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Synthesis and Insecticidal Activity of New Deoxypodophyllotoxin-Based Phenazine Analogues against *Mythimna separata* Walker

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ABSTRACT: In continuation of our program aimed at the discovery and development of natural-product-based insecticidal agents, a series of new deoxypodophyllotoxin-based phenazine analogues modified in their E-ring were prepared, and their structures were well characterized by ¹H NMR, HRMS, ESI-MS, IR, optical rotation, and mp. The absolute steric configuration of one key isomer was unambiguously confirmed by X-ray crystallography. Their insecticidal activity was examined against the pre-third-instar larvae of oriental armyworm, *Mythimna separata* (Walker) in vivo at the concentration of 1 mg/mL. All derivatives showed delayed insecticidal activity. Especially compound **9i**, containing *p*-methoxybenzoylamnio at the C-9' position of deoxypodophyllotoxin-based phenazine fragment, exhibited the most promising insecticidal activity with the final mortality rate of 72.4%. According to the symptoms of the tested *M. separata*, the derivatives likely displayed an antimolting hormone effect. In addition, preliminary structure–activity relationships were observed. These suggested that the proper length of the side chain of alkylacylamino might be important for their insecticidal activity, and introduction of the acylamino groups at the C-9' position of deoxypodophyllotoxin-based phenazine fragment usually afforded more potent compounds than those containing the same ones at the C-10' position. This will pave the way for further design, structural modification, and development of deoxypodophyllotoxin-based derivatives as insecticidal agents.

KEYWORDS: podophyllotoxin, deoxypodophyllotoxin, phenazine, structural modification, botanical insecticide, insecticidal activity, Mythimna separata Walker

INTRODUCTION

Repeat application of synthetic agrochemicals over years has resulted in the development of resistance in insect pest populations and environmental problems.^{1,2} On the other hand, plant secondary metabolites result from the interaction between plants and the environment (life and nonlife) during the long period of evolution in plants, and pesticides produced from plant secondary metabolites may lead to less or slower resistance development and lower pollution.³ Consequently, the discovery of new insecticidal compounds directly from plant secondary metabolites or by using them as the lead compounds for further structural modifications has recently become an important area of research and development of new pesticides.^{4–10} For example, nowadays some botanical insecticides such as nicotine, pyrethrum, and neem extracts are made by plants as defenses against insect pests.¹¹

Podophyllotoxin (1, Figure 1), a main secondary metabolite isolated from the roots and rhizomes of *Podophyllum* species such as *Podophyllum hexandrum* and *Podophyllum peltatum*, has been used as a unique lead compound for the preparation of potent antitumor drugs such as etoposide (VP-16), teniposide (VM-26), and etopophos against various cancers including small cell lung cancer, testicular carcinoma, lymphoma, and Kaposi's sarcoma.¹²⁻¹⁵ Additionally, extensive chemical modification of compound 1 has been reported leading to insecticidal and antifungal agents.¹⁶⁻²¹

More recently, we have studied the insecticidal activity of 2β chloropodophyllotoxin or $2\alpha/\beta$ -bromopodophyllotoxin derivatives modified in the C-ring and 4-deoxypodophyllotoxin (2, Figure 1) derivatives modified in the E-ring and found some



Figure 1. Chemical structures of podophyllotoxin (1) and deoxypodophyllotoxin (2).

compounds exhibited insecticidal activity equal to or higher than that of toosendanin, a commercial botanical insecticide isolated from *Melia azedarach*.^{22–27} On the other hand, a series of new phenazine derivatives of 4β -acyloxypodophyllotoxin modified in the E-ring were prepared, and some compounds showed potent insecticidal activity.²⁸ Encouraged by the abovementioned results, we herein wanted to prepare a series of 4deoxypodophyllotoxin-based phenazine derivatives by introduction of the phenazine fragment in the E-ring (Scheme 1). Their insecticidal activity was evaluated against the pre-thirdinstar larvae of oriental armyworm, *Mythimna separata* (Walker) in vivo.

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Scheme 1^{*a*}



"Reagents and conditions: (a) H₂, 10% Pd/C, 95 °C, 5 h, 66%; (b) concd HNO₃, 0 °C, 4 min, 73%; (c) 1,2-diaminobenzene, room temperature, 0.5 h, 82%; (d) 4-nitro-1,2-phenylenediamine, room temperature, 5 h, 29% (5) and 59% (6); (e) SnCl₂·2H₂O, N₂, reflux, 7 h, 81% (7) or 85% (8); (f) RCOCl (11a-e and h), Et₃N, 0 °C, room temperature, 1-9.5 h, 28-89%, or RCOCl (11f,g,i, and j), Et₃N, 0 °C, room temperature, reflux, 3-80 h, 32-92%.

MATERIALS AND METHODS

General. Podophyllotoxin was purchased from Gansu Gerui Medicinal Materials Co., Ltd. All reagents and solvents were of reagent grade or purified according to standard methods before use. Analytical thin-layer chromatography (TLC) and preparative thin-layer chromatography (PTLC) were performed with silica gel plates using silica gel 60 GF₂₅₄ (Qingdao Haiyang Chemical Co., Ltd.). Silica gel column chromatography was performed with silica gel 200-300 mesh (Qingdao Haiyang Chemical Co., Ltd.). Melting points (mp) were determined on an XT-4 digital melting point apparatus (Beijing Tech Instrument Co., Ltd.) and were uncorrected. Infrared spectra (IR) were recorded on a Bruker TENSOR 27 spectrometer. Proton nuclear magnetic resonance spectra (¹H NMR) were recorded on a Bruker

Avance DMX 400 or 500 MHz instrument in CDCl₃ using tetramethylsilane (TMS) as the internal standard. High-resolution mass spectra (HR-MS) were carried out with an IonSpec 4.7 T FTMS instrument.

Synthesis of 4-Deoxypodophyllotoxin (2). A mixture of 10% palladium/carbon (12 g) and podophyllotoxin (1, 16 g, 38.6 mmol) in acetic acid solution (150 mL) was stirred at 95 °C under 2 atm of hydrogen for 5 h. After the catalyst and solvent were removed, the residue was separated by silica gel column chromatography to give the crude product, which was further purified by recrystallization from methanol to afford 2 in 66% yield as a white solid: mp = 165-167 °C [literature, 166–168 °C];²⁹ $[\alpha]^{20}_{D} = -116$ (c 1 mg/mL, CHCl₃); IR 2892, 2831, 1763, 1587, 1482, 1457, 1223, 1120, 925, 768 cm⁻¹; ¹H

NMR (400 MHz, CDCl₃) δ 6.67 (s, 1H, H-5), 6.52 (s, 1H, H-8), 6.34 (s, 2H, H-2',6'), 5.93 (d, *J* = 8.0 Hz, 2H, OCH₂O), 4.60 (s, 1H, H-1), 4.43–4.48 (m, 1H, H-11), 3.89–3.95 (m, 1H, H-11), 3.79 (s, 3H, 4'-OCH₃), 3.75 (s, 6H, 3', 5'-OCH₃), 3.06–3.09 (m, 1H, H-4), 2.71–2.78 (m, 3H, H-2,3,4); MS (EI) *m*/*z* (%) 398 (M⁺, 100).

Synthesis of 4',5'-Didemethoxy-4',5'-dioxodeoxypodophyllotoxin (3). Concentrated nitric acid (1.2 mL) was rapidly added to a solution of 2 (60 mg, 0.15 mmol) in propionic acid (1.2 mL) at 0 °C. After stirring at 0 °C for 4 min, the dark red solution was poured into water (10 mL) and extracted with CH_2Cl_2 (2 × 20 mL). The organic layer was then washed with saturated aqueous NaHCO3 and brine, dried over Na2SO4, concentrated in vacuo, and purified by recrystallization from CH2Cl2 and petroleum ether to afford 3 in 73% yield as a crimson solid: mp = 184–186 °C [literature, 182–184 °C];³⁰ $[\alpha]_{D}^{20} = -35$ (c 2.4 mg/mL, CHCl₃); ¹H NMR (400 MHz, $CDCl_3$) δ 6.62 (s, 1H, H-6'), 6.51 (d, J = 1.2 Hz, 1H, H-5), 6.50 (s, 1H, H-8), 5.95 (dd, I = 1.2, 6.4 Hz, 2H, OCH₂O), 5.35 (s, 1H, H-2'), 4.59-4.63 (m, 1H, H-11), 4.26 (d, J = 5.6 Hz, 1H, H-1), 4.01-4.05 (m, 1H, H-11), 3.84 (s, 3H, 3'-OCH₃), 3.03-3.05 (m, 1H, H-4), 2.88-2.92 (m, 1H, H-2), 2.71-2.79 (m, 2H, H-3,4). HRMS (ESI) calcd for $C_{20}H_{17}O_7$ ([M + H]⁺), 369.0968; found, 369.0965.

Synthesis of Phenazine Derivative of Deoxypodophyllotoxin (4). A solution of *o*-phenylenediamine (35.6 mg, 0.33 mmol) in CHCl₃ (5 mL) was added dropwise to a solution of 3 (110 mg, 0.3 mmol) in CHCl₃ (5 mL) at room temperature. After 0.5 h, the mixture was concentrated in vacuo and purified by PTLC to afford 4 in 82% yield as a yellow solid: mp = 127–129 °C; $[\alpha]^{20}_{D}$ = +5.3 (*c* 2.2 mg/mL, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 8.38–8.40 (m, 1H), 8.10 (d, *J* = 8.0 Hz, 1H), 7.80–7.81 (m, 2H), 7.56 (s, 1H, H-6'), 7.06 (s, 1H, H-2'), 6.71 (s, 1H, H-5), 6.56 (s, 1H, H-8), 5.94 (d, *J* = 8.0 Hz, 2H, OCH₂O), 4.88 (d, *J* = 4.0 Hz, 1H, H-1), 4.41–4.44 (m, 1H, H-11), 4.24 (s, 3H, 3'-OCH₃), 3.95–3.99 (m, 1H, H-11), 3.13–3.15 (m, 1H, H-4), 2.80–2.96 (m, 3H, H-2,3,4). HRMS (ESI) calcd for C₂₆H₂₁N₂O₅ ([M + H]⁺), 441.1445; found, 441.1434.

Synthesis of Phenazine Derivative of Deoxypodophyllotoxin (5 and 6). A solution of 4-nitro-1,2-phenylenediamine (50.5 mg, 0.33 mmol) in CHCl₃ (5 mL) was added dropwise to a solution of 3 (110 mg, 0.3 mmol) in CHCl₃ (5 mL) at room temperature. After 5 h, the mixture was concentrated in vacuo and purified by PTLC to afford 5 and 6 in 29 and 59% yields, respectively.

Data for Compound 5: orange solid; mp = $174-176 \, ^{\circ}C$; $[\alpha]^{20}_{D}$ = +28 (c 3.1 mg/mL, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 9.37 (d, J = 2.4 Hz, 1H), 8.53 (dd, J = 2.4, 9.2 Hz, 1H), 8.24 (d, J = 9.6 Hz, 1H), 7.62 (d, J = 1.2 Hz, 1H, H-6'), 7.10 (d, J = 0.8 Hz, 1H, H-2'), 6.74 (s, 1H, H-5), 6.55 (s, 1H, H-8), 5.95 (dd, J = 1.2, 4.4 Hz, 2H, OCH₂O), 4.90 (d, J = 5.2 Hz, 1H, H-1), 4.43-4.47 (m, 1H, H-11), 4.26 (s, 3H, 3'-OCH₃), 3.98-4.02 (m, 1H, H-11), 3.15-3.18 (m, 1H, H-4), 2.97-3.01(m, 1H, H-2), 2.80-2.89 (m, 2H, H-3,4). HRMS (ESI) calcd for C₂₆H₂₀N₃O₇ ([M + H]⁺), 486.1295; found, 486.1287.

Data for Compound **6**: orange solid; mp = $272-274 \,^{\circ}$ C; $[\alpha]^{20}_{D}$ = +31 (*c* 4.4 mg/mL, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 9.06 (s, 1H), 8.53 (s, 2H), 7.65 (s, 1H, H-6'), 7.10 (s, 1H, H-2'), 6.75 (s, 1H, H-5), 6.55 (s, 1H, H-8), 5.97 (s, 2H, OCH₂O), 4.89 (d, *J* = 4.8 Hz, 1H, H-1), 4.44-4.48 (m, 1H, H-11), 4.26 (s, 3H, 3'-OCH₃), 3.98-4.03 (m, 1H, H-11), 3.17-3.19 (m, 1H, H-4), 2.97-3.01 (m, 1H, H-2), 2.84-2.89 (m, 2H, H-3, 4). HRMS (ESI) calcd for C₂₆H₂₀N₃O₇ ([M + H]⁺), 486.1295; found, 486.1286.

Synthesis of Compounds 7 and 8. A mixture of 5 or 6 (121 mg, 0.25 mmol) and $SnCl_2 \cdot 2H_2O$ (395 mg, 1.75 mmol) in ethyl acetate (15 mL) was refluxed under N_2 for 7 h. After cooling, the pH value of the mixture (the initial pH value of the mixture was 3–4) was adjusted to 8–9 by the addition of saturated aqueous NaHCO₃ (30 mL). Then the mixture was filtered, and the water layer was extracted with ethyl acetate (3 × 50 mL). Then the combined organic phase was dried over anhydrous Na₂SO₄, concentrated in vacuo, and purified by PTLC to give 7 in 81% yield or 8 in 85% yield.

Data for Compound 7: rufous solid; mp = $184-186 \,^{\circ}\text{C}$; $[\alpha]^{20}_{D}$ = +39 (c 3.5 mg/mL, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.88 (d, J = 9.2 Hz, 1H), 7.48 (s, 1H, H-6'), 7.34 (s, 1H), 7.29 (s, 1H), 6.94 (s, 1H, H-2'), 6.69 (s, 1H, H-5), 6.56 (s, 1H, H-8), 5.93 (d, J = 7.6 Hz, 1H), 7.6 (s, 1H), 7.6 (s,

2H, OCH₂O), 4.84 (s, 1H, H-1), 4.40–4.43 (m, 1H, H-11), 4.34 (s, 2H, NH₂), 4.19 (s, 3H, 3'-OCH₃), 3.94–3.98 (m, 1H, H-11), 3.10–3.13 (m, 1H, H-4), 2.77–2.94 (m, 3H, H-2,3,4). HRMS (ESI) calcd for $C_{26}H_{22}N_3O_5$ ([M + H]⁺), 456.1554; found, 456.1543.

Data for Compound **8**: rufous solid; mp = 190–192 °C; $[\alpha]^{20}_{D}$ = +27 (*c* 3.9 mg/mL, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.14 (d, *J* = 8.8 Hz, 1H), 7.40 (s, 1H, H-6'), 7.24 (s, 1H), 7.03 (s, 1H, H-2'), 6.90 (s, 1H), 6.69 (s, 1H, H-5), 6.56 (s, 1H, H-8), 5.92 (d, *J* = 7.6 Hz, 2H, OCH₂O), 4.83 (s, 1H, H-1), 4.40–4.43 (m, 3H, H-11, NH₂), 4.19 (s, 3H, 3'-OCH₃), 3.93–3.97 (m, 1H, H-11), 3.09–3.12 (m, 1H, H-4), 2.76–2.94 (m, 3H, H-2,3,4). HRMS (ESI) calcd for C₂₆H₂₂N₃O₅ ([M + H]⁺), 456.1554; found, 456.1548.

Synthesis of Compounds 9a-c,e-j, 10a-j, and 10g'. To a stirred mixture of 7 or 8 (45.5 mg, 0.1 mmol) and Et₃N (0.12 mmol) in CH₂Cl₂ (5 mL) at 0 °C, was added dropwise a solution of acyl chlorides RCOCl (11a-j, 0.11 mmol) in CH₂Cl₂ (5 mL). After this addition to 11a-e and h, the reaction was allowed to proceed at room temperature, and the mixture was stirred at room temperature for 1–9.5 h, whereas after the addition to 11f,g,i, and j, the reaction was first allowed to proceed at room temperature and then the mixture was refluxed for 3–80 h. Subsequently, CH₂Cl₂ (10 mL) and water (20 mL) were added to the mixture, and the organic phase was separated. The water phase was further extracted with CH₂Cl₂ (2 × 40 mL). Finally, the combined organic phase was washed by brine, dried over anhydrous Na₂SO₄, concentrated in vacuo, and purified by PTLC to give the target compounds.

Data for Compound **9a**: yield = 74%; yellow solid; mp = 280–282 °C; $[\alpha]^{21}_{D}$ = +20 (*c* 3.1 mg/mL, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.13 (d, *J* = 8.8 Hz, 2H), 7.90 (s, 1H), 7.51 (s, 1H, H-6'), 7.05 (s, 1H, H-2'), 6.71 (s, 1H, H-5), 6.61 (s, 1H, H-8), 5.95 (d, *J* = 3.2 Hz, 2H, OCH₂O), 4.87 (d, *J* = 3.6 Hz, 1H, H-1), 4.43–4.46 (m, 1H, H-11), 4.10 (s, 3H, 3'-OCH₃), 3.95–3.99 (m, 1H, H-11), 3.12–3.16 (m, 1H, H-4), 2.79–2.97 (m, 3H, H-2,3,4), 2.11 (s, 3H, CH₃). HRMS (ESI) calcd for C₂₈H₂₄N₃O₆ ([M + H]⁺), 498.1659; found, 498.1652.

Data for Compound **9b**: yield = 87%; yellow solid; mp = 265–267 °C; $[\alpha]^{21}_{D} = +25$ (c 2.2 mg/mL, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 8.65 (s, 1H, NH), 8.57 (s, 1H), 8.09 (d, *J* = 9.5 Hz, 1H), 7.99 (d, *J* = 9.5 Hz, 1H), 7.55 (s, 1H, H-6'), 7.03 (s, 1H, H-2'), 6.71 (s, 1H, H-5), 6.56 (s, 1H, H-8), 5.94 (d, *J* = 8.0 Hz, 2H, OCH₂O), 4.87 (d, *J* = 4.5 Hz, 1H, H-1), 4.41–4.44 (m, 1H, H-11), 4.28 (s, 2H, CH₂Cl), 4.22 (s, 3H, 3'-OCH₃), 3.95–3.99 (m, 1H, H-11), 3.13–3.15 (m, 1H, H-4), 2.80–2.96 (m, 3H, H-2,3,4). HRMS (ESI) calcd for C₂₈H₂₃N₃O₆Cl ([M + H]⁺), 532.1269; found, 532.1258.

Data for Compound **9c**: yield = 80%; yellow solid; mp = 274–276 °C; $[\alpha]^{21}_{D} = +27$ (*c* 4.5 mg/mL, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.20 (d, *J* = 8.8 Hz, 2H), 7.97 (s, 1H), 7.51 (s, 1H, H-6'), 7.04 (s, 1H, H-2'), 6.70 (s, 1H, H-5), 6.59 (s, 1H, H-8), 5.94 (d, *J* = 4.4 Hz, 2H, OCH₂O), 4.86 (d, *J* = 4.4 Hz, 1H, H-1), 4.42–4.45 (m, 1H, H-11), 4.14 (s, 3H, 3'-OCH₃), 3.94–3.99 (m, 1H, H-11), 3.11–3.14 (m, 1H, H-4), 2.78–2.96 (m, 3H, H-2,3,4), 2.41–2.50 (m, 2H, CH₃<u>CH₂</u>), 1.22–1.29 (m, 3H, <u>CH₃</u>CH₂). HRMS (ESI) calcd for C₂₉H₂₆N₃O₆ ([M + H]⁺), 512.1816; found, 512.1802.

Data for Compound **9e**: yield = 79%; yellow solid; mp = 167–169 °C; $[\alpha]^{21}_{D} = +16$ (*c* 2.0 mg/mL, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.34 (d, *J* = 2.0 Hz, 1H, NH), 8.20 (d, *J* = 9.2 Hz, 1H), 8.04 (d, *J* = 9.2 Hz, 1H), 7.61 (s, 1H), 7.54 (s, 1H, H-6'), 7.02 (s, 1H, H-2'), 6.71 (s, 1H, H-5), 6.56 (s, 1H, H-8), 5.94 (d, *J* = 6.0 Hz, 2H, OCH₂O), 4.86 (d, *J* = 4.8 Hz, 1H, H-1), 4.41–4.45 (m, 1H, H-11), 4.21 (s, 3H, 3'-OCH₃), 3.95–3.99 (m, 1H, H-11), 3.12–3.15 (m, 1H, H-4), 2.79–2.96 (m, 3H, H-2,3,4), 2.44 (t, *J* = 7.6 Hz, 2H, CH₂CONH), 1.75–1.81 (m, 2H, CH₂CH₂CONH), 1.32–1.41 (m, 6H, CH₃CH₂CH₂CH₂CH₂CH₂CONH), 0.87 (t, *J* = 6.8 Hz, 3H, CH₃). HRMS (ESI) calcd for C₃₃H₃₄N₃O₆ ([M + H]⁺), 568.2442; found, 568.2429.

Data for Compound **9f**: yield = 84%; yellow solid; mp = 215–217 °C; $[\alpha]^{20}_D$ = +34 (*c* 3.1 mg/mL, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 8.49 (s, 1H, NH), 8.28 (d, *J* = 9.5 Hz, 2H), 8.08 (s, 1H), 7.89–7.90 (m, 2H), 7.47–7.52 (m, 4H), 7.03 (s, 1H, H-2'), 6.70 (s, 1H, H-5), 6.57 (s, 1H, H-8), 5.93 (d, *J* = 8.5 Hz, 2H, OCH₂O), 4.86

(d, J = 4.5 Hz, 1H, H-1), 4.42-4.45 (m, 1H, H-11), 4.18 (s, 3H, 3'-OCH₃), 3.95-3.99 (m, 1H, H-11), 3.12-3.14 (m, 1H, H-4), 2.79-2.95 (m, 3H, H-2,3,4). HRMS (ESI) calcd for $C_{33}H_{26}N_3O_6$ ([M + H]⁺), 560.1816; found, 560.1804.

Data for Compound **9g**: yield = 92%; yellow solid; mp = 222–224 °C; $[α]^{21}_D$ = +17 (*c* 3.0 mg/mL, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.48 (s, 1H, NH), 8.34 (d, *J* = 9.6 Hz, 1H), 8.03–8.14 (m, 2H), 7.87 (d, *J* = 7.6 Hz, 1H), 7.52–7.55 (m, 2H), 7.37–7.41 (m, 1H), 7.09–7.12 (m, 1H), 7.02 (s, 1H, H-2'), 6.69 (s, 1H, H-5), 6.57 (s, 1H, H-8), 5.94 (d, *J* = 4.8 Hz, 2H, OCH₂O), 4.85 (d, *J* = 4.4 Hz, 1H, H-1), 4.43–4.46 (m, 1H, H-11), 4.19 (s, 3H, 3'-OCH₃), 3.95– 4.00 (m, 1H, H-11), 3.11–3.14 (m, 1H, H-4), 2.79–2.96 (m, 3H, H-2,3,4). HRMS (ESI) calcd for C₃₃H₂₅N₃O₆I ([M + H]⁺), 686.0782; found, 686.0771.

Data for Compound **9h**: yield = 86%; yellow solid; mp = 141–143 °C; $[\alpha]^{21}_{D}$ = +25 (*c* 1.5 mg/mL, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.52 (*d*, *J* = 2.0 Hz, 1H, NH), 8.24 (*dd*, *J* = 2.4, 9.6 Hz, 1H), 8.17 (s, 1H), 8.10 (*d*, *J* = 9.6 Hz, 1H), 7.55 (s, 1H, H-6'), 7.49 (s, 1H), 7.41–7.46 (m, 2H), 7.12–7.14 (m, 1H), 7.03 (s, 1H, H-2'), 6.72 (s, 1H, H-5), 6.57 (s, 1H, H-8), 5.94 (*d*, *J* = 5.6 Hz, 2H, OCH₂O), 4.87 (*d*, *J* = 4.4 Hz, 1H, H-1), 4.42–4.46 (m, 1H, H-11), 4.23 (s, 3H, 3'-OCH₃), 3.96–4.00 (m, 1H, H-11), 3.89 (s, 3H, OCH₃), 3.13–3.16 (m, 1H, H-4), 2.80–2.97 (m, 3H, H-2,3,4). HRMS (ESI) calcd for C₃₄H₂₈N₃O₇ ([M + H]⁺), 590.1921; found, 590.1910.

Data for Compound 9i: yield = 78%; yellow solid; mp = 196–198 °C; $[\alpha]^{21}_{D} = +21$ (*c* 2.0 mg/mL, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.45 (d, *J* = 2.0 Hz, 1H, NH), 8.29 (dd, *J* = 2.4, 9.6 Hz, 2H), 8.06 (d, *J* = 9.2 Hz, 1H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.53 (s, 1H, H-6'), 7.03 (s, 1H, H-2'), 6.96 (d, *J* = 8.0 Hz, 2H), 6.71 (s, 1H, H-5), 6.57 (s, 1H, H-8), 5.94 (d, *J* = 6.4 Hz, 2H, OCH₂O), 4.86 (d, *J* = 4.8 Hz, 1H, H-1), 4.42–4.45 (m, 1H, H-11), 4.20 (s, 3H, 3'-OCH₃), 3.95–4.00 (m, 1H, H-11), 3.87 (s, 3H, OCH₃), 3.12–3.15 (m, 1H, H-4), 2.79–2.97 (m, 3H, H-2,3,4). HRMS (ESI) calcd for C₃₄H₂₈N₃O₇ ([M + H]⁺), 590.1921; found, 590.1908.

Data for Compound **9***j*: yield = 68%; yellow solid; mp = 201–203 °C; $[\alpha]^{21}_{D} = +23$ (*c* 3.3 mg/mL, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.47 (s, 1H, NH), 8.35 (s, 1H), 8.27 (dd, *J* = 2.0, 9.2 Hz, 1H), 8.07 (d, *J* = 9.2 Hz, 1H), 7.53 (s, 2H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.03 (s, 1H, H-2'), 6.89 (d, *J* = 6.4 Hz, 1H), 6.70 (s, 1H, H-5), 6.56 (s, 1H, H-8), 5.93 (d, *J* = 6.4 Hz, 2H, OCH₂O), 4.86 (d, *J* = 4.8 Hz, 1H, H-1), 4.42–4.45 (m, 1H, H-11), 4.19 (s, 3H, 3'-OCH₃), 3.93–3.99 (m, 7H, H-11, 2 × OCH₃), 3.12–3.15 (m, 1H, H-4), 2.79–2.96 (m, 3H, H-2,3,4). HRMS (ESI) calcd for C₃₅H₃₀N₃O₈ ([M + H]⁺), 620.2027; found, 620.2013.

Data for Compound **10a**: yield = 68%; yellow solid; mp = 214– 216 °C; $[\alpha]^{20}_{D}$ = +22 (*c* 3.1 mg/mL, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 8.44 (s, 1H, NH), 8.27 (d, *J* = 9.5 Hz, 1H), 8.03 (s, 1H), 7.85 (d, *J* = 9.0 Hz, 1H), 7.50 (s, 1H, H-6'), 7.01 (s, 1H, H-2'), 6.67 (s, 1H, H-5), 6.54 (s, 1H, H-8), 5.92 (d, *J* = 8.5 Hz, 2H, OCH₂O), 4.85 (d, *J* = 4.0 Hz, 1H, H-1), 4.40–4.43 (m, 1H, H-11), 4.21 (s, 3H, 3'-OCH₃), 3.94–3.98 (m, 1H, H-11), 3.08–3.11 (m, 1H, H-4), 2.77– 2.95 (m, 3H, H-2,3,4), 2.25 (s, 3H, CH₃). HRMS (ESI) calcd for C₂₈H₂₄N₃O₆ ([M + H]⁺), 498.1659; found, 498.1655.

Data for Compound **10b**: yield = 89%; yellow solid; mp = 191– 193 °C; $[\alpha]^{20}_{D}$ = +29 (c 3.9 mg/mL, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 8.57 (s, 2H), 8.35 (d, *J* = 9.0 Hz, 1H), 7.80 (d, *J* = 9.5 Hz, 1H), 7.53 (s, 1H, H-6'), 7.03 (s, 1H, H-2'), 6.71 (s, 1H, H-5), 6.56 (s, 1H, H-8), 5.94 (d, *J* = 7.0 Hz, 2H, OCH₂O), 4.86 (d, *J* = 5.0 Hz, 1H, H-1), 4.42–4.44 (m, 1H, H-11), 4.27 (s, 2H, CH₂Cl), 4.23 (s, 3H, 3'-OCH₃), 3.95–3.99 (m, 1H, H-11), 3.13–3.15 (m, 1H, H-4), 2.79– 2.96 (m, 3H, H-2,3,4). HRMS (ESI) calcd for C₂₈H₂₃N₃O₆Cl ([M + H]⁺), 532.1269; found, 532.1263.

Data for Compound **10c**: yield = 84%; yellow solid; mp = 204– 206 °C; $[\alpha]^{21}_{D}$ = +21 (*c* 2.0 mg/mL, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 8.38 (*s*, 1H, NH), 8.29 (d, *J* = 9.5 Hz, 1H), 7.89 (d, *J* = 9.5 Hz, 1H), 7.61 (*s*, 1H), 7.51 (*s*, 1H, H-6'), 7.01 (*s*, 1H, H-2'), 6.69 (*s*, 1H, H-5), 6.55 (*s*, 1H, H-8), 5.94 (d, *J* = 6.5 Hz, 2H, OCH₂O), 4.86 (d, *J* = 4.5 Hz, 1H, H-1), 4.41–4.44 (m, 1H, H-11), 4.22 (*s*, 3H, 3'-OCH₃), 3.94–3.98 (m, 1H, H-11), 3.11–3.13 (m, 1H, H-4), 2.80– 2.95 (m, 3H, H-2,3,4), 2.46 (q, *J* = 7.5 Hz, 2H, CH₃<u>CH</u>₂), 1.27 (t, *J* = 7.5 Hz, 3H, \underline{CH}_3CH_2). HRMS (ESI) calcd for $C_{29}H_{26}N_3O_6$ ([M + H]⁺), 512.1816; found, 512.1803.

Data for Compound **10d**: yield = 28%; yellow solid; mp = 200–202 °C; $[\alpha]^{21}_{D}$ = +23 (*c* 3.3 mg/mL, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ : 8.45 (s, 1H, NH), 8.31 (d, *J* = 9.2 Hz, 1H), 7.87–7.91 (m, 2H), 7.51 (s, 1H, H-6'), 7.02 (s, 1H, H-2'), 6.71 (s, 1H, H-5), 6.56 (s, 1H, H-8), 5.94 (d, *J* = 4.8 Hz, 2H, OCH₂O), 5.86 (s, 1H, C=CH₂), 5.54 (s, 1H, C=CH₂), 4.86 (d, *J* = 3.6 Hz, 1H, H-1), 4.41–4.45 (m, 1H, H-11), 4.22 (s, 3H, 3'-OCH₃), 3.95–3.99 (m, 1H, H-11), 3.12–3.16 (m, 1H, H-4), 2.78–2.95 (m, 3H, H-2,3,4), 2.10 (s, 3H, CH₃). HRMS (ESI) calcd for C₃₀H₂₆N₃O₆ ([M + H]⁺), 524.1816; found, 524.1807.

Data for Compound **10e**: yield = 81%; yellow solid; mp = 166– 168 °C; $[\alpha]^{20}_{D}$ = +22 (c 3.0 mg/mL, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 8.39 (s, 1H, NH), 8.29 (d, *J* = 9.0 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 1H), 7.70 (s, 1H), 7.50 (s, 1H, H-6'), 7.00 (s, 1H, H-2'), 6.68 (s, 1H, H-5), 6.55 (s, 1H, H-8), 5.93 (d, *J* = 7.5 Hz, 2H, OCH₂O), 4.85 (d, *J* = 4.0 Hz, 1H, H-1), 4.40–4.42 (m, 1H, H-11), 4.21 (s, 3H, 3'-OCH₃), 3.94–3.98 (m, 1H, H-11), 3.10–3.12 (m, 1H, H-4), 2.80–2.95 (m, 3H, H-2,3,4), 2.42 (t, *J* = 7.0 Hz, 2H, <u>CH₂CONH</u>), 1.73–1.76 (m, 2H, <u>CH₂CH₂CH₂CONH</u>), 1.29–1.36 (m, 6H, CH₃<u>CH₂CH₂CH₂CH₂CH₂CONH), 0.85 (t, *J* = 6.5 Hz, 3H, CH₃). HRMS (ESI) calcd for C₃₃H₃₄N₃O₆ ([M + H]⁺), 568.2442; found, 568.2430.</u>

Data for Compound **10f**: yield = 82%; yellow solid; mp = 213–215 °C; $[\alpha]^{20}_{D}$ = +27 (c 3.2 mg/mL, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 8.53 (s, 1H, NH), 8.34 (d, *J* = 9.5 Hz, 2H), 8.04–8.09 (m, 1H), 7.90 (d, *J* = 7.5 Hz, 2H), 7.46–7.57 (m, 4H), 7.03 (s, 1H, H-2'), 6.68 (s, 1H, H-5), 6.55 (s, 1H, H-8), 5.92 (d, *J* = 11.0 Hz, 2H, OCH₂O), 4.85 (d, *J* = 4.0 Hz, 1H, H-1), 4.41–4.43 (m, 1H, H-11), 4.21 (s, 3H, 3'-OCH₃), 3.94–3.98 (m, 1H, H-11), 3.10–3.13 (m, 1H, H-4), 2.77–2.95 (m, 3H, H-2,3,4). HRMS (ESI) calcd for C₃₃H₂₆N₃O₆ ([M + H]⁺), 560.1816; found, 560.1809.

Data for Compound **10g**: yield = 32%; yellow solid; mp = 204– 206 °C; $[\alpha]^{21}_{D}$ = +19 (*c* 4.7 mg/mL, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.51 (s, 1H, NH), 8.36 (d, *J* = 9.2 Hz, 1H), 8.02–8.04 (m, 2H), 7.89 (d, *J* = 7.6 Hz, 1H), 7.53–7.56 (m, 2H), 7.40–7.43 (m, 1H), 7.13–7.16 (m, 1H), 7.02 (s, 1H, H-2'), 6.68 (s, 1H, H-5), 6.56 (s, 1H, H-8), 5.93 (d, *J* = 6.4 Hz, 2H, OCH₂O), 4.87 (d, *J* = 3.2 Hz, 1H, H-1), 4.42–4.45 (m, 1H, H-11), 4.23 (s, 3H, 3'-OCH₃), 3.95– 3.99 (m, 1H, H-11), 3.10–3.13 (m, 1H, H-4), 2.78–2.95 (m, 3H, H-2,3,4). HRMS (ESI) calcd for C₃₃H₂₅N₃O₆I ([M + H]⁺), 686.0782; found, 686.0769.

Data for Compound **10g**': yield = 57%; yellow solid; mp = 172–174 °C; $[\alpha]^{21}_{D}$ = +9 (*c* 4.8 mg/mL, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.35 (d, *J* = 8.8 Hz, 1H), 8.25 (s, 1H), 7.90 (d, *J* = 8.8 Hz, 1H), 7.71 (d, *J* = 8.0 Hz, 2H), 7.52–7.56 (m, 3H), 7.23–7.20 (m, 2H), 7.04 (s, 1H, H-2'), 6.94–6.97 (m, 2H), 6.72 (s, 1H, H-5), 6.55 (s, 1H, H-8), 5.95 (s, 2H, OCH₂O), 4.87 (d, *J* = 4.0 Hz, 1H, H-1), 4.43–4.46 (m, 1H, H-11), 4.22 (s, 3H, 3'-OCH₃), 3.96–4.00 (m, 1H, H-11), 3.13–3.16 (m, 1H, H-4), 2.79–2.98 (m, 3H, H-2,3,4). HRMS (ESI) calcd for C₄₀H₂₈N₃O₇I₂ ([M + H]⁺), 916.0011; found, 915.9999.

Data for Compound **10h**: yield = 81%; yellow solid; mp = 145– 147 °C; $[\alpha]^{21}_{D}$ = +13 (*c* 2.9 mg/mL, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.53 (s, 1H, NH), 8.34 (d, *J* = 9.2 Hz, 1H), 8.27 (s, 1H), 7.98 (dd, *J* = 2.4, 9.2 Hz, 1H), 7.51 (d, *J* = 2.0 Hz, 1H, H-6'), 7.45– 7.46 (m, 1H), 7.36–7.44 (m, 2H), 7.08–7.10 (m, 1H), 7.02 (d, *J* = 0.8 Hz, 1H, H-2'), 6.70 (s, 1H, H-5), 6.56 (s, 1H, H-8), 5.93 (dd, *J* = 1.2, 6.8 Hz, 2H, OCH₂O), 4.86 (d, *J* = 4.8 Hz, 1H, H-1), 4.41–4.45 (m, 1H, H-11), 4.22 (s, 3H, 3'-OCH₃), 3.95–3.99 (m, 1H, H-11), 3.86 (s, 3H, OCH₃), 3.12–3.15 (m, 1H, H-4), 2.78–2.96 (m, 3H, H-2,3,4). HRMS (ESI) calcd for C₃₄H₂₈N₃O₇ ([M + H]⁺), 590.1921; found, 590.1905.

Data for Compound **10***i*: yield = 81%; yellow solid; mp = 217–219 °C; $[\alpha]^{21}_{D}$ = +35 (*c* 3.2 mg/mL, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.48 (s, 1H, NH), 8.32 (d, *J* = 9.2 Hz, 1H), 8.26 (s, 1H), 8.01 (d, *J* = 9.2 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 2H), 7.50 (s, 1H, H-6'), 7.01 (s, 1H, H-2'), 6.94 (d, *J* = 8.0 Hz, 2H), 6.69 (s, 1H, H-5), 6.55 (s, 1H, H-8), 5.92 (d, *J* = 8.0 Hz, 2H, OCH₂O), 4.85 (d, *J* = 4.0 Hz, 1H), H-1), 4.41–4.44 (m, 1H, H-11), 4.21 (s, 3H, 3'-OCH₃), 3.94–3.99 (m, 1H, H-11), 3.86 (s, 3H, OCH₃), 3.11–3.14 (m, 1H, H-4), 2.77–2.95 (m, 3H, H-2,3,4). HRMS (ESI) calcd for $C_{34}H_{28}N_3O_7$ ([M + H]⁺), 590.1921; found, 590.1906.

Data for Compound **10***j*: yield = 70%; yellow solid; mp = 202–204 °C; $[\alpha]^{21}_{D} = +32$ (*c* 4.2 mg/mL, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.51 (*s*, 1H, NH), 8.32–8.35 (m, 2H), 7.99 (d, *J* = 9.2 Hz, 1H), 7.50 (d, *J* = 4.4 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.02 (*s*, 1H, H-2'), 6.88 (d, *J* = 8.0 Hz, 1H), 6.69 (*s*, 1H, H-5), 6.55 (*s*, 1H, H-8), 5.92 (d, *J* = 8.0 Hz, 2H, OCH₂O), 4.85 (d, *J* = 3.6 Hz, 1H, H-1), 4.41–4.44 (m, 1H, H-11), 4.21 (*s*, 3H, 3'-OCH₃), 3.93–3.99 (m, 7H, H-11, 2 × OCH₃), 3.11–3.14 (m, 1H, H-4), 2.78–2.96 (m, 3H, H-2,3,4). HRMS (ESI) calcd for C₃₅H₃₀N₃O₈ ([M + H]⁺), 620.2027; found, 620.2015.

Biological Assay. The insecticidal activity of 1-8, 9a-c and 9e-i, 10a-j, and 10g' against the pre-third-instar larvae of oriental armyworm, *M. separata* (Walker), was assessed by leaf-dipping method, as described previously.²⁶ For each compound, 30 larvae (10 larvae per group) were used. Acetone solutions of compounds 1-8, 9a-c, 9e-j, 10a-j, 10g', and toosendanin (used as a positive control) were prepared at a concentration of 1 mg/mL. Meanwhile, the acetone solution of dibenzo[a,c]phenazine (12, mp = 226–228 °C (literature, 224.8–225.7 °C)³¹) at a concentration of 1 mg/mL was used as a control. Fresh wheat leaves were dipped into the corresponding solution for 3 s and 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, where every 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 relative humidity (RH) 65–80% and on a 12 h/12 h light/dark photoperiod. The insecticidal activity of the tested compounds against the pre-third-instar larvae of M. separata was calculated by using 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.

RESULTS AND DISCUSSION

Synthesis. A series of new phenazine derivatives of 4deoxypodophyllotoxin (9a-c, 9e-j, 10a-j, and 10g') modified in the E-ring were synthesized as shown in Scheme 1. First, podophyllotoxin (1) was smoothly transformed to 4-deoxypodophyllotoxin (2) in the presence of 10% palladium/ carbon.²⁹ By treatment of 2 with concentrated nitric acid, 4',5'-didemethoxy-4',5'-dioxodeoxypodophyllotoxin (3) was obtained in 73% yield.³² Then 3 rapidly reacted with 1,2phenylenediamine to give the phenazine derivative 4.³² When 3 reacted with 4-nitro-1,2-phenylenediamine for 5 h, two regioisomers, 5 and 6, were afforded in 29 and 59% yields, respectively. Additionally, to confirm the absolute threedimensional configuration of 5 and 6, a single crystal of 6 was obtained and its steric structure was illustrated by X-ray crystallography (Figure 2). It obviously demonstrated that the nitro group of 6 was unambiguously at the C-10' position and its trans lactone was retained, so the nitro group of the other isomer, 5, was at the C-9' position. Interestingly, it was noteworthy that two nitrogen atoms of the phenazine fragment of 6 were obviously at the C-4' and C-5' positions; therefore, the *o*-quinone moiety of **3** should also be at the C-4' and C-5' positions. Subsequently, reduction of 5 or 6 in the presence of SnCl₂ easily afforded 7 or 8. Finally, 9a-c, 9e-j, 10a-j, and 10g' were obtained by reaction of 7 or 8 with acyl chlorides (11a-j) in the presence of Et₃N. The structures of all target compounds were well characterized by ¹H NMR, HRMS,



Figure 2. X-ray crystal structure of 6.

optical rotation, and mp. Crystallographic data (excluding structure factors) for the structure of **6** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 908384. 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; e-mail, deposit@ccdc.cam.ac.uk].

Insecticidal Activity. The insecticidal activity of 1-8, 9ac, 9e-j, 10a-j, and 10g' against the pre-third-instar larvae of M. separata, tested by the leaf-dipping method at a concentration of 1 mg/mL, was expressed as mortality rates. As indicated in Table 1, the corresponding mortality rates caused by these compounds after 35 days were generally higher than those after 10 and 20 days. That is, these compounds as opposed to other quick-acting conventional neurotoxic insecticides such as organophosphates, carbamates, and pyrethroids, showed delayed insecticidal activity.²⁶⁻²⁸ In the meantime, the symptoms of the tested M. separata were also characterized in the same way as in our previous papers.²⁶⁻²⁸ Due to feeding too much treated leaves during the first 48 h, some larvae died slowly with slim and wrinkled bodies during the larval period (Figure 3). On the other hand, many larvae of the treated groups molted to malformed pupae and died during the stage of pupation (Figure 4). Malformed moths with imperfect wings also appeared in the treated groups (Figure 5). On the basis of the above-mentioned symptoms, the derivatives likely exhibited an antimolting hormone effect.

Compounds 4, 7, 9c, 9f, 9g, 9i, 9j, and 10c exhibited insecticidal activity equal to or higher than that of toosendanin. Especially 9i displayed the best promising insecticidal activity with a final mortality rate of 72.4%. In addition, preliminary structure—activity relationships were studied. When compound 2 was oxidized to the quinone 3, the final mortality rate of 3 was decreased to 24.1% (41.4% for 2). Whereas 3 reacting with 1,2-phenylenediamine gave the phenazine 4, the final mortality rate of 4 was increased to 55.2%. In general, introduction of the acylamino groups at the C-9' position of 4 could afford more potent compounds than those containing the same ones at the C-10' position. As shown in Table 1, the insecticidal activity of

Table 1. Insecticidal Activity of Some Deoxypodophyllotoxin-Based Phenazine Derivatives against *M. separata* on Leaves Treated with a Concentration of 1 mg/mL

	corrected mortality rate (%)		
compd	10 days	20 days	35 days
1	13.3 ± 4.7	24.1 ± 4.7	34.5 ± 4.7
2	20.0 ± 8.2	27.6 ± 0	41.4 ± 4.7
3	6.7 ± 9.4	13.8 ± 4.7	24.1 ± 9.4
4	6.7 ± 9.4	17.2 ± 8.2	55.2 ± 12.5
5	16.7 ± 4.7	24.1 ± 4.7	31.0 ± 12.5
6	13.3 ± 4.7	13.8 ± 4.7	20.7 ± 12.5
7	13.3 ± 4.7	34.5 ± 4.7	55.2 ± 4.7
8	13.3 ± 4.7	20.7 ± 4.7	34.5 ± 4.7
9a	13.3 ± 9.4	24.1 ± 4.7	44.8 ± 9.4
9b	6.7 ± 9.4	10.3 ± 4.7	48.3 ± 8.2
9c	13.3 ± 4.7	24.1 ± 9.4	62.1 ± 4.7
9e	10.0 ± 8.2	20.7 ± 9.4	41.4 ± 4.7
9f	13.3 ± 9.4	34.5 ± 9.4	62.1 ± 9.4
9g	13.3 ± 4.7	20.7 ± 4.7	55.2 ± 4.7
9h	13.3 ± 4.7	17.2 ± 8.2	44.8 ± 4.7
9i	26.7 ± 4.7	31.0 ± 9.4	72.4 ± 4.7
9j	16.7 ± 9.4	27.6 ± 8.2	51.7 ± 9.4
10a	16.7 ± 4.7	27.6 ± 8.2	34.5 ± 4.7
10b	3.3 ± 4.7	0 ± 4.7	17.2 ± 0
10c	13.3 ± 9.4	31.0 ± 9.4	58.6 ± 8.2
10d	13.3 ± 4.7	24.1 ± 9.4	31.0 ± 12.5
10e	13.3 ± 9.4	31.0 ± 12.5	34.5 ± 9.4
10f	13.3 ± 4.7	24.1 ± 4.7	31.0 ± 4.7
10g	13.3 ± 4.7	17.2 ± 8.2	27.6 ± 8.2
10g'	16.7 ± 4.7	20.7 ± 9.4	27.6 ± 0
10h	3.3 ± 4.7	24.1 ± 4.7	48.3 ± 8.2
10i	3.3 ± 4.7	6.9 ± 0	37.9 ± 0
10j	23.3 ± 4.7	20.7 ± 4.7	44.8 ± 9.4
12	0 ± 0	-3.4 ± 0	51.7 ± 4.7
toosendanin	26.7 ± 4.7	34.5 ± 4.7	48.3 ± 8.2



Figure 3. Representative abnormal larvae pictures of 4, 9c, and 10d during the larval period (CK, blank control group).

5, 7, 9a-c, and 9e-j was usually more pronounced than that of 6, 8, 10a-c, and 10e-j (except 9h and 10h). The proper length of the side chain of alkylacylamino was essential for their insecticidal activity. For example, the final mortality rates of 9a (R = Me), 9b (R = CH₂Cl), and 9e (R = *n*-hexyl) were only 44.8, 48.3, and 41.4%, respectively, whereas the final mortality rate of 9c (R = Et) was 62.1%. Similarly, the final mortality rates of 10a (R = Me), 10b (R = CH₂Cl), 10d (R = isopropenyl), and 10e (R = *n*-hexyl) were only 34.5, 17.2, 31,



Figure 4. Representative malformed pupae pictures of 4, 9b, 9g, 9h, and 10c during the pupation period (CK, blank control group).



Figure 5. Representative malformed moth pictures of 4, 9i, 9j, 10c, and 10h during the emergence period (CK, blank control group).

and 34.5%, respectively; however, the final mortality rate of 10c (R = Et) was 58.6%. Interestingly, when the methoxy group was introduced at the para position of **9f** to give **9i**, the final mortality rate of **9i** was increased to 72.4%; on the contrary, introduction of the methoxy group at the meta position of **9f** afforded **9h**, the final mortality rate of which was decreased to 44.8%. On the other hand, the insecticidal activity of the prepared derivatives was different from that of phenazine **12**. No larvae died for the group treated by compound **12**.

In conclusion, a series of new deoxypodophyllotoxin-based phenazine analogues modified in their E-ring were prepared and tested for their insecticidal activity against the pre-thirdinstar larvae of *M. separata* in vivo at a concentration of 1 mg/ mL. Of all the derivatives, compound 9i, containing pmethoxybenzoylamino at the C-9' position of the deoxypodophyllotoxin-based phenazine fragment, exhibited a more promising insecticidal activity than toosendanin. The derivatives likely displayed an antimolting hormone effect. This suggested that the proper length of the side chain of alkylacylamino was important for their insecticidal activity, and introduction of the acylamino groups at the C-9' position of deoxypodophyllotoxin-based phenazine fragment generally afforded more potent compounds than those containing the same ones at the C-10' position. Hence, other substituents could be introduced at the C-9' position of the deoxypodophyllotoxin-based phenazine fragment and will be reported in due course. This will pave the way for further design, structural modification, and development of deoxypodophyllotoxin-based derivatives as insecticidal agents.

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REFERENCES

(1) Ishtiaq, M.; Saleem, M. A.; Razaq, M. Monitoring of resistance in *Spodoptera exigua* (Lepidoptera: Noctuidae) from four districts of the Southern Punjab, Pakistan to four conventional and six new chemistry insecticides. *Crop Prot.* **2012**, *33*, 13–20.

(2) Rios-Diez, J. D.; Saldamando-Benjumea, C. I. Susceptibility of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) strains from central colombia to two insecticides, methomyl and lambda-cyhalothrin: A study of the genetic basis of resistance. *J. Econ. Entomol.* **2011**, *104*, 1698–1705.

(3) Isman, M. B. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol.* **2006**, *51*, 45–66.

(4) Chen, J. T.; Su, H. J.; Huang, J. W. Isolation and identification of secondary metabolites of *Clitocybe nuda* responsible for inhibition of zoospore germination of *Phytophthora capsici*. J. Agric. Food Chem. **2012**, 60, 7341–7344.

(5) Zhou, Y. Y.; Luo, S. H.; Yi, T. S.; Li, C. H.; Luo, Q.; Hua, J.; Liu, Y.; Li, S. H. Secondary metabolites from *Glycine soja* and their growth inhibitory effect against *Spodoptera litura*. J. Agric. Food Chem. **2011**, *59*, 6004–6010.

(6) Goel, M.; Dureja, P.; Rani, A.; Uniyal, P. L.; Laatsch, H. Isolation, characterization and antifungal activity of major constituents of the Himalayan lichen *Parmelia reticulata* Tayl. *J. Agric. Food Chem.* **2011**, *59*, 2299–2307.

(7) Caballero-Gallardo, K.; Olivero-Verbel, J.; Stashenko, E. E. Repellent activity of essential oils and some of their individual constituents against *Tribolium castaneum* Herbst. *J. Agric. Food Chem.* **2011**, *59*, 1690–1696.

(8) Huang, Z. Q.; Liu, Y. X.; Li, Y. Q.; Xiong, L. X.; Cui, Z. P.; Song, H. J.; Liu, H. L.; Zhao, Q. Q.; Wang, Q. M. Synthesis, crystal structures, insecticidal activities, and structure-activity relationships of novel N'-tert-butyl-N'-substituted-benzoyl-N-[di(octa)hydro]benzofuran{(2,3-dihydro)benzo[1,3]([1,4])dioxine}carbohydrazide derivatives. J. Agric. Food Chem. 2011, 59, 635-644.

(9) Song, H. J.; Liu, Y. X.; Xiong, L. X.; Li, Y. Q.; Yang, N.; Wang, Q. M. Design, synthesis, and insecticidal activity of novel pyrazole derivatives containing α -hydroxymethyl-N-benzyl carboxamide, α -chloromethyl-N-benzyl carboxamide, and 4,5-dihydrooxazole moieties. *J. Agric. Food Chem.* **2012**, *60*, 1470–1479.

(10) Sun, R. F.; Wang, Z. W.; Li, Y. Q.; Xiong, L. X.; Liu, Y. X.; Wang, Q. M. Design, synthesis, and insecticidal evaluation of new benzoylureas containing amide and sulfonate groups based on the sulfonylurea receptor protein binding site for diflubenzuron and glibenclamide. J. Agric. Food Chem. 2013, 61, 517–522.

(11) Dayan, F. E.; Cantrell, C. L.; Duke, S. O. Natural products in crop protection. *Bioorg. Med. Chem.* **2009**, *17*, 4022–4034.

(12) Xu, H.; Lv, M.; Tian, X. A review on hemisynthesis, biosynthesis, biological activities, mode of action, and structure-activity

relationship of podophyllotoxins: 2003–2007. *Curr. Med. Chem.* 2009, *16*, 327–349.

(13) You, Y. J. Podophyllotoxin derivatives: current synthetic approaches for new anticancer agents. *Curr. Pharm. Des.* **2005**, *11*, 1695–1717.

(14) Gordaliza, M.; Garcia, P. A.; Miguel Del Corral, J. M.; Castro, M. A.; Gomez-Zurita, M. A. Podophyllotoxin: distribution, sources, applications and new cytotoxic derivatives. *Toxicon* **2004**, *44*, 441–459.

(15) Renouard, S.; Lopez, T.; Hendrawati, O.; Dupre, P.; Doussot, J.; Falguieres, A.; Ferroud, C.; Hagege, D.; Lamblin, F.; Laine, E.; Hano, C. Podophyllotoxin and deoxypodophyllotoxin in *Juniperus bermudiana* and 12 other *Juniperus* species: optimization of extraction, method validation, and quantification. *J. Agric. Food Chem.* **2011**, *59*, 8101–8107.

(16) Kozawa, M.; Baba, K.; Matsuyama, Y.; Kido, T.; Sakai, M.; Takemoto, T. Components of the root of anthriscus sylvestris HOFFM. II. Insecticidal activity. *Chem. Pharm. Bull.* **1982**, *30*, 2885–2888.

(17) Inamori, Y.; Kubo, M.; Kato, Y.; Tsujibo, H.; Sakai, M.; Kozawa, M. Changes of tissue histopathology and uric acid excretion in silkworm larvae intoxicated with deoxypodophyllotoxin and race-momycin-D, and during starvation. *Chem. Pharm. Bull.* **1986**, *34*, 2542–2549.

(18) Miyazawa, M.; Fukuyama, M.; Yoshio, K.; Kato, T.; Ishikawa, Y. Biologically active components against *Drosophila melanogaster* from *Podophyllum hexandrum. J. Agric. Food Chem.* **1999**, *47*, 5108–5110.

(19) Gao, R.; Gao, C. F.; Tian, X.; Yu, X. Y.; Di, X. D.; Xiao, H.; Zhang, X. Insecticidal activity of deoxypodophyllotoxin, isolated from *Juniperus sabina L.*, and related lignans against larvae of *Pieris rapae L. Pest Manag. Sci.* **2004**, *60*, 1131–1136.

(20) Carpenter, C. D.; O'Neill, T.; Picot, N.; Johnson, J. A.; Robichaud, G. A.; Webster, D.; Gray, C. A. Anti-mycobacterial natural products from the Canadian medicinal plant *Juniperus communis. J. Ethnopharmacol.* **2012**, *143*, 695–700.

(21) Kumar, K. A.; Singh, S. K.; Kumar, B. S.; Doble, M. Synthesis, anti-fungal activity evaluation and QSAR studies on podophyllotoxin derivatives. *Cent. Eur. J. Chem.* **2007**, *5*, 880–897.

(22) Xu, H.; Xiao, X. Natural products-based insecticidal agents 4. semisynthesis and insecticidal activity of novel esters of 2-chloropodophyllotoxin against *Mythimna separata* Walker in vivo. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5415–5418.

(23) Xu, H.; Wang, Q. T.; Guo, Y. Stereoselective synthesis of 4α -alkyloxy-2- α/β -bromopodophyllotoxin derivatives as insecticidal agents. *Chem.-Eur. J.* **2011**, *17*, 8299–8303.

(24) Xu, H.; Xiao, X.; Zhao, X. F.; Guo, Y.; Yao, X. J. Unexpectedly convenient and stereoselective synthesis of 4α -acyloxy-2-chloropodo-phyllotoxins in the presence of BF₃·Et₂O. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4008–4012.

(25) Xu, H.; Xiao, X.; Wang, Q. T. Natural products-based insecticidal agents 7. Semisynthesis and insecticidal activity of novel 4α -alkyloxy-2-chloropodophyllotoxin derivatives against *Mythimna separata* Walker *in vivo. Bioorg. Med. Chem. Lett.* **2010**, 20, 5009–5012. (26) Xu, H.; Wang, J. J.; Sun, H. J.; Lv, M.; Tian, X.; Yao, X. J.; Zhang, X. Semisynthesis and quantitative structure-activity relationship (QSAR) study of novel aromatic esters of 4'-demethyl-4-deoxypodophyllotoxin as insecticidal agents. J. Agric. Food Chem. **2009**, 57, 7919–7923.

(27) Xu, H.; Wang, J. J. Natural products-based insecticidal agents. 5. Design, semisynthesis and insecticidal activity of novel 4'-substituted benzenesulfonate derivatives of 4-deoxypodophyllotoxin against *Mythimna separata* Walker in vivo. *Bioorg. Med. Chem. Lett.* **2010**, 20, 2500–2502.

(28) Zhi, X.; Yang, C.; Zhang, R.; Hu, Y.; Ke, Y.; Xu, H. Natural products-based insecticidal agents 13. Semisynthesis and insecticidal activity of novel phenazine derivatives of 4β -acyloxypodophyllotoxin modified in the E-ring against *Mythimna separata* Walker in vivo. *Ind. Crops Prod.* **2013**, *42*, 520–526.

(29) Gensler, W. J.; Judge, J. F. X.; Leeding, M. V. 2-Carboxydeoxypicropodophyllin. J. Org. Chem. 1972, 37, 1062–1064. (30) Kamil, W. M.; Dewick, P. M. Biosynthesis of the lignans α - and β -peltatin. Phytochemistry 1986, 25, 2089–2092.

(31) Markgraf, J. H.; Katt, R. J. Strained heterocyclic systems. VI. Basicities of some quinoxalines. J. Org. Chem. 1972, 37, 717–718.

(32) Castro, M. A.; Miguel del Corral, J. M.; Gordaliza, M.; Gómez-Zurita, M. A.; de la Puente, M. L.; Betancur-Galvis, L. A.; Sierra, J.; San Feliciano, A. Synthesis, cytotoxicity and antiviral activity of podophyllotoxin analogues modified in the E-ring. *Eur. J. Med. Chem.* **2003**, *38*, 899–911.