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Communication

Synthesis, bioactivity, action mode and 3D-QSAR of novel anthranilic diamide derivatives

Weijie Liu, Jiao Li, Kai He, Fangfang Huang, Yi Ma, Yuxin Li, Qingshan Li*, Fengbo Xu*

State Key Laboratory of Elemento-Organic Chemistry, College of Chemistry, Nankai University, Tianjin 300071, China

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ABSTRACT

To study the pesticide effect, action mode, structure-activity relationships (SARs) of anthranilic diamide insecticide and screen highly active pesticides, novel anthranilic diamide derivatives were synthesized. Bioassays indicated that all of the title compounds displayed 100% mortality against diamondback moth and oriental armyworm at 100 mg/L, among which **12v** and **12 w** showed 100% insecticidal acitvity at 5 mg/L. Surprisingly compound **12 w** exhibited better insecticidal acitvity than commercialized chlorantraniliprole against *Pyrausta nubilalis* (0.1 mg/L) and *Cnaphalocrocis Medinalis* (2 mg/L). 3D-QSAR and SARs statistical analysis revealed that title compounds with R^2 fixed as methoxy had the highest probability possessing high activity. The calcium fluorescence measurements on neurons revealed that **E** series compounds containing pyrazinyl may have a molecular target different from caffeine on ryanodine receptors rather than the voltage-gated calcium channel present on cytomembran.

To overcome resistance and ecological problems involved with conventional insecticides, there was an urgent need to discover novel potent insecticides with a new mode of action [1]. Ryanodine receptors (RyR) are members of a family of intra-cellular Ca^{2+} release channel proteins present on endoplasmic reticulum (ER) or sarcoplasmic reticulum (SR) membranes, deriving the name from its high affinity with ryania [2,3]. Ryanodine receptors insecticides had already become a focus of pesticide discovery and research because of its good insecticidal activity and high safety for non-target creatures such as mammal [4–6]. Natural ryanodine receptors insecticides include caffeine, spiganthine, 9,21-dehydroryanodine, ryanodol, *etc.* [7]. Commercial ryanodine receptors inscticides include phthaldiamide such as flubendiamide (**A**) discovered by Nihon Nohyaku in 1993, anthranilic diamide such as cyantraniliprole (**B**) and chlorantraniliprole (**C**) discovered by DuPont [8–11] (Fig. 1).

In this article, we divided the chemical structure of chlorantraniliprole (Fig. 1**D**) into three parts: *N*-pyridylpyrazole moiety (a), anthraniloyl moiety (b), and aliphatic moiety (c). In our previous work, *o*-carboxamidobenzamide compounds containing 2-(substituted phenyl)oxazole group (Fig. 1**C**) in part a were synthesized, but unfortunately most of the compounds did not showed higher bioactivity than commercialized chlorantraniliprole [12]. Based on this and encouraged by the extensive and excellent biological activity of pyrazine derivants in the fields of medicine [13], pesticide [14,15], flavouring [16], *etc.*, *N*-pyrazinylpyrazole was introduced into part a according to the principle of bioelectronics to give the series of **E** compounds (Fig. 1**E**). SARs analysis revealed that **E** series containing *N*-pyrazinylpyrazole in part a exhibited inferior bioactivity compared with chlorantraniliprole containing *N*-pyridylpyrazole, in addition, title compounds with methoxylcarbamoyl in patrt c had the highest probability possessing high activity, so the series of **F** compounds (**12v** and **12 w**) containing methoxylcarbamoyl on part c and *N*-pyridylpyrazole in part a were further designed (Fig. 1**F** and Scheme 1). Three dimensional quantitative structure-activity relationship (3D-QSAR) and action mode of title compounds were also investigated in this paper.

The synthesis of compounds **12a–w** is shown in Scheme 1. The pure product of 2-chloro-6-hydrazinylpyrazine (**2a**) can easily be obtained after filtration instead of extraction and distillation by the reaction of 2,6-dichloropyraine with 80% hydrazine hydrate at present of ethanol. Compound **4a** was synthesized according the reported method for chlorantraniliprole synthesis with minor improvements [5]. During which process, it was found that 2-hydrazinylpyrazine and 2-chloro-6-hydrazinylprazine can successfully be reacted with diethyl maleate by controlling the reaction temperature, but 3-chloro-6-hydrazinylpyrazine cannot be reacted. Compound **4a** was bromized with phosphorus oxybromide to give compound **5a.** Then **5a** was hydrolysed with

* Corresponding authors.
E-mail addresses: qli@nankai.edu.cn (Q. Li), xufb@nankai.edu.cn (F. Xu).

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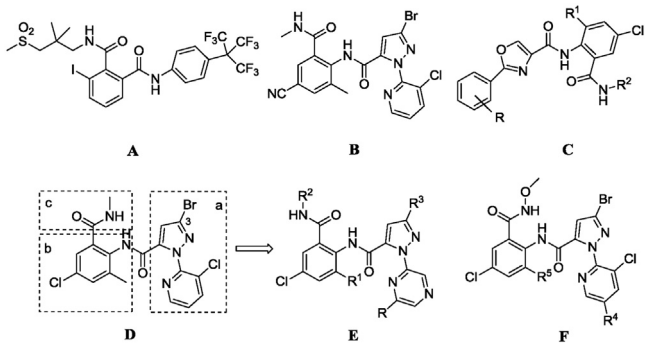


Fig. 1. Chemical structures of compounds **A–F**.

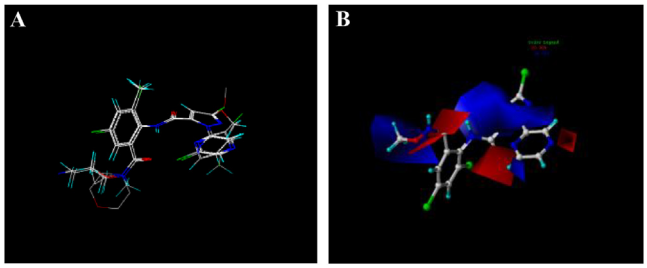


Fig. 2. (A) Superimposition of the training and test sets. (B) Electrostatic map form CoMFA model.

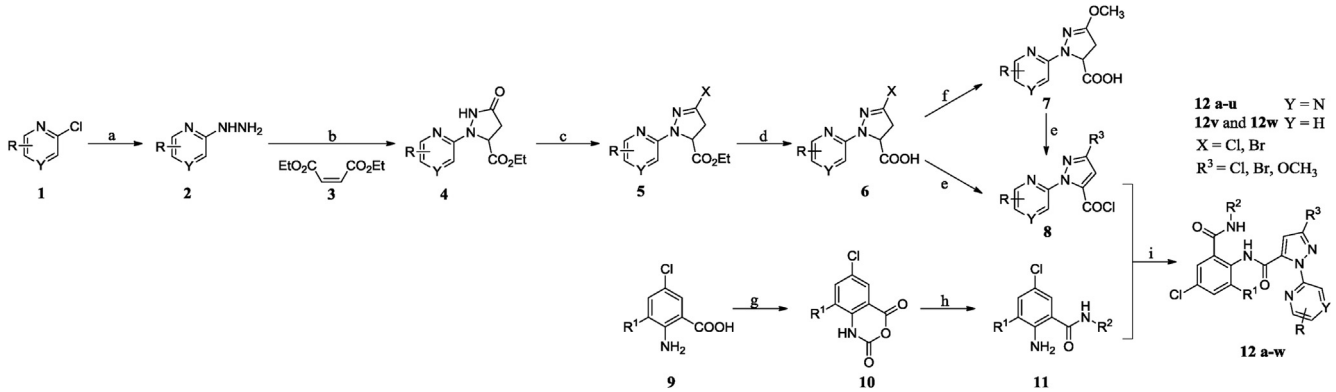
NaOH to give the corresponding carboxylic acid **6a**. Compound **6a** was converted into the acyl chloride **8a** by treatment with thionyl chloride and pyrazolone ring was constructed at the same time. Methoxy substituted intermediates **7a** can directly be obtained by refluxing compound **6a** in methanol, which was not reported before.

Compound **9a** was cyclized by triphosgene to give the corresponding six-membered lactam **10a** at a high yield of 98%. Amine hydrochloride derivatives were firstly deprotonated with triethylamine to release their nucleophilicity, and then were used for ring opening reaction of lactam **10a** to give 2-amino-5-chlorobenzamide derivatives **11**. Title compounds **12a-w** were synthesized with acyl chloride **8** and amine **11** throungh nucleophilic substitution reaction in the presence of organic base triethylamine.

The insecticidal activities of **12a-w** against diamondback moth and oriental armyworm were shown in Table 1. All of title compounds showed 100% insecticidal activity against diamondback moth and oriental armyworm at 100 mg/L. Title compounds **12c**, **12v** and **12 w** exhibited 100% mortality at 50 mg/L, but when the concentration was reduced to 5 mg/L, only **12 w** keep 100% mortality against both diamondback moth and oriental armyworm. For compound **12 w** with excellent activity, its insecticidal activity against *Mythimnaseparata walker*, *Pyrausta nubilalis*, *Cnaphalocrocis Medinalis* at concentrations below 5 mg/l were further investaged. The results were listed in Table 2, it was worth mentioning that **12 w** showed better activity than contrast compound chlorantraniliprole against *Pyrausta nubilalis* (0.1 mg/L) and *Cnaphalocrocis Medinalis* (2 mg/L).

In order to evaluate the SARs of title compounds systematically, according to the insecticidal activity of **E** series bearing *N*-pyrazinylpyrazole against diamondback moth at 50 mg/L, **12a-u** were categoried and sorted into three types: H (high activity with percentage 70–100), M (moderate activity with percentage 40–70), L (low activity with percentage 0–40). As shown in Table S1 and Fig. S1 in Supporting information, seven of **E** series fell into H category when R^2 fixed as OCH_3, so 87.5% were H, similarly, 75% are H as CH_3, 40% as OEt and 0% as cyclopropyl, consequengtly the order from high to low was OCH_3, CH_3, OEt, cyclopropyl. As a result, title compounds with methoxylcarbamoyl in patrt c had the highest probability possessing high activity, in addition, keeping R, R^1, R^3 same with each other, compounds with the highest activity in each series were **12c**, **12d**, **12e**, **12f**, **12k**, **12l** and **12n**, respectively. It was found that 5/7 of these had a methoxylcarbamoyl in part a. Bulky substituent in part a could lead to a decrease activity, for example, **12 m** with R^2 fixed as CH_3 exhibited 80% mortality while **12t** only showed 10% with cyclopropyl. The results analysed above did explain that the later designed **12v** and **12 w** bearing methoxylcarbamoyl in part c and *N*-pyridylpyrazole in part a could show relatively higher activity than **12a-u** and why keeping the other chemical structure consistent with each other **12 w** could exhibit higher actvity than chlorantraniliprole. For R^3, the corresponding sequence was OCH_3 > Br > Cl, for R^1, it was Cl > CH_3. It was also found that title compound with R and R^1 fixed as Cl and Cl showed higher activity than those fixed as Cl and CH_3 respectively, which was demonstrated by the sequence **12d** > **12 l, 12e** > **12s, 12f** > **12p, 12 g** > **12n, 12i** > **12 u.**

Comparative molecular field analysis (CoMFA) is an effective computer implemented methodology of 3D-QSAR employing both the interactive graphics and statistical techniques for correlating structure of molecules with observed biological properties, and thus is widely used in drug design [17,18]. The results of CoMFA computation was summarized in Table S2 (Supporting information). The cross-validated coefficient q^2 obtained by leave-one-out method is 0.658, where a value over 0.5 indicates the model is a



Scheme 1. Synthetic route of **12a-w**. Reagent and conditions: (a) NH_2NH_2, EtOH, 80 °C; (b) Na, EtOH, HAc, 50 °C; (c) $P(O)X_3$, CH_3CN; (d) NaOH, H_2O, EtOH 65 °C; (e) $SOCl_2$, 65 °C; (f) triphosgene, 1,4-dioxane, 40 °C; (g) R_2NH_2, CH_3COOEt, 40 °C; (h) Et_3N, CH_3CN, r.t.; (i) CH_3CN.

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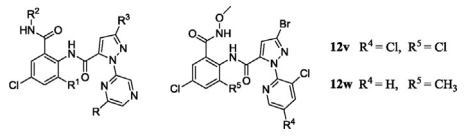
Table 1
Insecticidal activity (%) against diamondback moth and oriental armyworm.



Compd.	R	R^1	R^2	R^3	Insecticidal activity (%)				
					Diamondback moth			Oriental armyworm	
					100[b]	50[b]	5[b]	100[b]	5[b]
12a	H	Cl	OCH_3	Br	100	90	30	100	–
12b	H	CH_3	CH_3	Br	100	100	50	100	–
12c	H	CH_3	OCH_3	Br	100	100	60	100	40
12d	Cl	Cl	OCH_3	OCH_3	100	100	60	100	–
12e	Cl	Cl	OCH_3	Cl	100	100	80	100	–
12f	Cl	Cl	OEt	Br	100	95	50	100	–
12 g	Cl	Cl	OCH_3	Br	100	95	40	100	–
12h	Cl	Cl	CH_2CN	Br	100	90	30	100	–
12i	Cl	Cl	OEt	OCH_3	100	80	20	100	–
12j	Cl	Cl	OEt	Cl	100	50	0	100	–
12k	Cl	CH_3	CH_3	Cl	100	100	60	100	–
12 l	Cl	CH_3	OCH_3	OCH_3	100	90	30	100	–
12 m	Cl	CH_3	CH_3	OCH_3	100	80	0	100	–
12n	Cl	CH_3	OCH_3	Br	100	80	0	100	–
12o	Cl	CH_3	CH_3	Br	100	60	0	100	–
12p	Cl	CH_3	OEt	Br	100	60	0	100	–
12q	Cl	CH_3	Morpholin-1-yl	Br	100	60	0	100	–
12r	Cl	CH_3	Cyclopropyl	Br	100	55	0	100	–
12s	Cl	CH_3	OCH_3	Cl	100	50	0	100	–
12 t	Cl	CH_3	Cyclopropyl	OCH_3	100	10	–	100	–
12 u	Cl	CH_3	OEt	OCH_3	100	10	–	100	–
12v					100	100	100	100	60
12 w					100	100	100	100	100
Control[a]					100	100	100	100	100

-: Not tested.
[a] Chlorantraniliprole.
[b] Concentration of tested compound (mg/L).

Table 2
Insecticidal activity (%) of **12 w** against different insects.

Insects	Compd.	Concentration (mg/L)				
		0.1	0.5	1	2	4
Mythimnaseparata walker	**12 w**	100	100	100	100	100
	Control[a]	100	100	100	100	100
Pyrausta nubilalis	**12 w**	60	100	100	100	100
	Control[a]	50	100	100	100	100
Cnaphalocrocis medinalis	**12 w**	–	–	–	26	59
	Control[a]	–	–	–	19	28

[a]Chlorantraniliprole.
-: Not tested.

great predictor of the insecticidal activity. The results of structural alignment and isocontour diagrams of the electrostatic field contributions obtained from the CoMFA analysis were illustrated in Fig. 2, in which a postive charged group in the blue region will increase the activity but a negative charged substituent in the red region will be favorable.

Title compounds bearing an electron-withdrawing group at 3- or 4- position and an electron-donating group at 6- position of the pyrazine or pyridine ring, such as **12v**, **12 w**, and **12c**, displayed higher activity. At the 3-position of the imidazole ring (R^3), compounds bearing an electron-donating groups would display higher activity, as shown in the sequence $OCH_3 > Br > Cl$ discussed above. Alkoxycarbamoyl in part c displayed higher activity because they occupy the red region, and a relatively positive-charged alkyl part in alkoxycarbamoyl was favorable for the increase of activity.

Title compounds **12c**, **12d**, **12e** and chlorantraniliprole at a concentration of 100 mg/L were applied to neurons of beet armyworm, after which the effect of that on free cytosolic calcium concentration ($[Ca^{2+}]_c$) in beet armyworm neurons was shown in Fig. 3A. It was observed that the application of **12c**, **12d** and **12e** can induce a rapid increase of $[Ca^{2+}]_c$ in single neuron. Previous studies suggested that calcium signal was generated by calcium ion store present on ER in neurons of insects. Ryanodine receptors, which locates at ER membranes of insect nerve cells, is the ligand-gated calcium channel regraluting release of calcium ion [2]. It was reported that the transfer of calcium from ER to cytosol in insect neurons can indirectly evoke the muscle contraction [19]. In view of the description above, the action mode could be correctly conjectured (Fig. 3B). The combination of tested compounds with RyR resulted in the sustained release of calcium ion from ER to cytoplasm, which induced the stress contraction of insect muscle fiber. Ultimately, pests died from symptoms of body contraction, paralysis, feeding cessation, *etc.* [20]. It was concluded that the target of **E** series of compounds is the ligand-gated calcium channel present on ER, and thus they may be RyR activators.

To explore the effect of **E** series of compounds on voltage-gated calcium channel present on cytomembrane of neurons. Title compound **12e** (100 mg/L) was applied to beet armyworm neurons at the presence and absence of Ca^{2+} in extracellular fluid and its effect on $[Ca^{2+}]_c$ was recorded (Fig. 3C). After beet armyworm neurons were loaded by incubation with 100 μL Fluo-3-AM for 3 h, F/F_0 (%) reached a maximum of about 109 % at 5 min after application under both calcium-rich and calcium-free conditon in extracellular fluid. As a result, title compound **12e** cannot activate the voltage-gated calcium channel present on cytomembrane of neurons and have no molecular target on it.

After beet armyworm neurons were loaded by incubation with 100 μL Fluo-3-AM for 3 h, under calcium-free condition in extracellular fluid, the effect of the application of **12e** (100 mg/L)

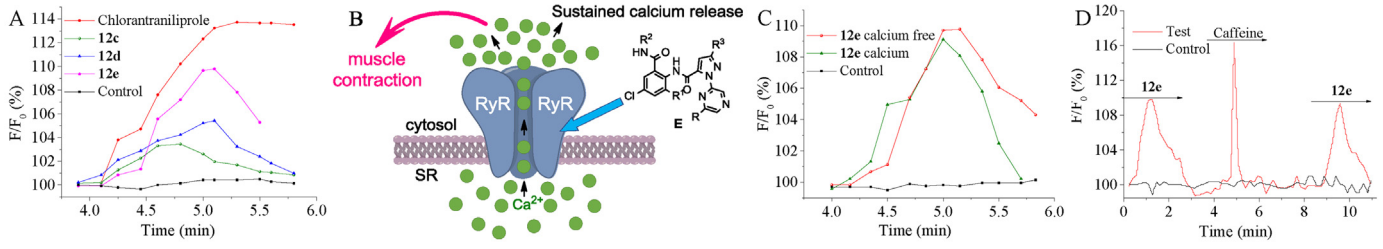


Fig. 3. (A) Effect of **12c**, **12d**, **12e** and chlorantraniliprole (100 mg/L) on $[Ca^{2+}]_c$ in beet armyworm neurons. (B) Schematic illuatration of the molecular target and action mode of **E** series of compounds. (C) Effect of Ca^{2+} in extracellular fluid on $[Ca^{2+}]_c$ in beet armyworm neurons after the application of **12e** (100 mg/L). (D) Effect of the application of **12e** (100 mg/L) and caffeine (30 μmol/L) during different time intervals on $[Ca^{2+}]_c$ in beet armyworm neurons.

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and caffeine (30 μmol/L) during different intervals on $[Ca^{2+}]_c$ in beet armyworm neurons was recorded (Fig. 3D). It was observed that the prior application of **12e** had no effect on $[Ca^{2+}]_c$ change of caffeine application and the subsequent $[Ca^{2+}]_c$ change of **12e** application was not affected by prior application of caffeine neither. If the molecular target of caffeine was occupied by the priorly applied **12e**, a rise on $[Ca^{2+}]_c$ would not be observed, in other words, title compound **12e** has a molecular target on RyR different from natural ryanodine receptors insecticide caffeine.

In summary, we synthesized 21 novel anthranilic diamide derivatives containing pyrazinyl, 2 derivatives containing methoxylcarbamoyl in the aliphatic part of chlorantraniliprole. Bioassays indicated that all of the title compounds displayed 100% mortality against diamondback moth and oriental armyworm at 100 mg/L, based on analysis, we obtained title compound **12w** with better insecticidal activity than chlorantraniliprole against *Pyrausta nubilalis* (0.1 mg/L) and *Cnaphalocrocis Medinalis* (2 mg/L). The 3D-QSAR results provided significant information for the structure optimization of anthranilic diamide insecticides, especially, **12w** can serve as lead compound to screen highly active pesticide and have the value of further research. The calcium fluorescence measurements results revealed that **E** series compounds containing pyrazinyl may possess a molecular target different from caffeine on RyR rather than the voltage-gated calcium channel present on cytomembrane.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi: https://doi.org/10.1016/j.cclet.2018.05.023.

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