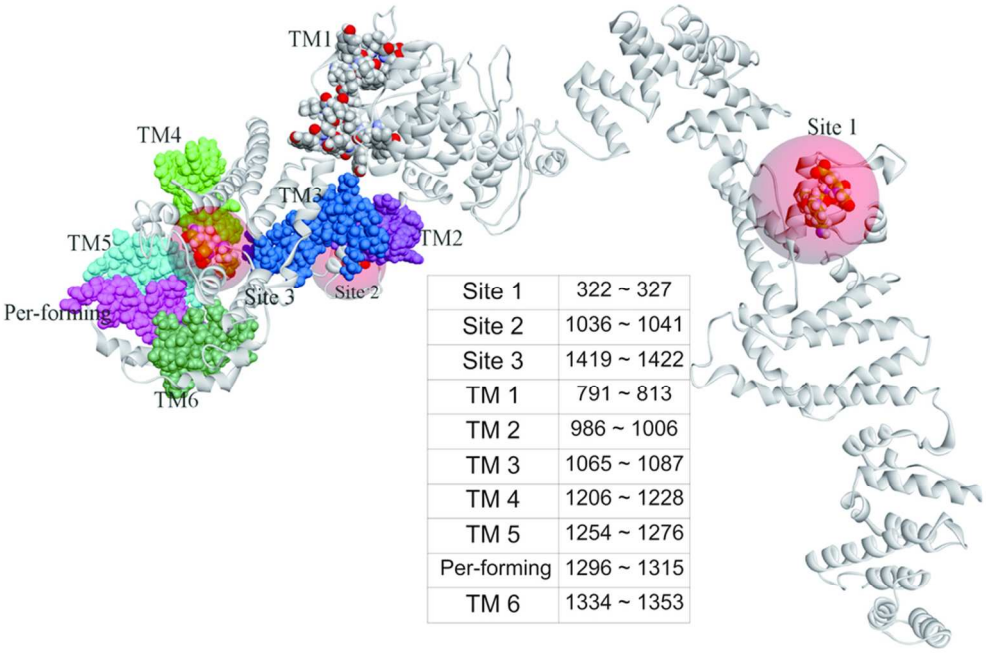
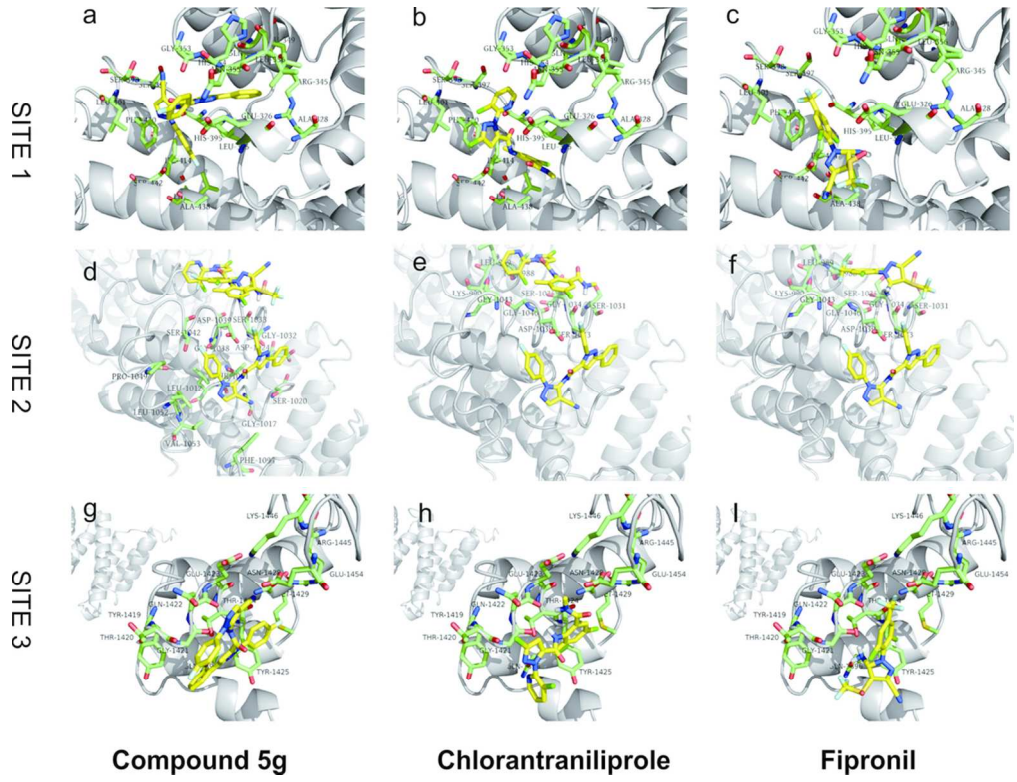


Site 1	322 ~ 327
Site 2	1036 ~ 1041
Site 3	1419 ~ 1422
TM 1	791 ~ 813
TM 2	986 ~ 1006
TM 3	1065 ~ 1087
TM 4	1206 ~ 1228
TM 5	1254 ~ 1276
Per-forming	1296 ~ 1315
TM 6	1334 ~ 1353

88x61mm (300 x 300 DPI)



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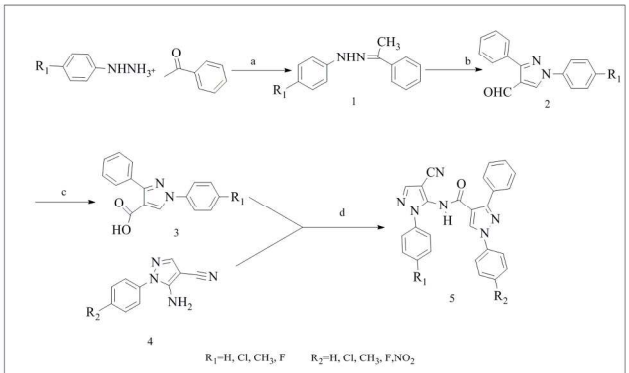

Scheme 1. General synthesis of compounds 5a-5s.

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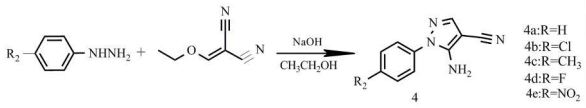

Scheme 2 General synthesis of compounds 4a-4e.

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