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Synthesis and insecticidal evaluation of novel *N*-pyridylpyrazolecarboxamides containing cyano substituent in the *ortho*-position

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ABSTRACT

In an attempt to search for potent insecticides targeting the ryanodine receptor (RyR), a series of novel *N*-pyridylpyrazolecarboxamides containing cyano substituent in the *ortho*-position were designed and synthesized. Their insecticidal activities of target compounds against oriental armyworm (*Mythimna separata*) and diamondback moth *(Plutella xylostella*) indicated that most of the compounds showed moderate to high activities at the tested concentrations. In particular, compound **6l** and **6o** showed 86% larvicidal activities against *Plutella xylostella* at the concentration of 0.1 mg/L, while the activity of compound **6h** against *Mythimna separate* was 80% at 1 mg/L. The calcium imaging technique was applied to investigate the effects of some title compounds on the intracellular calcium ion concentration ($[Ca^{2+}]_i$), experimental results demonstrated that compound **6h** stimulates a transient elevation in $[Ca^{2+}]_i$ in the absence of external calcium after the central neurons dye loading with fluo-3 AM. However, when the central neurons were dyed with fluo-5 N and incubated with 2-APB, $[Ca^{2+}]i$ decreased transiently by treated of compound **6h.** All of the calcium imaging technique experiments demonstrated that these novel compounds deliver calcium from endoplasmic reticulum to cytoplasm, which proved that the title compounds were the possible activators of insect RyR.

In order to overcome resistance and ecobiological problems associated with conventional insecticides, there is an urgent need to discover novel potent insecticides with a new mode of action. In recently years, Dupont discovered chlorantraniliprole[1] (Fig. 1A), which has an anthranilic diamide structure, exhibits exceptional broad-spectrum activity, high potency and low mammalian toxicity, and proves itself to be selective activators of the insect ryanodine receptor.[2] Due to its unique modes of action and good environmental profiles, anthranilic diamides have attracted considerable attention.

Most modifications in chlorantraniliprole structure in following researches preserve the anthranilic amide moiety, indicating that anthranilic amide is a key pharmacophore in this kind of compounds.[3–5] The introduction of a cyano group to replace the 4-halo substituent led to the discovery of cyantraniliprole[4] (Fig. 1B), which had improved plant mobility and increased spectra of insect control. However, there were also reported for the structural modification of the amides in the *ortho*-position, such as hydrazone,[6] heterocyclic groups[7,8] and cyano-containing amides.[9,10] The compound **C** reported by Li et al, showed excellent larvicidal activity against beet armyworm (*Spodoptera exigua*).[9] In view of the above information, introducing the cyano group into the structure of chlorantraniliprole skeleton would improve plant mobility and insecticidal activities. In order to obtain compounds with higher larvicidal activity and study the structure-activity relationship, a series of novel *N*-pyridylpyrazolecarboxamides (Fig. 1, D) containing cyano at the *ortho*-position were designed and synthesized. The larvicidal activities against oriental armyworms and diamondback moths were evaluated and the relating structure-activity relationships were also discussed. To further explore the mode of action for the target compounds, the effect of some target compounds on $[Ca^{2+}]_i$ in the central neurons isolated from the third instar of *Spodoptera exigua* was studied by calcium imaging techniques.

2-Amino-5-substuituted-3-methylbenzoic acid (**1b–d**) were synthesized by referring to the known procedure.[11,12] Compounds **2a–d** were prepared according to the reported method with minor improvements as shown in Scheme 1,[13] the pure product were easily obtained after filtration instead of extraction using sodium sulfate decahydrate as the quencher. Subsequent reaction with 8 equiv of manganese dioxide yielded compounds **3a–d** in excellent yields. 2-Amino-5-cyano-3-methylbenzaldehyde was obtained from **3d** in the presence of cuprous cyanide in DMF at 140 °C.[12]

Compounds **4a–c** were synthesized by the method reported by literature.[11,12,14] The key intermediates **5a–i** were achieved according to our previous work (Scheme 2).[13,14] Nevertheless, attempts to synthesize compound **5m** with same procedure failed, probably

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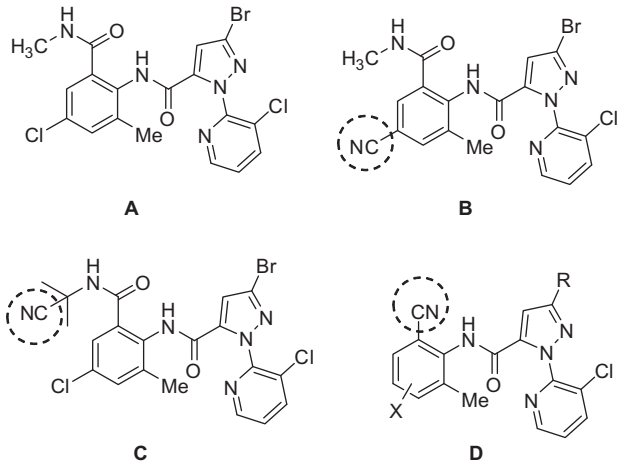


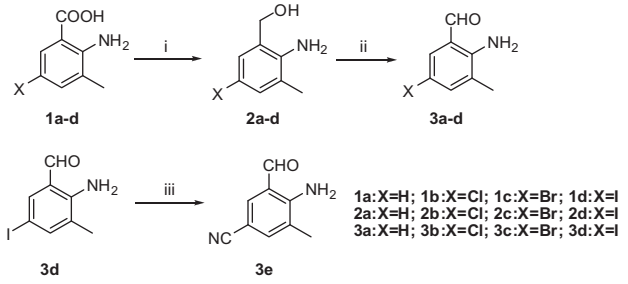
Figure 1. Chemical structures of compounds **A**–**D**.

due to the strong electron-withdrawing cyano group resulting in a poor reactivity of amino moiety. Instead, **5m** was obtained via reflux in acetonitrile (Scheme 3).[15] The nitro-containing intermediates (**5l**, **5j**, **5k**) were synthesized as shown in Scheme 4, and the compounds (**5a**, **5c**, **5g**) were treated with fuming HNO_3 in concentrated H_2SO_4 to yield corresponding nitro-containing products in good yields and high regioselectivity.[16]

The target compounds **6a–m** were synthesized from **5a–m** as shown in Schemes 2–4. We attempted to treat **5a–m** with hydroxylamine hydrochloride in DMF at 50–55 °C to afford the corresponding cyano-containing products with CN in the *ortho*-position. Unfortunately, the aldehyde group of compound **5** were converted into oxime. In the presence of iodine and aqueous NH_3 in THF, the target compounds **6a–m** were achieved with satisfactory yields and purity.[17]

The alternative synthetic route to prepare the target compounds **6m–o** with cyano group in the 4-position of the benzene ring from **8a–c** as shown in Scheme 5**.** Intermediates **7a–c** and **8a–c** were synthesized following the previously reported procedure with minor improvements.[4,18] No reaction occurred using 2,4,6-trichloro-1,3,5-triazine as dehydrant to synthesize **6m–o** from **8a–c.**[19] However, the dehydration reaction proceeded smoothly with thionyl dichloride and the products were obtained in excellent yields.[20]

Most of the intermediates were determined by ^1H NMR, and all new target compounds were characterized with ^1H NMR, ^{13}C NMR and elemental analysis (or HRMS) (see Supplementary data). Compounds **6b** was selected to further investigate the IR spectrum characterization of this kind of compounds. The characteristic stretching vibration ν (C≡N) appears at 2235 cm^{-1}.



Scheme 1. Reagents and conditions: (i) $LiAlH_4$, THF, 0 °C, then $NaSO_4{\cdot}10H_2O$, room temperature; (ii) MnO_2, CH_2Cl_2, room temperature; (iii) CuCN, DMF, 140 °C

The larvicidal activity of compounds **6a–o** against oriental armyworms is summarized in Table 1. The bioassay results indicated that most compounds have excellent larvicidal activities against oriental armyworm. For example, the larvicidal activities of **6a**, **6e**, **6h** and **6i** against oriental armyworm at 1.0 mg L^{-1} were 60%, 80%, 50%, 20%, respectively. Activities varied significantly depending upon the types of substituents on the 3-position pyrazole. Compared with 3-Br and 3-CF_3 in pyrazole, compounds with 3-OCH_2CF_3 substituents showed higher insecticidal activities against oriental armyworm, with the sequence of **6h** > **6a** > **6e**, **6i** > **6f** > **6b** and **6o** > **6m** > **6n**, which suggests that the introduction of the 2,2,2-trifluoroethoxy groups in the 3-position of pyrazole has a positive effect on the larvicidal activities. Furthermore, different substituents in benzene ring had various influence on activity. When R was fixed as Br, the bioactivity of compounds with different X indicated the sequence of Cl > Br > CN > I > NO_2 > H, while compounds with 3-CF_3 and 3-OCH_2CF_3 in pyrazole showed a similar trend. However, the compounds with cyano group in 4-position of the benzene ring did not exhibited higher activities as we expected. For example, the larvicidal activities of **6m** and **6n** at a concentration of 10 mg L^{-1} were 70% and 20%, respectively. In addition, the introduction of nitro group at the 5-position of the benzene ring led to a significant decrease in activity, such as **6l**.

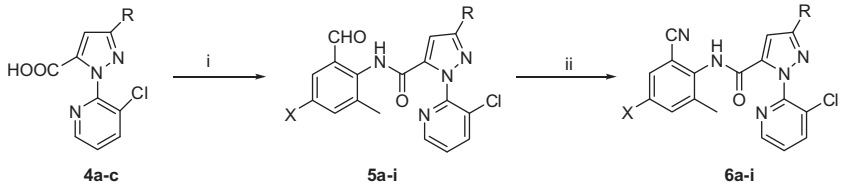
The larvicidal activity of compounds **6a–o** against diamondback moth were evaluated as shown in Table 2. Most of them had excellent larvicidal activity against diamondback moth. In particular, compounds **6l** and **6o** had around 86% mortality at the concentration of 0.1 mg/L, approaching closer to chlorantraniliprole. Surprisingly, compound **6d** (X = 4-I) showed good activity against diamondback moth (86% death rate at 1 mg/L).

Figure 2 illustrated the change of $[Ca^{2+}]_i$ versus recording time when the neurons were treated with **6b**, **6c**, **6e**, **6h**, **6k**, **6l, 6n** and chlorantraniliprole. The peak of $[Ca^{2+}]_i$ were elevated to 117.38 ± 4.21% (n = 18), 111.71 ± 3.29% (n = 13), 119.29 ± 3.47% (n = 13), 114.63 ± 4.11% (n = 9), 114.43 ± 3.78% (n = 9), 109.23 ± 2.37% (n = 9) and 122.06 ± 2.54% (n = 18) of the initial value when the cells were treated with 1000 mg/L of **6b**, **6c**, **6e**, **6h**, **6k**, **6l, 6n** and chlorantraniliprole, respectively. Compared with the control (99.91 ± 2.56%), these compounds induced $[Ca^{2+}]_i$ increase without extracellular Ca^{2+}. It indicated that compounds could activate the calcium release channel in the endoplasmic reticulum (ER) membrane. Figure 2 also indicated that the recorded $[Ca^{2+}]_i$ (F/F_0) had a good positive correlation with bioactivities.

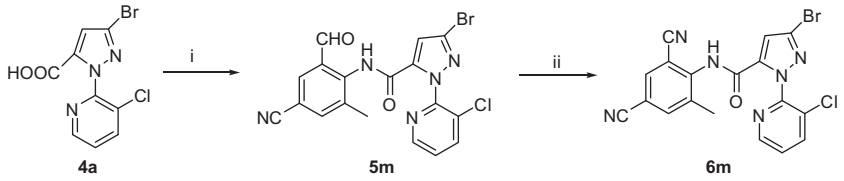
As shown in Figure 3, brief application of compound **6h** continued to stimulate a transient elevation in $[Ca^{2+}]_i$ in the absence of external calcium. Reintroduction of standard saline allowed depleted calcium stores to become refilled and thereby available for the next **6h** challenge but resulted in an attenuated response.

To test why compound **6h** and chlorantraniliprole can cause $[Ca^{2+}]_i$ elevation, the primary cultured neurones were dyed loading with fluo-5 N. Figure 4 illustrated the change of $[Ca^{2+}]_i$ versus recording time when the neurons were treated with **6h** and chlorantraniliprole. Compound **6h** and chlorantraniliprole decrease $[Ca^{2+}]i$ to 95.12 ± 2.06% (n = 12) and 90.34 ± 3.64% (n = 18), respectively. These data indicated that $[Ca^{2+}]_i$ decreased by 1000 mg/L of **6h** and chlorantraniliprole. It means that compound **6h** and chlorantraniliprole could deliver calcium from endoplasmic reticulum (ER) to cytoplasm.

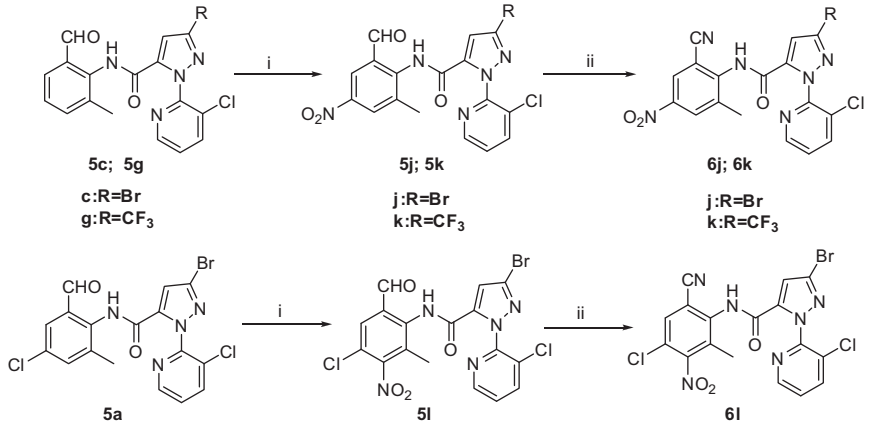
There were two kinds of calcium release channels in the ER membrane, namely RyR and IP_3R Ca^{2+} channels. To test which pathway was involved in the elevation of $[Ca^{2+}]_i$, the primary cultured neurones were dyed loading with fluo-5 N (low-affinity calcium indicator, accurately tracks the dynamic changes in calcium in the ER and SR), and then incubated with 2-aminoethoxydiphenyl borate (2-APB 50 μM, a chemical that acts to inhibit both IP_3



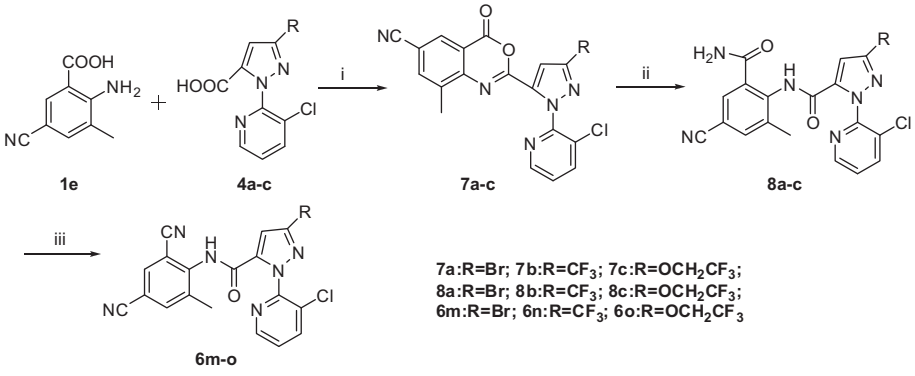
Scheme 2. Reagents and conditions: (i) CH_2Cl_2, $(COCl)_2$, DMF; CH_2Cl_2,pyridine, **3a**–**d**, 0 °C, then room temperature; (ii) THF, I_2/NH_3 H_2O, room temperature.



Scheme 3. Reagents and conditions: (i) CH_2Cl_2, $(COCl)_2$, DMF; CH_3CN, **3e**, reflux; (ii) THF, I_2/NH_3 H_2O, room temperature.



Scheme 4. Reagents and conditions: (i) concentrated sulfuric acid, fuming nitric acid, 0 °C, then room temperature; (ii) THF, I_2/NH_3 H_2O, room temperature.



Scheme 5. Reagents and conditions: (i) CH_3CN, pyridine, CH_3SO_2Cl, room temperature; (ii) CH_3CN, $NH_3{\cdot}H_2O$, room temperature; (iii) DMF, $SOCl_2$, 0 °C, then room temperature.

receptors and TRP channels) for 20 min. As shown in Figure 5, when external Ca^{2+} was free, IP_3 receptors were blocked using 2-APB, the decrease of $[Ca^{2+}]_i$ was only attributed to compound **6h** (1000 mg/L). After the central neurons were incubated with 2-APB, compound **6h** decrease $[Ca^{2+}]i$ to 94.23 ± 3.48% (n = 12) and there was no statistically difference in the calcium response. It

Table 1
Insecticidal activities of compounds **6a–o** and chlorantraniliprole against oriental armyworms

Compound	Larvicidal activity (%) at a concentration of (mg/L)				
	25	10	5	2.5	1
6a	100	100	100	100	60
6b	100	80			
6c	40				
6d	100	60			
6e	100	100	100	100	50
6f	100	100	100	80	
6g	100	100	40		
6h	100	100	100	100	80
6i	100	100	100	100	20
6j	100	20			
6k	100	100	60		
6l	100	100	60		
6m	100	70			
6n	100	20			
6o	100	100	60		
Control[a]	100	100	100	100	100

[a] Chlorantraniliprole.

Table 2
Insecticidal activities of compounds **6a–o** and chlorantraniliprole against diamond-back moth

Compound	Larvicidal activity (%) at a concentration of (mg/L)		
	10	1	0.1
6a	100	43	0
6b	86	43	0
6c	43	29	0
6d	100	86	14
6e	100	29	0
6f	57	29	0
6g	71	29	0
6h	100	71	14
6i	100	43	0
6j	100	57	14
6k	71	29	0
6l	100	100	86
6m	100	71	29
6n	100	57	14
6o	100	100	86
Control[a]	100	100	100

[a] Chlorantraniliprole.

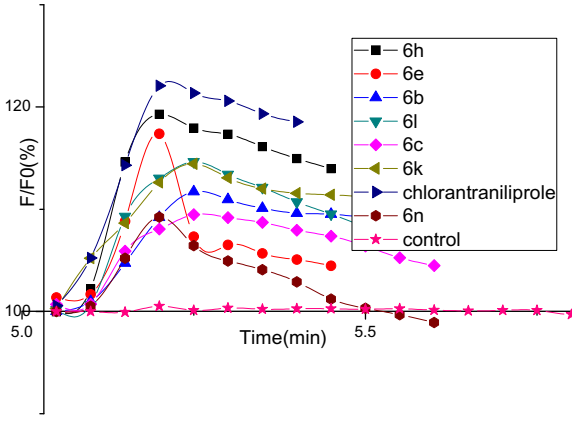


Figure 2. The change of $[Ca^{2+}]_i$ versus recording time when the neurons treated with 0.1 mg/L **6b**, **6c**, **6e**, **6h**, **6k**, **6l**, **6n** and chlorantraniliprole.

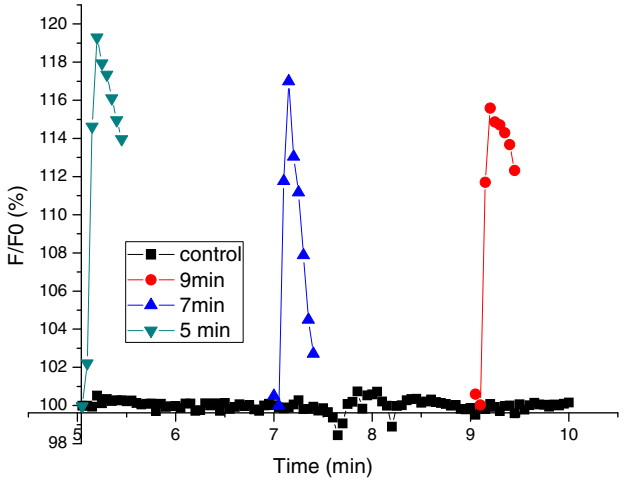


Figure 3. Characterization of compound **6h** stimulated calcium responses in the central neurons of *S. exgua* third larvae. Repeated challenges with compound **6h** in calcium-free saline (1 mM EGTA with $CaCl_2$ omitted).

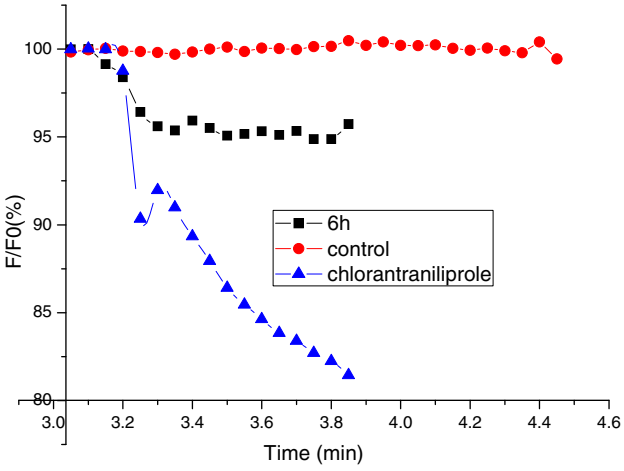


Figure 4. Effect of treatments with of **6h** and chlorantraniliprole on intracellular Ca^{2+} at different time when extracellular Ca^{2+} was in absence (EGTA replace Ca^{2+}). The central neurons of *S. exigua* third larvae dye loading with fluo-5 N.

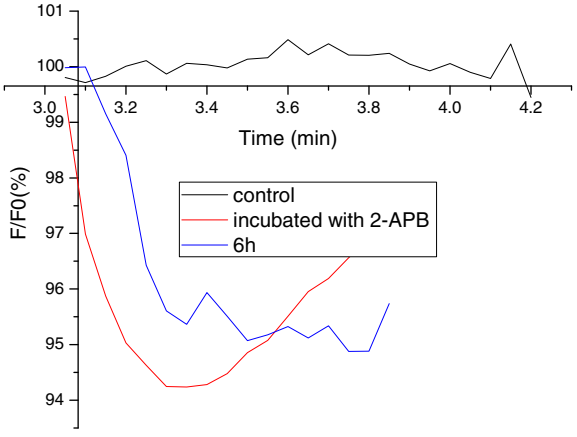


Figure 5. Effect of treatments with of **6h** on intracellular Ca^{2+} at different time when extracellular Ca^{2+} was in absence (EGTA replace Ca^{2+}). The central neurons of *S. exigua* third larvae dye loading with fluo-5 N, then incubated with 2-aminoethoxydiphenyl borate for 20 min.

means that compound **6h** only has weak influence on TRP channels. More importantly, these data demonstrated that compound **6h** delivers calcium from endoplasmic reticulum (ER) to cytoplasm by RyRs.

In conclusion, a series of novel *N*-pyridylpyrazolecarboxamides containing cyano in the *ortho*-position were designed and synthesized. The bioassays showed that some of the compounds exhibited excellent insecticidal activities against oriental armyworm (*Mythimna separata*) and diamondback moth *(Plutella xylostella*). In particular, compound **6l** and **6o** showed 86% larvicidal activities against *Plutella xylostella* at the concentration of 0.1 mg/L, while the activity of compound **6h** against *Mythimna separate* was 80% at 1 mg/L. The calcium imaging techniques were used to investigate the effects of some title compounds on the $[Ca^{2+}]_i$, which indicated that the title compounds were the possible activators of the RyR. The results of the present study provide useful information for further structural optimization of these compounds and a rapid detection for the activity of the target compounds.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.11.045.

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