JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

Novel Anthranilic Diamides Scaffolds Containing N-Substituted Phenylpyrazole as Potential Ryanodine Receptor Activators

Jingbo Liu, Yuxin Li, xiulan zhang, Xue-Wen Hua, Changchun Wu, Wei Wei, Yingying Wan, Dandan Cheng, Lixia Xiong, Na Yang, Haibin Song, and Zheng-Ming Li

J. Agric. Food Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jafc.6b00380 • Publication Date (Web): 24 Apr 2016

Just Accepted

ACS Publications

Journal of Agricultural and Food Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Novel Anthranilic Diamides Scaffolds Containing *N*-Substituted Phenylpyrazole as Potential Ryanodine Receptor Activators

Jing-Bo Liu, Yu-Xin Li*, Xiu-Lan Zhang, Xue-Wen Hua, Chang-Chun Wu, Wei Wei, Ying-Ying Wan, Dan-Dan Cheng, Li-Xia Xiong, Na Yang, Hai-Bin Song, Zheng-Ming Li*

State Key Laboratory of Elemento-Organic Chemistry, Research Institute of Elemento-Organic Chemistry, Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Nankai University, Tianjin 300071, China

*Corresponding Author: Zheng-Ming Li (nkzml@vip.163.com, Tel.: 0086-22-23503732, Fax: 0086-22-23505948.); Yu-Xin Li (liyx128@nankai.edu.cn, Tel.: 0086-22-23503798)

ABSTRACT: In order to discover potent insecticides targeting at ryanodine receptors (RyRs), a series of novel anthranilic diamides analogs (**12a-u**) containing *N*-substituted phenylpyrazole were designed and synthesized. These compounds were characterized by ^{1}H NMR, ^{13}C NMR and HRMS, and the structure of compound **12u** was confirmed by X-ray diffraction. Their insecticidal activities indicated that these compounds displayed moderate to excellent activities. In particular, **12i** showed 100% and 37% larvicidal activities against oriental armyworm (*Mythimna separata*) at 0.25 and 0.05 mg L^{-1}, equivalent to that of chlorantraniliprole (100%, 0.25 mg $L^{-1}$and 33 %, 0.05 mg L^{-1}). The activity of **12i** against diamondback moth (*Plutella xylostella*) was 95% at 0.05 mg L^{-1}, while the control was 100% at 0.05 mg L^{-1}. The calcium-imaging technique experiment results showed that the effects of **12i** on the intracellular calcium ion concentration ($[Ca^{2+}]_i$) in neurons were concentration-dependent. After the central neurons of *Helicoverpa Armigera* were dyed by loading with fluo-5N and treated with **12i**, the free calcium released in endoplasmic reticulum indicated the target of compound **12i** is RyRs or IP_3Rs. The activation of the RyRs by natural ryanodine completely blocked the calcium release induced by **12i**, which indicated that RyRs in the central neurons of *Helicoverpa Armigera* third-instar larvae is the possible target of compound **12i**.

Keywords: Insectical activity, *N*-substituted phenylpyrazole, Nitro, Chlorine, Calcium channel

INTRODUCTION

The increasing resistance to insecticides has been a major factor in insect control and pest management due to the frequent and widespread use of conventional pesticides with the same mode of action.[1-3] Therefore, to cope with the issue of pest resistance discovering novel potent insecticides with a new mode of action has become an urgent task for scientists. In recent years, chlorantraniliprole (Figure 1A),[4] the first commercial anthranilic diamide insecticide discovered by Dupont, has been proved to bind selectively to insect ryanodine receptor and the binding mode of action is different from that of other traditional insecticides. It causes unregulated release of internal calcium stores leading to Ca^{2+} deletion, feeding cessation, lethargy, muscle paralysis, and ultimately death of insect.[5] Chlorantraniliprole has opened a new era of synthetic insecticides and the insect ryanodine receptor has been considered as one of the key targets to discover novel insecticides.

Chlorantraniliprole has aroused interests worldwide due to its high efficiency, low toxicity and broad insecticidal spectra. Most modifications for chlorantraniliprole were focused on the anthraniloyl moiety,[6-10] the aliphatic amide moiety[11-14] and the pyrazole moiety.[15-17] Nevertheless, the modifications about the pyridine part were seldom reported in the previous work.[18] Recently, our research group has reported novel anthranilic diamides containing *N*-substituted nitrophenylpyrazole with high insecticidal activities against Lepidoptera,[19] indicating that the nitrobenzene moiety can be considered as a bioisostere of pyridine moiety. Moreover, the nitrobenzene moiety will provide more structural modification from the point of synthesis. Enlightened by the descriptions above, in order to

38 systematically explore relationship of structural modification and insecticidal activity, a series of
39 novel anthranilic diamides analogues were designed and synthesized by introducing *N*-
40 phenylpyrazole substructure and exploring the favorable position of nitro and chloro groups on
41 benzene ring (Figure. 1B). To further investigate the mechanism of action, the effects of **12i** on
42 intracellular calcium ion concentration ($[Ca^{2+}]_i$) in the central neurons isolated from the third instar
43 of *M. Separata* and *H. armigera* were studied by the calcium imaging techniques after the neurons
44 dyed with fluo-3 AM and fluo-5N AM. The current research also explored the possible effect of **12i**
45 on the calcium signaling pathway in the neurons.

46 **MATERIALS AND METHODS**

47 **Chemicals** Deuterotrichloromethane ($CDCl_3$), Petroleum ether (60-90), Ethyl acetate,
48 Dichloromethane, Ethanol, n-Hexane, *N*,*N*-dimethylformamide (DMF), Glacial acetic acid, Sodium
49 nitrite, Sodium hydroxide, Tin (II) chloride, Sodium bicarbonate, Sodium sulfate, Potassium
50 carbonate, and Sodium dihydrogen phosphate (Heowns, Tianjin, China); Dimethylsulfoxid-d_6
51 (DMSO-d_6), Acetone and Potassium permanganate (J&K Scientific, China); Oxalyl chloride,
52 Trichloroisocyanuric acid (TCCA), 3-Nitroaniline (**1a**), 2-Fluoro-5-nitroaniline (**1b**), 2-Chloro-5-
53 nitroaniline (**1c**) and 4-Chloro-3-nitroaniline (**1f**) (Aladdin, Shanghai, China); Silica gel (200−300
54 mesh), Ammonium sulfide (20%), 1,3-Dinitrobenzene (**2**), 1-Fluoro-2-nitrobenzene (**8a**) and 1-
55 Fluoro-4-nitrobenzene (**8b**) (Energy Chemical, Shanghai, China).

56 **Instruments and Materials**. The melting points were determined on an X-4 binocular

microscope melting point apparatus (Beijing Tech Instrument Co., Beijing, China) and were uncorrected. ^{1}H NMR spectra were recorded at 400 MHz using a Bruker AV 400 spectrometer (Bruker Co., Switzerland) in $CDCl_3$ or DMSO-d_6 solution with tetramethylsilane as the internal standard, and chemical shift values (δ) were given in parts per million (ppm). High-resolution mass spectrometry (HRMS) data were obtained on a Varian QFT-ESI instrument. Flash chromatography was performed with silica gel (200−300 mesh). Reagents were all analytically pure. All solvents and liquid reagents were dried by standard methods in advance and distilled before use.

2-Bromo-5-nitroaniline **1d** and 2-chloro-3-nitroaniline **1g** were synthesized according to the references.[20,21] 3-chloro-5-nitroaniline **1e** was prepared by referring to literature with some modification as shown in Figure 2.[22,23] Compounds **5** and **9** were synthesized according to the method reported by our previous work.[24] 2-Amino-3,5-disubstituted benzamide derivatives **10a−f** were synthesized with 2-amino-3,5-disubstituted-benzoic acids as materials in moderate yield referring to the literature.[10] Commercial insecticide chlorantraniliprole was used only as contrast compounds and synthesized referring to the literature.[26]

General Synthetic Procedure for Substituted Phenylhydrazine (4a−g).[25] Phenylamine (**1a−g**, 25.0 mmol) was added to an ice-cold aqueous solution of concentrated hydrochloric acid (20 mL), followed by the dropwise addition of sodium nitrite (1.52 g, 22.0 mmol) in distilled water (5 mL). After stirring for 0.5 h at 0 °C, tin (II) chloride (11.28 g, 50 mmol) in concentrated hydrochloric acid (30 mL) was added dropwise to the resulting mixture. The reaction mixture was stirred for 4 h at

0 °C and filtered and successively washed with concentrated hydrochloric acid (10 mL). The precipitate was dissolved in distilled water and filtered. When the filtrate was adjusted to pH 13 by adding 6 mol/L sodium hydroxide under stirring, a large amount of solid formed and was filtered and dried to obtain compounds **4a–g**: yields 66.2%–75.5%.

General Synthetic Procedure for Compounds 6a–g.[10] A mixture of phenylhydrazine (**4a-g**, 20.0 mmol), 1,1,1-trifluoro-4-(furan-2-yl)-4-hydroxybut-3-en-2-one (**5**, 4.12 g, 20.0 mmol) and glacial acetic acid (15 mL) was refluxed for 5-6 h. The reaction mixture was condensed under reduced pressure and ethyl acetate (35 mL) was added. The mixture was washed with saturated sodium bicarbonate solution (15 mL) and brine (10 mL) and dried with anhydrous sodium sulfate. The solvent was evaporated in a vacuum, and the residue was purified on silica gel with petroleum/ethyl acetate (v/v, 50:1) to obtain compounds **6a–g**: yields 59.1%–71.9%.

General Synthetic Procedure for Compounds 6h, 6i.[19] Potassium carbonate (4.12 g, 30 mmol) was added to a solution of 5-(furan-2-yl)-3-(trifluoromethyl)-1H-pyrazole (**9**, 4.04 g, 20.0 mmol) in *N*,*N*-dimethylformamide (15mL) and water (2 mL). The reaction mixture was stirred at 55–60 °C for 1 h. Then 1-fluoro-2-nitrobenzene (**8a**, 3.38 g, 24 mmol) or 1-fluoro-4-nitrobenzene (**8b**, 3.38 g, 24 mmol) in 5 mL of *N*,*N*-dimethylformamide was added dropwise to the mixture. The reaction mixture was further stirred at 120–130 °C for 4 h and concentrated in vacuum. The residue was extracted with ethyl acetate (30 mL × 3) and then washed with dilute hydrochloric acid (15 mL), saturated sodium bicarbonate solution (15 mL) and brine (10 mL) successively, dried with anhydrous sodium

sulfate. The solvent was evaporated in a vacuum, and the residue was purified on silica gel with petroleum/ethyl acetate (v/v, 50:1) to obtain compounds **6h**, **6i**.

General Synthetic Procedure for Compounds 7a–i.[15] Potassium permanganate (22.12 g, 50.0 mol) was added in portion to a stirring mixture of 5-(furan-2-yl)-1-phenyl-3-(trifluoromethyl)-1*H*-pyrazole (**6a–i**, 10.0 mmol) and sodium dihydrogen phosphate (1.20 g, 10.0 mmol) in 25 mL of acetone and 25 mL of distilled wate. The reaction was slowly heated to reflux and kept for 0.5 h. The resulting mixture was filtered and washed with ethyl acetate (35 mL). The filtrate was acidified with dilute hydrochloric acid (5 mL) and dried with anhydrous sodium sulfate. The solvent was evaporated to obtain compounds **7a–i**, which were used directly in the next step without further purification.

General Synthetic Procedure for Title Compounds 12a–u. To a solution of 1-phenyl-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxylic acid (**7a–i**, 0.5 mmol) in 5 mL of anhydrous dichloromethane were added successively oxalyl chloride (0.127 g, 1.0 mmol) and one drop of *N*,*N*-dimethylformamide (DMF) at room temperature. After stirring for 1.5 h, the solvent was evaporated. The resulting carbonyl chloride was dissolved in 5 mL of anhydrous tetrahydrofuran and added to an ice-cold solution of triethylamine (0.101 g, 1.0 mmol) and 2-amino-3-methylbenzamide derivatives (**10a–f**, 0.5 mmol) in 5 mL of anhydrous tetrahydrofuran. After stirring at room temperature overnight, the reaction mixture was added dichloromethane (20 mL), and washed with dilute hydrochloric acid (10 mL × 2) and saturated sodium bicarbonate solution (10 mL× 2). The organic

114 layer was dried and evaporated. The residue was purified on silica gel with petroleum/ethyl acetate
115 (v/v, 4:1) to obtain compounds **12a–u**.

116 **X-ray Diffraction**. Compound **12u** was recrystallized from a mixture of dichloromethane and n-
117 Hexane (v:v＝5:1) to colorless crystals with dimensions of 0.20 mm × 0.18 mm × 0.12 mm suitable
118 for X-ray single crystal diffraction. The diffraction data were collected on a Rigaku Saturn 724 CCD
119 diffractometer Mokα radiation (λ = 0.71073 Å). The crystal is a monoclinic system, space group
120 *P2(1)/n*, with crystallographic parameters: a =15.081 (3) Å, b = 33.486 (7) Å, c = 21.263 (4) Å, α =
121 90°, β = 93.41 (3)°, γ = 90°, V = 10719(4) Å^3, Z = 16, D_c = 1.494 g cm^{-3}, μ= 1.692 mm^{-1}, F(000) =
122 4864, R = 0.1126, T = 113 (2) K, wR= 0.1789, final R factor = 6.50%. The structure was solved by
123 direct methods with the *SHELXL-97* program.[27] All the non-H atoms were refined anisotropically by
124 full-matrix least-squares, and hydrogen atoms were located from a difference Fourier map and were
125 placed at calculated positions and were included in the refinements in the riding mode with isotropic
126 thermal parameters.

127 **Biological Assay**. All biological assays were performed on test organisms reared in a greenhouse.
128 The bioassay was replicated at 25 ± 1 °C according to statistical requirements. Assessments were
129 made on a dead/alive basis, and mortality rates were corrected applying Abbott's formula.[28]
130 Evaluation was based on a percentage scale of 0–100, in which 0 equals no activity and 100 equals
131 total kill. The standard deviations of the tested biological values were ±5%. LC_{50} and LC_{95} values
132 were calculated by probit analysis.[29]

Larvicidal Activity against Oriental Armyworm (*M. separata*). The larvicidal activity of title compounds **12a–u** and chlorantraniliprole against oriental armyworm was tested according to the leaf-dip method using the reported procedure.[30] The insecticidal activity is summarized in Table 1. LC_{50} and LC_{95} values of compound **12i** and chlorantraniliprole against oriental armyworm are listed in Table 2.

Larvicidal Activity against Diamondback Moth (*P. xylostella*). The larvicidal activities of compounds **12h–12j, 12o–12p** and chlorantraniliprole were tested by the leaf-dip method using the reported procedure.[31] The insecticidal activity is summarized in Table 3.

Calcium Imaging Experiment. The insecticideal mechanism of these novel compounds was investigated according to calcium imaging experiment reported by our previous work.[13,32]

RESULT AND DISCUSSION

Chemistry. Compound **1e** were prepared by referring to literature with some modification as shown in Figure 2.[22,23] Compounds **4a–g** were prepared from different substituted amines **1a–g** through diazotization and reduction in 66.2%–78.5% yields,[25] in which the reaction temperature was strictly controlled -5 °C – 0 °C to reduce the generation of byproducts.

In order to find the SAR, different substituted phenylpyrazole carboxylic acid (**7a–i**) were synthesized. Firstly, compounds **4a–g** were condensed with intermediate **5** to regioselectively give compounds **6a–g**, and compounds **6h**, **6i** were prepared according to the reported method.[21] Then the furan moiety of compounds **6a–i** was oxidized with potassium permanganate at reflux to afford the

corresponding carboxylic acid **7a–i**. Compounds **7a–i** were converted to the acyl chlorides and coupled with compounds **10a–f** to obtain the title compounds **12a–u** as shown in Figure 5 in 56.6%–78.9% yields.

Crystal Structure Analysis. The molecular stucture of **12u** is shown in Figure 6. Generally, the average bond lengths and bond angles of benzene ring were normal.[33-36] Due to the *p*-π conjugate effect, the bond lengths of N (4)－C (5) and N (5)－C (13) were 1.353(7) Å and 1.347(7) Å, respectively, much shorter than the normal C－N (1.47 Å), but close to C＝N (1.33 Å).[37-39] The sum of C (5)－N (4)－C (6), C (5)－N (4)－H (4) and C (6)－N (4)－H (4) angles was 360.00°, indicating the sp^2 hybridization state of the N (4) atom. Similarly, the N (5) atom also adopted a sp^2 hybridization state. The benzene ring (C18 to C23) and pyrazole ring (N1/N2/C2/C3/C4) are non-planar with a dihedral angle (θ) of 44.2°. The packing structure demonstrates the existence of intermolecular hydrogenbond: N－H···O, N－H···F and N－H···Br. Additionally, weak π-π interactions occurred among the benzene and pyrazole rings of the adjacent molecules. These interactions are estimated to stengthen the stability of the crystal strucuture.

Structure–Activity Relationship (SAR). *Larvicidal Activity against Oriental Armyworm*. The larvicidal activity of target compounds **12a**-**12u** against oriental armyworm is summarized in Table 1. The bioassay results indicated that most compounds have moderate to excellent larvicidal activities against oriental armyworm. For example, the larvicidal activity of **12h**, **12i** and **12j** against oriental armyworm was 100% at 1 mg L^{-1}, same with that of chlorantraniliprole (1 mg L^{-1}, 100%).

Furthermore, **12i** showed 37% larvicidal activity at 0.05 mg L^{-1}, which was a little effective than the standard chlorantraniliprole (0.05 mg L^{-1}, 33%) against oriental armywarm.

To explore the relationship between activities of target molecules with the position of nitro on benzene ring of phenylpyrazole moiety, compounds **12a** (R'_x = 4-NO_2), **12b** (R'_x = 2-NO_2) and **12c** (R'_x = 3-NO_2) were designed and synthesized. The larvicidal activity of **12a** (R'_x = 4-NO_2), **12b** (R'_x = 2-NO_2) and **12c** (R'_x = 3-NO_2) at concentration of 10 mg L^{-1} were 0%, 20% and 90%, respectively, indicating that the position of nitro on benzene ring of phenylpyrazole moiety has an important effect on the larvicidal activity. What is more, the sequence of the larvicidal activities was **12c** (R'_x =3-NO_2) > **12b** (R'_x =2-NO_2) > **12a** (R'_x =4-NO_2). Based on the above research, in order to further investigate the effect of the position of subsitituent Cl on benzene ring of the phenylpyrazole moiety, compounds **12h–k** (R'_x = 2-Cl, 5-NO_2), **12l–n** (R'_x = 3-Cl, 5-NO_2), **12o–q** (R'_x = 2-Cl, 3-NO_2) and **12r–u** (R'_x = 4-Cl, 3-NO_2) were designed and synthesized. Generally, the sequence of the larvicidal activities of these compounds was **12h–k** (R'_x = 2-Cl, 5-NO_2) > **12l–n** (R'_x = 2-Cl, 3-NO_2) >> **12o–q** (R'_x = 4-Cl, 3-NO_2) ≥ **12r–u** (R'_x = 3-Cl, 5-NO_2). Moreover, compounds **12h–k** (R'_x = 2-Cl, 5-NO_2) and **12o–q** (R'_x = 2-Cl, 3-NO_2) showed much higher insecticidal activities against oriental armywarm than that of **12c-g** (R'_x = 3-NO_2). For example, **12h** (R'_x = 2-Cl, 5-NO_2) and **12o** (R'_x = 2-Cl, 3-NO_2) showed 100% and 80% insecticidal activity at 0.25 mg L^{-1}, respectively, whereas **12c** (R'_x = 3-NO_2) gave a death rate of 30% at only 5 mg L^{-1}. These observations clearly showed that the substituent Cl in the 2-position of benzene ring had a positive effect on the larvicidal activities, and is a key

pharmacophore. However, the larvicidal activities of **12o–q** (R'_x = 4-Cl, 3-NO_2) and **12r–u** (R'_x = 3-Cl, 5-NO_2) were lower than that of **12c-g** (R'_x = 3-NO_2). For instance, the larvicidal activity of **12e** (R'_x = 3-NO_2) at 10 mg L^{-1} were 50%, whereas **12n** (R'_x = 3-Cl, 5-NO_2) and **12t** (R'_x = 4-Cl, 3-NO_2) were almost inactive at the same dosage. The results indicated that the existence of 3-Cl or 4-Cl on benzene ring of the phenylpyrazole moiety had a negative effect on the larvicidal activities. Hence, the position of substituent Cl on benzene ring of phenylpyrazole moiety has a significant effect on the larvicidal activity. In addition , as shown in Table 1, the bioactivity of target compounds **12a-u**, when R_1 and R'_x was fixed, indicated the sequence of larvicidal activity was *i*-propyl > *t*-Bu ≥ CH_3 in the aliphatic amide moiety (R_2); and when R_2 and R'_x was fixed, indicated the sequence of larvicidal activity was Cl > Br in the anthraniloyl moiety (R_1).

From Table 2, it is noteworthy that the LC_{50} and LC_{95} values of **12i** were 0.0772 and 0.4064 mg L^{-1}, respectively, slightly higher than that of chlorantraniliprole (0.0664 mg L^{-1} and 0.3237 mg L^{-1}), indicating that the larvicidal activity of **12i** was comparable to that of chlorantraniliprole.

Larvicidal Activity against Diamondback Moth. The larvicidal activity of target compounds **12h-j**, **12o** and **12p** against diamondback moth is showed in Table 3. Most of them had excellent insecticidal activity against diamondback moth. At 1 mg L^{-1} all of the compounds showed 100% larvicidal activity. It is worth noting that **12i** showed a death rate of 95% at 0.05 mg L^{-1}, equal to that of chlorantraniliprole (0.05 mg L^{-1}, 100%). The activities of target compounds, when R'_x and R_1 were fixed, indicated the sequence of larvicidal activity was *i*-propyl > CH_3 in the R_2; and when R'_x

209 and R_2 were fixed, indicated the trend Cl > Br in the R_1, which was consistent with the above
210 research.

211 **Calcium Imaging.** For studying the insecticidal mechanism of these novel compounds, we
212 examined the effects on the calcium concentration by using central neurons of *M. Separata* and *H.*
213 *Armigera* with calcium imaging technique after the neurons loading with fluo-3AM and fluo-5N AM
214 (the low-affinity Ca^{2+} indicator of ER). Figure 7A and 7B show the change of $[Ca^{2+}]_i$ versus
215 recording time when the *M. Separata* and *H. Armigera* central neurons were treated with different
216 concentrations of compound **12i** and chlorantraniliprole from 10 ppm, 5pm, 2ppm to 1 ppm in the
217 absence of extracellular calcium, respectively. The experiment results indicated that there is no
218 apparent difference effect on the calcium concentrations elevated by **12i** between the central neurons
219 of *M. Separata* and *H. Armigera.* Based on the calcium cencentrations elevated by different
220 concentrations of **12i** and chlorantraniliprole, it is concluded there is slight difference between the
221 insecticidal activities against *M. Separata* and *H. Armigera.*

222 Figure 8A shows that the enhancement of free calcium concentration in neurons in the presence of
223 extracellular calcium is the same as that in the absence of extracellular calcium because the peaks of
224 $[Ca^{2+}]_i$ were all elevated to 105% of the initial value when the *H. Armigera* neurons were treated
225 with 10 ppm **12i**. It indicates that elevation of Ca^{2+} concentrations is attributed to the release of
226 calcium ions from the endoplasmic reticulum and **12i** could not activate the calcium channel on the
227 plasma membrane. To further determine the paths that induced the elevation of extracellular calcium

228 in the neuron cells, the central neurons of *H. Armigera* were loaded with fluo-5N AM, and the results
229 show that **12i** could release the calcium from the endoplasmic reticulum by RyRs or IP3Rs (Figure
230 8B).

231 Figure 9 shows that after application of caffeine (30 mM) and the co-application of **12i** together
232 with ryanodine (3 μM), caffeine (30 mM) and **12i** could not induce the calcium responses. The
233 results demonstrate that **12i** could activate ryanodine receptor, the same as caffeine.

234 Figure 10 shows the impact of 12i on the calcium response after the neurons pre-incubated by
235 thapsigargin. In our experiments, after the intracellular calcium store in the neurons of *H. Armigera*
236 was depleted by thapsigargin (30 μM), **12i** could not cause any calcium responses, meaning that
237 there are no other targets except ryanodine receptor.

238 In summary, a series of novel anthranilic diamides analogs (**12a-u**) containing *N*-substituted
239 phenylpyrazole were designed and synthesized. The premilinary structure-activity relationship (SAR)
240 of the target compounds indicated that the position of nitro and chlorine on benzene ring of
241 phenylpyrazole moiety exerted different effects on activity following the sequence **12h**–**k** (R'_x = 2-Cl,
242 5-NO_2) > **12l**–**n** (R'_x = 2-Cl, 3-NO_2) > **12c** (R'_x =3-NO_2) > **12b** (R'_x =2-NO_2) > **12a** (R'_x =4-NO_2) >
243 **12o**–**q** (R'_x = 4-Cl, 3-NO_2) ≥ **12r**–**u** (R'_x = 3-Cl, 5-NO_2), and indicating that R'_x (2-Cl, 5-NO_2) on
244 benzene ring of the phenylpyrazole moiety was a key pharmacophore for the maintenance of
245 insecticidal activity. Especially, compound **12i** showed 100% and 37% larvicidal activities against
246 oriental armyworm at 0.25 mg L^{-1} and 0.05 mg L^{-1}, respectively, equal to that of chlorantraniliprole

(100%, 0.25 mg L^{-1} and 33%, 0.05 mg L^{-1}). The activity of **12i** against diamondback moth was 95% at 0.05% mg L^{-1}, while the control was 100% at 0.05% mg L^{-1}. More importantly, the calcium imaging technique experiments indicated that RyRs in the central neurons of *H. Armige*ra is the biochemical target, meaning that the title compounds were ryanodine receptor activators.

Associated Content

Supporting Information. The data and characterization of the intermediates **3**, **1d**, **1e**, **1g**, **4a**–**4g**, **6a**–**6i** and the target compounds **12a**–**u** is available free of charge via the Internet at http:// pubs. acs.org

Author Information

Corresponding Author

Tel.: 0086-22-23503732 (Z. Li.), 0086-22-23503798 (Y. Li.), Fax: 0086-22-23505948.

E-mail: nkzml@vip.163.com (Z. Li), liyx128@nankai.edu.cn (Y. Li).

Acknowledgment

This work was supported by the National Science Foundation of China (No. 21272129, No. 31370039) and the “111” Project of Ministry of Education of China (No. B06005).

Notes

The authors declare no competing financial interest.

References

(1) Tabashnik, B. E.; Mota-Sanchez, D.; Whalon, M. E.; Hollingworth, R. M.; Carriere, Y. Defining terms for proactive management of resistance to Bt crops and pesticides. *J. Econ. Entomol.* **2014**, *107*, 496–507.

(2) Forgash. A. J. History, evolution, and consequences of insecticide resistance. *Pestic. Biochem. Physiol.* **1984**, *22*, 178–186.

(3) Whalon, M. E.; Mota-Sanchez, D.; Hollingworth, R. M. *Analysis of global pesticide resistance in arthropods*; Global Pesticide Resistance in Arthropods, CAB International, Wallingford, UK, **2008**; pp 5–31.

(4) Lahm, G. P.; Selby, T. P.; Frendenberger, J. H.; Stevenson, T. M.; Myers, B. J.; Seburyamo, G.; Smith, B. K.; Flexner, L.; Clark, C. E.; Cordova, D. Insecticidal anthranilic diamides: a new class of potent ryanodine receptor activators. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4898–4906.

(5) Lahm, G. P.; Stevenson, T. M.; Selby, T. P.; Frendenberger, J. H.; Cordova, D.; Flexner, L.; Bellin, C. A.; Dubas, C. M.; Smith, B. K.; Hughes, K. A.; Hollingshaus, J. G.; Clark, C. E.; Benner, E. A. Rynaxypyr™: a new insecticidal anthranilic diamide that acts as a potent and selective ryanodine receptor Activator. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6274–6279.

(6) Loiseleur, O.; Hall, R. G.; Stoller, A. D.; Graig, G. W.; Jeanguenat, A.; Edmunds, A. Novel insecticides. WO Patent 2009024341 A1, **2009**.

(7) Loiseleur, O.; Durieux, P.; Trah, S.; Edmunds, A.; Jeanguenat, A.; Stoller, A.; Hughes, D. J. Pesticides containing a bicyclic bisamide structure. WO Patent 2007093402 A1, **2007**.

(8) Mao, M. Z.; Li, Y. X.; Liu, Q. X.; Zhou, Y. Y.; Zhang, X. L.; Xiong, L. X.; Li, Y. Q.; Li, Z. M. Synthesis and insecticidal evaluation of novel *N*-pyridylpyrazolecarboxamides containing cyano substituent in the ortho-position. *Bioorg*. *Med. Chem*. *Lett*. **2013**, *23*, 42–46.

(9) Chen, Y. B.; Li, J. N.; Shao, X. S.; Xu, X. Y.; Li, Z. Design, synthesis and insecticidal activity of novel anthranilic diamides with benzyl sulfide scaffold. *Chin. Chem. Lett.* **2013**, *24*, 673–676.

(10) Zhang, J. F.; Xu, J. Y.; Wang, B. L.; Li, Y. X.; Xiong, L. X.; Li, Y. Q.; Ma, Y.; Li. Z. M. Synthesis and insecticidal activities of novel anthranilic diamides containing acylthiourea and acylurea. *J. Agric. Food Chem.* **2012**, *60*, 7565−7572.

(11) Kruger, B.; Hense, A.; Alig, B.; et al. Dioxazine- and oxadiazinesubstituted arylamides. WO Patent 2007031213 A1, **2007**.

(12) Liu, Q.; Chen, K.; Wang, Q.; Ni, J. P., Li, Y. F., Zhu, H. J.; Ding, Y. Synthesis, insecticidal activity, structure-activity relationship (SAR) and density functional theory (DFT) of novel anthranilic diamides analogs containing 1,3,4-oxadiazole rings. *RSC Adv.* **2014**, *4*, 55445–55451.

(13) Mao, M. Z.; Li, Y. X.; Zhou, Y. Y.; Zhang, X. L.; Liu, Q. X.; Di, F. J.; Song, H. B.; Xiong, L. X.; Li, Y. Q.; Li, Z. M. Synthesis and insecticidal evaluation of novel *N*-pyridylpyrazolecarboxamides containing an amino acid methyl ester and their analogues. *J. Agric. Food Chem.* **2014**, *62*, 1536−1542.

(14) Luo, M.; Chen, Q.; Wang, J.; Hu, C.; Lu, J.; Luo, X.; Sun, D. Novel chlorantraniliprole derivatives as potential insecticides and probe to chlorantraniliprole binding site on ryanodine receptor. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 1987–1992.

(15) Feng, Q.; Liu, Z. L.; Xiong, L. X.; Wang, M. Z.; Li, Y. Q.; Li, Z. M. Synthesis and insecticidal activities of novel anthranilicdiamides containing modified N-pyridylpyrazoles. *J. Agric. Food Chem.* **2010**, *58*, 12327−12336.

(16) Zhao, Y.; Li, Y. Q.; Xiong, L. X.; Wang, H. X.; Li, Z. M. Design, synthesis and biological activities of novel anthranilic diamide insecticide containing trifluoroethyl ether. *Chin. J. Chem.* **2012**, *30*, 1748−1758.

(17) Chen, K.; Liu, Qi.; Ni, J. P.; Zhu, H. J.; Li, Y. F.; Wang, Q. Synthesis, insecticidal activities and structure-activity relationship studies of novel anthranilic diamides containing pyridylpyrazole-4-carboxamide. *Pest Manag. Sci.* **2015**, *71*, 1503−1512.

(18) Kaiser, F.; Deshmukh, P.; Körber, K.; Von Deyn, W.; Dickhaut, J.; Narine, A.; Bandur, N. G.; Culbertson, D. L.; Anspaugh, D. D. Carbamoylmethoxy- and carbamoylmethylthio- and carbamoylmethylamino benzamides for combating invertebrate pests. WO Patent 2013024170 A1, **2013**.

(19) Zhang, X. L.; Li, Y. X.; Ma, J. L.; Zhu, H. W.; Wang, B. L.; Mao, M. Z.; Xiong, L. X.; Li, Y. Q.; Li, Z. M. Synthesis and insecticidal evaluation of novel anthranilic diamides containing *N*-substitued nitrophenylpyrazole. *Bioorg. Med. Chem.* **2014**, *22*, 186−193.

322 (20) Gan, Z. J.; Hu, B.; Song, Q.; Xu, Y. G. Convenient chlorination of some special aromatic
323 compounds using *N*-chlorosuccinimide. *Synthesis* **2012**, *44*, 1074–1078.
324 (21) Bartoli, S; Cipollone, A; Squarcia, A; Madami, A; Fattori, D. Electrophilic bromination of meta-
325 substituted anilines with *N*-bromosuccinimide: regioselectivity and solvent effect. *Synthesis* **2009**, *8*,
326 1305–1308.
327 (22) Mendonça, G. F.; Senra, M. R.; Esteves, P. M.; de Mattos, M. C. S. Trichloroisocyanuric acid in
328 98% sulfuric acid: A superelectrophilic medium for chlorination of deactivated arenes. *Appl. Catal.*
329 *A: Gen.* **2011**, *401*, 176–181.
330 (23) Venkateshappa, C.; Sivanandhan, D.; Bakthavatchalam, R.; Kethiri, R. R.; Viswanadhan, V.;
331 Giri, S. Substituted pyrimidine compounds, compositions and medicinal applications thereof. WO
332 Patent 2015025197 A1, **2015**.
333 (24) Wang, B. L.; Zhu, H. W.; Ma, Y.; Xiong, L. X.; Li, Y. Q.; Yu Zhao, Y,; Ji-Feng Zhang, J. F.;
334 Chen, Y. W.; Zhou, S.; Li. Z. M. Synthesis, insecticidal activities, and sar studies of novel
335 pyridylpyrazole acid derivatives based on amide bridge modification of anthranilic diamide
336 insecticides. *J. Agric. Food Chem.* **2013**, *61*, 5483−5493.
337 (25) Gima, H. Jin.; Li, H.; Jeong, J. H. Lee, Su. J.; Sung, M. K.; Song, M. Y.; Park, B. H.; Oh, S. Jin.;
338 Ryu, J. H.; Jeon, R. Design, synthesis, and biological evaluation of a series of alkoxy-3-indolylacetic
339 acids as peroxisome proliferator-activated receptor c/d agonists. *Bioorg. Med. Chem.* **2015**, *23*,
340 3322–3336.

(26) Dong, W. L.; Xu, J. Y.; Xiong, L. X.; Liu, X. H.; Li, Z. M. Synthesis, structure and biological activities of some novel anthranilic acid esters containing *N*-pyridylpyrazole. *Chin. J. Chem.* **2009**, *27*, 579−586.

(27) Sheldrick, G. M. *SHELXS97 and SHELXL9*7; University of Göttingen, Germany, **1997**.

(28) Abbott, W. S. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* **1925**, *18*, 265−267.

(29) Raymond, M. Presentation d'um programme basicd'analyse log-probit pour miero-ordinateur. *Cah. Orstom Ser. Entomol. Med. Parasitol.* **1985**, *23*, 117−121.

(30) Wu, Y. D.; Shen, J. L.; Chen, J.; Lin, X. W.; Li, A. M. Evaluation of two resistance monitoring methods in Helicoverpa armigera: topical application method and leaf dipping method. *Plant Prot.* **1996**, *22* (5), 3−6.

(31) Ma, H.; Wang, K. Y.; Xia, X. M.; Zhang, Y.; Guo, Q. L. The toxicity testing of five insecticides to different instar larvae of Spodoptera exigua. *Mod. Agrochem.* **2006**, *5*, 44−46.

(32) Chen, Y. W.; Li, Y. X.; Pan, L.; Liu, J. B.; Wan, Y. Y.; Chen, W.; Xiong, L. X.; Yang, N.; Song, H. B.; Li, Z. M. Synthesis, insecticidal activities and SAR of novel phthalamidestargeting calcium channel. *Bioorg. Med. Chem.* **2014**, *22*, 6366–6379.

(33) Zhou, S.; Gu, Y. C.; Liu, M.; Wu, C. C.; Zhou, S.; Zhao, Y.; Jia, Z. H.; Wang, B. L.; Xiong, L. X.; Yang, N.; Li, Z. M. Insecticidal activities of chiral *N*-trifluoroacetyl sulfilimines as potential ryanodine receptor modulators. *J. Agric. Food Chem.* **2014**, *62*, 11054−11061.

(34) Liu, X. H.; Sun, Z. H.; Yang, M. Y.; Tan, C. X.; Weng, J. Q.; Zhang, Y. G.; Ma, Y. Microwave assistant one pot synthesis, crystal structure, antifungal activities and 3D-QSAR of novel 1,2,4-triazolo[4,3-a]pyridines. *Chem. Biol. Drug Des.* **2014**, *84*, 342–347.

(35) Liu, X. H.; Zhai, Z. W.; Xu, X. Y.; Yang, M. Y.; Sun, Z. H.; Weng, J. Q.; Tan, C. X.; Chen, J. Facile and efficient synthesis and herbicidal activity determination of novel 1,2,4-triazolo[4,3-a]pyridin-3(2*H*)-one derivatives via microwave irradiation. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 5524–5528.

(36) Liu, X. H.; Xu, X. Y.; Tan, C. X.; Weng, J. Q.; Xin, J. H.; Chen, J. Synthesis, crystal structure, herbicidal activities and 3D-QSAR study of some novel 1,2,4-triazolo[4,3-*a*]pyridine derivatives. *Pest Manag. Sci.* **2015**, *71*, 292–301.

(37) Ye, Z. J.; Shi, L. N.; Shao, X. S.; Xu, X. Y.; Xu, Z. P.; Li, Z. Pyrrole- and dihydropyrrole-fused neonicotinoids: design, synthesis, and insecticidal evaluation. *J. Agric. Food Chem.* **2013**, *61*, 312−319.

(38) Shen, Z. H.; Shi, Y. X.; Yang, M. Y.; Sun, Z. H.; Weng, J. Q.; Tan, C. X.; Liu, X. H.; Li, B. J.; Zhao, W. G. Synthesis, crystal structure, DFT studies and biological activity of a novel schiff base containing triazolo[4,3-*a*]pyridine moiety. *Chin. J. Struct. Chem.* **2016**, *35*, 457–464.

(39) Sun, G. X.; Shi, Y. X.; Zhai, Z. W.; Yang, M. Y.; Weng, J. Q.; Tan, C. X.; Liu, X. H.; Li, B. J. Synthesis, crystal structure and biological activity of (E)-3-chloro-2-(2-(2-methylbenzylidene)-hydrazinyl)pyridine. *Chin. J. Struct. Chem.* **2016**, *35*, 640–644.

Figure Captions

Figure 1 Chemical structures of the design of target compounds

Figure 2 Synthetic Route of Compound **1e**

Figure 3 Synthetic Route of Compounds **6a–g**

Figure 4 Synthetic Route of Compounds **6h** and **6i**

Figure 5 Synthetic Route of Title Compounds **12a–u**

Figure 6 Crystal structure of compound **12u**

Figure 7 Effects of different concentration of **12i** and chlorantaniliprole on $[Ca^{2+}]_i$ in the central neurons of *M. Separata* (**A**) and *H. Armigera* (**B**) when extracellular calcium was in absence (EGTA replaced calcium). The central neurons of *M. Separata* and *H. Armigera* third-instar larvae were dyed by loading with fluo-3 AM.

Figure 8 Effect of treatments with 10 ppm of **12i** in isolated *H. Armigera* in the presence or absence of extracellular calcium. The central neurons of *H. Armigera* third-instar larvae were dyed by loading with fluo-3 AM (**A**). Effect of treatments with 10 ppm of **12i** on intracellular Ca^{2+} at different time in the absence of extracellular Ca^{2+} (EGTA replaced Ca^{2+}). The central neurons of *H. Armigera* third-instar larvae were dyed by loading with fluo-5N (**B**).

Figure 9 Characterization of **12i** stimulated calcium responses in the central neurons of *H. Armigera* third-instar larvae. Repeated challenges with caffeine, **12i**, co-application of ryanodine together with

12i, caffeine and **12i** in standard saline. The central neurons of *H. Armigera* third-instar larvae were dyed by loading with fluo-3 AM.

Figure 10 Effects of 100 ppm of 12i on $[Ca^{2+}]_i$ in the central neurons of *H. Armigera* when the neurons preincubated thapsigargin. The central neurons of *H. Armigera* third larvae were dyed by loading with fluo-3 AM.

Figure 1

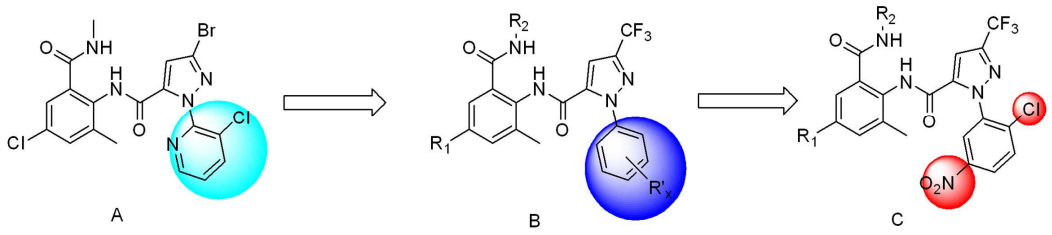


Figure 2

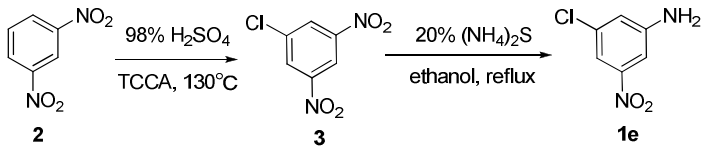
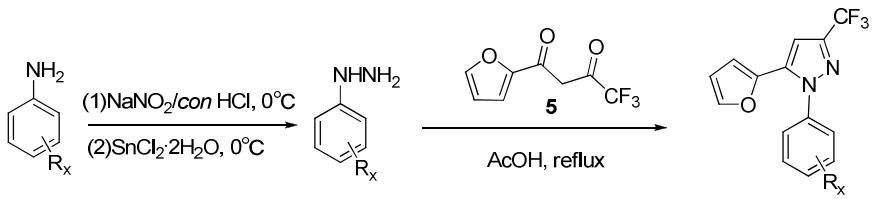
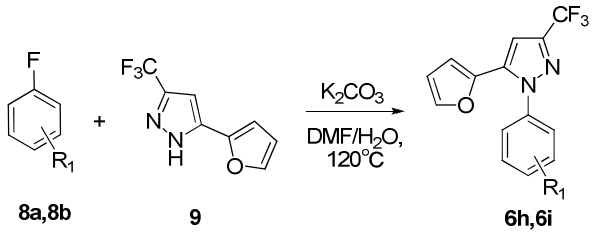


Figure 3



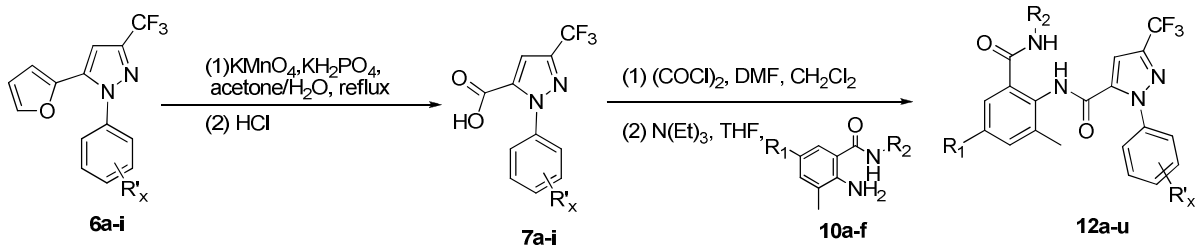
1a, **4a**, **6a**, : R_X= 3-NO_2; **1b**, **4b**, **6b**: R_X= 2-F, 5-NO_2; **1c**, **4c**, **6c**: R_X=2-Cl, 5-NO_2;
1d, **4d**, **6d**: R_X= 2-Br, 5-NO_2; **1e**, **4e**, **6e**: R_X= 3-Cl, 5-NO_2; **1f**, **4f**, **6f**: R_X= 4-Cl,3-NO_2;
1g, **4g**, **6g**: R_X= 2-Cl, 3-NO_2

Figure 4



6h, **8a**: R_1= 2-NO_2; **6i**, **8b**: R_1= 4-NO_2

Figure 5



6a, **7a**: R_X= 3-NO_2; **6b**, **7b**: R_X= 2-F, 5-NO_2; **6c**, **7c**: R_X=2-Cl, 5-NO_2; **6d**, **7d**: R_X= 2-Br, 5-NO_2; **6e**, **7e**: R_X= 3-Cl, 5-NO_2;
6f, **7f**: R_X= 4-Cl, 3-NO_2; **6g**, **7g**: R_X= 2-Cl, 3-NO_2; **6h**, **7h**: R_X= 2-NO_2; **6i**, **7i**: R_X= 4-NO_2;

12a: R_1= Cl, R_2=CH_3, R'$_x$= 4-NO_2; **12b:** R_1= Cl, R_2=CH_3, R'$_x$= 2-NO_2; **12c:**R_1= Cl, R_2=CH_3, R'$_x$= 3-NO_2;
12d: R_1= Cl, R_2=cyclopropyl, R'$_x$= 3-NO_2; **12e:** R_1= Br, R_2=CH_3, R'$_x$= 3-NO_2; **12f:** R_1= Cl, R_2=CH_3, R'$_x$= 2-F, 5-NO_2;
12g: R_1= Cl, R_2=CH_3, R'$_x$= 2-Br, 5-NO_2; **12h:** R_1= Cl, R_2=CH_3, R'$_x$= 2-Cl, 5-NO_2; **12i:** R_1= Cl, R_2=*i*-Pr, R'$_x$= 2-Cl, 5-NO_2;
12j: R_1= Cl, R_2=*t*-Bu, R'$_x$= 2-Cl, 5-NO_2; **12k:** R_1= Br, R_2=CH_3, R'$_x$= 2-Cl, 5-NO_2; **12l:** R_1= Cl, R_2=CH_3, R'$_x$= 3-Cl, 5-NO_2;
12m: R_1= Cl, R_2=*i*-Pr, R'$_x$= 3-Cl, 5-NO_2; **12n:** R_1= Br, R_2=CH_3, R'$_x$= 3-Cl, 5-NO_2;**12o:** R_1= Cl, R_2=CH_3, R'$_x$= 2-Cl, 3-NO_2;
12p: R_1= Cl, R_2=*i*-Pr, R'$_x$= 2-Cl, 3-NO_2; **12q:** R_1= Br, R_2=CH_3, R'$_x$= 2-Cl, 3-NO_2; **12r:** R_1= Cl, R_2=CH_3, R'$_x$= 4-Cl, 3-NO_2;
12s: R_1= Cl, R_2=*i*-Pr, R'$_x$= 4-Cl, 3-NO_2; **12t:** R_1= Br, R_2=CH_3, R'$_x$= 4-Cl, 3-NO_2; **12u:** R_1= Br, R_2=*t*-Bu, R'$_x$= 4-Cl, 3-NO_2;

Figure 6

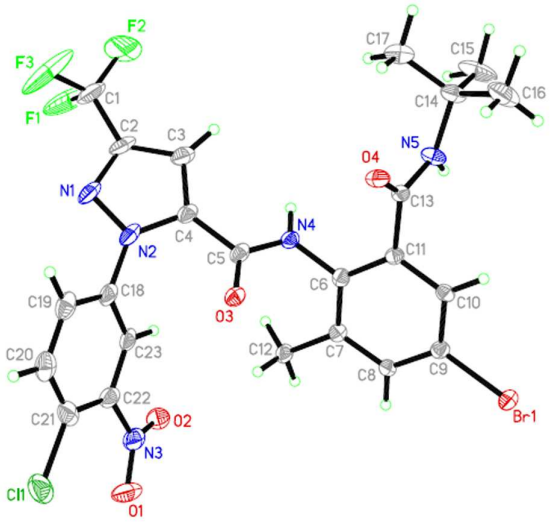


Figure 7

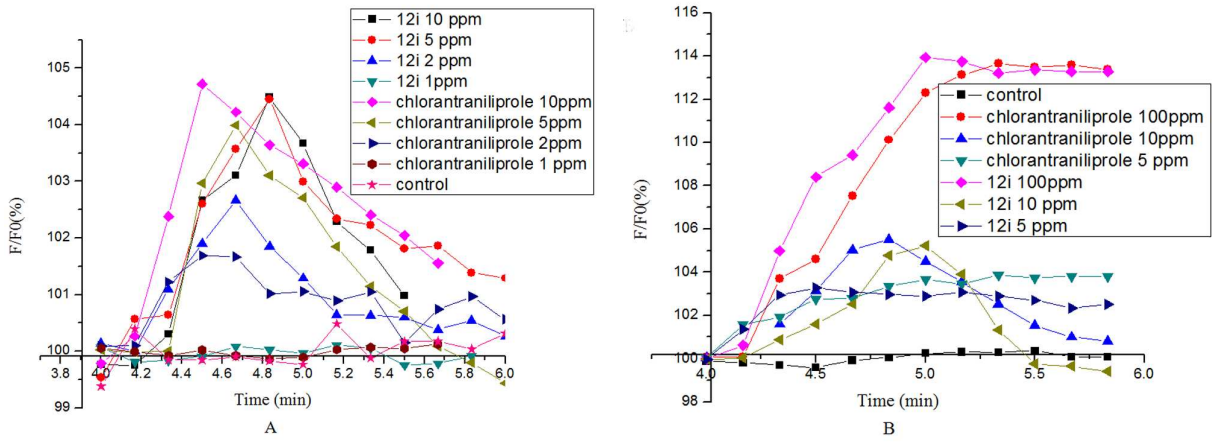


Figure 8

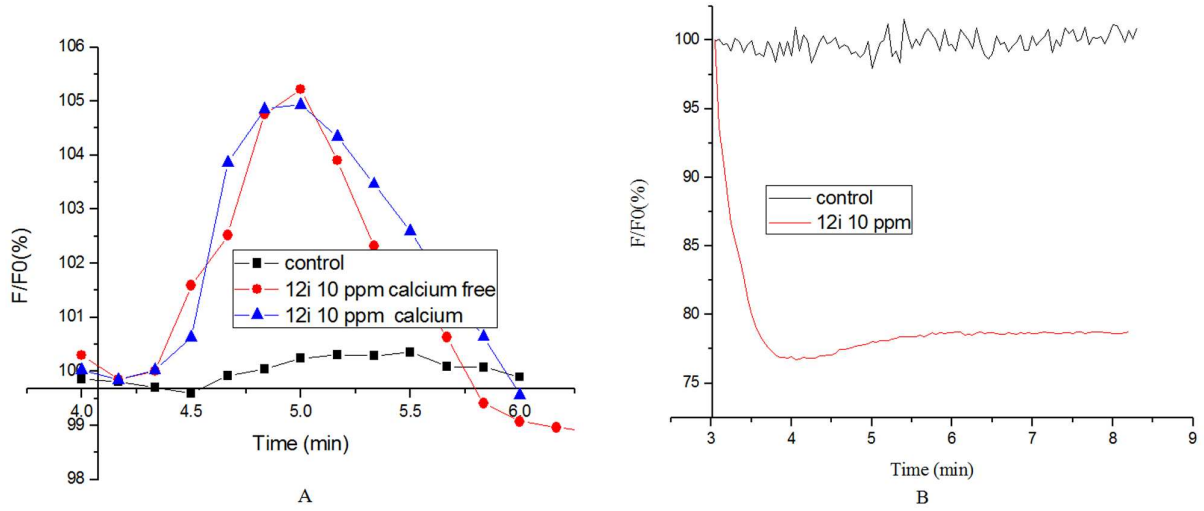


Figure 9

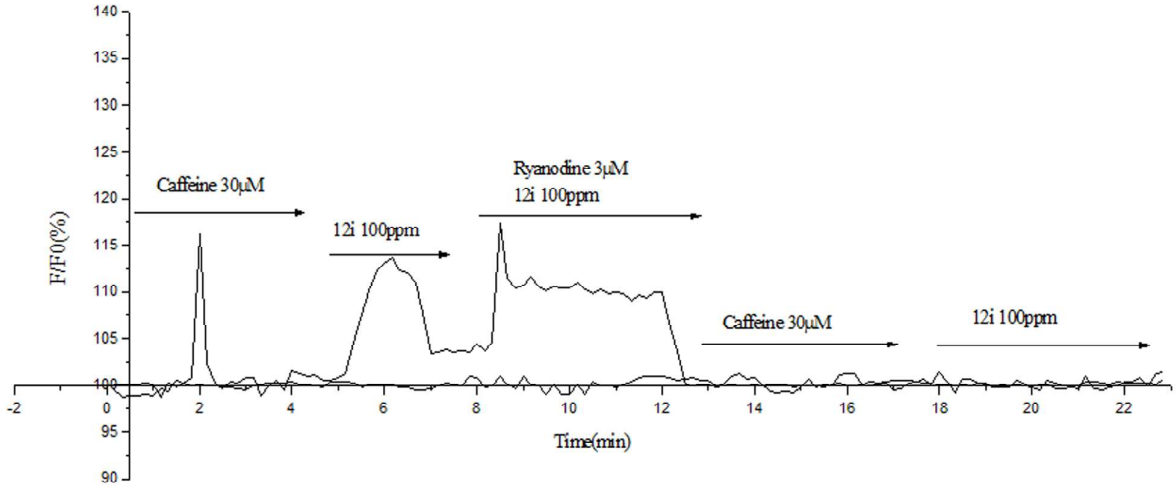


Figure 10

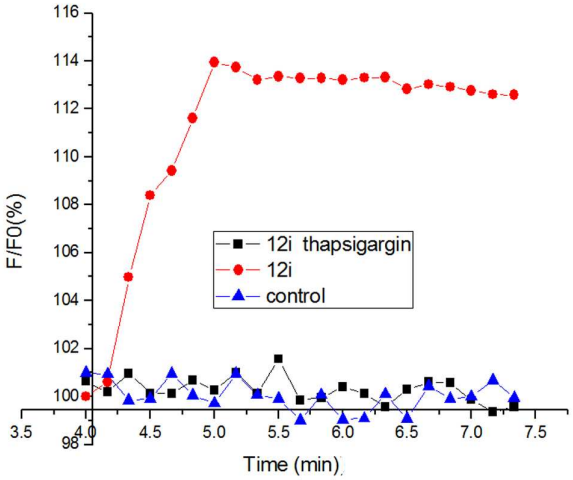
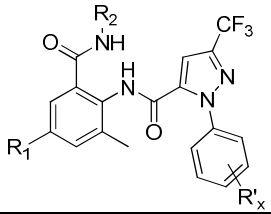


Table 1 Insecticidal Activities of Compounds **12a–u** and Chlorantraniliprole against Oriental Armyworm



				larvicidal activity (%) at a concentration of (mg/L)									
Compd.	R'_x	R_1	R_2	200	100	50	25	10	5	1	0.25	0.1	0.05
12a	4-NO_2	Cl	CH_3	100	100	100	80						
12b	2-NO_2	Cl	CH_3	100	100	100	100	20					
12c	3-NO_2	Cl	CH_3	100	100	100	100	90	30				
12d	3-NO_2	Cl	cyclopropyl	100	100	100	100	100	60				
12e	3-NO_2	Br	CH_3	100	100	100	100	50					
12f	2-F, 5-NO_2	Cl	CH_3	100	100	100	100	20					
12g	2-Br, 5-NO_2	Cl	CH_3	100	100	100	100	100	40				
12h	2-Cl, 5-NO_2	Cl	CH_3	100	100	100	100	100	100	100	100	40	
12i	2-Cl, 5-NO_2	Cl	*i*-propyl	100	100	100	100	100	100	100	100	53	37
12j	2-Cl, 5-NO_2	Cl	*t*-butyl	100	100	100	100	100	100	100	100	45	
12k	2-Cl, 5-NO_2	Br	CH_3	100	100	100	100	100	100	60			
12l	3-Cl, 5-NO_2	Cl	CH_3	100	100	40							
12m	3-Cl, 5-NO_2	Cl	*i*-propyl	100	100	70							
12n	3-Cl, 5-NO_2	Br	CH_3	100	100	20							
12o	2-Cl, 3-NO_2	Cl	CH_3	100	100	100	100	100	100	100	80		
12p	2-Cl, 3-NO_2	Cl	*i*-propyl	100	100	100	100	100	100	100	90		
12q	2-Cl, 3-NO_2	Br	CH_3	100	100	100	100	100	100	70			
12r	4-Cl,3-NO_2	Cl	CH_3	100	100	100	10						
12s	4-Cl,3-NO_2	Cl	*i*-propyl	100	100	100	30						
12t	4-Cl,3-NO_2	Br	CH_3	100	100	50							
12u	4-Cl,3-NO_2	Br	*t*-butyl	100	100	70							
chlorantraniliprole				100	100	100	100	100	100	100	100	60	33

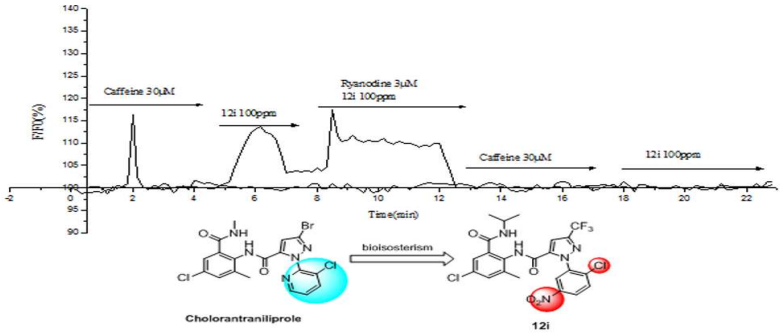
Table 2 LC_{50} Values of Compound **12i** and Chlorantraniliprole against Oriental Armyworm

Compd.	y = a + bx	R	LC_{50} (mg/L)	LC_{95} (mg/L)
12i	y=7.5368+2.2809x	0.9888	0.0772	0.4064
chlorantraniliprole	y=7.8167+2.3918x	0.9831	0.0664	0.3237

Table 3 Insecticidal Activities of Compounds **12h-j**, **12o** and **12p** and Chlorantraniliprole against Diamondback Moth

	larvicidal activity (%) at a concentration of (mg/L)							
Compd.	200	100	50	25	10	1	0.1	0.05
12h	100	100	100	100	100	100	70	
12i	100	100	100	100	100	100	100	95
12j	100	100	100	100	100	100	30	
12o	100	100	100	100	100	100	10	
12p	100	100	100	100	100	100	60	
chlorantraniliprole	100	100	100	100	100	100	100	100

TOC graphic


Caffeine 30μM
12i 100ppm
Ryanodine 3μM
12i 100ppm
Caffeine 30μM
12i 100ppm
F/F0(%)
Time(min)
bioisosterism
Cholorantraniliprole
12i