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Synthesis and quantitative structure−activity relationship (QSAR) studies of novel rosin-based diamide insecticides

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Abstract

In a continuing effort to develop novel natural product-based insecticidal agents endowed with low mammalian toxicity, two series of rosin-based diamides have been synthesized, and their insecticidal activities against *Plutella xylostella* and *Mythimna separate* were evaluated. Most of synthesized compounds exhibited moderate to significant insecticidal activity. Among them, thiadiazole-containing diamides **6a-6n** displayed better activity than others, especially compounds **6f** and **6n** exhibited excellent insecticidal activity against *P. xylostella*, with LC_{50} values of 0.223 and 0.214 mg/L, respectively, which approximate to or lower than that of the control flubendiamide (0.222 mg/L). The preliminary structure–activity relationship analysis indicated that rosin-based diamides with electron-withdrawing groups on the benzene ring showed better insecticidal activity than those with electron-donating groups. Via a best multilinear regression analysis, the generated QSAR model (R^2 = 0.9566) revealed a strong correlation of insecticidal activity against *P. xylostella* with molecular structures of these compounds. These consequences can be expected to instruct the design and development of new rosin-based insecticides.

Keywords: *rosin, synthesis, diamide, insecticidal activity, QSAR*

1. Introduction

Synthetic insecticides that protect crops from plant insects and diseases are still play important roles in the current agricultural practice.[1] However, the continual and abuse application of all these conventional insecticides over years has lead to environmental problems and undesired development of resistance in pest.[2, 3] Therefore, the search of potential insecticides with new targets and low mammalian toxicity remains desirable in the field of crop protection.[4, 5]

The diamides constitute a new class of insecticides for the control of lepidopteron pest.[6, 7] Studies have shown that diamides are activators of the ryanodine receptors (RyRs) which regulate the Ca^{2+} release from intracellular stores located in the sarcoplasmic reticulum.[8] Owing to their exceptional activity, unique mode of action, and low mammalian toxicity, the diamides have received considerable attention.[9] So far, three of diamide insecticides, i.e., flubendiamide, chlorantraniliprole (Rynaxypyr) and cyantraniliprole (Cyazypyr) were discovered and commercialized (**Figure 1**).[10-12] However, chemical synthesis of these diamides insecticides is time-consuming and expensive because of their complicated molecular structure and chiral centers. As a feasible solution, a class of secondary metabolites from natural sources can be chemically modified with similar structures and chiral centers.

The plant secondary metabolites are obtained in the process of coevolution between plants and the environment.[13] Currently, a range of secondary metabolites from natural sources such as flavonoids, alkaloids and terpenoids have been developed as the lead compounds for preparation of potent insecticides.[14] With advantage of good environmental profile and rare development of resistance, these botanical insecticides have been considered as attractive alternatives to synthetic agrochemicals for pest management.

Rosin, a major component of secretion from pine trees, is considered as one of the most abundant and popular natural terpenoids resources in China.[15] The broad-spectrum biological activities of rosin and its derivatives, such as antitumor, antibacterial, antivirus, and hormone regulation have been well documented.[16] Even so, investigations reporting on

the systematic study of the quantitative structure-activity relationship (QSAR) of rosin-based insecticidal agents are few. Due to the rising cost, synthesizing all possible compounds and screening a few candidates from thousands of compounds become economically and practically impossible. One approach to improve this time-consuming and cost expensive process is to apply QSAR. QSAR methodology is an essential tool, which enables the calculation of numerous quantitative descriptors solely on the basis of molecular structural information. Based on the constructed QSAR models, some vital features responsible for the insecticidal activity can be identified, and the potential target sites can even be speculated. Meanwhile, the use of computational approaches and methodologies of the QSAR study may provide further guidance to the design of novel potent insecticides.[17-22]

In order to obtain novel natural product-based insecticides, series of rosin-based diamides were synthesized on the basis of molecular similarity. In addition, the thiadiazole heterocyclic group, an important pharmacophore, was introduced into the backbone structure of diamides for designing insecticides with higher activity. The insecticidal activities of the title compounds against diamondback moth (*P. xylostella*) and oriental armworm (*M. separata*) were evaluated. Moreover, the QSAR was also performed on all of the title compounds using the Gaussian and CODESSA software package, which can account for their structural features responsible for the insecticidal activity. This exploration is expected to improve the application of rosin as an insecticide material.

2. Experimental sections

2.1 General considerations in chemistry

Gum rosin (Grade one) was obtained from a commercial source (Wu Zhou Pine Chemicals Ltd., Guangxi, China) and used without further purification. All other chemicals used were of reagent grade. The IR spectra were taken on a Nicolet IS10 FT-IR (Nicolet, Madison, USA) spectrophotometer. The ^{1}H NMR spectra were recorded on a Bruker AV-300 (Bruker, Karlsruhe, Germany) nuclear magnetic resonance spectrometer with $CDCl_3$ or DMSO-d_6 as solvent and TMS as an internal standard. The MS spectra were taken on an Agilent-5973

(Agilent, Santa Clara, USA) spectrophotometer. The melting points were determined using XT-5 (Saiao, Beijing, China) melting point apparatus. The elemental analysis (C, H, and N) was done on a Vario EL-III (Elementar, Hanau, Germany) elemental analyzer and the results were in good agreement with the calculated values. All reactions were traced by thin layer chromatography (TLC).

2.1.1 Syntheses of 1b. Following the procedures described in previous literature,[23] 1000 g gum rosin and 5 g hydroquinone were added to a flask equipped with a stirrer, dropping funnel, N_2 inlet, thermometer, and water trap topped with a water-cooled condenser. The rosin was first heated under a slow stream of nitrogen and then stirred after melting had occurred. The temperature was adjusted to 220°C, and the acrylic acid was added dropwise within 0.3 h. The mixture was then heated to 230°C for a residence time of 4 h and the products were collected after cooling to 170°C. Two isomers of the rosin-acrylic acid adduct (RAAA) (**1a** and **1b** in **Scheme 1**, with contents of 15% and 55%, respectively, in the products) were found by gas chromatography. The target chemical **1b** was obtained by recrystallization with ethanol to give colorless crystals (0.30 mm × 0.20 mm × 0.20 mm) suitable for X-ray single crystal diffraction as shown in **Figures 2** and **3** with the following crystallographic parameters: a =12.682 (3) Å, b = 12.476 (3) Å, c = 14.629 (3) Å, β =90.12 (3) °, λ = 0.71073 Å, θ = 9–12 °, V = 2314.6 (12) Å^3, Z = 4, μ= 0.08 mm^{-1}, F_{000} = 868, R = 0.0498, T = 293 K, wR (F^2) = 0.194, S = 1.02, final R factor = 7.70%.[24]

2.1.2 Syntheses of 16-isopropyl-5, 9-dimethyltetracyclo [10.2.2.0$^{1,\ 10}$.0$^{4,\ 9}$] hexadec-15-ene-5, 14-dicarbonyl chloride (2). A solution of **1b** (7.50 g; 20 mmol) and 80 mL dichloromethane (DCM) were added to a 250 mL flask equipped with a water-cooled condenser, thermometer, drying tube, and dropping funnel. The solution was stirred until the solid was dissolved. After that, thionyl chloride (5.95 g; 50 mmol) was added dropwise through a dropping funnel within 1 h. After refluxing for 4 h at 65 ℃, acrylpimaryl chloride (**2**) was obtained as yellow oil after removing the DCM and excess thionyl chloride under reduced pressure. Yield: 7.40 g (90%). IR (cm^{-1}): 2926, 2854 (-CH_3, -CH_2); 1741 (C=O). ^{1}H NMR ($CDCl_3$. δ/ppm. 300 MHz), 5.50 (S, H, C=CH-); 2.52-1.41 (m, 5H, -CH-); 1.86-1.24

(m, 14H, -CH_2-); 1.27-1.10 (m, 12H, CH_3). ESI-MS m/z = 412 [M + H] $^+$. Anal. Calcd for $C_{23}H_{32}Cl_2O_2$: C, 67.15; H, 7.84. Found: C, 67.21; H, 7.89.

2.1.3 Syntheses of 16-isopropyl-5, 9-dimethyltetracyclo [10.2.2.0$^{1,\ 10}$.0$^{4,\ 9}$] hexadec-15-ene-5, 14-dicarbonylamide (3). A solution of the compound **2** (7.40 g; 18 mmol) in 15 mL of tetrahydrofuran (THF) was added dropwise to a solution of 200 mL ammonium water (80%) in 40 mL THF within 30 minutes at 0 ℃. After reacting for 12 h, the THF solvent and excess ammonium water were removed under vacuum. Then, the mixture was washed with deionized water three times and recrystallized with ethyl acetate to give yellow solid **3**. Yield: 4.71 g (70.4%); yellow powder; m.p. 152.8-153.6°C. IR (cm^{-1}): 3359 (N-H); 2933, 2849 (-CH_3, -CH_2); 1663 (N-C=O). ^{1}H NMR ($CDCl_3$. δ/ppm. 300 MHz), 5.78 (m, 4H, $CONH_2$); 5.49 (S, H, C=CH-); 2.61 (m, H, -C<u>H</u>-$(Me)_2$); 2.42 (S, H, -CH-C=O-); 2.12-1.43 (m, 3H, -CH-); 1.82-1.26 (m, 14H, -CH_2-); 1.34-1.06 (m, 12H, CH_3). ESI-MS m/z = 373 [M + H] $^+$. Anal. Calcd for $C_{23}H_{36}N_2O_2$: C, 74.15; H, 9.74; N, 7.52. Found: C, 74.11; H, 9.69; N, 7.56.

2.1.4 Syntheses of 16-isopropyl-5, 9-dimethyltetracyclo [10.2.2.0$^{1,\ 10}$.0$^{4,\ 9}$] hexadec-15-ene-5, 14-dicarboxamides (4a-4n). A solution of the compound **2** (7.40 g; 18 mmol) in 15 mL of DCM was added dropwise to a solution of 60 mmol of amine and 60 mmol of triethylamine (Et_3N) in 40 mL of DCM within 30 minutes at room temperature. After reacting for 12 h, the mixture was washed using 50 mL of 0.1M hydrochloric acid and deionized water three times. Purification of the residue by silica gel chromatography [V (ethyl acetate)/ V (petroleum ether) = 1:10] gave the fourteen resulting derivatives, **4a** to **4n**. The example data of compounds **4a** and **4b** are shown as follows, whereas data of compounds **4c-4n** can be found in the Supporting Information.

*Data for compound **4a***. Yield: 7.77 g (70.4%); white powder; m.p. 140.8-141.6°C. IR (cm^{-1}): 3363, 3259 (N-H); 2926, 2854 (-CH_3, -CH_2); 1659 (N-C=O); 738, 697 (Ar-H). ^{1}H NMR (DMSO-d_6. δ/ppm. 300MHz): 9.61, 9.06 (m, 2H, CONH-); 7.61-6.96 (m, 10H, Ar-H); 5.29 (S, H, C=CH-); 4.71-4.30 (m, 4H, Ar-CH_2-); 2.57 (S, H, -CH-C=O-); 2.01-1.83 (m, 3H, -CH-); 1.82-1.24 (m, 14H, -CH_2-); 1.53 (m, H, -CH-$(Me)_2$); 1.14-0.61 (m, 12H, CH_3).

ESI-MS m/z = 553 $[M + H]^+$. Anal. Calcd for $C_{37}H_{48}N_2O_2$: C, 80.39; H, 8.75; N, 5.07. Found: C, 80.27; H, 9.00; N, 4.86.

*Data for compound **4b***. Yield: 7.55 g (72%); white powder; m.p. 135.6-137.4°C. IR (cm^{-1}): 3354 (N-H); 2961, 2861 (-CH_3, -CH_2); 1663 (N-C=O); 754, 691 (Ar-H). ^{1}H NMR (DMSO-d_6. δ/ppm. 300MHz): 8.08, 7.95 (m, 2H, CONH-); 7.29-7.20 (m, 10H, Ar-H); 5.25 (S, H, C=CH-); 2.68 (S, H, -CH-C=O-); 2.50-1.28 (m, 14H, -CH_2-); 2.11-1.90 (m, 3H, -CH-); 1.57 (m, H, -CH-$(Me)_2$); 1.17-0.55 (m, 12H, CH_3). ESI-MS m/z = 525 $[M + H]^+$. Anal. Calcd for $C_{35}H_{44}N_2O_2$: C, 80.11; H, 8.45; N, 5.34. Found: C, 79.90; H, 8.54; N, 5.08.

2.1.5 Syntheses of 16-isopropyl-5, 9-dimethyltetracyclo [10.2.2.0$^{1,\ 10}$.0$^{4,\ 9}$] hexadec-15-ene-5, 14-dicarbonylamide (5). A mixture of **1b** (3.75 g; 10 mmol) and thiosemicarbazide (1.90 g; 20 mmol) in phosphorus oxychloride (25 ml) was refluxed. After reacting for 2 h, the mixture was cooled and adjusted to PH=10 with aqueous sodium hydroxide. The solid was collected by filtration and then recrystallized with ethanol to give the compound **5**. Yield: 4.60 g (95%); white powder; m.p. 135.9-136.7 ℃. IR (cm^{-1}): 3412, 3298, 1614 (N-H); 1635 (C=N); 1099 (C-S-C). ^{1}H NMR (DMSO-d_6. δ/ppm. 300 MHz), 5.44 (s, H, C=CH-); 4.00 (m, 4H, -NH_2); 2.74-1.78 (m, 5H, -CH-); 1.80-1.25 (m, 14H, -CH_2-); 1.33-1.05 (m, 12H, CH_3). ESI-MS m/z = 485 $[M + H]^+$. Anal. Calcd for $C_{25}H_{36}N_6S_2$: C, 61.95; H, 7.49; N, 17.34. Found: C, 61.93; H, 7.51; N, 17.05.

2.1.6 Syntheses of 16-isopropyl-5, 9-dimethyltetracyclo [10.2.2.0$^{1,\ 10}$.0$^{4,\ 9}$] hexadec-15-ene-5, 14- [1,3,4]thiadiazol-2-yl]-diamide (6a-6n). A solution of the chloride (20 mmol) in 15 mL of DCM was added dropwise to a solution of (**5**) (4.85 g; 10 mmol) and 60 mmol of Et_3N in 40 mL of DCM within 30 minutes at 0 ℃. After reacting for 12 h, the mixture was washed using 50 mL of 0.1M hydrochloric acid and deionized water three times. Purification of the residue by silica gel chromatography [V (ethyl acetate)/ V (petroleum ether) = 1:5] gave the fourteen resulting derivatives, **6a** to **6n**. The example data of compounds **6a** and **6b** are shown as follows, whereas data of compounds **6c-6n** can be found in the Supporting Information.

*Data for compound **6a***. Yield: 3.98 g (70%); white powder; m.p. 235.9-236.7 ℃. IR

(cm^{-1}): 3370, 3159 (N-H); 2926, 2854 (-CH_3, -CH_2); 1660 (N-C=O); 1633 (C=N); 1080 (C-S-C). ^{1}H NMR (DMSO-d_6. δ/ppm. 300 MHz), 8.01, 7.95 (S, 2H, -CONH-); 5.44 (s, H, C=CH-); 2.80-1.80 (m, 5H, -CH-); 2.02, 1.95 (m, 6H, -$COCH_3$); 1.86-1.24 (m, 14H, -CH_2-); 1.34-0.98 (m, 12H, -CH_3). ESI-MS m/z = 569 $[M + H]^+$. Anal. Calcd for $C_{29}H_{40}N_6O_2S_2$: C, 61.24; H, 7.09; N, 14.78. Found: C, 61.33; H, 7.01; N, 14.75.

Data for compound ***6b***. Yield: 4.50 g (65%); white powder; m.p. 222.3-223.2 ℃. IR (cm^{-1}): 3329, 3100 (N-H); 2961, 2880 (-CH_3, -CH_2); 1659 (N-C=O); 1632 (C=N); 1079 (C-S-C); 751, 690 (Ar-H). ^{1}H NMR (DMSO-d_6. δ/ppm. 300 MHz): 8.18, 8.02 (m, 2H, -CONH-); 7.59-7.44 (m, 10H, Ar-H); 5.25 (s, H, C=CH-); 2.81-1.79 (m, 5H, -CH-); 1.95-1.28 (m, 14H, -CH_2-); 1.37-0.55 (m, 12H, CH_3). ESI-MS m/z = 693 $[M + H]^+$. Anal. Calcd for $C_{39}H_{44}N_6O_2S_2$: C, 67.60; H, 6.40; N, 12.13. Found: C, 67.63; H, 6.41; N, 12.05.

2.2 Biological assay

The *P. xylostella* and *M. separate* used in bioassay were provided by research & development center of biorational pesticide, Northwest A&F University. The bioassays were performed in artificial greenhouse with the constant temperature (25 ± 0.5 ℃), relative humidity (75 ± 5%), and photoperiod (L/D = 14/10). All test compounds were dissolved in DMSO and diluted with sterile water to obtain required concentrations. Assessments were made on a dead/alive basis, and mortality rates were corrected using Abbott's formula. Evaluations were based on a percentage scale of 0−100, where 0 = no activity and 100 = complete eradication. The standard deviations of the tested biological values were ±5%. LC_{50} values were calculated by probit analysis. For comparative purposes, the commercial product flunbendiamide were tested under the same conditions.[25, 26]

2.2.1 Larvicidal activity against *P. xylostella*. The larvicidal activity of the title compounds and contrast compound flunbendiamide against *P. xylostella* were estimated according to the leaf-dip method using the reported procedure.[27-30] Briefly, leaf disks (6 cm × 2 cm) of fresh cabbage leaves were dipped into the test solution for 3-5 s and dried. The treated leaf disks were placed individually into glass tubes. Each dried treated leaf disk was infested with 30 second-instar *P. xylostella*. Leaves treated with DMSO and sterile water

were provided as control. All experiments were conducted in triplicate to ensure reproducibility at a given concentration.

2.2.2 Larvicidal activity against *M. separata*. The larvicidal activity of the title compounds and contrast compound flunbendiamide against *M. separata* were also estimated according to the leaf-dip method using the reported procedure.[26, 31, 32] Briefly, leaf disks (about 5 cm diameter) of fresh corn leaves were dipped into the test solution for 10-15 s. After air drying, the treated leaf disks were placed on plates. Each dried treated leaf disk was infested with 10 third-instar *M. separata*. Percentage mortalities were evaluated 72 h after treatment. Leaves treated with DMSO and sterile water were provided as control. All experiments were conducted in triplicate to ensure reproducibility at a given concentration.

2.3 Building and validation of QSAR model

Firstly, the optimal conformers and lowest energy of the title compounds were performed at the DFT/6-31G (d) level using Gaussian 03W package of programs.[30] Secondly, the calculated results were changed into the form compatible with CODESSA 2.7.15[33] using Ampac 9.1.3.[34] Finally, all the molecular descriptors involved in these compounds were calculated by CODESSA 2.7.15. In order to find out which structural features play an important role in insecticidal activity against *P. xylostella*, the best multiple regression analysis was selected to generate the QSAR model equation. In this equation, the statistical criteria were indicated by the squared correction coefficient (R^2), the squared standard error of the estimates (S^2), and the Fisher significance ratio (F). Tested LC_{50} values were converted into the corresponding log LC_{50} and used as dependent variables in the QSAR studies. The quality of the final model was determined using both an internal validation and the "leave-one-out" cross-validation methods.

3. Results and discussion

3.1 Synthesis

The syntheses of two series of rosin-based diamide derivatives developed in the present work are illustrated in **Scheme 1** and **2** respectively. As a dicarboxylic acid, RAAA was obtained by the Diels-Alder addition reaction between rosin and acrylic acid; the content of

RAAA in the products was 70%. Additional purifications were required to remove other reactants and separate the two isomers **1a** and **1b** from RAAA. According to the literature, the target isomer **1b** was separated by recrystallization with a overall yield of 95%.[15] Acrylopimaryl chloride **2** was prepared from the reaction of **1b** with thionyl chloride and used without purification. The target compounds **3** and **4a-4n** were prepared by a simple and convenient three-step procedure starting from rosin. At room temperature, compounds **4a-4n** were synthesized in high yields (60-90%) by the reaction of acrylopimaryl chloride (**2**) with various aromatic amines in the DCM using Et_3N as the acid acceptor. However, the compound **3** was prepared by the reaction of $NH_3{\cdot}H_2O$ with acrylopimaryl chloride **2** at 0°C in the THF with satisfactory yield (70%). As shown in **Scheme 2**, the compound **1b** and thiosemicarbazide refluxed in the $POCl_3$ to give corresponding thiadiazole substituted amine **5** via a cyclization reaction. The reaction of **5** with various chlorides can give thiadiazole-containing amides, **6a** to **6n**. The structures of the title compounds were well-characterized by IR, ^{1}H NMR, MS, and elemental analysis.

3.2 Biological activity and structure-activity relationships

3.2.1 Larvicidal activity against *P. xylostella*. All of the compounds were initially tested at a concentration of 10 mg/L, and consequently the compounds with high insecticidal potency were investigated further at low concentration. The result of larvicidal activity of the title compounds against *P. xylostella* was summarized in **Table 1**, from which we can see that compounds **3**, **4a-4n** and **6a-6n** exhibited moderate to significant insecticidal activity. The introduction of thiadiazole heterocyclic group increased the insecticidal activity of the title compounds. From **Table 1**, we can see that at 5 mg/L, compounds **6c-6f**, **6h** and **6k-6n** exhibited 90-100% larvicidal activities, and the eight compounds still possessed 50-80% activities at 1 mg/L, respectively. It was worth noting that **6f** and **6n** showed a death rate of 77% at 0.25 mg/L, which is more effective than flubendiamide (70%) against *P. xylostella*. Its LC_{50} values correspondingly were 0.223 and 0.214 mg/L, respectively, which were similar to or lower than that of the control flubendiamide (0.222 mg/L). On the one hand, most compounds with electron-withdrawing substituents F, Cl, Br,

and -CF_3 displayed higher larvicidal activity against *P. xylostella* (the compound **6j**, possessed electron-withdrawing substituent NO_2, did not display higher larvicidal activity, which needs further study), while compounds with electron-donating substituents -OCH_3 and -OCF_3 led to significant decrease in activity (**4k-4n** > **4h-4j**; **6d-6f**, **6h**, **6k-6n** > **6g**, **6i** and **6j**). It can be concluded that the electronic effect of substituent on the benzene ring is important in the insecticidal activity of amide groups.[25] On the other hand, the steric effect should be considered, when substituents of -OCH_3 and -OCF_3 were introduced, their activities were comparably low, which indicated that the larger substituents in the position have negative effect on the activity. These observations revealed that substitution patterns on the benzene ring have an important influence on the larvicidal activity.[11, 35]

Table 1. Insecticidal activity of compounds against *P. xylostella*

		Larvicidal activity (%) at a concentration of (mg/L)											
No.	Compound	10	5	2.5	1	0.5	0.25	0.1	0.05	LC_{50}	y=a+bx	R^2	log LC_{50}
1	3	100	67	40	20	0	/	/	/	2.759	y=-1.210+2.746x	0.985	0.441
2	4a	100	73	40	7	0	/	/	/	2.949	y=-1.672+3.560x	1.000	0.470
3	4b	100	73	40	7	0	/	/	/	2.949	y=-1.672+3.560x	1.000	0.470
4	4c	100	77	40	7	0	/	/	/	2.879	y=-1.681+3.659x	1.000	0.459
5	4d	100	73	37	10	0	/	/	/	2.923	y=-1.563+3.355 x	0.992	0.466
6	4e	100	77	37	10	0	/	/	/	2.853	y=-1.567+3.443x	0.986	0.455
7	4f	100	80	57	7	0	/	/	/	2.506	y=-1.463+3.667x	0.974	0.399
8	4g	100	73	40	7	0	/	/	/	2.949	y=-1.672+3.560x	1.000	0.470
9	4h	70	40	17	3	0	/	/	/	6.193	y=-1.948+2.460x	0.998	0.792
10	4i	70	37	17	3	0	/	/	/	6.379	y=-1.968+2.446x	0.995	0.805
11	4j	67	40	20	3	0	/	/	/	6.339	y=-1.837+2.290x	0.998	0.802
12	4k	100	83	50	23	3	0	/	/	2.131	y=-0.924+2.813x	0.983	0.329
13	4l	100	80	50	20	3	0	/	/	2.246	y=-0.988+2.812x	0.993	0.351
14	4m	100	77	50	23	3	0	/	/	2.244	y=-0.931+2.652x	0.980	0.351
15	4n	100	77	50	27	3	0	/	/	2.185	y=-0.875+2.578x	0.964	0.339
16	6a	100	87	50	30	3	0	/	/	2.076	y=-0.922+2.906x	0.982	0.317
17	6b	100	87	57	30	3	0	/	/	1.982	y=-0.874+2.942x	0.992	0.297
18	6c	100	90	57	27	3	0	/	/	1.879	y=-0.816+2.977x	0.983	0.274
19	6d	100	90	70	57	23	7	0	/	1.096	y=-0.084+2.115x	0.972	0.040
20	6e	100	90	70	53	23	7	0	/	1.123	y=-0.107+2.125x	0.981	0.050
21	6f	100	100	90	80	77	60	33	7	0.223	y=1.004+1.543x	0.953	-0.652
22	6g	100	70	40	10	0	/	/	/	2.927	y=-1.516+3.250x	1.000	0.466
23	6h	100	90	70	50	20	7	0	/	1.182	y=-0.155+2.136x	0.974	0.073
24	6i	100	67	37	10	0	/	/	/	3.068	y=-1.559+3.201x	0.999	0.487

25	6j	100	73	40	7	0	/	/	/	2.949	y=-1.672+3.560x	1.000	0.470
26	6k	100	90	70	53	23	7	0	/	1.123	y=-0.107+2.125x	0.981	0.050
27	6l	100	90	67	50	20	7	0	/	1.211	y=-0.178+2.147x	0.983	0.083
28	6m	100	90	70	57	23	7	0	/	1.096	y=-0.084+2.115x	0.972	0.040
29	6n	100	100	90	80	77	60	33	13	0.214	y=1.001+1. 494x	0.966	-0.670
	flubendiamide	100	100	97	80	73	60	30	10	0.222	y=1.125+1.722x	0.977	-0.654

3.2.2 Larvicidal activity against *M. separata*. The results of larvicidal activity of the title compounds against *M. separate* are listed in **Table 2**, from which we can see that compounds **3**, **4a-4n**, **and 6a-6n** exhibited moderate larvicidal activity against *M. separate*. The death rate of all these compounds at 50 mg/L was about 30-40%, which was less effective than larvicidal activity against *P. xylostella*.

Table 2. Insecticidal activity of compounds against *M. separata*

Compd.	Larvicidal activity (%) at a concentration of (mg/L)			Compd.	Larvicidal activity (%) at a concentration of (mg/L)		
	50	20	10		50	20	10
3	40	0	/	**6a**	37	0	/
4a	30	0	/	**6b**	37	0	/
4b	40	0	/	**6c**	20	0	/
4c	40	0	/	**6d**	40	0	/
4d	37	0	/	**6e**	17	0	/
4e	37	0	/	**6f**	17	0	/
4f	27	0	/	**6g**	20	0	/
4g	40	0	/	**6h**	40	0	/
4h	17	0	/	**6i**	30	0	/
4i	17	0	/	**6j**	40	0	/
4j	20	0	/	**6k**	40	0	/
4k	40	0	/	**6l**	37	0	/
4l	30	0	/	**6m**	37	0	/
4m	40	0	/	**6n**	30	0	/
4n	40	0	/	**flubendiamide**	100	100	100

3.3 QSAR

There are many regression approaches available for CODESSA 2.7.15 software, such as best multi-linear regression, multi-linear regression, principal component analysis, partial least-square regression, and heuristic regression.[36] Taking into account the number of samples and descriptors used in this study, the best multi-linear regression was selected for developing the QSAR model. The “breaking point” rule for determination of the number of

the descriptors was employed as described in **Figure 4**. The best multi-linear regression showed significant increase in R^2 when the number of the descriptors was no more than 5. However, there was negligible change in R^2 when the number of descriptors increased from 5 to 6. Descriptors with high t values were accepted and those with low t values were rejected. A "breaking point" indicates that the improvement of the regression model has become insignificant (ΔR^2 < 0.02-0.04).[37] In addition, the number of the descriptors complies to the linear regressions equation (1).

$$N \geq 3\ (K+1) \qquad (1)$$

Where N is the number of sample compounds and K is the number of descriptors. Therefore, the final model with five descriptors was selected as the best model. The values of all five descriptors were found in the Supporting Information (**Table S1**).

The statistically optimized QSAR equation for log LC_{50} data has the following statistical characteristics: R^2 = 0.9566, F = 101.46, s^2 = 0.0040 (**Table 3**). This model includes five descriptors in descending order according to their statistical significance (t values), where X and ΔX are the regression coefficients and their standard errors. The comparison between experimental and predicted log LC_{50} was listed in **Table 4**. **Figure 5** shows the plot of predicted versus experimental activity of 29 compounds. The five-descriptor QSAR model equation and corresponding statistical criteria were described in following equation (2).

$$\log LC_{50} = 2.8005 + 3.4038 \times \mathrm{HOMO} - 9.2784 \times \mathrm{DM} + 2.7178 \times q^{O}_{max} - 2.3561 \times q^{N}_{min} + 2.4803 \times \mu_h \qquad (2)$$

N = 29, R^2 = 0.9566, F = 101.46, s^2 = 0.0040

Table 3. The best five-descriptor model

Descriptor No.	X	$\pm\Delta X$	t-Test	Descriptor
0	2.8005e+00	4.9272e-01	5.6838	Intercept
1	3.4038e+00	3.0849e-01	11.0338	HOMO[a]
2	-9.2784e+00	9.1786e-01	-10.1086	DM[b]
3	2.7178e+00	4.0462e-01	6.7169	q^{O}_{max}[c]
4	-2.3561e+00	2.6446e-01	-8.9090	q^{N}_{min}[d]
5	2.4803e+00	7.6091e-01	3.2596	μ_h[e]

[a] Energy of the highest occupied molecular orbit in atomic units. [b] Dipole moment. [c] Max net atomic charge for a O atom. [d] Min net atomic charge for a N atom. [e] Tot hybridization composite of the molecular dipole.

Table 4. The difference between experimental log LC_{50} and predicted log LC_{50}

No.	Compd.	Calc. log LC_{50}	Exp. log LC_{50}	difference	No.	Compd.	Calc. log LC_{50}	Exp. log LC_{50}	difference
1	**3**	0.508	0.441	0.067	**16**	**6a**	0.268	0.317	-0.049
2	**4a**	0.463	0.470	-0.007	**17**	**6b**	0.228	0.297	-0.069
3	**4b**	0.431	0.470	-0.039	**18**	**6c**	0.270	0.274	-0.004
4	**4c**	0.469	0.459	0.010	**19**	**6d**	0.139	0.040	0.099
5	**4d**	0.469	0.466	0.003	**20**	**6e**	0.118	0.050	0.068
6	**4e**	0.474	0.455	0.019	**21**	**6f**	-0.619	-0.652	0.033
7	**4f**	0.443	0.399	0.044	**22**	**6g**	0.563	0.466	0.097
8	**4g**	0.505	0.470	0.035	**23**	**6h**	0.051	0.073	-0.022
9	**4h**	0.779	0.792	-0.013	**24**	**6i**	0.418	0.487	-0.069
10	**4i**	0.749	0.805	-0.056	**25**	**6j**	0.500	0.470	0.030
11	**4j**	0.803	0.802	0.001	**26**	**6k**	0.083	0.050	0.033
12	**4k**	0.269	0.329	-0.060	**27**	**6l**	0.154	0.083	0.071
13	**4l**	0.257	0.351	-0.094	**28**	**6m**	0.094	0.040	0.053
14	**4m**	0.248	0.351	-0.103	**29**	**6n**	-0.766	-0.670	-0.096
15	**4n**	0.355	0.339	0.016					

The internal validation and "leave-one-out" cross-validation methods were used to validate the developed QSAR model.[37, 38] The internal validation was carried out by dividing the compounds data into three subsets A, B and C, with 10, 10, and 9 compounds respectively. The compounds: **1, 4, 7, 10,** *etc*., went into subset (A), **2, 5, 8, 11,** *etc*., went into subset (B), and **3, 6, 9, 12,** *etc*., went into the third subset (C). Two of three subsets, (A and B), (A and C), and (B and C), made up the training set while the remaining subset was treated as a test set. The correlation equations were derived from each of the training sets using the same descriptors and then applied to predict values for the corresponding test set. Internal validation results were presented in **Table 5**. The $R^2_{Training}$ and R^2_{Test} are within 5% for all three sets, and the average values of $R^2_{Training}$ = 0.9575 and R^2_{Test} = 0.9530 were close to the overall R^2. Therefore, the QSAR model obtained demonstrated the predictive power of 3-fold cross-validation. The "leave-one-out" method was completed in a similar manner to the internal validation. In the "leave-one-out" method, a set of seven compounds (**4**, **8**, **12**, *etc*.) were used as the external test set and the remaining compounds were left in the training subset. The QSAR model containing the same five descriptors was obtained with R^2 = 0.9868 from the training set. When the same QSAR model was applied on the test set,

R^2 = 0.9793 was observed. Therefore, the "leave-one-out" cross-validation results were also satisfactory.

Table 5. Internal validation of the QSAR model

Training set	N	R^2	F	S^2	Test set	N	R^2	F	S^2
A+B	20	0.9600	100.26	0.0053	C	9	0.9644	112.34	0.0037
B+C	19	0.9599	98.44	0.0031	A	10	0.9433	100.52	0.0039
A+C	19	0.9526	118.20	0.0048	B	10	0.9515	108.75	0.0048
Average		0.9575	105.63	0.0044	Average		0.9530	107.20	0.0041

Compds. A: **1**, **4**, **7**, **10**, **13**, **16**, **19**, **22**, **25**, **28**. Compds. B: **2**, **5**, **8**, **11**, **14**, **17**, **20**, **23**, **26**, **29**.

Compds. C: **3**, **6**, **9**, **12**, **15**, **18**, **21**, **24**, **27**.

By interpreting the descriptors involved in the model, we gained some insight into structural features influencing insecticidal activity. The foremost important descriptor was the HOMO energy (the energy of the highest occupied molecular orbital). This descriptor has a significant effect on the activity as the energy of the HOMO is directly related to the ionization potential of the compounds and characterizes the susceptibility of the molecule to electrophilic attack.[39, 40] The negative contribution of HOMO energy also suggested that the electron withdrawing substitution groups of rosin-based diamides are favorable for the insecticidal activity against *P. xylostella*.

The second important descriptor was the dipole moment. This descriptor was important in modulating insecticidal activity against *P. xylostella* may because of the presence of C=O, N-H, and thiadiazole groups, which existed permanent polarization due to an electronegativity difference between the atoms.[37, 39] The O (C=O), N (N-H/thiadiazole group), and S (thiadiazole group) atoms may be involved in binding interactions with insect cells present at the target site. The dipole moment thus played a critical role in modulating the insecticidal activity of test compounds.[41, 42]

The 3rd and 4th descriptors obtained in the model were max net atomic charge for a O atom and min net atomic charge for a N atom. These two descriptors belonged to electrostatic descriptors, and reflected characteristics of the charge distribution of the molecules.[39, 41-43] The 5th descriptor obtained in the model was tot hybridization composite of the molecular dipole, which belonged to quantum-chemically descriptors. This descriptor

reflected the quantitative measure of the lipophilic and hydrophobic properties of the compounds, and was essential for the penetration and distribution of the compounds as well as the interaction of compounds with receptors.[44, 45] In equation (2), appearance with a negative sign in the model indicate that molecule with lower descriptor value has a higher log LC_{50}, and contrary to that a positive sign in the model indicate that molecule with higher descriptor value has a higher log LC_{50}.

4. Conclusions

In summary, 29 rosin-based diamides **3**, **4a-4n** and **6a-6n** were synthesized and their insecticidal activities against *P. xylostella* and *M. separate* were evaluated. Thiadiazole-containing diamides **6a–6n** displayed better activity than others, especially the compounds **6f** and **6n** exhibited excellent insecticidal activities against *P. xylostella*. QSAR study indicated the involved descriptors for rosin-based diamides may account for their structural features responsible for the insecticidal activity. These promising results are of significant importance to the development of insecticide from common, inexpensive, and non-toxic natural products.

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Notes and references

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† Electronic Supplementary Information (ESI) available: IR, ^{1}H NMR, MS, and elemental analysis data for the target compounds. The values of all descriptors of rosin-based diamides with insecticidal activity against *P. xylostella* are also available as Supplementary Information. See DOI: 10.1039/b000000x/

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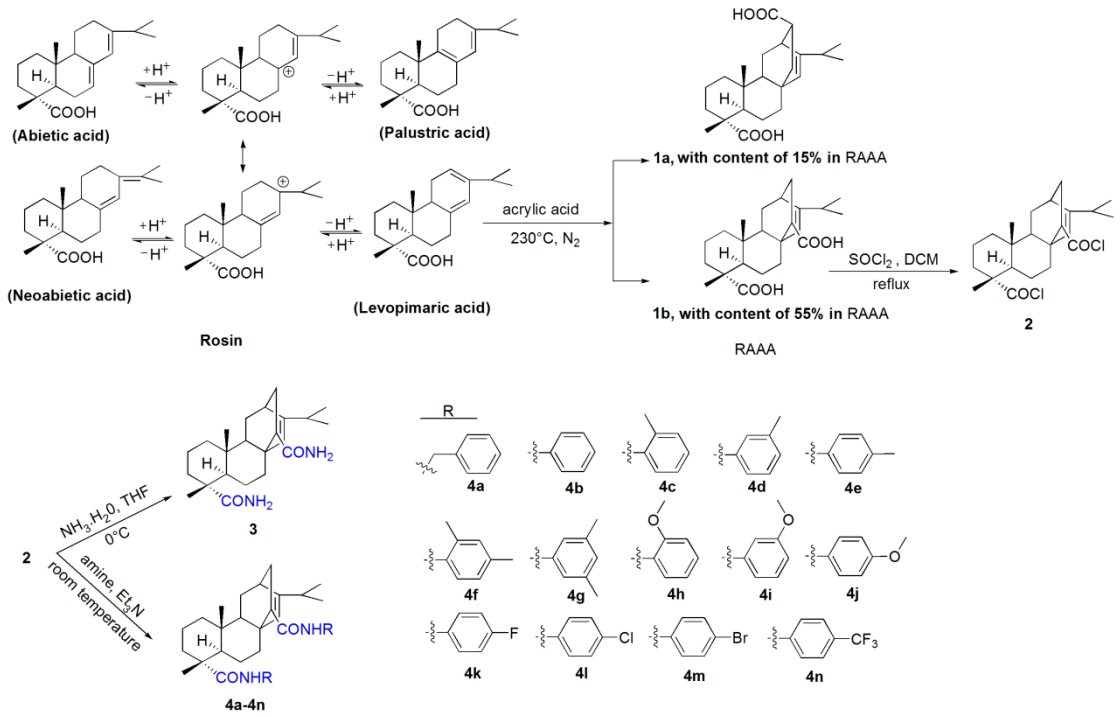
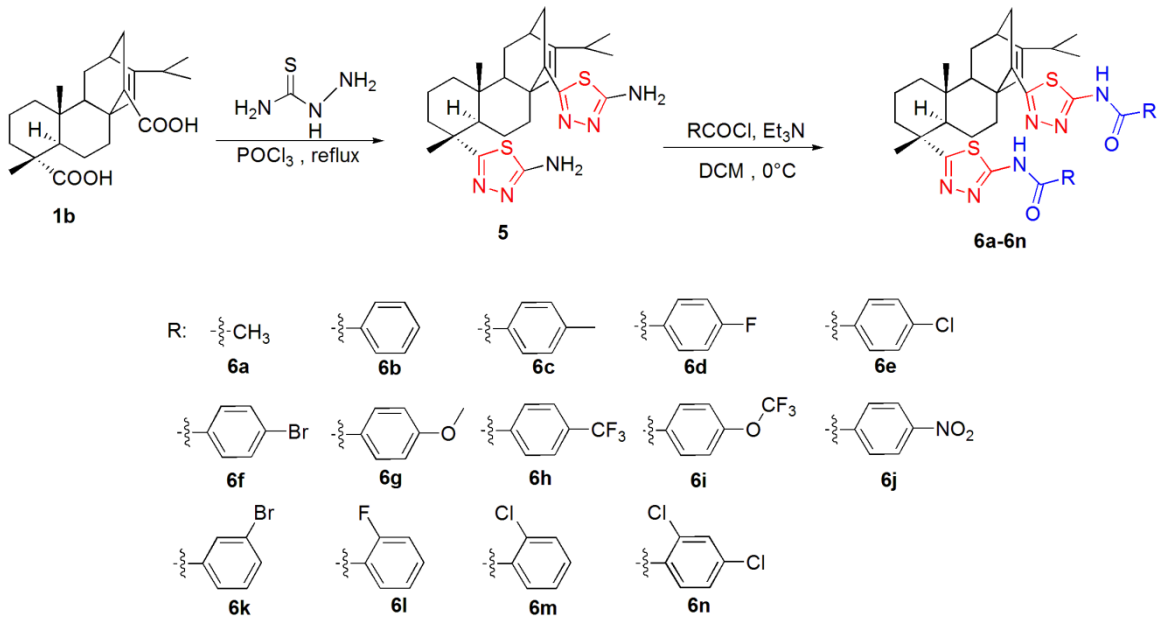
Schemes



Scheme 1. Preparation of compounds 3, 4a-4n



Scheme 2. Preparation of compounds 6a-6n

Figures

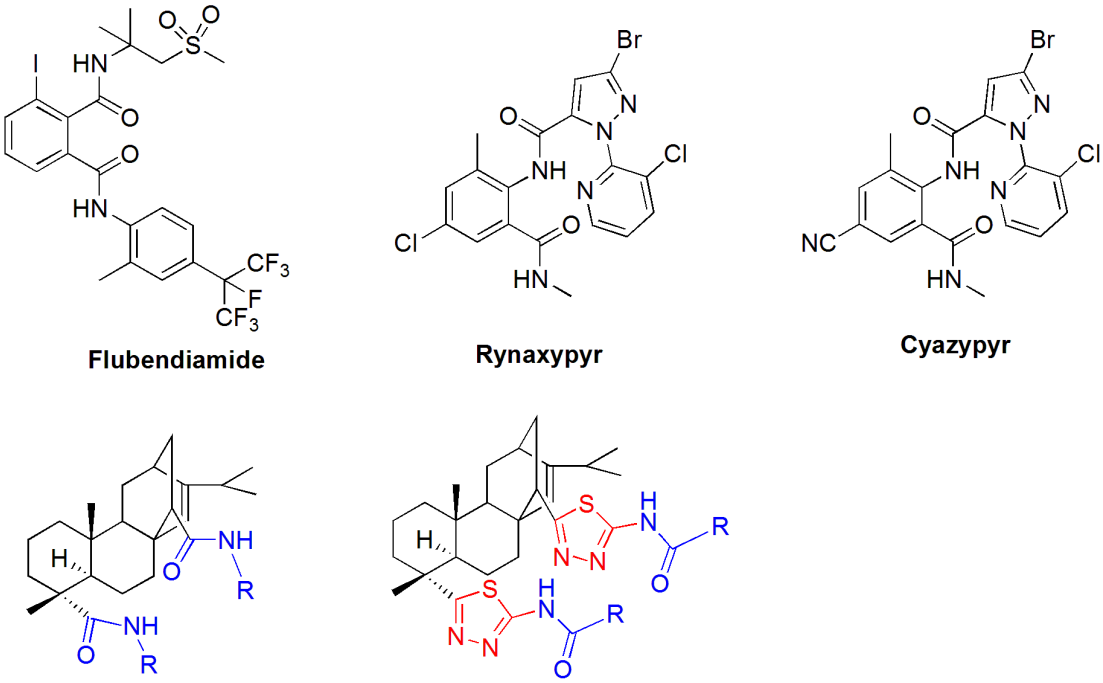


Fig. 1. Chemical structures of diamide insecticides

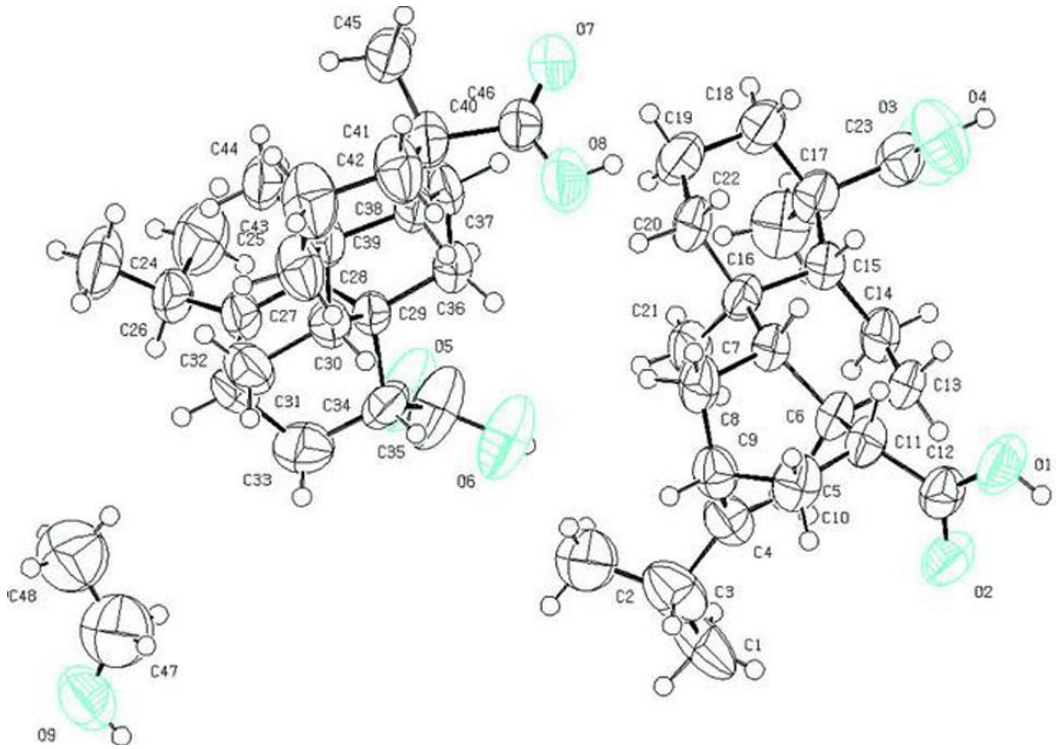


Fig. 2. Molecular structure of the compound 1b

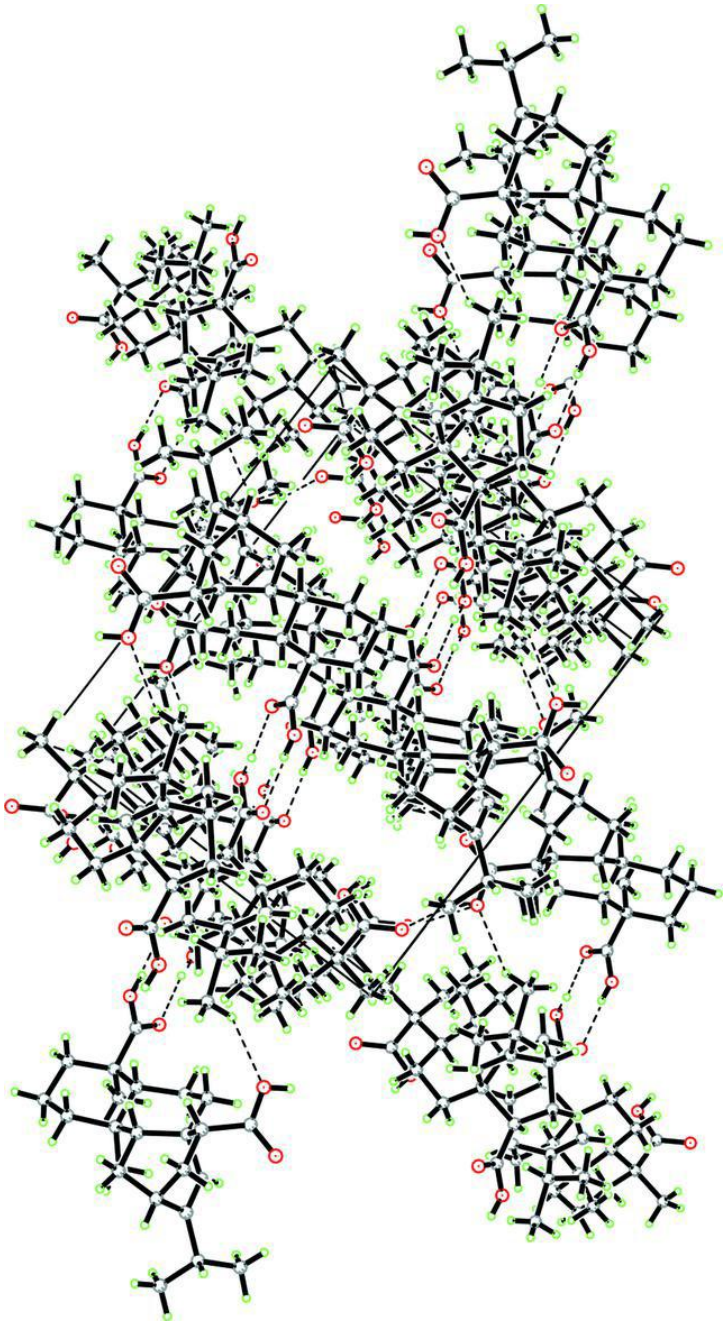


Fig. 3. Packing diagram of the compound 1b

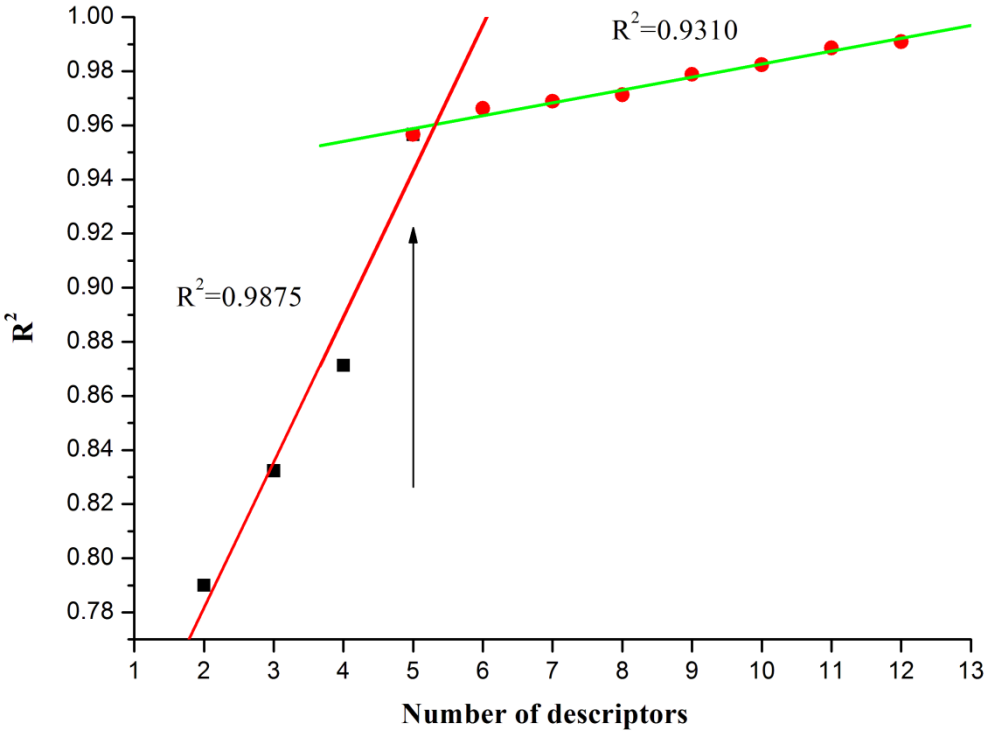


Fig. 4. The "breaking point" rule results

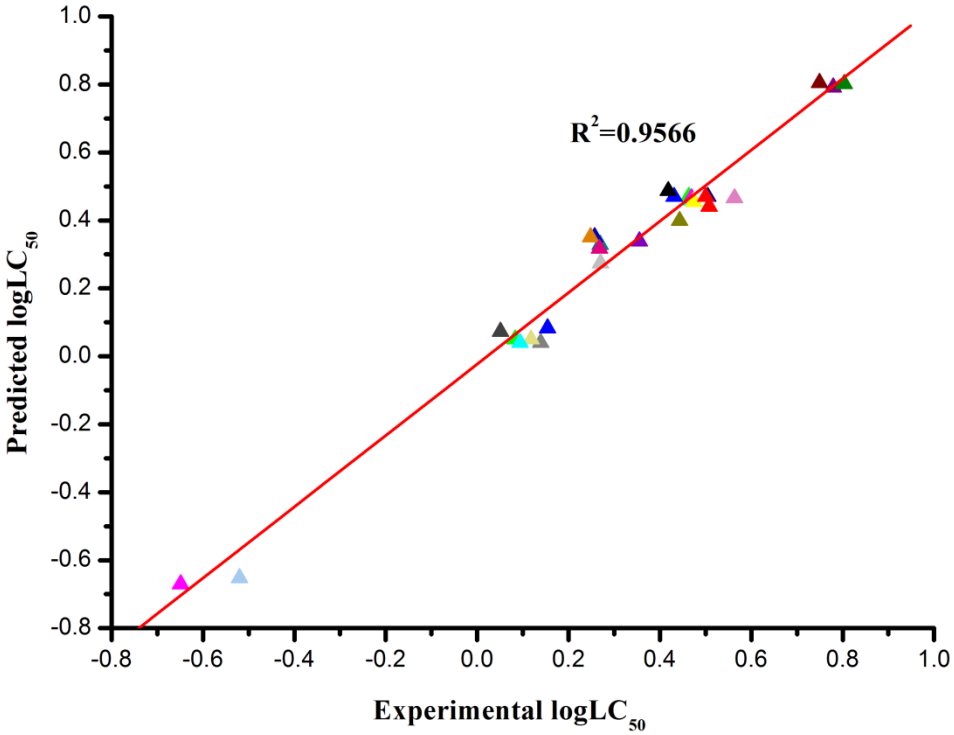
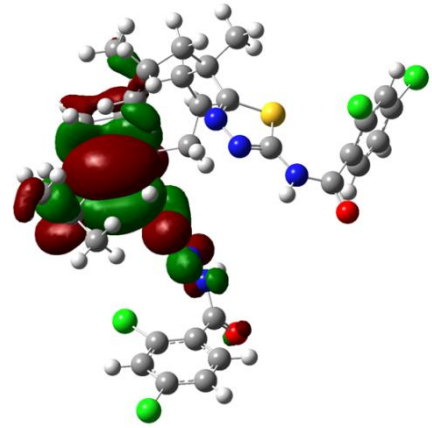
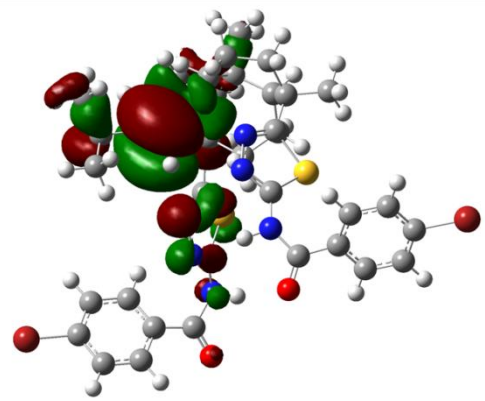


Fig. 5. Experimental log LC_{50} *versus* predicted log LC_{50}



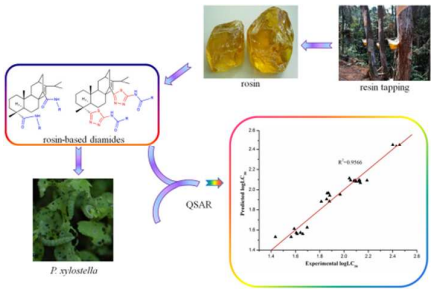
HOMO (6f)



HOMO (6n)

Fig. 6. HOMO energy maps for compounds 6f and 6n from DFT calculation of Gaussian 03W. The green parts represent positive molecular orbital, and the red parts represent negative molecular orbital.

Table of contents entry:



The quantitative structure-activity relationship (QSAR) of two series of rosin-based diamides with insecticidal activity against *P. xylostella* was studied.