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Synthesis and insecticidal activity study of novel anthranilic diamides analogues containing a diacylhydrazine bridge as effective Ca^{2+} modulators

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Abstract: Anthranilic diamides is a class of insecticides target at ryanodine receptors (RyRs). To discover potent insecticides targeting at RyRs, a series of novel anthranilic diamides with a diacylhydrazine bridge were designed and synthesized. Their insecticidal activities were evaluated and a preliminary structure-activity relationship (SAR) was summarized. In particular, compound **5g** exhibited good lethality against oriental armyworm (*Mythimna separata*) at a concentration of 5 mg/L.

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The calcium-imaging experimental results indicated that the compound **5g** can serve as effective insect Ca^{2+} level modulators by disrupting the cellular calcium homeostasis in *Mythimna separata (Walker)* and *Spodoptera exigua (Hübner)*, which probably activated the RyRs on the ER membrane.

Keywords: anthranilic diamides, diacylhydrazine, Ryanodine receptors, Ca^{2+} modulator

The anthranilic diamides are a new category of insecticides. In particular, two of representative compounds chlorantraniliprole and cyantraniliprole were widely applied over the world due to their ultra-high efficiency, low toxicity, broad insecticidal spectra.[1, 2] They have also been verified as the modulators of the insect Ryanodine receptors (RyRs), a non-voltage calcium channel on the endoplasmic reticulum/sarcoplasmic reticulum (ER/SR) with different binding sites.[3] The binding of the anthranilic diamides would cause the unregulated release of Ca^{2+} from ER/SR lumen and the subsequent break of the intracellular calcium homeostasis, which led to further uncontrolled contraction of skeletal muscle and the final death of the insect.[4-6]

During the past decade, chemical biologists have been devoted to clarify the structure-activity relationship (SAR) of anthranilic diamide insecticides. Many new compounds with high insecticidal activities were found through structural optimization of chlorantraniliprole.[7-10] Most research focused on the aliphatic amide moiety (**Fig. 1a**, part A), the functional groups including cyano[11], hydrazine[8] and heterocyclic[12,13] groups were found to be highly effective. Yet exploration on amide bridge moiety (**Fig. 1a**, part B) were relatively rare and there has not been sufficiently comprehensive research on it. In our previous studies, the acylhydrazine group was found to be a potential effective substituent.[14,15] Actually, the acylhydrazine fragment have been reported to display a variety of biological activities, such as antimicrobial,[16, 17] insecticidal[18, 19], anti-HIV[20, 21], anti-HCV[22], antitumor[23, 24], analgesic[25], antitrypanosomal[26], antioxidant[27]. In addition, some diacylhydrazines (**Fig. 1b**) such as tebufenozide are potent ecdysone receptor agonists with high insect growth regulatory activity.[28,29]

In hope of finding novel potent lead compounds, a series of diamdes was designed and synthesized by introducing a diacylhydrazine moiety to modify the amide bridge (**Fig. 1a**, part B), the strategy of which was seldom reported.[30] We also attempted to replace the 1-(3-chloropyridyl) pyrazole moiety (**Fig. 1a**, part C) with substituted phenyl group for further SAR study. Their insecticidal activities against oriental armyworms (*Mythimna separata*) were tested, some compounds exhibited excellent insecticidal activities. The structure–activity relationship (SAR) was discussed as well. The calcium imaging technique was also adopted to investigate the effects on calcium channels in the central neurons of *M. separata* and *S. exigua.*

Synthetic routes for compound series **5** were shown in **Scheme 1**. Intermediates **2** were obtained by treated anthranilic acid derivatives with triphosgene, after that intermediates **3** were synthesized *via* a nucleophilic substitution at carbonyl group of **2** using hydrazine hydrate at room temperature. At first,

the compound **4** was prepared with trimethylamine as acid binding reagent and dichloromethane as the solvent. The yield was low with a couple of byproducts, because there were two competitive reactions between NH_2 of aniline and benzoyl hydrazine in the reaction process. With the improvement of reaction conditions, compound **3** were selectively acylated by 1-(3-chloropyridyl) pyrazole-5-carbonyl chlorides in a THF/pyridine system through controlling 1.0 equivalent of the reagent and pyridyl pyrazole chlorides very slowly added to the mixture. Finally, compounds **5** were prepared in good yields by another acylation reaction of the aniline derivatives **4** with corresponding acyl chlorides. Because of multiple reaction sites in the compound **4**, it was necessary to keep the reaction temperature at 0 □ during the acylation process to increase the yield and minimize the byproducts. The substituents of compounds **4a-h** and **5a-u** were summarized in **Table 1**.

Table 1. Substituent pattern of compounds **4a-h** and **5a-u**.

Compd.	**R_1**	**R_2**	**R_3**	**Compd.**	**R_1**	**R_2**	**R_3**
4a	3-CH_3	Br	-	**5h**	3-CH_3,5-Cl	Br	OCH_2CH_3
4b	4-Cl	Br	-	**5i**	3-CH_3,5-Cl	Br	$OCH(CH_3)_2$
4c	5-Cl	Br	-	**5j**	3-CH_3,5-Cl	Br	$COOCH_3$
4d	3-CH_3,5-Cl	Br	-	**5k**	3-CH_3,5-Cl	Br	$COOCH_2CH_3$
4e	3-CH_3,5-Cl	CF_3	-	**5l**	3-CH_3,5-Cl	Br	$COOCH_2CH{=}CH_2$
4f	3-CH_3,5-Cl	Cl	-	**5m**	3-CH_3,5-Cl	CF_3	OCH_3
4g	3-CH_3,5-Br	Br	-	**5n**	3-CH_3,5-Cl	CF_3	OCH_2CH_3
4h	3-CH_3,5-I	Br	-	**5o**	3-CH_3,5-Cl	CF_3	$OCH(CH_3)_2$
5a	3-CH_3	Br	OCH_3	**5p**	3-CH_3,5-Cl	CF_3	$COOCH_3$
5b	3-CH_3	Br	OCH_2CH_3	**5q**	3-CH_3,5-Cl	Cl	OCH_3
5c	4-Cl	Br	OCH_3	**5r**	3-CH_3,5-Br	Br	OCH_3
5d	4-Cl	Br	OCH_2CH_3	**5s**	3-CH_3,5-Br	Br	OCH_2CH_3
5e	5-Cl	Br	OCH_3	**5t**	3-CH_3,5-I	Br	OCH_3
5f	5-Cl	Br	OCH_2CH_3	**5u**	3-CH_3,5-I	Br	OCH_2CH_3
5g	3-CH_3,5-Cl	Br	OCH_3				

Table 2. Insecticidal activities of compounds **4** and **5** against oriental armyworms

Compd.	Larvicidal activity (%) at conc. (mg/L)					Compd.	Larvicidal activity (%) at conc. (mg/L)				
	200	100	50	10	5		200	100	50	10	5
4a	40					**5j**	50				
4b	50					**5k**	100	40			
4c	30					**5l**	100	40			
4d	100	100	60			**5m**	100	70			
4e	30					**5n**	100	60			
4f	100	100	100	20		**5o**	40				
4g	40					**5p**	10				
4h	60					**5q**	100	100	40		
5a	10					**5r**	100	100	50		
5b	75					**5s**	100	60			
5c	10					**5t**	45				
5d	20					**5u**	65				
5e	10					**5v**	10				
5f	10					**5w**	30				
5g	100	100	100	100	60	**control**[a]	100	100	100	100	100
5h	100	30				**control**[b]	100	100	100	100	70
5i	60										

[a]: chlorantraniliprole; [b]: tebufenozide

The insecticidal activities of compounds **4** and **5** against oriental armyworms were evaluated and the results were shown in **Table 2**. Chlorantraniliprole and tebufenozide were selected as positive controls. A part of compounds **4** and **5** exhibited good insecticidal activity at 100 mg/L concentration especially **4f** and **5g**, which showed lethality rates of 20 % at 10 mg/L and 60 % at 5 mg/L, respectively. The potency of compound **5g** was comparable to tebufenozide (70 % at 5 mg/L), however, was less effective than chlorantraniliprole.

The preliminary SAR was summarized. Activities varied significantly with respect to the structures. The substitution pattern of R_1 was crucial to the insecticidal activity of title compounds. The compounds bearing 3-CH_3, 5-Cl substituted phenyl ring displayed superior activities (**4d**, **4f**, **5g** and **5q**) than those containing Br (**4i** and **5s**), I (**5t** and **5u**) or H (**4a** and **5a**). The position of R_2 exhibited

minor effect on the insecticidal activity and could tolerate some different groups, in which Br and Cl (**4d**, **4f**, **5g** and **5q**) are slightly better than CF_3 (**5m** and **5n**). In addition, we observed that the volume of the R_3 group was an important factor for the activity. The methyl ester (**5g**, **5q** and **5r**) showed preferable efficacy than other compounds. The compounds containing ethyl esters (**5n** and **5s**) showed little loss of activities. And the activities decreased significantly along with the volume augmentation of the substituent groups such as isopropyl (**5i** and **5o**) or methoxalyl group (**5j**, **5k**, **5l** and **5p**). We attempted to replace the complicated 1-(3-chloropyridyl) pyrazole moiety (**Fig. 1a**, part C) with more easy available phenyl analogues (**5v** and **5w**) but the compounds displayed limited insecticidal activities. These results indicated that the pyridyl-pyrazole moiety played an important role in insecticidal activity.

Previously, it was reported that insecticidal diamides targeted the ryanodine receptors (RyRs), resulting in the calcium disorder of the cells.[5] To explore the insecticidal mechanism of our novel active structures, we turn to study their impacts on the cellular calcium homeostasis of the central neurons of *M. separata* and *S. exigua*., using calcium imaging technique with Fluo-3 AM (for cytosolic calcium) or Fluo-5N AM (for ER lumen calcium) as fluorescent dyes.

Fig. 2 showed that the application of 1000 mg/L **5g** and **5h** to isolated *M. separata* central neurons could obviously increase the cytosolic calcium concentration. The peak of $[Ca^{2+}]_i$ was increased to 104.91 ± 1.57 % (n = 9) and 103.33 ± 1.24 % (n = 9) of the initial value after the neurons were treated with compounds **5g** and **5h**, respectively. Thus, calcium released from the calcium store elevated the cytosolic calcium levels. In addition, the extent of calcium change was positively correlated with the insecticidal activities of the compounds. In addition, it was consistent with our expectation that tebufenozide did not activate ryanodine receptor. **Fig. 3** showed that the impact of **5h** on calcium response almost unchanged after the neurons had been pre-incubated with tebufenozide, demonstrating tebufenozide has no adverse or positive effects on the activation of the ryanodine receptor by our compounds and there was no synergistic effect between compound **5h** and tebufenozide. These results indicated that these novel compounds indeed have the ability to deliver calcium from endoplasmic reticulum to cytoplasm.

We speculated that the elevated cytosolic calcium was released from the ER lumen, the internal calcium store. Therefore, the effect of compound **5g** on the calcium level of the ER lumen in *S. exigua* central neuron was determined and the result was shown in **Fig. 4**. We observed that **5g** could cause continuous release of calcium from the ER lumen at the concentration of 200 mg/L. 7 min later, the $[Ca^{2+}]_i$ was decreased to 61 %. In other words, 39 % $[Ca^{2+}]_i$ was released through the ryanodine receptor (or three trisphosphate receptor), causing the calcium to be overloaded in the cytoplasm. It also showed from the fluorescence photo that the fluorescence intensity was obviously reduced after the compound **5g** was added, because of the decrease of calcium in ER lumen. This indicated that compound **5g** was an effective calcium modulator for insect cells which targeted RyRs to exhibit this biological effect.

In conclusion, a novel series of anthranilic diamides containing diacylhydrazine bridge was synthesized and their insecticidal SAR against oriental armyworm was preliminarily summarized. The bioassays showed that compound **5g** exhibited good lethality against oriental armyworm at 5 mg/L. Calcium imaging technique was applied to detect cellular calcium level for evaluating the impact of **5g** on the cellular calcium homeostasis in the central neurons of *M. separata* and *S. exigua*. The results indicated that compounds **5g** could effectively modulate the calcium level in the insect cells, possibly by activating the ryanodine receptor on the ER.

Figure 1. Chemical structures of anthranilic diamide, Tebufenozide and the target compounds.

Figure 2. Effects of compounds **5g**, **5h** and tebufenozide on $[Ca^{2+}]_i$ in the central neurons of *M. separata* at 1000 mg/L concentration. The central neurons of *M. separata* third-instar larvae were dyed by Fluo-3 AM.

Figure 3. Effects of **5h** at 1000 mg/L concentration on $[Ca^{2+}]_i$ in the central neurons of *M. separata* when the neurons were preincubated by tebufenozide. The central neurons of *M. separata* third-instar larvae were dyed by loading with Fluo-3 AM.

Figure 4. Effects of compounds **5g** on $[Ca^{2+}]_i$ in the central neurons of *S. exigua* at 200 mg/L concentration. The central neurons of *S. exigua* third-instar larvae were dyed by Fluo-5N AM.

Figure 5. Fluorescence photos of compounds **5g** on $[Ca^{2+}]_i$ in the central neurons of *S. exigua* at 200 mg/L concentration. The central neurons of *S. exigua* third-instar larvae were dyed by Fluo-5N AM. The neurons in the left one show the fluorescence before the compound **5g** was added; the right one shows the fluorescence of the neurons affected 7 min by compound **5g** (the excitation wavelength was 488 nm).

Scheme 1. Synthetic routes of compounds **5a-w**. Reagents and conditions: (i) triphosgene, THF, rt; (ii) $NH_2NH_2 \cdot H_2O$, EtOH; (iii) 1-(3-chloropyridyl) pyrazole-5-carbonyl chlorides, pyridine, THF, ice bath; (iv) chloroformates, pyridine, THF, ice bath; (v) benzoyl chloride, pyridine, THF, ice bath; (vi) methyl chloroformate; pyridine, THF, ice bath.

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Conflict of interest

The authors declare no competing financial interest.

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Supplementary data

Supplementary data associated with this article can be found in the online version.