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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

Running Title: **4-Acyloxypodophyllotoxin Derivatives Modified in the A and C rings as Insecticidal Agents**

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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 6,7-methylenedioxy group of podophyllotoxin will lead to the less active compound and the C-4 position of podophyllotoxin was the important modification location. Quantitative structure-activity relationship (QSAR) model was developed by genetic algorithm combined with multiple linear regression (GA-MLR). For this model, the square correlation coefficient (R^2) is 0.914, the leave-one-out cross-validation correlation coefficient (Q^2_{Loo}) is 0.881 and root mean square error (RMSE) is 0.024. Five descriptors such as BEHm2, Mor14v, Wap, G1v and RDF020e, are likely to influence the biological activity of these compounds. Among them, two important ones are the BEHm2 and Mor14v. It will pave the way for further design, structural modification and development of podophyllotoxin derivatives as insecticidal agents.

KEYWORDS: Podophyllotoxin; Acyloxy; A and C rings 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 could 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 pests populations.[3-5] Development of new effective, selective and safe pesticides, therefore, is still a challenging task. As plant secondary metabolites result from the interaction between plants and 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,[6] consequently, the discovery and development of new insecticidal compounds directly from plant secondary metabolites, or by using them as the lead-compounds for further modifications has recently been one of the important ways for 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 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 products-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 A and C rings and the configuration of acyloxy at the C-4 position on the insecticidal activity. Meanwhile, quantitative structure-activity relationship (QSAR) studies were 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 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 TMS (tetramethylsilane) as the internal standard. High-resolution mass spectra (HR-MS) were carried out with IonSpec 4.7 Tesla FTMS instrument. The purities of the tested compounds were determined by reverse phase high performance liquid chromatography (RP-HPLC), which were 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, 1mL) precooled at -70 ^{o}C was added dropwise podophyllotoxin (**1**) (0.1 g, 0.25 mmol) in dichloromethane (5 mL) over 15 min. After the mixture was stirred at -70 ^{o}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 aq. HCl (2 M) until 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 a 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 ^{o}C, methyl iodide (0.071 g, 0.5 mmol) was added. 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 a 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 ^{o}C, a solution of $BF_3{\cdot}Et_2O$ (0.378 mL, 1.4 mmol) in CH_3CN (3 mL) was added dropwise. After adding, 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,

115 acetone-water (5 mL, 10 mL) and $BaCO_3$ (395 mg, 2 mmol) was added. Then the mixture
116 was reacted at room temperature. When the reaction was complete according to TLC analysis,
117 the mixture was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were
118 washed with water and aq. $Na_2S_2O_3$ (10%, 25 mL), dried over anhydrous Na_2SO_4,
119 concentrated *in vacuo*, and purified by silica gel column chromatography to give **7** in a 69%
120 yield as a white solid.
121 *Synthesis of 6,7-O,O-Demethylene-6,7-O,O-dibenzylpodophyllotoxin (**8**):* To a mixture of **5**
122 (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
123 mL), benzyl bromide (0.103 g, 0.6 mmol) was added. Then the mixture was stirred at room
124 temperature. When the reaction was complete according to TLC analysis, the mixture was
125 filtered. The filtrate was evaporated in vacuo to give the residue, which was diluted with
126 CH_2Cl_2 (30 mL), washed by water, dried over anhydrous sodium sulfate, and purified by
127 PTLC eluting with petroleum ether/ethyl acetate (1:2, v/v) to give **8** in a 36% yield as a white
128 solid.
129 *General Procedure for the Synthesis of*
130 *6,7-O,O-Demethylene-6,7-O,O-dimethyl-4α-acyloxypodophyllotoxin Derivatives (**9a-m**),*
131 *6,7-O,O-Demethylene-6,7-O,O-dimethyl-4β-acyloxypodophyllotoxin Derivatives* (***10c**, **d**, **f**, **g***
132 *and **l***), *and 6,7-O,O-Demethylene-6,7-O,O-dibenzyl-4α-acyloxypodophyllotoxin Derivatives*
133 *(**11d, f, g** and **l**):* A mixture of the corresponding acids (0.32 mmol), diisopropylcarbodiimide
134 (DIC, 0.32 mmol), 4-dimethylaminopyridine (DMAP, 0.045 mmol), and **6**, **7** or **8** (0.23 mmol)
135 in dry CH_2Cl_2 (10 mL) was stirred at room temperature. When the reaction was complete
136 according to TLC analysis, the mixture was diluted by CH_2Cl_2 (30 mL), washed by aq. HCl
137 (0.1 M, 20 mL), aq. $NaHCO_3$ (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** and **l**, and **11d**, **f**, **g** and **l**. Their structures were well characterized by ^{1}H NMR, HRMS, optical rotation, IR, and mp. The 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 ^{o}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 ^{o}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 ^{o}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. ^1H 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), 2.95-3.00 (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. ^1H 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. ^1H 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,

184 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),
185 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),
186 2.70-2.78 (m, 1H, H-3). HRMS: calcd for $C_{43}H_{40}O_9$ ($[M]^+$), 700.2667; found, 700.2674.
187 *Biological Assay.* The insecticidal activity of three series of 4-acyloxypodophyllotoxin
188 derivatives (**9a**-**m**, **10c**, **d**, **f**, **g** and **l**, and **11d**, **f**, **g** and **l**) against the pre-third-instar larvae of
189 *M. separata* was assessed by leaf-dipping method, as described previously.[23] For each
190 compound, 30 pre-third-instar larvae (10 larvae per group) were used. Acetone solutions of
191 compounds **9a**-**m**, **10c**, **d**, **f**, **g** and **l**, **11d**, **f**, **g** and **l**, and toosendanin (used as a positive
192 control) were prepared at the concentration of 1 mg/mL. Fresh wheat leaves were dipped into
193 the corresponding solution for 3 s, then taken out, and dried in a room. Leaves treated with
194 acetone alone were used as a blank control group. Several treated leaves were kept in each
195 dish, where every 10 larvae were raised. If the treated leaves were consumed, additional
196 treated leaves were added to the dish. After 48 h, untreated fresh leaves were added to all
197 dishes until adult emergence. The experiment was carried out at 25 ± 2 ^oC and relative
198 humidity (RH) 65-80%, and on 12 h/12 h (light/dark) photoperiod. The insecticidal activity
199 of the tested compounds against the pre-third-instar larvae of *M. separata* was calculated by
200 the following formula:

201 $$\text{corrected mortality rate (\%)} = (T - C) \times 100/(1 - C)$$

202 Where T is the mortality rate in the treated group expressed as a percentage and C is the
203 mortality rate in the untreated group expressed as a percentage.

204 **QSAR Model Development.**

205 *Data Set.* The experimental data used in this study contained 27 compounds (**1**, **5-8**, **9a**-**m**,
206 **10c**, **d**, **f**, **g** and **l**, and **11d**, **f**, **g** and **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 pre-optimized by using MM+ molecular mechanics force field. The final lowest energy conformation of the compound was obtained by using the semi-empirical 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 groups 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 could be found in the references of the DRAGON package. The detailed calculation procedure could be found in the *Handbook of Molecular Descriptors.*[29] Constant values and descriptors found to be pairwise correlated with a correlation coefficient greater than 0.99 were excluded to reduce the redundant information. Thus, 605 molecular descriptors were left after pretreatment step. The quadratic term of the descriptors was also added to the descriptors pool; thus, a total of 1210 descriptors would subject to the descriptors selection procedure.

Descriptors Selection, Model Development and Validation. The genetic algorithm[30] (GA)

method was used for descriptors 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 variable 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 are achieved. In this paper, GA-MLR calculation was performed by using the 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 square correlation coefficient R^2, leave-one-out cross-validation (Q^2_{LOO}) and root mean square error (RMSE).

Applicability Domain of the QSAR Model. The analysis of the applicability domain (AD) of the developed QSAR model allows to verify the prediction reliability and to identify the possible outliers in the QSAR model. 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 with 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 number of the chemical used to build model), it should keep in mind that the prediction for the compound is extrapolated and may be considered less reliable. A compound is considered as a Y outlier if the compound with cross validated standardized residuals greater than three standard deviation units.

253 **RESULTS AND DISCUSSION**

254 **Synthesis.** Three series of novel 4-acyloxypodophyllotoxin derivatives (**9a-m**, **10c**, **d**,
255 **f**, **g** and **l**, and **11d**, **f**, **g** and **l**) modified in the A and C rings were synthesized as shown in
256 Scheme 1. First, the intermediate **5** was prepared via selective removing the
257 6,7-methylenedioxy group of **1** by treating with boron trichloride (BCl_3), followed by weak
258 basic hydrolysis with calcium carbonate ($CaCO_3$).[33] Methylation and benzylation of **5** were
259 then achieved by using methyl iodide and benzyl bromide in the presence of K_2CO_3 or
260 Cs_2CO_3 to afford **6** and **8**, respectively.[34] Compound **6** reacted with $NaI/BF_3{\cdot}Et_2O$ to give **7**.[35]
261 A series of 6,7-*O,O*-demethylene-6,7-*O,O*-dimethyl-4α-acyloxypodophyllotoxin derivatives
262 (**9a-m**) were obtained by reaction of **6** with the corresponding carboxylic acids (**12a-m**) in the
263 presence of *N*,*N*′-diisopropylcarbodiimide (DIC) and 4-*N*,*N*-dimethylaminopyridine
264 (DMAP).[19] Similarly, a series of
265 6,7-*O,O*-demethylene-6,7-*O,O*-dimethyl-4β-acyloxypodophyllotoxin derivatives (**10c**, **d**, **f**, **g**
266 and **l**) were produced by reaction of **7** with **12c**, **d**, **f**, **g** and **l** in the presence of DIC and
267 DMAP. Finally, a series of 6,7-*O,O*-demethylene-6,7-*O,O*
268 -dibenzyl-4α-acyloxypodophyllotoxin derivatives (**11d**, **f**, **g** and **l**) were afforded by reaction
269 of **8** with **12d**, **f**, **g** and **l** in the presence of DIC and DMAP.

270 The assignment of configuration at the C-4 position of the above three series of
271 4-acyloxypodophyllotoxin derivatives was based on $J_{3,4}$ coupling constants: the
272 C-4β-substituted compounds have a $J_{3,4} \approx 4.0$ Hz due to a *cis* relationship between H-3 and
273 H-4; if $J_{3,4} \geqq 10.0$ Hz, it indicates that H-3 and H-4 is *trans* relationship, and the substituent
274 at the C-4 position of podophyllotoxin is α configuration.[36] For example, as shown in Figure
275 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 position of three series of 4-acyloxypodophyllotoxins and their $J_{3,4}$ coupling constants were described in Table 1.

Insecticidal Activity. The insecticidal activity of three series of 4-acyloxypodophyllotoxin derivatives (**9a-m**, **10c**, **d**, **f**, **g** and **l**, and **11d**, **f**, **g** and **l**) against the pre-third-instar larvae of *M. separata* was tested by the leaf-dipping method at the concentration of 1 mg/mL. The purities of all target compounds were all greater than 95% measured with reverse phase high performance liquid chromatography (RP-HPLC) (see 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 *M. separata* after 10 days was only 10%, 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 of 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 *M. separata* were also characterized in the same way as our previous reports.[23] Due to feeding too much treated leaves during the first 48 h, some larvae died slowly with the slim and wrinkled bodies during the larval period. In the meantime, many larvae of the treated groups moulted to malformed pupae, and died during the stage of pupation. Malformed moths with imperfect wings were also appeared in the treated groups. Compounds **9e**, **9g**, **9j**, and **10g** exhibited equal or higher insecticidal activity than toosendanin. Especially **9g**, bearing α-naphthylacetyloxy at the C-4

299 position, displayed the best promising insecticidal activity with the final mortality rate of
300 60%. In general, cleavage of 6,7-methylenedioxy group of **1** will lead to the less active
301 compound **5**. For example, when compound **1**, whose final mortality rate was 40%, was
302 selectively removed the 6,7-*O,O*-methylene group to give **5**, the final mortality rate of **5** was
303 decreased to 23.3%. However, introduction of methyloxy or benzyloxy group at the C-6 and
304 C-7 positions of **5** afforded **6** and **8**, respectively, the corresponding mortality rates of **6**
305 (36.7%) and **8** (33.3%) were increased as compared with that of **5**. The C-4 position of
306 podophyllotoxin was the important modification location; that is, introduction of appropriate
307 substituents at the C-4 position of **6** could give the more active derivatives (*e.g.*, **9e**, **9g**, **9j**,
308 and **10g**) than **6**. However, the effect of the configuration of acyloxy at the C-4 position on
309 the insecticidal activity was not very obvious. For example, to 4α-acyloxypodophyllotoxin
310 series, the final mortality rates of **9c**, **9d**, **9f**, **9g** and **9l** were 33.3%, 36.7%, 26.7%, 60.0% and
311 36.7%, respectively; to the corresponding 4β-acyloxypodophyllotoxin series, the final
312 mortality rates of **10c**, **10d**, **10f**, **10g** and **10l** were 36.3%, 43.3%, 33.3%, 50.0% and 36.7%,
313 respectively. Interestingly, when the 6,7-dimethoxy group of **8g** was substituted by the
314 6,7-dibenzyloxy one to afford **12g**, the corresponding mortality rates of **12g** was sharply
315 decreased to 33.3%. To alkylacyloxy series of **9a-e**, introduction of *n*-heptylacyloxy at the
316 C-4 position of **6** resulted in the more potent compound **9e**. For example, the final mortality
317 rates of **9a** (R = Me), **9b** (R = CH_2Cl), **9c** (R = *n*-Pr) and **9d** (R = *n*-pentyl) were 36.7%,
318 26.7%, 33.3% and 36.7%, respectively; whereas the final mortality rate of **9e** (R = *n*-heptyl)
319 was 53.3%. Introduction of α-naphthylacetyloxy at the C-4 position of **6** could lead to the
320 more potent compound **9g** by the same way as described in our previous paper.[19,21]

321 **QSAR Model.** In order 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 adding another descriptor did not improve the statistics parameter (Q^2_{LOO}) of a model significantly, it is means that the best descriptors combination has been achieved. On the basis of this principle, the 5-descriptor model was selected, whose regression equation and the statistical items were as follows:

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

Where Y is the final mortality rate.

$n_{dataset} = 27$, $R^2 = 0.914$, RMSE = 0.024, $Q^2_{LOO} = 0.881$, $RMSE_{LOO} = 0.028$

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

By a deep analysis of the descriptors in derived QSAR model, it allows us to find some factors that are likely to influence the biological activity of these compounds. From the 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 the BEHm2 (highest eigenvalue n.2 of Burden matrix / weighted by atomic masses) encoding the Burden eigenvalues descriptor, which is calculated based on 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) were obtained based on 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 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 1st 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 a RDF descriptor representing Radial Distribution Function – 2.0/weighted by atomic Sanderson electronegativities. The applicability domain of the derived model described by Williams plot (the cross-validated standardized residual versus hat values) was shown in Figure 4. It obviously suggested that there is no Y outlier, and only compound **11g** (No.26) is 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 *M. separata* in vivo at the concentration of 1 mg/mL. Especially **9g** exhibited the more promising and pronounced insecticidal activity than toosendanin. It suggested that cleavage of 6,7-methylenedioxy group of podophyllotoxin will lead to the less active compound and the C-4 position of podophyllotoxin was the important modification location. QSAR model demonstrated that five descriptors, such as BEHm2, Mor14v, Wap, G1v and RDF020e, are likely to influence the biological activity of these compounds. Among them, two important ones are the BEHm2 and Mor14v. It 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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REFERENCES

(1) Scoble, M.J. The Lepidoptera: Form, Function and Diversity (2nd ed.); Oxford University Press: Oxford, 1995, pp 4-5.

(2) Sharma, H. C.; Sullivan, D. J.; Bhatnagar, V. S. Population dynamics and natural mortality factors of the Oriental armyworm, *Mythimna separata* (Lepidoptera: Noctuidae), in South-Central India. *Crop Prot*. **2002**, *21*, 721-732.

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

(4) Lai, T.; Su, J. Assessment of resistance risk in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) to chlorantraniliprole. *Pest Manag. Sci.* **2011**, *67*, 1468-1472.

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

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

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

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

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

(10) Xu, H.; Lv, M.; Tian, X. A review on hemisynthesis, biosynthesis, biological activities, mode of action, and structure-activity relationship of podophyllotoxins: 2003-2007. *Curr. Med. Chem.* **2009**, *16*, 327-349.

(11) You, Y. J. Podophyllotoxin derivatives: Current synthetic approaches for new anticancer. *Curr. Pharm. Des*. **2005**, *11*, 1695-1717.

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

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

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

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

(16) Gao, R.; Gao, C. F.; Tian, X.; Yu, X. Y.; Di, X. D.; Xiao, H.; Zhang, X. Insecticidal activity of deoxypodophyllotoxin, isolated from *Juniperus sabina* L, and related lignans

against larvae of *Pieris rapae* L. *Pest Manag. Sci.* **2004**, *60*, 1131-1136.

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

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

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

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

(21) Xu, H.; Xiao, X.; Zhao, X. F.; Guo, Y.; Yao, X. J. Unexpectedly convenient and stereoselective synthesis of 4 alpha-acyloxy-2-chloropodophyllotoxins in the presence of $BF_3{\cdot}Et_2O$. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4008-4012.

(22) Xu, H.; Xiao, X.; Wang, Q.T. Natural products-based insecticidal agents 7. Semisynthesis and insecticidal activity of novel 4α-alkyloxy-2-chloropodophyllotoxin derivatives against *Mythimna separata* Walker *in vivo*. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5009-5012.

(23) Xu, H.; Wang, J. J.; Sun, H. J.; Lv, M.; Tian, X.; Yao, X. J.; Zhang, X. Semisynthesis and quantitative structure-activity relationship (QSAR) study of novel aromatic esters

of 4′-demethyl-4-deoxypodophyllotoxin as insecticidal agents. *J. Agric. Food Chem*. **2009**, *57*, 7919-7923.

(24) Hypercube, Inc. *HyperChem 7.0*; Hypercube, Inc.: Gainesville, FL, 2002.

(25) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. Development and use of quantum-mechanical molecular models. 76. AM1 – A new general purpose quantum mechanical molecular model. *J. Am. Chem. Soc.* **1985,** *107*, 3902-3909.

(26) Talete srl, DRAGON for windows (software for molecular descriptor calculations). Version 5.4, 2006. http://www.talete.mi.it.

(27) Todeschini, R.; Gramatica, P. New 3D molecular descriptors: The WHIM theory and QSAR applications. *Persp. Drug Discov. Des.* **1998,** *9-11*, 355-380.

(28) Consonni, V.; Todeschini, R.; Pavan, M. Structure/response correlations and similarity/diversity analysis by GETAWAY descriptors. 1. Theory of the novel 3D molecular descriptors. *J. Chem. Inform. Computer Sci.* **2002,** *42*, 682-692.

(29) Todeschini, R.; Consonni, V. *Handbook of Molecular Descriptors: Methods and Principles in Medicinal Chemistry.* Wiley-VCH, Weinheim, Germany, 2000.

(30) Leardi, R.; Boggia, R.; Terrile, M. Genetic algorithms as a strategy for feature-selection. *J. Chemometrics* **1992,** *6* (5), 267-281.

(31) Todeschini, R.; Consonni, V.; Pavan, M. *MOBY DIGS***,** *Version 1.2 for Windows, Software for Multilinear Regression Analysis and Variable Subset Selection by Genetic Algorithm;* Talete Srl: Milan, Italy, 2002.

(32) Tropsha, A.; Gramatica, P.; Gombar, V. K. The importance of being earnest: Validation is the absolute essential for successful application and interpretation of QSPR models. *QSAR Comb. Sci.* **2003,** *22*, 69-77.

(33) Wang, Z. Q.; Hu, H.; Chen, H. X.; Cheng, Y. C.; Lee, K. H. Antitumor agents. 124. New 4β-substituted aniline derivatives of 6,7-*O,O*-demethylene-4'-*O*-demethylpodophyllotoxin and related-compounds as potent inhibitors of human DNA topoisomerase-II. *J. Med. Chem.* **1992**, *35*, 871-877.

(34) Roy, S.; Eastman, A.; Gribble, G. W. Synthesis of N-alkyl substituted bioactive indolocarbazoles related to Go6976. *Tetrahedron* **2006**, *62*, 7838-7845.

(35) Kamal, A.; Kumar, B. A.; Arifuddin, M. A one-pot, efficient and facile synthesis of 4β-arylaminopodophyllotoxins: synthesis of NPF and GL-331 as DNA topoisomerase II inhibitors. *Tetrahedron Lett*. **2003**, *44*, 8457-8459.

(36) Lee, K. H.; Imakura, Y.; Haruna, M.; Beers, S. A.; Thursto, L. S.; Dai, H. J.; Chen, C. H.; Liu, S. Y.; Cheng, Y. C. Antitumor agents, 107. New cytotoxic 4-alkylamino analogues of 4'-demethyl-epipodophyllotoxin as inhibitors of human DNA topoisomerase II. *J. Nat. Prod*. **1989**, *52*, 606-613.

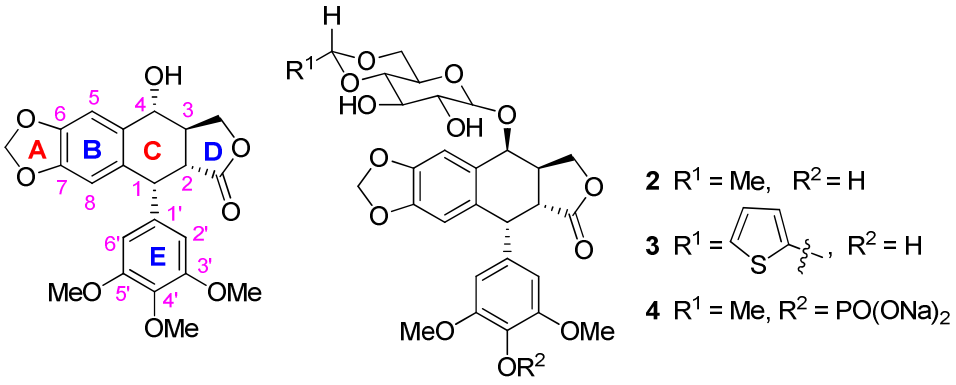


Figure 1. Chemical structures of podophyllotoxin (**1**), etoposide (**2**), teniposide (**3**) and etopophos (**4**).

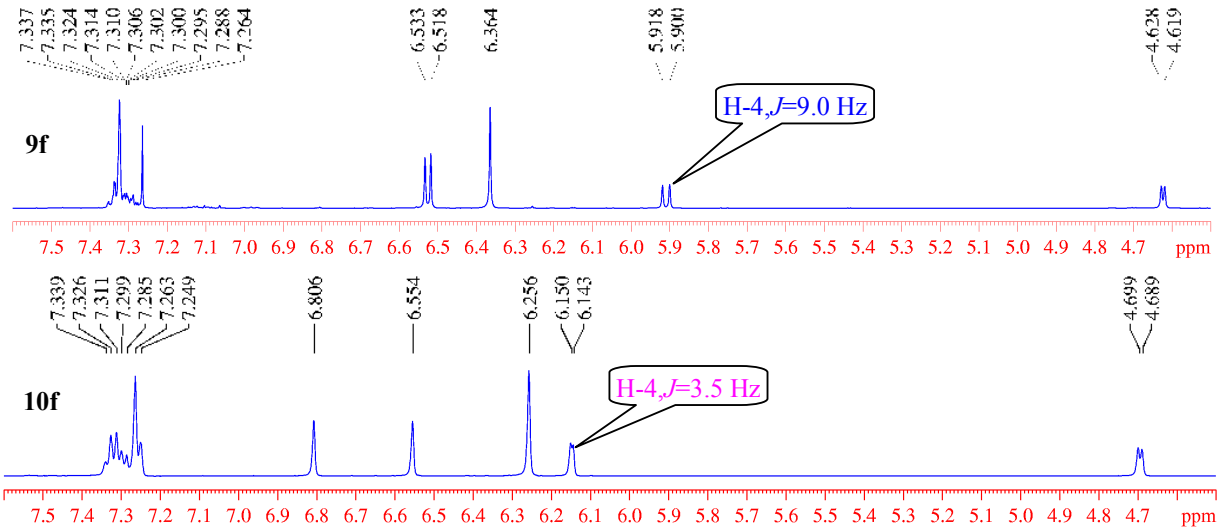


Figure 2. The partial ^{1}HNMR spectra of **9f** and **10f**.

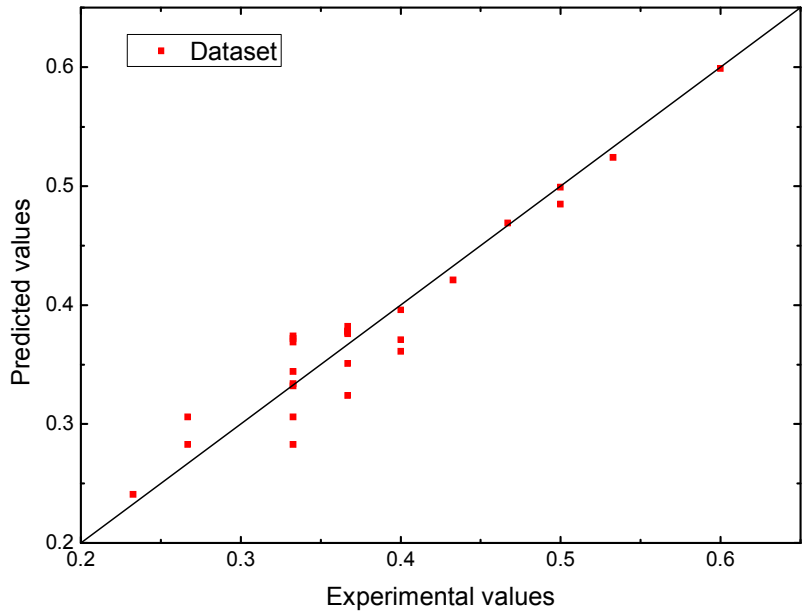


Figure 3. Plot of experimental and predicted biological activity values of 27 compounds by GA-MLR model.

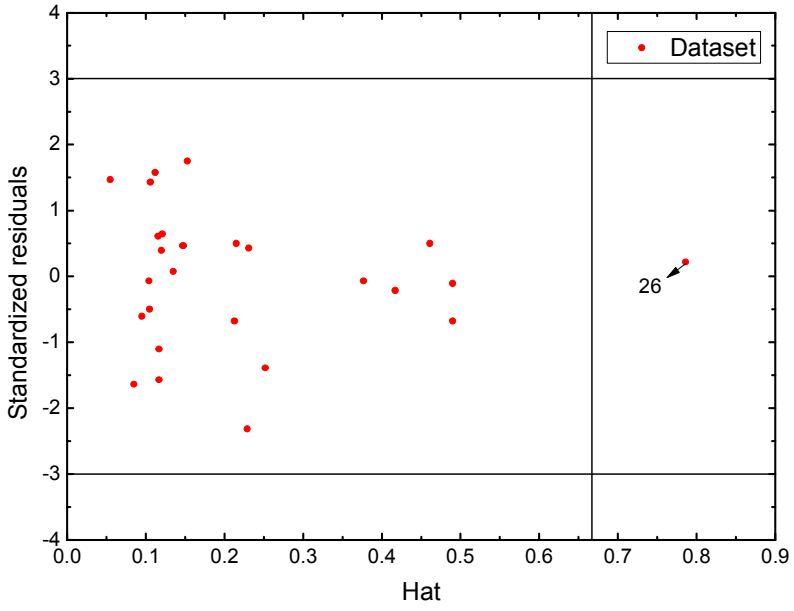
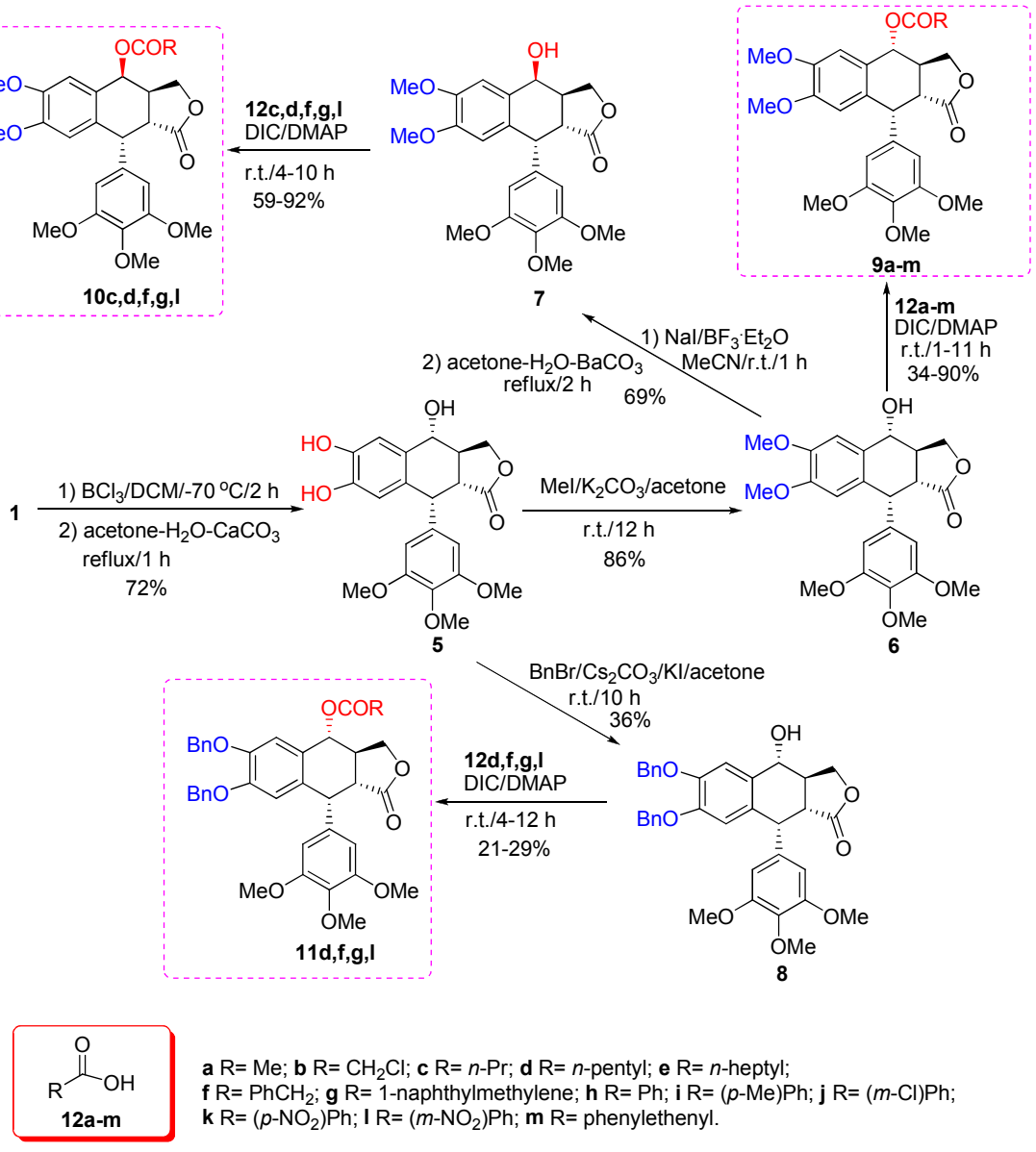


Figure 4. Williams plot for the GA-MLR model with five descriptors.



Scheme 1. The route for the synthesis of three series of 4-acyloxypodophyllotoxin derivatives (**9a-m**, **10c**, **d**, **f**, **g** and **l**, and **11d**, **f**, **g** and **l**).

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

compound	$\delta_{H\text{-}4}$ (ppm)	$J_{3,4}$ (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

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

compound	corrected mortality rate (%)		
	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

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

number	compound	experimental 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

TOC

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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