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Synthesis and Insecticidal Activities of Novel Anthranilic Diamides Containing Acylthiourea and Acylurea

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Supporting Information

ABSTRACT: Two series of anthranilic diamides containing acylthiourea and acylurea linkers were designed and synthesized, with changed length and flexibility of the linkers to compare to known anthranilic diamide insecticides. In total, 26 novel compounds were synthesized, and all compounds were characterized by ^{1}H nuclear magnetic resonance and high-resolution mass spectrometry. Their insecticidal activities against oriental armyworm (*Mythimna separata*), mosquito larvae (*Culex pipiens pallens*), and diamondback moth (*Plutella xylostella*) were evaluated. The larvicidal activities against oriental armyworm indicated that the introduction of acylthiourea into some structures could retain their insecticidal activity; 8 of the 15 compounds (**13a**−**13e**, **14a**−**14e**, and **15a**−**15e**) exhibited 100% larvicidal activity at 10 mg/L. However, the introduction of acylurea decreased the insecticidal activity; only 3 of the 11 compounds (**17a**−**17k**) exhibited 100% larvicidal activity at 200 mg/L. The whole-cell patch-clamp technique indicated that compound **13b** and chlorantraniliprole exhibited similar effects on the voltage-gated calcium channel. The calcium-imaging technique was also applied to investigate the effects of compounds **13b** and **15a** on the intracellular calcium ion concentration ($[Ca^{2+}]_i$), which indicated that they released stored calcium ions from endoplasmic reticulum. Experimental results denoted that several new compounds are potential activators of the insect ryanodine receptor (RyR).

KEYWORDS: *Anthranilic diamide, insecticidal activity, acylthiourea, acylurea, calcium channel*

INTRODUCTION

For years, scientists have been dedicated in searching for insecticides with new mechanisms to cope with the global food shortage. Recently, the anthranilic diamides chlorantraniliprole (Rynaxypyr; DPX-E2Y45) (compound **A** in Figure 1) and cyantraniliprole (Cyazypyr) (compound **B**) were marketed by Dupont, targeting at the insect ryanodine receptor,[1,2] which exhibit their action by activating the uncontrolled release of the calcium store.[1] Their broad insecticidal spectra, high efficiency, and low toxicity aroused interests worldwide. Some modified structures (compound **C**) have been reported, which mainly focused on the 1-(3-chloropyridyl) pyrazole moiety (I), the aliphatic amide moiety (II), and the anthraniloyl moiety (III).[2−11] Nevertheless, the modifications about the amide bridge part as a linker of two aryl rings were seldom reported.[12]

In crop protection and bioactive chemicals, acylthioureas and acylureas have been reported to display a variety of biological activities, such as insecticidal, fungicidal, herbicidal, antimicrobial, antitumor, etc.[13−18] With this in mind, two series of novel anthranilic diamide derivatives (compound **D**) were designed and synthesized in this paper by introducing acylthiourea and acylurea moieties to increase the linker length and flexibility. Their synthetic routes were shown in Schemes 1−5. Their insecticidal activities against oriental armyworms, mosquito larvae, and diamondback moths were tested accordingly. The preliminary structure−activity relationship (SAR) was discussed. To provide insight to further study the biological effect of target compounds, the whole-cell patch-clamp and calcium-imaging techniques were used to investigate the effects of compounds **13b** and **15a** on calcium channels in the central neurons of *Spodoptera exigua*.

MATERIALS AND METHODS

Instruments. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded at 300 MHz using a Bruker AC-P300 spectrometer or 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 on CombiFlash Companion (Teledyne Isco, Inc., Lincoln, NE) with silica gel (300−400 mesh). The melting points were determined on a X-4 binocular microscope melting point apparatus (Beijing Tech Instruments Co., Beijing, China) and were uncorrected. The whole-cell patch clamp was performed using a patch-clamp amplifier (EPC-10, HEKA Electronik, Lambrecht, Germany). Reagents were all analytically or chemically pure. All solvents and liquid reagents were dried by standard methods in advance and distilled before use. Chlorantraniliprole used in this work was synthesized according to the literature only for bioassay reference.[19]

General Synthetic Procedure for Compounds 6a and 6b. The title compounds **6a** and **6b** were prepared in our laboratory

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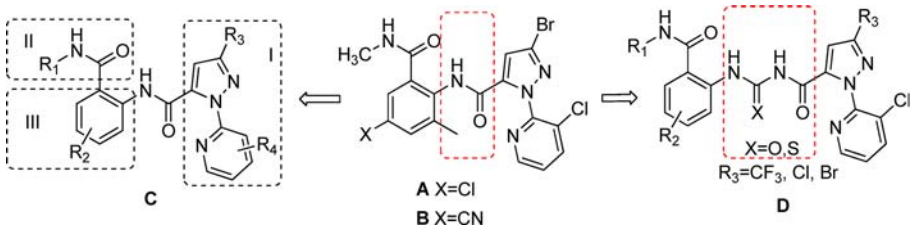
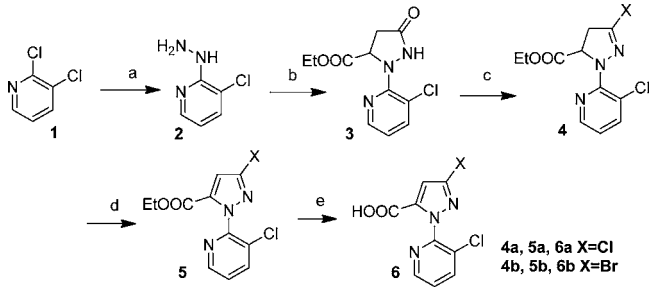


Figure 1. Chemical structures of compounds **A**–**D**.

according to the methods reported by Dong et al. (Scheme 1), with some modifications. The melting points and ^{1}H NMR data of all of the compounds were consistent with the literature.[19,20]

Scheme 1. Synthesis of Title Compounds 6a and 6b[a]



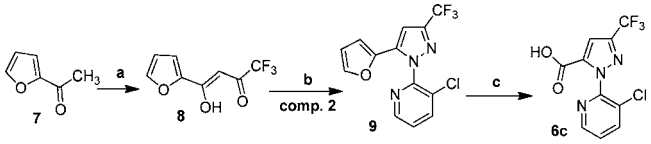
[a]Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$ (80%), reflux, (b) NaOEt, EtOH, reflux, (c) $POBr_3$ or $POCl_3$, MeCN, 80 °C, (d) $K_2S_2O_8$, H_2SO_4, MeCN, reflux, and (e) (i) aqueous NaOH, MeOH and (ii) aqueous HCl.

1,1,1-Trifluoro-4-(furan-2-yl)-4-hydroxybut-3-en-2-one (8). Sodium (1.38 g, 60 mmol) was dissolved in 30 mL of anhydrous methanol slowly. Methanol was evaporated to dryness under reduced pressure to give the white solid sodium methylate. Then, diethyl ether (50 mL), ethyl trifluoroacetate (8.52 g, 60 mmol), and 2-acetylfuran (6.60 g, 60 mmol) were successively added dropwise, stirred at room temperature for 5 h, and filtered, which was acidized with 1 M sulfuric acid to give a red liquid product. Yield = 64.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.67 (br s, 1H, OH), 7.67 (s, 1H, Ar–H), 7.33 (d, 1H, J = 3.6 Hz, Ar–H), 6.63 (d, 1H, J = 3.6 Hz, Ar–H), 6.48 (s, 1H, CH).

3-Chloro-2-(5-(furan-2-yl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)pyridine (9). In a three-neck 1000 mL round-bottomed flask, 1,1,1-trifluoro-4-(furan-2-yl)-4-hydroxybut-3-en-2-one (**8**, 27.1 g, 130 mmol) and 3-chloro-2-hydrazinylpyridine (18.9 g, 130 mmol) were added to 300 mL of acetic acid. The mixture was refluxed for 8 h, and then the excess acetic acid was removed under reduced pressure. The residue was further purified by flash column chromatography on silica gel with petroleum ether/ethyl acetate (5:1) to give intermediate **9** as a red oil. Yield = 42.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.58 (d, J = 4.8 Hz, 1H, pyridyl-H), 7.96 (d, J = 8.0 Hz, 1H, pyridyl-H), 7.51 (dd, J = 8.0, 4.8 Hz, 1H, pyridyl-H), 7.36 (s, 1H, pyrazolyl-H), 6.93 (s, 1H, furanyl-H), 6.41–6.30 (s, 1H, furanyl-H), 6.01 (d, J = 3.4 Hz, 1H, furanyl-H).

1-(3-Chloropyridin-2-yl)-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxylic acid (6c). To a solution of 3-chloro-2-(5-(furan-2-yl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)pyridine (**9**, 3.88 g, 12.4 mmol) in acetone (35 mL) and water (35 mL), potassium permanganate (9.80 g, 62 mmol) was added in portion. When the addition was complete, the reaction was refluxed for 30 min. The resulting mixture was filtered. The filtrate was acidified with 2 M HCl and extracted with ethyl acetate (50 mL × 3). The extracts were combined, washed with water (30 mL × 2) and brine (30 mL), then dried with anhydrous sodium sulfate, and evaporated to give compound **6c** as a white solid (1.85 g). Yield = 51.3%. Melting point (mp) = 176–179 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 14.14 (s, 1H), 8.61 (dd, J = 4.8, 1.5 Hz, 1H, pyridyl-H), 8.31 (dd, J = 8.0, 1.5 Hz, 1H, pyridyl-H), 7.74 (dd, J = 8.0, 4.8 Hz, 1H, pyridyl-H), 7.61 (s, 1H, pyrazolyl-H) (Scheme 2).

Scheme 2. Synthesis of Compound 6c[a]



[a]Reagents and conditions: (a) $NaOCH_3$, ethyl trifluoroacetate, (b) AcOH, reflux, and (c) $KMnO_4$, acetone/H_2O, reflux.

General Synthetic Procedure for Compounds 11a–11m. The compounds **11a**–**11m** were prepared in our laboratory according to the methods reported by Dong et al. (Scheme 3 and Table 1). The melting points and ^{1}H NMR data of all of the compounds were consistent with the literature.[19,20]

Scheme 3. Synthesis of Compounds 11a–11m

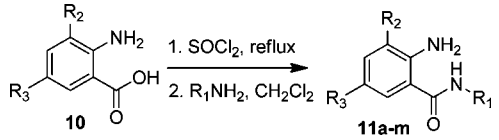


Table 1. Structure of Compounds 11a–11m

compound	R_1	R_2	R_3
11a	Me	Me	Cl
11b	Et	Me	Cl
11c	i-Pr	Me	Cl
11d	i-Pr	H	Cl
11e	*n*-Pr	H	Cl
11f	*n*-Bu	Me	Cl
11g	*t*-Bu	Me	Cl
11h	cyclopropyl	Me	Cl
11i	cyclohexyl	Me	Cl
11j	i-Pr	Me	Br
11k	benzyl	Me	Br
11l	i-Pr	Me	H
11m	i-amyl	Me	H

General Synthetic Procedure for Compounds 13a–13e, 14a–14e, and 15a–15e. To a suspension of 1-(3-chloropyridin-2-yl)-1*H*-pyrazole-5-carboxylic acid derivative (**6a**–**6c**) (1 mmol) in 25 mL of dichloromethane, oxalyl chloride (2.0 mmol) and a drop of *N*,*N*-dimethylformamide (DMF) were added. After stirring at room temperature for 3 h, the solution was evaporated. The resulting acyl chloride was dissolved in 20 mL of acetonitrile and added to a solution of potassium thiocyanate in 15 mL of acetonitrile with two drops of polyethylene glycol-400 (PEG-400). After stirring at room temperature for 30 min, the mixture was filtered to give the acyl isothiocyanate derivatives **12a**–**12c**, which were used without further

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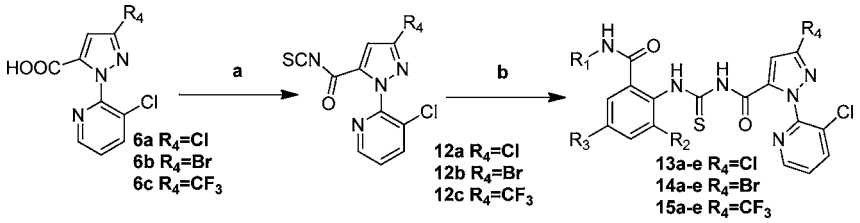
Scheme 4. Synthesis of Compounds 13a−13e, 14a−14e, and 15a−15e[a]



[a]Reagents and conditions: (a) (i) oxalyl chloride, CH_2Cl_2 and (ii) KSCN, PEG-400, CH_3CN and (b) **11a−11m**, CH_3CN.

purification, to which the 2-amino-3-methylbenzamide derivatives (**11a−11m**) (1.0 mmol) were added and stirred overnight, then filtered, and further purified by recrystallization using methanol or by a silica-gel column eluted with petroleum ether/ethyl acetate (4:1) to obtain the title compounds **13a−13e**, **14a−14e**, and **15a−15e**.

1-(3-Chloropyridin-2-yl)-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxamide (16). To a suspension of 3-trifluoromethyl-1-(3-chloropyridin-2-yl)-1*H*-pyrazole-5-carboxylic acid (**6c**) (2.40 g, 8.23 mmol) in 50 mL of dichloromethane, oxalyl chloride (17.0 mmol) and a drop of DMF were added. After stirring at room temperature for 3 h, the solution was evaporated. The resulting acyl chloride was dissolved in 50 mL of tetrahydrofuran (THF) and added to aqueous ammonia (25%) (5.76 g, 41.15 mmol) at 0 °C. After stirring overnight, a large amount of solid formed and was filtered, which was further purified by a silica-gel column eluted with petroleum ether/ethyl acetate (2:1) to give the title compound as a white solid (1.85 g). Yield = 77.3%. mp = 183−185 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.56 (d, J = 4.8 Hz, 1H, pyridyl-H), 8.00 (d, J = 8.0 Hz, 1H, pyridyl-H), 7.53 (dd, J = 8.0, 4.8 Hz, 1H, pyridyl-H), 7.38 (s, 1H, pyrazolyl-H), 6.78 (s, 1H, NH), 5.77 (s, 1H, NH).

General Synthetic Procedure for Compounds 17a−17k. A suspension of compound **16** (0.29 g, 1 mmol) in 20 mL of 1,2-dichloroethane and oxalyl chloride (3.0 mmol) was heated to reflux and kept for 5 h. Then, the mixture was evaporated, and the residue was dissolved in acetonitrile, to which the 2-amino-3-methylbenzamide derivatives (**11a−11m**) (1 mmol) were added and stirred overnight. Then, the produced precipitate was filtered and washed with acetonitrile (5 mL × 3) to give the title compounds **17a−17k**.

Larvicidal Activity against Oriental Armyworm (*M. separata*). The larvicidal activities of compounds **13a−13e**, **14a−14e**, **15a−15e**, **17a−17k**, and chlorantraniliprole were evaluated using the reported procedure.[19] The insecticidal activity against oriental armyworms was tested by foliar application; individual corn (Tangyu 10, *Zea mays* L.) leaves were placed on moistened pieces of filter paper in Petri dishes. The leaves were then sprayed with the test solution and allowed to dry. The dishes were infested with 10 fourth-instar oriental armyworm larvae. Percentage mortalities were evaluated 2 days after treatment. Each treatment was replicated 3 times.

Larvicidal Activity against Mosquito Larvae (*C. pipiens pallens*). The larvicidal activities of compounds **13a−13e**, **14a−14e**, and chlorantraniliprole against mosquito larvae were evaluated by the reported procedure.[22] The compounds **13a−13e**, **14a−14e**, and chlorantraniliprole were prepared to different concentrations by dissolving compounds in acetone and adding distilled water. Then, 20 fourth-instar mosquito larvae were placed in 10 mL of test solution and raised for 3 days. Each treatment was performed 3 times, and the average value of the three tested values was calculated. The results were expressed by death percentage.

Larvicidal Activity against Diamondback Moth (*P. xylostella*). The larvicidal activity of compounds **15a−15e** and chlorantraniliprole was tested by the leaf-dip method. At first, a solution of each test sample in DMF (AR, purchased from Alfa Aesar) at a concentration of 200 mg/L was prepared and then diluted to the required concentration with water (distilled). Leaf disks (6 × 2 cm) were cut from fresh cabbage leaves and then sprayed with the test solution for 3 s and allowed to dry. The resulting leaf disks were placed individually into glass tubes. Each disk was infested with 30 s-instar diamondback moth larvae. Percentage mortalities were evaluated 2 days after treatment. Each treatment was performed 3 times.

Electrophysiological Recording and Calcium Imaging. The currents of calcium (I_{Ca}) were recorded using the reported procedure.[23] Calibration of the fluorescence signal was achieved using the method by Iakahashi et al., with some modifications.[24] The attached neurons were rinsed in standard physiological saline [150 mM NaCl, 4 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, and 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), buffered to pH 6.9] and then incubated in the dark at 28 °C in standard external saline containing the dye fluo-3-AM (Sigma, 10 μM) for 45 min or incubated in the dark at 28 °C in external saline [150 mM NaCl, 4 mM KCl, 2 mM $MgCl_2$, 2 mM ethylene glycol bis(2-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), and 10 mM HEPES, buffered to pH 6.9] containing the dye fluo-5N (Invitrogen, 5 μM) for 5 h. After dye loading, cells were again rinsed in physiological saline twice.

For full depletion of thapsigargin-sensitive stores, cells were incubated with 1 μM thapsigargin for 10 min. Calcium ratio imaging studies were conducted using the imaging system coupled to an inverted fluorescence microscope with a Fluor 40× oil immersion objective (Olympus IX71). Cells which excited at 488 and 530 nm under fluorescence emission were acquired using a charge-coupled device (CCD) camera (Image Pri-6.0).

Each experiment was repeated at least 6 times. The data were analyzed using SPSS, Inc., version 17.0, and Microcal Origin, version 8.0 (Origin Lab Corp., Northampton, MA). Results were expressed as the mean ± standard deviation (SD) (n = number of cells). Statistical significance was determined by Student's paired or unpaired t tests. Fluorescence values were expressed as F/F_0, with F_0 being the resting (or baseline) fluorescence and F being the change in fluorescence from baseline after the drug application.

RESULTS AND DISCUSSION

Synthesis. The carboxylic acids **6a** and **6b** were prepared using the procedure reported by Dong et al. (Scheme 1),[19,20] with some modifications, for the synthesis of intermediates. With the reported procedure, intermediate **2** was prepared in 69% yield by refluxing 2,3-dichloropyridine (**1**) and hydrazine hydrate (50%) in ethanol for 36 h. In our experiments, hydrazine hydrate (80%) was used to directly reflux with compound **1** for 5−6 h without solvent ethanol. By the improvement, compound **2** could be obtained in a 93% high yield.

The title compounds **13a−13e**, **14a−14e**, and **15a−15e** were synthesized, as shown in Scheme 4. Carboxylic acid (**6a−6c**) was treated with oxalyl chloride and then added to potassium thiocyanate in acetonitrile with two drops of PEG-400. The corresponding acyl isothiocyanate derivative was filtered after stirring at room temperature for 30 min and reacted with 1 equiv of amines **11a−11m** to afford the title compounds **13a−13e**, **14a−14e**, and **15a−15e**, without further purification. Unlike the reported procedure that a reflux condition was usually needed,[14] this reaction was worked out

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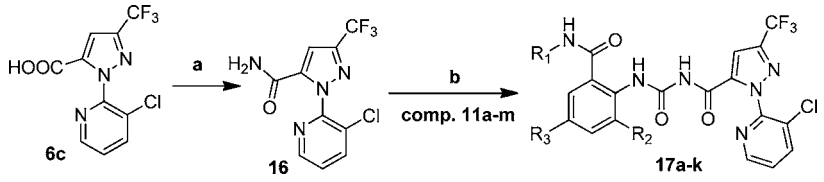
Scheme 5. Synthesis of Compounds 17a−17k[a]



[a]Reagents and conditions: (a) (i) oxalyl chloride, CH_2Cl_2 and (ii) $NH_3{\cdot}H_2O$ and (b) (i) oxalyl chloride, $ClCH_2CH_2Cl$, reflux and (ii) **11a−11m**, CH_3CN.

Table 2. Insecticidal Activities of Compounds 13a−13e, 14a−14e, 15a−15e, 17a−17k, and Chlorantraniliprole against Oriental Armyworms

					larvicidal activity (%) at a concentration of mg/L					
compound	R_1	R_2	R_3	R_4	200	100	50	20	10	5
13a	Me	Me	Cl	Cl	100	100	100	100	40	
13b	i-Pr	Me	Cl	Cl	100	100	100	100	100	40
13c	i-Pr	Me	Br	Cl	100	80	40			
13d	i-Pr	Me	H	Cl	100	100	100	100	100	0
13e	i-amyl	Me	H	Cl	60					
14a	Me	Me	Cl	Br	100	100	100	100	100	50
14b	i-Pr	Me	Br	Br	100	100	100	100	100	0
14c	*t*-Bu	Me	Cl	Br	100	100	100	100	100	40
14d	cyclohexyl	Me	Cl	Br	0					
14e	i-amyl	Me	H	Br	100	30				
15a	Me	Me	Cl	CF_3	100	100	100	100	100	50
15b	Et	Me	Cl	CF_3	100	100	100	100	100	0
15c	cyclohexyl	Me	Cl	CF_3	40					
15d	cyclopropyl	Me	Cl	CF_3	100	100	100	100	100	30
15e	benzyl	Me	Br	CF_3	100	100	100	100	0	
17a	Me	Me	Cl	CF_3	100	20				
17b	cyclopropyl	Me	Cl	CF_3	20					
17c	*n*-Bu	Me	Cl	CF_3	40					
17d	*t*-Bu	Me	Cl	CF_3	60					
17e	cyclohexyl	Me	Cl	CF_3	0					
17f	i-Pr	Me	Cl	CF_3	70					
17g	cyclopropyl	Me	Br	CF_3	100	0				
17h	*n*-Pr	H	Cl	CF_3	0					
17i	cyclopropyl	Me	H	CF_3	100	80				
17j	cyclopropyl	H	Cl	CF_3	30					
17k	*n*-Pr	Me	H	CF_3	60					
chlorantraniliprole					100	100	100	100	100	100

at room temperature under the catalysis of a small amount of PEG-400 with high yield.

The title compounds **17a−17k** were synthesized, as shown in Scheme 5. Carboxylic acid **6c** was converted to amide **16** by reacting with oxalyl chloride and then aqueous ammonia. Compound **16** was then refluxed with oxalyl chloride in 1,2-dichloroethane to give its corresponding acyl isocyanate derivative, which coupled with amines **11a−11m** to afford the title compounds **17a−17k**.

The ^{1}H NMR data of acylthiourea and acylurea derivatives were characteristic in all of the target compounds. In compounds **13a−13e** and **14a−14e**, the two active proton signals of NHCS and NHCO on carbonyl thiourea moiety were observed in DMSO-d_6 at 12.15−12.04 and 11.48−11.37 ppm, respectively. In compounds **15a−15e**, the introduction of a trifluoromethyl group increased the hydrophobicity of the whole molecule; therefore, the ^{1}H NMR determination was carried out in $CDCl_3$. The chemical shifts of these two active protons were at 11.34−11.21 and 10.10−9.77 ppm. In compounds **17a−17k**, when R_2 = Me, the two active proton signals of CONHCO and NHCO on the carbonyl acylurea moiety appeared at 11.55−11.46 and 9.93−9.72 ppm, respectively. Whereas their values shifted to 11.54−11.52 and 11.16−11.11 ppm, respectively, when R_2 = H (**17h** and **17j**) in DMSO-d_6.

SAR. *Larvicidal Activities against Oriental Armyworms (M. separata).* The larvicidal activities of compounds **13a−13e**, **14a−14e**, **15a−15e**, **17a−17k**, and commercial chlorantraniliprole against oriental armyworms were summarized in Table 2. In general, most of the compounds exist in moderate to good pesticidal activities. Particularly, eight compounds (**13a−13e**, **14a−14e**, and **15a−15e**) exhibited 100% larvicidal activities at 10 mg/L against oriental armyworm. From the activities against oriental armyworms, we could obviously find that the compounds containing the acylthiourea moiety (**13a−13e**, **14a−14e**, and **15a−15e**) gave higher insecticidal activities than the corresponding acylurea derivatives (**17a−17k**). When compounds **15a** and **15d** are compared to compounds **17a**

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and **17b**, we could find that one atom difference between acylthiourea and acylurea can cause a great change in its insecticidal activity. When different substitutions in the aliphatic amide moiety of title compounds **13a−13e**, **14a−14e** and **15a−15e** are compared, it was found that large substituents, such as i-amyl and cyclohexyl (**13e**, **14d**, **14e**, and **15c**) decreased the insecticidal activity against oriental armyworms. Furthermore, compounds with the i-propyl and *t*-butyl substituents showed the best larvicidal activity against ariental armyworms in our research, which was consistent with the results reported in previous SARs.[2,25]

Biological Assay. Insecticidal activities against oriental armyworms (*Mythimna separata*), mosquito larvae (*Culex pipiens pallens*), and diamondback moths (*Plutella xylostella*) were performed on test organisms reared in a greenhouse. The bioassay was replicated at 25 ± 1 °C according to statistical requirements. Assessments were made on a dead/alive basis, and mortality rates were corrected applying Abbott's formula.[21] Evaluation was based on a percentage scale of 0−100, where 0 equals no activity and 100 equals total kill. Error of the experiments was 5%. For comparative purposes, chlorantraniliprole was tested as a reference. The insecticidal activity was summarized in Tables 2−4.

Larvicidal Activities against Mosquito Larvae (C. pipiens pallens). The larvicidal activities of compounds **13a−13e** and **14a−14e** against mosquito larvae were shown in Table 3. Most of the compounds tested showed good larvicidal activities, while compounds with big substituents i-amyl and cyclohexyl in the aliphatic amide moiety revealed somewhat less activity.

Table 3. Insecticidal Activities of Compounds 13a−13e, 14a−14e, and Chlorantraniliprole against Mosquito Larvae

	larvicidal activity (%) at a concentration of mg/L		
compound	2	1	0.5
13a	70	30	
13b	40		
13c	90	60	20
13d	20		
13e	10		
14a	60	20	
14b	90	70	30
14c	100	90	30
14d	40		
14e	20		
chlorantraniliprole	100	100	100

Larvicidal Activity against Diamondback Moths (P. xylostella). The larvicidal activities against diamondback moths of compounds **15a−15e** and chlorantraniliprole were shown in Table 4. From it, we can see that most of the compounds tested showed excellent larvicidal activities. Compounds **15d** and **15e** showed the best activities (both 100% at 0.01 mg/L). Similarly, compound **15e** with a large substituent (R_2 = benzyl) also showed good larvicidal activity against diamondback moths.

The toxicity profile (LC_{50} values) of compounds **15d** and **15e**, which were found to be the most active insecticides of these two series of compounds against diamondback moths (100% at 0.01 mg/L), was shown in Table 5. The LC_{50} values of compounds **15d** and **15e** were 0.000 25 and 0.000 65 mg/L, respectively, but still higher than that of chlorantraniliprole (0.000 012 3 mg/mL; Table 5).

Table 4. Insecticidal Activities of Compounds 15a−15e and Chlorantraniliprole against Diamondback Moths

	larvicidal activity (%) at a concentration of mg/L				
compound	20	1	0.1	0.01	0.001
15a	100	100	100	71	43
15b	100	100	100	86	29
15c	30	0			
15d	100	100	100	100	71
15e	100	100	100	100	57
chlorantraniliprole	100	100	100	100	100

Effects of Compounds 13b and 15a on Calcium Channels of Neurons from *S. exigua*. Figure 2 shows the change rate of peak current amplitude versus recording time in 0.1 and 0.001 mmol/L compound **13b**- and 0.1 mmol/L chlorantraniliprole-treated and control neurons. The peak currents were reduced to 54.71 ± 4.95% (n = 6), 74.63 ± 5.37% (n = 5), 39.43 ± 5.02% (n = 8), and 85.44 ± 1.14% (n = 6) of the initial value by the end of the 10 min recording, when the neurons were treated with 0.1 and 0.001 mmol/L compound **13b**, 0.1 mmol/L chlorantraniliprole, and the control, respectively. The peak current was reduced to 54.71 ± 4.95% (n = 6) when the neurons were treated with 0.1 mmol/L compound **13b**. In comparison to the control (85.44 ± 1.14%), compound **13b** at 0.001 mmol/L has a weak effect on $Ca^{2+}{}_{(L)}$ currents. The recorded peak currents of calcium channels treated with compound **13b** were in a concentration-dependent manner (Figure 2 and Table 6).

There was a significant change in maximal value of the peak current when the neurons were held at −70 mV with the treatment of 0.1 mmol/L compound **13b** (Figure 3). The calcium current decreased when compound **13b** was injected in the surrounding of the neuron cells. The maximal value of I_{Ca} (−2.1303 ± 0.0237 nA) shifted to the negative direction by approximately 10 mV after the neurons were treated with compound **13b** for 1 min. By the end of the 5 and 10 min recordings, I_{Ca} decreased to −1.6356 ± 0.0641 and −1.2738 ± 0.882 nA, respectively, and the I−V curves were also shifted to the negative direction by approximately 10 mV during the recording. Unlike the results for I_{Ca} described above, the maximal value of I_{Ca} did not shift to a negative direction when the neurons were treated with 0.001 mM compound **13b**, from which we concluded that, when the concentration was ≤0.001 mM, there was no effect on calcium channels in the central neurons of *S. exigua* third larvae.

These results indicated that $Ca^{2+}{}_{(L)}$ channels of *S. exigua* neurons were modulated by compound **13b** and partly closed after the action. Although there was no notable change on activation voltage, the negatively shifted I−V relationship curves indicate that voltage dependence was influenced by compound **13b**. All of these results suggest that the $Ca^{2+}{}_{(L)}$ channels of *S. exigua* neurons were the possible target of compound **13b**.

Figure 4 illustrated the change of $[Ca^{2+}]_i$ versus the recording time when the neurons were treated with compounds **13b**, **15a**, and chlorantraniliprole. The peak of $[Ca^{2+}]_i$ was 116.863 ± 2.33% (n = 10), 112.408 ± 1.26% (n = 8), and 123.292 ± 2.17% (n = 21) of the initial value by the end of the 10 min recording when the cells were treated with 1000 mg/L compound **13b**, 1000 mg/L compound **15a**, and 1000 mg/L

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Table 5. LC_{50} Values of Compounds 15d, 15e, and Chlorantraniliprole against Diamondback Moths

compound	$y = a + bx$	LC_{50} (mg/L)	R	95% confidence interval
15d	y =11.04 + 1.68x	0.00025	0.9974	0.00023−0.00028
15e	y =12.90 + 2.48x	0.00065	0.9723	0.00053−0.00081
chlorantraniliprole	y = 11.50 + 1.32x	0.0000123	0.9995	1.16×10^{-5}−1.31×10^{-5}

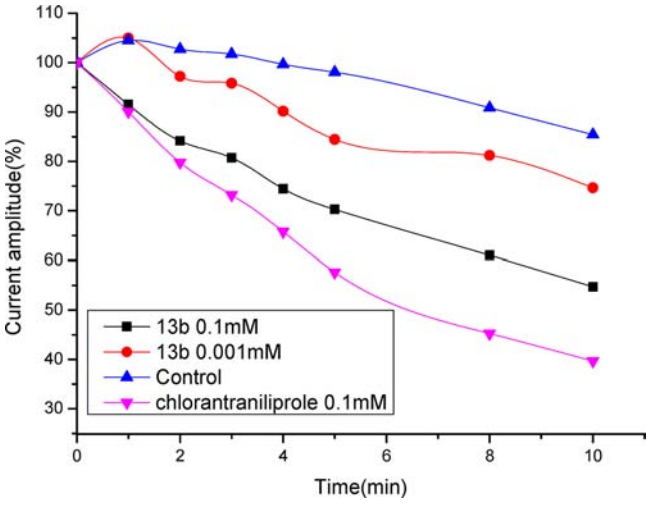

Figure 2. Variation of the peak current for the whole-cell calcium channels in neurons (treated with different concentrations of compound **13b** and chlorantraniliprole) at different times during patch-clamp recording in comparison to the peak value at 0 min.

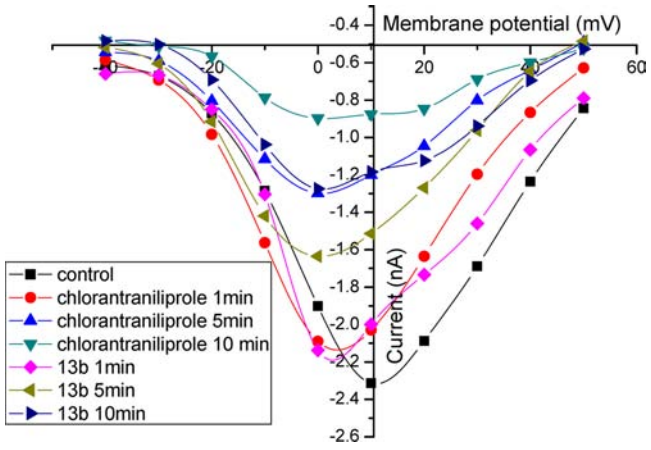

Figure 3. Current−voltage relationship curves of whole-cell calcium channels recorded in neurons of *S. exigua* (treated with 0.1 mmol/L compound **13b** and chlorantraniliprole) at different intervals (in minutes).

chlorantraniliprole, respectively. In comparison to the control (99.91 ± 2.56%), compounds **13b** and **15a** induced a $[Ca^{2+}]_i$ increase without extracellular Ca^{2+}. It indicated that compounds **13b** and **15a** could activate the calcium release channel in the endoplasmic reticulum (ER) membrane. Figure 3 also indicated that the recorded $[Ca^{2+}]_i$ (F/F_0) had a good positive correlation with bioactivities.

There were two kinds of calcium release channels in the ER membrane, namely, RyR and IP_3R Ca^{2+} channels.[26] To test which pathway was involved in the elevation of $[Ca^{2+}]_i$, the primary cultured neurons were dyed, loading with fluo-5N, then treated with heparin (10 mg/mL, a competitive antagonist of IP_3) for 20 min, and incubated with 1 μM thapsigargin for 10 min. When external Ca^{2+} was free, IP_3 receptors were blocked using heparin and the intracellular calcium store was depleted with thapsigargin; the decrease of $[Ca^{2+}]_i$ was only attributed to compound **13b** (1000 mg/L). These data indicated that RyRs would be the possible action target of this series of novel compounds.

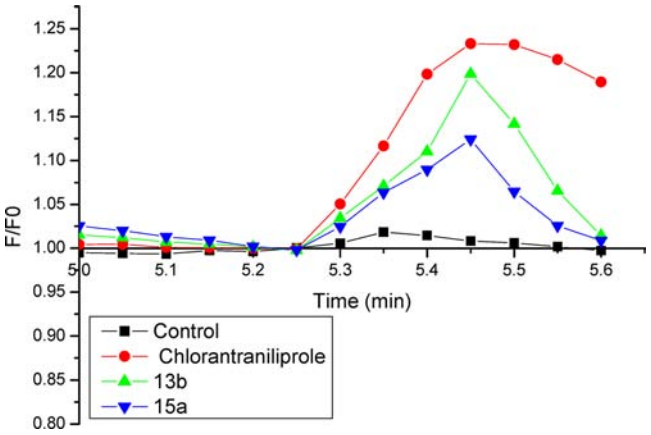

Figure 4. Change of $[Ca^{2+}]_i$ versus recording time when the neurons were treated with 1000 mg/L compounds **13b**, **15a**, and chlorantraniliprole.

In summary, two novel series of anthranilic diamides containing acylthiourea and acylurea moieties were synthesized,

Table 6. Analysis of the Peak Current Change Rate with Time in the Neurons of *S. exigua* (Treated by Compound 13b and Chlorantraniliprole)[a]

time (min)	0.1 mM compound **13b**	0.001 mM compound **13b**	0.1 mM chlorantraniliprole	control
0	100	100	100	100
1	91.492 ± 3.47[b]	104.964 ± 1.34[c]	89.62 ± 2.16[b]	104.47 ± 2.33[b]
2	84.128 ± 2.16[b]	97.215 ± 7.58[b]	74.01 ± 8.01[b]	102.75 ± 2.45[c]
3	80.727 ± 4.77[b]	95.803 ± 7.26[b]	69.57 ± 9.43[b]	101.76 ± 2.7[c]
4	74.437 ± 5.31[b]	90.134 ± 6.41[b]	63.36 ± 7.71[b]	99.66 ± 1.64[c]
5	70.252 ± 8.48[b]	84.442 ± 5.48[b]	55.79 ± 4.73[b]	98.06 ± 1.01[c]
8	61.013 ± 3.28[b]	81.209 ± 3.69[b]	44.31 ± 2.62[b]	90.85 ± 0.65[b]
10	54.715 ± 4.95[b]	74.632 ± 6.12[b]	39.685 ± 5.02[b]	85.44 ± 1.14[b]

[a]Values are the mean ± SD. [b]Significant difference at $p < 0.01$. [c]Significant difference at $p < 0.05$.

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and their larvicidal activities against oriental armyworm, mosquito larvae, and diamondback moth were evaluated. The results indicated that the introduction of acylthiourea moiety into some structures could retain their insecticidal activity; 8 of the 15 compounds (**13a−13e**, **14a−14e**, and **15a−15e**) exhibited 100% larvicidal activity at 10 mg/L against oriental armyworm. However, the introduction of acylurea moiety decreased the insecticidal activity; only 3 of the 11 compounds (**17a−17k**) exhibited 100% larvicidal activity at 200 mg/L against oriental armyworm. We speculated that it might be the solubility factor involved because compounds containing acylthiourea moiety exhibited better solubility in organic solvents during our experiments. The effects on calcium channels of neurons from *S. exigua* indicated that the title compounds influenced the same target RyRs as chlorantraniliprole. Also, the experiment of intracellular calcium of neurons provided us a rapid detection for the activity of the target compound.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H NMR, HRMS, and melting point data for compounds **13a−13e**, **14a−14e**, **15a−15e**, and **17a−17k**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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