Synthesis of Novel Oxime Sulfonate Derivatives of 2′(2′,6′)-(di)Chloropicropodophyllotoxins as Insecticidal Agents

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Abstract

To discover novel natural-product-based pesticidal agents, we prepared a series of oxime sulfonate derivatives of 2′(2′,6′)-(di)chloropicropodophyllotoxins by structural modification of podophyllotoxin. Their structures were well characterized by ^{1}H NMR, HRMS, optical rotation, and melting point. Moreover, the key steric structure of **5f** was unambiguously determined by single-crystal X-ray diffraction. Additionally, their insecticidal activity was evaluated at 1 mg/mL against the pre-third-instar larvae of oriental armyworm (*Mythimna separata* Walker), a typical lepidopteran pest. Among all derivatives, compounds **4c**, **5c** and **5d** exhibited more promising insecticidal activity with the final mortality rates greater than 60%, when compared with their precursor podophyllotoxin and the positive control, toosendanin. It demonstrated that introduction of the chlorine atom at the C-2′ or C-2′,6′ position on the E-ring of picropodophyllotoxin or oxime sulfonate derivatives of picropodophyllotoxin was important for the insecticidal activity; and introduction of a halogen (e.g., fluorine, chlorine, or bromine) atom-substituted phenylsulfonyl group on the oxime fragment of 2′(2′,6′)-(di)chloropicropodophyllones could lead to more promising compounds.

KEYWORDS: podophyllotoxin, oxime sulfonate, insecticidal activity, natural-product-based insecticide, structural modification, *Mythimna separata* Walker

INTRODUCTION

Oriental armyworm, *Mythimna separata* Walker, is a typical lepidopteran pest. The intermittent outbreaks of its larvae at very high densities can terribly result in complete crop loss.[1] For example, in 2012, the severe outbreak of 3rd-generation larvae of *M. separata* widely occurred in China, and approximately 4 million hectares of crops (e.g., corn, rice, wheat, etc.) were affected.[2] Currently, chemical pesticides are still the effective method to control insect pests in agriculture. However, increasing and long-term application of synthetic agrochemicals has resulted in the development of resistance in pest populations and environmental problems.[3-7] Therefore, search of the potential alternatives to control insect pests has recently received much attention in the agricultural field.[8-15]

Podophyllotoxin (**1**, Figure 1), a naturally occurring aryltetralin cyclolignan, is isolated from the roots and rhizomes of some *Podophyllum* and *Juniperus* species. Compound **1** has been widely used as the lead molecule for chemical modifications to discover more potent antitumor, insecticidal and antifungal agents.[16] Additionally, Hu *et al.* found that once a chlorine atom was introduced at the C-2' position of podophyllotoxin derivatives, the corresponding compounds showed no significant cytotoxicity.[17] More recently, we have prepared a series of oxime sulfonate derivatives of picropodophyllotoxin[18] (**2**, Figure 1) and 4α/β-acyloxy-2′(2′,6′)-(di)halogenopodophyllotoxin derivatives[19,20] (**3**, Figure 1) as insecticidal agents, and found some derivatives exhibited more potent insecticidal activity than toosendanin, a commercial botanical insecticide isolated from *Melia azedarach*. Based upon the above-mentioned results, and in continuation of our program to discover new potential natural-product-based insecticidal agents, consequently, in the present paper we prepared a series of novel oxime sulfonate derivatives of 2′-chloropicropodophyllotoxin and

2′,6′-dichloropicropodophyllotoxin (**4** and **5**, Figure 1). Their insecticidal activity against *M. separata* was also presented.

MATERIALS AND METHODS

Chemicals and Reagents. *N*-Chlorosuccinimide (NCS), sodium hydride, hydroxylamine hydrochloride, and sulfonyl chlorides were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Chromium trioxide was purchased from Tianli Chemical Reagent Co., Ltd. (Tianjin, China). *N,N*-Dimethylformamide, ethyl acetate, petroleum ether, pyridine, dichloromethane, absolute ethanol, tetrahydrofuran were analytical grade and purchased from Bodi Chemical Co., Ltd. (Tianjin, China). Sodium carbonate (Na_2CO_3), anhydrous sodium sulfate (Na_2SO_4), and sodium hydrogen sulfite ($NaHSO_3$) were purchased from Kelong Chemical Reagent Co., Ltd. (Chengdu, China). Sodium bicarbonate ($NaHCO_3$) was purchased from Guangdong Guanghua Chemical Factory Co., Ltd. (Shantou, China). Podophyllotoxin was purchased from Gansu Gerui Medicinal Materials Co., Ltd. (Lanzhou, China). Analytical thin-layer chromatography (TLC) and preparative thin-layer chromatography (PTLC) were performed with silica gel plates using silica gel 60 GF_{254} (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). Picropodophyllotoxin (**6**, 72% yield, Scheme 1) was prepared in the same way as in our previous report.[18]

Instrumentation. Melting points (mp) were determined on a XT-4 digital melting point apparatus (Beijing Tech Instrument Co., Ltd., Beijing, China) and were uncorrected. Optical rotation was measured on a Rudolph Research Analytical Autopol III automatic polarimeter. Proton nuclear magnetic resonance spectra (^{1}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 (HR-MS) were carried out with IonSpec 4.7 Tesla FTMS instrument.

General Procedure for Synthesis of 2′-Chloropicropodophyllotoxin (7), 2′,6′-Dichloropicropodophyllotoxin (8). To a solution of **6** (2 mmol) in dry *N,N*-dimethylformamide (DMF, 5 mL) at 0 ^{o}C, a solution of *N*-chlorosuccinimide (NCS, 1.15 or 2.0 equiv.) in dry DMF (4 mL) was added dropwise for 10 min. During one hour, the solution was allowed to warm slowly from 0 ^{o}C to 28 ^{o}C. When the reaction was complete checked by TLC analysis, the reaction mixture was diluted with water (15 mL), and extracted with ethyl acetate (30 mL × 3). Subsequently, the combined organic phase was washed by saturated aq. Na_2CO_3 (30 mL × 3) and brine (30 mL), dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by silica gel column chromatography eluting with petroleum ether/ethyl acetate (1:1, v/v) to afford **7** (747 mg, 90% yield) or **8** (705 mg, 85% yield).

*Data for **7***: CAS: 1458601-21-2; Yield: 90%, white solid, m.p. 115-117 ^{o}C [lit. 116-117 ^{o}C][20]; $[\alpha]^{20}_{D}$ = 5 (*c* 3.2 mg/mL, acetone); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.09 (s, 1H, H-5), 6.61 (s, 1H, H-8), 6.18 (s, 1H, H-6′), 5.94 (s, 2H, OCH_2O), 4.66 (d, *J* = 9.5 Hz, 1H, H-11), 4.47 (d, *J* = 9.5 Hz, 1H, H-4), 4.40-4.43 (m, 1H, H-11), 4.38 (d, *J* = 5.0 Hz, 1H, H-1), 3.92 (s, 3H, 3′-OCH_3), 3.91(s, 3H, 5′-OCH_3), 3.80 (s, 3H, 4′-OCH_3), 3.27-3.30 (m, 1H, H-2), 2.90 (s, 1H, 4-OH), 2.60-2.65 (m, 1H, H-3); HRMS (ESI): Calcd for $C_{22}H_{25}ClNO_8$ ($[M+NH_4]^+$), 466.1263; found, 466.1271.

*Data for **8***: CAS: 1458601-23-4; Yield: 85%, pale yellow solid, m.p. 155-157 ^{o}C [lit. 159-160 ^{o}C][20]; $[\alpha]^{20}_{D}$ = 29 (*c* 3.8 mg/mL, acetone); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.15 (s, 1H, H-5),

6.05 (s, 1H, H-8), 5.93 (s, 2H, OCH_2O), 5.01 (d, J = 6.5 Hz, 1H, H-1), 4.77 (d, J = 10.0 Hz, 1H, H-4), 4.44-4.46 (m, 2H, H-11), 3.99 (s, 3H, 3′-OCH_3), 3.96 (s, 3H, 5′-OCH_3), 3.89 (s, 3H, 4′-OCH_3), 3.49 (dd, J = 9.0, 6.5 Hz, 1H, H-2), 2.59-2.64 (m, 1H, H-3); HRMS (ESI): Calcd for $C_{22}H_{24}Cl_2NO_8$ ($[M+NH_4]^+$), 500.0873; found, 500.0876.

General Procedure for Synthesis of 2′-Chloropicropodophyllone (9) and 2′,6′-Dichloropicropodophyllone (10). A mixture of **7** or **8** (1 mmol), chromium trioxide (CrO_3, 5 mmol), and pyridine (10 mmol) in dry dichloromethane (20 mL) was stirred at room temperature. When the reaction was complete after 4 h, checked by TLC analysis, the mixture was diluted by dichloromethane (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 (1:1, v/v) to afford **9** (386 mg, 86% yield) or **10** (383 mg, 79% yield).

*Data for **9***: Yield: 86%, yellow solid, m.p. 105-107 ^oC; $[\alpha]^{20}_D$ = -30 (*c* 3.4 mg/mL, acetone); ^1H NMR (500 MHz, $CDCl_3$) δ: 7.51 (s, 1H, H-5), 6.68 (s, 1H, H-8), 6.07 (d, J = 1.0 Hz, 2H, OCH_2O), 5.85 (s, 1H, H-6′), 5.21 (d, J = 1.5 Hz, 1H, H-1), 4.75 (d, J = 9.0 Hz, 1H, H-11), 4.32-4.35 (m, 1H, H-11), 3.95 (s, 3H, 3′-OCH_3), 3,85 (s, 3H, 5′-OCH_3), 3.54 (s, 3H, 4′-OCH_3), 3.42 (dd, J = 8.0, 2.0 Hz, 1H, H-2), 3.15-3.18 (m, 1H, H-3); HRMS (ESI): Calcd for $C_{22}H_{20}O_8Cl$ ($[M+H]^+$), 447.0841; Found, 447.0835.

*Data for **10***: Yield: 79%, yellow solid, m.p. 90-92 ^oC, $[\alpha]^{20}_D$ = -67 (*c* 3.3 mg/mL, acetone); ^1H NMR (500 MHz, $CDCl_3$) δ: 7.48 (s, 1H, H-5), 6.32 (s, 1H, H-8), 6.01 (s, 2H, OCH_2O), 5.68 (d, J = 4.5 Hz, 1H, H-1), 4.91 (dd, J = 9.0, 3.5 Hz, 1H, H-11), 4.50 (dd, J = 9.5, 7.0 Hz, 1H, H-11), 3.97 (s, 3H, 3′-OCH_3), 3.96 (s, 3H, 5′-OCH_3), 3.86 (s, 3H, 4′-OCH_3), 3.65-3.69 (m, 1H, H-2), 3.43-3.45 (m, 1H, H-3); HRMS (ESI): Calcd for $C_{22}H_{19}O_8Cl_2$ ($[M+H]^+$), 481.0451;

Found, 481.0455.

General Procedure for Synthesis of Oximes of 2′-Chloropicropodophyllone and 2′,6′-Dichloropicropodophyllone (11 and 12). A mixture of **9** or **10** (0.5 mmol), hydroxylamine hydrochloride (0.75 mmol), and pyridine (2 mmol) in absolute ethanol (20 mL) was refluxed. When the reaction was complete after 72 h, 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 petroleum ether/ethyl acetate (1:1, v/v) to afford **11** (193 mg, 83% yield) or **12** (214 mg, 89% yield).

*Data for **11***: Yield: 83%, pale yellow solid, m.p. 106-107 °C, $[\alpha]^{20}_D$ = -27 (*c* 3.4 mg/mL, acetone); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.27 (s, 1H, H-5), 6.70 (s, 1H, H-8), 5.99 (s, 2H, OCH_2O), 5.95 (s, 1H, H-6′), 5.07 (d, J = 2.0 Hz, 1H, H-1), 4.47-4.53 (m, 2H, H-11), 3.93 (s, 3H, 3′-OCH_3), 3.83-3.86 (m, 4H, H-3 and 5′-OCH_3), 3.52 (s, 3H, 4′-OCH_3), 3.43 (dd, J = 8.5, 2.5 Hz, 1H, H-2); HRMS (ESI): Calcd for $C_{22}H_{21}O_8NCl$ ($[M+H]^+$), 462.0950; Found, 462.0949.

*Data for **12***: Yield: 89%, white solid, m.p. 118-120 °C, $[\alpha]^{20}_D$ = 54 (*c* 5.3 mg/mL, acetone); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.63 (s, 1H, OH), 7.41 (s, 1H, H-5), 6.21 (s, 1H, H-8), 5.93 (s, 2H, OCH_2O), 5.24 (d, J = 10.5 Hz, 1H, H-1), 5.02-5.08 (m, 1H, H-11), 4.28-4.33 (m, 2H, H-11, H-3), 3.99 (s, 3H, 4′-OCH_3), 3.93 (s, 3H, 3′-OCH_3), 3.90(s, 3H, 5′-OCH_3), 3.59 (dd, J = 10.5, 6.5 Hz, 1H, H-2); HRMS (ESI): Calcd for $C_{22}H_{20}O_8NCl_2$ ($[M+H]^+$), 496.0560; Found, 496.0555.

General Procedure for Synthesis of Oxime Sulfonate Derivatives of

2′(2′,6′)-(di)Chloropicropodophyllotoxins (4a-j, and 5c,d,f-k). To a stirred solution of NaH (1.4 mmol) in dry tetrahydrofuran (8 mL) at −10 °C was slowly added compound **11** or **12** (0.2 mmol). After the completion of addition, the reaction mixture was stirred at −10 °C for 0.5 h. Then the corresponding sulfonyl chlorides RSO_2Cl (**13**, 0.8 mmol) were added to the above mixture. The reaction process was checked by TLC analysis. When the reaction mixture was stirred for 3 h at −10 °C (for methanesulfonyl chloride, the reaction mixture was stirred at room temperature for 48 h), saturated aq. $NaHCO_3$ (15 mL) was added to the mixture, which was extracted with dichloromethane (3×30 mL). The combined organic phase was dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure, and purified by PTLC to give target products **4a-j**, and **5c,d**,**f-k** in 37−91% yields. The example data of **4a-e**, and **5c,d** are described as follows, whereas the data of **4f-j**, and **5f-k** are shown in the Supporting Information.

*Data for **4a***: Yield: 82%, white solid, m.p. 102-104 °C, $[\alpha]^{20}_D = 4$ (*c* 4.2 mg/mL, acetone); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.02-8.04 (m, 2H, H-2″, H-6″), 7.73 (t, J = 7.5 Hz, 1H, H-4"), 7.59-7.62 (m, 2H, H-3″, H-5″), 7.14 (s, 1H, H-5), 6.71 (s, 1H, H-8), 6.02 (s, 2H, OCH_2O), 5.77 (s, 1H, H-6′), 5.08 (d, J = 2.0 Hz, 1H, H-1), 4.54 (dd, J = 10.0, 7.0 Hz, 1H, H-11), 4.31 (d, J = 10.0 Hz, 1H, H-11), 3.92 (s, 3H, 3′-OCH_3), 3.89-3.91 (m, 1H, H-3), 3.84 (s, 3H, 5′-OCH_3), 3.40 (dd, J = 8.5, 2.5 Hz, 1H, H-2), 3.36 (s, 3H, 4′-OCH_3); HRMS (ESI): Calcd for $C_{28}H_{25}O_{10}NClS$ ($[M+H]^+$), 602.0882; Found, 602.0885.

*Data for **4b***: Yield: 48%, yellow solid, m.p. 112-114 °C, $[\alpha]^{20}_D = 6$ (*c* 4.0 mg/mL, acetone); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.90 (s, 1H, H-2″), 8.59 (d, J = 7.5 Hz, 1H, H-6″), 8.36 (d, J = 7.5 Hz, 1H, H-4"), 7.87 (t, J = 7.5 Hz, 1H, H-5″), 7.09 (s, 1H, H-5), 6.73 (s, 1H, H-8), 6.03 (s, 2H, OCH_2O), 5.77 (s, 1H, H-6'), 5.09 (s, 1H, H-1), 4.54-4.57 (m, 1H, H-11), 4.31 (d, J = 10.0

Hz, 1H, H-11), 3.93 (s, 3H, 3′-OCH_3), 3.89-3.90 (m, 1H, H-3), 3.85 (s, 3H, 5′-OCH_3), 3.42-3.46 (m, 4H, 4′-OCH_3, H-2); HRMS (ESI): Calcd for $C_{28}H_{24}O_{12}N_2ClS$ ($[M+H]^+$), 647.07; Found, 647.07.

Data for ***4c***: Yield: 75%, yellow solid, m.p. 106-108 °C, $[\alpha]^{20}_D$ = 10 (*c* 3.6 mg/mL, acetone) ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 8.04-8.07 (m, 2H, H-2″, H-6″), 7.27-7.30 (m, 2H, H-3″, H-5″), 7.12 (s, 1H, H-5), 6.72 (s, 1H, H-8), 6.03 (s, 2H, OCH_2O), 5.77 (s, 1H, H-6′), 5.08 (d, *J* = 2.0 Hz, 1H, H-1), 4.55 (dd, *J* = 10.0, 7.0 Hz, 1H, H-11), 4.32 (d, *J* = 9.5 Hz, 1H, H-11), 3.92 (s, 3H, 3′-OCH_3), 3.90 (m, 1H, H-3), 3.84 (s, 3H, 5′-OCH_3), 3.39-3.41 (m, 4H, 4′-OCH_3 and H-2); HRMS (ESI): Calcd for $C_{28}H_{24}O_{10}NClFS$ ($[M+H]^+$), 620.0788; Found, 620.0784.

Data for ***4d***: Yield: 82%, white solid, m.p. 88-90 °C, $[\alpha]^{20}_D$ = 15 (*c* 4.0 mg/mL, acetone); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.96 (d, *J* = 7.0 Hz, 2H, H-2", H-6"), 7.59 (dd, *J* = 6.5, 2.0 Hz, 2H, H-3", H-5"), 7.13 (s, 1H, H-5), 6.72 (s, 1H, H-8), 6.03 (s, 2H, OCH_2O), 5.75 (s, 1H, H-6'), 5.08 (d, *J* = 2.5 Hz, 1H, H-1), 4.55 (dd, *J* = 9.5, 7.0 Hz, 1H, H-11), 4.30-4.32 (m, 1H, H-11), 3.92 (s, 3H, 3′-OCH_3), 3.87-3.90 (m, 1H, H-3), 3.84 (s, 3H, 5′-OCH_3), 3.41 (dd, *J* = 8.5, 2.5 Hz, 1H, H-2), 3.38 (s, 3H, 4′-OCH_3); HRMS (ESI): Calcd for $C_{28}H_{24}O_{10}NCl_2S$ ($[M+H]^+$), 636.0492; Found, 636.0498.

Data for ***4e***: Yield: 57%, yellow solid, m.p. 116-118 °C, $[\alpha]^{20}_D$ = 21 (*c* 3.0 mg/mL, acetone); ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 8.22-8.24 (m, 1H, H-6″), 7.79-7.81 (m, 1H, H-3″), 7.52-7.59 (m, 2H, H-4", H-5"), 7.00 (s, 1H, H-5), 6.71 (s, 1H, H-8), 5.99 (d, *J* = 3.5 Hz, 2H, OCH_2O), 5.76 (s, 1H, H-6'), 5.07 (d, *J* = 2.0 Hz, 1H, H-1), 4.52-4.61 (m, 2H, H-11), 3.94 (s, 3H, 3′-OCH_3), 3.89-3.92 (m, 1H, H-3), 3.87 (s, 3H, 5′-OCH_3), 3.44-3.47 (m, 1H, H-2), 3.40 (s, 3H, 4′-OCH_3); HRMS (ESI): Calcd for $C_{28}H_{24}O_{10}NBrClS$ ($[M+H]^+$), 679.9987; Found, 679.9985.

184 *Data for **5c**:* Yield: 42%, yellow solid, m.p. 98-100 ^{o}C, $[\alpha]^{20}_{D}$ = 16 (*c* 3.0 mg/mL, acetone)
185 ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.07-8.09 (m, 2H, H-2″, H-6″), 7.29-7.30 (m, 2H, H-3″, H-5″),
186 7.27 (s, 1H, H-5), 6.22 (s, 1H, H-8), 5.99 (d, *J* = 10.0 Hz, 2H, OCH_2O), 5.25 (d, *J* = 10.5 Hz,
187 1H, H-1), 4.92-4.97 (m, 1H, H-11), 4.21-4.27 (m, 2H, H-11, H-3), 3.97 (s, 3H, 4′-OCH_3),
188 3.91 (s, 3H, 3′-OCH_3), 3.87 (s, 3H, 5′-OCH_3), 3.48-3.52 (m, 1H, H-2); HRMS (ESI): Calcd
189 for $C_{28}H_{23}O_{10}NCl_2FS$ ($[M+H]^+$), 654.0398; Found, 654.0407.
190 *Data for **5d***: Yield: 44%, yellow solid, m.p. 96-98 ^{o}C, $[\alpha]^{20}_{D}$ = 20 (*c* 3.8 mg/mL, acetone) ^{1}H
191 NMR (500 MHz, $CDCl_3$) δ: 7.98-8.00 (m, 2H, H-2″, H-6″), 7.58-7.59 (m, 2H, H-3″, H-5″),
192 7.30 (s, 1H, H-5), 6.22 (s, 1H, H-8), 5.99 (d, *J* = 9.5 Hz, 2H, OCH_2O), 5.25 (d, *J* = 10.0 Hz,
193 1H, H-1), 4.91-4.97 (m, 1H, H-11), 4.24-4.27 (m, 2H, H-11, H-3), 3.97 (s, 3H, 4′-OCH_3),
194 3.92 (s, 3H, 3′-OCH_3), 3.87(s, 3H, 5′-OCH_3), 3.48-3.51 (m, 1H, H-2); HRMS (ESI): Calcd
195 for $C_{28}H_{23}O_{10}NCl_3S$ ($[M+H]^+$), 670.0103; Found, 670.0100.
196 **Biological Assay.** The insecticidal activity of compounds (**1**, **4a-j**, **5c,d,f-k** and **6-12**) was
197 evaluated as the mortality rate values by leaf-dipping method as described previously,[18]
198 against the pre-third-instar larvae of oriental armyworm (*Mythimna separata* Walker). For
199 each compound, 30 pre-third-instar larvae (10 larvae per group) were used. Acetone solutions
200 of compounds **1**, **4a-j**, **5c,d,f-k**, **6-12** and toosendanin (a positive control) were prepared at 1
201 mg/mL. Fresh wheat leaf discs (1×1 cm) were dipped into the corresponding solution for 3 s,
202 then taken out and dried. Leaf discs treated with acetone alone were used as a blank control
203 group. Several pieces of treated leaf discs were kept in each dish (10 larvae were raised in
204 each dish), which was then placed in a conditioned room (25 ± 2 ^{o}C, 65-80% relative
205 humidity (RH), 12 h/12 h (light/dark) photoperiod). If the treated leaf discs were consumed,
206 additional treated ones were added to the dish. After 48 h, compound-soaked leaves were

removed, and the larvae were fed with untreated fresh wheat leaves thereafter until adult emergence. The corrected mortality rate values were obtained by the formula

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

Where T is the mortality rate in the tested compounds group, and C is the mortality rate in the blank control group (T and C were expressed as percentages).

RESULTS AND DISCUSSION

Synthesis. As described in Scheme 1, 2′-chloropicropodophyllotoxin (**7**) and 2′,6′-dichloropicropodophyllotoxin (**8**) were smoothly prepared by reaction of **6** with the different amount of *N*-chlorosuccinimide (NCS). Further oxidation of the hydroxy group at the C-4 position of **7** or **8** in the presence of CrO_3 and pyridine gave 2′-chloropicropodophyllone (**9**) and 2′,6′-dichloropicropodophyllone (**10**), respectively. Subsequently, oximes of 2′-chloropicropodophyllone and 2′,6′-dichloropicropodophyllone (**11** and **12**) were obtained by reaction of hydroxylamine hydrochloride with **9** and **10**, respectively. Finally, oxime sulfonate derivatives of 2′(2′,6′)-(di)chloropicropodophyllotoxins (**4a-j**, and **5c,d,f-k**) were afforded by reaction of sulfonyl chlorides with **11** and **12**, respectively. The structures of all target compounds were well characterized by ^{1}H NMR, HRMS, optical rotation, and melting point. Moreover, the single-crystal structure of **5f** was further confirmed by X-ray crystallography (Figure 2). It clearly demonstrated that the substituents on the C=N double bond of **5f** were present in *trans* configuration; two hydrogen atoms at the C-2 and C-3 positions all adopted α configuration, that is, the configuration of lactone (D-ring) of **5f** was *cis;* and two chlorine atoms were at the C-2’ and C-6’ positions of **5f**. Crystallographic data (excluding structure factors) for the structure of **5f** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication

number CCDC 1055791. 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].

Insecticidal Activity. As shown in Table 1, the insecticidal activity of **1**, **4a-j**, **5c,d,f-k** and **6-12** against the pre-third-instar larvae of *M. separata* in vivo was evaluated as the mortality rates at 1 mg/mL. Toosendanin was used as a positive control at 1 mg/mL, and leaves treated with acetone alone were used as a blank control group. The corresponding mortality rates of the treated groups after 35 days were higher than those after 5, 10, 15, 20 and 25 days as we reported in our previous papers.[18-20] As described in Figure 3, the lethal time for 50% mortality of **8**, **4c-g**, and **5c,d,f,g** were 32, 23, 26, 31, 29, 29, 24, 25, 27, and 28 days, respectively. In addition, the symptoms of *M. separata* in the treated groups were characterized as follows: some larvae with the slim and wrinkled bodies died at the larval stage due to feeding too much treated leaves during the first 48 h (Figure 4); some larvae molted to malformed pupae or died during the pupation period (Figure 5), and it was noteworthy that more than half of the final mortality rates were generally occupied by the mortality rates at this stage; malformed moths also appeared with imperfect wings (Figure 6). It suggested that the podophyllotoxin derivatives possibly exhibited the anti-molting hormone effect. Among all derivatives, compounds **7**, **8**, **4c-g**, and **5c,d,f,g** exhibited insecticidal activity equal to, or higher than, that of the positive control toosendanin. Especially **4c**, **5c** and **5d** exhibited more promising insecticidal activity with the final mortality rates greater than 60%. For example, the final mortality rates of **4c**, **5c** and **5d** were 62.1%, 65.5% and 62.1%, respectively; whereas the final mortality rates of the precursor **1** and toosendanin were 37.9% and 48.3%, respectively. Introduction of the chlorine atom at the C-2′ or C-2′,6′ position on

the E-ring of **6** afforded the potent compounds **7** and **8** (48.3% for **7** and 51.7% for **8**, and 34.5% for **6**). Similarly, introduction of the chlorine atom at the C-2′ or C-2′,6′ position on the E-ring of oxime sulfonate derivatives of picropodophyllotoxin (**2**) could also produce more potent compounds as compared with **2**.[18] For example, the final mortality rates of **4a,b,d,g,i,j** were 37.9%, 41.4%, 58.6%, 55.2%, 41.4% and 37.9%, respectively; whereas the final mortality rates of the corresponding oxime sulfonate derivatives of picropodophyllotoxin were 24.1%, 13.8%, 55.2%, 27.6%, 44.8% and 27.6%, respectively.[18] To oxime sulfonate derivatives of 2′-chloropicropodophyllotoxins (**4a-j**), introduction of a halogen atom (such as a fluorine, chlorine, or bromine atom) on the phenylsulfonyl group of **4a** resulted in more promising compounds **4c-g**. The final mortality rates of **4c-g** were 62.1%, 58.6%, 51.7%, 55.2%% and 55.2%, respectively; whereas the final mortality rate of **4a** was only 37.9%. Interestingly, to oxime sulfonate derivatives of 2′,6′-dichloropicropodophyllotoxins (**5c,d,f-k**), introduction of a fluorine, chlorine, or bromine atom on the phenylsulfonyl group could also give potent compounds **5c,d,f,g**, the final mortality rates of which were 65.5%, 62.1%, 58.6%, and 58.6%, respectively. On the contrary, to **4a-j** and **5c,d,f-k**, whether introduction of nitro group (electron-withdrawing group) or methyl/ethyl group (electron-donating group) on the phenylsulfonyl fragment all afforded less potent compounds **4b**, **4i**, **4j**, **5i** and **5j**. When the methylsulfonyl group was introduced at the oxime fragment of **12**, the corresponding compound **5k** showed less potent insecticidal activity as compared with **5c** (44.8% for **5k** and 65.5% for **5c**).

In conclusion, a series of oxime sulfonate derivatives of 2′(2′,6′)-(di)chloropicropodophyllotoxins were prepared by structural modification of podophyllotoxin. Their insecticidal activity was evaluated at 1 mg/mL against the

pre-third-instar larvae of *M. separata*. The key steric structure of **5f** was unambiguously determined by single-crystal X-ray diffraction. Among all derivatives, compounds **4c**, **5c** and **5d** exhibited more potent insecticidal activity with the final mortality rates greater than 60%. It demonstrated that introduction of the chlorine atom at the C-2′ or C-2′,6′ position on the E-ring of picropodophyllotoxin or oxime sulfonate derivatives of picropodophyllotoxin was necessary for the insecticidal activity; and introduction of a halogen (e.g., fluorine, chlorine, or bromine) atom-substituted phenylsulfonyl group on the oxime fragment of 2′(2′,6′)-(di)chloropicropodophyllones led to more promising compounds. It will pave the way for further design and chemical modification of podophyllotoxin as botanical insecticidal agents.

ASSOCIATED CONTENT

Supporting Information

Data on ^{1}H NMR, HRMS, optical rotation, and melting point for the target compounds. The Supporting Information is available free of charge on the ACS Publications website at DOI:

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Notes

The authors declare no competing financial interest.

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Figure Captions

Figure 1. Chemical structures of podophyllotoxin (**1**) and its derivatives (**2-5**).

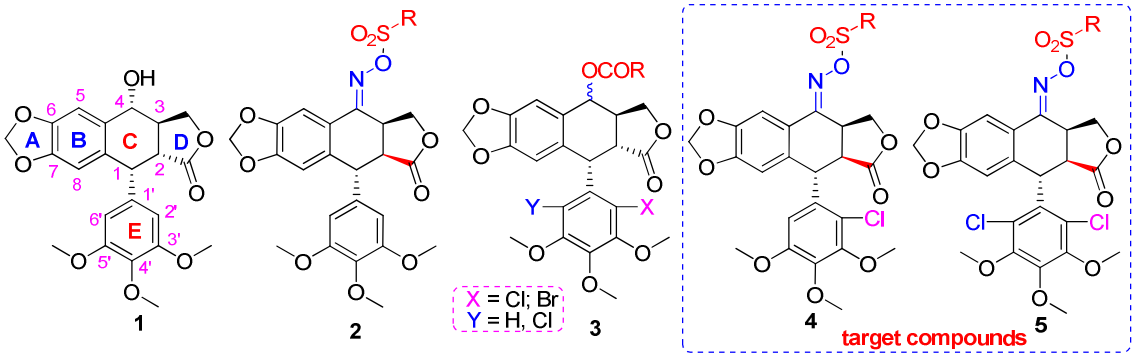
Figure 2. X-ray crystal structure of **5f**.

Figure 3. The lethal time for 50% mortality of **8**, **4c-g**, and **5c,d,f,g**.

Figure 4. The representative abnormal larvae pictures of **4g** (**WR-015**), **4d** (**WR-026**), **4c** (**WR-039**), **5g** (**WR-022**), **5j** (**WR-043**), **5d** (**WR-038**) and **5c** (**WR-041**) during the larval period (**CK**: blank control group).

Figure 5. The representative malformed pupae pictures of **4g** (**WR-015**), **4d** (**WR-026**), **4f** (**WR-046**), **5j** (**WR-043**), **5g** (**WR-022**), **5d** (**WR-038**) and **5c** (**WR-041**) during the pupation period (**CK**: blank control group).

Figure 6. The representative malformed moth pictures of **4g** (**WR-015**), **4d** (**WR-026**), **4a** (**WR-030**), **5k** (**WR-036**), **5d** (**WR-038**), **5c** (**WR-041**), and **4f** (**WR-046**) during the stage of adult emergence (**CK**: blank control group).



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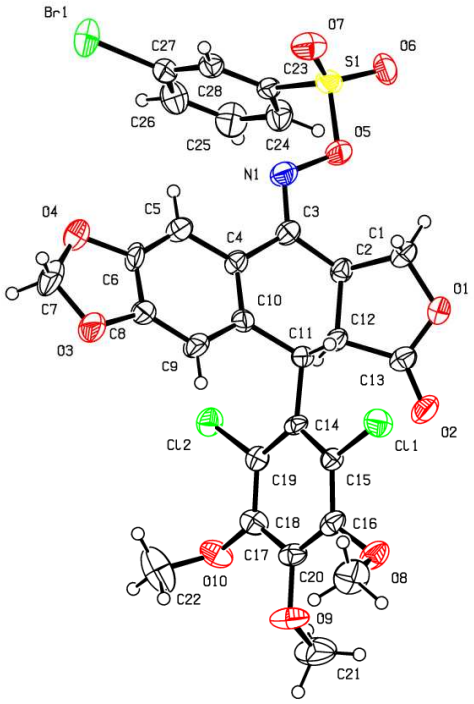
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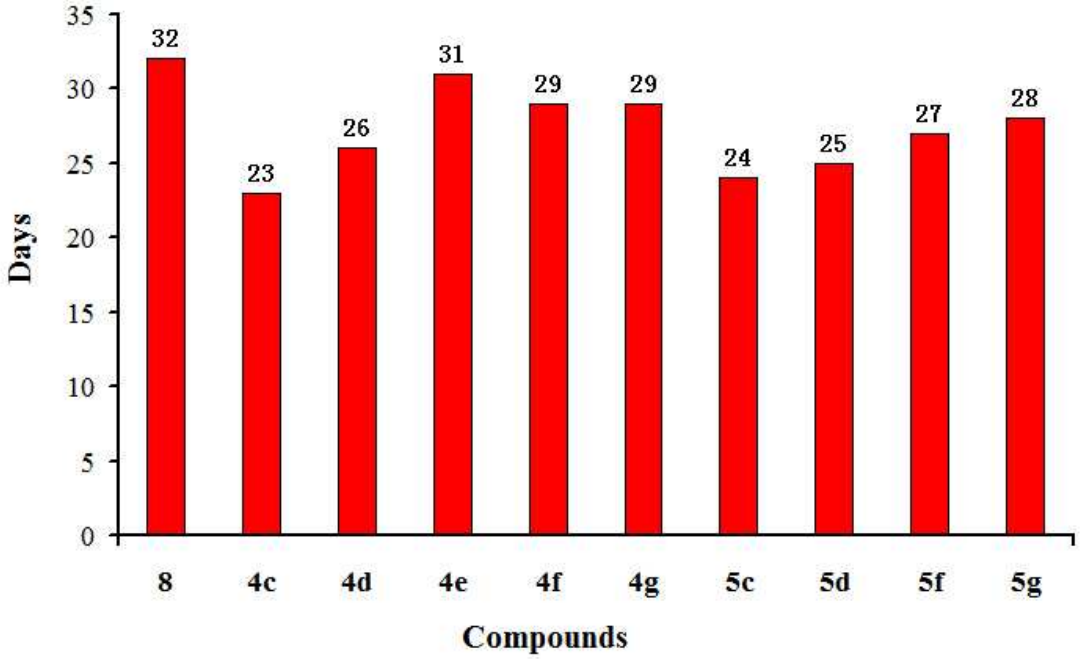
Figure 2.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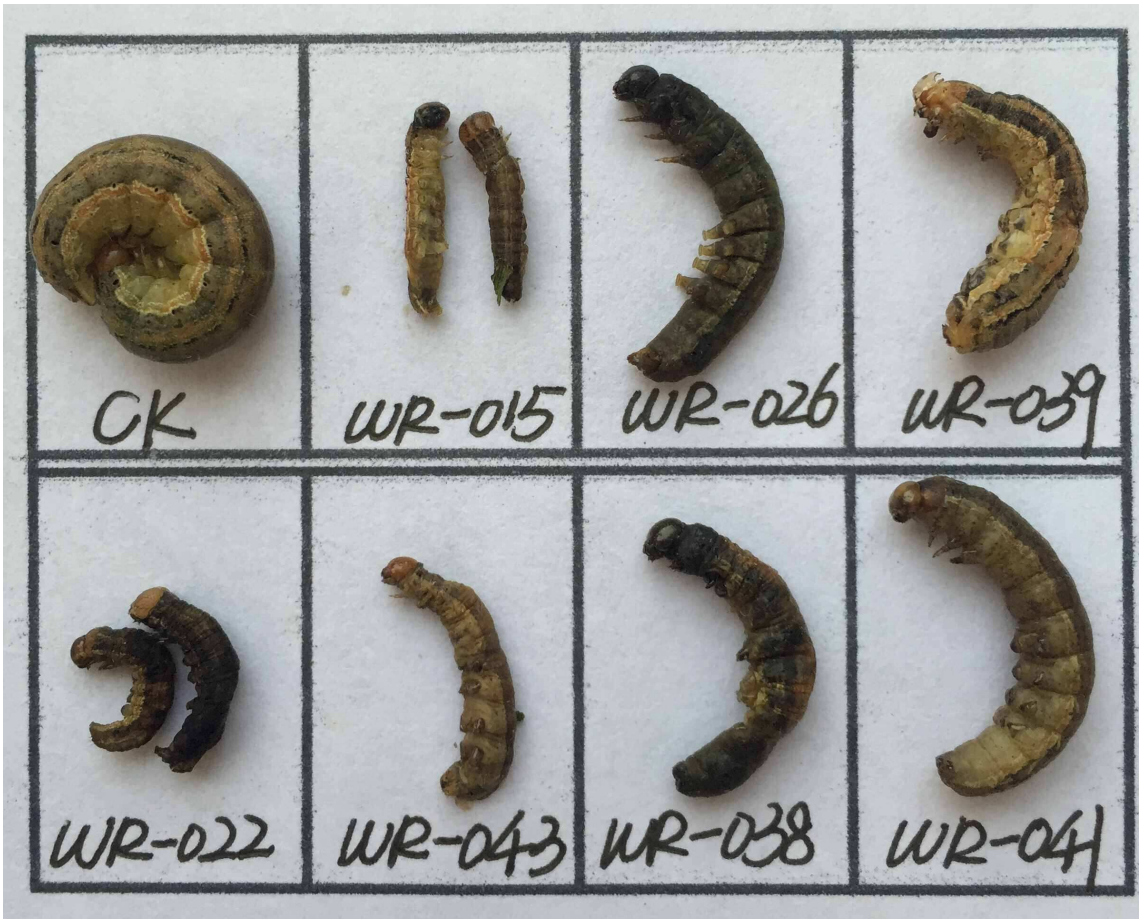
Compounds

Figure 3.



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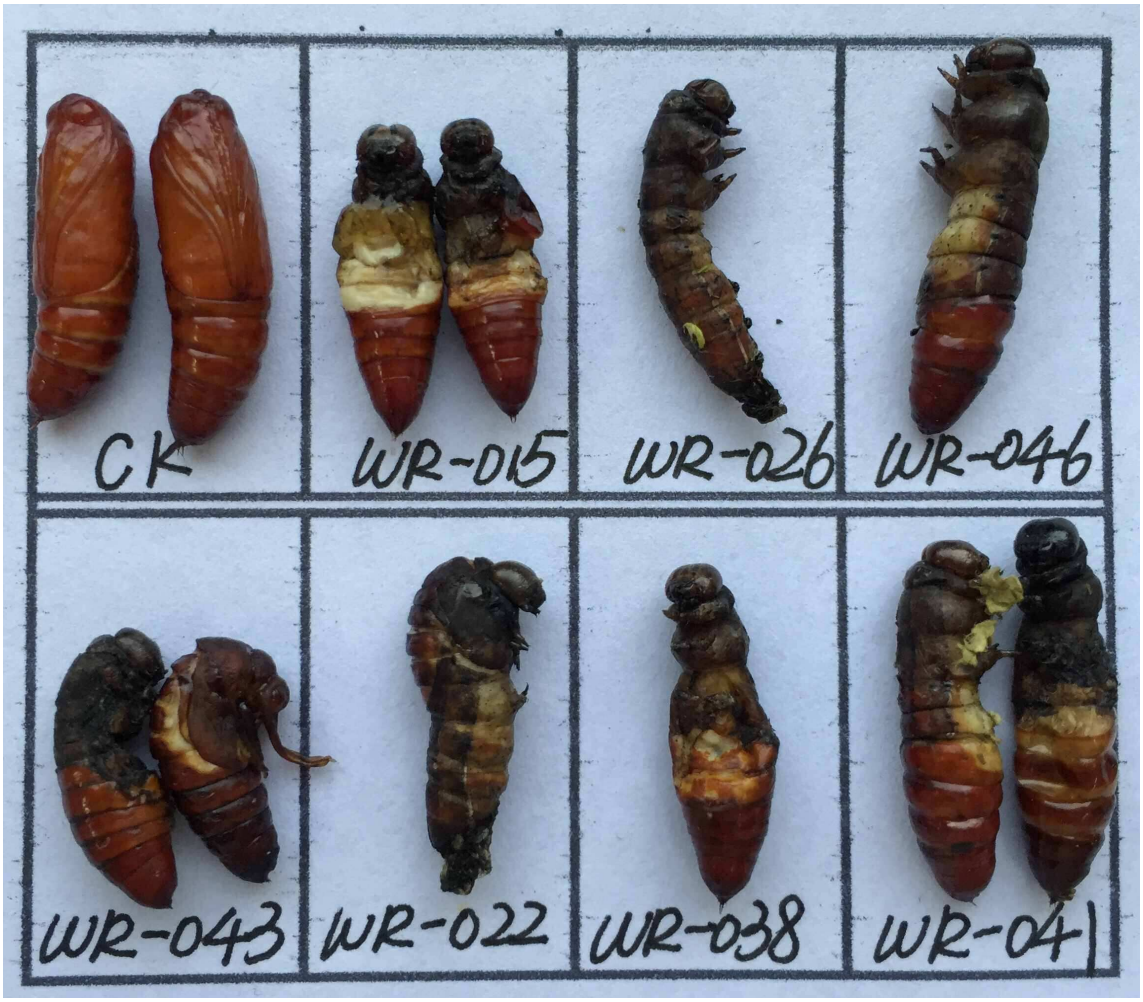


Figure 5.

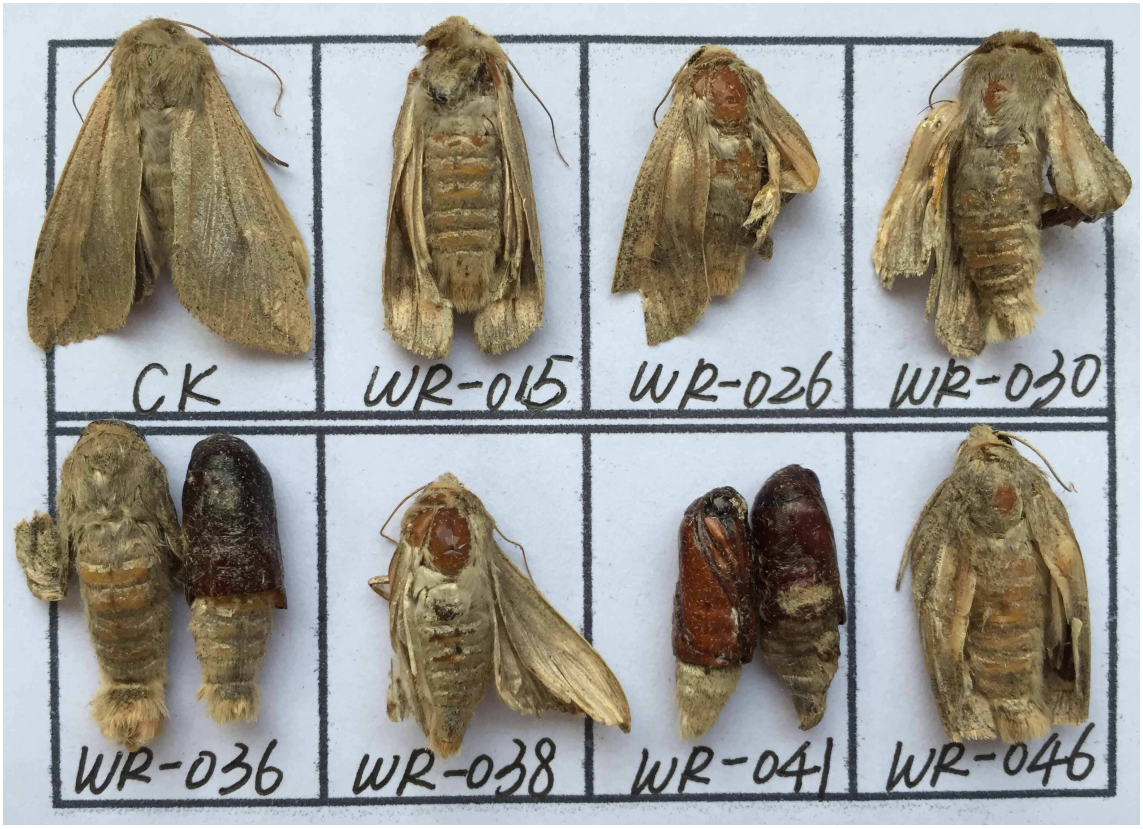
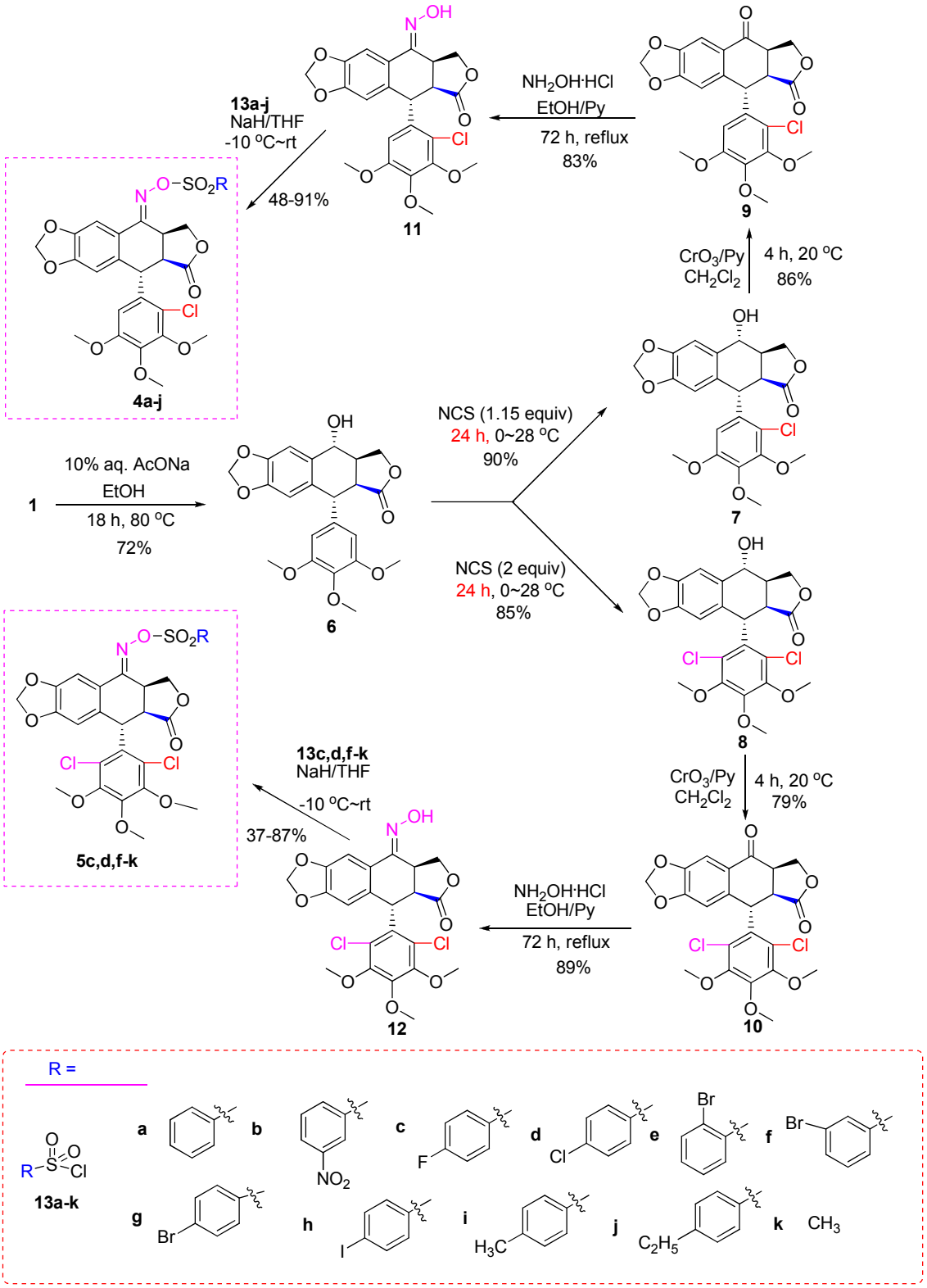


Figure 6.



Scheme 1. Synthesis of oxime sulfonate derivatives of 2′(2′,6′)-(di)chloropicropodophyllotoxins (**4a-j**, and **5c,d,f-k**).

Table 1. Insecticidal Activity of Oxime Sulfonate Derivatives of 2′(2′,6′)-(di)Chloropicropodophyllotoxins (4a-j, and 5c,d,f-k) against *M. separata* on Leaves Treated with a Concentration of 1 mg/mL.

compound	corrected mortality rate (%)		
	10 days	20 days	35 days
1	13.3 ± 3.3	16.7 ± 3.3	37.9 ± 0
6	10.0 ± 0	16.7 ± 3.3	34.5 ± 3.3
7	16.7 ± 3.3	33.3 ± 3.3	48.3 ± 0
8	20.0 ± 0	30.0 ± 0	51.7 ± 3.3
9	13.3 ± 3.3	20.0 ± 0	37.9 ± 5.8
10	10.0 ± 0	16.7 ± 3.3	34.5 ± 3.3
11	13.3 ± 3.3	20.0 ± 0	34.5 ± 3.3
12	6.7 ± 3.3	13.3 ± 3.3	31.0 ± 3.3
4a	3.3 ± 3.3	16.7 ± 3.3	37.9 ± 5.8
4b	13.3 ± 3.3	26.7 ± 3.3	41.4 ± 3.3
4c	16.7 ± 3.3	40.0 ± 0	62.1 ± 3.3
4d	20.0 ± 0	33.3 ± 3.3	58.6 ± 5.8
4e	16.7 ± 3.3	30.0 ± 0	51.7 ± 3.3
4f	20.0 ± 0	26.7 ± 3.3	55.2 ± 3.3
4g	16.7 ± 3.3	30.0 ± 0	55.2 ± 3.3
4h	16.7 ± 3.3	30.0 ± 0	44.8 ± 3.3
4i	13.3 ± 3.3	20.0 ± 0	41.4 ± 3.3
4j	6.7 ± 3.3	13.3 ± 3.3	37.9 ± 0
5c	16.7 ± 3.3	46.7 ± 3.3	65.5 ± 3.3
5d	16.7± 3.3	36.7 ± 3.3	62.1± 3.3
5f	20.0 ± 0	33.3 ± 3.3	58.6 ± 5.8
5g	16.7 ± 3.3	33.3 ± 3.3	58.6 ± 0
5h	16.7 ± 3.3	23.3 ± 3.3	44.8 ± 3.3
5i	16.7 ± 3.3	23.3 ± 3.3	44.8 ± 3.3
5j	16.7 ± 3.3	23.3 ± 3.3	41.4 ± 3.3
5k	10.0 ± 0	20.0 ± 0	44.8 ± 3.3
toosendanin	13.3 ± 3.3	20.0 ± 0	48.3 ± 0
blank control	0 ± 0	0 ± 0	3.3 ± 3.3

TOC graphic