Bioorganic & Medicinal Chemistry Letters xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of some ester derivatives of 4′-demethoxyepipodophyllotoxin/2′-chloro-4′-demethoxyepipodophyllotoxin as insecticidal agents against oriental armyworm, *Mythimna separata* Walker

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ARTICLE INFO

Article history:
Received 19 September 2016
Revised 23 November 2016
Accepted 8 December 2016
Available online xxxx

Keywords:
Podophyllotoxin
4′-Demethoxyepipodophyllotoxin
2′-Chloro-4′-demethoxyepipodophyllotoxin
Insecticidal activity
Mythimna separata Walker

ABSTRACT

Podophyllotoxin is a naturally occurring non-alkaloid toxin isolated from the roots and rhizomes of *Podophyllum peltatum* and *P. hexandrum*. In continuation of our program aimed at the discovery and development of natural product-based insecticides, two series of ester derivatives of 4′-demethoxyepipodophyllotoxin/2′-chloro-4′-demethoxyepipodophyllotoxin were prepared. The structures of the target compounds were well characterized by 1H NMR, IR, optical rotation and mp. The precise three-dimensional structural information of **8j** was further determined by single-crystal X-ray diffraction. Their insecticidal activity was tested against *Mythimna separata* Walker. These compounds showed delayed insecticidal activity. Among all derivatives, some compounds showed more potent insecticidal activity than toosendanin against *M. separata*; especially compounds **8k** and **9k** exhibited the most potent activity with the final mortality rates of 71.4%. Their structure–activity relationships were discussed.

Podophyllotoxin (**1**, Fig. 1) and desoxypodophyllotoxin (**2**, Fig. 1) containing four consecutive chiral centers (labeled C-1–C-4) and five rings (labeled A–E), are two naturally occurring non-alkaloid toxins isolated from the roots and rhizomes of *Podophyllum peltatum* and *P. hexandrum*.[1–3] Compound **1** has been used as a lead compound for preparation of potent anticancer drugs such as etoposide, teniposide and etoposide phosphate.[2–6] Additionally, compounds **1** and **2**, and their derivatives also showed the interesting insecticidal and antifungal activities in the agricultural field.[7–12] On the other hand, the long-term, repeated and unreasonable application of synthetic agrochemicals has resulted in the development of resistance in insect pest populations and environmental problems.[13] Recently, for new pesticides originated from plant secondary metabolites may cause less or slower emerging resistance and lower environmental pollution, discovery of new pesticides directly or indirectly from plant secondary metabolites has been one of the important fields in research and development of agrochemicals.[14–20]

More recently, by structural modifications of compound **1**, a series of ester derivatives of 2′(2′,6′)-(di)halogenopodophyllotoxins (**3**, Fig. 1) and 2α-chloro-2′(2′,6′)-(di)halogenopicropodophyllotoxins (**4**, Fig. 1) have been prepared.[21,22] In addition, a series of ester derivatives (**5**, Fig. 1) of compound **2** were also synthesized.[23] To our delight, some compounds showed more potent insecticidal activity than toosendanin, a commercial botanical insecticide isolated from *Melia azedarach*.[21–23] In our previous reports, we found that when a chlorine atom was introduced at the C-2′ position on the E-ring of 4′-demethoxyepipodophyllotoxin (**6**, Fig. 1), the corresponding 2′-chloro-4′-demethoxyepipodophyllotoxin (**7**, Fig. 1) showed more promising insecticidal activity than compound **6** against early 3rd-instar larvae of *Mythimna separata* Walker;[24] however, 2′,6′-dichloro-4′-demethylepipodophyllotoxin (**7′**, Fig. 1) was not obtained by dichlorination of compound **6** in the presence of NCS.[24] Based upon the above results, herein we synthesized two series of ester derivatives (**8** and **9**, Scheme 1) of 4′-demethoxyepipodophyllotoxin/2′-chloro-4′-demethoxyepipodophyllotoxin as insecticidal agents against *M. separata*.

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http://dx.doi.org/10.1016/j.bmcl.2016.12.026
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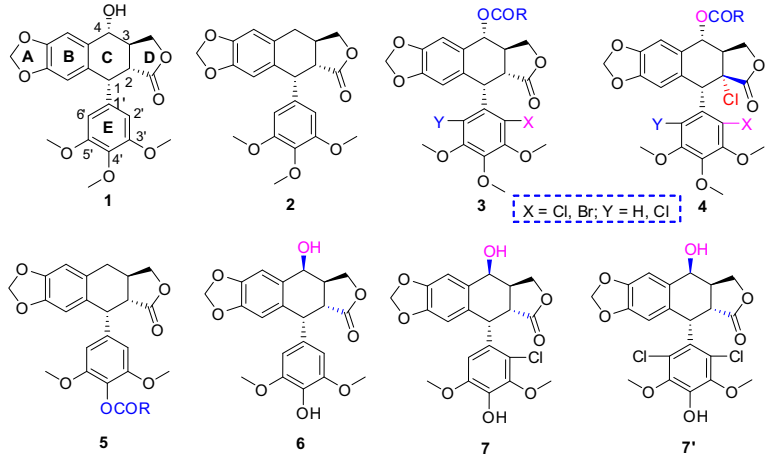
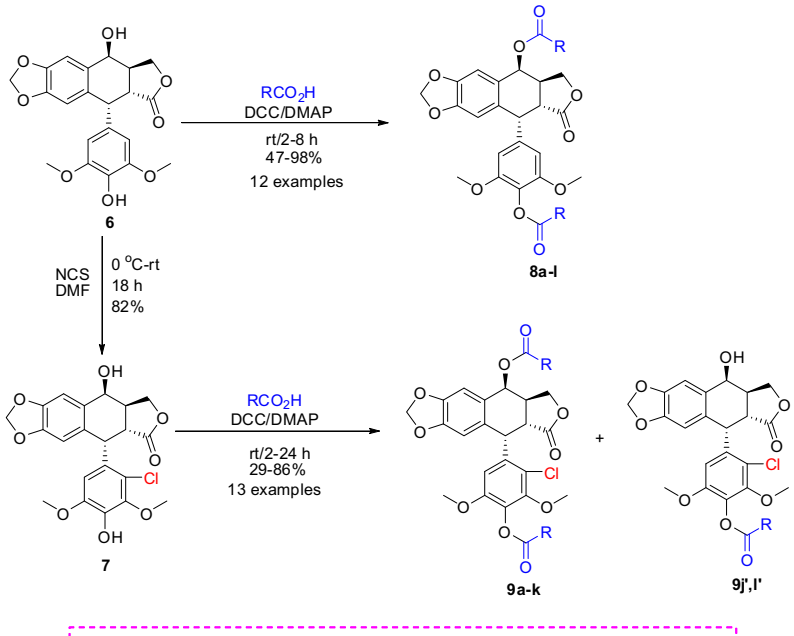


Fig. 1. Chemical structures of podophyllotoxin (**1**), desoxypodophyllotoxin (**2**) and their derivatives (**3**–**7′**).



Scheme 1. Preparation of some ester derivatives of 4′-demethoxyepipodophyllotoxin/2′-chloro-4′-demethoxyepipodophyllotoxin (**8a–l, 9a–k** and **9j′,l′**).

As shown in Scheme 1, first, 2′-chloro-4′-demethoxyepipodophyllotoxin (**7**, mp: 142–144 °C; lit.:[24], 140–142 °C) was smoothly synthesized by reaction of **6** with 1.15 equiv. of *N*-chlorosuccinimide (NCS).[24] Then, target compounds **8a–l** were smoothly prepared by reaction of **6** with the corresponding carboxylic acids in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 4-*N*,*N*-dimethylaminopyridine (DMAP). However, when compound **7** reacted with the corresponding carboxylic acids in the presence of DCC and DMAP, in addition to 4,4′-diester derivatives (**9a–k**), 4′-monoester derivatives (**9j′** and **9l′**) were obtained. The structures of the target compounds were well characterized by ^{1}H NMR, IR, optical rotation and mp (See the supplementary data).[25] The three-dimensional structural information of **8j** was unambiguously confirmed by single-crystal X-ray diffraction (Fig. 2). It obviously demonstrated that two 4-fluorobenzoyloxy groups were at the C-4 and C-4′ positions of **8j**; 4-fluorobenzoyloxy at the C-4

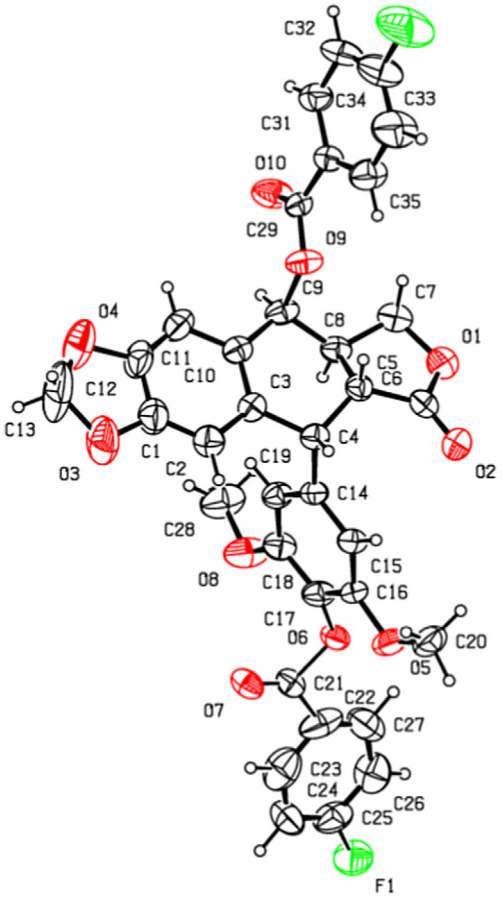


Fig. 2. X-ray crystal structure of compound **8j**.

position was in β configuration; and its lactone was also *trans* configuration. Crystallographic data (excluding structure factors) for the structure of **8j** has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 1503846. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

The assignment of substituent position of monoester derivatives **9j′** and **9l′** was based on the chemical shifts of H-4. As shown in Fig. 3, once the C4-OH groups of **8j**, **8l**, and **9j** were substituted by the acyloxy ones, their corresponding chemical shifts of H-4 moved to low field with the values of 6.38, 6.38, and 6.42 ppm, respectively. Here the chemical shifts of H-4 of **9j′** and **9l′** were at 4.91 ppm, so their C4-OH groups were free. Obviously, the C4′-OH groups of **9j′** and **9l′** were substituted by the acyloxy ones.

The insecticidal activity of some ester derivatives of 4′-demethoxyepipodophyllotoxin/2′-chloro-4′-demethoxyepipodophyllotoxin (**8a–l, 9a–k** and **9j′,l′**) against the pre-third-instar larvae of *M. separata* was tested by the leaf-dipping method at the concentration of 1 mg/mL.[26] Toosendanin was used as the positive control at 1 mg/mL. As shown in Table 1, the corresponding mortality rates caused by these compounds after 36 days were higher than those after 10 and 25 days. For example, the corrected mortality rate of **6** against *M. separata* after 10 days was only 3.3%, and after 25 days, the corresponding mortality rate of **6** against *M. separata* was increased to 26.7%; however, after 36 days, the corresponding mortality rate of **6** against *M. separata* was 42.8%, which was nearly 13-fold of that after 10 days. That is, these compounds showed delayed insecticidal activity as in our previous reports.[21–24] Among all derivatives, compounds **7**; **8a**,**b**,**d–h**,**k**,**l**; **9a**,**b**,**e–g**,**k**; and **9j′**,**l′** showed more potent insecticidal activity than toosendanin against *M. separata*; especially compounds **8k** and **9k** exhibited the most potent insecticidal activity with the final mortality rates (FMRs) of 71.4%. Meanwhile, times for 50% mortality of **7**; **8a**,**b**,**d–h**,**k**,**l**; **9a**,**b**,**e–g**,**k**; and **9j′**,**l′** against *M. separata* were 27, 17, 30, 36, 33, 36, 36, 33, 19, 36, 19, 17, 19, 16, 36, 30, 28 and 28 days, respectively (Fig. 4). Obviously, after structural modifications of **7**, the times for 50% mortality of **9a**,**b**,**e**,**f** were shortened to 16–19 days; whereas the times for 50% mortality of **9g** were prolonged to 36 days when compared with that of **7**. On the other hand, the symptoms of the tested *M. separata* were also characterized as follows: due to feeding too much treated leaves during the first 48 h, some larvae died slowly with the slim and wrinkled bodies (Fig. 5); some larvae of the treated groups molted to malformed pupae and died during the stage of pupation (Fig. 6); malformed moths with imperfect wings also appeared in the treated groups (Fig. 7).

As described in Table 1, when two acetyloxy or propionyloxy were introduced at the C-4 and C-4′ positions of **6** and **7**, the FMRs of the corresponding compounds **8a**, **8b**, **9a**, and **9b** were 64.3%, 60.7%, 64.3% and 60.7%, respectively; whereas when two *n*-butyryloxy were introduced at the C-4 and C-4′ positions of **6** and **7**, the FMRs of the corresponding compounds **8c**, and **9c** were 46.4% and 42.8%, respectively. It demonstrated that the length of side chain of the alkylacyloxy at the C-4 and C-4′ positions was very important for their insecticidal activity. On the other hand, to the arylacyloxy series **8d–k**, **9d–k** and **9j′**,**l′**, the electronic effects on their activity was not obvious. Introduction of the acyloxy at the C-4 and C-4′ positions of **6** led to more promising derivatives as compared with **6**; however, when the acyloxy groups were introduced at the C-4 and C-4′ positions of **7**, only compounds **9a,b,e,f,k** showed more potent insecticidal activity than **7**. Introduction of the acyloxy groups containing a heterocyclic ring at the C-4 and C-4′ positions of **6** or **7** could not afford the most potent compounds (*e.g.*, **8h,i** and **9h,i**). Interestingly, when two 4-nitrobenzoyloxy groups were introduced at the C-4 and C-4′ positions of **6** or **7**, the FMRs of the corresponding compounds **8k** and **9k** were all 71.4%. Compound **9j′** containing 4-fluorobenzoyloxy group at its C-4′ position, showed more potent insecticidal activity than that of **9j** containing two 4-fluorobenzoyloxy groups at its C-4 and C-4′ positions.

In summary, two series of ester derivatives of 4′-demethoxyepipodophyllotoxin/2′-chloro-4′-demethoxyepipodophyllotoxin were prepared, and well characterized by ^{1}H NMR, IR, optical rotation and mp. Especially the precise three-dimensional structural information of **8j** was determined by single-crystal X-ray diffraction. These compounds showed delayed insecticidal activity against *M. separata*. Among all derivatives, compounds **8k** and **9k** exhibited the most potent insecticidal activity with the final mortality rates of 71.4%. Their structure-insecticidal activity relationship was also observed. This will lay the foundation for further design and structural modification of podophyllotoxin derivatives as insecticidal agents.

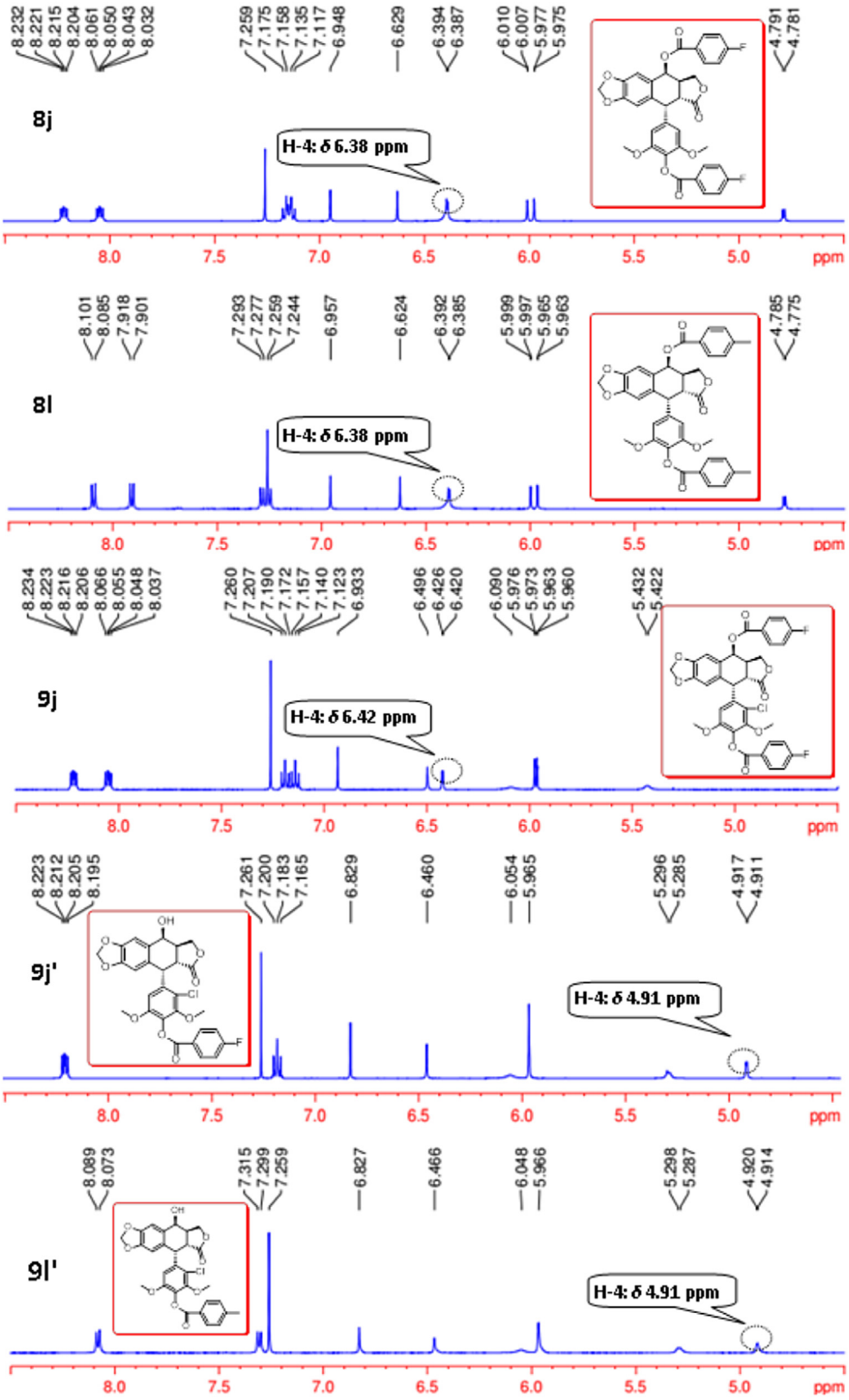


Fig. 3. Comparison of partial ^{1}H NMR spectra of **8j**,**l** and **9j** with **9j′**,**l′**.

Table 1
Insecticidal activity of compounds **6**, **7**, **8a–l**, **9a–k** and **9j′,l′** against the pre-third-instar larvae of *M. separata* on leaves treated with a concentration of 1 mg/mL.

Compound	Corrected mortality rate (%)[a]		
	10 days	25 days	36 days
6	3.3 ± 3.3	26.7 ± 3.3	42.8 ± 6.7
7	30.0 ± 0	46.7 ± 3.3	53.6 ± 3.3
8a	36.7 ± 3.3	53.3 ± 3.3	64.3 ± 3.3
8b	23.3 ± 3.3	36.7 ± 3.3	60.7 ± 3.3
8c	20.0 ± 5.8	33.3 ± 3.3	46.4 ± 0
8d	16.7 ± 3.3	30.0 ± 5.8	57.1 ± 0
8e	30.0 ± 5.8	43.3 ± 3.3	64.3 ± 3.3
8f	20.0 ± 0	33.3 ± 3.3	53.6 ± 3.3
8g	13.3 ± 6.7	36.7 ± 3.3	53.6 ± 3.3
8h	13.3 ± 3.3	36.7 ± 3.3	53.6 ± 3.3
8i	20.0 ± 5.8	33.3 ± 6.7	50.0 ± 3.3
8j	3.3 ± 3.3	30.0 ± 5.8	50.0 ± 6.7
8k	30.0 ± 0	53.3 ± 3.3	71.4 ± 6.7
8l	20.0 ± 5.8	33.3 ± 6.7	57.1 ± 5.8
9a	36.7 ± 3.3	53.3 ± 3.3	64.3 ± 3.3
9b	30.0 ± 5.8	50.0 ± 5.8	60.7 ± 3.3
9c	20.0 ± 0	40.0 ± 0	42.8 ± 3.3
9d	13.3 ± 6.7	30.0 ± 5.8	42.8 ± 3.3
9e	26.7 ± 3.3	53.3 ± 3.3	67.8 ± 5.8
9f	30.0 ± 5.8	50.0 ± 5.8	64.3 ± 6.7
9g	13.3 ± 6.7	26.7 ± 3.3	57.1 ± 5.8
9h	16.7 ± 3.3	30.0 ± 5.8	46.4 ± 5.8
9i	23.3 ± 3.3	33.3 ± 3.3	50.0 ± 6.7
9j	36.7 ± 3.3	43.3 ± 3.3	46.4 ± 5.8
9k	30.0 ± 0	46.7 ± 3.3	71.4 ± 3.3
9j′	36.7 ± 6.7	43.3 ± 3.3	53.6 ± 3.3
9l′	20.0 ± 5.8	46.7 ± 6.7	60.7 ± 3.3
Toosendanin	20.0 ± 5.8	36.7 ± 3.3	46.4 ± 0
Blank control	0 ± 0	0 ± 0	6.7 ± 3.3[b]

[a] Values are the mean ± SD of three replicates.
[b] After 33 days.

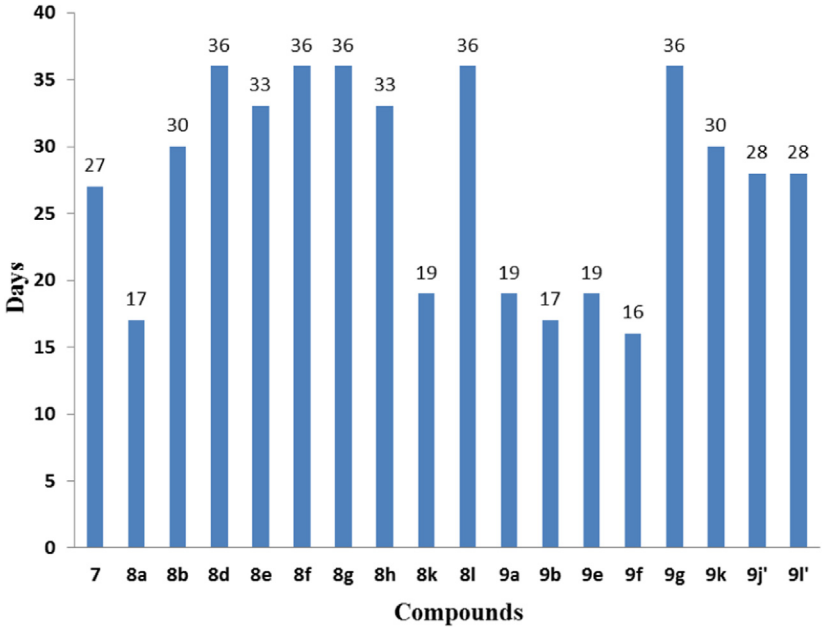


Fig. 4. Lethal times for 50% mortality of compounds **7**; **8a**,**b**,**d–h**,**k**,**l**; **9a**,**b**,**e–g**,**k**; and **9j′**,**l′** against *M. separata*.

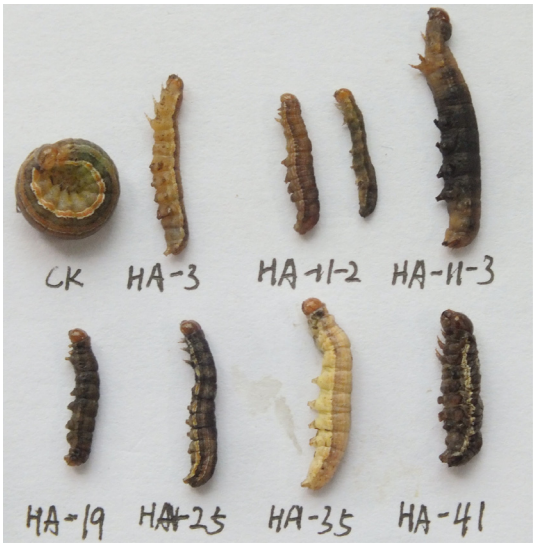


Fig. 5. The representative abnormal larvae pictures of **9a** (HA-3), **9j** (HA-11-2), **9j′** (HA-11-3), **9f** (HA-19), **8a** (HA-25), **8k** (HA-35) and **9k** (HA-41) during the larval period (**CK**: blank control group).

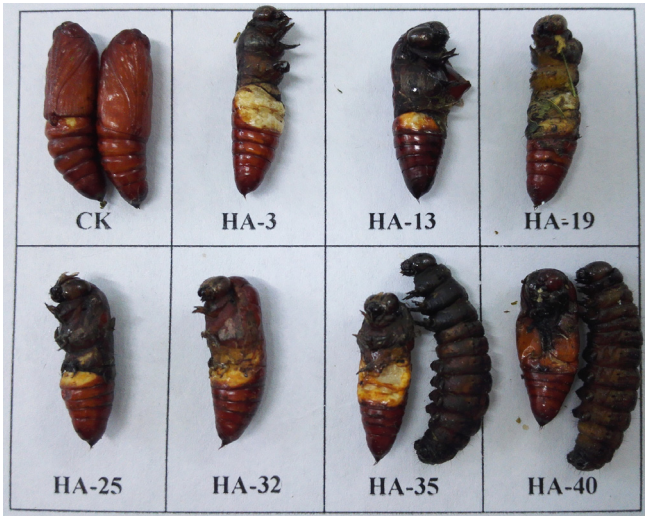


Fig. 6. The representative malformed pupae pictures of **9a** (HA-3), **9d** (HA-13), **9f** (HA-19), **8a** (HA-25), **8h** (HA-32), **8k** (HA-35) and **9e** (HA-40) during the pupation period (**CK**: blank control group).

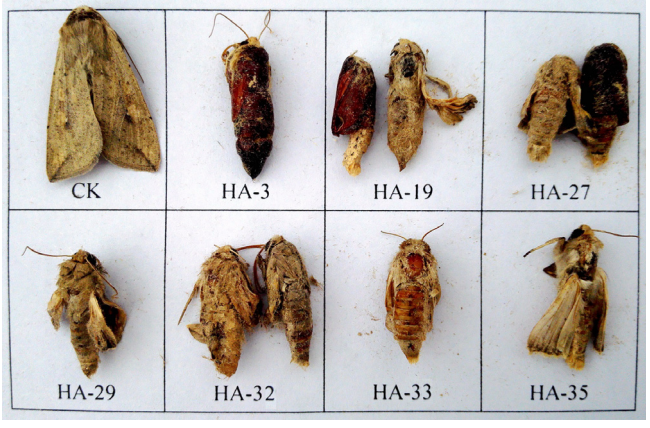


Fig. 7. The representative malformed moth pictures of **9a** (HA-3), **9f** (HA-19), **8c** (HA-27), **8g** (HA-29), **8h** (HA-32), **8i** (HA-33) and **8k** (HA-35) during the emergence period (**CK**: blank control group).

Acknowledgments

The present research was partly supported by National Natural Science Foundation of China (No. 31672071), and Special Funds of Central Colleges Basic Scientific Research Operating Expenses (No. 2452015096) to H.X.

A. Supplementary data

Supplementary data (experimental procedures and spectral data for all new products) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.12.026.

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25. Representative spectral data for **8j**, **8l**, **9j**, **9j′** and **9l′**: Compound **8j**: Yield: 74%, white solid, mp = 135–137 °C; $[\alpha]_D^{20} = -37$ (*c* 2.8 mg/mL, $CHCl_3$); IR cm^{-1} (KBr): 3080, 2943, 1779, 1746, 1704, 1602, 1484, 1267, 1130, 857, 761; ^1H NMR (500 MHz, $CDCl_3$) *δ*: 8.20-8.23 (m, 2H, Ar-H), 8.03-8.06 (m, 2H, Ar-H), 7.11–7.17 (m, 4H, Ar-H), 6.94 (s, 1H, H-5), 6.62 (s, 1H, H-8), 6.38–6.39 (m, 3H, H-4, H-2′, 6′), 5.97 (dd, *J* = 1.5, 16.5 Hz, 2H, OCH_2O), 4.78 (d, *J* = 5.0 Hz, 1H, H-1), 4.40–4.44 (m, 1H, H-11), 3.94–3.98 (m, 1H, H-11), 3.70 (s, 6H, 3′, 5′-OCH_3), 3.34 (dd, *J* = 5.0, 14.0 Hz, 1H, H-2), 3.06–3.14 (m, 1H, H-3). Compound **8l**: Yield: 65%, white solid, mp = 253–255 °C; $[\alpha]_D^{20} = -35$ (*c* 2.5 mg/mL, $CHCl_3$); IR cm^{-1} (KBr): 3037, 2911, 1779, 1742, 1704, 1606, 1485, 1267, 1131, 858, 749; ^1H NMR (500 MHz, $CDCl_3$) *δ*: 8.08–8.10 (m, 2H, Ar-H), 7.90–7.91 (m, 2H, Ar-H), 7.24–7.29 (m, 4H, Ar-H), 6.95 (s, 1H, H-5), 6.62 (s, 1H, H-8), 6.38–6.39 (m, 3H, H-4, H-2′, 6′), 5.96 (dd, *J* = 1.0, 17.0 Hz, 2H, OCH_2O), 4.77 (d, *J* = 5.0 Hz, 1H, H-1), 4.39–4.43 (m, 1H, H-11), 3.96–4.00 (m, 1H, H-11), 3.69 (s, 6H, 3′, 5′-OCH_3), 3.36 (dd, *J* = 5.0, 14.0 Hz, 1H, H-2), 3.06–3.13 (m, 1H, H-3), 2.43 (s, 3H, PhC$\underline{H}_3$), 2.42 (s, 3H, PhC$\underline{H}_3$). Compound **9j**: Yield: 34%, white solid, mp = 158–160 °C; $[\alpha]_D^{20} = -34$ (*c* 3.2 mg/mL, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) *δ*: 8.20–8.23 (m, 2H, Ar-H), 8.03–8.06 (m, 2H, Ar-H), 7.17–7.20 (m, 2H, Ar-H), 7.12–7.15 (m, 2H, Ar-H), 6.93 (s, 1H, H-5), 6.49 (s, 1H, H-8), 6.42 (d, *J* = 3.0 Hz, 1H, H-4), 6.09 (s, 1H, H-6′), 5.96 (dd, *J* = 1.5, 6.5 Hz, 2H, OCH_2O), 5.42 (d, *J* = 5.0 Hz, 1H, H-1), 4.46–4.49 (m, 1H, H-11), 3.96–4.00 (m, 1H, H-11), 3.88 (s, 3H, OCH_3), 3.59 (s, 3H, OCH_3), 3.47 (dd, *J* = 6.0, 15.0 Hz, 1H, H-2), 3.26–3.32 (m, 1H, H-3). Compound **9j′**: Yield: 29%, white solid, mp = 153–155 °C; $[\alpha]_D^{20} = -19$ (*c* 2.8 mg/mL, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) *δ*: 8.19–8.22 (m, 2H, Ar-H), 7.16–7.26 (m, 2H, Ar-H), 6.82 (s, 1H, H-5), 6.46 (s, 1H, H-8), 6.05 (s, 1H, H-6′), 5.96 (s, 2H, OCH_2O), 5.28 (d, *J* = 5.5 Hz, 1H, H-1), 4.91 (d, *J* = 3.0 Hz, 1H, H-4), 4.35–4.42 (m, 2H, H-11), 3.87 (s, 3H, OCH_3), 3.55 (s, 3H, OCH_3), 3.49 (s, 3H, OCH_3), 3.45 (dd, *J* = 6.5, 14.5 Hz, 1H, H-2), 3.01–3.08 (m, 1H, H-3). Compound **9l′**: Yield: 65%, white solid, mp = 161–163 °C; $[\alpha]_D^{20} = -21$ (*c* 2.6 mg/mL, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) *δ*: 8.07 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.29 (d, *J* = 8.0 Hz, 2H, Ar-H), 6.82 (s, 1H, H-5), 6.46 (s, 1H, H-8), 6.04 (s, 1H, H-6′), 5.96 (s, 2H, OCH_2O), 5.28 (d, *J* = 5.5 Hz, 1H, H-1), 4.91 (d, *J* = 3.0 Hz, 1H, H-4), 4.36–4.43 (m, 2H, H-11), 3.87 (s, 3H, OCH_3), 3.55 (s, 3H, OCH_3), 3.44 (dd, *J* = 7.0, 14.5 Hz, 1H, H-2), 3.04–3.08 (m, 1H, H-3), 2.45 (s, 3H, PhC$\underline{H}_3$).
26. *Biological assay*: The insecticidal activity of compounds **6**, **7**, **8a–l**, **9a–k** and **9j′**,**l′** against the pre-third-instar larvae of *Mythimna separata* was assessed by leaf-dipping method. For each compound, 30 pre-third-instar larvae (10 larvae per group) were used. Acetone solutions of compounds **6**, **7**, **8a–l**, **9a–k** and **9j′**,**l′**

were prepared at 1 mg/mL. Toosendanin was used as the positive control at 1 mg/mL. Fresh wheat leaves were dipped into the corresponding solution for 3 s, then taken out, and dried in a room. Leaves treated with acetone alone were used as a blank control group. Several treated leaves were kept in each dish, in which 10 larvae were raised. If the treated leaves were consumed, additional treated leaves were added to the dish. After 48 h, untreated fresh leaves were added to all dishes until adult emergence. The experiment was carried out at 25 ± 2 °C and on 12 h/12 h (light/dark) photoperiod. Their corrected mortality rate values were calculated by the formula:Corrected mortality rate (%) = $(T - C) \times 100/(100\% - C)$where T is the mortality rate in the treated group expressed as a percentage and C is the mortality rate in the untreated group expressed as a percentage.

Please cite this article in press as: Huang J., et al. Bioorg. Med. Chem. Lett. (2016), http://dx.doi.org/10.1016/j.bmcl.2016.12.026