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Preparation of Matrinic/Oxymatrinic Amide Derivatives as Insecticidal/Acaricidal Agents, and Study on the Mechanisms of Action against *Tetranychus cinnabarinus*

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Abstract

In continuation of our program to develop natural-product-based pesticidal candidates, matrinic/oxymatrinic amides were obtained through structural optimization of matrine. *N'*-(4-Fluoro)phenyl-*N*-(4-bromo)phenylsulfonyloxymatrinic amide (**IIm**) showed the potent insecticidal activity against *Mythimna separata*. *N*-(Un)submitted phenylsulfonylmatrinic acids (**3a**–**c**) exhibited the promising acaricidal activity against *Tetranychus cinnabarinus*. By qRT-PCR analysis of nAChR subunits and AChE genes, and determination of AChE activity of (un)treated *T. cinnabarinus*, it suggested that the open lactam ring of matrine, and carboxyl group and (4-methyl)phenylsulfonyl of *N*-(4-methyl)phenylsulfonylmatrinic acid (**3b**) were necessary for action with α2, α4, α5 and β3 *nAChR* subunits; compound **3b** was an inhibitor of AChE in *T. cinnabarinus*, and AChE was one possible target of action in *T. cinnabarinus* against **3b**; and compound **3b** may be an antagonist of *nAChR* and AChE in *T. cinnabarinus*.

KEYWORDS: Matrine, Structural modification, Amide derivatives, Pesticidal activity, Antagonist

INTRODUCTION

To reduce pests threats to crop production, although lots of chemical pesticides were extensively used, insect pests resistance, and negative impacts on human health and environmental safety usually emerged.[1-7] Recently, much attention has been paid to develop new potential alternatives for efficiently controlling pests direct or indirect from plant secondary metabolites.[8-18] Matrine (**1**, Figure 1), and oxymatrine (**1'**, Figure 1) are two quinolizidine alkaloids isolated from the roots of *Sophora flavescens* (Kushen).[19,20] Matrine, oxymatrine and their derivatives not only displayed a variety of biological properties (e.g., anti-inflammatory,[21-23] anticancer,[24-26] and antiviral activities[27-29]), but also possessed potential agrochemical activities.[30-34]

Although matrine has been registered as a botanical pesticide in China, its pesticidal activities were much lower in magnitude than those of commercially chemical pesticides. So many works need to be done to improve its pesticidal activities. Additionally, optimization of natural-based products for the development of pesticides has received much attention.[35-41] To explore potent pesticidal candidates, herein a series of matrinic/oxymatrinic amide derivatives (**I** and **II**, Figure 1) were prepared through opening the lactam ring of matine. Their pesticidal activities were tested against *Tetranychus cinnabarinus* Boisduval and *Mythimna separata* Walker. Meanwhile, their mechanisms of action were evaluated against *T. cinnabarinus*.

MATERIALS AND METHODS

Synthesis of Compounds 2a–c. Matrine (**1**, 12 mmol) in 6 M aq. hydrochloric acid (45 mL) was refluxed for 4 h. Then MeOH (50 mL) was added to the mixture, and it was stirred at room temperature for 3 h. After the solvent was evaporated, the residue was dissolved in

CH_2Cl_2 (30 mL), and KOH (36 mmol) and benzenesulfonyl chlorides (18 mmol) were added to the solution. Subsequently, the mixture was stirred at room temperature for 12–18 h, and it was washed with saturated aq. Na_2CO_3 (20 mL × 2), and dried over anhydrous Na_2SO_4. Finally, it was concentrated *in vacuo*, and purified by silica gel column chromatography eluting with petroleum ether/ethyl acetate (v/v =1/1 or 1/2) to give **2a–c** (49–80% yields).

*Data for Compound **2a**:* Yield: 49%, white solid, mp 80–82 °C; $[\alpha]^{25}_D$ = -2 (*c* 0.32 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.86 (d, *J* = 7.0 Hz, 2H), 7.53–7.48 (m, 3H), 3.64 (s, 3H), 3.60–3.54 (m, 2H), 3.31 (t, *J* = 11.0 Hz, 1H), 2.65 (d, *J* = 11.0 Hz, 1H), 2.60 (d, *J* = 10.0 Hz, 1H), 2.24–2.19 (m, 2H), 2.04–2.00 (m, 2H), 1.87–1.82 (m, 6H), 1.68-1.65 (m, 2H), 1.56–1.54 (m, 2H), 1.47–1.43 (m, 2H), 1.36–1.34 (m, 3H); HRMS (ESI): calcd for $C_{22}H_{33}N_2O_4S$ ($[M + H]^+$) 421.2155; found, 421.2179.

*Data for Compound **2b**:* Yield: 75%, white solid, mp 76–78 °C; $[\alpha]^{25}_D$ = -1 (*c* 0.30 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.73 (d, *J* = 7.0 Hz, 2H), 7.27 (d, *J* = 6.0 Hz, 2H), 3.64 (s, 3H), 3.55–3.53 (m, 2H), 3.27 (t, *J* = 12.0 Hz, 1H), 2.67 (d, *J* = 10.5 Hz, 1H), 2.62 (d, *J* = 11.0 Hz, 1H), 2.41 (s, 3H), 2.28–2.25 (m, 1H), 2.20–2.16 (m, 1H), 2.05–2.00 (m, 2H), 1.88–1.83 (m, 6H), 1.71–1.70 (m, 1H), 1.58–1.52 (m, 3H), 1.48–1.43 (m, 2H), 1.36–1.34 (m, 3H); HRMS (ESI): calcd for $C_{23}H_{35}N_2O_4S$ ($[M + H]^+$) 435.2312; found, 435.2304.

*Data for Compound **2c**:* Yield: 80%, white solid, mp 80–82 °C; $[\alpha]^{25}_D$ = -2 (*c* 0.29 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.72 (d, *J* = 8.5 Hz, 2H), 7.66 (d, *J* = 9.0 Hz, 2H), 4.43–4.40 (m, 1H), 4.16 (t, *J* = 13.5 Hz, 1H), 3.84 (dd, *J* = 4.0 Hz, 14.0 Hz, 1H), 3.60 (s, 3H), 3.53 (t, *J* = 11.5 Hz, 2H), 3.27 (d, *J* = 10.5 Hz, 1H), 2.67–2.60 (m, 2H), 2.56–2.53 (m, 2H), 2.35–2.33 (m, 1H), 2.20–2.14 (m, 3H), 1.87–1.83 (m, 2H), 1.73–1.69 (m, 3H), 1.65–1.63 (m, 1H), 1.58–1.51 (m, 2H), 1.40–1.35 (m, 2H); HRMS (ESI): calcd for $C_{22}H_{32}BrN_2O_4S$ ([M +

H]$^+$) 499.12600, 501.1242; found, 499.1232, 501.1209.

Synthesis of Compounds 3a–c. Compounds **2a–c** (5 mmol) in saturated solution of NaOH in MeOH (20 mL) was refluxed for 2 h. Then at room temperature, its pH value was adjusted to 7 by 30% aq. H_2SO_4. The residue was obtained by concentration *in vacuo*, and it was extracted with CH_2Cl_2 (20 mL × 3). Finally, the organic layer was combined and concentrated *in vacuo* to produce **3a–c** (80–86% yields).

*Data for Compound **3a**:* Yield: 80%, white solid, mp 78–80 °C; $[\alpha]^{25}_D$ = -3 (*c* 0.24 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.83 (d, *J* = 7.5 Hz, 2H), 7.54–7.47 (m, 3H), 3.83 (t, *J* = 8.5 Hz, 1H), 3.72–3.69 (m, 1H), 3.49–3.44 (m, 1H), 3.23 (s, 2H), 2.66 (s, 1H), 2.31 (s, 2H), 2.15–2.05 (m, 4H), 1.98–1.91 (m, 2H), 1.70–1.67 (m, 3H), 1.63–1.50 (m, 3H), 1.43–1.34 (m, 4H); HRMS (ESI): calcd for $C_{21}H_{31}N_2O_4S$ ([M + H]$^+$) 407.1999; found, 407.2014.

*Data for Compound **3b**:* Yield: 85%, white solid, mp 76–78 °C; $[\alpha]^{25}_D$ = -2 (c 0.20 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.72 (d, *J* = 8.0 Hz, 2H), 7.28 (d, *J* = 7.5 Hz, 2H), 3.79 (t, *J* = 9.5 Hz, 1H), 3.69–3.67 (m, 1H), 3.43 (t, *J* = 12.5 Hz, 1H), 3.16–3.08 (m, 2H), 2.52–2.49 (m, 1H), 2.40 (s, 3H), 2.24 (s, 2H), 2.11–2.00 (m, 4H), 1.94–1.86 (m, 2H), 1.70–1.64 (m, 3H), 1.59–1.56 (m, 1H), 1.47–1.34 (m, 6H); HRMS (ESI): calcd for $C_{22}H_{33}N_2O_4S$ [M + H]$^+$) 421.2155; found, 421.2161.

*Data for Compound **3c**:* Yield: 86%, white solid, mp 85–87 °C; $[\alpha]^{25}_D$ = -1 (*c* 0.29 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.71 (d, *J* = 8.0 Hz, 2H), 7.61 (d, *J* = 8.0 Hz, 2H), 3.74 (t, *J* = 9.0 Hz, 1H), 3.61–3.59 (m, 1H), 3.37 (t, *J* = 12.5 Hz, 1H), 2.99 (s, 2H), 2.39 (s, 1H), 2.14–2.10 (m, 4H), 1.99–1.96 (m, 2H), 1.88–1.82 (m, 2H), 1.72–1.69 (m, 1H), 1.60–1.52 (m, 3H), 1.44–1.36 (m, 6H); HRMS (ESI): calcd for $C_{21}H_{30}BrN_2O_4S$ ([M + H]$^+$) 485.1104, 487.1085; found, 485.1103, 487.1082.

General Procedure for Preparation of Compounds Ia–o. A mixture of compounds **3a–c** (0.3 mmol), different aromatic amines (**4a–e**, 0.4 mmol), EDCI (0.3 mmol) and HOBt (0.3 mmol) in CH_2Cl_2 (10 mL) at 0 °C was stirred for 24–30 h. Then the mixture was diluted with CH_2Cl_2 (30 mL). It was washed by 5% aq. hydrochloric acid (20 mL × 2), saturated aq. Na_2CO_3 (20 mL) and brine (20 mL), and dried over anhydrous Na_2SO_4. Finally, it was concentrated *in vacuo*, and purified by preparative thin-layer chromatography eluting with petroleum ether/ethyl acetate (v/v = 1/3 or 1/4) to afford target compounds **Ia–o** (65–92% yields).

*Data for Compound **Ia**:* Yield: 87%, white solid, mp 68–70 °C; $[\alpha]^{25}_D$ = -6 (*c* 0.30 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.87 (d, J = 6.0 Hz, 2H), 7.65 (s, 1H), 7.59–7.54 (m, 3H), 7.49–7.48 (m, 2H), 7.31–7.29 (m, 2H), 7.09–7.06 (m, 1H), 3.64–3.60 (m, 1H), 3.53–3.50 (m, 1H), 3.24 (t, J = 11.5 Hz, 1H), 2.61 (d, J = 9.5 Hz, 1H), 2.52 (d, J = 10.5 Hz, 1H), 2.45–2.43 (m, 1H), 2.33–2.28 (m, 1H), 1.99 (s, 1H), 1.95–1.94 (m, 1H), 1.90–1.88 (m, 3H), 1.80–1.76 (m, 5H), 1.52–1.42 (m, 3H), 1.37–1.28 (m, 4H); HRMS (ESI): calcd for $C_{27}H_{36}N_3O_3S$ ($[M + H]^+$) 482.2471; found, 482.2461.

*Data for Compound **Ib**:* Yield: 89%, white solid, mp 64–66 °C; $[\alpha]^{25}_D$ = -7 (*c* 0.30 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.87 (d, J = 6.5 Hz, 2H), 7.54–7.53 (m, 1H), 7.49–7.46 (m, 3H), 7.43 (d, J = 6.5 Hz, 2H), 7.11 (d, J = 6.5 Hz, 2H), 3.63–3.59 (m, 1H), 3.54–3.50 (m, 1H), 3.24 (t, J = 12.0 Hz, 1H), 2.61 (d, J = 9.5 Hz, 1H), 2.53 (d, J = 9.5 Hz, 1H), 2.42–2.38 (m, 1H), 2.30 (s, 4H), 2.00 (s, 1H), 1.88–1.78 (m, 8H), 1.61 (s, 1H), 1.52–1.50 (s, 1H), 1.44–1.31 (m, 6H); HRMS (ESI): calcd for $C_{28}H_{38}N_3O_3S$ ($[M + H]^+$) 496.2628; found, 496.2632.

*Data for Compound **Ic**:* Yield: 65%, white solid, mp 69–71 °C; $[\alpha]^{25}_D$ = -4 (*c* 0.28 mg/mL,

$CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ: 7.87 (d, J = 7.5 Hz, 2H), 7.77 (s, 1H), 7.57–7.47 (m, 5H), 7.00–6.97 (m, 2H), 3.64–3.63 (m, 1H), 3.52 (dd, J = 6.0, 12.5 Hz, 1H), 3.22 (t, J = 11.5 Hz, 1H), 2.60 (d, J = 11.0 Hz, 1H), 2.50–2.43(m, 2H), 2.35–2.32 (m, 1H), 1.99 (s, 1H), 1.96–1.86 (m, 4H), 1.81–1.77 (m, 5H), 1.72 (s, 1H), 1.51–1.43 (m, 2H), 1.38–1.30 (m, 4H); HRMS (ESI): calcd for $C_{27}H_{35}FN_3O_3S$ ($[M + H]^+$) 500.2390; found, 500.2377.

General Procedure for Preparation of Compounds IIa–o. A mixture of compounds **Ia–o** (0.23 mmol), and *m*-chloroperoxybenzoic acid (*m*-CPBA, 0.36 mmol) in CH_2Cl_2 (10 mL) at 0 °C was stirred for 2 h. Then the mixture was diluted with CH_2Cl_2 (30 mL). It was washed by 20% aq. KOH (20 mL × 2) and dried over anhydrous Na_2SO_4. Finally, it was concentrated *in vacuo*, and purified by silica gel column chromatography eluting with CH_2Cl_2/MeOH (v/v = 10/1 to 7/1) to afford target compounds **IIa–o** (73–94% yields).

*Data for Compound **IIa**:* Yield: 88%, white solid, mp 215–217 °C; $[\alpha]^{25}_D$ = -1 (*c* 0.20 mg/mL, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ: 8.37 (s, 1H), 7.81 (d, J = 7.0 Hz, 2H), 7.60 (d, J = 7.0 Hz, 2H), 7.53–7.47 (m, 3H), 7.29–7.27 (m, 2H), 7.07–7.04 (m, 1H), 5.25 (s, 1H), 4.48–4.43 (m, 1H), 3.64 (dd, J = 3.0, 11.0 Hz, 1H), 3.17–3.12 (m, 5H), 2.62–2.60 (m, 1H), 2.39–2.38 (m, 1H), 2.32–2.25 (m, 4H), 2.05 (d, J = 14.0 Hz, 1H), 1.88–1.86 (m, 2H), 1.74–1.71 (m, 2H), 1.58–1.44 (m, 5H); HRMS (ESI): calcd for $C_{27}H_{36}N_3O_4S$ ($[M + H]^+$) 498.2421; found, 498.2425.

*Data for Compound **IIb**:* Yield: 75%, white solid, mp 236–238 °C; $[\alpha]^{25}_D$ = -1 (*c* 0.22 mg/mL, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ: 8.16 (s, 1H), 7.81 (d, J = 7.5 Hz, 2H), 7.54–7.51 (m, 1H), 7.49–7.45 (m, 4H), 7.09 (d, J = 8.0 Hz, 2H), 5.40 (s, 1H), 4.60–4.55 (m, 1H), 3.66 (dd, J = 5.0, 12.0 Hz, 1H), 3.12–3.07 (m, 5H), 2.69–2.66 (m, 1H), 2.45–2.42 (m, 1H), 2.29–2.25 (m, 7H), 2.06 (d, J = 15.0 Hz, 1H), 1.91–1.87 (m, 2H), 1.73–1.68 (m, 2H), 1.61–1.41 (m, 5H);

HRMS (ESI): calcd for $C_{28}H_{38}N_3O_4S$ ([M + H]$^+$) 512.2577; found, 512.2577.

*Data for Compound **IIc**:* Yield: 73%, white solid, mp 235–237 ^oC; $[\alpha]^{25}_D$ = -1 (*c* 0.20 mg/mL, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ: 8.61 (s, 1H), 7.80 (d, *J* = 7.5 Hz, 2H), 7.60 –7.58 (m, 2H), 7.55–7.52 (m, 1H), 7.49–7.46 (m, 2H), 6.98 (t, *J* = 8.5 Hz, 2H), 5.42 (s, 1H), 4.54–4.49 (m, 1H), 3.65 (dd, *J* = 4.5, 12.0 Hz, 1H), 3.15–3.10 (m, 5H), 2.64–2.62 (m, 2H), 2.35–2.31 (m, 3H), 2.27–2.21 (m, 1H), 2.06 (d, *J* = 15.0 Hz, 1H), 1.94–1.91 (m, 1H), 1.84–1.82 (m, 1H), 1.73–1.70 (m, 2H), 1.62–1.43 (m, 5H); HRMS (ESI): calcd for $C_{27}H_{35}FN_3O_4S$ ([M + H]$^+$) 516.2326; found, 516.2325.

Biological Assay.

Growth Inhibitory Activity of Compounds **1**–**3**, **Ia**–**o** and **IIa**–**o** *against Mythimna separata*. The growth inhibitory activity of compounds **1**–**3**, **Ia**–**o** and **IIa**–**o** against early 3rd-instar larvae of *M. separata* was assessed by leaf-dipping method.[40]

Acaricidal Activity of Compounds **1**–**3**, **Ia**–**o** and **IIa**–**o** *against Tetranychus cinnabarinus.* The acaricidal activity of compounds **1**–**3**, **Ia**–**o** and **IIa**–**o** against the female adults of *T. cinnabarinus* was evaluated by slide-dipping method.[41,42]

Mechanisms of Action against *T. cinnabarinus*. The female adults of *T. cinnabarinus* were treated with **1** and **3b** (at 0.25 mg/mL in 0.1 g/L aq. Tween-80), and imidacloprid and isoprocarb (at 0.055 and 0.50 mg/mL in 0.1 g/L aq. Tween-80), respectively. The procedures for quantitative real-time PCR (qRT-PCR) analysis of nicotinic acetylcholine receptor (nAChR) subunits and acetylcholinesterase (AChE) genes of *T. cinnabarinus* were the same as previous reports.[43,44]

Acetylcholinesterase (AChE) Activity Assays. The total protein concentration was assayed by Bradford's method (bovine serum albumin (BSA) as a standard).[45] Acetylcholinestrase

(AChE) activity was tested according to the Ellman's procedures.[46] AChE inhibition activity was determined using the AChE assay kit. Each replication had 120 female adults of *T. cinnabarinus*. The change in absorbance at 412 nm was recorded in a multi-detection microplate fluorescence reader after 2 min.

RESULTS AND DISCUSSION

Preparation of Matrine Derivatives. First, *N*-phenylsulfonylmatrinic methyl esters (**2a**–**c**) were produced by reaction of **1** with 6 M aq. hydrochloric acid, followed by methanol, and phenylsulfonyl chlorides (Figure 2).[47] Then hydrolysis of **2a**–**c** afforded *N*-phenylsulfonylmatrinic acids (**3a**–**c**).[48] Finally, compounds **3a**–**c** reacting with different aromatic amines (**4a**–**e**) gave matrinic amides (**Ia**–**o**),[49] which were further oxidized by *m*-chloroperoxybenzoic acid (*m*-CPBA) to give oxymatrinic amides (**IIa**–**o**).[50] Chemical structures of target compounds **Ia**–**o** and **IIa**–**o** were described Figure 3, and were characterized by optical rotation, HRMS, melting points, and ^{1}H NMR. X-ray crystallographies of **Ig**–**j**,**o**, and **IIc,m** were described in Figures 4 and 5. The Cambridge Crystallographic Data Centre (CCDC) numbers of **Ig**–**j**,**o**, and **IIc,m** were 1938773, 1938774, 1938775, 1938772, 1938777, 1938776, and 1938778, respectively.

Pesticidal Activities. As shown in Table 1, compounds **Im**, **IIc**, **IIh**, **IIm** and **IIn** exhibited higher growth inhibitory activity than toosendanin. The final mortality rates (FMRs) of **Im**, **IIc**, **IIh**, **IIm** and **IIn** were 55.2%, 51.7%, 58.6%, 65.5%, and 55.2%, respectively; but the FMR of **1** was only 24.1%. Notably compound **IIm** displayed the most pronounced growth inhibitory activity. For FMRs of compounds **1**–**3**, **Ia**–**o** and **IIa**–**o** against *M. separata* at 1 mg/mL after 35 days were greater than those after 10 or 20 days (Table 1), these derivatives may show delayed insecticidal activity. Meanwhile, the symptoms of *M. separata* treated by

derivatives were the same as previous reports:[40,41] at the larval stage, the dead larvae were showed with thin and wrinkled bodies in Figure S1; at the pupation stage, some dead and malformed pupae appeared in Figure S2; at the adult emergence stage, some malformed moths were found in Figure S3. On the other hand, the percentages of FMRs at the larval, pupation and adult emergence stages of toosendanin, **Im**, **IIc**, **IIh**, **IIm**, and **IIn** were showed in Figure 6. Greater than 50% of FMRs of toosendanin, **Im**, **IIc**, **IIh**, **IIm**, and **IIn** were at the larval stage. These results were the same with those of oximino esters of fraxinellone[40] and some matrine ethers,[51] however, they were different with those of acids, alcohols, and esters of matrine.[41]

In general, the insecticidal activity of oxymatrinic amide derivatives (**IIa**–**o**) was more potent than that of matrinic amide derivatives (**Ia**–**o**). Obviously, the oxygen atom at the N-1 position of **IIa**–**o** was very vital for the growth inhibitory activity. Compounds **3a**–**c** exhibited better growth inhibitory activity than **2a**–**c**, and it suggested that the carboxyl group of **3a**–**c** was important for the growth inhibitory activity. Among compounds **2a**–**c** and **3a**–**c**, compounds **2b** and **3b** displayed potent activity, so R^1 as 4-methyl of *N*-phenylsulfonylmatrinic methyl ester and *N*-phenylsulfonylmatrinic acid was vital for the growth inhibitory activity. To derivatives **Ia**–**o** and **IIa**–**o**, compounds **Im** and **IIm** (R^1 = 4-Br; R^2 = 4-F) displayed excellent growth inhibitory activity. To derivatives **IIa**–**o**, compounds **IIc**, **IIh** and **IIm** (R^2 = 4-F) all showed good activity, and it demonstrated that R^2 as 4-fluorine atom was necessary for the growth inhibitory activity.

The results of acaricidal activity of **1**–**3**, **Ia**–**o** and **IIa**–**o** were shown in Table 2. Unlike the insecticidal activity, compounds **Ia**–**o** and **IIa**–**o** all showed low acaricidal activity, moreover, they exhibited less potent acaricidal activity than matrinic acid/alcohol/ester

derivatives.[41] Interestingly, compounds **3a**–**c** (72 h mortality rates (MRs): 36.2% (**3a**), 43.5% (**3b**) and 40.3% (**3c**)) displayed better acaricidal activity than **1** (72 h MR: 13.6%). Furthermore, LC_{50} values of **3a**–**c** were 0.65, 0.55 and 0.63 mg/mL, respectively; but the LC_{50} value of **1** was 4.01 mg/mL (Table 3). Obviously, compounds **3a**–**c** displayed > 6 folds more potent acaricidal activity than **1**. However, compounds **2a**–**c** showed the low acaricidal activity. So the carboxyl group of **3a**–**c** was also necessary for the acaricidal activity.

Mechanisms of Action against *T. cinnabarinus*. The primers of *nAChR* subunits and *AChE* of *T. cinnabarinus* for qRT-PCR were shown in Table 4. Alignment of amino acid sequences in transmembrane domain of *nAChR* subunits was described in Figure 7a . Expression changes of *nAChR* subunits against **1**, **3b** and imidacloprid (IMI) were tested by qRT-PCR (Figure 7b–d).

As shown in Figure 7b, α1, α2, α4, α5 and β3 *nAChR* subunits against IMI were up-regulated to 4.61, 3.84, 5.41, 31.63 and 10.25 folds, respectively. Because IMI was an agonist for *nAChR* α or β subunits,[52] IMI may interact with α1, α2, α4, α5 and β3 subunits of *T. cinnabarinus*. Matrine (**1**) regulated α1, α5, α7 and β3 subunits to 0.31, 0.41, 7.47 and 3.17 folds, respectively (Figure 7c); whereas **3b** up-regulated α5 subunit to 4.87 folds, and down-regulated α2, α4 and β3 subunits to 0.25, 0.24, and 0.33 folds, respectively (Figure 7d). It suggested that the open lactam ring of **1**, and carboxyl group and *N*-(4-methyl)phenylsulfonyl of compound **3b** were necessary for acting with α2, α4, α5 and β3 *nAChR* subunits.

Meanwhile, compound **1** can target insect acetylcholine (ACh) receptors and then affects AChE production,[53] so AChE maybe also one target enzyme. In *Bemisia tabaci*, AChE activity was decreased after treatment with compound **1**.[45] After successive oral

administration of compound **1** to mice for three days at doses of 0.4, 2, and 10 mg/kg, compound **1** significantly improved SCOP-induced learning and memory deficits via inhibition of AChE/BuChE and oxidative stress mechanisms.[54] In *Carassius auratus*, AChE activity was inhibited after treatment with chlorpyrifos and isoprocarb (a carbamate insecticide targeting AChE).[55]

In our experiment, we only found one AChE gene of *T. cinnabarinus*. AChE gene expressions against **1**, **3b** and isoprocarb were significantly down-regulated to 0.26, 0.11 and 0.09 folds, respectively (Figure 8a). On the other hand (Figure 8b), compounds **1**, **3b** and isoprocarb all inhibited the AChE activity, and relative inhibition ratios of **1**, **3b** and isoprocarb on CK were 0.44, 0.36, and 0.38, respectively. It demonstrated that **3b** was an inhibitor of AChE in *T. cinnabarinus*, and AChE was one possible target of action to **1** and **3b**.

In conclusion, matrinic/oxymatrinic amides were semisynthesized by using matrine as a lead compound. Seven target molecules were further confirmed by crystal structures. Oxymatrinic amide **IIm** (R^1 = 4-Br; R^2 = 4-F) displayed the most pronounced growth inhibitory activity. Especially compounds **3a**–**c** displayed > 6 folds more promising acaricidal activity than matrine. Generally, the growth inhibitory activity of oxymatrinic amides was more potent than that of matrinic amides. Notably the oxygen atom at the N-1 position of **IIa**–**o** was very important for the insecticidal activity, and the carboxyl group of **3a**–**c** was necessary for the acaricidal activity. By qRT-PCR analysis of nAChR subunits and AChE genes, and determination of AChE activity of (un)treated *T. cinnabarinus*, it suggested that the open lactam ring of matrine, and carboxyl group and *N*-(4-methyl)phenylsulfonyl of **3b** were vital for acting with α2, α4, α5 and β3 *nAChR* subunits; compound **3b** was an inhibitor

of AChE in *T. cinnabarinus*, and AChE was one possible target of action in *T. cinnabarinus* against **3b**. So compound **3b** may be an antagonist of *nAChR* and AChE in *T. cinnabarinus*. These results will pave the basis for future optimization and application of matrine derivatives as agrochemicals.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: Chemicals and instruments; data on ^{1}H NMR, HRMS, optical rotation and melting points of target compounds; the pictures of the treated *Mythimna separata* at three growth stages; methods for the biological assay and mechanisms of action.

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Notes

The authors declare no competing financial interest.

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Figure Captions.

Figure 1. Design strategy for preparation of target compounds (**I**–**II**).

Figure 2. Synthetic route for matrine and oxymatrine derivatives (**Ia**–**o** and **IIa**–**o)**.

Figure 3. Chemical structures of matrine and oxymatrine derivatives (**Ia**–**o** and **IIa**–**o)**.

Figure 4. Four X-ray crystal structures of matrinic ester derivatives (**Ig**: top left; **Ih**: top right; **Ii**: bottom left; **Ij**: bottom right).

Figure 5. Three X-ray crystal structures of matrine and oxymatrine derivatives (**Io**: top left; **IIc**: top right; **IIm**: bottom).

Figure 6. The percentages of FMRs at three different growth stages of compounds **Im**, **IIc**, **IIh**, **IIm**, **IIn** and toosendanin against *M. separata*.

Figure 7. The expression patterns of *nAChR* subunits in female adults of *T. cinnabarinus* collected 72 h post-treatment with 0.25 mg/mL of each compound (imidacloprid: 0.055 mg/mL). (a): Alignment of the amino acid sequences in transmembrane (TM) domain of nAChR subunits in *T. cinnabarinus*. (b–d): The *nAChR* subunits were evaluated quantitative real-time PCR (qRT-PCR). The mRNA expressions of *nAChR* subunits were normalized to β-actin expression (mean ± SD, n = 3). Asterisks indicate significant differences (*$P < 0.05$; **$P < 0.01$) compared with CK. CK: blank control group. Dα1: *Drosophila melanogaster nAChR* subunit α1; Tcα1: *T. cinnabarinus nAChR* subunit α1; Tcα2: *T. cinnabarinus nAChR* subunit α2; Tcα4: *T. cinnabarinus nAChR* subunit α4; Tcα5: *T. cinnabarinus nAChR* subunit α5; Tcα7: *T. cinnabarinus nAChR* subunit α7; Tcβ3: *T. cinnabarinus nAChR* subunit β3; TM: Transmembrane domain.

Figure 8. The expression patterns of acetylcholinesterase (AChE) and AChE enzyme activity of female adults of *T. cinnabarinus* collected 72 h post-treatment with 0.25 mg/mL of each

compound (isoprocarb: 0.50 mg/mL). (a): The expression patterns of the AChE in *T. cinnabarinus* against matrine (**1**) and **3b** tested by qRT-PCR (mean ± SD, n = 3). Asterisks indicate significant differences (**$P< 0.01$) compared with CK. CK: blank control group. (b): AChE enzyme activity values of *T. cinnabarinus* against matrine (**1**) and **3b**, respectively. Relative ratio: AChE activity values of *T. cinnabarinus* treated by compounds/AChE activity value of *T. cinnabarinus* of CK. Multiple range test using Duncan's test ($p < 0.05$). The same letters denote treatments not significantly different from each other.

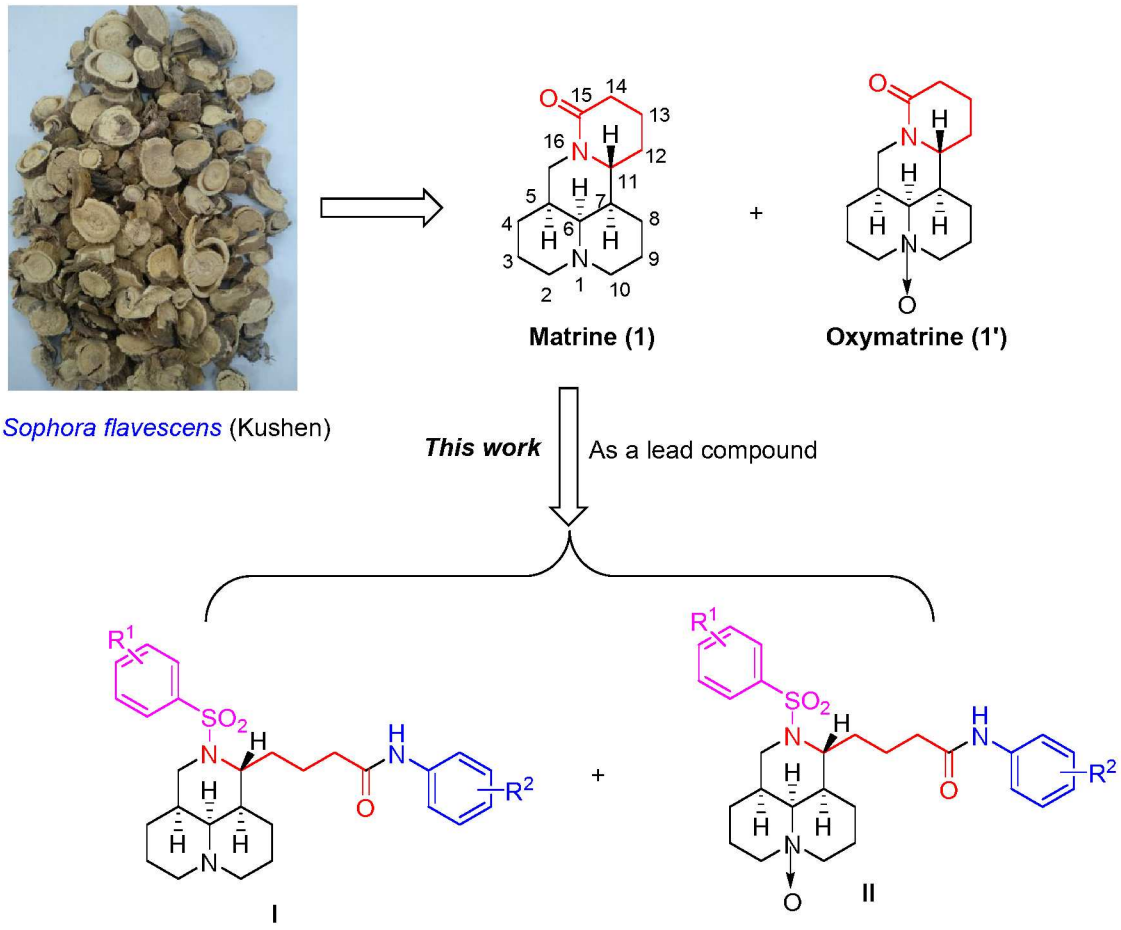


Figure 1. Design strategy for preparation of target compounds **(I–II)**.

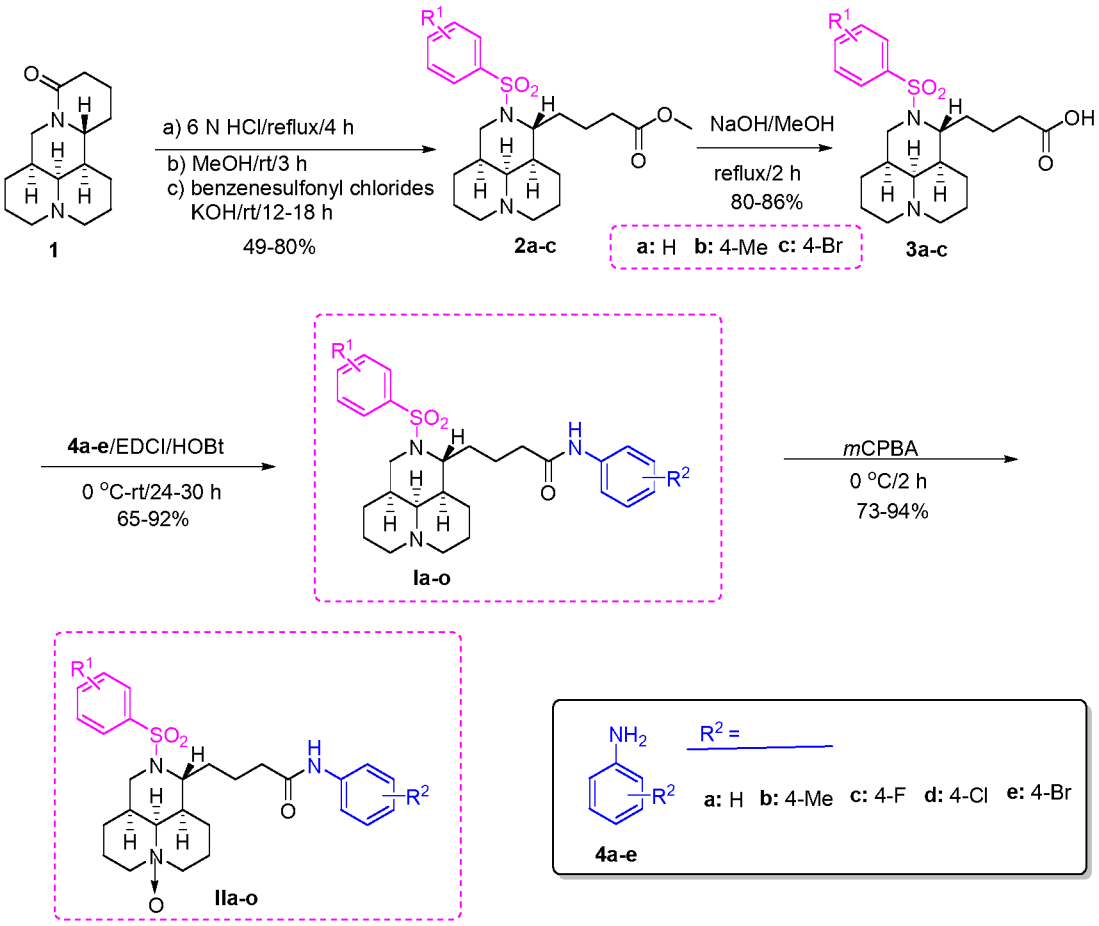


Figure 2. Synthetic route for matrine and oxymatrine derivatives (**Ia**–**o** and **IIa**–**o**).

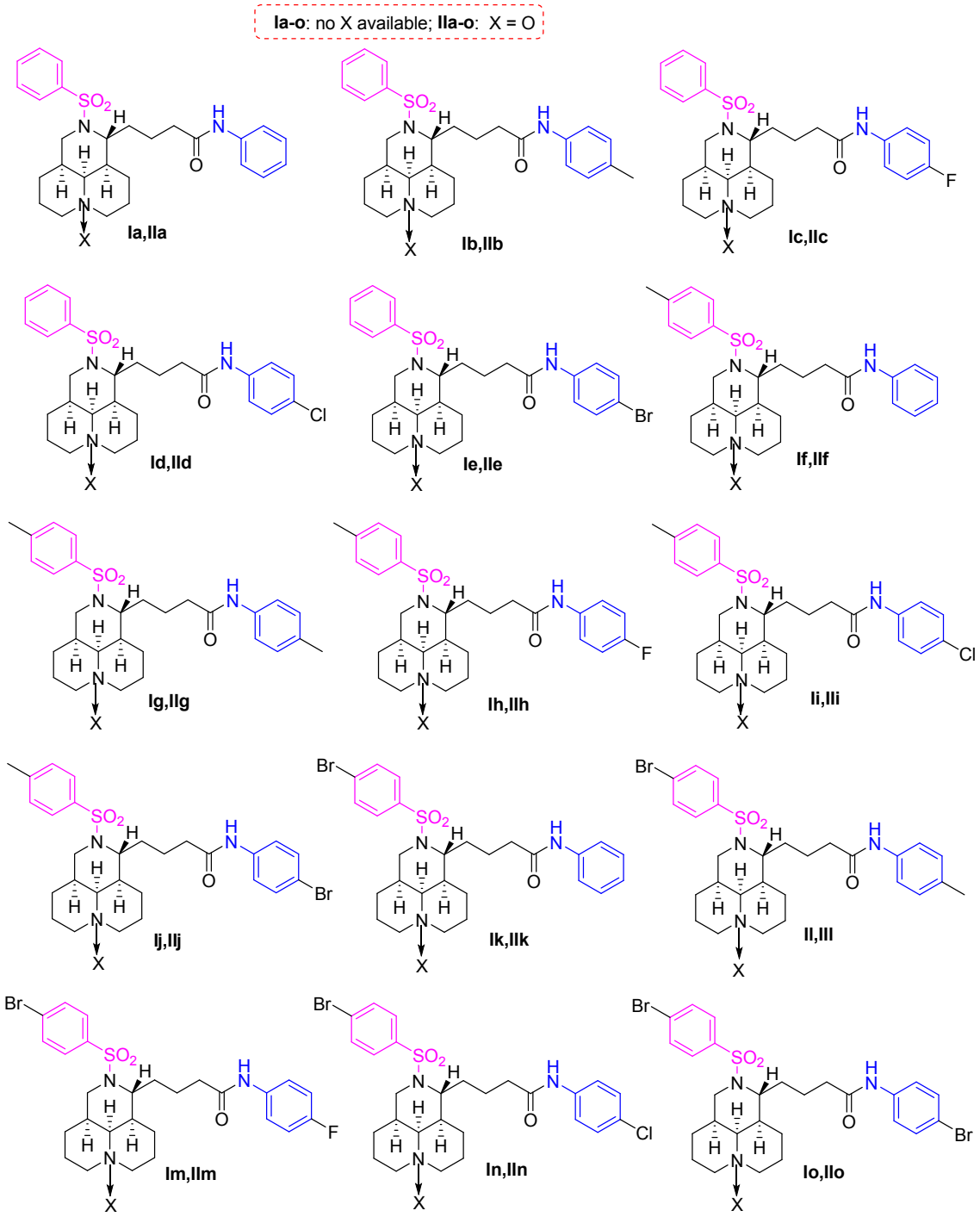
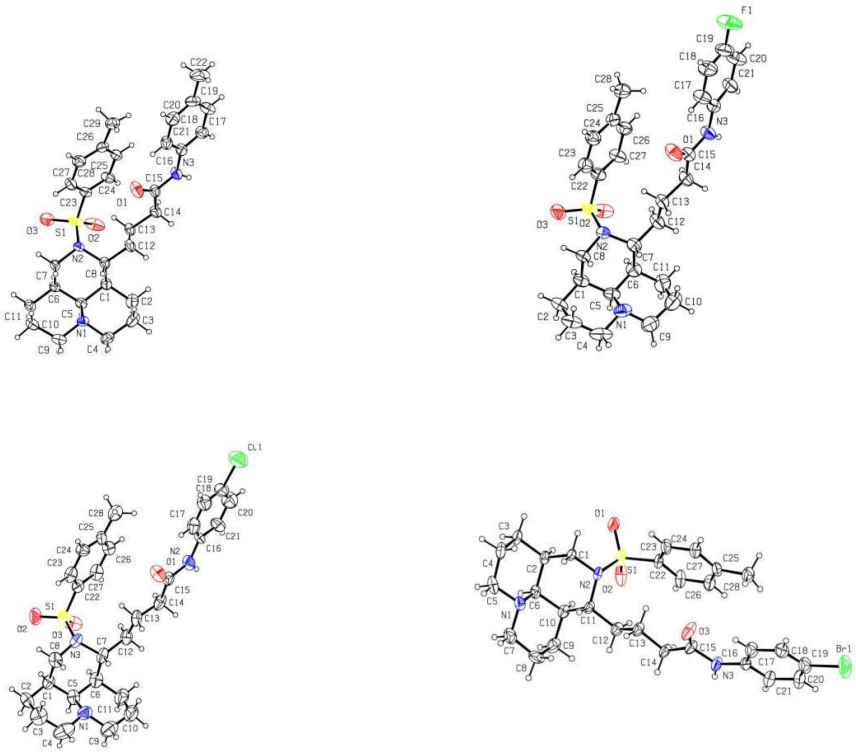


Figure 3. Chemical structures of matrine and oxymatrine derivatives (**Ia–o** and **IIa–o)**.



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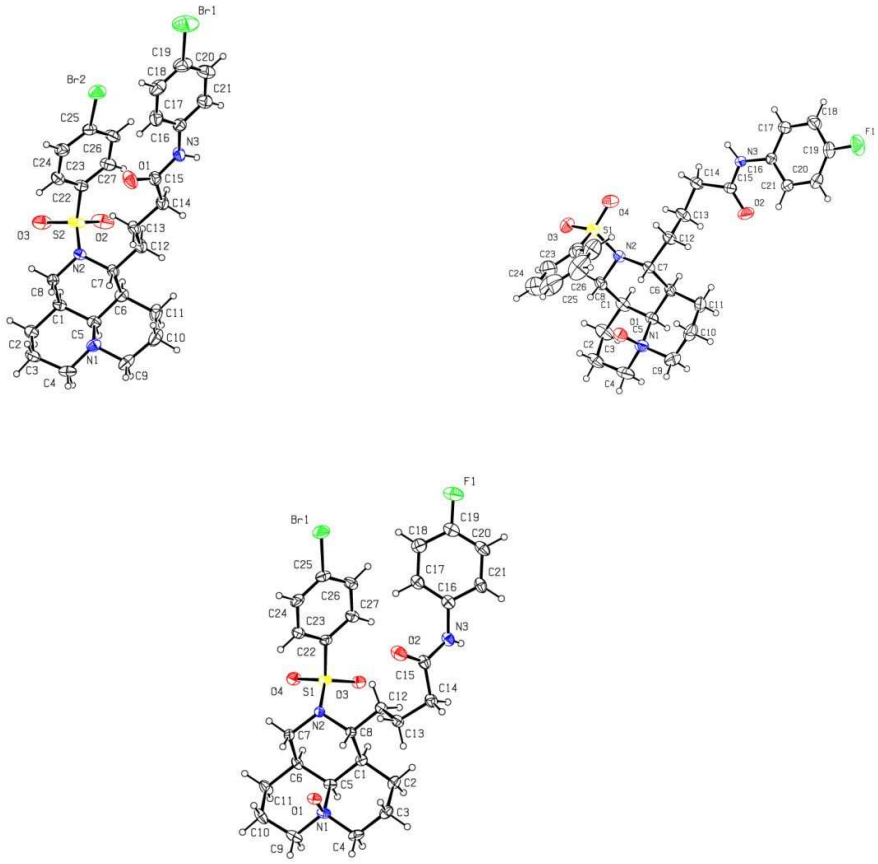


Figure 5. Three X-ray crystal structures of matrine and oxymatrine derivatives (**Io**: top left; **IIc**: top right; **IIm**: bottom).

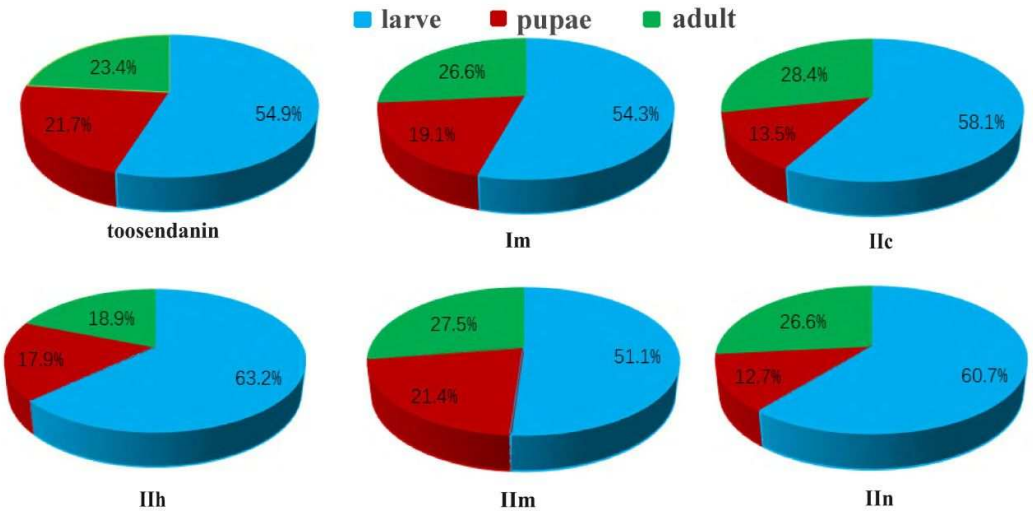


Figure 6. The percentages of FMRs at three different growth stages of compounds **Im**, **IIc**, **IIh**, **IIm**, **IIn** and toosendanin against *M. separata*.

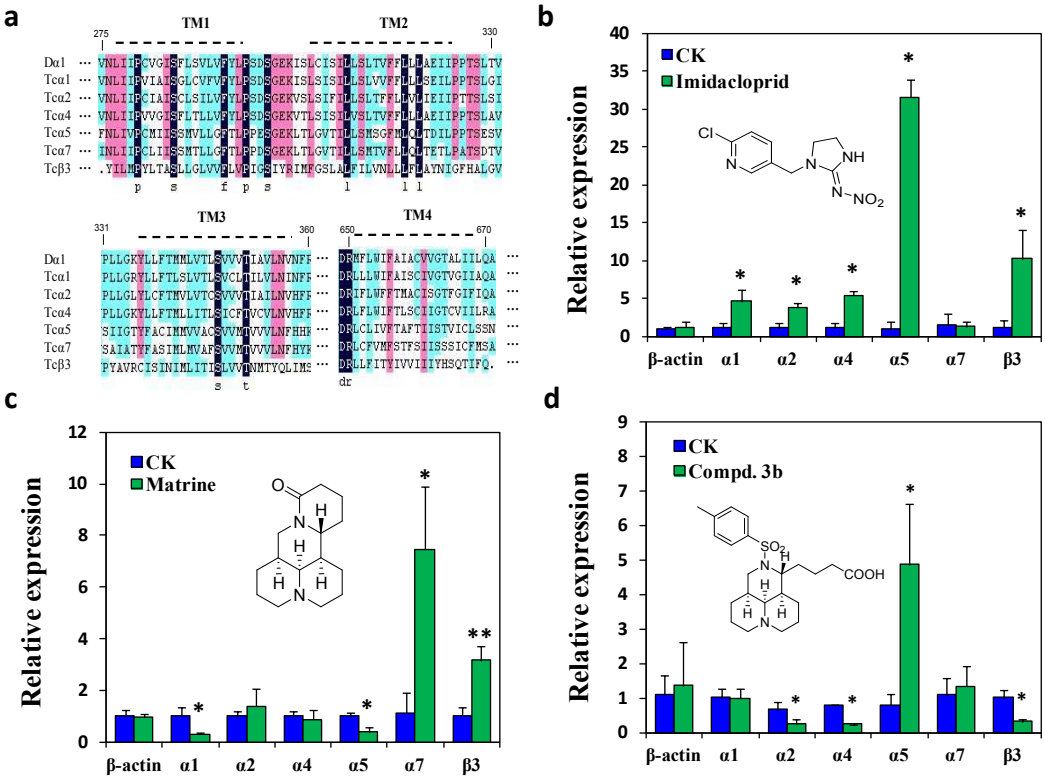
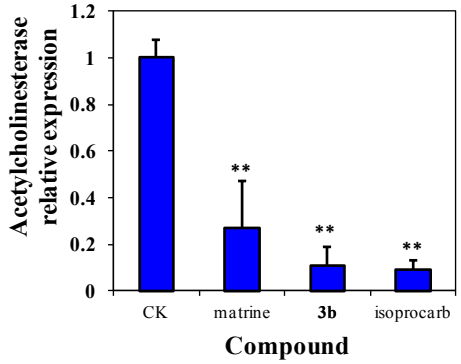

a
TM1
TM2
TM3
TM4
b
CK
Imidacloprid
Relative expression
β-actin α1 α2 α4 α5 α7 β3
c
CK
Matrine
Relative expression
d
CK
Compd. 3b
COOH
Relative expression

Figure 7.

a



b

AChE activity of *T. cinnabarinus* after 72h treatment with different compounds

compound	concentration (ppm)	AChE activity value (U/(mg.min))	relative ratio
CK	0	43.05 ± 7.18 a	1
matrine	250	18.78 ± 5.37 b	0.44
3b	250	15.35 ± 9.46 b	0.36
isoprocarb	500	16.45 ± 6.89 b	0.38

Figure 8.

Table 1. Growth Inhibitory Activity of Compounds **1**–**3**, **Ia**–**o** and **IIa**–**o** against *M. separata* on Leaves Treated with a Concentration of 1 mg/mL

compound	corrected mortality rate (mean ± SD, %)		
	10 days	20 days	35 days
1	3.3 ± 4.7	10.4 ± 4.7	24.1 ± 4.7
2a	3.3 ± 4.7	13.8 ± 4.7	27.6 ± 8.2
2b	3.3 ± 4.7	10.4 ± 4.7	31.0 ± 4.7
2c	6.7 ± 4.7	13.8 ± 4.7	27.6 ± 8.2
3a	6.7 ± 4.7	20.7 ± 4.7	41.4 ± 4.7
3b	6.7 ± 4.7	24.2 ± 4.7	48.3 ± 8.2
3c	13.3 ± 4.7	24.2 ± 4.7	44.8 ± 4.7
Ia	3.3 ± 4.7	10.4 ± 4.7	24.1 ± 4.7
Ib	3.3 ± 4.7	13.8 ± 4.7	37.9 ± 8.2
Ic	13.3 ± 4.7	20.7 ± 4.7	34.5 ± 4.7
Id	13.3 ± 4.7	17.3 ± 0	31.0 ± 4.7
Ie	10.0 ± 0	13.8 ± 4.7	34.5 ± 4.7
If	3.3 ± 4.7	10.4 ± 4.7	31.0 ± 4.7
Ig	6.7 ± 4.7	13.8 ± 4.7	34.5 ± 4.7
Ih	13.3 ± 4.7	17.3 ± 8.2	44.8 ± 4.7
Ii	6.7 ± 4.7	13.8 ± 4.7	34.5 ± 4.7
Ij	6.7 ± 4.7	13.8 ± 4.7	37.9 ± 8.2
Ik	3.3 ± 4.7	13.8 ± 4.7	27.6 ± 8.2
Il	3.3 ± 4.7	10.4 ± 4.7	31.0 ± 9.4
Im	13.3 ± 4.7	24.2 ± 4.7	55.2 ± 9.4
In	13.3 ± 4.7	20.7 ± 4.7	44.8 ± 4.7
Io	6.7 ± 4.7	17.3 ± 8.2	37.9 ± 8.2
IIa	3.3 ± 4.7	17.3 ± 0	41.4 ± 4.7
IIb	3.3 ± 4.7	13.8 ± 4.7	41.4 ± 4.7
IIc	13.3 ± 4.7	24.2 ± 4.7	51.7 ± 4.7
IId	13.3 ± 0	17.3 ± 8.2	41.4 ± 4.7
IIe	6.7 ± 4.7	24.2 ± 4.7	44.8 ± 4.7
IIf	6.7 ± 4.7	13.8 ± 4.7	34.5 ± 4.7
IIg	13.3 ± 4.7	20.7 ± 4.7	41.4 ± 9.4
IIh	13.3 ± 4.7	31.1 ± 4.7	58.6 ± 8.2
IIi	3.3 ± 4.7	20.7 ± 4.7	44.8 ± 4.7
IIj	3.3 ± 4.7	20.7 ± 4.7	37.9 ± 8.2
IIk	3.3 ± 4.7	17.3 ± 0	34.5 ± 4.7
IIl	3.3 ± 4.7	20.7 ± 4.7	44.8 ± 4.7
IIm	13.3 ± 4.7	41.4 ± 4.7	65.5 ± 4.7
IIn	13.3 ± 8.2	34.5 ± 4.7	55.2 ± 4.7
IIo	6.7 ± 4.7	24.2 ± 9.4	44.8 ± 4.7
toosendanin	13.3 ± 4.7	34.5 ± 4.7	48.3 ± 8.2

Table 2. Acaricidal Activity of Compounds **1**–**3**, **Ia**–**o** and **IIa**–**o** against *T. cinnabarinus* Treated at a Concentration of 0.5 mg/mL

compound	corrected mortality rate (mean ± SD, %)	
	48 hours	72 hours
1	8.7 ± 0.4	13.6 ± 1.9
2a	4.3 ± 2.5	11.5 ± 1.5
2b	4.8 ± 2.0	11.3 ± 2.4
2c	4.9 ± 1.2	10.3 ± 3.7
3a	11.0 ± 2.3	36.2 ± 0.8
3b	12.8 ± 1.5	43.5 ± 2.0
3c	10.9 ± 3.4	40.3 ± 2.9
Ia	9.4 ± 0.8	17.0 ± 3.2
Ib	5.3 ± 1.7	16.1 ± 2.3
Ic	9.7 ± 2.6	25.2 ± 6.1
Id	6.3 ± 3.2	24.8 ± 5.5
Ie	6.6 ± 3.2	19.1 ± 3.7
If	9.3 ± 3.2	19.9 ± 5.6
Ig	8.2 ± 3.4	25.0 ± 3.2
Ih	8.0 ± 1.4	21.9 ± 6.8
Ii	9.1 ± 1.9	18.7 ± 3.7
Ij	8.2 ± 1.7	14.6 ± 2.0
Ik	6.0 ± 0.4	11.8 ± 2.5
Il	7.6 ± 4.0	15.3 ± 3.9
Im	11.6 ± 4.4	25.6 ± 5.8
In	8.5 ± 2.8	18.7 ± 2.7
Io	9.7 ± 4.0	23.8 ± 4.8
IIa	8.0 ± 2.3	12.9 ± 2.6
IIb	9.4 ± 2.1	14.9 ± 3.7
IIc	10.2 ± 0.4	20.8 ± 1.5
IId	10.6 ± 4.9	21.5 ± 2.9
IIe	6.4 ± 2.3	15.0 ± 4.2
IIf	7.4 ± 3.3	14.2 ± 4.6
IIg	5.2 ± 1.9	15.8 ± 2.7
IIh	5.4 ± 2.0	16.4 ± 0.9
IIi	4.2 ± 1.5	17.8 ± 6.2
IIj	5.8 ± 2.5	15.2 ± 3.4
IIk	9.1 ± 4.6	16.8 ± 0.8
IIl	6.1 ± 3.1	11.3 ± 2.0
IIm	9.5 ± 1.1	15.8 ± 2.4
IIn	7.1 ± 2.6	15.0 ± 1.7
IIo	7.8 ± 4.4	21.2 ± 2.1
spirodiclofen	41.5 ± 3.8	68.5 ± 4.1

Table 3. LC_{50} Values of Compounds **1** and **3a–c** at 72 h against *T. cinnabarinus*

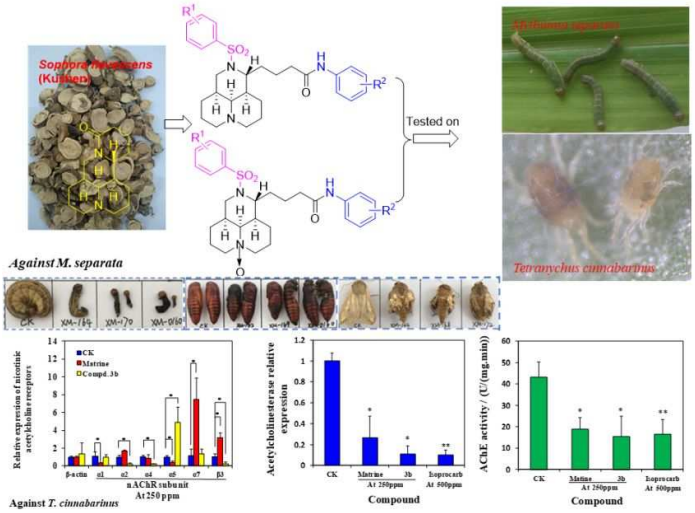
compound	LC_{50} (mg/mL)	regression equation	r
1	4.01	Y = 4.4924 + 0.8420X	0.9942
3a	0.65	Y = 5.3475 + 1.8782X	0.9499
3b	0.55	Y = 5.2900 + 1.1108X	0.9746
3c	0.63	Y = 5.2699 + 1.3386X	0.9553
spirodiclofen	0.33	Y = 5.6900 + 1.4426X	0.9990

Table 4. Primers of *nAChR* Subunits and *AChE* of *T. cinnabarinus* Used for qRT-PCR.

gene	sequence (5'–3')[b]	annealing temperature (°C)	product size (bp)
β-actin	F-gtttggatttggctggtcgt R-tgctcaaagtcaagggcaac	60	145
α1	F-tgtctctccttcgcctcttg R-ctcggtgagtcaacattggc	60	158
α2	F-tggtgacttgttccgttgtg R-ggcggtttcatgagcagaat	60	136
α4	F-caacatcccttgcagttccc R-tgagtcgatggtgaacggaa	60	121
α5	F-gtcgttgcctgttcagttgt R-ttgaacttggtgaggcttgc	60	181
α7	F-ccagccactttcaccacaaa R-ggcaacaagagcaaacctga	60	119
β3	F-gcccatcatctaacaaaccca R-agccgtaaaagtagagccca	60	170
AChE[a]	F- cctcgactcgctctgtacat R- ggttccctcatcacgattgc	60	218

[a]AChE: acetylcholinesterase. [b]F: forward primer; R: reverse primer.

TOC graphic


Sophora flavescens (Kushen)
Tested on
Against M. separata
Tetranychus cinnabarinus
CK
XM-1l5
XM-1l9
XM-0l6
Relative expression of nicotinic acetylcholine receptors
CK
Matrine
Compd.3b
β-actin
α1
α2
α3
α6
α7
β3
nAChR subunit
At 250 ppm
Against T. cinnabarinus
Acetylcholinesterase relative expression
CK
Matrine
3b
Isoprocarb
At 250ppm
At 500ppm
Compound
AChE activity / (U/(mg.min))
CK
Matrine
3b
Isoprocarb
At 250ppm
At 500ppm
Compound