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Synthesis and Quantitative Structure−Activity Relationship (QSAR) Study of Novel Isoxazoline and Oxime Derivatives of Podophyllotoxin as Insecticidal Agents

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Supporting Information

ABSTRACT: In continuation of our program aimed at the discovery and development of natural-product-based insecticidal agents, 33 isoxazoline and oxime derivatives of podophyllotoxin modified in the C and D rings were synthesized and their structures were characterized by Proton nuclear magnetic resonance (^{1}H NMR), high-resolution mass spectrometry (HRMS), electrospray ionization−mass spectrometry (ESI−MS), optical rotation, melting point (mp), and infrared (IR) spectroscopy. The stereochemical configurations of compounds **5e**, **5f**, and **9f** were unambiguously determined by X-ray crystallography. Their insecticidal activity was evaluated against the pre-third-instar larvae of northern armyworm, *Mythimna separata* (Walker), *in vivo*. Compounds **5e**, **9c**, **11g**, and **11h** especially exhibited more promising insecticidal activity than toosendanin, a commercial botanical insecticide extracted from *Melia azedarach*. A genetic algorithm combined with multiple linear regression (GA−MLR) calculation is performed by the MOBY DIGS package. Five selected descriptors are as follows: one two-dimensional (2D) autocorrelation descriptor (GATS4e), one edge adjacency indice (EEig06x), one RDF descriptor (RDF080v), one three-dimensional (3D) MoRSE descriptor (Mor09v), and one atom-centered fragment (H-052) descriptor. Quantitative structure−activity relationship studies demonstrated that the insecticidal activity of these compounds was mainly influenced by many factors, such as electronic distribution, steric factors, etc. For this model, the standard deviation error in prediction (SDEP) is 0.0592, the correlation coefficient (R^2) is 0.861, and the leave-one-out cross-validation correlation coefficient (Q^2_{loo}) is 0.797.

KEYWORDS: *Podophyllotoxin, insecticidal activity, botanical insecticide, structural modification, QSAR, Mythimna separata Walker*

INTRODUCTION

The use of synthetic pesticides in agriculture worldwide is still the most widespread method for the control of insect pests. It is estimated that, without pesticides, 30% of the world's crops would be lost before they were harvested.[1] However, the extensive application of those agrochemicals over the years has led to the development of resistance in pest populations and environmental problems.[2−6] It is well-known that plant secondary metabolites result from the interaction between plants and the environment (life and non-life) during the long period of evolution in plants, and pesticides produced from plant secondary metabolites may result in less or slower resistance development and lower pollution.[7] Hence, botanical insecticides have been considered as attractive alternatives to synthetic agrochemicals for pest management.[8] Some botanical insecticides, such as nicotine, pyrethrum, and neem extracts, are made by plants as defenses against insects.[9]

Podophyllotoxin (**1**, Figure 1), a naturally occurring cyclolignan, is the main secondary metabolite isolated from the roots and rhizomes of the *Podophyllum* genus, such as *Podophyllum hexandrum* and *Podophyllum peltatum*. Besides its use as the lead compound for the preparation of potent anticancer drugs, such as etoposide and teniposide,[10−12] compound **1** also showed interesting insecticidal and antifungal activities.[13−16] Recently, structural modifications of compound **1** have been reported, which show more potent insecticidal activity.[17−23] To the best of our knowledge, almost all of structural modifications of compound **1** were focused on the C

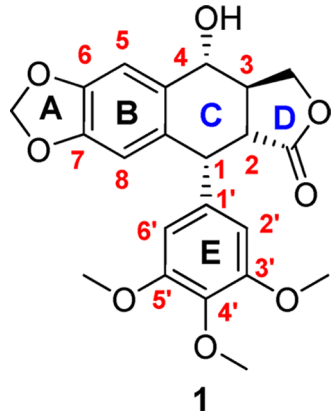

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Figure 1. Chemical structure of podophyllotoxin (**1**).

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ring and little attention has been paid to structural modifications of the D ring of compound **1** as insecticidal agents by opening its lactone. Meanwhile, del Corral et al. found that some isoxazoline and oxime derivatives of podophyllotoxin displayed promising cytotoxic activities.[24] Encouraged by the above-mentioned interesting results and in continuation of our program aimed at the discovery and development of natural-product-based insecticidal agents,[20−23,25] in this study, 33 isoxazoline and oxime derivatives of podophyllotoxin modified in the C and D rings were synthesized. Among them, 26 derivatives were new compounds. Their insecticidal activity was evaluated against the pre-third-instar larvae of northern armyworm, *Mythimna separata* (Walker), *in vivo*. In addition, quantitative structure−activity relationship (QSAR) studies were also performed on all of the derivatives using the MOBY DIGS package.

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 GF_{254} (Qingdao Haiyang Chemical Co., Ltd.). Melting points were determined on a XT-4 digital melting-point (mp) apparatus (Beijing Tech Instrument Co., Ltd.) and were uncorrected. Infrared (IR) spectra were recorded on a Bruker TENSOR 27 spectrometer. Optical rotation was measured on Rudolph Research Analytical Autopol III automatic polarimeter. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker Avance III 500 MHz instrument in $CDCl_3$ using tetramethylsilane (TMS) as the internal standard. High-resolution mass spectrometry (HRMS) and electrospray ionization−mass spectrometry (ESI−MS) were carried out with an IonSpec 4.7 T FTMS instrument and an Agilent 1100 LC/MSD SL instrument, respectively.

*Synthesis of Picropodophyllotoxin (**2**).* A mixture of podophyllotoxin (0.83 g, 2.0 mmol), absolute ethanol (12 mL), and 10% aqueous CH_3CO_2Na (8 mL) was refluxed. When the reaction was complete after 15 h, checked by TLC analysis, the mixture was cooled at 0 °C and filtered to give the solid, which was further recrystallized from absolute ethanol to afford compound **2** (0.605 g, 73% yield) as a white solid. mp = 222−223 °C [literature, 222−224 °C].[26] $[\alpha]^{20}_D$ = +5 (*c* 3.2 mg/mL, $CHCl_3$). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.06 (s, 1H, H-5), 6.60 (s, 2H, H-2′, H-6′), 6.00 (s, 1H, H-8), 5.91−5.95 (m, 3H, H-4 and OCH_2O), 4.51 (d, *J* = 9.0 Hz, 1H, H-1), 4.34−4.41 (m, 2H, H-11), 3.92 (d, *J* = 7.5 Hz, 1H, H-2), 3.74 (s, 6H, 3′-OCH_3, 5′-OCH_3), 3.69 (s, 3H, 4′-OCH_3), 3.40−3.44 (m, 1H, H-3). MS (ESI): *m*/*z* (%) 432 ([M + NH_4]$^+$, 100).

*Synthesis of Picropodophyllone (**3**).* A mixture of picropodophyllotoxin (**2**, 414 mg, 1 mmol), CrO_3 (0.5 g, 5 mmol), and pyridine (0.91 mL, 10 mmol) in dry dichloromethane (DCM, 20 mL) was stirred at room temperature. When the reaction was complete after 3 h, checked by TLC analysis, the mixture was diluted by DCM (60 mL), washed by saturated aqueous $NaHSO_3$ (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 compound **3** (0.39 g, 95% yield) as a white solid. mp = 152−154 °C [literature, 182−183 °C].[27] $[\alpha]^{20}_D$ = −164 (*c* 3.4 mg/mL, $CHCl_3$). IR (cm^{-1}): 3004, 2971, 2924, 2825, 1784, 1762, 1662, 1589, 1502, 1476, 1455, 1327, 1252, 1125, 1019, 994, 895, 813, 713. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.49 (s, 1H, H-5), 6.68 (s, 1H, H-8), 6.23 (s, 2H, H-2′, H-6′), 6.04 (d, *J* = 3.5 Hz, 2H, OCH_2O), 4.75 (d, *J* = 9.0 Hz, 1H, H-11), 4.69 (s, 1H, H-1), 4.33−4.36 (m, 1H, H-11), 3.80 (s, 3H, 4′-OCH_3), 3.75 (s, 6H, 3′-OCH_3, 5′-OCH_3), 3.30−3.32 (m, 2H, H-2, H-3). MS (ESI): *m*/*z* (%) 413 ([M + H]$^+$, 32).

*Synthesis of Oxime of Picropodophyllone (**4**).* A mixture of picropodophyllone (**3**, 206 mg, 0.5 mmol), hydroxylamine hydrochloride (52 mg, 0.75 mmol), and pyridine (0.18 mL, 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 aqueous $NaHCO_3$ (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 (3:2, v/v) to afford compound **4** (0.181 g, 85% yield) as a white solid. mp = 114−116 °C [literature, no reported].[24] $[\alpha]^{20}_D$ = −53 (*c* 3.0 mg/mL, $CHCl_3$). IR (cm^{-1}): 3409, 2935, 2907, 2837, 1766, 1589, 1503, 1481, 1420, 1373, 1242, 1123, 1022, 962, 821. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.27 (s, 1H, H-5), 6.69 (s, 1H, H-8), 6.25 (s, 2H, H-2′, H-6′), 5.98 (d, *J* = 2.5 Hz, 2H, OCH_2O), 4.57 (d, *J* = 2.0 Hz, 1H, H-1), 4.51−4.52 (m, 2H, H-11), 3.98−4.02 (m, 1H, H-3), 3.79 (s, 3H, 4′-OCH_3), 3.74 (s, 6H, 3′-OCH_3, 5′-OCH_3), 3.24 (dd, *J* = 2.5, 8.5 Hz, 1H, H-2). MS (ESI): *m*/*z* (%) 428.0 ([M + H]$^+$, 43).

*General Procedure for the Synthesis of Oxime Sulfonates of Picropodophyllone (**5a**−**5i**).* To a stirred solution of NaH (33.6 mg, 1.4 mmol) in dry tetrahydrofuran (THF, 10 mL) at −10 °C was slowly added compound **4** (85.4 mg, 0.2 mmol). After adding, the reaction mixture was stirred at −10 °C for 0.5 h. Then, the corresponding sulfonyl chlorides R^1SO_2Cl (0.8 mmol) were added. The reaction process was checked by TLC analysis. When the reaction mixture was stirred at −10 °C for 3 h (in the case of methanesulfonyl chloride, the reaction mixture was stirred at room temperature for 48 h), saturated aqueous $NaHCO_3$ (15 mL) was added to the mixture, which was extracted with DCM (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 compounds **5a**−**5i** in 53−91% yields. The example data of compounds **5a** and **5b** are shown as follows, whereas data of compounds **5c**−**5i** can be found in the Supporting Information.

*Data for Compound **5a**.* R_f = 0.26 (petroleum ether/ethyl acetate = 2:1). Yield = 66%, white solid. mp = 110−112 °C. $[\alpha]^{20}_D$ = 13 (*c* 3.0 mg/mL, $CHCl_3$). IR (cm^{-1}): 2919, 2848, 1771, 1588, 1503, 1482, 1375, 1245, 1191, 1124, 1027, 932, 810, 721. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.03 (d, *J* = 7.5 Hz, 2H, H-2″, H-6″), 7.70 (t, *J* = 7.5 Hz, 1H, H-4″), 7.59 (d, *J* = 7.5 Hz, 2H, H-3″, H-5″), 7.15 (s, 1H, H-5), 6.70 (s, 1H, H-8), 6.13 (s, 2H, H-2′, H-6′), 6.00 (d, *J* = 2.5 Hz, 2H, OCH_2O), 4.54−4.59 (m, 2H, H-1, H-11), 4.33−4.35 (m, 1H, H-11), 4.00 (t, *J* = 7.5 Hz, 1H, H-3), 3.78 (s, 3H, 4′-OCH_3), 3.63 (s, 6H, 3′-OCH_3, 5′-OCH_3), 3.23 (dd, *J* = 2.0, 8.5 Hz, 1H, H-2). MS (ESI): *m*/*z* (%) 590 ([M + Na]$^+$, 100). HRMS (ESI): calcd for $C_{28}H_{25}NO_{10}SNa$ ([M + Na]$^+$), 590.1091; found, 590.1089.

*Data for Compound **5b**.* R_f = 0.25 (petroleum ether/ethyl acetate = 2:1). Yield = 56%, white solid. mp = 108−110 °C. $[\alpha]^{20}_D$ = 50 (*c* 3.2 mg/mL, $CHCl_3$). IR (cm^{-1}): 2917, 2840, 1772, 1589, 1504, 1482, 1374, 1244, 1191, 1177, 1124, 1028, 933, 815, 716. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.90 (d, *J* = 8.0 Hz, 2H, H-2″, H-6″), 7.38 (d, *J* = 8.0 Hz, 2H, H-3″, H-5″), 7.16 (s, 1H, H-5), 6.70 (s, 1H, H-8), 6.14 (s, 2H, H-2′, H-6′), 6.00 (dd, *J* = 1.0, 4.0 Hz, 2H, OCH_2O), 4.53−4.57 (m, 2H, H-1, H-11), 4.31−4.36 (m, 1H, H-11), 4.00 (t, *J* = 7.5 Hz, 1H, H-3), 3.78 (s, 3H, 4′-OCH_3), 3.63 (s, 6H, 3′-OCH_3, 5′-OCH_3), 3.22 (dd, *J* = 2.0, 8.5 Hz, 1H, H-2), 2.48 (s, 3H, CH_3). MS (ESI): *m*/*z* (%) 604 ([M + Na]$^+$, 100). HRMS (ESI): calcd for $C_{29}H_{27}NO_{10}SNa$ ([M + Na]$^+$), 604.1247; found, 604.1244.

*Synthesis of Podophyllotoxone (**6**).* A mixture of compound **1** (414 mg, 1 mmol), CrO_3 (0.5 g, 5 mmol), and pyridine (0.91 mL, 10 mmol) in dry DCM (20 mL) was stirred at room temperature. When the reaction was complete after 3 h, checked by TLC analysis, the mixture was diluted by DCM (60 mL), washed by saturated aqueous $NaHSO_3$ (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 (3:1, v/v) to afford compound **6** (0.35 g, 85% yield) as a white solid. mp = 174−176 °C [literature, 174 °C].[28] $[\alpha]^{20}_D$ = −132 (*c* 2.8 mg/mL, $CHCl_3$). IR (cm^{-1}): 3125, 2975, 2869, 1754, 1680, 1502, 1462, 1359, 1261, 1193, 1129, 1041, 978, 957, 873, 743. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.55 (s, 1H, H-5), 6.70 (s, 1H, H-8), 6.38 (s, 2H, H-2′, H-6′), 6.08 (d,

J = 8.5 Hz, 2H, OCH_2O), 4.84 (d, J = 4.5 Hz, 1H, H-1), 4.54−4.57 (m, 1H, H-11), 4.33 (t, J = 9.5 Hz, 1H, H-11), 3.82 (s, 3H, 4′-OCH_3), 3.75 (s, 6H, 3′-OCH_3, 5′-OCH_3), 3.48−3.55 (m, 1H, H-3), 3.26−3.30 (m, 1H, H-2). MS (ESI): m/z (%) 413 ([M + H]$^+$, 30).

*Synthesis of Oxime of Podophyllotoxone (**7**) and Isoxazolopodophyllic Acid (**8**).* A mixture of podophyllotoxone (**6**, 206 mg, 0.5 mmol), hydroxylamine hydrochloride (44.5 mg, 0.64 mmol), and pyridine (0.14 mL) 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 aqueous $NaHCO_3$ (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 compound **7** (16.6 mg, 7.7% yield) and compound **8** (168.7 mg, 79% yield).

*Data for Compound **7**.* White solid. mp = 104−106 °C [literature, 138−140 °C].[24] $[\alpha]^{20}_D$ = −31 (c 3.2 mg/mL, acetone). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.43 (s, 1H, H-5), 6.68 (s, 1H, H-8), 6.28 (s, 2H, H-2′, H-6′), 6.06 (d, J = 9.5 Hz, 2H, OCH_2O), 4.50 (t, J = 9.0 Hz, 1H, H-11), 4.56 (d, J = 6.0 Hz, 1H, H-1), 3.82−3.86 (m, 1H, H-11), 3.80 (s, 3H, 4′-OCH_3), 3.72 (s, 6H, 3′-OCH_3, 5′-OCH_3), 3.53−3.62 (m, 2H, H-2, H-3). MS (ESI): m/z (%) 428 ([M + H]$^+$, 28).

*Data for Compound **8**.* White solid. mp = 242−244 °C [literature, 246−248 °C].[24] $[\alpha]^{20}_D$ = −66 (c 3.8 mg/mL, acetone). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.42 (s, 1H, H-5), 6.56 (s, 1H, H-8), 6.21 (s, 2H, H-2′, H-6′), 6.00 (s, 2H, OCH_2O), 4.76 (s, 1H, H-11), 4.66 (s, 1H, H-1), 3.76 (s, 2H, H-3, H-11), 3.65 (s, 3H, 4′-OCH_3), 3.62 (s, 6H, 3′-OCH_3, 5′-OCH_3), 3.13 (s, 2H, H-2). MS (ESI): m/z (%) 428 ([M + H]$^+$, 98).

*General Procedure for the Synthesis of Isoxazolopodophyllic-Acid-Based Esters (**9a**−**9i**).* A mixture of the corresponding alcohols R^2OH (0.28 mmol), diisopropylcarbodiimide (DIC, 0.2 mmol), 4-dimethylaminopyridine (DMAP, 0.04 mmol), and compound **8** (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), aqueous HCl (0.1 mol/L, 20 mL), saturated aqueous $NaHCO_3$ (20 mL), and brine (20 mL), dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by PTLC to give compounds **9a**−**9i** in 74−89% yields. The example data of compounds **9a** and **9b** are shown as follows, whereas data of compounds **9c**−**9i** can be found in the Supporting Information.

*Data for Compound **9a**.* Yield = 86%, white solid. mp = 150−152 °C [literature, 82−84 °C].[24] $[\alpha]^{20}_D$ = −77 (c 3.4 mg/mL, acetone). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.46 (s, 1H, H-5), 6.56 (s, 1H, H-8), 6.13 (s, 2H, H-2′, H-6′), 6.00 (s, 2H, OCH_2O), 4.82 (t, J = 8.5 Hz, 1H, H-11), 4.63 (d, J = 5.0 Hz, H-1), 3.88−3.95 (m, 1H, H-11), 3.78−3.82 (m, 4H, H-3, 4′-OCH_3), 3.73 (s, 3′-OCH_3, 5′-OCH_3), 3.67 (s, 3H, −CO_2CH_3), 3.19 (dd, J = 5.0, 12.5 Hz, 1H, H-2). MS (ESI): m/z (%) 442.27 ([M + H]$^+$, 100).

*Data for Compound **9b**.* Yield = 75%, white solid. mp = 181−182 °C. $[\alpha]^{20}_D$ = −59 (c 3.3 mg/mL, acetone). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.46 (s, 1H, H-5), 6.57 (s, 1H, H-8), 6.15 (s, 2H, H-2′, H-6′), 6.00 (s, 2H, OCH_2O), 4.82 (t, J = 8.5 Hz, 1H, H-11), 4.64 (d, 1H, J = 5.0 Hz, H-1), 4.02−4.15 (m, 2H, −$CO_2CH_2CH_3$), 3.87−3.96 (m, 1H, H-11), 3.79−3.83 (m, 4H, H-3, 4′-OCH_3), 3.73 (s, 3′-OCH_3, 5′-OCH_3), 3.17 (dd, J = 5.0, 12.5 Hz, 1H, H-2), 1.21 (t, J = 7.0 Hz, 3H, CH_3). HRMS (ESI): calcd for $C_{24}H_{26}NO_8$ ([M + H]$^+$), 456.1653; found, 456.1646.

*Synthesis of Isoxazolopodophyllol (**10**).* Compound **9a** (100 mg, 0.23 mmol) in dry THF (5 mL) was slowly added to a suspension of $LiAlH_4$ (120 mg, 3.16 mmol) in dry THF. The reaction mixture was stirred at room temperature under nitrogen for 3 h. Then, ethyl acetate (10 mL) was added to the mixture, which was filtered, dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by silica gel column chromatography eluting with petroleum ether/ethyl acetate (2:5, v/v) to afford compound **10** (76 mg, 80% yield) as a white solid. mp = 200−202 °C [literature, 146−148 °C].[24] $[\alpha]^{20}_D$ = −44 (c 3.1 mg/mL, acetone). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.46 (s, 1H, H-5), 6.56 (s, 1H, H-8), 6.24 (s, 2H, H-2′, H-6′), 5.98 (s, 2H, OCH_2O), 4.63 (t, J = 8.5 Hz, 1H, H-11), 4.37 (s, 1H, H-1), 3.85−3.89 (m, 1H, H-11), 3.81 (s, 3H, 4′-OCH_3), 3.75 (s, 6H, 3′-OCH_3, 5′-OCH_3), 3.43−3.51 (m, 2H, H-3, −CH_2OH), 3.36−3.39 (m, 1H, −CH_2OH), 2.37−2.38 (m, 1H, H-2). MS (ESI): m/z (%) 414.1 ([M + H]$^+$, 73).

*General Procedure for the Synthesis of Isoxazolopodophyllol-Based Esters (**11a**−**11h**).* A mixture of the corresponding carboxylic acids R^3CO_2H (0.28 mmol), DIC (0.28 mmol), DMAP (0.04 mmol), and compound **10** (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), aqueous HCl (0.1 mol/L, 20 mL), saturated aqueous $NaHCO_3$ (20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by PTLC to give compounds **11a**−**11h** in 75−98% yields. The example data of compounds **11a** and **11b** are shown as follows, whereas data of compounds **11c**−**11g** can be found in the Supporting Information.

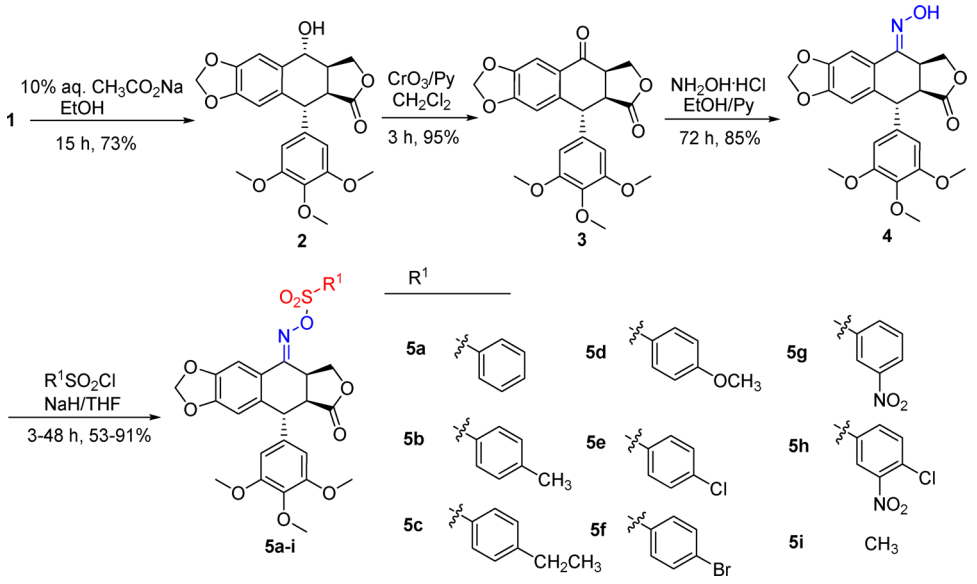
*Data for Compound **11a**.* Yield = 81%, white solid. mp = 136−137 °C. $[\alpha]^{20}_D$ = −56 (c 3.0 mg/mL, acetone). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.10 (s, 1H, −CHO), 7.47 (s, 1H, H-5), 6.56 (s, 1H, H-8), 6.16 (s, 2H, H-2′, H-6′), 5.99 (s, 2H, OCH_2O), 4.64 (t, J = 8.5 Hz, 1H, H-11), 4.31 (d, J = 4.5 Hz, 1H, H-1), 4.02−4.06 (m, 1H, −CH_2OCHO), 3.89−3.93 (m, 1H, H-11), 3.81−3.85 (m, 4H, −CH_2OCHO, 4′-OCH_3), 3.75 (s, 6H, 3′-OCH_3, 5′-OCH_3), 3.50−3.57 (m, 1H, H-3), 2.53−2.60 (m, 1H, H-2). HRMS (ESI): calcd for $C_{23}H_{24}NO_8$ ([M + H]$^+$), 442.1496; found, 442.1493.

*Data for Compound **11b**.* Yield = 98%, white solid. mp = 83−84 °C. $[\alpha]^{20}_D$ = −57 (c 3.2 mg/mL, acetone). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.47 (s, 1H, H-5), 6.55 (s, 1H, H-8), 6.15 (s, 2H, H-2′, H-6′), 5.99 (s, 2H, OCH_2O), 4.64 (t, J = 9.0 Hz, 1H, H-11), 4.28 (d, J = 4.0 Hz, 1H, H-1), 4.00−4.03 (m, 1H, −$CH_2OCOC_3H_7$), 3.88−3.92 (m, 1H, H-11), 3.81 (s, 3H, 4′-OCH_3), 3.69−3.74 (m, 7H, 3′-OCH_3, 5′-OCH_3, −$CH_2OCOC_3H_7$), 3.49−3.57 (m, 1H, H-3), 2.52−2.54 (m, 1H, H-2), 2.29 (t, J = 7.5 Hz, 2H, $OCOCH_2C_2H_5$), 1.62−1.69 (m, 2H, $OCOCH_2CH_2CH_3$), 0.95 (t, J = 7.0 Hz, 3H, CH_3). HRMS (ESI): calcd for $C_{26}H_{30}NO_8$ ([M + H]$^+$), 484.1966; found, 484.1958.

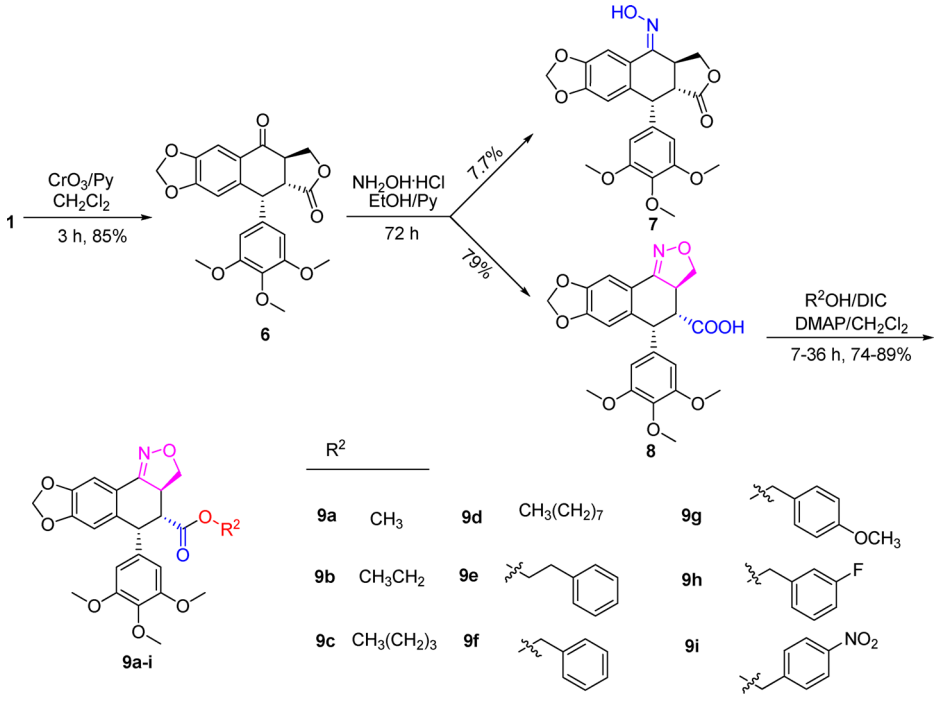
*Synthesis of Isoxazolopodophyllic-Acid-Based n-Butylamide (**12**).* A mixture of *n*-butylamine (0.28 mmol), DIC (0.20 mmol), DMAP (0.04 mmol), and compound **8** (0.2 mmol) in dry DCM (10 mL) was stirred at room temperature. When the reaction was complete after 14 h according to TLC analysis, the mixture was diluted by DCM (40 mL), washed by water (20 mL), aqueous HCl (0.1 mol/L, 20 mL), saturated aqueous $NaHCO_3$ (20 mL), and brine (20 mL), dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by PTLC to give compound **12** in 88% yield as a white solid. mp = 182−183 °C. $[\alpha]^{20}_D$ = −95 (c 3.1 mg/mL, acetone). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.45 (s, 1H, H-5), 6.55 (s, 1H, H-8), 6.12 (s, 2H, H-2′, H-6′), 6.00 (s, 2H, OCH_2O), 5.26 (s, 1H, NH), 4.75 (t, J = 7.5 Hz, 1H, H-11), 4.50 (d, J = 4.5 Hz, H-1), 3.79−3.88 (m, 4H, H-3, 4′-OCH_3), 3.65−3.69 (m, 7H, H-11, 3′-OCH_3, 5′-OCH_3), 3.15−3.19 (m, 1H, −NHCH_2), 3.04 (dd, J = 5.0, 12.0 Hz, 1H, H-2), 1.36−1.42 (m, 2H, −$CH_2CH_2CH_3$), 1.25−1.30 (m, 2H, −$CH_2CH_2CH_3$), 0.89 (t, 3H, J = 7.5 Hz, −$CH_2CH_2CH_3$). HRMS (ESI): calcd for $C_{26}H_{31}N_2O_7$ ([M + H]$^+$), 483.2125; found, 483.2116.

Biological Assay. The insecticidal activity of compounds **1**, **3**, **4**, **5a**−**5i**, **6**−**8**, **9a**−**9i**, **10**, **11a**−**11h**, and **12** against the pre-third-instar larvae of northern armyworm, *M. separata* (Walker), was assessed by the leaf-dipping method, as described previously.[25] For each compound, 30 larvae (10 larvae per group) were used. Acetone solutions of compounds **1**, **3**, **4**, **5a**−**5i**, **6**−**8**, **9a**−**9i**, **10**, **11a**−**11h**, **12**, and toosendanin (used as a positive control) were prepared at the concentration of 1 mg/mL. Fresh wheat leaves were dipped into the corresponding solution for 3 s, then taken out, and dried in a room. Leaves treated with acetone alone were used as a blank control group. Several treated leaves were kept in each dish, 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) of 65−80% on a 12 h/12 h (light/dark) photoperiod. The insecticidal activity of the tested

Scheme 1. Preparation of Compounds 5a−5i



Scheme 2. Preparation of Compounds 9a−9i



compounds against the pre-third-instar larvae of *M. separata* was calculated by the following formula:

$$\text{corrected mortality rate } (\%) = (T - C) \times 100/(1 - C)$$

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.

Descriptor Generation and QSAR Model Development by a Genetic Algorithm Combined with Multiple Linear Regression (GA−MLR). Here, the two-dimensional (2D) structures of 33 compounds (compounds **3**, **4**, **5a−5i**, **6−8**, **9a−9i**, **10**, **11a−11h**, and **12**) were sketched in the HyperChem 7.0 program[29] and pre-optimized using MM+ molecular mechanics force field with a convergence criterion of 0.01 kcal/mol, and final geometries of the minimum energy conformations were obtained by more precise optimization by the semi-empirical AM1 method.

All of the optimized structures were then exported to DRAGON package 5.0[30] to generate molecular descriptors. A total of 1664 descriptors were computed for each compound. They include (a) zero-dimensional (0D) constitutional descriptors, (b) one-dimensional (1D) functional groups, atom-centered fragments, (c) 2D topological descriptors, walk and path counts, connectivity index, information index, various autocorrelations from the molecular graph, edge adjacency indices, descriptors of Burden eigenvalues, topological charge indices, eigenvalue-based indices, (d) three-dimensional (3D) Randic molecular profiles, geometrical descriptors, weighted holistic invariant molecular descriptors (WHIMs), geometry, topology, and atom-weight assembly (GETAWAY) descriptors, (e) charge descriptors, and (f) molecular properties. Constant and near-constant descriptors were excluded for the sake of containing useless information, and descriptors whose pairwise correlation coefficient

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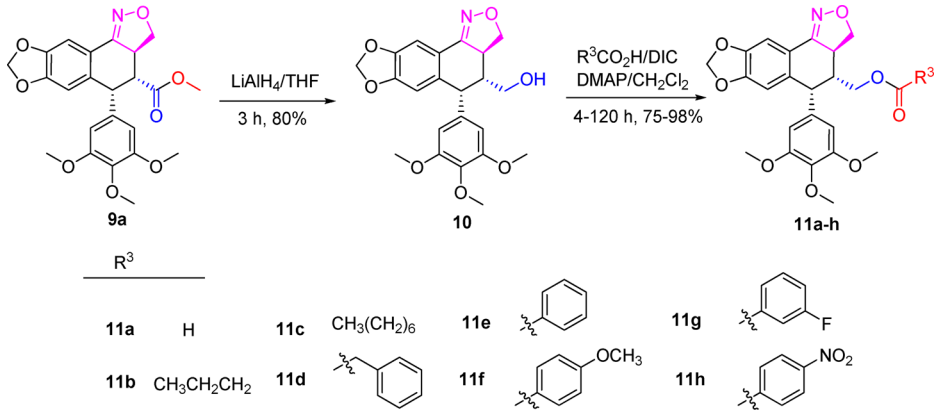
Scheme 3. Preparation of Compounds 11a−11h



was greater than 0.98 were also removed to reduce redundant information. A total of 567 descriptors were remained.

After descriptor calculation, a GA−MLR is employed to select the most important features related to the biological activity values (final mortality rates of 33 compounds) and to build the linear QSAR model. GA has been proven to be a very effective tool in the feature selection for the QSAR study. The used fitness function here is the Friedman LOF function, defined as

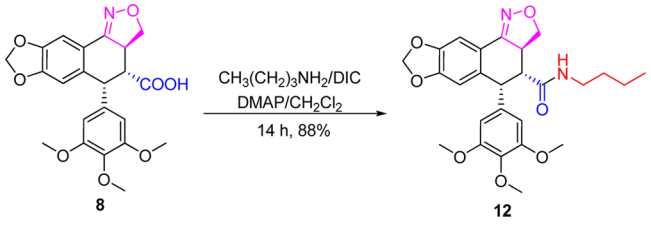
$$\mathrm{LOF} = \{\mathrm{SSE}/(1 - (c + dp/n))\}^2$$

where SSE is the sum of squares errors, c is the number of basic functions, d is the smoothness factor (default 0.5), p is the number of descriptors in the model, and n is the number of samples in data sets. The important parameters that controlled the GA performance were set as follows: population size, 100; maximum generations, 5000; and mutation probability, 0.1. The correlation coefficient (R^2) and leave-one-out cross-validation correlation coefficient (Q^2_{loo}) were adopted to assess the performance of developed models. The GA−MLR calculation is performed by the MOBY DIGS package.[31]

RESULTS AND DISCUSSION

Synthesis. As shown in Scheme 1, oxime sulfonates of picropodophyllone (**5a−5i**) were easily obtained by the reaction of sulfonyl chlorides with oxime of picropodophyllone (**4**), which was prepared from picropodophyllone (**3**) with hydroxylamine hydrochloride. Interestingly, as described in Scheme 2, when podophyllotoxone (**6**) reacted with hydroxylamine hydrochloride, besides oxime of podophyllotoxone (**7**, 7.7% yield), isoxazolopodophyllic acid (**8**, 79% yield) was obtained as the major product. Finally, as shown in Schemes 2−4, isoxazolopodophyllic-acid-based esters (**9a−9i**), isoxazolopodophyllol-based esters (**11a−11h**), and isoxazolopodophyllic-acid-based *n*-butylamide (**12**) were synthesized by the reaction of isoxazolopodophyllic acid or isoxazolopodophyllol with alcohols, carboxylic acids, or *n*-butylamine in the presence of DIC and DMAP. The structures of the target compounds were well-characterized by ^{1}H NMR, HRMS, ESI−MS, optical rotation, mp, and IR. Additionally, to confirm the 3D structural information of compounds **5a−5i**, the single-crystal structures of compounds **5e** and **5f** were determined by X-ray crystallography, as illustrated in Figures 2 and 3, respectively.

Scheme 4. Preparation of Compound 12



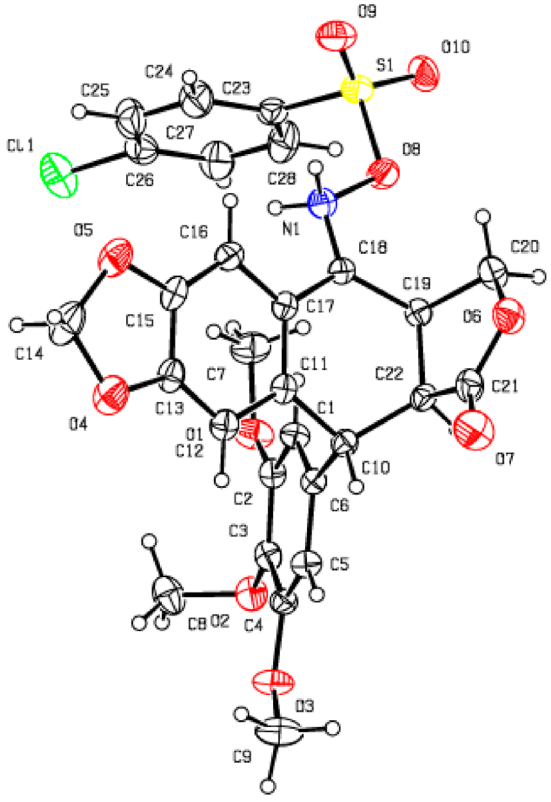


Figure 2. X-ray crystal structure of compound **5e**.

It demonstrated that the oxime fragments of compounds **5e** and **5f** all adopted *trans* configuration. The steric configuration of compound **9f** was unambiguously confirmed by X-ray crystallography, as illustrated in Figure 4. It suggested that methylene at the C-3 position and benzyloxycarbonyl at the C-2 position of compound **9f** adopted β and α configurations, respectively; that is, the configuration of the carboxy group of compound **8** was also α. It can be concluded that, although the *trans* lactone moiety of compound **6** was opened by the reaction of compound **6** with hydroxylamine hydrochloride to give compound **8**, the stereo configuration of C-2 and C-3

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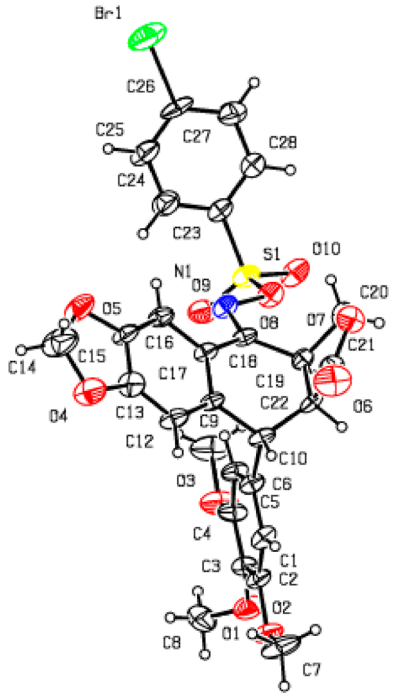

Figure 3. X-ray crystal structure of compound **5f**.

positions of compound **8** was the same as that of compound **6**. Crystallographic data (excluding structure factors) for the structures of compounds **5e**, **5f**, and **9f** have been deposited within the Cambridge Crystallographic Data Centre (CCDC) as supplementary publication numbers 882837, 883317, and 882720, respectively. Copies of the data can be obtained, free of charge, on application to CCDC, U.K.

Insecticidal Activity. The insecticidal activity of compounds **1**, **3**, **4**, **5a**−**5i**, **6**−**8**, **9a**−**9i**, **10**, **11a**−**11h**, and **12** against the pre-third-instar larvae of *M. separata in vivo* was tested by the leaf-dipping method at the concentration of 1 mg/mL. As shown in Table 1, the corresponding mortality rates after 33 days were generally higher than those after 5 and 20 days; therefore, these compounds, in a time-dependent manner, different from those conventional neurotoxic insecticides, such as organophosphates, carbamates, and pyrethroids, exhibited delayed insecticidal activity. Meanwhile, the symptoms of the treated *M. separata* were also characterized in the same way as our previous reports.[20−23,25] For example, because of eating too much treated leaves for the first 48 h, some larvae died slowly during the larval period, many malformed pupae of the treated groups appeared and died during the stage of pupation, and malformed moths with imperfect wings also appeared in the treated groups. Compounds **5b**, **5e**, **9b**, **9c**, **9g**, **11g**, and **11h** exhibited equal or higher insecticidal activity than toosendanin. Especially compounds **5e**, **9c**, **11g**, and **11h** exhibited the highest insecticidal activity. To compounds **5a**−**5i**, introduction of the chlorine atom or methyl on the phenyl ring of oxime sulfonates was very important for the insecticidal activity. For example, the final mortality rates of compound **5a** (containing phenyl), compound **5c** (containing 4-ethylphenyl), and compound **5d** (containing 4-methoxyphenyl) were only 24.1, 27.6, and 24.1%, respectively, whereas the final mortality rates of compound **5b** (containing 4-methylphenyl) was 44.8%. It was noteworthy that the introduction of the chlorine atom on the phenyl ring could lead to a more potent compound than those possessing bromine, nitro, or nitro and chlorine one (**5e** versus **5f**, **5g**, and **5h**). To compounds **9a**−**9i**, the proper length of the side chain of alkyloxycarbonyl at the C-2 position of podophyllotoxin was important for their insecticidal activity. When R^2 was the *n*-butyl group, the corresponding compound **9c**, whose final mortality rate was 58.6%, displayed a more promising activity than those containing methyl, ethyl, *n*-octyl, etc. The introduction of the electron-withdrawing group (e.g., F and NO_2) on the phenyl ring of acyloxy at the C-2 position of compounds **11a**−**11h** was important for the insecticidal activity. For example, the final mortality rates of compound **11g** (containing the fluorine atom) and compound **11h** (containing the nitro group) were 55.2 and 58.6%, respectively. Interestingly, when the oxygen atom of *n*-butyloxycarbonyl at

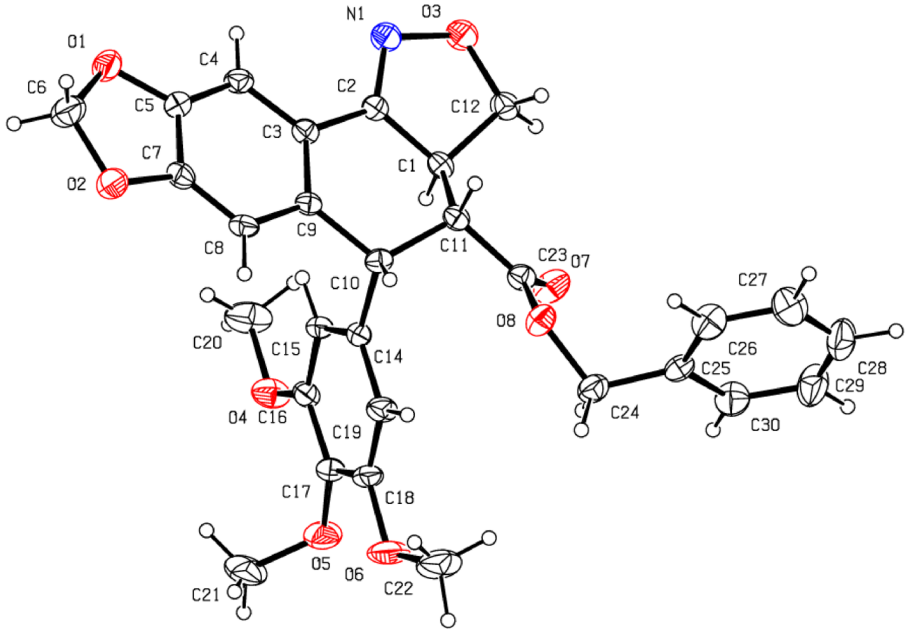

Figure 4. X-ray crystal structure of compound **9f**.

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Table 1. Insecticidal Activity of Compounds 5a−5i, 9a−9i, 11a−11h, and 12 against *M. separata* on Leaves Treated with a Concentration of 1 mg/mL[a]

compound	corrected mortality rate (%)		
	5 days	20 days	33 days
1	3.3 ± 4.7	10.3 ± 12.5	37.9 ± 0
3	16.7 ± 4.7	20.7 ± 9.4	27.6 ± 0
4	0 ± 0	6.9 ± 8.2	17.2 ± 0
5a	0 ± 0	6.9 ± 8.2	24.1 ± 12.5
5b	20.0 ± 8.2	34.5 ± 9.4	44.8 ± 4.7
5c	3.3 ± 4.7	17.2 ± 0	27.6 ± 8.2
5d	0 ± 0	3.5 ± 4.7	24.1 ± 12.5
5e	26.7 ± 4.7	37.9 ± 8.2	55.2 ± 4.7
5f	0 ± 0	10.3 ± 9.4	27.6 ± 0
5g	0 ± 0	3.5 ± 4.7	13.8 ± 4.7
5h	0 ± 0	3.5 ± 4.7	13.8 ± 4.7
5i	0 ± 0	17.2 ± 8.2	24.1 ± 9.4
6	6.7 ± 4.7	3.5 ± 4.7	17.2 ± 8.2
7	10.0 ± 0	24.1 ± 12.5	34.5 ± 4.7
8	20.0 ± 0	31.0 ± 4.7	41.4 ± 4.7
9a	23.3 ± 4.7	31.0 ± 4.7	41.4 ± 4.7
9b	20.0 ± 8.2	31.0 ± 9.4	44.8 ± 4.7
9c	23.3 ± 4.7	34.5 ± 4.7	58.6 ± 8.2
9d	30.0 ± 0	34.5 ± 4.7	37.9 ± 8.2
9e	20.0 ± 0	27.6 ± 8.2	41.4 ± 4.7
9f	23.3 ± 9.4	31.0 ± 9.4	37.9 ± 8.2
9g	26.7 ± 4.7	37.9 ± 8.2	44.8 ± 4.7
9h	23.3 ± 9.4	34.5 ± 9.4	34.5 ± 9.4
9i	23.3 ± 4.7	24.1 ± 9.4	27.6 ± 8.2
10	20.0 ± 0	20.7 ± 4.7	37.9 ± 8.2
11a	0 ± 0	10.3 ± 12.5	24.1 ± 4.7
11b	6.7 ± 4.7	13.8 ± 4.7	34.5 ± 4.7
11c	0 ± 0	6.9 ± 0	17.2 ± 8.2
11d	6.7 ± 4.7	6.9 ± 8.2	20.7 ± 4.7
11e	26.7 ± 4.7	27.6 ± 0	41.4 ± 4.7
11f	6.7 ± 4.7	13.8 ± 9.4	37.9 ± 8.2
11g	23.3 ± 4.7	34.5 ± 9.4	55.2 ± 4.7
11h	26.7 ± 4.7	37.9 ± 8.2	58.6 ± 8.2
12	0 ± 0	0 ± 4.7	10.3 ± 4.7
toosendanin	6.7 ± 4.7	13.8 ± 9.4	44.8 ± 4.7

[a]Values are the mean ± standard deviation (SD) of three replicate.

the C-2 position of compound **9c** was substituted by the nitrogen atom to afford compound **12**, the final mortality rate of compound **12** was sharply decreased to 10.3%.

QSAR. Many models have been obtained through the GA−MLR approach, and the best model is selected from all of these models based on the criterion that it should have the highest cross-validation correlation coefficient (Q^2). The selected best model has five descriptors. For this model, the standard deviation error in prediction (SDEP) is 0.0592, the correlation coefficient (R^2) is 0.861, and the leave-one-out Q^2 is 0.797. The descriptors of the best model together with their corresponding statistical items were shown in Table 2. The predicted values of the activity (final mortality rates) of 33 compounds by this model are listed in Table 3. Figure 5 shows the plot of the predicted versus experimental activities of 33 compounds.

Table 3. Experimental and Predicted Activity of 33 Compounds

number	compound	*Y* experimental	*Y* predicted
1	**3**	0.276	0.299
2	**4**	0.172	0.262
3	**5a**	0.241	0.151
4	**5b**	0.448	0.495
5	**5c**	0.276	0.254
6	**5d**	0.241	0.285
7	**5e**	0.552	0.513
8	**5f**	0.276	0.316
9	**5g**	0.138	0.127
10	**5h**	0.138	0.135
11	**5i**	0.241	0.251
12	**6**	0.172	0.220
13	**7**	0.345	0.262
14	**8**	0.414	0.395
15	**9a**	0.414	0.318
16	**9b**	0.448	0.482
17	**9c**	0.586	0.597
18	**9d**	0.379	0.305
19	**9e**	0.414	0.419
20	**9f**	0.379	0.399
21	**9g**	0.448	0.413
22	**9h**	0.345	0.391
23	**9i**	0.276	0.361
24	**10**	0.379	0.398
25	**11a**	0.241	0.225
26	**11b**	0.345	0.298
27	**11c**	0.172	0.197
28	**11d**	0.207	0.243
29	**11e**	0.414	0.462
30	**11f**	0.379	0.372
31	**11g**	0.552	0.505
32	**11h**	0.586	0.505
33	**12**	0.103	0.144

By interpreting the selected molecular descriptors, we could gain some insight into structural features influencing the biological activity. In this study, five descriptors have been selected: they include one 2D autocorrelation descriptor (GATS4e), one edge adjacency indice (EEig06x), one RDF descriptor (RDF080v), one 3D MoRSE descriptor (Mor09v), and one atom-centered fragment (H-052) descriptor. From the

Table 2. Best Five-Parameter Model

	variables	meaning of variables	regression coefficient	error coefficient	standardized coefficient
0	intercept	constant	−2.843	0.358	
1	GATS4e	Geary autocorrelation of lag 4/weighted by atomic Sanderson electronegativities	1.511	0.261	0.734
2	EEig06x	eigenvalue 06 from edge adjacency matrix weighted by edge degrees	0.505	0.094	0.787
3	RDF080v	radial distribution function 8.0/weighted by atomic van der Waals volumes	−0.062	0.005	−1.922
4	Mor09v	3D MoRSE Singal 09/weighted by atomic van der Waals volumes	−0.307	0.043	−0.777
5	H-052	H attached to CO (sp^3) with 1X attached to next C	0.081	0.015	0.502

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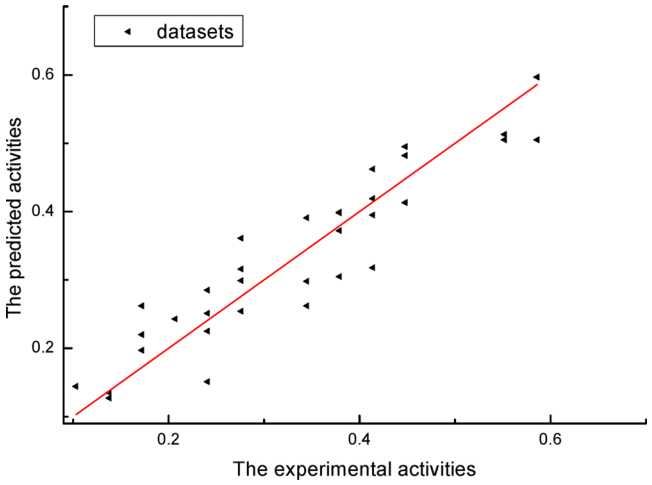

Figure 5. Plot of experimental and predicted activities of 33 compounds.

standardized coefficient of the descriptors shown in Table 2, it can be seen that the most important descriptor is EEig06x (Eigenvalue 06 from edge adjacency matrix weighted by edge degrees). It belongs to edge adjacency indices and is calculated from the edge adjacency matrix of a molecule. The second important parameter is GATS4e (Geary autocorrelation lag 4/ weighted by atomic Sanderson electronegativities), which is a 2D autocorrelation indice. It reflects the information on molecular dimension and Sanderson electronegativity. H-052 (H attached to CO (sp^3) with 1X attached to next C) belongs to atom-centered fragments. It is a simple molecular descriptor defined as the number of specific atom types in a molecule, and it is calculated by knowing the molecular composition and atom connectivity. The above three descriptors are positively correlated to the activity. RDF080v can be interpreted as the probability distribution of finding an atom in a spherical volume of radius. The descriptor Mor09v is 3D MoRSE (3D molecule representation of structure based on electron diffraction) descriptor, which is based on the idea of obtaining information from the 3D atomic coordinates by the transform. Mor09v is weighted by atomic van der Waal volumes. From the aforementioned discussion, it can be seen that the activities of these compounds were mainly influenced by many factors, such as electronic distribution, steric factors, etc.

In conclusion, 33 isoxazoline and oxime derivatives of podophyllotoxin were synthesized and evaluated for their insecticidal activity against the pre-third-instar larvae of *M. separata in vivo*. Compounds **5e**, **9c**, **11g**, and **11h** especially exhibited higher insecticidal activity than toosendanin. QSAR demonstrated that the involved descriptors for these compounds may account for their structural features responsible for the insecticidal activity, and their insecticidal activity was mainly influenced by many factors, such as electronic distribution, steric factors, etc. The above results will lay the foundation for further structural modification and development of podophyllotoxin as insecticidal agents.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H NMR, HRMS, optical rotation, mp, and IR data for the target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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