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Synthesis and Quantitative Structure–Activity Relationship (QSAR) Study of Novel 4-Acyloxypodophyllotoxin Derivatives Modified in the A and C Rings 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, we have synthesized three series of novel 4-acyloxy compounds derived from podophyllotoxin modified in the A and C rings, which is isolated as the main secondary metabolite from the roots and rhizomes of *Podophyllum hexandrum*. Their insecticidal activity was preliminarily evaluated against the pre-third-instar larvae of *Mythimna separata* in vivo. Compound **9g** displayed the best promising insecticidal activity. It revealed that cleavage of the 6,7-methylenedioxy group of podophyllotoxin will lead to a less active compound and that the C-4 position of podophyllotoxin was the important modification location. A quantitative structure–activity relationship (QSAR) model was developed by genetic algorithm combined with multiple linear regression (GA-MLR). For this model, the squared correlation coefficient (R^2) is 0.914, the leave-one-out cross-validation correlation coefficient (Q^2_{LOO}) is 0.881, and the root-mean-square error (RMSE) is 0.024. Five descriptors, BEHm2, Mor14v, Wap, G1v, and RDF020e, are likely to influence the biological activity of these compounds. Among them, two important ones are BEHm2 and Mor14v. This study will pave the way for further design, structural modification, and development of podophyllotoxin derivatives as insecticidal agents.

KEYWORDS: *podophyllotoxin, acyloxy, A and C ring modification, botanical insecticide, insecticidal activity, QSAR, Mythimna separata Walker*

INTRODUCTION

Lepidoptera are the most diverse pest insect order. The larvae of many lepidopteran species are major pests in agriculture and can cause extensive damage to certain crops.[1] For example, the oriental armyworm (*Mythimna separata* Walker), a typical lepidopteran pest, is widely distributed in China, Japan, Southeast Asia, India, eastern Australia, New Zealand, and some Pacific Islands, and sometimes its outbreaks result in widespread incidence and complete crop loss.[2] Although a wide variety of synthetic insecticides were introduced to control lepidopteran pests, the extensive application of synthetic agrochemicals over the years has resulted in the development of resistance in lepidopteran pest populations.[3−5] Development of new effective, selective, and safe pesticides, therefore, is still a challenging task. Plant secondary metabolites result from the interaction between plants and the environment (life and nonlife) during the long period of evolution in plants, and pesticides produced from plant secondary metabolites may result in less or slower resistance development and lower pollution;[6] consequently, the discovery and development of new insecticidal compounds directly from plant secondary metabolites, or by using them as lead compounds for further modifications, have recently been important areas of research and development of new pesticides.[7−9]

Podophyllotoxin (**1**, Figure 1), a naturally occurring aryltetralin lignan, besides its use as the lead compound for the preparation of potent anticancer drugs such as etoposide (VP-16, **2**, Figure 1), teniposide (VM-26, **3**, Figure 1), and

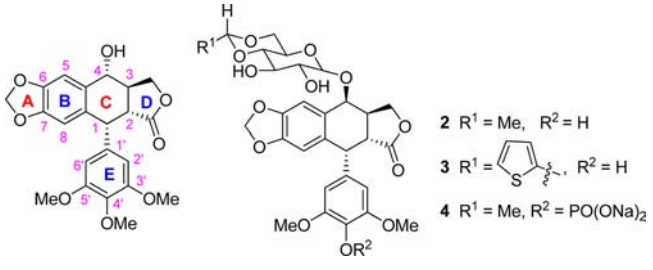

Figure 1. Chemical structures of podophyllotoxin (**1**), etoposide (**2**), teniposide (**3**), and etopophos (**4**).

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etopophos (etoposide phosphate, **4**, Figure 1),[10−12] has also received much research attention for its interesting insecticidal and antifungal activities.[13−18] Recently, we have studied the insecticidal activity of a series of 2β-chloropodophyllotoxin and 2α/β-bromopodophyllotoxin derivatives with modified C and D rings of **1** and found some compounds showed more potent insecticidal activity than toosendanin, a commercial botanical insecticide extracted from *Melia azedarach*.[19−22] Encouraged by the above-mentioned interesting results, and to find novel natural product-based insecticidal agents to control the lepidopteran pests, we herein designed and synthesized three series of 4-acyloxypodophyllotoxin derivatives modified in the A and C rings as insecticidal agents against the pre-third-instar larvae of *Mythimna separata* and wanted to investigate the influence of the A and C rings and the configuration of acyloxy at the C-4 position on the insecticidal activity. Quantitative structure−activity relationship (QSAR) studies are also described.

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 (mp) were determined on a digital melting point apparatus and were uncorrected. Infrared spectra (IR) were recorded on a Bruker TENSOR 27 spectrometer. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a Bruker Avance III 500 MHz instrument in $CDCl_3$ using tetramethylsilane (TMS) as the internal standard. High-resolution mass spectra (HR-MS) were carried out with an IonSpec 4.7 T FTMS instrument. The purities of the tested compounds were determined by reverse phase high-performance liquid chromatography (RP-HPLC), recorded on a Shimadzu LC-15C liquid chromatograph (SPD-15C UV−vis spectrophotometric detector (190−700 nm)) using a flow rate of 1.0 mL/min (MeOH/H_2O = 5:1) and a Hypersil ODS C_{18} column (5 μm, 4.6 × 150 mm) as the stationary phase.

*Synthesis of 6,7-O,O-Demethylenepodophyllotoxin (**5**).* To a solution of boron trichloride in dichloromethane (1 M, 1 mL) precooled at −70 °C was added dropwise podophyllotoxin (**1**) (0.1 g, 0.25 mmol) in dichloromethane (5 mL) over 15 min. After the mixture had been stirred at −70 °C for an additional 2 h, the mixture was poured into 20 mL of ice−water and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with brine until the pH was 6−7, dried over anhydrous sodium sulfate, and filtered. The filtrate was evaporated in vacuo to give a white solid, which was put into a mixture of acetone−water−calcium carbonate (1 mL, 1 mL, 0.3 g) and refluxed for 1 h. The white suspension was filtered off, and the filtrate was acidified by aqueous HCl (2 M) until the pH was 2−3 and extracted by ethyl acetate (5 × 30 mL). The combined organic layers were dried over anhydrous sodium sulfate and evaporated in vacuo to afford **5** in 72% yield as a white solid.

*Synthesis of 6,7-O,O-Demethylene-6,7-O,O-dimethylpodophyllotoxin (**6**).* To a mixture of **5** (0.05 g, 0.125 mmol) and K_2CO_3 (0.276 g, 2 mmol) in acetone (5 mL) at 0 °C was added methyl iodide (0.071 g, 0.5 mmol). Then the mixture was stirred at room temperature. When the reaction was complete according to TLC analysis, the mixture was filtered. The filtrate was evaporated in vacuo and purified by silica gel column chromatography to give **6** in 86% yield as a white solid.

*Synthesis of 6,7-O,O-Demethylene-6,7-O,O-dimethylepipodophyllotoxin (**7**).* To a mixture of **6** (430 mg, 1 mmol) and NaI (299 mg, 2 mmol) in CH_3CN (10 mL) at 0 °C was added dropwise a solution of $BF_3{\cdot}Et_2O$ (0.378 mL, 1.4 mmol) in CH_3CN (3 mL). After the addition, the mixture was stirred at room temperature. When the reaction was complete according to TLC analysis, the mixture was evaporated in vacuo to afford the residue. To the above residue were added acetone−water (5 mL, 10 mL) and $BaCO_3$ (395 mg, 2 mmol). Then the mixture was reacted at room temperature. When the reaction was complete according to TLC analysis, the mixture was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with water and aqueous $Na_2S_2O_3$ (10%, 25 mL), dried over anhydrous Na_2SO_4, concentrated in vacuo, and purified by silica gel column chromatography to give **7** in 69% yield as a white solid.

*Synthesis of 6,7-O,O-Demethylene-6,7-O,O-dibenzylpodophyllotoxin (**8**).* To a mixture of **5** (0.1 g, 0.25 mmol), Cs_2CO_3 (0.195 g, 0.6 mmol), and KI (12 mg, 0.075 mmol) in acetone (10 mL) as added benzyl bromide (0.103 g, 0.6 mmol). Then the mixture was stirred at room temperature. When the reaction was complete according to TLC analysis, the mixture was filtered. The filtrate was evaporated in vacuo to give the residue, which was diluted with CH_2Cl_2 (30 mL), washed by water, dried over anhydrous sodium sulfate, and purified by PTLC eluting with petroleum ether/ethyl acetate (1:2, v/v) to give **8** in 36% yield as a white solid.

*General Procedure for the Synthesis of 6,7-O,O-Demethylene-6,7-O,O-dimethyl-4α-acyloxypodophyllotoxin Derivatives (**9a−m**), 6,7-O,O-Demethylene-6,7-O,O-dimethyl-4β-acyloxypodophyllotoxin Derivatives (**10c,d,f,g,l**), and 6,7-O,O-Demethylene-6,7-O,O-dibenzyl-4α-acyloxypodophyllotoxin Derivatives (**11d, f, g,l**).* A mixture of the corresponding acids (0.32 mmol), diisopropylcarbodiimide (DIC, 0.32 mmol), 4-dimethylaminopyridine (DMAP, 0.045 mmol), and **6**, **7**, or **8** (0.23 mmol) in dry CH_2Cl_2 (10 mL) was stirred at room temperature. When the reaction was complete according to TLC analysis, the mixture was diluted by CH_2Cl_2 (30 mL), washed by aqueous HCl (0.1 M, 20 mL), aqueous $NaHCO_3$ (5%, 20 mL), and brine (20 mL), dried over anhydrous Na_2SO_4, concentrated in vacuo, and purified by PTLC to give the pure target products **9a−m**, **10c,d,f,g,l**, and **11d,f,g,l**. Their structures were well characterized by ^{1}H NMR, HRMS, optical rotation, IR, and mp. Example data of **9a**, **9b**, **10c**, **10d**, **11d**, and **11f** are shown as follows, whereas those of other compounds can be found in the Supporting Information.

*Data for **9a**:* yield = 69%, white solid, mp = 70−72 °C; $[\alpha]^{20}_D$ = −2 (*c* 3.2 mg/mL, $CHCl_3$); IR (cm^{-1}) 2959, 2921, 2837, 1772, 1726, 1588, 1514, 1465, 1236, 1125, 1008, 762; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.76 (s, 1H, H-5), 6.54 (s, 1H, H-8), 6.38 (s, 2H, H-2′, 6′), 5.91 (d, *J* = 9.0 Hz, 1H, H-4), 4.64 (s, 1H, H-1), 4.37−4.40 (m, 1H, H-11), 4.19−4.23 (m, 1H, H-11), 3.90 (s, 3H), 3.80 (s, 6H), 3.74 (s, 6H), 2.91−2.94 (m, 1H, H-2), 2.77−2.86 (m, 1H, H-3), 2.20 (s, 3H). HRMS calcd for $C_{25}H_{28}O_9$ ($[M]^+$), 472.1728; found, 472.1739.

*Data for **9b**:* yield = 34%, white solid, mp = 191−193 °C; $[\alpha]^{20}_D$ = −72 (*c* 3.2 mg/mL, $CHCl_3$); IR (cm^{-1}) 2955, 2921, 2837, 1780, 1752, 1590, 1518, 1420, 1263, 1243, 1226, 1193, 1169, 1125, 1104, 995, 866; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.80 (s, 1H, H-5), 6.56 (s, 1H, H-8), 6.38 (s, 2H, H-2′, 6′), 5.99 (d, *J* = 9.0 Hz, 1H, H-4), 4.66 (d, *J* = 3.5 Hz, 1H, H-1), 4.40−4.43 (m, 1H, H-11), 4.21−4.26 (m, 1H, H-11), 4.16 (d, *J* = 8.0 Hz, 2H, CH_2Cl), 3.90 (s, 3H), 3.82 (s, 6H), 3.74 (s, 6H), 2.85−2.98 (m, 2H, H-2, 3). HRMS calcd for $C_{25}H_{27}O_9Cl$ ($[M]^+$), 506.1338; found, 506.1345.

*Data for **10c**:* yield = 59%, white solid, mp = 76−78 °C; $[\alpha]^{20}_D$ = −92 (*c* 3.5 mg/mL, $CHCl_3$); IR (cm^{-1}) 2963, 2936, 2837, 1777, 1729, 1589, 1515, 1461, 1249, 1172, 1127, 1110, 1047, 997, 853, 745; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.90 (s, 1H, H-5), 6.56 (s, 1H, H-8), 6.27 (s, 2H, H-2′, 6′), 6.20 (d, *J* = 2.5 Hz, 1H, H-4), 4.70 (d, *J* = 5.0 Hz, 1H, H-1), 4.34−4.38 (m, 1H, H-11), 3.91−3.95 (m, 1H, H-11), 3.89 (s, 3H), 3.81 (s, 3H), 3.80 (s, 3H), 3.72 (s, 6H), 3.24 (dd, *J* = 14.0, 4.5 Hz, 1H, H-2), 2.97−3.01 (m, 1H, H-3), 2.33 (t, *J* = 7.5 Hz, 2H, $\underline{CH_2}CH_2CH_3$), 1.64−1.71 (m, 2H, $CH_2\underline{CH_2}CH_3$), 0.94 (t, *J* = 7.5 Hz, 3H, $CH_2CH_2\underline{CH_3}$). HRMS calcd for $C_{27}H_{32}O_9$ ($[M]^+$), 500.2041; found, 500.2052.

*Data for **10d**:* yield = 84%, white solid, mp = 66−68 °C; $[\alpha]^{20}_D$ = −80 (*c* 3.2 mg/mL, $CHCl_3$); IR (cm^{-1}) 2955, 2836, 1779, 1728, 1589, 1515, 1463, 1248, 1127, 1110, 997, 856, 747; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.90 (s, 1H, H-5), 6.56 (s, 1H, H-8), 6.27 (s, 2H, H-2′, 6′), 6.19 (d, *J* = 3.0 Hz, 1H, H-4), 4.70 (d, *J* = 4.5 Hz, 1H, H-1), 4.34−4.37 (m, 1H, H-11), 3.91−3.95 (m, 1H, H-11), 3.89 (s, 3H), 3.81 (s, 3H), 3.80 (s, 3H), 3.72 (s, 6H), 3.24 (dd, *J* = 14.5, 4.5 Hz, 1H, H-2), 2.95−

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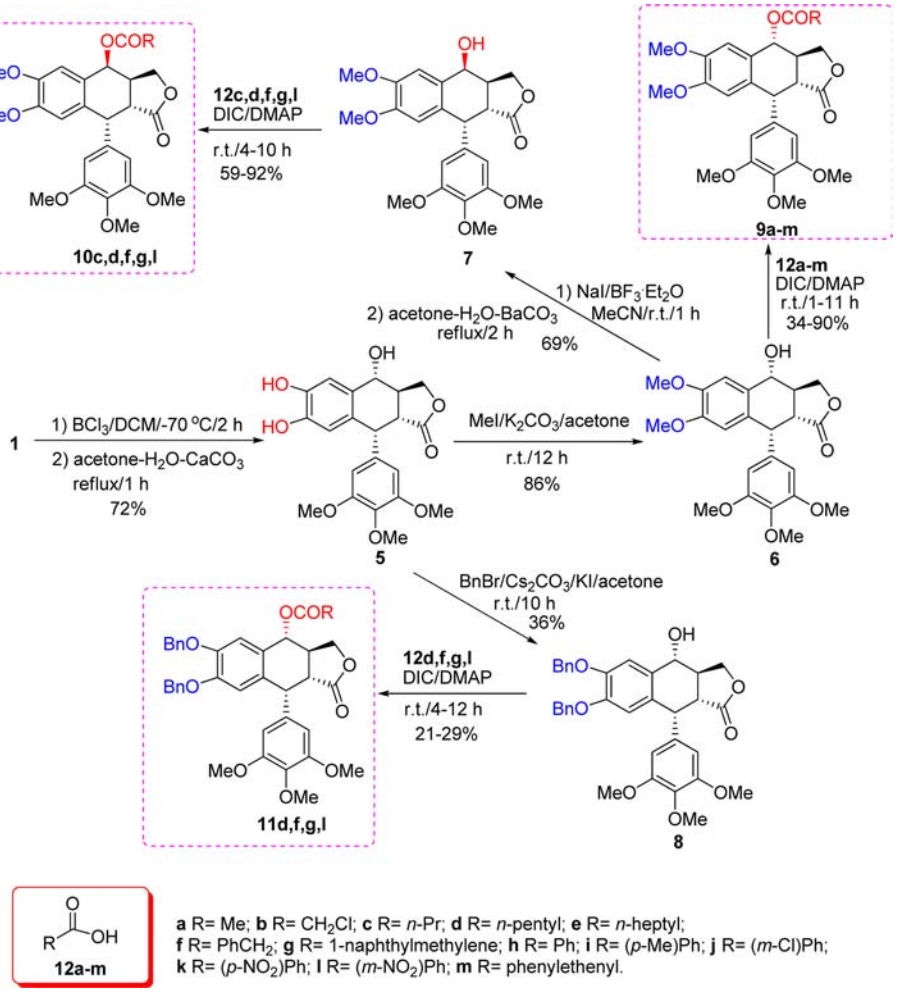
Scheme 1. Route for the Synthesis of Three Series of 4-Acyloxypodophyllotoxin Derivatives (9a−m, 10c,d,f,g, l, and 11d,f,g,l)



3.00 (m, 1H, H-3), 2.34 (t, J = 7.5 Hz, 2H, $\underline{CH_2}(CH_2)_3CH_3$), 1.61−1.67 (m, 2H, $CH_2\underline{CH_2}(CH_2)_2CH_3$), 1.25−1.31 (m, 4H, $CH_2CH_2\underline{(CH_2)_2}CH_3$), 0.87 (t, J = 7.0 Hz, 3H, $CH_2(CH_2)_3\underline{CH_3}$). HRMS, calcd for $C_{29}H_{36}O_9$ ([M]$^+$), 528.2354; found, 528.2358.

Data for ***11d****:* yield = 23%, white solid, mp = 50−52 °C; $[\alpha]^{20}_D$ = −49 (*c* 3.2 mg/mL, $CHCl_3$); IR (cm^{-1}) 2928, 2859, 1778, 1728, 1587, 1510, 1454, 1331, 1244, 1167, 1127, 1002, 997, 739; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27−7.43 (m, 10H, 2 × $OCH_2\underline{C_6H_5}$), 6.82 (s, 1H, H-5), 6.64 (s, 1H, H-8), 6.31 (s, 2H, H-2′, 6′), 5.85 (d, J = 9.0 Hz, 1H, H-4), 5.07−5.16 (m, 4H, 2 × $O\underline{CH_2}C_6H_5$), 4.58 (d, J = 4.0 Hz, 1H, H-1), 4.33−4.36 (m, 1H, H-11), 4.16−4.20 (m, 1H, H-11), 3.82 (s, 3H), 3.70 (s, 6H), 2.88 (dd, J = 14.5, 4.0 Hz, 1H, H-2), 2.71−2.80 (m, 1H, H-3), 2.33 (t, J = 7.5 Hz, 2H, $\underline{CH_2}(CH_2)_3CH_3$), 1.62−1.68 (m, 2H, $CH_2\underline{CH_2}(CH_2)_2CH_3$), 1.25−1.34 (m, 4H, $CH_2CH_2\underline{(CH_2)_2}CH_3$), 0.90 (t, J = 6.0 Hz, 3H, $CH_2(CH_2)_3\underline{CH_3}$). HRMS calcd for $C_{41}H_{44}O_9$ ([M]$^+$), 680.2980; found, 680.2989.

Data for ***11f****:* yield = 21%, white solid, mp = 69−70 °C; $[\alpha]^{20}_D$ = −55 (*c* 3.6 mg/mL, $CHCl_3$); IR (cm^{-1}) 2924, 2881, 1777, 1732, 1508, 1455, 1245, 1126, 992, 742; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27−7.40 (m, 15H, 2 × $OCH_2\underline{C_6H_5}$, $CH_2\underline{C_6H_5}$), 6.66 (s, 1H, H-5), 6.61 (s, 1H, H-8), 6.29 (s, 2H, H-2′, 6′), 5.83 (d, J = 9.0 Hz, 1H, H-4), 5.05 (s, 2H, $O\underline{CH_2}C_6H_5$), 4.96 (s, 2H, $O\underline{CH_2}C_6H_5$), 4.56 (d, J = 4.0 Hz, 1H, H-1), 4.24−4.27 (m, 1H, H-11), 4.12−4.16 (m, 1H, H-11), 3.82 (s, 3H), 3.69 (s, 6H), 3.67 (s, 2H, $\underline{CH_2}C_6H_5$), 2.86 (dd, J = 14.5, 4.5 Hz, 1H, H-2), 2.70−2.78 (m, 1H, H-3). HRMS calcd for $C_{43}H_{40}O_9$ ([M]$^+$), 700.2667; found, 700.2674.

Biological Assay. The insecsticidal activity of three series of 4-acyloxypodophyllotoxin derivatives (**9a−m**, **10c,d,f,g,l**, and **11d,f,g,l**) against the pre-third-instar larvae of *Mythimna separata* was assessed by leaf-dipping method, as described previously.[23] For each compound, 30 pre-third-instar larvae (10 larvae per group) were used. Acetone solutions of compounds **9a−m**, **10c,d,f,g,l**, **11d,f,g,l**, 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, in which 10 larvae were raised. If the treated leaves were consumed, additional treated leaves were added to the dish. After 48 h, untreated fresh leaves were added to all dishes until adult emergence. The experiment was carried out at 25 ± 2 °C and a relative humidity (RH) of 65−80% and on 12 h/12 h (light/dark) photoperiod. The insecticidal activity of the tested compounds against the pre-third-instar larvae of *Mythinma separata* was calculated by the 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.

QSAR Model Development. *Data Set.* The experimental data used in this study contained 27 compounds (**1**, **5−8**, **9a−m**, **10c,d,f,g,l**, and **11d,f,g,l**). The biological activity of 27 compounds was expressed by final mortality rate values and used as dependent variable in the following analyses.

Molecular Descriptor Calculation and Filtering. The 2D structures of 27 compounds were drawn into HyperChem[24] software and preoptimized by using MM+ molecular mechanics force field. The final lowest energy conformation of the compound was obtained by

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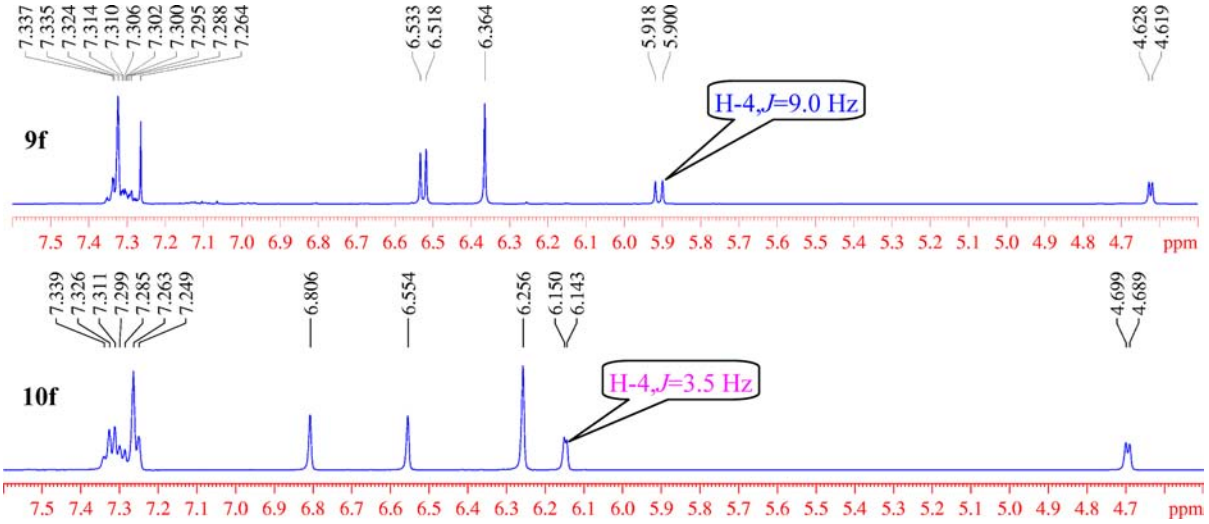

Figure 2. Partial ^{1}H NMR spectra of **9f** and **10f**.

using the semiempirical AM1 method.[25] The theoretical molecular descriptors for the optimized geometries were calculated using DRAGON5.4 software.[26] In DRAGON, a total of 1664 0D−3D molecular descriptors were calculated to describe structural features for each compound. The types of descriptors include (a) 0D molecular descriptors (constitutional descriptors), (b) 1D molecular descriptors (functional group counts and atom-centered fragments), (c) 2D molecular descriptors (topological descriptors, walk and path counts, connectivity indices, information indices, 2D autocorrelations, edge adjacency indices, Burden eigenvalues, topological charge index, and eigenvalue-based index), (d) 3D molecular descriptors (3D-Randic molecular profiles, geometrical descriptors, RDF descriptors, 3D-MoRSE descriptors, WHIM descriptors,[27] and GETAWAY descriptors[28]), and (e) other molecular descriptors (charge descriptors and molecular properties). The list and meanings of different types of descriptors can be found in the references of the DRAGON package. The detailed calculation procedure can be found in the *Handbook of Molecular Descriptors*.[29] Constant values and descriptors found to be pairwise correlated with a correlation coefficient >0.99 were excluded to reduce the redundant information. Thus, 605 molecular descriptors were left after the pretreatment step. The quadratic term of the descriptors was also added to the descriptors pool; thus, a total of 1210 descriptors would be subject to the descriptor selection procedure.

Descriptor Selection, Model Development, and Validation. The genetic algorithm[30] (GA) method was used for descriptor selection. Multiple linear regression (MLR) was used for the QSAR model development based on the descriptors selected by GA. The genetic algorithm was applied to the pool of 1210 molecular descriptors to extract the molecular descriptors relevant to the studied biological activity. In general, the genetic algorithm selects variables by a mechanism of selection, crossover, and mutation operation by mimicking the biological population evolution theory. The three operations were repeated until the stopping conditions were achieved. In this paper, GA-MLR calculation was performed by using Moby Digs software.[31] The fitness function was correlation coefficient of leave-one-out (LOO) cross-validation (Q^2_{LOO}). The parameters used in the GA process were as follows: population size, 100; maximum allowed descriptors in a model, 5; and reproduction/mutation trade-off, 0.5. The default values of the other parameters in the Moby Digs software were used. Several different parameters were applied to assess the performance of the developed QSAR model such as squared correlation coefficient R^2, leave-one-out cross-validation (Q^2_{LOO}), and root-mean-square error (RMSE).

Applicability Domain (AD) of the QSAR Model. Analysis of the AD of the developed QSAR model allows verification of the prediction reliability and identification of the possible outliers in the QSAR model. A Williams plot[32] (the plot of cross-validated standardized residuals versus hat values) was used to describe the applicability domain of the QSAR model. When the compound has a high hat value (h) ($h>h^*$, the waning hat being $h^* = 3p'/n$, where p' is the number of model parameters plus one and n is the number of the chemical used to build model), it should be kept in mind that the prediction for the compound is extrapolated and may be considered to be less reliable. A compound is considered as a Y outlier if the compound has cross-validated standardized residuals >3 standard deviation units.

RESULTS AND DISCUSSION

Synthesis. Three series of novel 4-acyloxypodophyllotoxin derivatives (**9a−m**, **10c,d,f,g,l**, and **11d,f,g,l**) modified in the A and C rings were synthesized as shown in Scheme 1. First, the intermediate **5** was prepared via selective removal of the 6,7-methylenedioxy group of **1** by treatment with boron trichloride (BCl_3), followed by weak basic hydrolysis with calcium carbonate ($CaCO_3$).[33] Methylation and benzylation of **5** were then achieved by using methyl iodide and benzyl bromide in the presence of K_2CO_3 or Cs_2CO_3 to afford **6** and **8**, respectively.[34] Compound **6** reacted with NaI/BF_3·Et_2O to give **7**.[35] A series of 6,7-*O*,*O*-demethylene-6,7-*O*,*O*-dimethyl-4α-acyloxypodophyllotoxin derivatives (**9a−m**) were obtained by reaction of **6** with the corresponding carboxylic acids (**12a−m**) in the presence of *N*,*N*′-diisopropylcarbodiimide (DIC) and 4-*N*,*N*-dimethylaminopyridine (DMAP).[19] Similarly, a series of 6,7-*O*,*O*-demethylene-6,7-*O*,*O*-dimethyl-4β-acyloxypodophyllotoxin derivatives (**10c,d,f,g,l**) were produced by reaction of **7** with **12c,d,f,g,l** in the presence of DIC and DMAP. Finally, a series of 6,7-*O*,*O*-demethylene-6,7-*O*,*O*-dibenzyl-4α-acyloxypodophyllotoxin derivatives (**11d,f,g,l**) were afforded by reaction of **8** with **12d,f,g,l** in the presence of DIC and DMAP.

The assignment of configuration at the C-4 position of the above three series of 4-acyloxypodophyllotoxin derivatives was based on $J_{3,4}$ coupling constants: the C-4β-substituted compounds have a $J_{3,4} \approx 4.0$ Hz due to a *cis* relationship between H-3 and H-4; $J_{3,4} \geq 10.0$ Hz indicates that H-3 and H-4 is a *trans* relationship, and the substituent at the C-4 position of podophyllotoxin is α configuration.[36] For example, as shown in Figure 2, the $J_{3,4}$ values of H-4 of **9f** and **10f** were 9.0 and 3.5 Hz, respectively; therefore, the phenylacetyloxy groups at the C-4 position of **9f** and **10f** were α and β configuration, respectively. The relationships of the configuration at the C-4

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position of three series of 4-acyloxypodophyllotoxins and their $J_{3,4}$ coupling constants are described in Table 1.

Table 1. Relationships of the Configuration of C-4 Position of Three Series of 4-Acyloxypodophyllotoxin Derivatives with Their $J_{3,4}$ Coupling Constants

compd	$\delta_{H\text{-}4}$	$J_{3,4}$ (Hz)	configuration
6	4.80	9.5	α
7	4.90	3.0	β
9a	5.92	9.0	α
9b	6.00	9.0	α
9c	5.95	9.0	α
9d	5.94	9.0	α
9e	5.94	9.0	α
9f	5.90	9.0	α
9g	5.89	9.0	α
9h	6.16	8.5	α
9i	6.14	8.5	α
9j	6.17	8.0	α
9k	6.20	8.5	α
9l	6.23	8.0	α
9m	6.07	9.0	α
10c	6.20	2.5	β
10d	6.20	3.0	β
10f	6.14	3.5	β
10g	6.13	3.0	β
10l	6.49	3.0	β
11d	5.86	9.0	α
11f	5.84	9.0	α
11g	5.83	9.5	α
11l	6.17	8.5	α

Insecticidal Activity. The insecticidal activity of three series of 4-acyloxypodophyllotoxin derivatives (**9a−m**, **10c,d,f,g,l**, and **11d,f,g,l**) against the pre-third-instar larvae of *Mythimna separata* was tested by the leaf-dipping method at the concentration of 1 mg/mL. The purities of all target compounds were >95% measured with RP-HPLC (see the Supporting Information).

As indicated in Table 2, the corresponding mortality rates caused by these compounds after 35 days were usually higher than those after 10 and 20 days. For example, the corrected mortality rate of **9g** against *Mythimna separata* after 10 days was only 10%, and after 20 days, the corresponding mortality rate was increased to 26.7%, but after 35 days, the corresponding mortality rate was sharply increased to 60%, which was 6-fold that after 10 days. That is, these compounds, in a time-dependent manner, different from other conventional neurotoxic insecticides such as organophosphates, carbamates, and pyrethroids, showed delayed insecticidal activity. Meanwhile, the symptoms of the tested *Mythimna separata* were also characterized in the same way as in our previous study.[23] Due to feeding too much treated leaves during the first 48 h, some larvae died slowly with slim and wrinkled bodies during the larval period. In the meantime, many larvae of the treated groups molted to malformed pupae and died during the stage of pupation. Malformed moths with imperfect wings also appeared in the treated groups. Compounds **9e**, **9g**, **9j**, and **10g** exhibited insecticidal activity equal to or higher than that of toosendanin. Especially **9g**, bearing α-naphthylacetyloxy at the C-4 position, displayed the best promising insecticidal activity with the final mortality rate of 60%. In general, cleavage of the

Table 2. Insecticidal Activity of Three Series of 4-Acyloxypodophyllotoxin Derivatives (9a−m, 10c,d,f,g,l, and 11d,f,g,l) at 1 mg/mL against *Mythimna separata*

	corrected mortality rate (%)		
compd	10 days	20 days	35 days
1	13.3 ± 3.3	23.3 ± 6.7	40.0 ± 5.8
5	0 ± 0	6.7 ± 6.7	23.3 ± 3.3
6	0 ± 0	23.3 ± 3.3	36.7 ± 3.3
7	16.7 ± 3.3	23.3 ± 3.3	40.0 ± 5.8
8	10.0 ± 5.8	33.3 ± 6.7	33.3 ± 6.7
9a	10.0 ± 5.8	23.3 ± 6.7	36.7 ± 3.3
9b	0 ± 0	13.3 ± 6.7	26.7 ± 3.3
9c	6.7 ± 3.3	20.0 ± 5.8	33.3 ± 3.3
9d	6.7 ± 3.3	23.3 ± 3.3	36.7 ± 3.3
9e	10.0 ± 0	30.0 ± 5.8	53.3 ± 3.3
9f	3.3 ± 3.3	13.3 ± 3.3	26.7 ± 3.3
9g	10.0 ± 5.8	26.7 ± 3.3	60.0 ± 0
9h	10.0 ± 0	26.7 ± 3.3	36.7 ± 3.3
9i	6.7 ± 6.7	16.7 ± 3.3	33.3 ± 3.3
9j	3.3 ± 3.3	23.3 ± 3.3	50.0 ± 5.8
9k	3.3 ± 3.3	13.3 ± 3.3	46.7 ± 3.3
9l	3.3 ± 3.3	16.7 ± 3.3	36.7 ± 3.3
9m	6.7 ± 3.3	23.3 ± 6.7	33.3 ± 3.3
10c	3.3 ± 3.3	10.0 ± 5.8	36.7 ± 3.3
10d	23.3 ± 3.3	26.7 ± 6.7	43.3 ± 3.3
10f	10.0 ± 0	16.7 ± 6.7	33.3 ± 3.3
10g	13.3 ± 6.7	20.0 ± 5.8	50.0 ± 5.8
10l	10.0 ± 5.8	26.7 ± 3.3	36.7 ± 3.3
11d	10.0 ± 0	13.3 ± 3.3	33.3 ± 3.3
11f	3.3 ± 3.3	16.7 ± 6.7	40.0 ± 5.8
11g	3.3 ± 3.3	26.7 ± 3.3	33.3 ± 3.3
11l	3.3 ± 3.3	6.7 ± 3.3	33.3 ± 3.3
toosendanin	10.0 ± 0	20.0 ± 0	50.0 ± 5.8

6,7-methylenedioxy group of **1** will lead to the less active compound **5**. For example, when the 6,7-*O*,*O*-methylene group was selectively removed from compound **1**, having a final mortality rate of 40%, to give **5**, the final mortality rate of **5** was decreased to 23.3%. However, introduction of a methyloxy or benzyloxy group at the C-6 and C-7 positions of **5** afforded **6** and **8**, respectively, and the corresponding mortality rates of **6** (36.7%) and **8** (33.3%) were increased as compared with that of **5**. The C-4 position of podophyllotoxin was the important modification location; that is, introduction of appropriate substituents at the C-4 position of **6** could give more active derivatives (e.g., **9e**, **9g**, **9j**, and **10g**) than **6**. However, the effect of the configuration of acyloxy at the C-4 position on the insecticidal activity was not very obvious. For example, for the 4α-acyloxypodophyllotoxin series, the final mortality rates of **9c**, **9d**, **9f**, **9g**, and **9l** were 33.3, 36.7, 26.7, 60.0, and 36.7%, respectively; for the corresponding 4β-acyloxypodophyllotoxin series, the final mortality rates of **10c**, **10d**, **10f**, **10g**, and **10l** were 36.3, 43.3, 33.3, 50.0, and 36.7%, respectively. Interestingly, when the 6,7-dimethoxy group of **9g** was substituted by 6,7-dibenzyloxy to afford **11g**, the corresponding mortality rate of **11g** was sharply decreased to 33.3%. For the alkylacyloxy series of **9a−e**, introduction of *n*-heptylacyloxy at the C-4 position of **6** resulted in the more potent compound **9e**. For example, the final mortality rates of **9a** (R = Me), **9b** (R = CH_2Cl), **9c** (R = *n*-Pr), and **9d** (R = *n*-pentyl) were 36.7, 26.7, 33.3, and 36.7%, respectively, whereas the final mortality rate of **9e** (R = *n*-heptyl) was 53.3%. Introduction of α-

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naphthylacetyloxy at the C-4 position of **6** could lead to the more potent compound **9g** in the same way as described in our previous papers.[19,21]

QSAR Model. To select the most relevant descriptors responsible for the biological activity, a total of 1210 molecular descriptors were subjected to the GA selection procedure. When the addition of another descriptor did not improve the statistics parameter (Q^2_{LOO}) of a model significantly, the best descriptor combination has been achieved. On the basis of this principle, the five-descriptor model was selected. The regression equation and the statistical items were

$$Y = 0.776\mathrm{BEHm2} + 0.338\mathrm{RDF020e} - (0.720\mathrm{Wap})^2 + 0.741(\mathrm{Mor14v})^2 + 0.471(\mathrm{G1v})^2 - 4.806 \quad (1)$$

where Y is the final mortality rate.

$$n_{\text{data set}} = 27,\ R^2 = 0.914,$$
$$\mathrm{RMSE} = 0.024,\ Q^2_{LOO} = 0.881,\ \mathrm{RMSE}_{LOO} = 0.028$$

From the above statistical parameters, it can be seen that the model was stable and robust. The predicted biological activity values by this model are listed in Table 3. Figure 3 shows the graph of the experimental versus predicted values of 27 compounds by the GA-MLR model.

A deep analysis of the descriptors in the derived QSAR model allows us to find some factors that are likely to influence the biological activity of these compounds. From eq 1, we can see that five descriptors were involved. The relative importance was determined by their standardized coefficient values. The most important descriptor is BEHm2 (highest eigenvalue n.2 of

Table 3. Experimental and Predicted Activity by the Developed QSAR Model

no.	compd	exptl activity	predicted activity
1	**1**	0.400	0.371
2	**5**	0.233	0.241
3	**6**	0.367	0.378
4	**7**	0.400	0.361
5	**8**	0.333	0.369
6	**9a**	0.367	0.324
7	**9b**	0.267	0.283
8	**9c**	0.333	0.372
9	**9d**	0.367	0.382
10	**9e**	0.533	0.524
11	**9f**	0.267	0.306
12	**9g**	0.600	0.599
13	**9h**	0.367	0.351
14	**9i**	0.333	0.332
15	**9j**	0.500	0.485
16	**9k**	0.467	0.469
17	**9l**	0.367	0.377
18	**9m**	0.333	0.306
19	**10c**	0.367	0.378
20	**10d**	0.433	0.421
21	**10f**	0.333	0.374
22	**10g**	0.500	0.499
23	**10l**	0.367	0.376
24	**11d**	0.333	0.283
25	**11f**	0.400	0.396
26	**11g**	0.333	0.334
27	**11l**	0.333	0.344

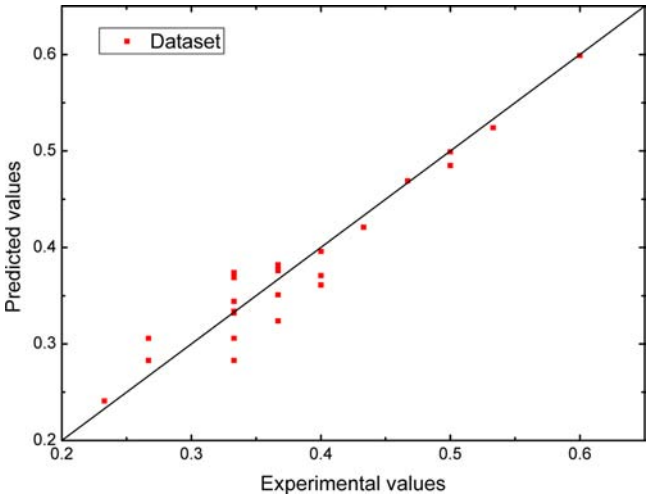

Figure 3. Plot of experimental and predicted biological activity values of 27 compounds by GA-MLR model.

Burden matrix/weighted by atomic masses) encoding the Burden eigenvalues descriptor, which is calculated on the basis of the hydrogen-included molecular graph weighted by atomic masses. Another important descriptor is Mor14v, which belongs to 3D-MoRSE descriptors (3D-Molecule Representation of Structure based on Electron diffraction) obtained on the basis of the idea of combination of the 3D atomic coordinates of the molecular and chemical atomic information. This descriptor represents the 3D-MoRSE signal 14/weighted by atomic van der Waals volumes. Wap is a topological descriptor based on the all-path Wiener index, and the Wiener index is calculated as the half-sum of all topological distances collected in the distance matrix. The topological descriptors are based on the graph representation of the molecule, which are sensitive to one or more structural features of the molecule such as size, shape, symmetry, branching, and cyclicity, and can also encode chemical information concerning atom type and bond multiplicity. G1v is a WHIM descriptor, which represents first-component symmetry directional WHIM index/weighted by atomic van der Waals volumes. WHIM descriptors are built in such a way as to capture relevant molecular 3D information regarding molecular size, shape, symmetry, and atom distribution with respect to invariant reference frames. RDF020e is an RDF descriptor representing Radial Distribution Function −2.0/weighted by atomic Sanderson electronegativities. The applicability domain of the derived model described by a Williams plot (the cross-validated standardized residual versus hat values) is shown in Figure 4. It obviously suggested that there is no Y outlier, and only compound **11g** (no. 26) is an outlier from molecular structure (with the hat value higher than the warning h^* value of 0.667).

In summary, three series of novel 4-acyloxypodophyllotoxin derivatives modified in the A and C rings were synthesized and evaluated for their insecticidal activity against the pre-third-instar larvae of *Mythimna separata* in vivo at the concentration of 1 mg/mL. Especially **9g** exhibited more promising and pronounced insecticidal activity than toosendanin. This suggested that cleavage of the 6,7-methylenedioxy group of podophyllotoxin will lead to less active compounds and the C-4 position of podophyllotoxin was the important modification location. The QSAR model demonstrated that five descriptors, BEHm2, Mor14v, Wap, G1v, and RDF020e, are likely to

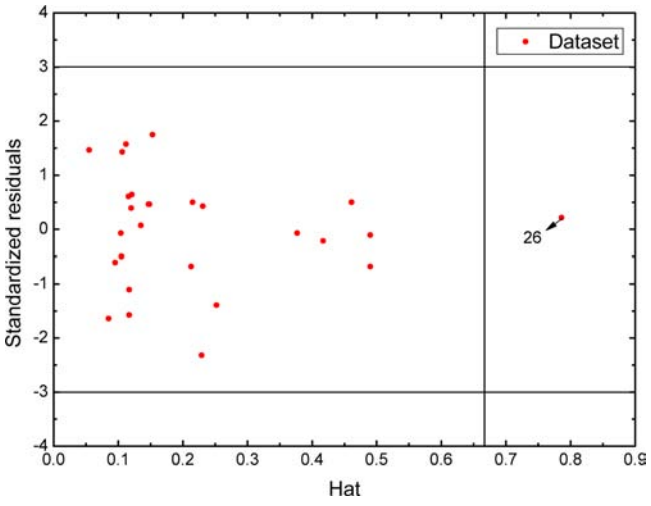

Figure 4. Williams plot for the GA-MLR model with five descriptors.

influence the biological activity of these compounds. Among them, two important ones are BEHm2 and Mor14v. This work will pave the way for further design, structural modification, and development of podophyllotoxin derivatives as insecticidal agents.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H NMR, HRMS, optical rotation, melting point, and IR data and HPLC spectra 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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