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Synthesis and insecticidal evaluation of novel sulfide-containing amide derivatives as potential ryanodine receptor modulators

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ABSTRACT

With the aim of discovering new bioactive pesticides for crop protection, a series of novel sulfide-containing amide derivatives **A** were efficiently synthesized *via* a strategy of modifying the "amide" structure of anthranilic diamide insecticides. The single-crystal structures of **A2-3** and **A4-5** were firstly reported. The bioassay results showed that most of the synthesized compounds display moderate to high insecticidal activities. Particularly, some sulfone-containing compounds, *e.g.,* **A2-3**, **A3-3** and **A6-3**, not only possessed favorable lethality rate (50%–100%) against *P. xylostella* at a concentration of 0.1 mg/L, but also held good activities towards a variety of agricultural pests such as *M. separata*, *C. pipiens pallen*, *H. armigera* and *O. nubilalis*; the larvicidal activities of **A4-1** and **A6-1** towards *P. xylostella* were close to that of chlorantraniliprole at 0.01 mg/L. The calcium imaging experiments revealed that the representative compounds **A2-3** and **A6-3** are potential ryanodine receptor (RyR) modulators. The structure–activity relationships were discussed in detail. These results provide useful information for further design and development of novel insecticides.

The discovery and development of new pesticides with novel structures and excellent biological activities are perpetual objectives for agrochemical researchers [1–5]. The phthalic diamides and anthranilic diamides which are two kinds of amide derivatives have been given great attention in agrochemistry area during the recent 15 years [6–9]. This is mainly because compounds with such structural features usually possess exceptional insecticidal activities with broad spectrum towards various pests, such as Lepidoptera, Coleoptera, Isoptera and Diptera pests; most importantly, some of which—flubendiamide, chlorantraniliprole and cyantraniliprole (Fig. 1, **I**–**III**) have been successfully developed as commercial insecticides by Nihon Nohyaku Co., Ltd. and DuPont, respectively [10–12]. These insecticides are known as highly potent and selective modulators of insect ryanodine receptor (RyR, also known as the calcium ion channel receptor) [11,13]. Recently, another anthranilic diamide insecticide cyclaniliprole (Fig. 1, **IV**) has also been successfully developed by Ishihara Sangyo Kaisha [14]. However, because of the overuse of these insecticides, resistance issue has been developed in some pests, such as *Tuta absoluta, Plutellidae Plutella* and *Spodoptera exigua* [15–17]. For example, from 2010 to 2011, there was a high-level resistance in *Plutellidae Plutella* populations reported in Philippines and China [16]. Moreover, food safety concerns about the application of chlorantraniliprole and flubendiamide have also been reported recently [18]. Accordingly, developing new insecticides to overcome resistance and safety issues associated with the diamide insecticides is urgently needed. The structural modification based on these diamide insecticides has been considered as one of the significant and feasible ways to do the insecticide innovations.

The common structural characteristic of these insecticides is that there are two amide groups in each of the molecules. For the structural modification on the anthranilic diamide insecticides, *e.g.*, chlorantraniliprole, there are a few reports about the investigations on the amide bond modifications compared to many explorations of phenyl group or pyridylpyrazole motif transformations [19–22]. The acyl thiourea [9,23], heterocycle such as aziridine [24], β-lactam [24] and benzotriazinone [25], and aminomethylphosphonate [26] motifs as new amide bond substitutes of such diamide structure have been reported, resulting in promising insecticidal or fungicidal activities of the corresponding new compounds. Therefore, more new forms of amide bond modifications based on anthranilic diamide structures are expected to produce potential breakthroughs for discovering novel agrochemicals.

Sulfides, an important class of agrochemicals, have attracted much attention because of their versatile bioactivities and easy derivatizable structures. Many sulfur-containing compounds have been applied in almost all kinds of agrochemicals, such as herbi-

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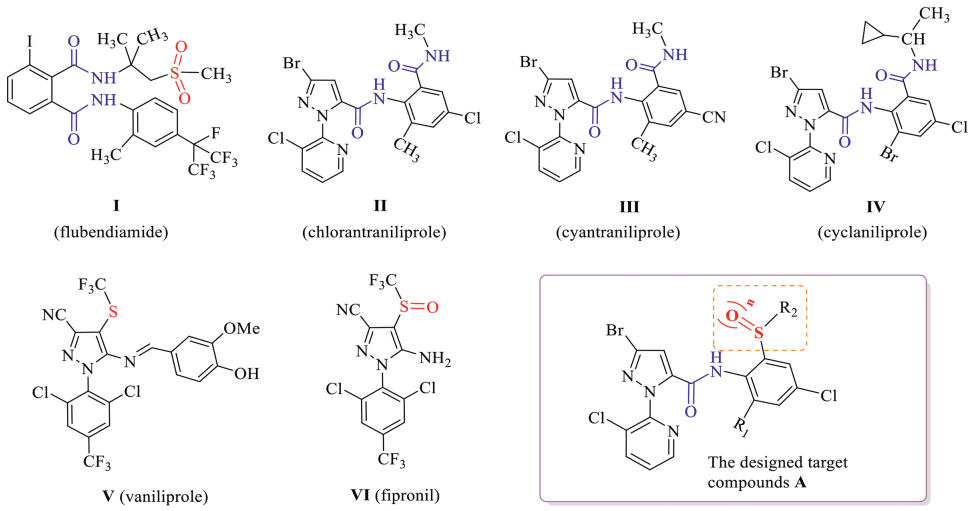
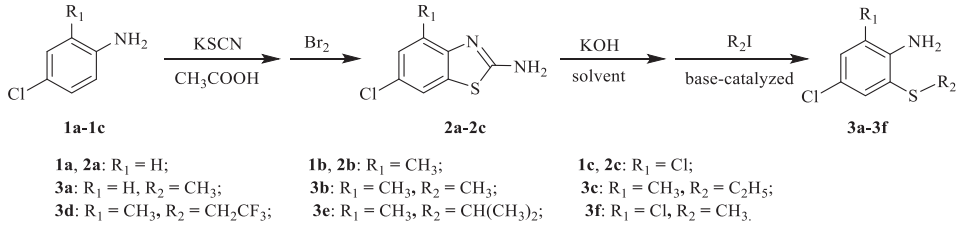


Fig. 1. The structures of some diamide- and sulfide-containing insecticides, and the designed target compounds **A** in this paper.



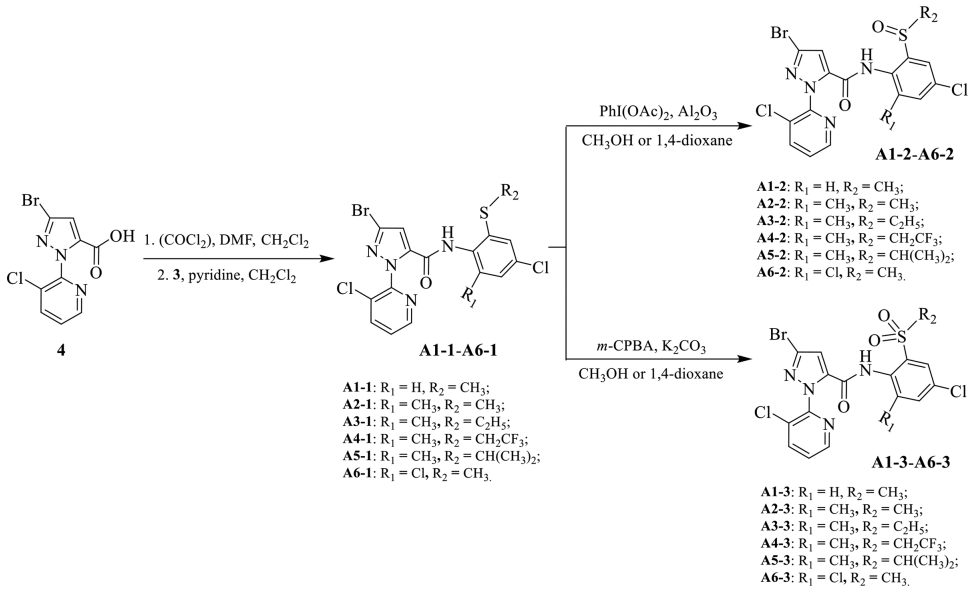
Scheme 1. Synthetic route of the intermediates **2** and **3**.

cides [27], insecticides [28,29] and fungicides [30]. It is mentioned by Ando [31] that the sulfur atom of organosulfur-containing compounds can offer reactive site for environmental degradation and for further derivatization, and ultimately lead to versatile bioactivities. It is also reported that more than 30% of all the agrochemicals contained at least one sulfur atom [32]. Therefore, developing new sulfur-containing compounds may be an important aspect in future pesticide research.

Thioethers, sulfoxides and sulfones are three kinds of sulfides. The sulfur-containing groups of which are important pharmacophores of many insecticides. For examples, vaniliprole (Fig. 1, **V**) [28] has a trifluoromethylthio moiety (thioether motif), fipronil (Fig. 1, **VI**) [29] and flubendiamide (Fig. 1, **I**) have sulfinyl and sulfonyl groups, respectively. With this in mind, based on a strategy of replacing one of the amide moieties of anthranilic diamide insecticides with sulfide/sulfinyl/sulfonyl group, a series of novel thioether-containing amide derivatives and their oxidation products-sulfoxide/sulfone-containing amide derivatives were synthesized in this paper. Their insecticidal activities and mode of action for the calcium ion modulations were investigated.

The intermediates **2** were synthesized referring to a reported similar procedure (Scheme 1) [33–35]. The synthesis for compound **3a** is according to a similar procedure in literature (Scheme 1) [36]: 6-chlorobenzo[*d*]thiazol-2-amine **2a** (20 mmol), KOH (5 times the weight of thiazole amine) and water (100 mL) were placed in a round-bottom flask and heated under reflux for 8 h. Upon completion of the reaction and cooling down to room temperature, the system was poured into ice water and adjusted pH value to 7 with 1 mol/L hydrochloric acid. After that, the mixture was extracted with ethyl acetate (3 × 30 mL) and the combined organic phase was dried over anhydrous Na_2SO_4. The solvent was removed to afford the corresponding aryl thiol. Without further purification, the crude aryl thiol was mixed with KOH (40 mmol) and water (50 mL), and the mixture was warmed up to reflux for 1 h, then cooled down to room temperature. After being added the methyl iodide (20 mmol), the system was further turned to reflux for about 3 h until completion of the reaction, then cooled down. The reaction system was poured into ice water and conducted extraction with ethyl acetate (3 × 20 mL). The combined organic phase was washed with brine and dried over Na_2SO_4. After removal of solvent, the residue was further purified using column chromatography with ethyl acetate and petroleum ether (1:2, v/v) as solvents to afford the intermediate 4-chloro-2-(methylthio)aniline (**3a**).

The synthetic procedure for compounds **3b**, **3c**, **3e** and **3f** was similar to that of **3a** in general. The difference is the reaction condition for the first step-thiazole amine **2** (**2a** or **2c**, 20 mmol), KOH (5 times the weight of thiazole amine) and ethylene glycol (100 mL) were mixed and heated under reflux for about 12 h. The synthesis for compound **3d**: compound **2b** (20 mmol), KOH (5 times the weight of thiazole amine), and ethylene glycol (100 mL) were mixed and the mixture were refluxed with stirring for about 12 h. Upon completion of the reaction and cooling down to room temperature, the system was poured into ice water and adjusted pH value to 7 with 1 mol/L hydrochloric acid. After that, the mixture was extracted with ethyl acetate (3 × 30 mL) and the combined organic phase was dried over anhydrous Na_2SO_4. The solvent was removed to afford the corresponding aryl thiol. Without further purification, the crude aryl thiol was mixed with K_2CO_3 (22 mmol) and *N*,*N*-dimethylformamide (DMF) (20 mL), and the mixture was warmed up to reflux for 3 h, then cooled down to room temperature. After being added trifluoroiodoethane (20 mmol), the system was further turned to reflux for about 7 h until completion of the reaction, then cooled down. The reaction system was



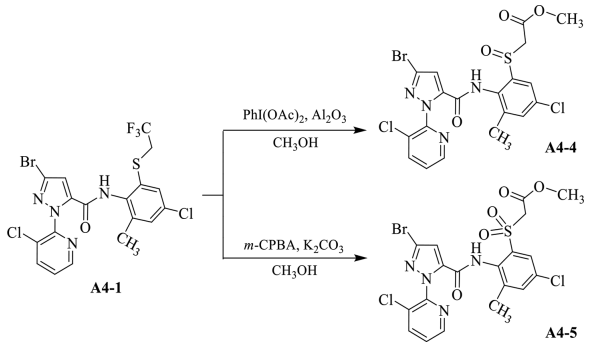
Scheme 2. Synthetic route of the title compounds **A**.

poured into ice water and conducted extraction with ethyl acetate (3 × 20 mL). The combined organic phase was washed with brine and dried over Na_2SO_4. After removal of solvent, the residue was further purified using column chromatography with ethyl acetate and petroleum ether (1:3, v/v) as solvents to afford the intermediate 4-chloro-2-methyl-6-((2,2,2-trifluoroethyl)thio)aniline (**3d**).

The synthesized alkylthioarylamine (**3**, 10 mmol), pyridine (10.1 mmol) and dichloromethane (20 mL) were placed in a 100 mL round-bottom flask and the system was cooled to 0 °C using an ice bath. A dichloromethane (10 mL) solution of pyrazole acyl chloride, which was freshly prepared by carboxylic acid **4** (10 mmol) according to a reported procedure [23,26], was added dropwise to the above cold system. After that, the reaction mixture was warmed to room temperature and stirred for about 5 h (monitored by TLC), then was concentrated under reduced pressure. The residue was redissolved with ethyl acetate (30 mL), and the solution was washed with brine. The organic phase was dried over anhydrous Na_2SO_4. After removal of the solvent, the residue was conducted for column chromatography with ethyl acetate and petroleum ether (1:3, v/v) as solvents to afford the thioether-containing amide compounds **A1-1**–**A6-1** (Scheme 2).

Methylthio-containing amide compound **A1-1** (1 mmol), $PhI(OAc)_2$ (3 mmol) and Al_2O_3 (1 mmol) were mixed in methanol (20 mL), the reaction system was stirred at room temperature for 3 h [monitored by thin layer chromatography (TLC)] and concentrated under reduced pressure. To the residue CH_2Cl_2 (20 mL) was added, the mixture was washed with brine and the organic phase was dried over anhydrous Na_2SO_4. After removal of the solvent, the residue was further purified by column chromatography with ethyl acetate and petroleum ether (1:2, v/v) as solvents to afford the sulfoxide-containing compound **A1-2**. Using the same procedure, the sulfoxide-containing compounds **A2-2**–**A6-2** (1,4-dioxane solvent used in the case of **A4-2**, Scheme 2) and **A4-4** (Scheme 3) were synthesized successfully.

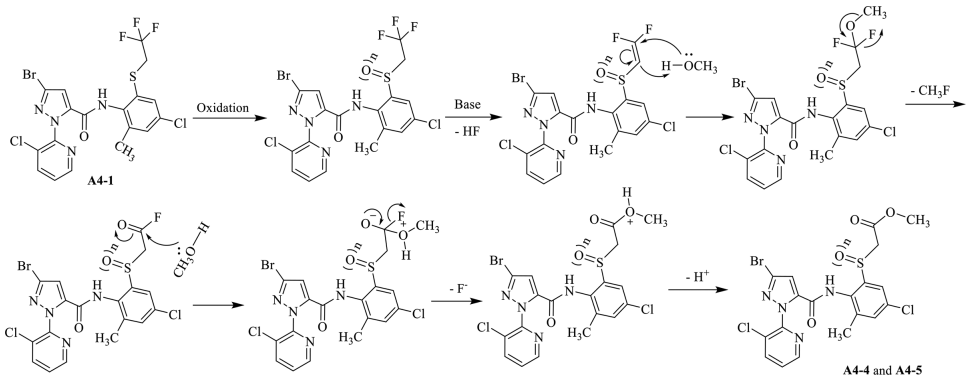
Methylthio-containing amide compound **A1-1** (1 mmol), *m*-CPBA (3 mmol) and K_2CO_3 (1 mmol) were mixed in methanol (20 mL), the reaction system was heated to reflux for 3 h (monitored by TLC) and then concentrated under reduced pressure. To the residue CH_2Cl_2 (20 mL) was added, the mixture was washed with brine and the organic phase was dried over anhydrous Na_2SO_4. After removal of the solvent, the residue was further purified by column chromatography with ethyl acetate and petroleum ether (1:1, v/v) as solvents to afford the sulfone-containing compound **A1-3**. Using the same procedure, the sulfone-containing compounds **A2-3**–**A6-3** (1,4-dioxane solvent used in the case of **A4-3**, Scheme 2) and **A4-5** (Scheme 3) were synthesized successfully.



Scheme 3. Synthetic route of the ester by-products **A4-4** and **A4-5**.

The insecticidal evaluation and calcium imaging experiments of the synthesized compounds were conducted according to the reported methods [19,21,23,37–39], the detailed procedures were presented in the Supporting information.

The key intermediates **2** can be efficiently prepared from the corresponding 2-substituted-4-chloroaniline (**1**) as the starting material *via* a synthetic route shown in Scheme 1. The intermediates alkylthio-containing arylamine **3** were prepared from a KOH-decomposition of benzothiazole amine **2**, followed by an alkylation of the generated *o*-aminophenthiol under base-catalyzing in moderate to high yield (45%–83%). For the substrates **2**, when R_1 is H, the first step reaction of the corresponding compound is easier than that of CH_3 or Cl, indicating an influence of the steric hindrance effect from substituent in 4-position of benzothiazole (R_1) on its reactivity. The following comparison of reaction temperature and time can well reflect this influence: boiling in water (b.p. 100 °C), 8 h (R_1 = H); boiling in ethylene glycol (b.p. 197 °C), 12 h (R_1 = CH_3 or Cl). Interestingly, for the alkylation reagents R_2I, when R_2 is alkyl group [CH_3, C_2H_5 and $CH(CH_3)_2$], the second step reaction for preparing **3** is much easier than that of trifluoroethyl group (CH_2CF_3), which may be due to the easy elimination side-reaction of the reagent CF_3CH_2I under refluxing conditions in



Scheme 4. Possible synthetic mechanism for compounds **A4-4** and **A4-5**.

strong base solution (KOH-H_2O) (while in K_2CO_3-DMF system, the reaction underwent smoothly).

The thioether-containing amide compounds **A1-1**–**A6-1** (Scheme 2) can be prepared *via* the reaction of alkylthio-containing arylamine (**3**) and pyridylpyrazole acyl chloride generated from pyridylpyrazole acid (**4**) using pyridine as acid binding agent with good yields. When using triethylamine as acid binding agent, it was found that such reaction will produce certain amount of disubstituted by-product, *e.g.*, compounds **A7** and **A8** (Scheme S1 in Supporting information) from their respective reactants **3b** and **3d**. This indicates the weaker base (pyridine) is better for synthesizing the monosubstituted target structures. Treated with different oxidants, the thioether-containing product (**A1-1**–**A6-1**) can be directly oxidized to the corresponding sulfoxide-containing product (**A1-2**–**A6-2** and **A4-4**) in $PhI(OAc)_2$-Al_2O_3 system or sulfone-containing product (**A1-3**–**A6-3** and **A4-5**) in *m*-CPBA-K_2CO_3 system. It is especially worth mentioning that the reflux condition for the sulfone-containing product was necessary in view of the situation that only sulfoxide-containing products can be obtained at room temperature. According to the literature [40], methanol was more beneficial to the oxidation reaction when a solvent was used. When using compound **A4-1** (R_2 = CH_2CF_3) as the reactant to do the oxidation in the methanol solvent, neither $PhI(OAc)_2$-Al_2O_3 nor *m*-CPBA-K_2CO_3 could efficiently give the desired target products (**A4-2** and **A4-3**). However, an abnormal side reaction was found to form the ester by-products (**A4-4** and **A4-5**, Scheme 3), which was further confirmed by a single crystal structure of **A4-5** (Fig. 2). By taking into account the reaction conditions, the mechanism of this abnormal reaction was suggested as shown in Scheme 4. We speculated that this is due to the extremely strong electron-withdrawing effect of the generated sulfinyl/sulfonyl group in the reaction led to the easy occurence of following elimination and alcoholysis of trifluoroethyl group in methanol. As a result, aprotic solvent—1,4-dioxane as an alternative of methanol was chosen to do such oxidation reactions, smoothly affording the target products (**A4-2** and **A4-3**).

The title compounds **A** were identified by melting points, ^1H nuclear magnetic resonance (NMR), ^{13}C NMR and ^{19}F NMR spectra. The measured elemental analysis or high resolution mass spectrometry (HRMS) data were also consistent with the corresponding calculated values. When R_1 and R_2 are the same, the characteristic proton peak of N-H in ^1H NMR spectra exhibited a chemical shift sequence of sulfoxide-containing amides (**A1-2**–**A6-2** and **A4-4**, δ 9.25–11.65 ppm) > sulfone-containing amides (**A1-3**–**A6-3** and **A4-5**, δ 8.71–10.36 ppm) > thioether-containing amides (**A1-1**–**A6-1**, δ 7.59–8.84 ppm). The pyrazole-H showed a similar sequence—sulfoxide-containing amides (δ 7.05–7.31 ppm) > sulfone-containing amides (δ 6.98–7.08 ppm) > thioether-containing amides (δ 6.89–6.98 ppm). While the trend of chemical shift for the protons on alkyl carbon attached to sulfur atom generally was sulfone-containing amides (δ 3.05–4.18 ppm) > sulfoxide-containing amides (δ 2.71–3.88 ppm) > thioether-containing amides (δ 2.32–3.34 ppm). These results are mainly due to the different electronic effect of –S–, –S(=O)– and –$S(=O)_2$– groups. Moreover, when R_2 = CH_2CF_3, the ^1H NMR and ^{13}C NMR spectra for compounds **A4-1, A4-2** and **A4-3** showed up chemical shifts of $\underline{CH_2}CF_3$ moiety at δ 3.34–3.97 ppm and δ 36.7–57.5 ppm, respectively, which were both split into quartets-$\underline{CH_2}$ (q, J = 9.3–9.6 Hz) and $\underline{CH_2}$ (q, J = 31.3–33.0 Hz) owing to the coupling-splitting of F to neighboring H or C. Meanwhile, the typical carbon chemical shift for the CF_3 group appeared at δ 119.6–125.1 ppm as quartets (J = 277.8–279.8 Hz). In the ^{19}F NMR spectra of the compounds, the fluorine signals were observed at δ −93.9–60.5 ppm (**A4-1**, **A4-2**, **A4-3** and **A8**), among which the F signal of sulfoxide-containing compound **A4-2** (δ −93.9 ppm) exhibited a much more upfield shift than that of the others (δ −66.0–−60.5 ppm).

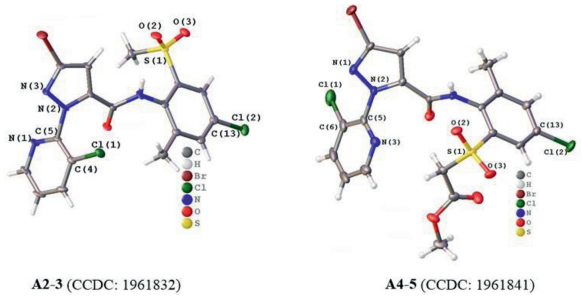


Fig. 2. The single-crystal structures of **A2-3** and **A4-5**.

The structures of compounds **A2-3** (CCDC No. 1961832) and **A4-5** (CCDC No. 1961841) were further confirmed by single crystal X-ray diffraction analysis, which are shown in Fig. 2. The corresponding crystal data were provided in the Supporting information. From the molecular structures, it can be seen that the dihedral angle between pyrazole ring and pyridine ring are 69.274(210)° (**A2-3**) and 51.603(75)° (**A4-5**), respectively, which revealed that the two heterocyclic rings are not coplanar. Furthermore, the torsion angles of N(3)-N(2)-C(5)-N(1) in **A2-3**, and N(1)-N(2)-C(5)-N(3) in **A4-5** are 69.926(758)° and −123.348(220)°, respectively, indicates that the pyridine ring flipped about 180° between the two molecular structures, which can be clearly seen from the Fig. 2 that the relative positions of the pyridine ring towards pyrazole ring in two molecules are reverse. This may be derived from maintaining the lowest energy optimal configuration of the molecular structure to reduce the steric hindrance of groups. Therefore, the electronic effect and large steric hindrance of substituent in benzene ring (**A4-5**: R_2 = $CH_2CO_2CH_3$; **A2-3**: R_2 = CH_3) were the main possible fac-

Table 1
Insecticidal activity of compounds **A** against *M. separata*.

Compd.	R_1	R_2	Larvicidal activity (%) at a concentration of (mg/L)				
			200	100	50	25	10
A1-1	H	CH_3	40 ± 3	n.t.*	n.t.	n.t.	n.t.
A1-2	H	CH_3	65 ± 1	n.t.	n.t.	n.t.	n.t.
A1-3	H	CH_3	20 ± 5	n.t.	n.t.	n.t.	n.t.
A2-1	CH_3	CH_3	98 ± 2	45 ± 1	n.t.	n.t.	n.t.
A2-2	CH_3	CH_3	100	60 ± 2	n.t.	n.t.	n.t.
A2-3	CH_3	CH_3	100	99 ± 1	100	100	60 ± 2
A3-1	CH_3	CH_2CH_3	100	50 ± 2	n.t.	n.t.	n.t.
A3-2	CH_3	CH_2CH_3	97 ± 3	30 ± 3	n.t.	n.t.	n.t.
A3-3	CH_3	CH_2CH_3	100	100	95 ± 5	98 ± 2	20 ± 0
A4-1	CH_3	CH_2CF_3	100	100	100	50 ± 2	n.t.
A4-2	CH_3	CH_2CF_3	100	60 ± 3	n.t.	n.t.	n.t.
A4-3	CH_3	CH_2CF_3	95 ± 5	20 ± 0	n.t.	n.t.	n.t.
A4-4	CH_3	$CH_2CO_2CH_3$	98 ± 2	35 ± 1	n.t.	n.t.	n.t.
A4-5	CH_3	$CH_2CO_2CH_3$	98 ± 2	50± 2	n.t.	n.t.	n.t.
A5-1	CH_3	$CH(CH_3)_2$	95 ± 5	50 ± 2	n.t.	n.t.	n.t.
A5-2	CH_3	$CH(CH_3)_2$	80 ± 3	0	n.t.	n.t.	n.t.
A5-3	CH_3	$CH(CH_3)_2$	95 ± 5	15 ±3	n.t.	n.t.	n.t.
A6-1	Cl	CH_3	98 ± 2	80 ± 0	n.t.	n.t.	n.t.
A6-2	Cl	CH_3	100	98 ± 2	50 ± 2	n.t.	n.t.
A6-3	Cl	CH_3	100	97 ± 3	100	95 ± 5	45 ± 1
A7	CH_3	CH_3	94 ± 6	55 ± 1	n.t.	n.t.	n.t.
A8	CH_3	CH_2CF_3	100	95 ± 5	60 ± 0	n.t.	n.t.
Chlorantraniliprole	-	-	100	100	98 ± 2	100	100

* n.t.: not test.

tors leading to this result, which also may be the same reason for the great difference of the bioactivity between the two compounds.

At an initial test concentration of 200 mg/L, all the title compounds **A** exhibited obvious insecticidal activities against *M. separata* (20%–100%). As shown in Table 1, we can get the following structure–activity relationship (SAR). When R_2 was fixed as CH_3, compounds bearing CH_3 or Cl group for R_1 (*e.g.*, **A2-3** and **A6-3**, 100%) exhibited much higher larvicidal activity than those of bearing non-substituent (R_1 = H) [*e.g.*, **A1-3**, (20 ± 5)%]. At lower concentrations, some of the compounds still showed good activity against *M. separata*. For examples, **A2-3**, **A3-3**, **A4-1** and **A6-3** held lethality rate of 50%–100% at 25 mg/L; particularly, **A2-3** and **A6-3** at 10 mg/L possessed lethality rate of (60 ± 2)% and (45 ± 1)%, respectively. Through SAR analysis, it was found that when fixing R_1 and R_2 as electron-donating groups, smaller substituents in R_2 produce better bioactivity [sequence: CH_3 > C_2H_5 > $CH(CH_3)_2$]. While when fixing R_2 as CH_3, the insecticidal activity trend corresponding to R_1 substituent was CH_3 ~ Cl. Furthermore, when R_1 and R_2 were fixed as CH_3 or Cl, and CH_3 or C_2H_5, respectively, it showed an activity sequence of sulfone-containing compounds > sulfoxide-containing compounds ≥ thioether-containing compounds in the same series of compounds, that is, **A2-3** > **A2-2** > **A2-1**; **A3-3** > **A3-2** ≈ **A2-1**; **A6-3** > **A6-2** > **A6-1**. This indicated that the stronger electron-withdrawing ability of the sulfur-substituents is favorable for the improvement of the bioactivity, in other words, the more O atoms the S atom connects, the higher insecticidal activity the corresponding compound has. However, when R_1 = CH_3 and R_2 = CH_2CF_3, the larvicidal activity sequence was thioether-containing compound [**A4-1**, (50 ± 2)% (25 mg/L)] > sulfoxide-containing compound [**A4-2**, (60 ± 3)% (100 mg/L)] > sulfone-containing compound [**A4-3**, (20 ± 0)% (100 mg/L)]. Compounds **A4-4** and **A4-5**, unexpectedly obtained from the oxidation reaction of thioether compound **A4-1** in methanol solvent, showed common larvicidal activity against *M. separata* at 100 mg/L [(35 ± 1)% and (50 ± 2)%]. So we speculated that bearing electron-withdrawing and bulky groups together for R_2 are unfavorable to the increase of bioactivity against *M. separata*, meanwhile the CF_3 moiety in the case of **A4-1** may also contribute to the bioactivity improvement, possibly due to the effect of fluorine atom (*e.g.*, lipophilicity and metabolic stability). In addition, from Table 1 the title compounds containing sulfone moiety were found to possess much better bioactivity than that of the others.

Table 2
Insecticidal activity of partial compounds **A** against *P. xylostella*.

Compd.	Larvicidal activity (%) at a concentration of (mg/L)					
	200	100	10	1	0.1	0.01
A2-1	100	100	100	100	50 ± 2	n.t.
A2-2	100	100	100	90 ± 1	60 ± 0	0
A2-3	100	100	100	100	50 ± 2	10 ± 5
A3-1	100	100	100	80 ± 1	n.t.	n.t.
A3-2	100	100	100	65 ± 1	40 ± 2	n.t.
A3-3	100	100	100	100	70 ± 2	20 ± 5
A4-1	100	100	100	100	100	80 ± 0
A4-2	100	100	100	90 ± 1	75 ± 1	n.t.
A4-3	100	100	90 ± 1	50 ± 1	20 ± 2	n.t.
A4-4	100	100	100	85 ± 1	70 ± 2	n.t.
A4-5	100	100	100	77 ± 1	55 ± 1	n.t.
A5-1	100	100	100	80 ± 0	n.t.	n.t.
A5-2	100	100	40 ± 2	n.t.*	n.t.	n.t.
A5-3	100	100	70 ± 1	30 ± 2	n.t.	n.t.
A6-1	100	100	100	100	100	80 ± 1
A6-2	100	100	100	100	100	40 ± 1
A6-3	100	100	100	100	100	60 ± 1
A7	100	100	80 ± 1	30 ± 2	n.t.	n.t.
A8	100	100	100	94 ± 1	80 ± 1	n.t.
Chlorantraniliprole	100	100	100	100	100	85 ± 1

* n.t.: not test.

From the insecticidal activity data of compounds **A** against *P. xylostella* listed in Table 2, we can see that all the tested compounds exhibited high larvicidal activity (40%–100%) towards *P. xylostella* at 10 mg/L, and most of compounds also showed obvious insecticidal activity (20%–100%) at lower concentration of 0.1 mg/L. Compounds **A4-1**, **A6-1**, **A6-2** and **A6-3** can still possess lethality rate of 40%–80% even at 0.01 mg/L concentration, especially the larvicidal activities of trifluoethylthio-containing compound **A4-1** [(80 ± 0)%] and methylthio-containing compound **A6-1** [(80 ± 1)%] were found very close to that of positive control chlorantraniliprole [(85

Table 3
LC_{50} values of partial compounds **A** against *P. xylostella*.

Compd.	$y = ax + b$	LC_{50} (mg/L)	*R*
A2-2	$y = 1.81x + 6.60$	0.1306	0.9938
A2-3	$y = 1.41x + 6.46$	0.0921	0.9939
A3-3	$y = 1.25x + 6.51$	0.0614	0.9798
A4-1	$y = 1.99x + 9.2887$	0.0070	0.9644
A6-1	$y = 2.55x + 10.96$	0.0046	0.9066
A6-2	$y = 2.64x + 9.92$	0.0137	0.8459
A6-3	$y = 1.34x + 7.68$	0.0099	0.9889
Chlorantraniliprole	$y = 2.25x + 10.64$	0.0031	0.8800

± 1)%] towards *P. xylostella*. Through SAR analysis, it was found that when fixing R_1 as CH_3, the activity trend of R_2 to the insecticidal activity of the compounds against *P. xylostella* was similar to that against *M. separata*, that is, smaller substituents in R_2 produce better bioactivity:-CH_3 > C_2H_5 > $CH(CH_3)_2$. However, when R_1 was fixed as CH_3, the tested compounds with CH_2CF_3 or $CH_2CO_2CH_3$ of R_2 almost possessed better bioactivity than those with CH_3, C_2H_5 and $CH(CH_3)_2$, which indicates a R_2 group with weaker electron-donating ability may be favorable for the bioactivity improvement of such kind of structures. Noticeably, all the compounds had favorable larvicidal activity when R_1 = Cl and $R_2 = CH_3$, showing a lethality rate of 40%–80% at 0.01 mg/L. When R_2 was fixed as CH_3, the bioactivity sequence corresponding to substituent R_1 was Cl > CH_3. Moreover, compounds **A2-2**, **A2-3**, **A3-3**, **A4-1**, **A6-1**, **A6-2** and **A6-3** were further tested lethal concentration 50% (LC_{50}) values towards *P. xylostella* and the results are shown in Table 3. These compounds possessed LC_{50} value of 0.0046–0.1306 mg/L, higher than that of control chlorantraniliprole (0.0031 mg/L); among which, thioether-containing compound **A6-1** with LC_{50} value of 0.0046 mg/L exhibited as the best insecticide, and had a great potential for further investigation.

Partial compounds with favorable activities in preliminary screening were selected to further test their insecticidal activities against *C. pipiens pallens, H. armigera* and *O. nubilalis*, and the results are shown in Table 4. We can see that towards *C. pipiens pallens*, the tested compounds showed high insecticidal activity (95%–100%) at 25 mg/L; however, when decreasing the test concentration to 10 mg/L, the compounds showed only moderate insecticidal activity (15%–55%). When fixing R_1 as CH_3, the insecticidal activity of sulfone-containing compounds displayed a trend of C_2H_5 [**A3-3**, (55 ± 1)%] > CH_3 [**A2-3**, (40 ± 0)%] for R_2 group. Meanwhile, when R_2 was fixed as CH_3, the insecticidal activity trend of R_1 was CH_3 [**A2-3**, (40 ± 0)%] > Cl [**A6-3**, (15 ± 3)%] for sulfone-containing compounds. Moreover, it also showed an activity trend of sulfoxide-containing compound [**A2-2**, (50 ± 2)%] > sulfone-containing compound [**A2-3**, (40 ± 0)%], when both bearing CH_3 for R_1 and R_2. All the four tested compounds displayed favorable larvicidal activities (95%–100%) towards *H. armigera* and *O. nubilalis* at 200 mg/L. In particular, sulfone-containing compounds **A2-3** and **A6-3** held lethality rate of 97%–100% at 100 mg/L, showing as the best insecticides among the four compounds. At lower concentration of 50 mg/L, both of the compounds merely exhibited moderate larvicidal activities against such two kinds of pests. The overall relationships between these compound structures and the insecticidal activities towards *H. armigera* and *O. nubilalis* illustrated the similar activity trends of sulfone-containing compound > sulfoxide-containing compound, CH_3 > Cl for R_1, and CH_3 > C_2H_5 for R_2.

To further study the mechanism of the novel sulfide-containing insecticidal compounds, the effects on the calcium channels of high bioactive compounds **A2-3** and **A6-3** were tested by using central neurons isolated from third-instar larvae of *M. separata* with the calcium imaging technique after neuron loading with fluo-3 AM. Fig. 3 showed the change in $[Ca^{2+}]_i$ *versus* recording time when the central neurons were treated with **A2-3**, **A6-3** and chlorantraniliprole in the absence of extracellular calcium. There are two intracellular calcium release channels in neurons named IP3 receptor (IP3R) and RyR. Free calcium could be released from calcium stores *via* RyR or IP3R into the endoplasmic reticulum. 2-Aminoethoxydiphenyl borate (2-APB) as IP3R antagonist has been used as blocker to probe aspects of calcium signaling. According to the experiment results, it was found that the peaks of $[Ca^{2+}]_i$ were all increased by treating the tested compounds with 5 mg/L **A2-3** and **A6-3** (Figs. 3A and C). These results demonstrated that the novel compounds could activate the calcium channels in the central neurons of the third-instar larvae of *M. separata*. For further study which is the main channel to release the calcium, RyR or IP3R blocker was used to preincubate the neurons about 5 min. The experiments showed that within the error range, the increasing of calcium had little effect after the blocking agents used (Figs. 3B and D). Therefore, ryanodine receptor in the central neurons of *M. separata* is the possible target—that is, these novel compounds may have the same target as that of chlorantraniliprole.

In summary, a series of novel sulfide-containing amide derivatives **A** were efficiently synthesized *via* a strategy of modifying the "amide" structure of anthranilic diamide insecticides, and their structures were identified by melting points, ^{1}H NMR, ^{13}C NMR, ^{19}F NMR and elemental analysis or HRMS. The molecular structures of **A2-3** and **A4-5** were further confirmed by the X-ray single-crystal diffraction analysis and firstly reported. The bioassay results showed that most of the compounds **A** possess moderate to high insecticidal activities. Especially, some sulfone-containing compounds exhibited favorable bioactivities, *e.g.*, compounds **A2-3**, **A3-3** and **A6-3** not only possessed high lethality rate against *P. xylostella* (50%–100%) at 0.1 mg/L, but also held good insecticidal activities towards a variety of agricultural pests such as *M. separata, C. pipiens pallen, H. armigera* and *O. nubilalis*. It is worth noting that the larvicidal activity towards *P. xylostella* for partial thioether-containing compounds [**A4-1**: (80 ± 0)%; **A6-1**: (80 ± 1)%] was close to that of chlorantraniliprole [(85 ± 1)%] at 0.01 mg/L. The calcium imaging experiments revealed that the highly insecticidal

Table 4
Insecticidal activities of partial compounds **A** against *C. pipiens pallens, H. armigera* and *O. nubilalis*.

Compd.	Larvicidal activity (%) at a concentration of (mg/L)							
	C. pipiens pallens		*H. armigera*			*O. nubilalis*		
	25	10	200	100	50	200	100	50
A2-2	100	50 ± 2	97 ± 3	40 ± 3	n.t.*	95 ± 5	10 ± 10	n.t.
A2-3	97 ± 3	40 ± 0	100	98 ± 2	35 ± 1	100	98 ± 2	25 ± 2
A3-3	100	55 ± 1	95 ± 5	10 ± 10	n.t.	100	20 ± 0	n.t.
A6-3	95 ± 5	15 ± 3	98 ± 2	100	30 ± 3	98 ± 2	97 ± 3	15 ± 3
Chlorantraniliprole	98 ± 2	100	100	97 ± 3	100	97 ± 3	100	98 ± 2

* n.t.: not test.

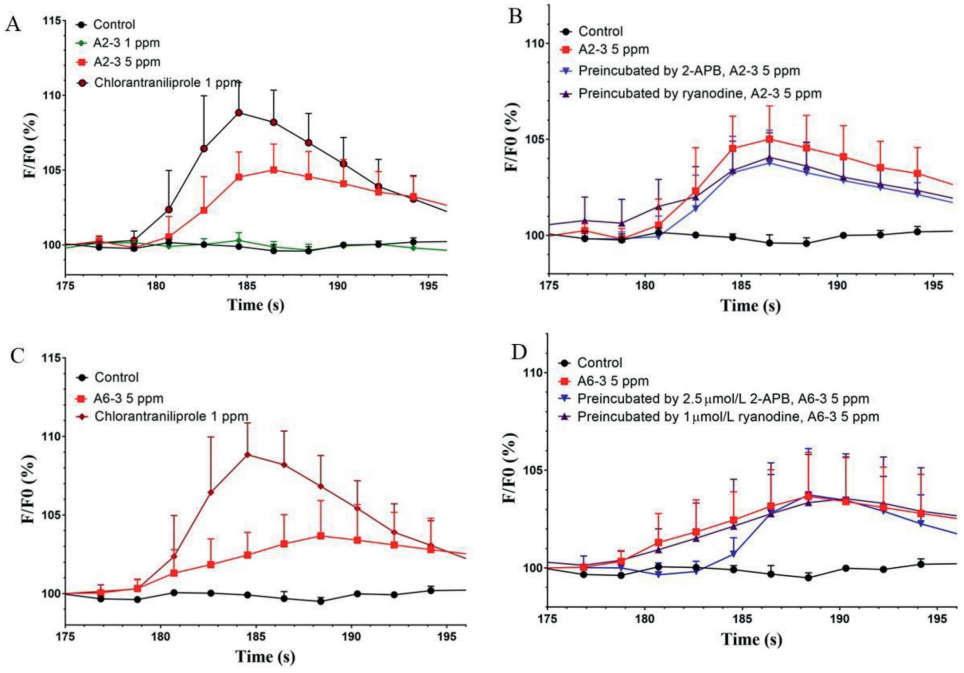


Fig. 3. Effects of **A2-3** and **A6-3** and chlorantaniliprole on $[Ca^{2+}]_i$ on the central neurons of *M. separata* when extracellular calcium was absent (EGTA replaced calcium).

A2-3 and **A6-3** as the representative compounds are potential RyR modulators. In addition, the structure–activity relationships were discussed in detail. The research results in this paper provide useful information for further design and development of novel compounds with promising insecticidal activities.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2021.05.027.

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