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Design, Synthesis and Evaluation of Novel Isoxazolines/Oxime Sulfonates of 2'(2',6')-(Di) Chloropodophyllotoxins as Insecticidal Agents

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A series of 2'(2',6')-(di)halogeno-isoxazolopodophyllin acids-based esters, and oxime sulfonates of 2'(2',6')-(di)halogenopodophyllones were prepared by structural modifications of podophyllotoxin as insecticidal agents against *Mythimna separata* Walker. It was found that when 2'(2',6')-(di) halogenopodophyllones or 2'(2',6')-(di)chloropodophyllones reacted with hydroxylamine hydrochloride, the desired products were related with the configuration of their lactones. Three key single-crystal structures of Ie, IIe and IIIb were determined by X-ray diffraction. Especially compounds IIc and Vc showed the highest insecticidal activity. Moreover, some interesting results of structure-insecticidal activity relationships of tested compounds were also observed.

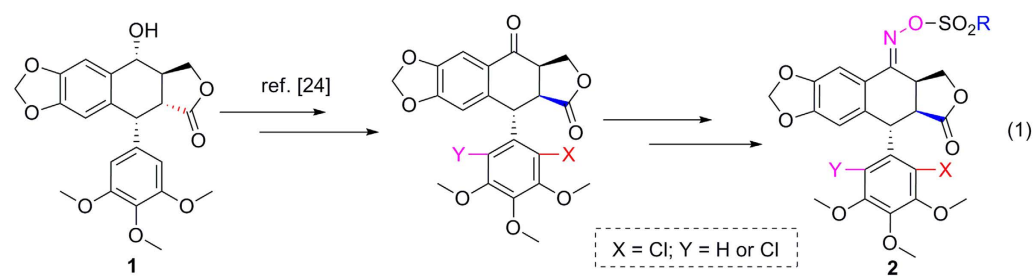
The oriental armyworm, *Mythimna separata* (Walker) (Lepidoptera: Noctuidae), a typical and key agricultural insect pest in China, Korea, Japan, Southeast Asia, Australia and Oceania, could extensively damage important cereal crops such as maize, wheat and rice^{1,2}. Currently, chemical control is still an essential component of crop protection in modern agriculture, and a variety of synthetic agrochemicals have been widely used to manage insect pests. However, a wide range of application of those agrochemicals has resulted in ecological disturbances and increasing resistance in pest populations, which will make the species more difficult to control³⁻⁸. Therefore, it is necessary to develop new potential alternatives to effectively and selectively control insect pests in the agricultural field⁴⁻⁸. In recent decades, the discovery and development of new pesticides directly or indirectly from plant secondary metabolites has been received much attention due to their less or slower resistance development and lower environmental pollution⁹⁻¹⁶.

Podophyllotoxin (**1**, Fig. 1), a naturally occurring cyclolignan, is isolated from the roots and rhizomes of some *Podophyllum* and *Juniperus* species. Compound **1** has been used as the lead compound for preparation of three clinical anticancer drugs such as etoposide (VP-16), teniposide (VM-26) and etoposide phosphate. Additionally, compound **1** also exhibits other interesting properties such as cytotoxic, insecticidal, antifungal, and antiviral activities¹⁷. Therefore, total synthesis¹⁸⁻²⁰ and structural modification²¹⁻²³ of **1** and its analogs is always the research topic.

More recently, a series of oxime sulfonate derivatives of 2'(2',6')-(di)chloropodophyllotoxins (**2**, Fig. 1, Eq. (1)) have been synthesized and some compounds showed more potent insecticidal activity than toosendanin, a commercial botanical insecticide isolated from *Melia azedarach*²⁴. Additionally, the *trans*-lactone was an important factor for podophyllotoxins exhibiting the good insecticidal activity²⁵. Especially when a chlorine atom was introduced at the C-2' position on the E-ring of podophyllotoxin derivatives, the corresponding compounds showed no significant cytotoxicity²⁶. Based upon the above results, in the present paper we wanted to prepare a series of oxime sulfonate derivatives of 2'(2',6')-(di)chloropodophyllotoxins (**3**, Fig. 1, Eq. (2)). Their insecticidal activity tested against a typical lepidopteran pest (*M. separata*) was also presented.

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Previous work



This work

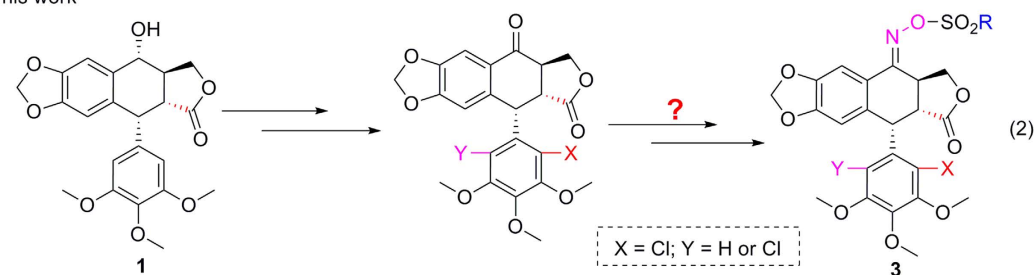


Figure 1. Chemical structures of podophyllotoxin (1) and its derivatives (2 and 3).

Methods

Materials and Instruments. Podophyllotoxin was purchased from Gansu Gerui Medicinal Materials Co., Ltd. (Lanzhou, China). All chemical reagents were purchased and utilized without further purification. Solvents were used directly or treated with 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., Qingdao, China). Silica gel column chromatography was performed with silica gel 200–300 mesh (Qingdao Haiyang Chemical Co., Ltd., Qingdao, China). Melting points (mp) were determined on a XT-4 digital melting point apparatus. Optical rotation was measured on a Rudolph Research Analytical Autopol III automatic polarimeter. Infrared spectra (IR) were recorded on a Bruker TENSOR 27 spectrometer. Proton nuclear magnetic resonance spectra ($^1\text{H NMR}$) were recorded in CDCl_3 on a Bruker Avance III 500 MHz instrument using tetramethylsilane (TMS) as the internal standard. High-resolution mass spectra (HRMS) were carried out with IonSpec 4.7 Tesla FTMS instrument.

General procedure for synthesis of 2'-(2',6')-(di)halogenopodophyllones (7–9). A mixture of 2'-(2',6')-(di)halogenopodophyllotoxin (4, 5, or 6, 1 mmol), CrO_3 (5 mmol), and pyridine (10 mmol) in dry dichloromethane (DCM, 20 mL) was stirred at room temperature. When the reaction was complete checked by TLC analysis, the mixture was diluted by DCM (60 mL), washed by saturated aq. NaHSO_3 (30 mL) and brine (30 mL), dried over anhydrous Na_2SO_4 , concentrated under reduced pressure, and purified by silica gel column chromatography eluting with petroleum ether/ethyl acetate (2:3, v/v) to afford compounds 7–9.

Data for 7: CAS: 1458601-16-5. Yield = 81%, white solid, m.p. 217–218 °C [lit. 218–219 °C]²⁷; $[\alpha]_{\text{D}}^{20} = -64$ (c 2.7 mg/mL, CHCl_3); IR cm^{-1} (KBr): 3075, 2933, 1787, 1685, 1487, 1391, 1117, 1020; $^1\text{H NMR}$ (500 MHz, CDCl_3) δ : 7.49 (s, 1H, H-5), 6.57 (s, 1H, H-8), 6.19 (s, 1H, H-6'), 6.05 (d, $J = 3.0$ Hz, 2H, OCH_2O), 5.42 (d, $J = 5.5$ Hz, 1H, H-1), 4.58–4.62 (m, 1H, H-11), 4.33–4.36 (t, $J = 9.5$ Hz, 1H, H-11), 3.92 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.76–3.80 (m, 1H, H-3), 3.65 (s, 3H, OCH_3), 3.40 (dd, $J = 16.0, 5.5$ Hz, 1H, H-2); HRMS (ESI): Calcd for $\text{C}_{22}\text{H}_{19}\text{ClO}_8\text{Na}$ ($[\text{M}+\text{Na}]^+$) 469.0671, found 469.0660.

Data for 8: Yield = 85%, white solid, m.p. 224–225 °C; $[\alpha]_{\text{D}}^{20} = -80$ (c 3.0 mg/mL, CHCl_3); IR cm^{-1} (KBr): 3084, 2937, 1789, 1686, 1479, 1395, 1249, 1062; $^1\text{H NMR}$ (500 MHz, CDCl_3) δ : 7.47 (s, 1H, H-5), 6.49 (s, 1H, H-8), 6.05 (s, 2H, OCH_2O), 5.73 (d, $J = 7.5$ Hz, 1H, H-1), 4.59–4.62 (m, 1H, H-11), 4.28–4.35 (m, 1H, H-11), 3.97 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 3.82–3.86 (m, 4H, H-3 and OCH_3), 3.52–3.57 (m, 1H, H-2); HRMS m/z calcd for $\text{C}_{22}\text{H}_{21}\text{O}_8\text{NCl}$ ($[\text{M}+\text{H}]^+$) 481.0451, found 481.0447.

Data for 9: CAS: 37158-57-9. Yield = 83%, white solid, m.p. 222–223 °C [lit. 220–221 °C]²⁷; $[\alpha]_{\text{D}}^{20} = -93$ (c 2.9 mg/mL, CHCl_3); IR cm^{-1} (KBr): 3074, 2934, 1789, 1685, 1479, 1391, 1196, 1075; $^1\text{H NMR}$ (500 MHz, CDCl_3) δ : 7.50 (s, 1H, H-5), 6.57 (s, 1H, H-8), 6.18 (s, 1H, H-6'), 6.06 (d, $J = 3.5$ Hz, 2H, OCH_2O), 5.52 (d, $J = 4.5$ Hz, 1H, H-1), 4.60–4.64 (m, 1H, H-11), 4.34–4.38 (m, 1H, H-11), 3.92 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.80–3.84 (m, 1H, H-3), 3.64 (s, 3H, OCH_3), 3.40 (dd, $J = 16.0, 5.5$ Hz, 1H, H-2); HRMS (ESI): Calcd for $\text{C}_{22}\text{H}_{19}\text{BrO}_8\text{Na}$ ($[\text{M}+\text{Na}]^+$) 513.0154, found 513.0155.

General procedure for synthesis of 2'-(2',6')-(di)halogeno-isoxazolopodophyllonic acids (10–12) and oximes of 2'-(2',6')-(di)halogenopodophyllones (13–15). A mixture of 2'-(2',6')-(di)

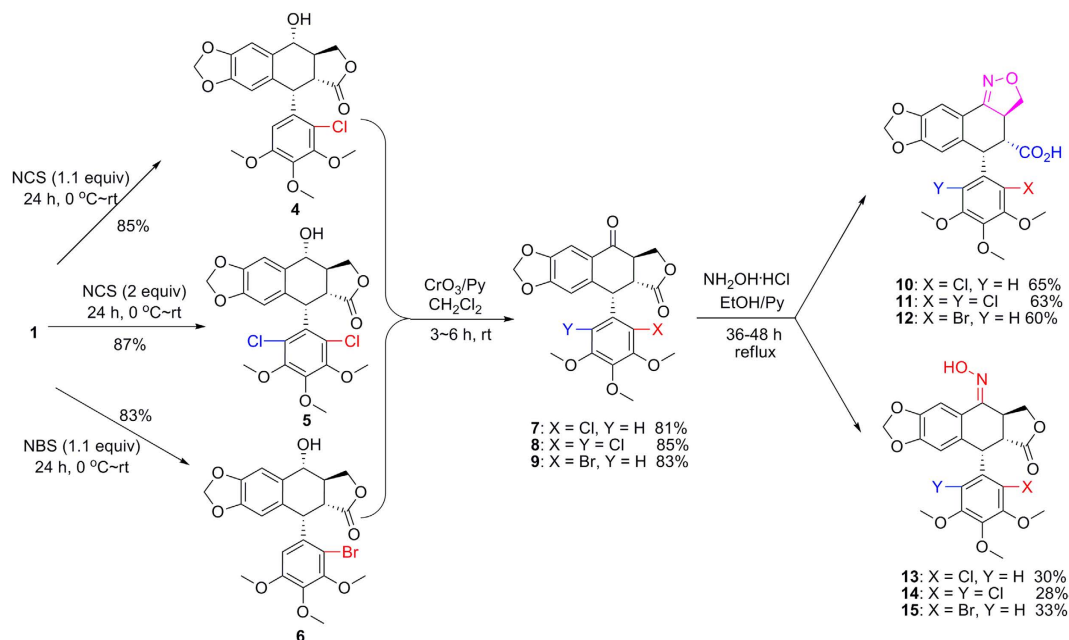


Figure 2. The synthetic route for the preparation of compounds 10–15.

halogenopodophyllone (7, 8 or 9, 1 mmol), hydroxylamine hydrochloride (1.5 mmol), and pyridine (4 mmol) in absolute ethanol (20 mL) was refluxed. When the reaction was complete checked by TLC analysis, the solvent was removed under reduced pressure, and saturated aq. NaHCO_3 (15 mL) was added to the residue, which was extracted with ethyl acetate (3×30 mL). The combined organic phase was dried over anhydrous Na_2SO_4 , filtered, concentrated under reduced pressure, and purified by silica gel column chromatography eluting with DCM/methanol (98:2, v/v) to afford compounds 10–15. For compounds 13–15 were not stable, they were used directly for the next step.

Data for 10: Yield = 65%, white solid, m.p. 174–175 $^\circ\text{C}$; $[\alpha]_D^{20} = -78$ (c 2.0 mg/mL, CHCl_3); IR cm^{-1} (KBr): 3447, 2930, 1713, 1483, 1583, 1233, 1036; $^1\text{H NMR}$ (500 MHz, CDCl_3) δ : 7.43 (s, 1H, H-5), 6.52 (s, 1H, H-8), 6.07 (s, 1H, H-6'), 5.99 (d, $J = 8.0$ Hz, 2H, OCH_2O), 5.35 (d, $J = 5.5$ Hz, 1H, H-1), 4.79 (t, $J = 9.0$ Hz, 1H, H-11), 4.01–4.09 (m, 1H, H-11), 3.87 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.79–3.84 (m, 1H, H-3), 3.62 (s, 3H, OCH_3), 3.28 (dd, $J = 13.0, 5.5$ Hz, 1H, H-2); HRMS m/z calcd for $\text{C}_{22}\text{H}_{21}\text{O}_8\text{NCl}$ ($[\text{M}+\text{H}]^+$) 462.0950, found 462.0943.

Data for 11: Yield = 63%, white solid, m.p. 200–201 $^\circ\text{C}$; $[\alpha]_D^{20} = -150$ (c 2.4 mg/mL, CHCl_3); IR cm^{-1} (KBr): 3437, 3108, 2938, 1711, 1480, 1230, 1096; $^1\text{H NMR}$ (500 MHz, CDCl_3) δ : 7.37 (s, 1H, H-5), 6.33 (s, 1H, H-8), 5.98 (d, $J = 3.5$ Hz, 2H, OCH_2O), 5.72 (d, $J = 8.5$ Hz, 1H, H-1), 4.74–4.78 (m, 1H, H-11), 4.51–4.56 (m, 1H, H-11), 3.94 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.76–3.80 (m, 4H, H-3 and OCH_3), 3.38 (dd, $J = 13.0, 8.5$ Hz, 1H, H-2); HRMS m/z calcd for $\text{C}_{22}\text{H}_{20}\text{O}_8\text{NCl}_2$ ($[\text{M}+\text{H}]^+$) 496.0560, found 496.0553.

Data for 12: Yield = 60%, white solid, m.p. 194–195 $^\circ\text{C}$; $[\alpha]_D^{20} = -109$ (c 2.7 mg/mL, CHCl_3); IR cm^{-1} (KBr): 3446, 3058, 2936.1709, 1482, 1234, 1105; $^1\text{H NMR}$ (500 MHz, CDCl_3) δ : 7.37 (s, 1H, H-5), 6.45 (s, 1H, H-8), 6.15 (s, 1H, H-6'), 5.98 (d, $J = 16.5$ Hz, 2H, OCH_2O), 5.24 (d, $J = 5.0$ Hz, 1H, H-1), 4.73 (t, $J = 8.5$ Hz, 1H, H-11), 3.98–4.05 (m, 1H, H-11), 3.81 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.71–3.76 (m, 1H, H-3), 3.59 (s, 3H, OCH_3), 3.03 (dd, $J = 12.5, 6.0$ Hz, 1H, H-2); HRMS m/z calcd for $\text{C}_{22}\text{H}_{21}\text{O}_8\text{NBr}$ ($[\text{M}+\text{H}]^+$) 506.0445, found 506.0440.

General procedure for synthesis of 2'-(2',6')-(di)halogeno-isoxazolopodophyllonic acids-based esters (Ia–c, e–g; IIa–f; and IIIa–g). A mixture of the corresponding alcohols R^1OH (0.28 mmol), N, N' -dicyclohexylcarbodiimide (DCC, 0.2 mmol), 4-dimethylaminopyridine (DMAP, 0.04 mmol), and 2'-(2',6')-(di)halogeno-isoxazolopodophyllonic acids (10, 11, or 12, 0.2 mmol) in dry DCM (10 mL) was stirred at room temperature. When the reaction was complete according to TLC analysis, the mixture was diluted by DCM (40 mL), washed by water (20 mL), aq. HCl (0.1 mol/L, 20 mL), saturated aq. NaHCO_3 (20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4 , concentrated in vacuo, and purified by PTLC to give compounds Ia–c, e–g; IIa–f; and IIIa–g in 47–93% yields. The example data of Ia–c; IIa–c; and IIIa–c are listed as follows, whereas data of Ie–g; IId–f; and IIId–g can be found in the Supporting Information.

Data for Ia: Yield = 63%, white solid, m.p. 155–156 $^\circ\text{C}$; $[\alpha]_D^{20} = -81$ (c 3.0 mg/mL, CHCl_3); IR cm^{-1} (KBr): 3094, 2937, 1736, 1484, 1233, 1109; $^1\text{H NMR}$ (500 MHz, CDCl_3) δ : 7.43 (s, 1H, H-5), 6.50 (s, 1H, H-8), 6.08 (s, 1H, H-6'), 5.99 (dd, $J = 8.0, 1.5$ Hz, 2H, OCH_2O), 5.31 (d, $J = 5.5$ Hz, 1H, H-1), 4.78–4.81 (m, 1H, H-11), 4.06–4.13 (m, 1H, H-11), 3.89 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.76–3.80 (m, 1H, H-3), 3.62 (s, 3H, OCH_3), 3.61 (s, 3H, CO_2CH_3), 3.27 (dd, $J = 13.0, 5.5$ Hz, 1H, H-2); HRMS m/z calcd for $\text{C}_{23}\text{H}_{23}\text{O}_8\text{NCl}$ ($[\text{M}+\text{H}]^+$) 476.1107, found 476.1099.

Data for Ib: Yield = 54%, white solid, m.p. 129–130 $^\circ\text{C}$, $[\alpha]_D^{20} = -91$ (c 3.0 mg/mL, CHCl_3); IR cm^{-1} (KBr): 3036, 2931, 1728, 1484, 1232, 1110; $^1\text{H NMR}$ (500 MHz, CDCl_3) δ : 7.43 (s, 1H, H-5), 6.51 (s, 1H, H-8), 6.11 (s, 1H,

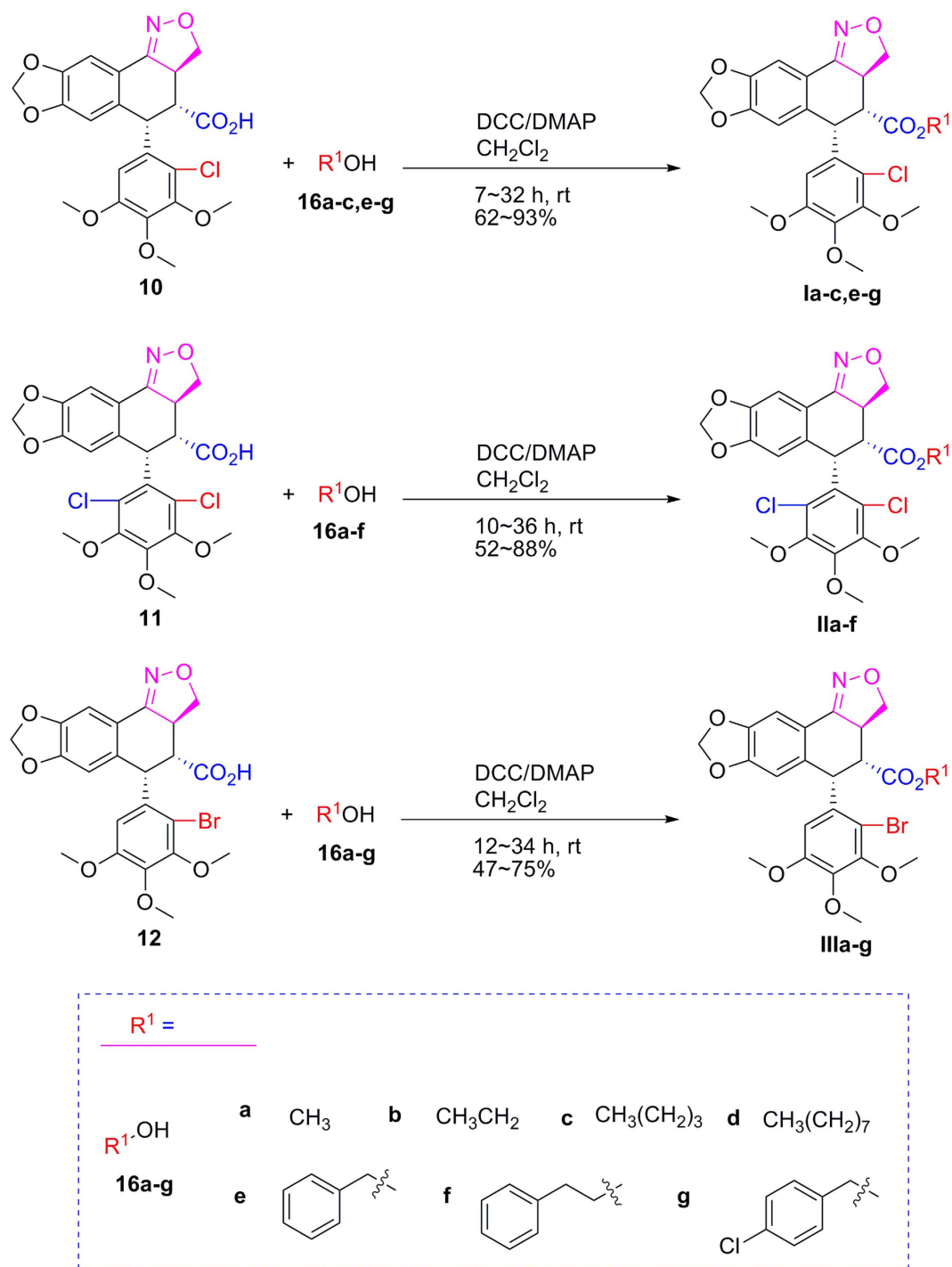


Figure 3. Preparation of 2'-(2',6')-(di)halogeno-isoxazolopodophyllinic acid-based esters (I–III).

H-6'), 5.99 (dd, $J = 9.0, 1.0$ Hz, 2H, OCH₂O), 5.34 (d, $J = 6.0$ Hz, 1H, H-1), 4.79–4.82 (m, 1H, H-11), 4.07–4.15 (m, 2H, CO₂CH₂CH₃), 3.94–3.97 (m, 1H, H-11), 3.89 (s, 3H, OCH₃), 3.85 (s, 3H, OCH₃), 3.77–3.81 (m, 1H, H-3), 3.63 (s, 3H, OCH₃), 3.25 (dd, $J = 13.0, 6.0$ Hz, 1H, H-2), 1.19 (t, $J = 7.0$ Hz, 3H, CO₂CH₂CH₃); HRMS m/z calcd for C₂₄H₂₅O₈NCl ([M+H]⁺) 490.1263, found 490.1255.

Data for Ic: Yield = 50%, white solid, m.p. 102–103 °C, $[\alpha]_D^{20} = -100$ (c 3.1 mg/mL, CHCl₃); IR cm⁻¹ (KBr): 3105, 2938, 1728, 1483, 1226, 1111; ¹H NMR (500 MHz, CDCl₃) δ : 7.43 (s, 1H, H-5), 6.51 (s, 1H, H-8), 6.10 (s, 1H, H-6'), 5.99 (dd, $J = 9.0, 1.0$ Hz, 2H, OCH₂O), 5.33 (d, $J = 5.5$ Hz, 1H, H-1), 4.77–4.81 (m, 1H, H-11), 4.10–4.14 (m, 1H, H-11), 4.02–4.09 (m, 2H, CO₂CH₂CH₂CH₂CH₃), 3.89 (s, 3H, OCH₃), 3.85 (s, 3H, OCH₃), 3.77–3.81 (m, 1H, H-3), 3.64 (s, 3H, OCH₃), 3.26 (dd, $J = 12.5, 6.0$ Hz, 1H, H-2), 1.47–1.58 (m, 2H, CO₂CH₂CH₂CH₂CH₃), 1.27–1.32 (m, 2H, CO₂CH₂CH₂CH₂CH₃), 0.91 (t, $J = 7.5$ Hz, 3H, CO₂CH₂CH₂CH₂CH₃); HRMS m/z calcd for C₂₆H₂₉O₈NCl ([M+H]⁺) 518.1576, found 518.1568.

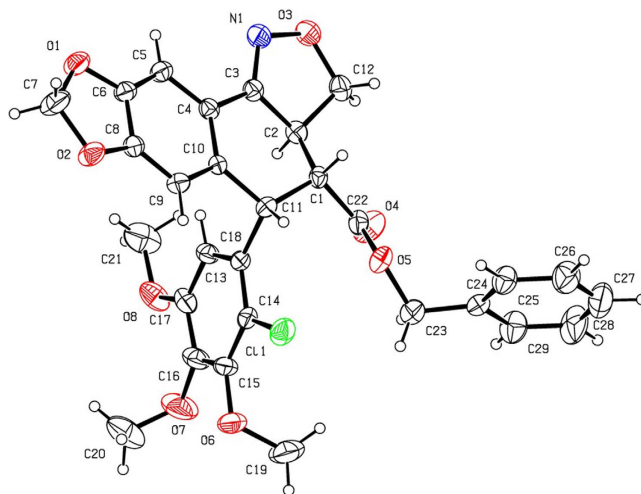


Figure 4. The X-ray crystal structure of **Ie**. Drawing by Hui Xu.

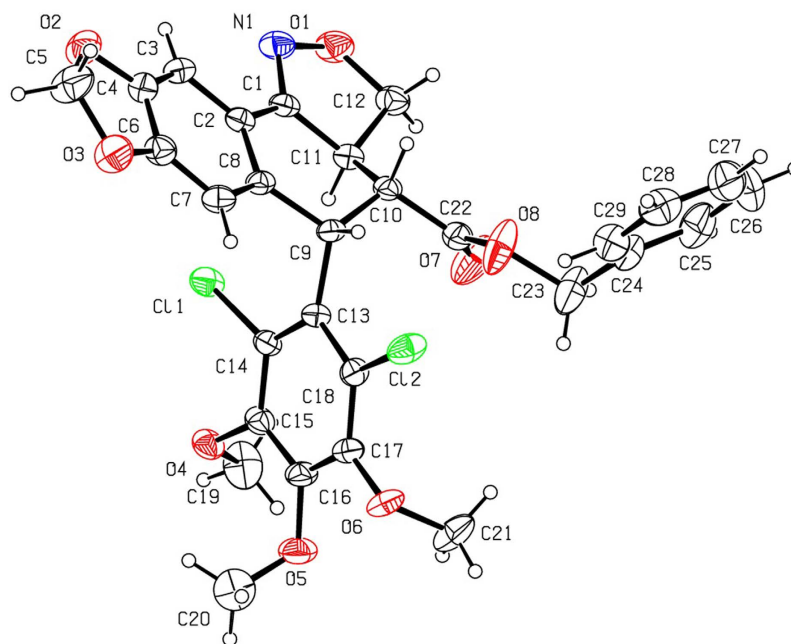


Figure 5. The X-ray crystal structure of **IIe**. Drawing by Hui Xu.

Data for IIa: Yield = 71%, white solid, m.p. 113–114 °C; $[\alpha]_D^{20} = -90$ (c 2.7 mg/mL, CHCl_3); IR cm^{-1} (KBr): 3078, 2935, 1734, 1483, 1230, 1109; $^1\text{H NMR}$ (500 MHz, CDCl_3) δ : 7.38 (s, 1H, H-5), 6.33 (s, 1H, H-8), 5.97 (dd, $J = 4.0, 1.5$ Hz, 2H, OCH_2O), 5.70 (d, $J = 9.0$ Hz, 1H, H-1), 4.76–4.79 (m, 1H, H-11), 4.58–4.64 (m, 1H, H-11), 3.94 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.73–3.78 (m, 1H, H-3), 3.46 (s, 3H, CO_2CH_3), 3.37 (dd, $J = 13.0, 9.0$ Hz, 1H, H-2); HRMS m/z calcd for $\text{C}_{23}\text{H}_{22}\text{O}_8\text{NCl}_2$ ($[\text{M}+\text{H}]^+$) 510.0717, found 510.0712.

Data for IIb: Yield = 52%, white solid, m.p. 168–169 °C, $[\alpha]_D^{20} = -69$ (c 2.7 mg/mL, CHCl_3); IR cm^{-1} (KBr): 3085, 2935, 1732, 1481, 1228, 1034; $^1\text{H NMR}$ (500 MHz, CDCl_3) δ : 7.38 (s, 1H, H-5), 6.31 (s, 1H, H-8), 5.97 (dd, $J = 3.5, 1.0$ Hz, 2H, OCH_2O), 5.72 (d, $J = 9.0$ Hz, 1H, H-1), 4.76–4.80 (m, 1H, H-11), 4.60–4.67 (m, 1H, H-11), 3.95–4.00 (m, 2H, $\text{CO}_2\text{CH}_2\text{CH}_3$), 3.93 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.75–3.78 (m, 1H, H-3), 3.34 (dd, $J = 13.0, 9.0$ Hz, 1H, H-2), 1.09 (t, $J = 7.0$ Hz, 3H, $\text{CO}_2\text{CH}_2\text{CH}_3$); HRMS m/z calcd for $\text{C}_{24}\text{H}_{24}\text{O}_8\text{NCl}_2$ ($[\text{M}+\text{H}]^+$) 524.0873, found 524.0869.

Data for IIc: Yield = 79%, white solid, m.p. 97–98 °C, $[\alpha]_D^{20} = -144$ (c 3.0 mg/mL, CHCl_3); IR cm^{-1} (KBr): 3078, 2933, 1731, 1479, 1226, 1021; $^1\text{H NMR}$ (500 MHz, CDCl_3) δ : 7.38 (s, 1H, H-5), 6.32 (s, 1H, H-8), 5.97 (d, $J = 3.0$ Hz, 2H, OCH_2O), 5.70 (d, $J = 8.5$ Hz, 1H, H-1), 4.75–4.79 (m, 1H, H-11), 4.59–4.66 (m, 1H, H-11), 3.94 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.78–3.80 (m, 4H, OCH_3 and H-3), 3.71–3.77 (m, 2H, $\text{CO}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 3.38 (dd, $J = 13.0, 8.0$ Hz, 1H, H-2), 1.38–1.47 (m, 2H, $\text{CO}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.23–1.29 (m, 2H,

Compound	Corrected mortality rate (%)		
	10 days	20 days	35 days
1	3.3 ± 3.3	23.3 ± 3.3	34.5 ± 3.3
4	23.3 ± 6.7	26.7 ± 8.8	44.8 ± 3.3
5	16.7 ± 3.3	36.7 ± 3.3	51.7 ± 3.3
6	26.7 ± 8.8	36.7 ± 3.3	41.4 ± 3.3
7	10.0 ± 5.8	33.3 ± 6.7	48.2 ± 5.8
8	13.3 ± 3.3	30.0 ± 5.8	41.4 ± 8.8
9	10.0 ± 5.8	33.3 ± 3.3	44.8 ± 3.3
10	13.3 ± 8.8	30.0 ± 5.8	41.4 ± 3.3
11	26.7 ± 3.3	33.3 ± 6.7	51.7 ± 3.3
12	16.7 ± 3.3	23.3 ± 6.7	37.9 ± 5.8
Ia	16.7 ± 3.3	20.0 ± 0	44.8 ± 3.3
Ib	10.0 ± 5.8	20.0 ± 5.8	37.9 ± 5.8
Ic	26.7 ± 3.3	36.7 ± 6.7	44.8 ± 3.3
Ie	10.0 ± 5.8	26.7 ± 3.3	37.9 ± 5.8
If	23.3 ± 3.3	30.0 ± 5.8	44.8 ± 3.3
Ig	20.0 ± 0	26.7 ± 6.7	31.0 ± 8.8
IIa	20.0 ± 0	30.0 ± 0	55.2 ± 3.3
IIb	16.7 ± 3.3	36.7 ± 8.8	55.2 ± 6.7
IIc	16.7 ± 3.3	36.7 ± 3.3	62.0 ± 3.3
IIId	10.0 ± 0	33.3 ± 6.7	48.2 ± 5.8
IIe	6.7 ± 3.3	30.0 ± 5.8	44.8 ± 3.3
IIIf	16.7 ± 3.3	20.0 ± 0	55.2 ± 3.3
IIIa	20.0 ± 5.8	33.3 ± 8.8	41.4 ± 6.7
IIIb	13.3 ± 3.3	20.0 ± 0	48.2 ± 3.3
IIIc	20.0 ± 0	26.7 ± 3.3	51.7 ± 3.3
IIId	13.3 ± 3.3	23.3 ± 3.3	41.4 ± 3.3
IIIe	6.7 ± 3.3	26.7 ± 6.7	51.7 ± 3.3
IIIIf	23.3 ± 3.3	36.7 ± 6.7	44.8 ± 8.8
IIIg	16.7 ± 3.3	26.7 ± 6.7	37.9 ± 5.8
IVa	13.3 ± 3.3	23.3 ± 3.3	34.5 ± 3.3
IVb	16.7 ± 3.3	33.3 ± 6.7	51.7 ± 3.3
IVc	20.0 ± 5.8	26.7 ± 3.3	58.6 ± 0
Va	16.7 ± 3.3	40.0 ± 5.8	44.8 ± 6.7
Vb	23.3 ± 8.8	33.3 ± 3.3	44.8 ± 3.3
Vc	16.7 ± 3.3	30.0 ± 5.8	65.2 ± 6.7
VIb	10.0 ± 0	20.0 ± 5.8	44.8 ± 3.3
VIc	23.3 ± 8.8	36.7 ± 3.3	55.2 ± 3.3
toosendanin	16.7 ± 3.3	33.3 ± 3.3	44.8 ± 3.3

Table 1. Insecticidal Activity of 1; 4–12; Ia–c,e–g; IIa–f; IIIa–g; IVa–c; Va–c; VIb; and VIc against *M. separata* on Leaves Treated with a Concentration of 1 mg/mL.

$\text{CO}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 0.88 (t, $J = 7.0$ Hz, 3H, $\text{CO}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$); HRMS m/z calcd for $\text{C}_{26}\text{H}_{28}\text{O}_8\text{NCl}_2$ ($[\text{M} + \text{H}]^+$) 552.1186, found 552.1176.

Data for IIIa: Yield = 55%, white solid, m.p. 147–148 °C, $[\alpha]_{\text{D}}^{20} = -85$ (c 2.1 mg/mL, CHCl_3); IR cm^{-1} (KBr): 3045, 2931, 1731, 1483, 1230, 1106; ^1H NMR (500 MHz, CDCl_3) δ : 7.43 (s, 1H, H-5), 6.52 (s, 1H, H-8), 6.14 (s, 1H, H-6'), 5.99 (dd, $J = 9.0, 1.0$ Hz, 2H, OCH_2O), 5.38 (d, $J = 6.0$ Hz, 1H, H-1), 4.80–4.83 (m, 1H, H-11), 4.10–4.17 (m, 1H, H-11), 3.88 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.76–3.81 (m, 1H, H-3), 3.63 (s, 3H, OCH_3), 3.60 (s, 3H, CO_2CH_3), 3.26 (dd, $J = 12.5, 6.0$ Hz, 1H, H-2); HRMS m/z calcd for $\text{C}_{23}\text{H}_{23}\text{O}_8\text{NBr}$ ($[\text{M} + \text{H}]^+$) 520.0602, found 520.0594.

Data for IIIb: Yield = 49%, white solid, m.p. 148–149 °C, $[\alpha]_{\text{D}}^{20} = -116$ (c 3.0 mg/mL, CHCl_3); IR cm^{-1} (KBr): 3058, 2933, 1726, 1482, 1229, 1106; ^1H NMR (500 MHz, CDCl_3) δ : 7.42 (s, 1H, H-5), 6.54 (s, 1H, H-8), 6.17 (s, 1H, H-6'), 5.99 (dd, $J = 10.5, 1.0$ Hz, 2H, OCH_2O), 5.41 (d, $J = 6.5$ Hz, 1H, H-1), 4.81–4.84 (m, 1H, H-11), 4.09–4.17 (m, 2H, $\text{CO}_2\text{CH}_2\text{CH}_3$), 3.92–3.96 (m, 1H, H-11), 3.88 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.78–3.82 (m, 1H, H-3), 3.64 (s, 3H, OCH_3), 3.25 (dd, $J = 12.5, 6.0$ Hz, 1H, H-2), 1.18 (t, $J = 7.0$ Hz, 3H, $\text{CO}_2\text{CH}_2\text{CH}_3$); HRMS m/z calcd for $\text{C}_{24}\text{H}_{25}\text{O}_8\text{NBr}$ ($[\text{M} + \text{H}]^+$) 534.0758, found 534.0749.

Data for IIIc: Yield = 47%, white solid, m.p. 129–130 °C, $[\alpha]_{\text{D}}^{20} = -109$ (c 2.3 mg/mL, CHCl_3); IR cm^{-1} (KBr): 3072, 2938, 1727, 1481, 1226, 1107; ^1H NMR (500 MHz, CDCl_3) δ : 7.42 (s, 1H, H-5), 6.54 (s, 1H, H-8), 6.16 (s, 1H, H-6'), 5.99 (dd, $J = 10.0, 1.0$ Hz, 2H, OCH_2O), 5.39 (d, $J = 6.0$ Hz, 1H, H-1), 4.79–4.82 (m, 1H, H-11), 4.11–4.18

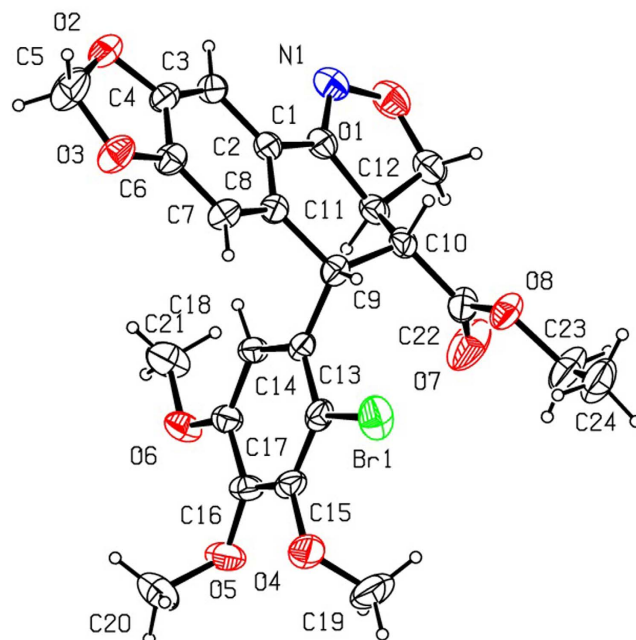


Figure 6. The X-ray crystal structure of IIIb. Drawing by Hui Xu.

(m, 1H, H-11), 4.01–4.05 (m, 1H, CO₂CH₂CH₂CH₂CH₃), 3.85–3.89 (m, 7H, 2 × OCH₃, CO₂CH₂CH₂CH₂CH₃), 3.78–3.82 (m, 1H, H-3), 3.64 (s, 3H, OCH₃), 3.26 (dd, *J* = 12.5, 6.0 Hz, 1H, H-2), 1.46–1.58 (m, 2H, CO₂CH₂CH₂CH₂CH₃), 1.26–1.30 (m, 2H, CO₂CH₂CH₂CH₂CH₃), 0.90 (t, *J* = 7.0 Hz, 3H, CO₂CH₂CH₂CH₂CH₃); HRMS *m/z* calcd for C₂₆H₂₉O₈NBr ([M+H]⁺) 562.1071, found 562.1057.

General procedure for synthesis of oxime sulfonates of 2'-(2',6')-(di)halogenopodophyllones (IVa–c; Va–c; and VIb,c).

To a stirred solution of NaH (1.4 mmol) in dry THF (8 mL) at –15 °C was slowly added compound 13, 14, or 15 (0.2 mmol). After addition, the reaction mixture was stirred at –15 °C for 0.5 h. Then, the corresponding sulfonyl chlorides (0.8 mmol) were added to the above mixture. After adding, the reaction temperature was raised from –15 °C to room temperature. When the reaction mixture was complete, checked by TLC analysis, saturated aq. NaHCO₃ (15 mL) was added to the mixture, which was extracted with DCM (3 × 30 mL). The combined organic phase was dried over anhydrous Na₂SO₄, filtered, concentrated under reduced pressure, and purified by PTLC to give compounds IVa–c; Va–c; and VIb,c in 43–72% yields. The example data of IVa; Va; and VIb are listed as follows, whereas data of IVb,c; Vb,c; and VIc can be found in the Supporting Information.

Data for IVa: Yield = 58%, white solid, m.p. 110–111 °C, [α]_D²⁰ = 5.5 (c 2.8 mg/mL, CHCl₃); IR cm⁻¹ (KBr): 3066, 2931, 1778, 1484, 1398, 1194, 1035, 759, 722; ¹H NMR (500 MHz, CDCl₃) δ: 8.02–8.04 (m, 2H, Ar-H), 7.60–7.63 (m, 3H, Ar-H), 7.14 (s, 1H, H-5), 6.71 (s, 1H, H-8), 6.03 (s, 2H, OCH₂O), 5.76 (s, 1H, H-6'), 5.07 (d, *J* = 2.0 Hz, 1H, H-1), 4.52–4.55 (m, 1H, H-11), 4.32 (d, *J* = 10.0 Hz, 1H, H-11), 3.92 (s, 3H, OCH₃), 3.88–3.91 (m, 1H, H-3), 3.84 (s, 3H, OCH₃), 3.36 (s, 3H, OCH₃), 3.41 (dd, *J* = 8.5, 2.0 Hz, 1H, H-2); HRMS *m/z* calcd for C₂₈H₂₅O₁₀NClS ([M+H]⁺) 602.0882, found 602.0877.

Data for Va: Yield = 45%, white solid, m.p. 107–108 °C, [α]_D²⁰ = 26 (c 3.0 mg/mL, CHCl₃); IR cm⁻¹ (KBr): 3067, 2936, 1780, 1481, 1377, 1193, 1021, 722, 686; ¹H NMR (500 MHz, CDCl₃) δ: 8.05–8.07 (m, 2H, Ar-H), 7.60–7.73 (m, 3H, Ar-H), 7.32 (s, 1H, H-5), 6.20 (s, 1H, H-8), 5.99 (dd, *J* = 10.5, 1.0 Hz, 2H, OCH₂O), 5.22 (d, *J* = 10.0 Hz, 1H, H-1), 4.95–4.98 (m, 1H, H-11), 4.21–4.26 (m, 1H, H-11), 3.98 (s, 3H, OCH₃), 3.87–3.94 (m, 7H, 2 × OCH₃ and H-3), 3.49–3.52 (m, 1H, H-2); HRMS *m/z* calcd for C₂₈H₂₄O₁₀NCl₂S ([M+H]⁺) 636.0492, found 636.0475.

Data for VIb: Yield = 44%, yellow solid, m.p. 111–112 °C, [α]_D²⁰ = 22 (2.9 mg/mL, CHCl₃); IR cm⁻¹ (KBr): 3083, 2933, 1781, 1483, 1390, 1194, 1035, 818; ¹H NMR (500 MHz, CDCl₃) δ: 7.92 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.40 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.15 (s, 1H, H-5), 6.71 (s, 1H, H-8), 6.02 (s, 2H, OCH₂O), 5.81 (s, 1H, H-6'), 5.08 (d, *J* = 2.5 Hz, 1H, H-1), 4.50–4.54 (m, 1H, H-11), 4.31 (d, *J* = 10.0 Hz, 1H, H-11), 3.91 (s, 3H, OCH₃), 3.86–3.89 (m, 1H, H-3), 3.84 (s, 3H, OCH₃), 3.42 (dd, *J* = 8.5, 2.5 Hz, 1H, H-2), 3.36 (s, 3H, OCH₃), 2.49 (s, 3H, CH₃); HRMS *m/z* calcd for C₂₉H₂₇O₁₀NBrS ([M+H]⁺) 660.0534, found 660.0516.

Biological assay. The pesticidal activity of 1; 4–12; Ia–c, e–g; IIa–f; IIIa–g; IVa–c; Va–c; VIb; and VIc was tested as the mortality rate values by using the leaf-dipping method²⁸, against the pre-third-instar larvae of *Mythimna separata*. For each compound, 30 pre-third-instar larvae (10 larvae per group) were used. Acetone solutions of 1; 4–12; Ia–c, e–g; IIa–f; IIIa–g; IVa–c; Va–c; VIb; VIc; and toosendanin (a positive control) were prepared at 1 mg/mL. Fresh wheat leaf discs (1 × 1 cm) were dipped into the corresponding solution for 3 s, then taken out and dried. Leaf discs treated with acetone alone were used as a blank control group. Several pieces

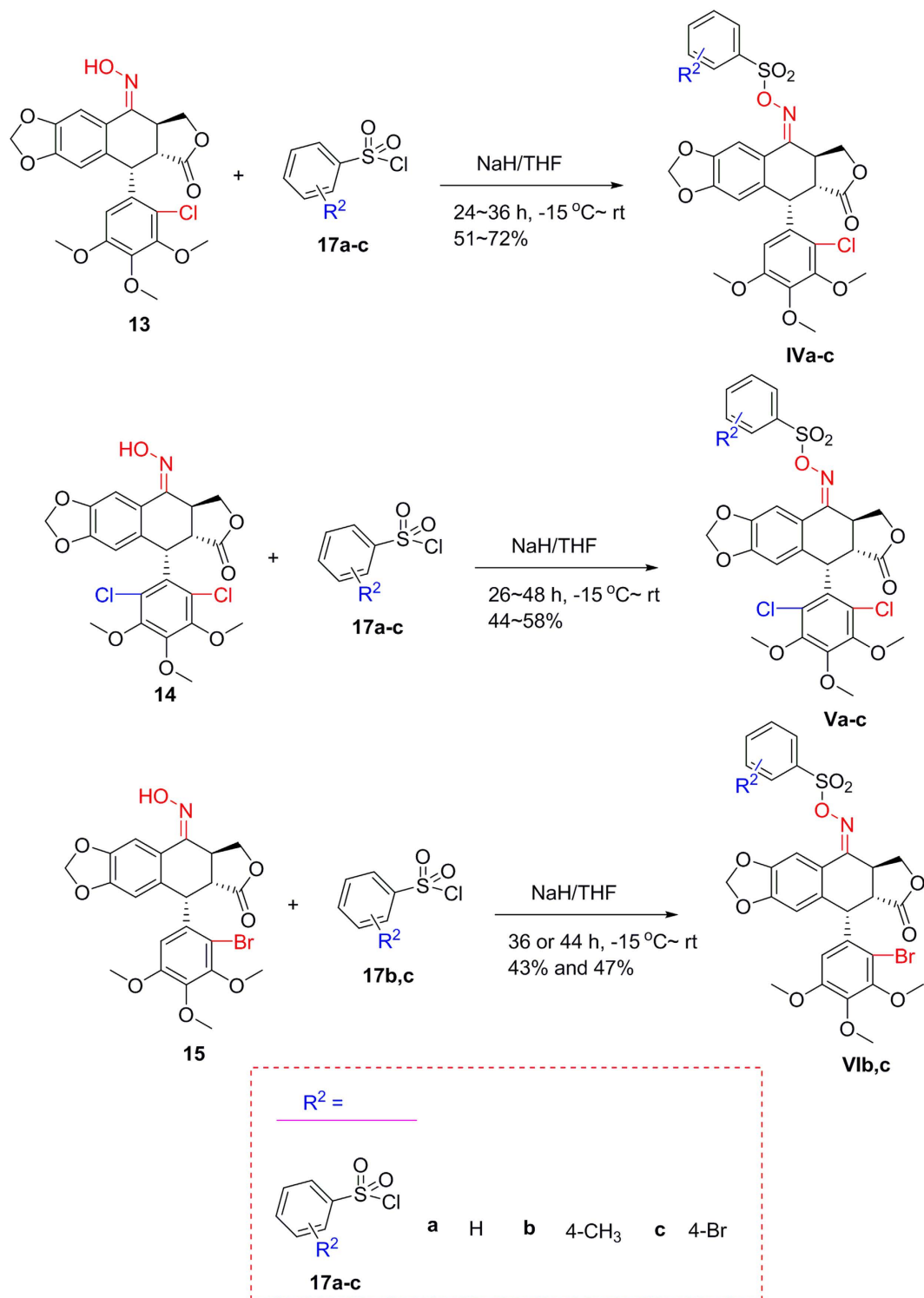


Figure 7. Preparation of oxime sulfonate derivatives of 2'(2',6')-(di)chloropodophyllotoxins (IV–VI).

of treated leaf discs were kept in each dish (10 larvae per dish), which was then placed in a conditioned room ($25 \pm 2^\circ\text{C}$, 65–80% relative humidity, 12h/12h (light/dark) photoperiod). If the treated leaf discs were consumed, additional treated ones were added to the dish. After 48 h, untreated fresh leaves were added to all dishes till the end of pupae. The corrected mortality rate values were obtained by the formula:

$$\text{corrected mortality rate (\%)} = (T - C) \times 100 / (100 - C)$$

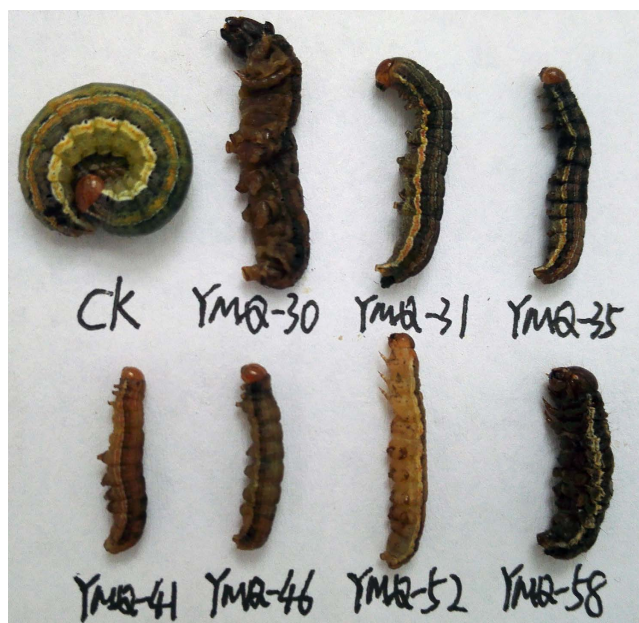


Figure 8. Representative abnormal larvae pictures of compounds Ic (YMQ-30), If (YMQ-31), IVb (YMQ-35), IIc (YMQ-41), IIIa (YMQ-46), IIIg (YMQ-52), and Va (YMQ-58) during the larval period (CK = blank control group).



Figure 9. Representative malformed pupae pictures of compounds Ic (YMQ-30), If (YMQ-31), IIc (YMQ-41), IIIa (YMQ-46), IIIg (YMQ-52), Vb (YMQ-55), and Va (YMQ-58) during the pupation period (CK = blank control group).

where T is the mortality rate in the group treated with the tested compounds, and C is the mortality rate in the blank control group (T and C were all expressed as the percentage).

Results and Discussion

As shown in Fig. 2, 2'-chloropodophyllotoxin (4), 2',6'-dichloropodophyllotoxin (5), and 2'-bromopodophyllotoxin (6) were firstly obtained as described previously²⁸. Then, 2'-(2',6')-(di)halogenopodophyllones (7–9) were easily obtained by oxidation of 4–6, respectively. Subsequently, when compounds 7–9 reacted with hydroxylamine hydrochloride, 2'-(2',6')-(di)halogeno-isoxazolopodophyllic acids (10–12) and oximes of 2'-(2',6')-(di)halogenopodophyllones (13–15) were all produced. However, in our previous paper, when 2'-(2',6')-(di)chloropodophyllones reacted with hydroxylamine hydrochloride, only oximes of 2'-(2',6')-(di)chloropodophyllotoxin were afforded²⁴. It indicated that when 2'-(2',6')-(di)halogenopodophyllones or 2'-(2',6')-(di)

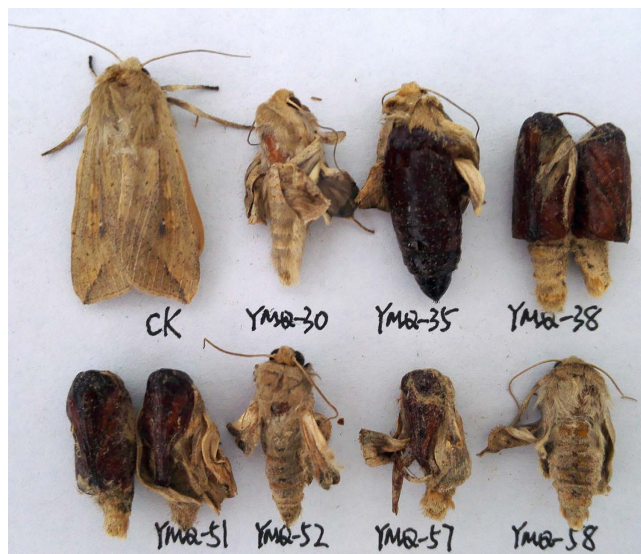


Figure 10. Representative malformed moth pictures of compounds **Ic** (YMQ-30), **IVb** (YMQ-35), **IIb** (YMQ-38), **IIIf** (YMQ-51), **IIIg** (YMQ-52), **IIId** (YMQ-57), and **Va** (YMQ-58) during the stage of adult emergence (CK = blank control group).

chloropicropodophyllones reacted with hydroxylamine hydrochloride, the desired products were related with the configuration of their lactones. Finally, as shown in Fig. 3, 2'-(2',6')-(di)halogeno-isoxazolopodophyllonic acids-based esters (**Ia-c,e-g**; **IIa-f**; and **IIIa-g**) were prepared by the reaction of **10-12** with different alcohols in the presence of DCC and DMAP, and well characterized by ^1H NMR, HRMS, optical rotation, mp and IR. Especially three single-crystal structures of **Ie**, **Ile** and **IIIb** were determined by X-ray crystallography as illustrated in Figs 4–6, respectively. It showed that the chlorine atom of **Ie** was at the C-2' position; two chlorine atoms of **Ile** was at the C-2' and C-6' position; the bromine atom of **IIIb** was at the C-2' position. Meanwhile, the two hydrogen atoms at C-2 and C-3 position of **Ie**, **Ile** and **IIIb** were all in β and α configuration, respectively. Crystallographic data (excluding structure factors) for the structures of **Ie**, **Ile** and **IIIb** have been deposited at the Cambridge Crystallographic Data Centre with supplementary publication number CCDC 1482635, 1482788, and 1482789, respectively. 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].

On the other hand, as described in Fig. 7, oxime sulfonates of 2'-(2',6')-(di)halogenopodophyllones (**IVa-c**; **Va-c**; and **VIb,c**) were smoothly obtained by reaction of **13-15** with the corresponding sulfonyl chlorides. Their structures were well characterized by ^1H NMR, HRMS, optical rotation, mp and IR.

As shown in Table 1, the insecticidal activity of **1;4-12**; **Ia-c,e-g**; **IIa-f**; **IIIa-g**; **IVa-c**; **Va-c**; **VIb**; and **VIc** against the pre-third-instar larvae of *M. separata* *in vivo* was evaluated at a concentration of 1 mg/mL. The corresponding mortality rates after 35 days were higher than those after 10 and 20 days. For example, the mortality rate of compound **Ic** against *M. separata* after 35 days was 62.0%, whereas the mortality rates of **Ic** against *M. separata* after 10 and 20 days were 16.7% and 36.7%, respectively. It suggested that these podophyllotoxin compounds showed delayed insecticidal activity^{24,28}. Meanwhile, the symptoms of the treated *M. separata* were observed in the same way as our previous reports^{24,28}. As shown in Fig. 8, many larvae of the treated groups died slowly during the larval stage; as shown in Fig. 9, some malformed pupae of the treated groups also appeared and died during the pupation stage; some malformed moths with imperfect wings also appeared in the treated groups (Fig. 10).

Among all derivatives, compounds **4**; **5**; **7**; **9**; **11**; **Ia**; **Ic**; **If**; **IIa-f**; **IIIb**; **IIIc**; **IIIe**; **IIIf**; **IVb**; **IVc**; **Va-c**; **VIb**; and **VIc** exhibited equal or higher insecticidal activity than toosendanin. Compounds **Ic** and **Vc**, especially, showed the highest insecticidal activity. For example, the final mortality rates (FMRs) of **Ic** and **Vc** were 62.0%, and 65.2%, respectively. Introduction of a halogen atom on the E ring of podophyllotoxin/podophyllotoxone was important for the insecticidal activity. For example, FMRs of **4** (containing a 2'-chlorine atom), **5** (containing two 2',6'-dichlorine atoms) and **6** (containing a 2'-bromine atom) were 44.8%, 51.7%, and 41.4%, respectively; whereas the FMR of **1** was only 34.5%. FMRs of **7** (containing a 2'-chlorine atom), **8** (containing two 2',6'-dichlorine atoms) and **9** (containing a 2'-bromine atom) were 44.8%, 51.7%, and 41.4%, respectively; whereas the FMR of podophyllotoxone was only 17.2%²⁹. In general, 2',6'-dichloro-isoxazolopodophyllonic acids-based esters exhibited more potent insecticidal activities than those of the corresponding 2'-chloro/bromo-isoxazolopodophyllonic acids-based ones. For example, FMRs of **IIa-c,e,f** were 55.2%, 55.2%, 62.0%, 44.8% and 55.2%, respectively; whereas FMRs of **Ia-c,e,f** were 44.8%, 37.9%, 44.8%, 37.9% and 44.8%, respectively. To oxime sulfonates of 2'-(2',6')-(di)halogenopodophyllones (**IVa-c**; **Va-c**; and **VIb,c**), introduction of a bromine atom on the phenyl ring of the sulfonate moiety led to more potent compounds than those containing methyl or hydrogen ones (**IVc** vs **IVa** and **IVb**; **Vc** vs **Va** and **Vb**; **VIc** vs **VIb**).

Conclusion

In summary, a series of 2'-(2',6')-(di)halogeno-isoxazolopodophyllin acids-based esters and oxime sulfonates of 2'-(2',6')-(di)halogenopodophyllones were prepared, and evaluated for their insecticidal activity against the pre-third-instar larvae of *M. separata* *in vivo*. It suggested that when 2'-(2',6')-(di)halogenopodophyllones or 2'-(2',6')-(di)chloropicropodophyllones reacted with hydroxylamine hydrochloride, the desired products were determined by the configuration of their lactones. Three key single-crystal structures of **Ie**, **IIf** and **IIIb** were confirmed by X-ray crystallography.

Among all derivatives, especially compounds **IIf** and **Vc** showed the highest insecticidal activity. Moreover, some interesting results of structure-insecticidal activity relationships of tested compounds were also observed. This will pave the way for further design and structural modifications of podophyllotoxin derivatives as insecticidal agents.

References

- Jiang, X. F., Zhang, L., Cheng, Y. X. & Luo, L. Z. Novel features, occurrence trends and economic impact of the oriental armyworm, *Mythimna separata* (Walker) in China. *Chin. J. Appl. Entomol.* **51**, 1444–1449 (2014).
- Li, F. B. *et al.* Complete mitochondrial genome of the oriental armyworm *Mythimna separata* (Walker) (Lepidoptera: Noctuidae). *Mitochondrial DNA* **26**, 881–882 (2015).
- Afzal, M. B. S. & Shad, S. A. Genetic analysis, realized heritability and synergistic suppression of indoxacarb resistance in *Phenacoccus solenopsis* Tinsley (Homoptera: Pseudococcidae). *Crop Prot.* **84**, 62–68 (2016).
- Kanga, L. H. B., Eason, J., Haseeb, M., Qureshi, J. & Stansly, P. Monitoring for insecticide resistance in Asian *Citrus Psyllid* (Hemiptera: Psyllidae) populations in Florida. *J. Econom. Entomol.* **109**, 832–836 (2016).
- Barros-Rios, J., Santiago, R., Jung, H. J. G. & Malvar, R. A. Covalent cross-linking of cell-wall polysaccharides through esterified diferulates as a maize resistance mechanism against corn borers. *J. Agric. Food Chem.* **63**, 2206–2214 (2015).
- Itokawa, K., Komagata, O., Kasai, S., Ogawa, K. & Tomita, T. Testing the causality between CYP9M10 and pyrethroid resistance using the TALEN and CRISPR/Cas9 technologies. *Sci. Rep.* **6**, 24652 (2016).
- Seong, K. M. & Sun, W., Clark, J. M. & Pittendrigh, B. R. Splice form variant and amino acid changes in MDR49 confers DDT resistance in transgenic *Drosophila*. *Sci. Rep.* **6**, 23355 (2016).
- Garzo, E. *et al.* Electrical penetration graph technique as a tool to monitor the early stages of aphid resistance to insecticides. *Pest Manage. Sci.* **72**, 707–718 (2016).
- Seiber, J. N., Coats, J., Duke, S. O. & Gross, A. D. Biopesticides: state of the art and future opportunities. *J. Agric. Food Chem.* **62**, 11613–11619 (2014).
- Qu, H., Lv, M., Yu, X., Lian, X. & Xu, H. Discovery of some piperine-based phenylsulfonylhydrazone derivatives as potent botanically narcotic agents. *Sci. Rep.* **5**, 13077 (2015).
- Zuo, Y. *et al.* Synthesis, herbicidal activity, and QSAR of novel *N*-benzothiazolyl-pyrimidine-2,4-diones as protoporphyrinogen oxidase inhibitors. *J. Agric. Food Chem.* **64**, 552–562 (2016).
- Engstrom, M. T. *et al.* Chemical structures of plant hydrolyzable tannins reveal their *in vitro* activity against egg hatching and motility of *haemonchus contortus* nematodes. *J. Agric. Food Chem.* **64**, 840–851 (2016).
- Song, Q. Y. *et al.* Antifungal, phytotoxic, and cytotoxic activities of metabolites from *Epichloe bromicola*, a fungus obtained from *Elymus tangutorum* grass. *J. Agric. Food Chem.* **63**, 8787–8792 (2015).
- Prasifka, J. R. *et al.* Sesquiterpene lactone composition of wild and cultivated sunflowers and biological activity against an insect pest. *J. Agric. Food Chem.* **63**, 4042–4049 (2015).
- Song, H. J. *et al.* Design, synthesis, anti-TMV, fungicidal, and insecticidal activity evaluation of 1,2,3,4-tetrahydro- β -carboline-3-carboxylic acid derivatives based on virus inhibitors of plant sources. *Bioorg. Med. Chem. Lett.* **24**, 5228–5233 (2014).
- Cao, J., Adler, B. & Perlmutter, P. Synthesis and evaluation of antibacterial activity of (*R*)-2-methylheptyl isonicotinate, a putative naturally occurring bioactive agent. *Bioorg. Med. Chem. Lett.* **25**, 5025–5027 (2015).
- Lv, M. & Xu, H. Recent advances in semisynthesis, biosynthesis, biological activities, mode of action, and structure-activity relationship of podophyllotoxins: an update (2008–2010). *Mini-Rev. Med. Chem.* **11**, 901–909 (2011).
- Abrams, J. N., Zhao, Q. & Ghiviriga, I. & Minaruzzaman. Palladium(II)-catalyzed enyne cyclization strategies toward the podophyllotoxin ring system. *Tetrahedron* **68**, 423–428 (2012).
- Takahashi, M., Suzuki, N. & Ishikawa, T. Enantioselective formal synthesis of (–)-podophyllotoxin from (2*S*,3*R*)-3-arylaziridine-2-carboxylate. *J. Org. Chem.* **78**, 3250–3261 (2013).
- Ting, C. P. & Maimone, T. J. C–H bond arylation in the synthesis of aryltetralin lignans: a short total synthesis of podophyllotoxin. *Angew. Chem. Int. Ed.* **53**, 3115–3119 (2014).
- Fan, L. L., Guo, Y., Zhi, X. Y., Yu, X. & Xu, H. Stereoselective synthesis of 2 α -chloropicropodophyllotoxins and insecticidal activity of their esters against oriental armyworm, *Mythimna separata* Walker. *J. Agric. Food Chem.* **62**, 3726–3733 (2014).
- Shankaraiah, N. *et al.* One-pot synthesis of podophyllotoxin–thiourea congeners by employing NH₂SO₃H/NaI: anticancer activity, DNA topoisomerase-II inhibition, and apoptosis inducing agents. *Bioorg. Med. Chem. Lett.* **25**, 4239–4244 (2014).
- Dunlap, N. *et al.* Synthesis and evaluation of ether-linked demethylepipodophyllotoxin dimmers. *Bioorg. Med. Chem. Lett.* **24**, 5627–5629 (2014).
- Wang, R., Zhi, X. Y., Li, J. & Xu, H. Synthesis of novel oxime sulfonate derivatives of 2'-(2',6')-(di)chloropicropodophyllotoxins as insecticidal agents. *J. Agric. Food Chem.* **63**, 6668–6674 (2015).
- Xu, H. & He, X. Q. Natural products-based insecticidal agents 6. design, semisynthesis, and insecticidal activity of novel monomethyl phthalate derivatives of podophyllotoxin against *Mythimna separata* Walker *in vivo*. *Bioorg. Med. Chem. Lett.* **20**, 4503–4506 (2010).
- Hu, H., Liu, S. Y., Cheng, Y. C., Lee, K. H. & Wang Z. Q. Antitumor agents. 123. Synthesis and human DNA topoisomerase II inhibitory activity of 2'-chloro derivatives of etoposide and 4 β -(arylamino)-4'-O-demethylpodophyllotoxins. *J. Med. Chem.* **35**, 866–871 (1992).
- Che, Z. P., Yu, X., Fan, L. L. & Xu, H. Insight into dihalogenation of E-ring of podophyllotoxins, and their acyloxylation derivatives at the C4 position as insecticidal agents. *Bioorg. Med. Chem. Lett.* **23**, 5592–5598 (2013).
- Che, Z. P., Yu, X., Zhi, X. Y., Fan, L. L. & Xu, H. Synthesis of novel 4 α -(acyloxy)-2'-(2',6')-(di)halogenopodophyllotoxin derivatives as insecticidal agents. *J. Agric. Food Chem.* **61**, 8148–8155 (2013).
- Wang, Y. *et al.* Synthesis and quantitative structure-activity relationship (QSAR) study of novel isoxazoline and oxime derivatives of podophyllotoxin as insecticidal agents. *J. Agric. Food Chem.* **60**, 8435–8443 (2012).

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Author Contributions

M.Y., G.L. and Y.Z. performed experiments, and analysed data; T.F. and M.X. analysed data; H.X. designed experiments, analysed data and wrote the manuscript.

Additional Information

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