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**NATURAL PRODUCTS-BASED INSECTICIDAL AGENTS 1. SEMI-SYNTHESIS AND INSECTICIDAL ACTIVITY OF 4 $\beta$ -BENZENE-SULFONAMIDE DERIVATIVES OF PODOPHYLLOTOXIN AGAINST *MYTHIMNA SEPARATA* WALKER**

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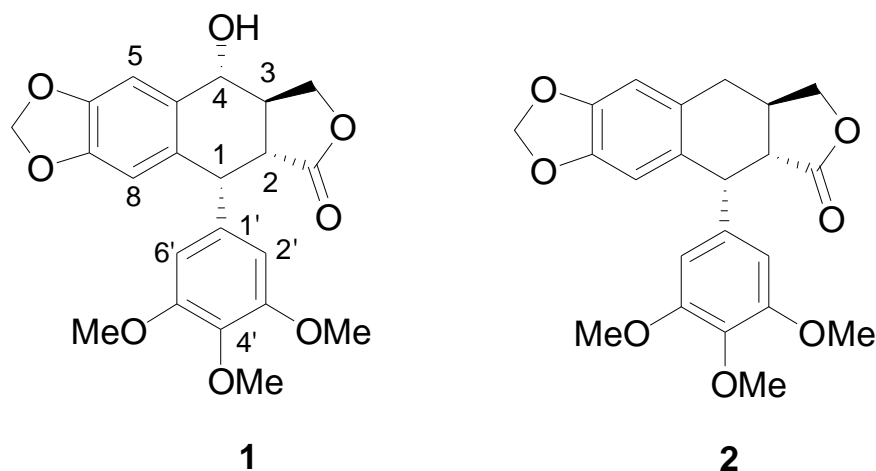
**Dedicated to Professor Emeritus Keiichiro Fukumoto on the occasion of his 75<sup>th</sup> birthday**

**Abstract**– Eleven 4 $\beta$ -benzenesulfonamide derivatives of podophyllotoxin were synthesized and evaluated for insecticidal activity against the third-instar larvae of *Mythimna separata* Walker *in vivo* at the concentration of 1 mg/mL. All derivatives showed delayed insecticidal activity, which was different from the traditional neurotoxic insecticides. Compounds **4a**–**4f** and **4i**–**4k** were found to be more potent than podophyllotoxin in the mortality rate after 20 d against *M. separata*. Especially compounds **4i**, the corresponding final mortality rate of which was 88.9%, exhibited more potent insecticidal activity than toosendanin (81.4%), a commercial insecticide derived from *Melia azedarach*. Furthermore, some preliminary qualitative structure–activity relationships were also observed.

## INTRODUCTION

Podophyllotoxin (**1**), one of the naturally occurring aryl tetrahydronaphthalene lignan lactones, is extracted as the main component from the roots and rhizomes of *Podophyllum* species such as *P. hexandrum* and *P. peltatum*, and has been used as a lead compound for drug design in the search for improved antitumor activity.<sup>1</sup> Consequently, many semisynthetic derivatives of podophyllotoxin have

been developed and tested for anticancer activity over about two decades, resulting in the commercial production of three antitumor drugs, such as etoposide, teniposide, and etoposide phosphate. On the other hand, although podophyllotoxin and deoxypodophyllotoxin (**2**), the another naturally occurring aryl tetrahydronaphthalene lignan lactone, were found to possess the insecticidal activity,<sup>2-6</sup> recently, the structural modifications of **1** and **2** have not yet been reported too much as insecticidal agents.<sup>7-9</sup> In our previous paper, twelve 4 $\beta$ -halogenated benzoylamide derivatives of podophyllotoxin were semisynthesized and tested against the 5th-instar larvae of *Pieris rapae* Linnaeus *in vivo*, and some compounds exhibited more potent insecticidal activity than podophyllotoxin.<sup>9</sup> In continuation of our ongoing project on the design and development of more potent compounds of podophyllotoxin, and as part of our program aimed at the discovery of bioactive molecules,<sup>9-12</sup> herein we want to report the semisynthesis and insecticidal activity of some 4 $\beta$ -benzenesulfonamide derivatives of podophyllotoxin (**4a—4k**).

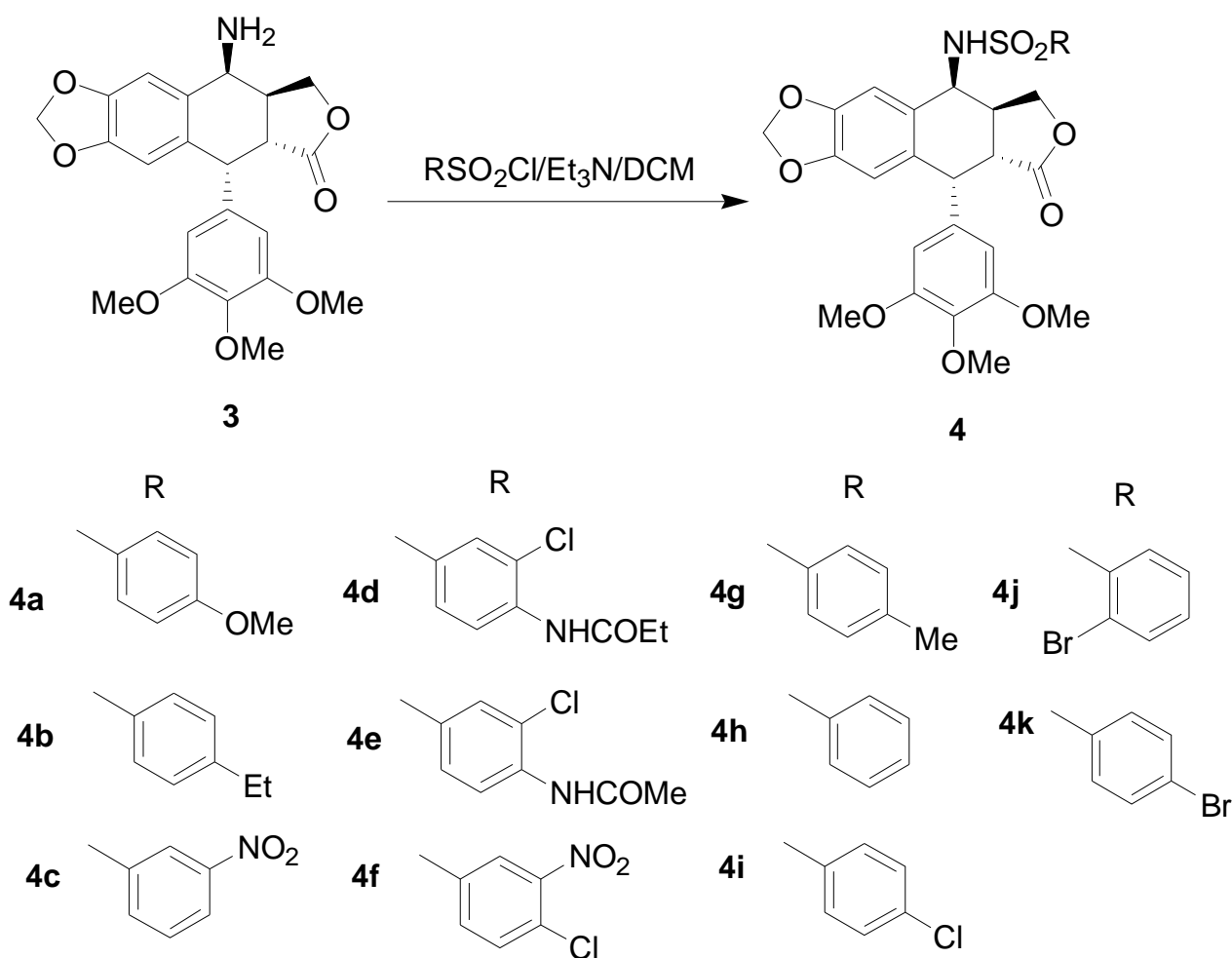


**Figure 1.** Structures of podophyllotoxin (**1**) and deoxypodophyllotoxin (**2**)

## RESULTS AND DISCUSSION

Eleven 4 $\beta$ -benzenesulfonamide derivatives of podophyllotoxin (**4a—4k**) were semisynthesized as shown in Scheme 1 and characterized by <sup>1</sup>H-NMR, MS HRMS, optical rotation and melting point. The insecticidal activity of compounds **1** and **4a—4k** against the third-instar larvae of *Mythimna separata* Walker *in vivo* was investigated by the leaf-dipping method at the concentration of 1 mg/mL. In addition, corrected mortality rates were calculated as shown in Table 1. Toosendanin, a commercial insecticide derived from *Melia azedarach*, was used as a positive control.

As shown in Table 1, the mortality rates caused by these compounds after 20 d were far higher than those after 5 and 15 d. For example, the mortality rate of **4i** against *M. separata* after 5 d was only 29.6%, but after 15 and 20 d, the corresponding mortality rates were increased to 51.9% and 88.9%, respectively.



**Scheme 1.** The synthetic route of **4a**—**4k**

That is, these compounds showed delayed insecticidal activity,<sup>9</sup> which was different from those conventional neurotoxic insecticides, such as organophosphates, carbamates and pyrethroids. Meanwhile, the spontaneous movement in the insects treated by these compounds was little different from that of control insects in 24 h after treatment. But after 48 h some insects of the treated groups were paralyzed, loss of body liquid and becoming immobilized. Immobilization was increased with the passage of time. Additionally, the pupation of the larvae and the adult emergence of *M. separata* were inhibited by these compounds, therefore, the stage from the larvae to adulthood of *M. separata* was prolonged as compared to the control group. Moreover, many larvae of the treated groups were unable to reach adulthood and died during the stage of pupation.

From the comparative study, it is possible to draw some structure-activity relationships as shown in Table 1. It was clear that the hydroxy group at C(4) of podophyllotoxin (**1**) substituted by  $4\beta$ -benzenesulfonamide moieties (**4a**—**4f** and **4i**—**4k**) could usually lead to increasing the final mortality rates except **4g** and **4h**. Especially **4i** exhibited the most potent insecticidal activity among all tested compounds. For example, the final mortality rate of **4i** against *M. separata* was 88.9%, which was even

higher than that of toosendanin (81.4%). Meanwhile, whether introducing electron-withdrawing (e.g., nitro group) or electron-donating groups (e.g., methoxyl group) on the benzene ring of 4 $\beta$ -benzenesulfonamide derivative of podophyllotoxin (**4h**) would give more potent compounds (e.g., **4c** and **4a**) than **4h**. For example, the final mortality rate of **4h** against *M. separata* was only 55.6%, on the contrary, the final mortality rates of **4c** and **4a** against *M. separata* were 74.1% and 77.8%, respectively.

**Table 1.** Insecticidal activity of **4a**—**4k** against *Mythimna separata* in Vivo

Compounds	Corrected Mortality Rate (%) <sup>a</sup>		
	5 d	15 d	20 d
<b>4a</b>	18.5 ( $\pm$ 7.2)	25.9 ( $\pm$ 9.8)	77.8 ( $\pm$ 14.1)
<b>4b</b>	22.2 ( $\pm$ 4.7)	33.3 ( $\pm$ 8.2)	74.1 ( $\pm$ 2.7)
<b>4c</b>	22.2 ( $\pm$ 4.7)	48.1 ( $\pm$ 5.4)	74.1 ( $\pm$ 2.7)
<b>4d</b>	14.8 ( $\pm$ 7.2)	22.2 ( $\pm$ 9.4)	70.3 ( $\pm$ 2.7)
<b>4e</b>	22.2 ( $\pm$ 0)	40.7 ( $\pm$ 5.4)	70.3 ( $\pm$ 7.2)
<b>4f</b>	18.5 ( $\pm$ 7.2)	29.6 ( $\pm$ 7.2)	70.3 ( $\pm$ 5.4)
<b>4g</b>	7.4 ( $\pm$ 7.2)	29.6 ( $\pm$ 7.2)	63.0 ( $\pm$ 10.9)
<b>4h</b>	0 ( $\pm$ 4.7)	11.1 ( $\pm$ 9.4)	55.6 ( $\pm$ 2.7)
<b>4i</b>	29.6 ( $\pm$ 5.4)	51.9 ( $\pm$ 15.2)	88.9 ( $\pm$ 0)
<b>4j</b>	25.9 ( $\pm$ 2.7)	44.4 ( $\pm$ 8.2)	77.8 ( $\pm$ 0)
<b>4k</b>	3.7 ( $\pm$ 2.7)	18.5 ( $\pm$ 2.7)	66.7 ( $\pm$ 2.5)
<b>1</b>	25.9 ( $\pm$ 7.2)	40.7 ( $\pm$ 7.2)	63.0 ( $\pm$ 5.0)
toosendanin <sup>b</sup>	22.2 ( $\pm$ 14.1)	51.9 ( $\pm$ 9.8)	81.4 ( $\pm$ 2.7)

<sup>a</sup> Concentration: 1 mg/mL; values are means of three experiments, standard deviation is given in parentheses; acetone as a control.

<sup>b</sup> Toosendanin as a positive control.

Especially when the chloro group was introduced at the *para* position on the benzene ring of **4h**, the final mortality rate of the corresponding compound (**4i**) against *M. separata* was increased sharply from 55.6% to 88.9%. Interestingly, once other functional group (e.g., methoxyl, ethyl or chloro group) was substituted for methyl group on the benzene ring of 4 $\beta$ -toluenesulfonamide derivative of podophyllotoxin (**4g**), the final mortality rates of the corresponding compounds **4a**, **4b**, and **4i** against *M. separata* were increased from 63.0% to 77.8%, 74.1%, and 88.9%, respectively.

Previously, we noticed that the introduction of bromo group at the *para* position on the benzene ring of 4 $\beta$ -benzoylamide derivative of podophyllotoxin afforded the compound, which was more potent than that containing the bromo group at the *ortho* position against *P. rapae*.<sup>9</sup> However, in this paper, it was found

that the substitution on the benzene ring of 4 $\beta$ -benzenesulfonamide derivative of podophyllotoxin with bromo group at the *para* position yielded compound (**4k**), which was less potent than that containing the bromo group at the *ortho* position against *M. separata* (**4j**) (66.7% vs. 77.8%).

This results demonstrated the possibility of further elaboration of the 4 $\beta$ -benzenesulfonamide derivatives of podophyllotoxin to obtain more potent and promising compounds. Furthermore, efforts to explain the reason why **4i** showed the most potent insecticidal activity of all tested compounds against *M. separata* are ongoing in our laboratory.

In conclusion, eleven 4 $\beta$ -benzenesulfonamide derivatives of podophyllotoxin were semisynthesized and tested for the insecticidal activity against the third-instar larvae of *Mythimna separata* Walker *in vivo* at the concentration of 1 mg/mL. Among all the tested compounds, especially **4i** showed the most promising and best insecticidal activity as displayed in Table 1. Based upon the above results, further structural modifications of podophyllotoxin will be conducted in our research group in order to find the more potent molecules as insecticidal agents.

## EXPERIMENTAL

All the solvents were of analytical grade and the reagents were used as purchased. Thin-layer chromatography (TLC) and silica gel column chromatography were used with silica gel 60 GF<sub>254</sub> and 200-300 mesh, respectively (Qingdao Haiyang Chemical Co., Ltd.). Melting points were determined on an X-4 micromelting-point apparatus and uncorrected. <sup>1</sup>H-NMR spectra were recorded on a Bruker Avance DMX 300 or 400 MHz instrument using TMS as internal standard and CDCl<sub>3</sub> as solvent. HRMS and EIMS were carried out with APEX II Bruker 4.7T AS and Thermo DSQ GC/MS instruments, respectively. Optical rotation was measured with a Perkin Elmer 341 polarimeter (PE Company, USA). 4 $\beta$ -Aminopodophyllotoxin (**3**, Scheme 1) was prepared according to our previous method.<sup>9</sup>

### General procedure for the synthesis of 4 $\beta$ -benzenesulfonamide derivatives of podophyllotoxin (**4a—4k**).

A mixture of **3** ( 0.5 mmol), benzenesulfonyl chlorides (1.0 mmol), triethylamine (1.0 mmol), and dry CH<sub>2</sub>Cl<sub>2</sub> (15 mL) was stirred at rt checked by TLC. When the reaction was complete, CH<sub>2</sub>Cl<sub>2</sub> (20 mL) was added to the reaction mixture. The mixture was washed by water (30 mL), 0.5 mol/L HCl (30 mL), 5% aq. NaHCO<sub>3</sub> (30 mL), dried over anhydrous NaSO<sub>4</sub>, concentrated *in vacuo*, and purified by silica gel column chromatography to give the pure 4 $\beta$ -benzenesulfonamide derivatives of podophyllotoxin.

**4a**: Yield: 75%, white solid, mp 135-136 °C; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -74° (C 6.0 mg/mL, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.88 (d, *J* = 8.8 Hz, 2H, H-2'', 6'' ), 7.48 (d, *J* = 9.2 Hz, 2H, H-3'', 5'' ), 6.42 (s, 1H, H-5), 6.20 (s, 2H, H-2', 6' ), 5.89 (s, 2H, OCH<sub>2</sub>O), 5.73 (s, 1H, H-8), 4.80 (d, *J* = 6.4 Hz, 1H, NH), 4.54 (m, 2H, H-1,

4), 4.31 (m, 2H, H-11), 3.95 (s, 3H, 4''-OCH<sub>3</sub>), 3.78 (s, 3H, 4'-OCH<sub>3</sub>), 3.72 (s, 6H, 3', 5'-OCH<sub>3</sub>), 2.92 (m, 2H, H-2, 3); ESI-MS *m/z*: 606 ([M+Na]<sup>+</sup>, 13). HRMS (ESI): *m/z* = 601.1853 (calcd. 601.1850 for C<sub>29</sub>H<sub>33</sub>O<sub>10</sub>N<sub>2</sub>S, [M+NH<sub>4</sub>]<sup>+</sup>).

**4b**: Yield: 84%, white solid, mp 130-132 °C; [α]<sup>20</sup><sub>D</sub> -71° (C 6.0 mg/mL, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.87 (d, *J* = 8.1 Hz, 2H, H-2'', 6''), 7.48 (d, *J* = 8.1 Hz, 2H, H-3'', 5''), 6.41 (s, 1H, H-5), 6.20 (s, 2H, H-2', 6'), 5.88 (s, 2H, OCH<sub>2</sub>O), 5.53 (s, 1H, H-8), 4.59 (d, *J* = 8.4 Hz, 1H, NH), 4.54 (m, 2H, H-1, 4), 4.32 (m, 2H, H-11), 3.78 (s, 3H, 4'-OCH<sub>3</sub>), 3.72 (s, 6H, 3', 5'-OCH<sub>3</sub>), 2.85 (m, 4H, H-2, 3 and CH<sub>2</sub>CH<sub>3</sub>), 1.30 (t, *J* = 7.5 Hz, 3H, CH<sub>2</sub>CH<sub>3</sub>); ESI-MS *m/z*: 604 ([M+Na]<sup>+</sup>, 13). HRMS (ESI): *m/z* = 599.2068 (calcd. 599.2058 for C<sub>30</sub>H<sub>35</sub>O<sub>9</sub>N<sub>2</sub>S, [M+NH<sub>4</sub>]<sup>+</sup>).

**4c**: Yield: 69%, pale yellow solid, mp 134-136 °C; [α]<sup>20</sup><sub>D</sub> -72° (C 5.6 mg/mL, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ: 8.81 (s, 1H, H-2''), 8.59 (dd, *J* = 1.2, 8.0 Hz, 1H, H-4''), 8.30 (d, *J* = 8.0 Hz, 1H, H-6''), 7.91 (m, 1H, H-5''), 6.44 (s, 1H, H-5), 6.20 (s, 2H, H-2', 6'), 5.88 (m, 2H, OCH<sub>2</sub>O), 5.62 (s, 1H, H-8), 5.08 (d, *J* = 7.2 Hz, 1H, NH), 4.58 (m, 1H, H-4), 4.54 (d, *J* = 3.6 Hz, 1H, H-1), 4.33 (m, 2H, H-11), 3.78 (s, 3H, 4'-OCH<sub>3</sub>), 3.74 (s, 6H, 3', 5'-OCH<sub>3</sub>), 2.92 (m, 2H, H-2, 3); EI-MS *m/z*: 598 (M<sup>+</sup>, 3). HRMS (ESI): *m/z* = 616.1601 (calcd. 616.1596 for C<sub>28</sub>H<sub>30</sub>O<sub>11</sub>N<sub>3</sub>S, [M+NH<sub>4</sub>]<sup>+</sup>).

**4d**: Yield: 40%, white solid, mp 146-148 °C; [α]<sup>20</sup><sub>D</sub> -60° (C 8.0 mg/mL, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ: 9.03 (d, *J* = 1.2 Hz, 1H, H-2''), 7.83 (d, *J* = 11.6 Hz, 1H, H-5''), 7.84 (m, 2H, H-5'', 4''-NH), 6.43 (s, 1H, H-5), 6.20 (s, 2H, H-2', 6'), 6.05 (s, 1H, H-8), 5.89 (m, 2H, OCH<sub>2</sub>O), 5.21 (d, *J* = 6.8 Hz, 1H, NH), 4.70 (m, 1H, H-4), 4.52 (d, *J* = 5.2 Hz, 1H, H-1), 4.26 (m, 2H, H-11), 3.79 (s, 3H, 4'-OCH<sub>3</sub>), 3.72 (s, 6H, 3', 5'-OCH<sub>3</sub>), 2.92 (m, 2H, H-2, 3), 2.51 (q, *J* = 7.2 Hz, 2H, CH<sub>2</sub>CH<sub>3</sub>), 1.27 (t, *J* = 7.2 Hz, 3H, CH<sub>2</sub>CH<sub>3</sub>); EI-MS *m/z*: 658 (M<sup>+</sup>, 8), 660 (M<sup>+</sup>, 4). HRMS (ESI): *m/z* = 676.1733 (calcd. 676.1726 for C<sub>31</sub>H<sub>35</sub>O<sub>10</sub>N<sub>3</sub>SCl, [M+NH<sub>4</sub>]<sup>+</sup>).

**4e**: Yield: 36%, white solid, mp 174-176 °C; [α]<sup>20</sup><sub>D</sub> -68° (C 5.9 mg/mL, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ: 8.76 (d, *J* = 9.2 Hz, 1H, H-5''), 7.98 (d, *J* = 1.6 Hz, 1H, H-2''), 7.89 (s, 1H, 4''-NH), 7.84 (dd, *J* = 1.6, 9.0 Hz, 1H, H-6''), 6.44 (s, 1H, H-5), 6.21 (s, 2H, H-2', 6'), 5.90 (s, 2H, OCH<sub>2</sub>O), 5.86 (s, 1H, H-8), 4.82 (d, *J* = 6.4 Hz, 1H, NH), 4.59 (m, 1H, H-4), 4.53 (d, *J* = 4.8 Hz, 1H, H-1), 4.30 (m, 2H, H-11), 3.79 (s, 3H, 4'-OCH<sub>3</sub>), 3.72 (s, 6H, 3', 5'-OCH<sub>3</sub>), 2.91 (m, 2H, H-2, 3), 2.33 (s, 3H, CH<sub>3</sub>); EI-MS *m/z*: 644 (M<sup>+</sup>, 7), 646 (M<sup>+</sup>, 2). HRMS (ESI): *m/z* = 662.1571 (calcd. 662.1570 for C<sub>30</sub>H<sub>33</sub>O<sub>10</sub>N<sub>3</sub>SCl, [M+NH<sub>4</sub>]<sup>+</sup>).

**4f**: Yield: 36%, pale yellow solid, mp 158-162 °C; [α]<sup>20</sup><sub>D</sub> -74° (C 4.7 mg/mL, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ: 8.44 (d, *J* = 2.0 Hz, 1H, H-2''), 8.07 (dd, *J* = 2.0, 8.2 Hz, 1H, H-6''), 7.85 (d, *J* = 8.4 Hz, 1H, 5''-H), 6.44 (s, 1H, H-5), 6.19 (s, 2H, H-2', 6'), 5.91 (d, 2H, *J* = 2.0 Hz, OCH<sub>2</sub>O), 5.84 (s, 1H, H-8), 5.24 (d, *J* = 7.6 Hz, 1H, NH), 4.65 (m, 1H, H-4), 4.52 (d, *J* = 3.6 Hz, 1H, H-1), 4.29 (m, 2H, H-11), 3.77 (s, 3H, 4'-OCH<sub>3</sub>), 3.72 (s, 6H, 3', 5'-OCH<sub>3</sub>), 2.93 (m, 2H, H-2, 3); EI-MS *m/z*: 632 (M<sup>+</sup>, 2), 634 (M<sup>+</sup>, 0.6). HRMS (ESI): *m/z* = 650.1215 (calcd. 650.1206 for C<sub>28</sub>H<sub>29</sub>O<sub>11</sub>N<sub>3</sub>SCl, [M+NH<sub>4</sub>]<sup>+</sup>).

**4g**: Yield: 98%, white solid, mp 209-211 °C (lit.<sup>13</sup> 209-212 °C); [α]<sup>20</sup><sub>D</sub> -80° (C 5.8 mg/mL, CHCl<sub>3</sub>);

<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.84 (d,  $J$  = 8.0 Hz, 2H, H-2'', 6''), 7.45 (d,  $J$  = 7.6, 2H, H-3'', 5''), 6.42 (s, 1H, H-5), 6.20 (s, 2H, H-2', 6'), 5.89 (s, 2H, OCH<sub>2</sub>O), 5.66 (s, 1H, H-8), 4.54 (m, 3H, H-1, 4 and NH), 4.31 (m, 2H, H-11), 3.79 (s, 3H, 4'-OCH<sub>3</sub>), 3.73 (s, 6H, 3', 5'-OCH<sub>3</sub>), 2.91 (m, 2H, H-2, 3), 2.52 (s, 3H, 4''-CH<sub>3</sub>); EI-MS  $m/z$ : 567 (M<sup>+</sup>, 5).

**4h**: Yield: 81%, white solid, mp 251-253 °C (lit.,<sup>13</sup> 233-235 °C); [ $\alpha$ ]<sub>D</sub><sup>20</sup> -67° (C 5.3 mg/mL, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.99 (d,  $J$  = 7.2 Hz, 2H, H-2'', 6''), 7.77 (dd,  $J$  = 7.2, 7.5 Hz, 1H, H-4''), 7.68 (dd, 2H,  $J$  = 7.2, 7.8 Hz, H-3'', 5''), 6.44 (s, 1H, H-5), 6.20 (s, 2H, H-2', 6'), 5.90 (s, 2H, OCH<sub>2</sub>O), 5.52 (s, 1H, H-8), 4.55 (m, 3H, H-1, 4, NH), 4.35 (m, 2H, H-11), 3.75 (s, 3H, 4'-OCH<sub>3</sub>), 3.72 (s, 6H, 3', 5'-OCH<sub>3</sub>), 3.43 (m, 2H, H-2, 3); EI-MS  $m/z$ : 553 (M<sup>+</sup>, 100).

**4i**: Yield: 79%, white solid, mp 226-228 °C; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -77° (C 4.0 mg/mL, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.90 (d,  $J$  = 6.6 Hz, 2H, H-2'', 6''), 7.63 (d,  $J$  = 6.6 Hz, 2H, H-3'', 5''), 6.44 (s, 1H, H-5), 6.20 (s, 2H, H-2', 6'), 5.91 (s, 2H, OCH<sub>2</sub>O), 5.77 (s, 1H, H-8), 4.85 (d,  $J$  = 7.2 Hz, 1H, NH), 4.58 (m, 1H, H-4), 4.52 (d,  $J$  = 4.4 Hz, 1H, H-1), 4.29 (m, 2H, H-11), 3.78 (s, 3H, 4'-OCH<sub>3</sub>), 3.72 (s, 6H, 3', 5'-OCH<sub>3</sub>), 2.92 (m, 2H, H-2, 3); EI-MS  $m/z$ : 587 (M<sup>+</sup>, 13), 589 (M<sup>+</sup>, 4). HRMS (ESI):  $m/z$  = 605.1363 (calcd. 605.1355 for C<sub>28</sub>H<sub>30</sub>O<sub>9</sub>N<sub>2</sub>SCl, [M+NH<sub>4</sub>]<sup>+</sup>).

**4j**: Yield: 32%, white solid, mp 232-236 °C; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -71° (C 5.3 mg/mL, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.81 (m, 4H, H-3''—6''), 6.44 (s, 1H, H-5), 6.20 (s, 2H, H-2', 6'), 5.92 (s, 2H, OCH<sub>2</sub>O), 5.76 (s, 1H, H-8), 4.77 (d,  $J$  = 6.4 Hz, 1H, NH), 4.59 (m, 1H, H-4), 4.53 (d,  $J$  = 4.4 Hz, 1H, H-1), 4.30 (m, 2H, H-11), 3.78 (s, 3H, 4'-OCH<sub>3</sub>), 3.72 (s, 6H, 3', 5'-OCH<sub>3</sub>), 2.90 (m, 2H, H-2, 3); EI-MS  $m/z$ : 631 (M<sup>+</sup>, 6), 633 (M<sup>+</sup>, 6). HRMS (ESI):  $m/z$  = 649.0860 (calcd. 649.0850 for C<sub>28</sub>H<sub>30</sub>O<sub>9</sub>N<sub>2</sub>SBr, [M+NH<sub>4</sub>]<sup>+</sup>).

**4k**: Yield: 60%, white solid, mp 245-247 °C; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -142° (C 5.0 mg/mL, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.83 (m, 4H, H-2'', 3'', 5'', 6''), 6.46 (s, 1H, H-5), 6.20 (s, 2H, H-2', 6'), 5.93 (s, 2H, OCH<sub>2</sub>O), 5.69 (s, 1H, H-8), 4.55 (brs, 3H, H-1, 4 and NH), 4.35 (m, 2H, H-11), 3.79 (s, 3H, 4'-OCH<sub>3</sub>), 3.72 (s, 6H, 3', 5'-OCH<sub>3</sub>), 2.92 (m, 2H, H-2, 3); EI-MS  $m/z$ : 631 (M<sup>+</sup>, 13), 633 (M<sup>+</sup>, 13). HRMS (ESI):  $m/z$  = 649.0840 (calcd. 649.0850 for C<sub>28</sub>H<sub>30</sub>O<sub>9</sub>N<sub>2</sub>SBr, [M+NH<sub>4</sub>]<sup>+</sup>).

## Bioassay

The insecticidal activity of compounds **1** and **4a—4k** against the third-instar larvae of *M. separata* were assessed by leaf-dipping method as described previously.<sup>9</sup> For each compound, 30 larvae (10 larvae per group) were used. Acetone solutions of compounds **1**, **4a—4k** and toosendanin (used as a positive control) were prepared at the concentration of 1 mg/mL. Fresh corn leaves were dipped into the solution for 3 s, then taken out and dried in a room. Leaves treated with acetone alone were used as a control group. Several treated leaves were kept in each dish, and every 10 larvae was raised in it. If the treated leaves were consumed, the corresponding ones were added to the dish. After 48 h, untreated fresh leaves were added to the all dish until the adult emergence. The experiment was carried out at 25 ± 2 °C and

relative humidity (R.H.) 65—80%, and on 12 h/12 h (light/dark) photoperiod. The insecticidal activity of the tested compounds against the third-instar larvae of *M. separata* was calculated by the formula:

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

where *T* is the percentage of mortality in the treated group, and *C* is the percentage of mortality in the untreated group.

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