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Natural-product-based pesticides: Semisynthesis, structural elucidation, and evaluation of new cholesterol–matrine conjugates as pesticidal agents

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ABSTRACT

To develop new potential pesticide candidates from low value-added natural bioactive products, a series of new cholesterol–matrine conjugates (**I(a–e)**–**IV(a–e)**) were prepared from two lead compounds cholesterol and matrine. Against *Mythimna separata* Walker, compound **IVa** exhibited 3.0 and 2.6 folds promising insecticidal activity of cholesterol and matrine, respectively; against *Aphis citricola* Van der Goot, compound **IVd** showed 4.3 and 2.2 folds potent aphicidal activity of their precursors; notably, it also showed good control effects in the greenhouse; against *Plutella xylostella* Linnaeus at a dose of 20 μg/nymph, compound **IIIe** exhibited 2.8 and 2.0 folds oral toxicity of cholesterol and matrine, respectively. Compounds **IIIe**, **IVd** and **IVe** can be used as the leads for further structural optimization as the insecticidal and aphicidal agents.

A steroidal natural product cholesterol (Fig. 1), a derivative of cyclopentane polyhydrophenanthrene, is an indispensable substance for human and animal cells. As an important component of mammalian cell membranes, for instance, cholesterol can efficiently regulate the related structures and functions of lipid bilayers.[1] On the other hand, cholesterol has recently been used as a lead compound for synthesis of cholesterol-type analogs which displayed lots of biological properties including antitumor activity,[2] antioxidant activity,[3,4] antileishmanial activity,[5,6] and antimicrobial activity.[7,8] In our previous paper, to our delight, we found that some hydrazones derivatives of cholesterol at the C-7 position showed more potent insecticidal activity than toosendanin; notably an intermediate, 3-acetyloxy-7-oxocholesterol (Fig. 1), also exhibited the promising insecticidal activity.[9] Matrine (Fig. 1) is isolated as the main component from *Sophora alopecuroides, Sophora subprostrata* and *Sophora flavescens* which are found in China, Japan and some European countries.[10] Although matrine was registered as a botanical pesticide in China, its pesticidal activities were much lower in magnitude than those of commercially agrochemicals.[11] To increase its pesticidal activities, therefore, structural optimization of matrine has been extensively carried out.[12–15] Interestingly, a series of matrinic acids (Fig. 1) were found to show the potent pesticidal activities.[14]

Meanwhile, a large number of pesticides were extensively sprayed to control pests, however pests resistance and negative impacts of pesticides residues on human health and environment accordingly appeared. Furthermore, structural optimization of natural products as lead compounds for the discovery and development of pesticide candidates has received much attention in recent years.[16–19] Based upon the above-mentioned interesting results, and to discover natural-product-based potential pesticide candidates, in this paper a series of new cholesterol–matrine conjugates (**I(a–e)**–**IV(a–e)**, Fig. 1) were designed by combination of 3-acetyloxy-7-oxocholesterol and matrinic acids fragments together via the oxime group at the C-7 position of cholesterol. Their agricultural activities were evaluated against three typically crop-threatening pests, *Mythimna separata* Walker, *Aphis citricola* Van der Goot and *Plutella xylostella* Linnaeus. Their control efficiency was tested against *A. citricola* in the greenhouse.

As described in Fig. 2, according to our previous reports,[9] cholesterol (**1**) was esterified with benzoyl chlorides R^1COCl to afford **2a–d**, which were then oxidized by CrO_3 and *t*-BuOOH to obtain **3a–d**. Next, compounds **3a–d** reacted with NH_2OH to give **4a–d**.[13,20] Compounds **6a–e** were obtained by opening the lactam of matrine (**5**), and subsequently esterifying and substitution reaction with benzyl chlorides/bromides.[14] Further hydrolysis of **6a–e** gave matric acids **7a–e**.[14] Finally, new cholesterol–matrine conjugates (**I(a–e)**–**IV(a–e)**) were produced by reaction of **4a–d** with **7a–e** in 11%–58% yields (Fig. 3).[21] The influence of the functional groups at the C-7 position on the chemical shift of H-6 of cholesterol derivatives was obvious. In Fig. 4, there was no obvious difference in the chemical shifts of H-3 of **3a** (δ = 4.956 ppm), **4a** (δ =

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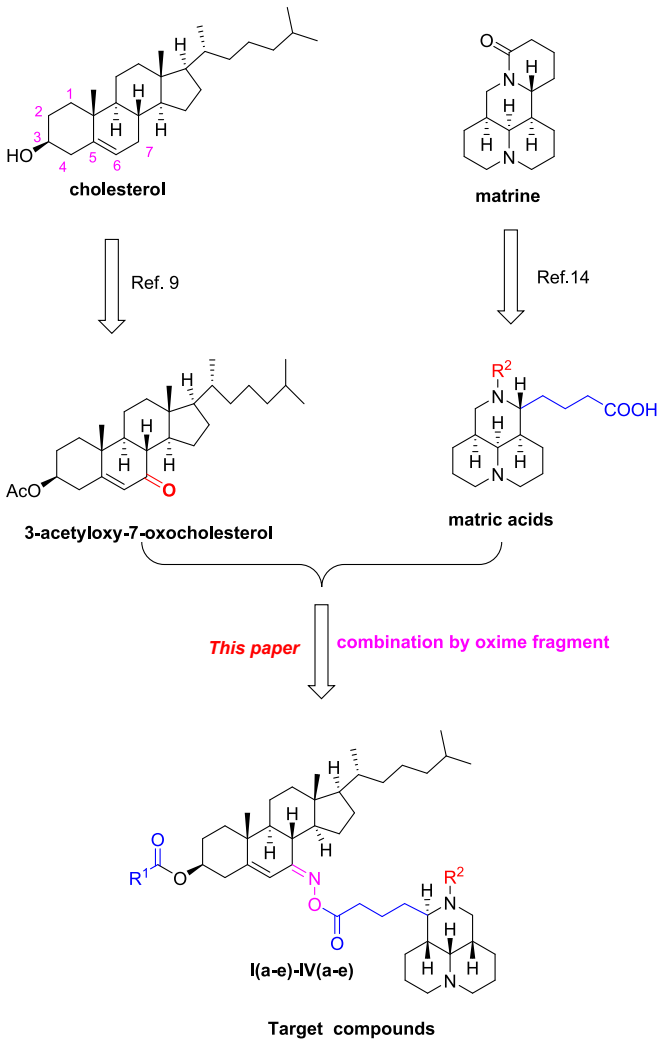

Fig. 1. Design of target compounds from cholesterol and matrine.

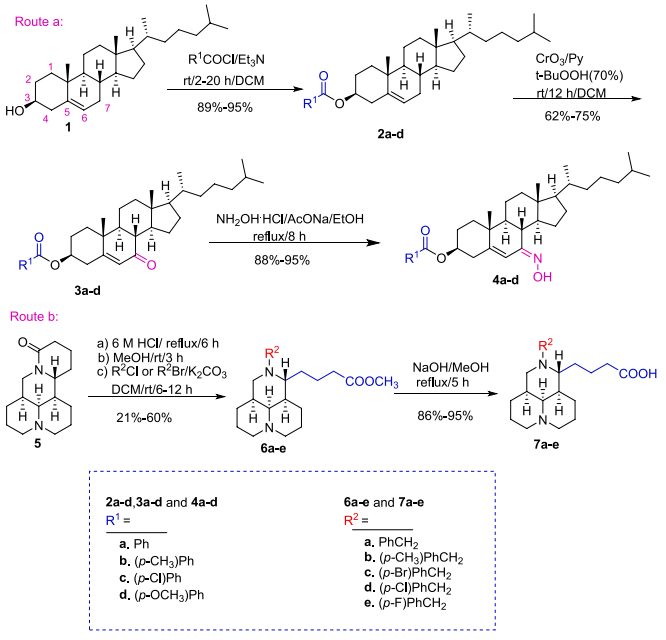

Fig. 2. Synthetic routes of (a) intermediates **4a**–**d**, and (b) intermediates **7a**–**e**.

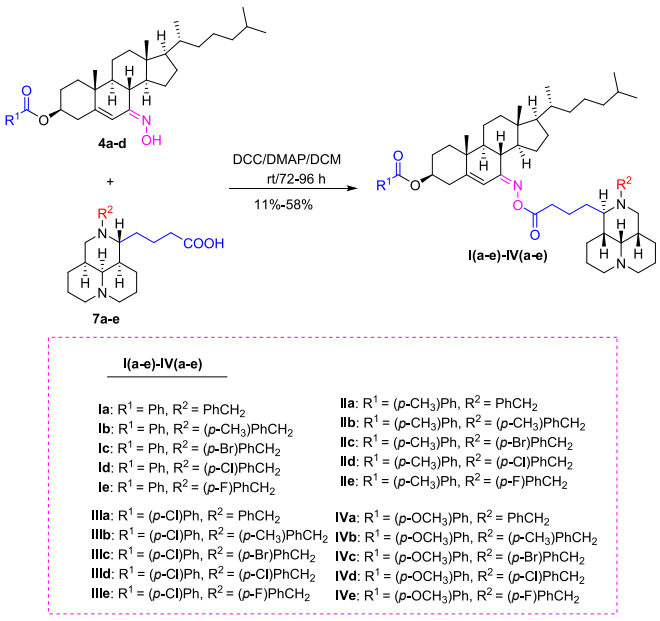

Fig. 3. Synthetic route of target compounds **I(a-e)**–**IV(a-e)**.

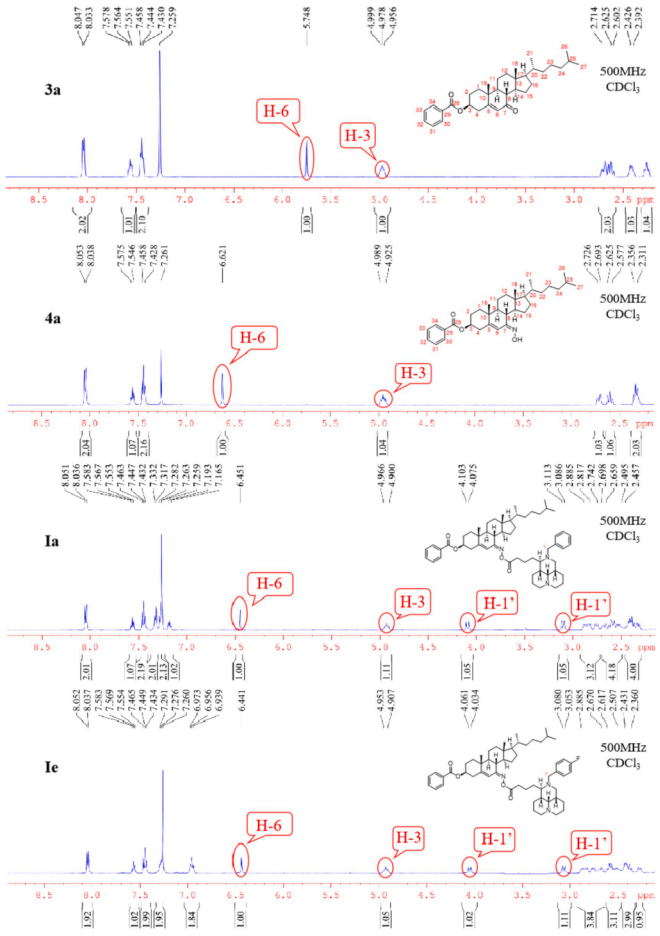

Fig. 4. Comparison of partial ^{1}H NMR spectra of **3a**, **4a**, **Ia** and **Ie**.

4.925 ppm), **Ia** ($\delta = 4.900$ ppm) and **Ie** ($\delta = 4.907$ ppm); in contrast, the difference in the chemical shifts of H-6 of **3a**, **4a**, **Ia** and **Ie** was significant, for example, the chemical shift of H-6 of **3a** was at 5.748 ppm, whereas the chemical shifts of H-6 of **4a**, **Ia** and **Ie** were at 6.621, 6.451

and 6.441 ppm. Due to the carbonyl group at the C-7 position of **3a** was substituted by the oxime, the corresponding chemical shifts of H-6 of **4a**, **Ia** and **Ie** were all moved to the low field. The chemical shifts of two hydrogen atoms of H-1′ of **Ia** and **Ie** were at 3.08/4.07 and 3.05/4.03 ppm, respectively. Their structures were characterized by optical rotation, IR, ^{1}H NMR or ^{13}C NMR, and mp (see Supplementary data). Compounds **4a** (CCDC: 2013575) and **IVe** (CCDC: 2024764) were further determined by X-ray crystallography (Fig. 5).

In Table 1, at 1 mg/mL against *M. separata*,[22] the results revealed that the insecticidal activity of all derivatives was improved when compared with their precursors matrine and cholesterol. The final mortality rates (FMRs) of **2d**, **IIb**, **IId**, **IVa**, **IVd** and **IVe** were 51.7%, 55.1%, 48.2%, 62.0%, 51.7% and 48.2%, respectively, which exhibited more potent insecticidal activity than toosendanin. The FMRs of **2d**, **IIb**, **IVa** and **IVd** were greater than 50%. Among them, compound **IVa** showed the best insecticidal activity. There was no significant difference in the insecticidal activity between **2a**–**c** and **3a**–**c**, and it generally demonstrated that introduction of a carbonyl group at the C-7 position of **2a-c** has no perceptible effect on the insecticidal activity. To conjugates **IIa**–**e**, compounds **IIb** (R^1 = *p*-methylphenyl, R^2 = *p*-methylbenzyl; FMR: 55.1%) and **IId** (R^1 = *p*-methylphenyl, R^2 = *p*-chlorobenzyl; FMR: 48.2%) displayed more promising insecticidal activity than their precursors and toosendanin. To conjugates **IVa**–**e**, compounds **IVa** (R^1 = *p*-methoxyphenyl, R^2 = benzyl), **IVd** (R^1 = *p*-methoxyphenyl, R^2 = *p*-chlorobenzyl), and **IVe** (R^1 = *p*-methoxyphenyl, R^2 = *p*-fluorobenzyl) showed the pronounced insecticidal activity with the FMRs of 62.0%, 51.7% and 48.2%, respectively.

In Fig. 6, the largest part of the percentages of FMRs at three growth

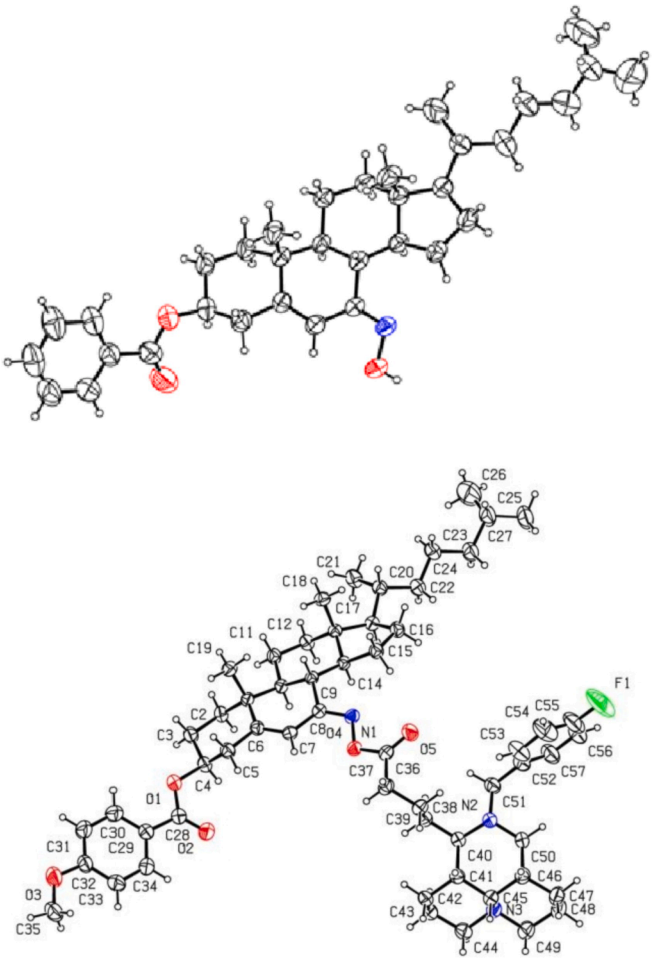

Fig. 5. X-ray crystal structures of **4a** (top) and **IVe** (bottom).

Table 1
Growth inhibitory activity of compounds **1**, **2a**–**d**, **3a**–**d**, **4a**–**d**, **5** and **I**(**a**–**e**)–**IV** (**a**–**e**) against *M. separata* at 1 mg/mL.

Compound	Corrected mortality rate (mean ± SE, %)		
	10 days	20 days	35 days
1	6.7 ± 3.3	16.7 ± 3.3	20.7 ± 3.3
5	10.0 ± 0	20.0 ± 0	24.1 ± 3.3
2a	36.7 ± 3.3	36.7 ± 3.3	44.8 ± 6.7
2b	13.3 ± 3.3	26.7 ± 3.3	27.6 ± 5.8
2c	36.7 ± 3.3	43.3 ± 3.3	44.8 ± 3.3
2d	36.7 ± 6.7	36.7 ± 6.7	51.7 ± 3.3
3a	30.0 ± 5.8	43.3 ± 3.3	41.4 ± 3.3
3b	20.0 ± 0	30.0 ± 5.8	31.0 ± 3.3
3c	30.0 ± 5.8	40.0 ± 5.8	41.4 ± 3.3
3d	16.7 ± 6.7	33.3 ± 6.7	37.9 ± 0
4a	26.7 ± 6.7	26.7 ± 6.7	31.0 ± 6.7
4b	36.7 ± 6.7	40.0 ± 5.8	44.8 ± 3.3
4c	23.3 ± 3.3	30.0 ± 5.8	31.0 ± 3.3
4d	33.3 ± 8.8	40.0 ± 5.8	44.8 ± 3.3
Ia	23.3 ± 6.7	30.0 ± 5.8	31.0 ± 3.3
Ib	23.3 ± 3.3	23.3 ± 3.3	31.0 ± 6.7
Ic	30.0 ± 5.8	33.3 ± 3.3	34.5 ± 6.7
Id	20.0 ± 0	30.0 ± 5.8	37.9 ± 5.8
Ie	36.7 ± 6.7	40.0 ± 5.8	44.8 ± 3.3
IIa	30.0 ± 5.8	36.7 ± 3.3	37.9 ± 0
IIb	20.0 ± 5.8	40.0 ± 5.8	55.1 ± 3.3
IIc	13.3 ± 3.3	26.7 ± 3.3	31.0 ± 6.7
IId	23.3 ± 6.7	23.3 ± 6.7	48.2 ± 0
IIe	33.3 ± 3.3	33.3 ± 3.3	37.9 ± 5.8
IIIa	23.3 ± 3.3	33.3 ± 3.3	37.9 ± 5.8
IIIb	30.0 ± 5.8	36.7 ± 3.3	44.8 ± 6.7
IIIc	36.7 ± 3.3	40.0 ± 5.8	37.9 ± 5.8
IIId	33.3 ± 6.7	33.3 ± 6.7	37.9 ± 0
IIIe	23.3 ± 3.3	26.7 ± 3.3	37.9 ± 5.8
IVa	43.3 ± 3.3	43.3 ± 3.3	62.0 ± 3.3
IVb	23.3 ± 6.7	26.7 ± 3.3	27.6 ± 0
IVc	16.7 ± 3.3	26.7 ± 3.3	31.0 ± 3.3
IVd	20.0 ± 5.8	30.0 ± 5.8	51.7 ± 3.3
IVe	33.3 ± 3.3	36.7 ± 3.3	48.2 ± 5.8
toosendanin	26.7 ± 3.3	30.0 ± 5.8	44.8 ± 3.3

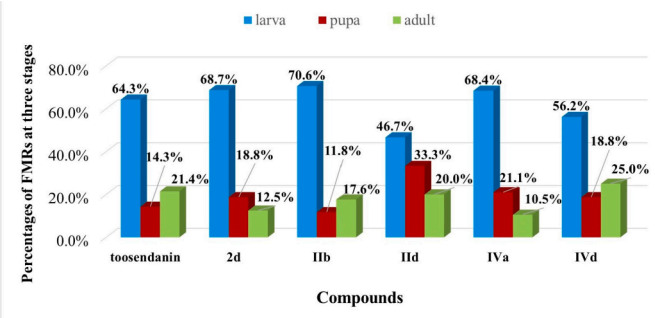

Fig. 6. The percentages of final mortality rates (FMRs) at three growth stages of *M. separata* treated with **2d**, **IIb**, **IId**, **IVa**, **IVd** and toosendanin.

stages of *M. separata* treated with **2d**, **IIb**, **IId**, **IVa** and **IVd** was at the larval stage, and it was in accordance with that of toosendanin. Compounds **2d**, **IIb**, **IId**, **IVa** and **IVd** may have the similar mechanism of action with toosendanin against *M. separata.* At the larval stage, some poisoned larvae were dehydrated and curled up eventually to die (Fig. 7). At the pupal stage, some deformed pupae appeared (Fig. 8). At the adult stage, malformed moths emerged with vestigial wings (Fig. 9).

In Table 2, at 0.04 μg/nymph against *A. citricola*,[23] the 48 h MRs of **2a**–**d**, **3a**–**d** and **4a**–**d** was higher than that of cholesterol. Among them, the 48 h MR of **2d** was 34.1%, which was twice as high as that of cholesterol (17.0%). To compounds **2a**–**d** and **4a**–**d**, the aphicidal activity of compounds containing *p*-methoxyphenyl group was slightly higher than those containing *p*-methylphenyl, *p*-chlorophenyl and

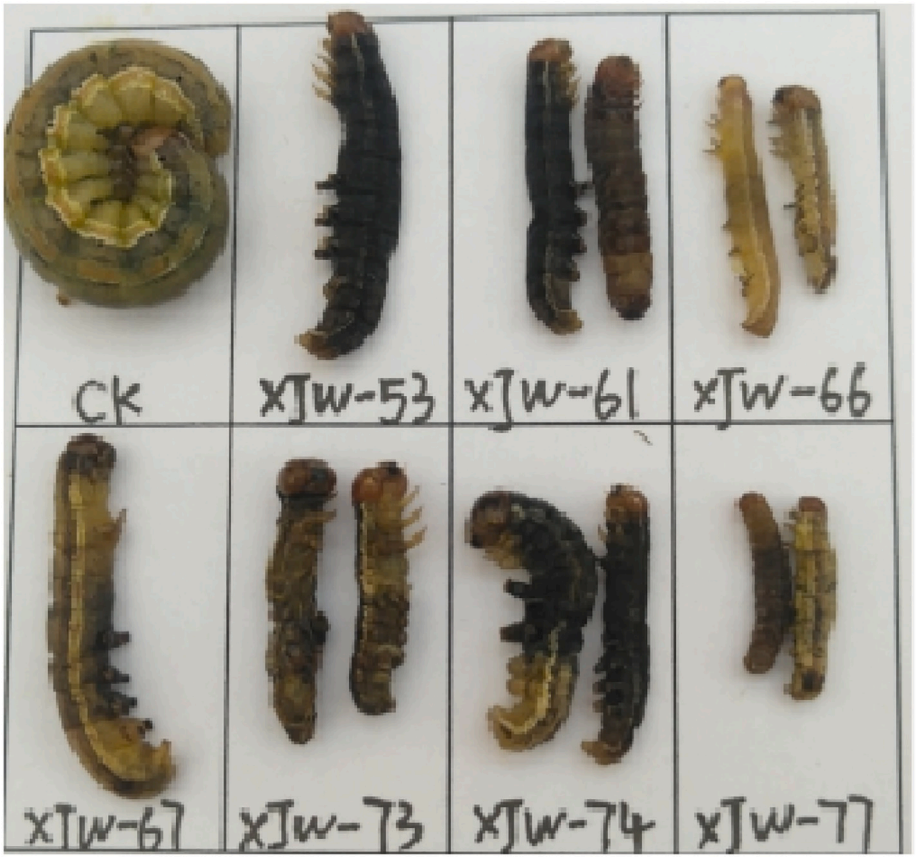

Fig. 7. The representative abnormal larvae pictures of *M. separata* treated with **2d** (**XJW-53**), **4d** (**XJW-61**), **Ie** (**XJW-66**), **IIa** (**XJW-67**), **IIIb** (**XJW-73**), **IIIc** (**XJW-74**) and **IVa** (**XJW-77**) during the larval period (CK: blank control group).

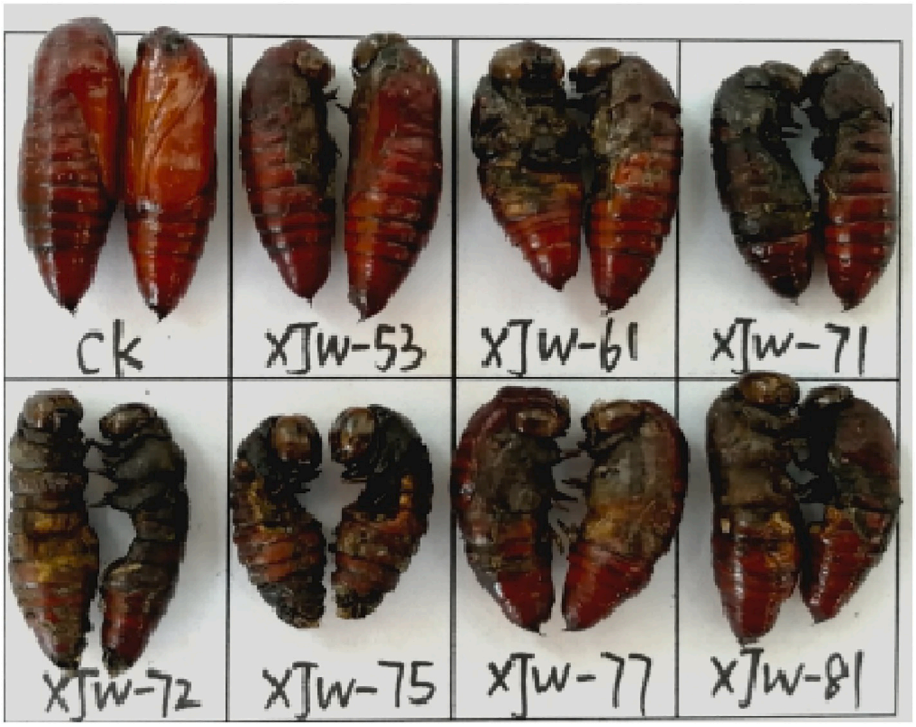

Fig. 8. The representative malformed pupae pictures of *M. separata* treated with **2d** (**XJW-53**), **4d** (**XJW-61**), **IIe** (**XJW-71**), **IIIa** (**XJW-72**), **IIId** (**XJW-75**), **IVa** (**XJW-77**) and **IVe** (**XJW-81**) during the pupation period (CK: blank control group).

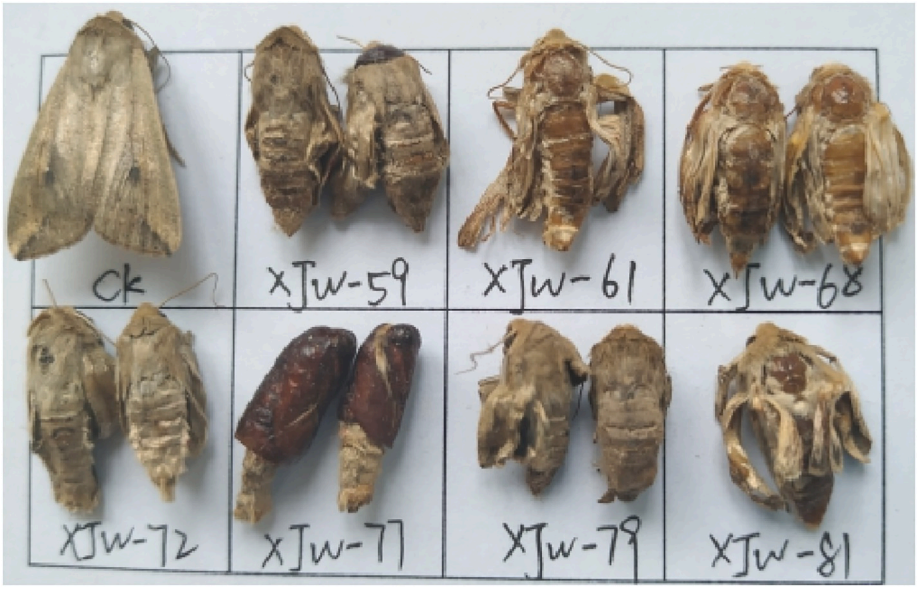

Fig. 9. The representative malformed moths pictures of *M. separata* treated with **4b** (**XJW-59**), **4d** (**XJW-61**), **IIb** (**XJW-68**), **IIIa** (**XJW-72**), **IVa** (**XJW-77**), **IVc** (**XJW-79**) and **IVe** (**XJW-81**) during the adult period (CK: blank control group).

Table 2
Aphicidal activity of compounds **1**, **2a–d**, **3a–d**, **4a–d**, **5** and **I(a–e)**–**IV(a–e)** against *A. citricola* at 0.04 μg/nymph.

Compound	Corrected mortality rate (mean ± SE, %)	
	24 h	48 h
1	7.9 ± 1.1	17.0 ± 1.1
5	14.6 ± 2.2	29.5 ± 2.9
2a	11.2 ± 1.1	20.5 ± 2.2
2b	16.9 ± 2.9	27.3 ± 4.4
2c	12.4 ± 1.9	25.0 ± 3.8
2d	15.7 ± 3.3	34.1 ± 2.2
3a	11.2 ± 1.1	27.3 ± 2.9
3b	14.6 ± 1.1	23.9 ± 4.0
3c	9.0 ± 1.9	33.0 ± 2.9
3d	13.4 ± 2.2	23.9 ± 4.0
4a	17.8 ± 2.9	32.2 ± 2.9
4b	14.4 ± 2.9	27.6 ± 1.9
4c	13.3 ± 1.9	29.9 ± 2.9
4d	12.2 ± 4.8	35.6 ± 2.2
Ia	11.1 ± 1.1	26.4 ± 2.9
Ib	15.6 ± 2.9	31.0 ± 3.8
Ic	7.9 ± 1.1	25.6 ± 2.2
Id	9.0 ± 1.9	23.3 ± 1.9
Ie	15.7 ± 1.9	33.7 ± 1.9
IIa	7.9 ± 2.2	36.1 ± 1.1
IIb	12.4 ± 1.9	34.9 ± 1.1
IIc	6.7 ± 1.1	39.5 ± 1.1
IId	14.6 ± 2.2	38.4 ± 1.1
IIe	9.0 ± 3.3	32.6 ± 4.0
IIIa	7.9 ± 2.2	24.4 ± 4.0
IIIb	10.1 ± 2.9	31.4 ± 1.1
IIIc	17.9 ± 4.0	19.8 ± 1.9
IIId	12.4 ± 1.9	33.7 ± 1.9
IIIe	25.8 ± 1.9	48.8 ± 4.0
IVa	6.8 ± 2.9	22.0 ± 1.1
IVb	11.4 ± 3.3	20.9 ± 2.9
IVc	10.2 ± 2.2	30.2 ± 1.9
IVd	19.3 ± 1.1	51.2 ± 1.9
IVe	14.8 ± 3.3	43.0 ± 1.1

phenyl ones. Compared to that of **4b** (FMR: 27.6%), the aphicidal activity of all its corresponding target compounds **IIa**–**IIe** (R^1 = *p*-methylphenyl; FMRs: greater than 39.5%) was increased. The 48 h MRs of **IIIa**–**IIIe** (R^1 = *p*-chlorophenyl) were 24.4%, 31.4%, 19.8%, 33.7% and 48.8%, respectively. **IIIe** showed two folds potent aphidicidal activity of **IIIa**. So introduction of a fluorine atom on the *N*-benzyl group of matrinic acid (**IIIa**) was necessary for the aphicidal activity. The 48 h MRs of **IVa**–**IVe** were 22.0%, 20.9%, 30.2%, 51.2%, and 43.0%, respectively. Obviously, introduction of a halogen atom on the *N*-benzyl fragment of **Iva** was vital for the aphicidal activity.

In Table 3, the 48 h LD_{50} values of **IIIe**, **IVd** and **IVe** against *A. citricola* were 0.045, 0.042, and 0.052 μg/nymph, respectively, so their aphicidal activity was 3.4–4.3 folds of that of cholesterol (LD_{50}: 0.179 μg/nymph), and 1.7–2.2 folds of that of matrine (LD_{50}: 0.091 μg/nymph). Especially compound **IVd** showed 4.3 and 2.2 folds potent aphicidal activity of cholesterol and matrine, respectively. Moreover, compound **IVd** showed good control effects in the greenhouse against *A. citricola* (Table 4). The control effect of **IVd** after 7 days was 65.8%, however, the control effects of **1** and **5** after 7 days were only 17.4% and 32.1%, respectively.

In Table 5, at 20 μg/nymph against *P. xylostella*,[22,24] when R^1 was *p*-chlorophenyl or *p*-methoxyphenyl, and R^2 was *p*-fluorobenzyl, the 48 h MRs of corresponding compounds **IIIe** and **IVe** were 51.2% and 46.5%, respectively, which were better or equivalent to that of toosendanin (46.5%). The 48 h MRs of **Ia**–**Ie** (R^1 = phenyl) against *P. xyllostella* were 25.0%, 35.7%, 40.5%, 35.7%, and 38.1%, respectively. The oral toxicity of **Ib**–**Ie** was higher than that of **Ia**, which indicated that introduction of electron-withdrawing or electron-donating groups on the *N*-benzyl fragment of **Ia** could lead to increasing the activity.

In summary, to discover new natural-product-based potential

Table 3
LD_{50} values of some compounds at 48 h against *A. citricola*.[a]

Compound	Linear regression equation	LD_{50} (μg/nymph)	Confidence interval 95% (μg/nymph)	*r*
1	Y = 1.714 + 2.292X	0.179	0.158–0.21	0.939
5	Y = 1.605 + 1.545X	0.091	0.074–0.13	0.966
IIIe	Y = 3.399 + 2.521X	0.045	0.040–0.052	0.983
IVd	Y = 3.626 + 2.632X	0.042	0.038–0.048	0.978
IVe	Y = 2.990 + 2.331X	0.052	0.046–0.064	0.956

[a] Regression analysis by IBM SPSS Statistics 20.0, $P < 0.05$.

Table 4
Control efficiency of compounds **1**, **5** and **IVd** against *A. citricola* in the greenhouse tests at a concentration of 1 mg/mL.

Compound	Control efficiency (mean ± SE, %)			
	1st day	3rd day	5th day	7th day
1	4.3 ± 1.0	11.5 ± 1.4	15.3 ± 1.3	17.4 ± 2.7
5	8.6 ± 1.0	27.5 ± 1.3	30.6 ± 1.3	32.1 ± 0.5
IVd	11.0 ± 1.7	54.0 ± 1.3	61.2 ± 1.3	65.8 ± 1.4

Table 5
Oral toxicity of compounds **1**, **2a–d**, **3a–d**, **4a–d**, **5** and **I(a–e)–IV(a–e)** against *P. xylostella* at 20 μg/nymph.

Compound	Corrected mortality rate (mean ± SE, %)	
	24 h	48 h
1	6.8 ± 2.2	18.6 ± 4.4
5	11.4 ± 0	25.6 ± 2.2
2a	11.4 ± 3.8	30.2 ± 0
2b	15.6 ± 2.2	27.3 ± 2.2
2c	8.9 ± 2.2	29.6 ± 2.2
2d	13.3 ± 0	34.1 ± 5.8
3a	13.3 ± 3.8	29.6 ± 5.8
3b	11.1 ± 4.4	34.1 ± 2.2
3c	13.3 ± 3.8	27.3 ± 5.8
3d	13.3 ± 6.6	34.1 ± 5.8
4a	9.1 ± 2.2	27.3 ± 2.2
4b	13.6 ± 2.2	29.6 ± 2.2
4c	18.2 ± 3.8	34.1 ± 5.8
4d	20.5 ± 4.4	38.6 ± 0
Ia	13.6 ± 5.8	25.0 ± 0
Ib	11.6 ± 4.4	35.7 ± 3.8
Ic	7.0 ± 2.2	40.5 ± 4.4
Id	9.3 ± 0	35.7 ± 3.8
Ie	7.0 ± 4.4	38.1 ± 5.8
IIa	11.6 ± 4.4	31.0 ± 2.2
IIb	14.0 ± 2.2	26.2 ± 4.4
IIc	14.0 ± 5.8	33.3 ± 4.4
IId	9.1 ± 4.4	35.7 ± 3.8
IIe	11.4 ± 3.8	38.1 ± 5.8
IIIa	11.4 ± 0	40.5 ± 2.2
IIIb	15.9 ± 5.8	42.9 ± 3.8
IIIc	13.6 ± 4.4	31.0 ± 2.2
IIId	20.5 ± 5.8	28.6 ± 3.8
IIIe	26.7 ± 3.8	51.2 ± 3.8
IVa	17.8 ± 4.4	34.9 ± 4.4
IVb	20.0 ± 3.8	41.9 ± 2.2
IVc	17.8 ± 2.2	39.5 ± 5.8
IVd	11.1 ± 4.4	30.2 ± 0
IVe	15.6 ± 5.8	46.5 ± 2.2
toosendanin	13.6 ± 2.2	46.5 ± 2.2

pesticide candidates, a series of new cholesterol–matrine conjugates were synthesized. Against *M. separata*, compounds **2d**, **IIb**, **IId**, **IVa**, **IVd** and **IVe** showed better insecticidal activity than toosendanin; especially compound **IVa** exhibited 3.0 and 2.6 folds promising insecticidal activity of cholesterol and matrine, respectively. Against *A. citricola*, compounds **IIIe**, **IVd** and **IVe** displayed good aphicidal activity with LD_{50} values of 0.042–0.052 μg/nymph; particularly, compound **IVd** showed 4.3 and 2.2 folds potent aphicidal activity of cholesterol and matrine, respectively, and it also displayed good control effects in the greenhouse (3.8 and 2.0 folds of cholesterol and matrine at 7th day). Against *P. xylostella*, compound **IIIe** exhibited 2.8 and 2.0 folds good oral toxicity of cholesterol and matrine, respectively. These results will provide a foundation for future structural modifications and applications of matrine and cholesterol analogs as pesticides in agriculture.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128350.

References

1 Johnson BB, Anguita J, Heuck AP. *Biophys J*. 2013;104:86a.
2 Cui J, Lin Q, Gan C, et al. *Steroids*. 2014;79:14–18.
3 Choteau F, Durand G, Ranchon-Cole I, et al. *Bioorg Med Chem Lett*. 2010;20:7405–7409.
4 Kumar M, Padmini T, Ponnuvel K. *J Saudi Chem Soc*. 2017;21:S322–S328.
5 Bazin MA, Loiseau PM, Bories C, et al. *Eur J Med Chem*. 2006;41:1109–1116.
6 Ghosh P, Ghosh A, Mandal A, et al. *Steroids*. 2016;107:65–73.
7 Sribalan R, Padmini V, Lavanya A, Ponnuvel K. *Saudi Pharm J*. 2016;24:658–668.
8 Banday MR, Farshori NN, Ahmad A, et al. *Eur J Med Chem*. 2010;45:1459–1464.
9 Yang C, Shao Y, Zhi X, et al. *Bioorg Med Chem Lett*. 2013;23:4806–4812.
10 Huang JL, Xu H. *Curr Top Med Chem*. 2016;16:3365–3378.
11 Zhong B, Lu C, Sun X, et al. *Agrochemicals*. 2010;49:924–926.
12 Ni W, Li C, Liu Y, et al. *J Agric Food Chem*. 2017;65:2039–2047.
13 Li S, Sun Z, Zhang B, et al. *Ind Crop Prod*. 2019;131:134–141.
14 Zhang B, Sun Z, Lv M, Xu H. *J Agric Food Chem*. 2018;66:12898–12910.
15 Xu H, Xu M, Sun Z, Li S. *J Agric Food Chem*. 2019;67:12182–12190.
16 Ji X, Guo J, Liu Y, et al. *J Agric Food Chem*. 2018;66:4062–4072.
17 Lin L, Mulholland N, Wu QY, et al. *J Agric Food Chem*. 2012;60:4480–4491.
18 Yang R, Guo Y, Zhang Y, Xu H. *Chin Chem Lett*. 2018;29:995–997.
19 Lv M, Sun ZQ, Li SC, et al. *Bioorg Med Chem Lett*. 2020;30, 127346.
20 Cui JG, Fan L, Huang LL, et al. *Steroids*. 2009;74:62–72.
21 General procedure for synthesis of target compounds I(a−e)−IV(a−e): A mixture of 4a−d (0.30 mmol), 7a−e (0.36 mmol), DCC (0.36 mmol) and DMAP (0.06 mmol) in dry CH2Cl2 (5 mL) was stirred at room temperature. After 72−96 h, the mixture was diluted with 20 mL of CH2Cl2, washed with 10 mL of H2O, 10 mL of 0.1 N aq. HCl, 10 mL of 5% aq. Na2CO3 and 10 mL of brine, and dried by anhydrous Na2SO4. Finally, it was purified by PTLC eluting with CH2Cl2/MeOH (15:1, v/v) to afford I(a−e)−IV(a−e) in 11%–58% yields. Representative data for compounds Ia–IVa are as follows: Compound Ia: Yield: 29%, white solid; mp 151−152 °C; [α]20D = -19 (c 1.8 mg/mL, CHCl3); IR cm-1 (KBr): 2933, 2860, 1762, 1717, 1631, 1539, 1454, 1272, 1118, 707. 1H NMR (500 MHz, CDCl3) δ: 8.05 (d, J = 7.5 Hz, 2H, Ar-H), 7.55−7.58 (m, 1H, Ar-H), 7.43−7.46 (m, 2H, Ar-H), 7.31−7.33 (m, 2H, Ar-H), 7.26−7.28 (m, 2H, Ar-H), 7.16−7.19 (m, 1H, Ar-H), 6.45 (s, 1H, -CH=C), 4.90−4.96 (m, 1H, -OCH), 4.10 (d, J = 14.0 Hz, 1H), 3.11 (d, J = 13.5 Hz, 1H), 2.74-2.88 (m, 3H), 2.49−2.69 (m, 4H), 2.31−2.45 (m, 4H), 2.03−2.09 (m, 3H), 1.88−1.99 (m, 4H), 1.73−1.86 (m, 6H), 1.64−1.70 (m, 2H), 1.50−1.57 (m, 7H), 1.30−1.41 (m, 9H), 1.25 (s, 2H), 1.01−1.18 (m, 9H), 0.93 (d, J = 6.5 Hz, 3H), 0.87 (d, J = 2.5 Hz, 3H), 0.86 (d, J = 2.5 Hz, 3H), 0.70 (s, 3H). Compound IIa: Yield: 16%, white solid; mp 121−122 °C; [α]20D = -16 (c 2.9 mg/mL, CHCl3); IR cm-1 (KBr): 2933, 2857, 1761, 1717, 1627, 1575, 1453, 1273, 1111, 845. 1H NMR (500 MHz, CDCl3) δ: 8.05 (d, J = 7.0 Hz, 2H, Ar-H), 7.55−7.58 (m, 1H, Ar-H), 7.43−7.46 (m, 2H, Ar-H), 7.21 (d, J = 7.5 Hz, 2H, Ar-H), 7.08 (d, J = 7.5 Hz, 2H, Ar-H), 6.45 (s, 1H, -CH=C), 4.91−4.95 (m, 1H, -OCH), 4.06 (d, J = 13.5 Hz, 1H), 3.06 (d, J = 13.0 Hz, 1H), 2.81−2.85 (m, 2H), 2.67−2.76 (m, 2H), 2.49−2.63 (m, 4H), 2.36−2.46 (m, 3H), 2.30 (s, 3H), 2.02−2.13 (m, 3H), 1.92−1.99 (m, 4H), 1.77−1.85 (m, 5H), 1.63−1.74 (m, 4H), 1.50−1.56 (m, 7H), 1.29−1.41 (m, 8H), 1.25 (s, 3H), 1.18 (s, 3H), 1.11−1.15 (m, 4H), 1.01−1.05 (m, 1H), 0.93 (d, J = 6.5 Hz, 3H), 0.87 (d, J = 2.0 Hz, 3H), 0.86 (d, J = 2.0 Hz, 3H), 0.70 (s, 3H). Compound IIIa: Yield: 11%, white solid; mp 188−190 °C; [α]20D = -22 (c 2.1 mg/mL, CHCl3); IR cm-1 (KBr): 2937, 2861, 1761, 1719, 1632, 1589, 1454, 1273, 1112, 849. 1H NMR (500 MHz, CDCl3) δ: 7.98 (d, J = 8.5 Hz, 2H, Ar-H), 7.43 (d, J = 8.5 Hz, 2H, Ar-H), 7.32−7.33 (m, 2H, Ar-H), 7.27−7.28 (m, 2H, Ar-H), 7.18−7.19 (m, 1H, Ar-H), 6.44 (s, 1H, -CH=C), 4.89−4.94 (m, 1H, -OCH), 4.10 (d, J = 13.5 Hz, 1H), 3.11 (d, J = 13.0 Hz, 1H), 2.75−2.88 (m, 2H), 2.50−2.67 (m, 4H), 2.32−2.42 (m, 3H), 2.03−2.08 (m, 3H), 1.89−1.99 (m, 5H), 1.74−1.86 (m, 6H), 1.55−1.70 (m, 8H), 1.47−1.54 (m, 3H), 1.31−1.41 (m, 8H), 1.22−1.25 (m, 3H), 1.18 (s, 3H), 1.11−1.15 (m, 4H), 1.01−1.05 (m, 1H), 0.93 (d, J = 6.5 Hz, 3H), 0.87 (d, J = 2.0 Hz, 3H), 0.86 (d, J = 2.5 Hz, 3H), 0.69 (s, 3H). Compound IVa: Yield: 14%, white solid; mp 191−193 °C; [α]20D = -15 (c 2.2 mg/mL, CHCl3); IR cm-1 (KBr): 2934, 2856, 1757, 1709, 1624, 1511, 1456, 1257, 1113, 848. 1H NMR (500 MHz, CDCl3) δ: 8.00 (d, J = 8.5 Hz, 2H, Ar-H), 7.31−7.33 (m, 2H, Ar-H), 7.26−7.28 (m, 2H, Ar-H), 7.16−7.19 (m, 1H, Ar-H), 6.93 (d, J = 9.0 Hz, 2H, Ar-H), 6.44 (s, 1H, -CH=C), 4.86−4.92 (m, 1H, -OCH), 4.10 (d, J = 13.5 Hz, 1H), 3.86 (s, 3H), 3.11 (d, J = 13.0 Hz, 1H), 2.74−2.88 (m, 2H), 2.49−2.67 (m, 4H), 2.32−2.45 (m, 4H), 2.03−2.08 (m, 3H), 1.88−1.98 (m, 4H), 1.73−1.86 (m, 5H), 1.57−1.71 (m, 8H), 1.48−1.54 (m, 3H), 1.31−1.41 (m, 8H), 1.25 (s, 3H), 1.17 (s, 3H), 1.08−1.15 (m, 5H), 1.01−1.05 (m, 1H), 0.93 (d, J = 6.5 Hz, 3H), 0.87 (d, J = 1.5 Hz, 3H), 0.86 (d, J = 2.0 Hz, 3H), 0.70 (s, 3H).

22 Li Q, Huang X, Li S, et al. *J Agric Food Chem*. 2016;64:5472–5478.
23 Hao M, Sun Z, Xu J, et al. *J Agric Food Chem*. 2020;68:4131–4143.
24 Yang R, Lv M, Xu H. *J Agric Food Chem*. 2018;66:11254–11264.