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Novel chlorantraniliprole derivatives as potential insecticides and probe to chlorantraniliprole binding site on ryanodine receptor

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ABSTRACT

The lepidopteran pests such as diamondback moth are the regularly harmful pests of crops in the world, which brings enormous losses in crop production. Chlorantraniliprole is an anthranilic diamide insecticide registered for the control of lepidopteran pests with high insecticidal activity, however with uncertain binding site action target of chlorantraniliprole on ryanodine receptor, a series of new chlorantraniliprole derivatives were synthesized and the insecticidal activities of these compounds against diamondback moth were evaluated with chlorantraniliprole and indoxacarb as control. All compounds except **8h**, **8p** and **8t** exhibited varying degree of activities against diamondback moth. Especially, compounds **8c**, **8i**, **8k** and **8l** displayed good insecticidal activities against diamondback moth and the activities are even better than that of indoxacarb during 72 h period. The K_i values of all synthesized compounds were calculated through autodocking program respectively. The relationship between calculation value of molecular docking and results of insecticidal activities indicated that the proposed specific receptor, the membrane-spanning domain protein of diamondback moth ryanodine receptor in our study might have chlorantraniliprole binding sites.

The diamondback moth is the harmful pest of crops in the world. They have become the difficult pests to control because of their resistance to various types of traditional insecticides, thus bringing about enormous losses in crop production.[1–3] Recently two classes of synthetic chemicals, the phthalic diamides (flubendiamide[4–9] Fig. 1) and the anthranilic diamides (chlorantraniliprole[10,11] and cyantraniliprole,[12] Fig. 1) emerge resulting in commercial insecticides that target insect ryanodine receptors.[13,14] They have exceptional insecticidal activities on a range of Lepidopteran pests and other orders, such as Coleoptera, Diptera, Isoptera and Hemiptera. Especially, chlorantraniliprole, the first anthranilic diamide insecticide registered for the control of lepidopteran pests has high selectivity, which accounts for its low mammalian toxicity and favorable environmental profile. It acts by activating the insect ryanodine receptor (Fig. 2), which is a non-voltage-gated calcium channel to affect calcium release from intracellular stores by locking channels in a partially opened state, an assignment based on electrophysiological and Ca^{2+}-release studies.[15,16] Therefore, chlorantraniliprole analogues have attracted considerable research attention and the ryanodine receptor has been regarded as one of the targets for novel insecticide discovery.

In the previous study,[17–21] several modifications around chlorantraniliprole skeleton have demonstrated the discovery of novel insecticides and showed high insecticidal activities. Most modifications focused on three parts: the pyrazole moiety (Fig. 3A), amide moiety (Fig. 3B), anthraniloyl moiety (Fig. 3C). Zheng-ming Li research group[17] reported several derivatives with good insecticidal activities after modification of the pyrazole moiety. Yang S. group[18] synthesized several derivatives containing hydrazone structures. The studies[22–25] found that amide group is a highly efficient and key pharmacophore used widely in insecticides design. Anthraniloyl moiety is modified by incorporation of cyan group instead of chlorine group since cyan group sometimes can affects bioactivities considerably.[26–28] However, all these studies focusing on the synthesis and activity study of chlorantraniliprole derivatives hadn't explored the chlorantraniliprole binding site(s) on molecular level and the exact chlorantraniliprole binding site(s) on insect ryanodine receptor have not been confirmed and are still unclear. Such situation impedes the rational design of potential leading compounds.

Nauen and co-workers[29] reported that a point mutation in C-terminal membrane-spanning domain of some the ryanodine receptor led to give a glycine with a glutamic acid substitution (G4946E) and suggested that this single amino acid substitution is associated with the diamide insecticides resistance. Lei Guo

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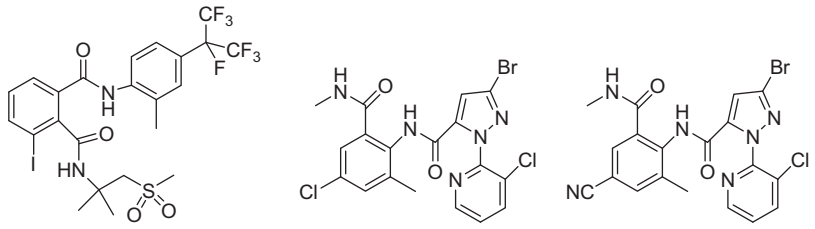


Figure 1. Insecticides acting on the insect ryanodine receptor.

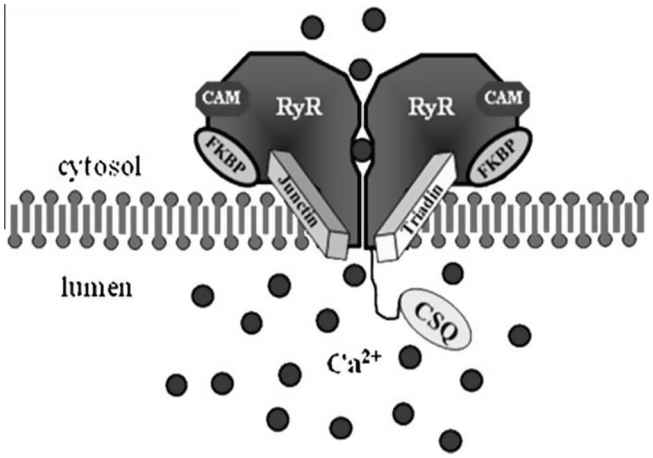


Figure 2. Schematic representation of the ryanodine receptor and the important associated proteins.

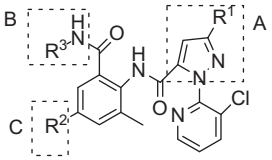


Figure 3. Chemical structures of anthranilic diamides.

et al.[30] revealed that the G4946E mutation was strongly associated with the chlorantraniliprole resistance in Plutella xylostella, and reduced binding affinity of PxRyR to the chlorantraniliprole through the analysis of K_d values. Therefore, we hypothesized and proposed that the membrane-spanning domain protein of the ryanodine receptor, a tetramer of four identical subunits, which regulates flow of calcium ions, might have the scpecific binding site for chlorantraniliprole. In this work, we choosed the membrane-spanning domain protein as the possible acceptor for study. Through studying the relationship between the K_i values of molecular docking and results of insecticidal activities, we wanted to provid some experimental envidence for the scpecific binding site for chlorantraniliprole.

Starting from 2,3-dichloropyridine (Scheme 1), the important intermediate **7** was obtained in six steps by the similar methods in literature.[11,31–33] Compounds **8a**–**8t** were conveniently obtained by treatment of **7** with different amines without any other catalyst (or strong base) in room temperature. It was found that both tetrahydrofuran and dichloromethane were suitable solvents for most of reactions, except that acetone was needed for preparation of compound **8m** due to its good solubility in acetone.

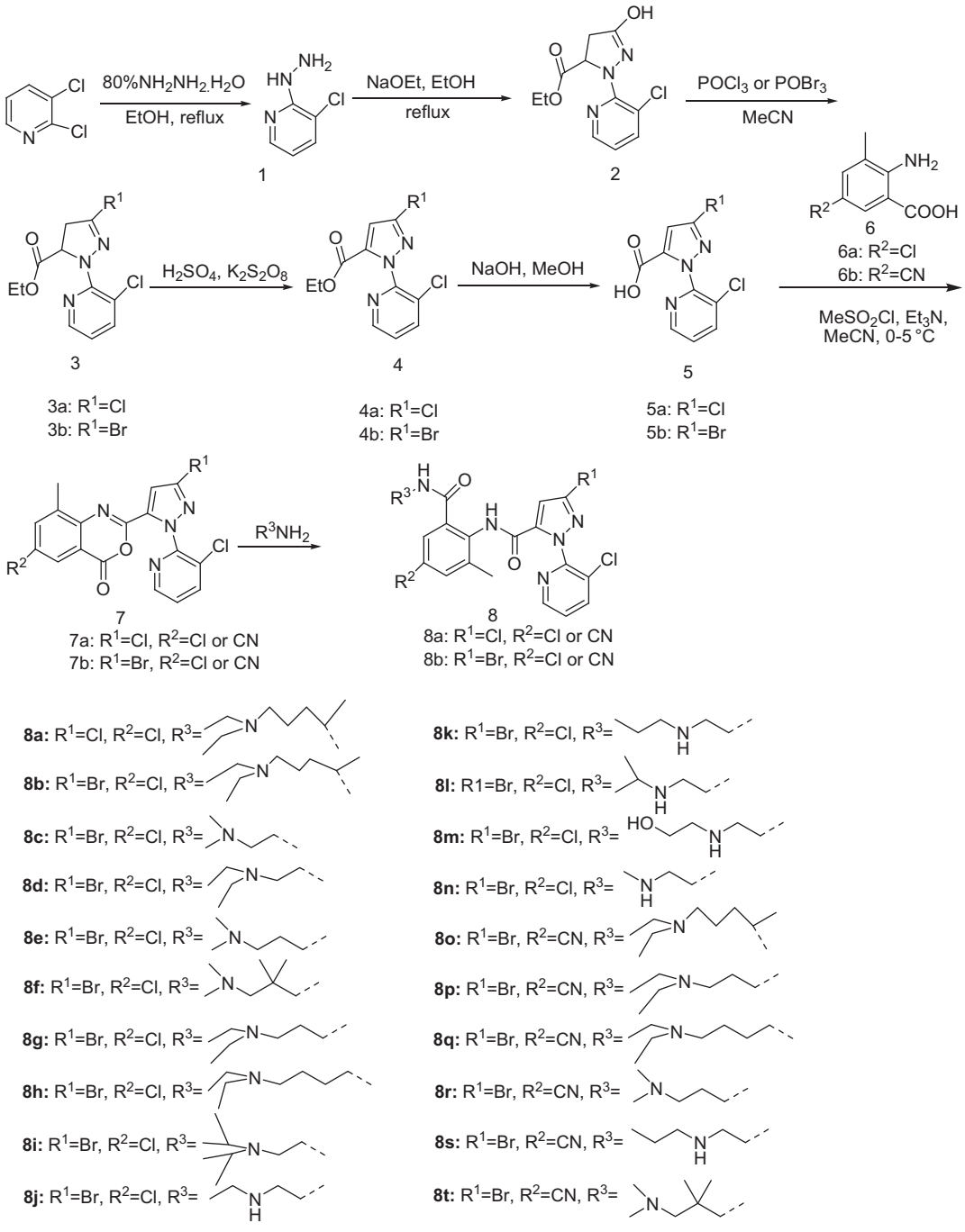
Compounds were tested against Diamondback Moth under standard laboratory procedures. The insecticidal activities of compounds **8a**–**8t**, chlorantraniliprole and indoxacarb were tested by the leaf dip method.[34] All compounds except **8h**, **8p** and **8t** exhibited varying degree of activity against diamondback moth at the concentration of 4 mg/L. Especially, compounds **8c**, **8i**, **8k** and **8l** exhibited similar insecticidal activities with chlorantraniliprole and indoxacarb at the concentration of 4 mg/L after 120 h. While at the concentration of 2 mg/L and 1 mg/L, they still showed low to middle activities after 120 h (Table 1).

LC_{50} values (Table 2) of compounds **8c**, **8i**, **8k** and **8l** (6.882, 2.599, 2.835 and 2.393 mg/L respectively) were much lower than that of indoxacarb (11.187 mg/L) after 72 h, which means that the four compounds could be the better fast-acting insecticide than indoxacarb. After 96 h and 120 h, compound **8l** still has similar LC_{50} value with that of indoxacarb. The LC_{50} values of compounds **8i**, **8k** and **8l** were about double of that of chlorantraniliprole during different action periods. However, LC_{50} value of compound **8c** decreased dramaticlly from 6.882 to 1.833 mg/L when action period changed from 72 to 120 h.

The preliminary structure–activity relationship (SAR) was discussed. All compounds with cyano group (R^2 = CN) instead of chloride group in the anthraniloyl moiety commonly had very low insecticidal activities. For example, compounds **8o**, **8p**, **8q**, **8r**, **8s** and **8t** (Table 1) had activities less than 25%.

Most of researches preserve the anthranilic amide moiety, which indicated that this structure is a key pharmacophore in this kind of compounds.[35] The number of methylenes group in amide moiety was an important factor for insecticidal activities and two methylenes group is the most favour for high activity (Table 3). For example, compounds **8c**, **8i**, **8k**, **8l** and **8n** (n = 2) had activities approximately 90–97%, while compounds **8e**, **8g** and **8h** (n = 3 or 4) showed much lower insecticidal activities. Obviously, when R^4 and R^5 have the same substitution groups, for example, **8c**/**8e** (R^4 = R^5 = methyl group), **8d**/**8g**/**8h** (R^4 = R^5 = ethyl group), the insecticidal activities reduced dramatically when number of methylenes group changed from 2 to 3 and 4. Besides the number of methylenes group, the substitution of R^4 or R^5 at the tail of amide moiety is also the important element for insecticidal activities. When one of the R^4 or R^5 was substituted by hydrogen, all compounds, such as **8j**, **8k**, **8l** and **8n** displayed relatively high activities, which indicated that secondary amine at the tail of the amide moiety was a necessary pharmacophore of insecticidal activity. While, both R^4 and R^5 were substituted by alkyl groups to generate tertiary amine at end of amide moiety, most of compounds (**8d**, **8e**, **8g** and **8h**) except **8i** with high activity displayed relatively lower activities. It indicated that the number of methylenes may be the main reason for influencing insecticidal activities. And more compounds with high activities could be designed based on the number of methylenes and the substitution condition in the terminal of amide moiety.

We hypothesized that the membrane-spanning domain protein of the ryanodine receptor has the binding site(s), and it might be the scpecific receptor for chlorantraniliprole. In our study, two second amino acid sequences of the membrane-spanning domain



Scheme 1. General synthetic route for the title compound **8**.

protein of diamondback moth ryanodine receptor were selected from GenBank accession no.JN801028[36] and GenBank accession no.JF927788[37] as two possible specific recepotors, which were proposed to bind with a series of small active moleculars to calculate K_i value through autodock software. The K_i values were used to evaluate the binding energy between small molecule and the possible receptor. The analysis of the relationship between K_i and insecticidal activities of compounds could afford the information of specific recepotor.

In Table 4, entry 1, seven compounds (**8d**, **8m**, **8a**, **8t**, **8n**, **8c** and **8b**) except **8t** with K_i values exhibited moderate to high insecticidal activities against diamondback moth. The other thirteen compounds could not afford K_i values. Eight of them showed no to low activities, however, five of them (**8g**, **8i**, **8j**, **8k** and **8l**) exhibited moderate to high insecticidal activities. The changes of K_i values and insecticidal activities show no reasonable rhythmicity. While in Table4, entry 2, eleven compounds (**8h**, **8j**, **8f**, **8b**, **8g**, **8d**, **8p**, **8i**, **8n**, **8k** and **8c**) with K_i values except three of them (**8h**, **8f** and **8p)** exhibited from moderate to high activities against diamondback moth. The other nine compounds (**8a**, **8e**, **8l**, **8m**, **8o**, **8q**, **8r**, **8s** and **8t**) with unavailable K_i values except three of them (**8a**, **8l** and **8m)** displayed very low biological activities. This result could imply that the selected membrane-spanning domain protein of the diamondback moth ryanodine receptor might have specific binding site(s) for the chlorantraniliprole (Fig. 4) and its derivatives. This point is worth further studying. However, the docking

Table 1
Insecticidal activities of compounds **8a**–**8t**, chlorantraniliprole and indoxacarb against diamondback moth

Concentrations	Insecticidal activities (%) at different concentrations								
	4 mg/L			2 mg/L			1 mg/L		
Time (h) Compounds	72	96	120	72	96	120	72	96	120
8a	51.58	60.92	68.61						
8b	74.44	88.89	88.89						
8c	29.91	54.62	94.87	6.67	43.33	50	3.03	9.7	16.06
8d	57.91	60.84	65.97						
8e	18.76	23.92	32.11						
8f	2.78	2.78	8.89						
8g	28.79	46.67	56.67						
8h	0	0	0						
8i	74.55	86.67	96.97	35.03	49.13	60.1	6.36	12.73	22.73
8j	63.32	75.91	78.94						
8k	77.58	90.61	90.61	23.33	40	43.33	0	10	19.09
8l	81.72	87.53	94.44	41.11	65.93	70	3.03	20	36.67
8m	50.25	64.91	82.58						
8n	47.22	83.33	88.89						
8o	8.33	11.67	13.33						
8p	0	0	0						
8q	6.67	16.67	24.17						
8r	8.33	8.33	14.14						
8s	12.42	15.21	21.26						
8t	0	0	5.59						
Chlorantraniliprole	94.84	97.22	100	53.89	80.34	82.91	32.12	52.12	68.18
Indoxacarb	16.85	87.31	97.22	14.52	58.39	79.88	0	24.24	45.45

Table 2
LC_{50} Values of Compounds **8c**, **8i**, **8k**, **8l**, chlorantraniliprole and indoxacarb

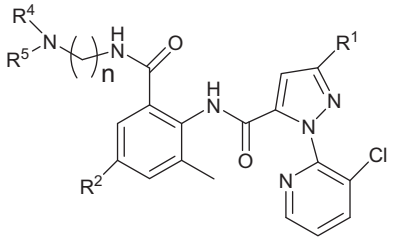
Compounds	LC_{50}[a]	LC_{50}[b]	LC_{50}[c]
8c	6.882	3.108	1.833
8i	2.599	2.022	1.598
8k	2.835	2.124	1.937
8l	2.393	1.672	1.308
Chlorantraniliprole	1.546	0.979	0.723
Indoxacarb	11.187	1.698	1.092

[a] 72 h.
[b] 96 h.
[c] 120 h.

software program could affect the accuracy of K_i values, meanwhile, biological diversity of pests played an important role in experimental activities test. These coefficients could explain why compounds **8h**, **8f** and **8p** with certain K_i values had low insecticidal activities and compounds **8a**, **8l** and **8m** with unavailable K_i values showed relatively good activities.

In summary, a series of novel chlorantraniliprole derivatives containing different amide groups and anthraniloyl moiety were designed and synthesized. The insecticidal activities of the compounds against diamondback moth were evaluated. The results indicated that compounds except compounds **8h**, **8p** and **8t** exhibited favorable insecticidal activities against diamondback moth. Especially, compounds **8c**, **8i**, **8k** and **8l** dispayed high insecticidal activities. The preliminary structure–activity relationship of the title compounds indicated that compounds with cyano group (R^2 = CN) had low insecticidal activities. The two methylenes groups and secondary amine in the amide moiety were necessary factors for increasing insecticidal activities. Furthermore, the molecular docking result revealed that most of the compounds with available K_i values exhibited moderate to high activities against diamondback moth and this relationship between K_i value

Table 3
SAR study of several chlorantraniliprole derivatives



Compound	The number of methylenes (n)	R^4	R^5	Insecticidal activities (%) at the concentration of 4 mg/L during 120 h
8c	2	CH_3	CH_3	94.87
8e	3	CH_3	CH_3	32.11
8d	2	CH_3CH_2	CH_3CH_2	65.97
8g	3	CH_3CH_2	CH_3CH_2	56.67
8h	4	CH_3CH_2	CH_3CH_2	0
8i	2	$(CH_3)_2CH$	$(CH_3)_2CH$	96.97
8j	2	CH_3CH_2	H	78.94
8k	2	$CH_3CH_2CH_2$	H	90.61
8l	2	$(CH_3)_2CH$	H	94.44
8n	2	CH_3	H	88.89

Table 4
K_i values and insecticidal activities against diamondback moth

Entry 1 (compd)	K_i[a]	Insecticidal activities[c] (%)	Entry 2 (compd)	K_i[b]	Insecticidal activities[c] (%)
8d	5.29 nM	65.97	**8h**	3.64 nM	0
8m	8.87 nM	82.58	**8j**	5.98 nM	78.94
8a	9.96 nM	68.61	**8f**	8.25 nM	8.89
8t	68.77 nM	5.59	**8b**	14.4 nM	88.89
8n	387.88 nM	88.89	**8g**	15.16 nM	56.67
8c	7.48 mM	94.87	**8d**	17.68 nM	65.97
8b	8.44 mM	88.89	**8p**	17.85 nM	0
Control[d]	50.12 nM	100	**8i**	28.27 nM	96.97
8e	Unavailable[e]	32.11	**8n**	64.92 nM	88.89
8f	Unavailable	8.89	**8k**	99.57 nM	90.61
8g	Unavailable	56.67	**8c**	93.86 nM	94.87
8h	Unavailable	0	Control[d]	50.12 nM	100
8i	Unavailable	96.97	**8a**	Unavailable	68.61
8j	Unavailable	78.94	**8e**	Unavailable	32.11
8k	Unavailable	90.61	**8l**	Unavailable	94.44
8l	Unavailable	94.44	**8m**	Unavailable	82.58
8o	Unavailable	13.33	**8o**	Unavailable	13.33
8p	Unavailable	0	**8q**	Unavailable	24.17
8q	Unavailable	24.17	**8r**	Unavailable	14.14
8r	Unavailable	14.14	**8s**	Unavailable	21.26
8s	Unavailable	21.26	**8t**	Unavailable	5.59

[a] K_i values (receptor selected from GenBank accession no.JN801028).
[b] K_i values (receptor selected from GenBank accession no. JF927788).
[c] The activity was determined at the concentration of 4 mg/L during 120 h.
[d] Chlorantraniliprole.
[e] Unavailable: compounds couldn't interact with the selected receptor and K_i values couldn't be calculated.

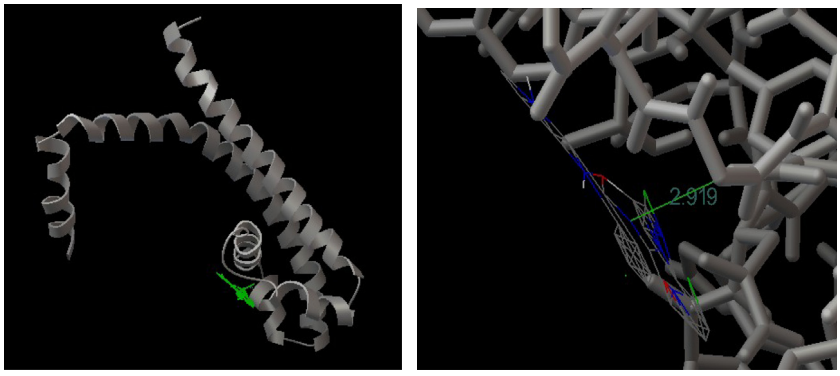


Figure 4. (a) Interaction of chlorantraniliprole with the transmembrance region protein of the diamondback moth ryanodine receptor (receptor selected from GenBank accession no. JF927788); (b) Zoomed-in view of the interaction between chlorantraniliprole and amino acids from the active site of the transmembrance region protein.

of molecular docking and insecticidal activities suggested that the proposed membrane-spanning domain protein of the diamondback moth ryanodine receptor might have the special binding site(s), which is worth of further studying.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.02.053.

References and notes

1. Sayyed, A. H.; Omar, D.; Wright, D. J. *Pest Manag. Sci.* **2004**, *60*, 827.
2. Sarfraz, M.; Keddie, B. A. *J. Appl. Entomol.* **2005**, *129*, 149.
3. Cao, G.; Han, Z. J. *Pest Manag. Sci.* **2006**, *62*, 746.
4. Tohnishi, M.; Nakao, H.; Kohno, E.; Nishida, T.; Furuya, T.; Shimizu, T.; Seo, A.; Sakata, K.; Fujioka, S.; U. S. Patent 6,559,341, 2003.
5. Tohnishi, M.; Nakao, H.; Furuya, T.; Seo, A.; Kodama, H.; Tsubata, K. *Pesticide Chemistry: Crop Protection, Public Health, Environmental Safety*; Wiley-VCH, 2007. Chapter 13.
6. Masaki, T.; Yasokawa, N.; Fujioka, S.; Motoba, K.; Tohnishi, M.; Hirooka, T. *J. Pest. Sci.* **2009**, *34*, 37.
7. Masaki, T. *J. Pest. Sci.* **2008**, *33*, 271.
8. Masali, T.; Yasokawa, N.; Tohnishi, M.; Nishimatsu, T.; Tsubata, K.; Inoue, K.; Motoba, K.; Hirooka, T. *Mol. Pharmacol.* **2006**, *69*, 1733.
9. Sattelle, D. B.; Cordova, D.; Cheek, T. R. *Invert. Neurosci.* **2008**, *8*, 107.
10. Lahm, G. P.; Cordova, D.; Barry, J. D. *Bioorg. Med. Chem.* **2009**, *17*, 4127.
11. Lahm, G. P.; Selby, T. P.; Freudenberger, J. H.; Stevenson, T. M.; Myers, B. J.; Seburyamo, G.; Smith, B. K.; Flexner, L.; Clark, C. E.; Cordova, D. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4898.
12. Hughes, K. A.; Lahm, G. P.; Selby, T. P.; Stevenson, T. M. WO 2004/067528, 2004.
13. Isaacs, A. K.; Qi, S. Z.; Sarpong, R.; Casida, J. E. *Chem. Res. Toxicol.* **2012**, *25*, 1571.

14. Lahm, G. P.; Stevenson, T. M.; Selby, T. P.; Freudenberger, J. H.; Cordova, D.; Flexner, L.; Bellin, C. A.; Dubas, C. M.; Smith, B. K.; Hughes, K. A.; Hollingshaus, J. G.; Clark, C. E.; Benner, E. A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6274.
15. Li, Y. X.; Mao, M. Z.; Li, Y. M.; Xiong, L. X.; Li, Z. M.; Xu, J. Y. *Physiol. Entomol.* **2011**, *36*, 230.
16. Cordova, D.; Benner, E. A.; Sacher, M. D.; Rauh, J. J.; Sopa, J. S.; Lahm, G. P.; Selby, T. P.; Stevenson, T. M.; Flexner, L.; Gutteridge, S.; Rhoades, D. F.; Wu, L.; Smith, R. M.; Tao, Y. *Pestic. Biochem. Physiol.* **2006**, *84*, 196.
17. Feng, Q.; Liu, Z. L.; Xiong, L. X.; Wang, M. Z.; Li, Y. Q.; Li, Z. M. J. *Agric. Food. Chem.* **2010**, *58*, 12327.
18. Wu, J.; Song, B. A.; Hu, D. Y.; Yue, M.; Yang, S. *Pest Manag. Sci.* **2012**, *68*, 801.
19. Jeanguenat. A.; O'sullivan, A. C. WO 2006/061200, 2006.
20. Dong, W. L.; Xu, J. Y.; Xiong, L. X.; Liu, X. H.; Li, Z. M. *Chin. J. Chem.* **2009**, *27*, 579.
21. Bruce, L. F.; Stephen, F. M. WO2007/024833, 2007.
22. Gnamm, C.; Jeanguenat, A.; Dutton, A. C.; Grimm, C.; Kloer, D. P.; Crossthwaite, A. J. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3800.
23. Zhao, Y.; Zhao, L. P.; Tong, J.; Li, Y. Q.; Xiong, L. Y.; Li, F.; Peng, L. N.; Li, Z. M. *Mol. Diversity* **2012**, *16*, 711.
24. Ou, J. J.; Zhu, S. K.; Wang, L.; Xu, C.; Liu, F.; Ren, L.; Xu, X.; Wang, Y.; Rui, C. H.; Liu, S. Z. *J. Agric. Food Chem.* **2012**, *60*, 10942.
25. Jeanguenat, A. *Pest Manag. Sci.* **2013**, *69*, 7.
26. Hughes, K. A.; Lahm, G. P.; Selby, T. P.; Stevenson, T. M. WO2004/067528, 2004.
27. Annan, I. B.; Hughes, K. A.; Lahm, G. P.; Selby, T. P.; Stevenson, T. M. WO2006/068669, 2006.
28. Schwarz, T.; Snow, T. A.; Santee, C. J.; Mulligan, C. C.; Class, T.; Wadsley, M. P.; Nanita, S. C. *J. Agric. Food Chem.* **2011**, *59*, 814.
29. Troczka, B.; Zimmer, C. T.; Elias, J.; Schorn, C.; Bass, C.; Davies, T. G. E.; Field, L. M.; Williamson, M. S.; Slater, R.; Nauen, R. *Insect Biochem. Mol. Biol.* **2012**, *42*, 837.
30. Guo, L.; Wang, Y.; Zhou, X.; Li, Z.; Liu, S.; Liang Peia, L.; Gaoa, X. *Pest Manag. Sci.* **2013**. doi: 10.1002/ps.3651.
31. Lahm, G. P.; Mccann, S. F. WO03/015518, 2003.
32. Berger, R. A.; Flexner, J. L. WO03/024222, 2003.
33. Liu, C. L.; Chai, B. S.; Guang, A. Y.; Zhang, H.; Peng, Y. W.; Wang, J. F.; Li, H. C.; Li, Z. N. WO2008/134970, 2008.
34. Zhao, Q. Q.; Li, Y. Q.; Xiong, L. X.; Wang, Q. M. *J. Agric. Food Chem.* **2010**, *58*, 4992.
35. Hall, R.G.; Loiseleur, O.; Pabba, J.; Pal, S.; Jeanguenat, A.; Edmunds, A.; Stoller, A. U.S. Patent 8,399,490, 2013.
36. Wang, X. L.; Wu, S. W.; Yang, Y. H.; Wu, Y. D. *Pestic. Biochem. Physiol.* **2012**, *102*, 204.
37. Sun, L.; Rui, C. H.; Yuan, H. Z. *Plant Prot.* **2012**, *38*, 118.