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SYNTHESIS OF 2′(2′,6′)-(DI)HALOGENOISOXAZOLOPODOPHYLLIC ACIDS-BASED AMIDES DERIVED FROM A NATURALLY OCCURRING LIGNAN PODOPHYLLOTOXIN AND THEIR ACARICIDAL ACTIVITY

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Dedicated to Professor Dr. Kiyoshi Tomioka on the occasion of his 70th birthday

Abstract – In continuation of our program to discover natural product-based pesticides, a series of 2′(2′,6′)-(di)halogenoisoxazolopodophyllic acids-based amides were prepared by structural modification of a naturally occurring lignan podophyllotoxin. Meanwhile, their acaricidal activity was evaluated against a typically crop-threatening agricultural insect pest, *Tetranychus cinnabarinus*. Among all derivatives, especially compounds **10**−**12** displayed 4.8-7.4 folds more potent acaricidal activity than podophyllotoxin against *T. cinnabarinus*. This demonstrated that the carboxyl group at their C-2 position of 2′(2′,6′)-(di)halogenoisoxazolopodophyllic acids was very important for the acaricidal activity.

Podophyllotoxin (**1**, Figure 1) containing four consecutive chiral centers (labeled C-1–C-4) and four almost planar fused rings (labeled A–D), is isolated from the roots and rhizomes of *Podophyllum hexandrum* and *Juniperus sabina*.[1,2] Besides compound **1** as a lead compound for preparation of potent anticancer drugs such as etoposide, teniposide and etoposide phosphate,[3-5] it displayed a large number of interesting properties including insecticidal activity,[6-8] antifungal activity,[9] antiviral activity,[10,11] anti-inflammatory activity,[12] immunosuppressive activity,[13] and antirheumatic activity.[14] On the other

hand, we investigated halogenation of E-ring of podophyllotoxins, and found some 2′(2′,6′)-(di)halogenopodophyllotoxin derivatives (**2**, Figure 1) showed more pronounced insecticidal activity than toosendanin.[15-17] Meanwhile, 2′(2′,6′)-(di)halogenoisoxazolopodophyllic acids-based esters (**3**, Figure 1) were also prepared by modified in the C and D rings, and some compounds exhibited more promising insecticidal activity than toosendanin.[18] Meanwhile, it is particularly notorious that *Tetranychus cinnabarinus* (Spider mite), a crop-threatening insect pest, is extremely hard to prevent and control. Due to the long-term and unreasonable use of synthetic agrochemicals to control this serious insect pest, insect resistance and environmental problems have occurred.[19,20] Therefore, discovery of the potential alternatives to efficiently control *T. cinnabarinus* is highly desirable.[21,22] Based on the above interesting results, and in continuation of our program aimed at the discovery of biorenewable natural product-based pesticides, herein a series of 2′(2′,6′)-(di)halogenoisoxazolopodophyllic acids-based amides were prepared by structural modification of compound **1**. Their acaricidal activity was tested against *T. cinnabarinus* in vivo.

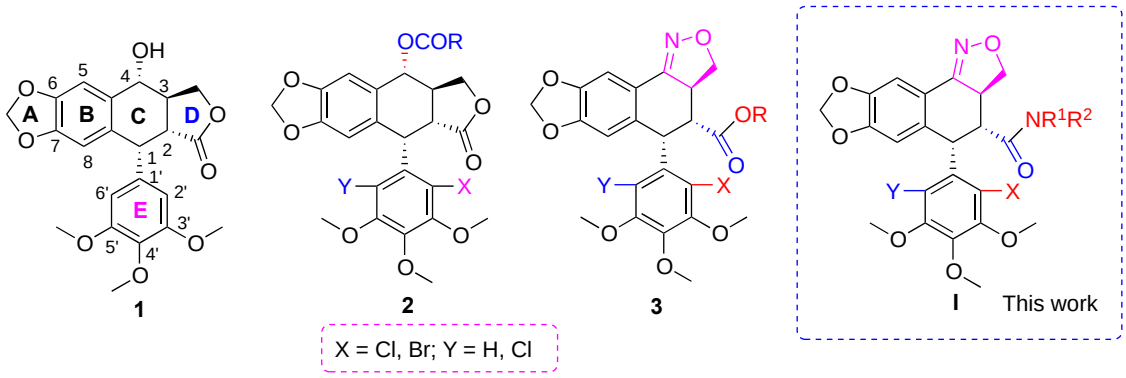
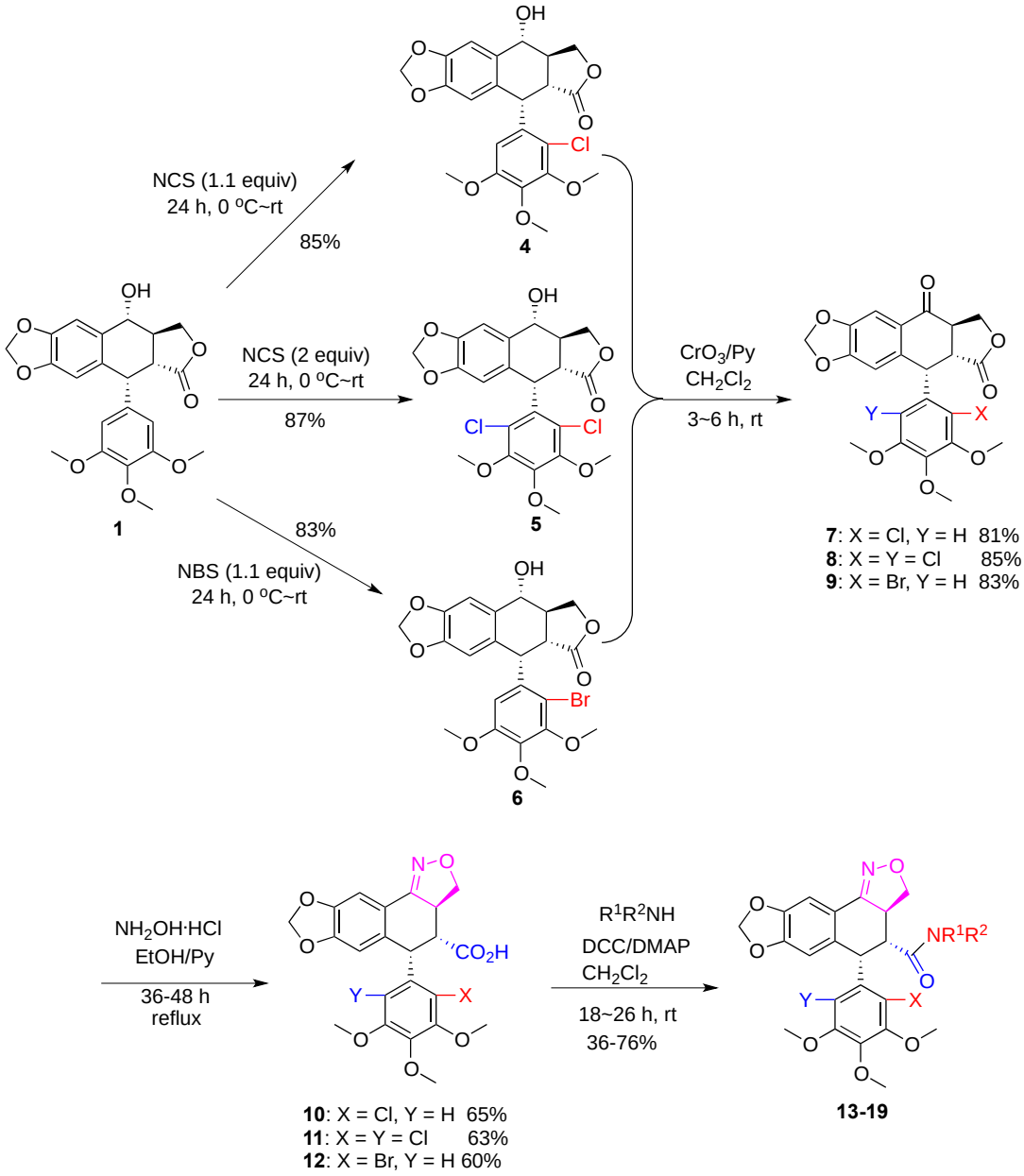


Figure 1. Chemical structures of podophyllotoxin (**1**), esters of 2′(2′,6′)-(di)halogen-substituted podophyllotoxins (**2**), 2′(2′,6′)-(di)halogenoisoxazolopodophyllic acids-based esters (**3**), and the target compounds (**I**)

As shown in Scheme 1, firstly, podophyllotoxin (**1**) reacted with *N*-chlorosuccinimide (NCS) or *N*-bromosuccinimide (NBS) to afford 2′-chloropodophyllotoxin (**4**), 2′,6′-dichloropodophyllotoxin (**5**), and 2′-bromopodophyllotoxin (**6**).[15] Then, 2′(2′,6′)-(di)halogenopodophyllones (**7**–**9**) were prepared by oxidation of **4**–**6**. Subsequently, 2′(2′,6′)-(di)halogenoisoxazolopodophyllic acids (**10**–**12**) were produced by reaction of compounds **7**–**9** with hydroxylamine hydrochloride.[23] Finally, in the presence of DCC and DMAP, target compounds **13**–**19** were obtained in 36-76% yields by reaction of compounds **10**–**12** with the corresponding amines. The chemical structures of compounds **13**–**19** (Figure 2) were well characterized by ^{1}H NMR, HRMS, optical rotation, and mp.



Scheme 1. The synthetic route for the preparation of 2′(2′,6′)-(di)halogenoisoxazolopodophyllic acids-based amides (**13**–**19**)

The acaricidal activity of compounds **1**, **4**–**6** and **10**–**19** against the female adults of *Tetranychus cinnabarinus* was tested by slide-dipping method at a concentration of 0.5 mg/mL. Spirodiclofen was used as a positive control at 0.5 mg/mL. As displayed in Table 1, compound **1** nearly had no acaricidal activity against *T. cinnabarinus*, and its mortality rate (MR) at 72 h was only 6.7%. After introduction of the halogen atom on the E-ring of compound **1**, the acaricidal activity of the corresponding compounds was increased; for example, the MRs at 72 h of compounds **4**–**6** were 13.3%, 22.0%, and 13.3%, respectively.

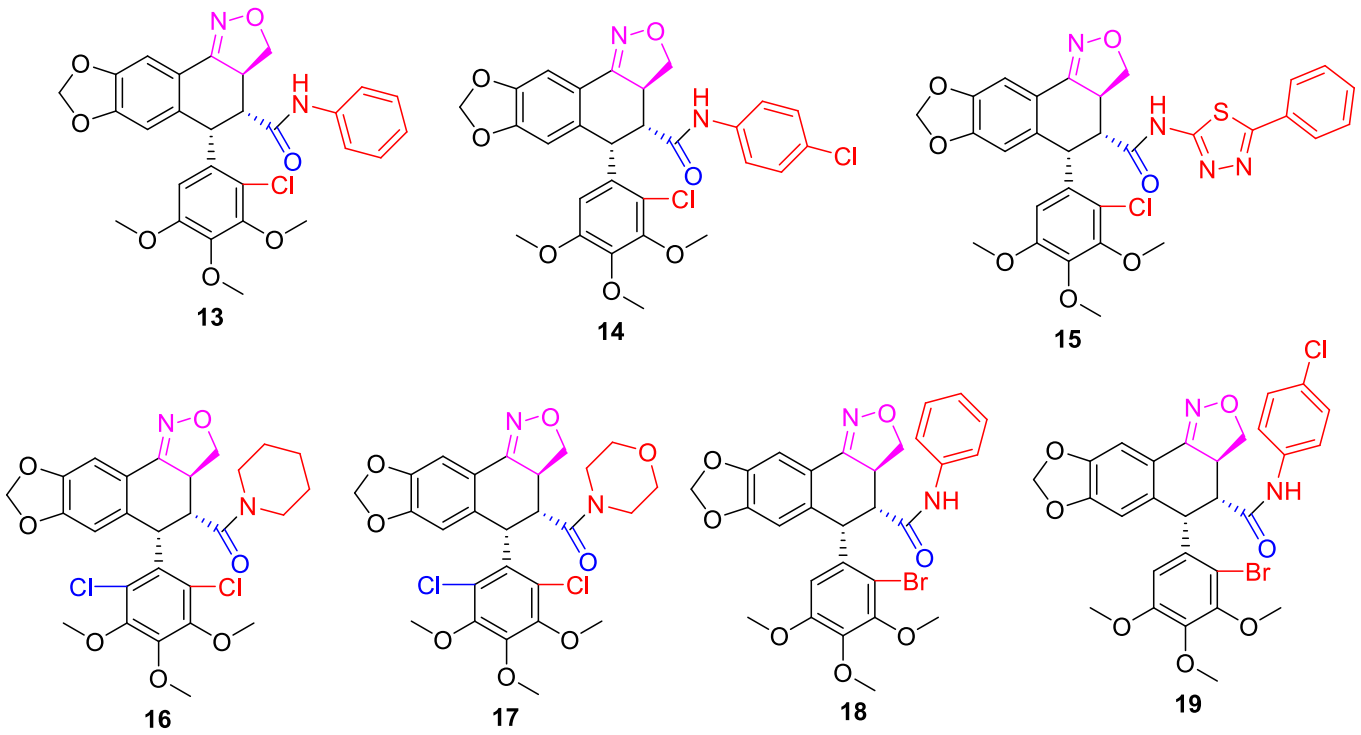


Figure 2. Chemical structures of 2′(2′,6′)-(di)halogenoisoxazolopodophyllic acids-based amides (**13**–**19**)

Table 1. Acaricidal activity of compounds **1**, **4**–**6**, and **10**–**19** against the female adults of *Tetranychus cinnabarinus* treated at a concentration of 0.5 mg/mL

Compound	Corrected mortality rate (mean ± SD, %)[a]	
	48 hours	72 hours
Spirodiclofen	41.2 ± 2.2	64.0 ± 0.6
1	3.3 ± 1.9	6.7 ± 1.3
4	11.0 ± 0.4	13.3 ± 0.5
5	15.4 ± 3.1	22.0 ± 3.0
6	12.3 ± 4.9	13.3 ± 5.2
10	13.8 ± 1.7	40.4 ± 4.2
11	21.3 ± 0.4	32.1 ± 2.3
12	31.2 ± 3.9	49.4 ± 2.6
13	11.2 ± 3.9	24.4 ± 3.0
14	12.6 ± 2.6	16.3 ± 0.3
15	8.1 ± 0.6	13.7 ± 2.7
16	6.8 ± 1.3	17.1 ± 1.9
17	4.2 ± 2.7	9.8 ± 3.5
18	10.9 ± 0.2	18.6 ± 4.1
19	6.8 ± 1.3	15.9 ± 2.4

[a]Values are means of three replicate.

It was noteworthy that 2′(2′,6′)-(di)halogenoisoxazolopodophyllic acids (**10**–**12**) exhibited more promising acaricidal activity than other compounds; and especially compounds **10**–**12** displayed 4.8-7.4 folds more potent acaricidal activity than podophyllotoxin (**1**) against *T. cinnabarinus*. For example, the MRs at 72 h of compounds **10**–**12** were 40.4%, 32.1%, and 49.4%, respectively; whereas the MR at 72 h of compound **1** was only 6.7%. However, once the carboxyl groups of compounds **10**–**12** were changed to the amides, the acaricidal activity of the corresponding compounds was decreased, and the range of MRs at 72 h of 2′(2′,6′)-(di)halogenoisoxazolopodophyllic acids-based amides (**13**–**19**) was only 9.8%–24.4%. For example, the MR at 72 h of compound **12** was 49.4%, whereas the MRs at 72 h of its amides (compounds **18** and **19**) were only 18.6% and 15.9%, respectively. In a word, it interestingly demonstrated that the carboxyl group at their C-2 position of 2′(2′,6′)-(di)halogenoisoxazolopodophyllic acids (**10**–**12**) was very essential for the acaricidal activity.

In conclusion, we have prepared a series of 2′(2′,6′)-(di)halogenoisoxazolopodophyllic acids-based amides by structural modification of a naturally occurring lignan podophyllotoxin (**1**). And their acaricidal activity was evaluated against a typically crop-threatening agricultural insect pest, *Tetranychus cinnabarinus*. Among all derivatives, three 2′(2′,6′)-(di)halogenoisoxazolopodophyllic acids exhibited the most potent acaricidal activity. It suggested that the carboxyl group at their C-2 position of 2′(2′,6′)-(di)halogenoisoxazolopodophyllic acids was very important for the acaricidal activity. This will pave the way for further structural modifications of podophyllotoxin (**1**) as potentially botanical acaricidal agents in crop protection.

EXPERIMENTAL

All chemical reagents were purchased and utilized without further purification. Solvents were used directly or treated with 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 GF254 (Qingdao Haiyang Chemical Co., Ltd., Qingdao, China). 2′(2′,6′)-(Di)halogenopodophyllotoxin (**4**–**6**, Scheme 1) were prepared in the same way as in our previous report.[15] 2′(2′,6′)-(Di)halogenopodophyllones (**7**–**9**, Scheme 1) and 2′(2′,6′)-(di)halogenoisoxazolopodophyllic acids (**10**–**12**, Scheme 1) were prepared according to our previous method.[23] Melting points (mp) were determined on a XT-4 digital melting point apparatus. Optical rotation was measured on a Rudolph Research Analytical Autopol III automatic polarimeter. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded in $CDCl_3$ on a Bruker Avance III 500 MHz instrument using tetramethylsilane (TMS) as the internal standard. High-resolution mass spectra (HR-MS) were carried out with IonSpec 4.7 Tesla FTMS instrument.

General procedure for synthesis of 2′(2′,6′)-(di)halogenoisoxazolopodophyllic acids-based amides

(13–19)

A mixture of 2′(2′,6′)-(di)halogenoisoxazolopodophyllic acids (**10**–**12**, 0.2 mmol), the corresponding amines (0.28 mmol), DCC (0.2 mmol), and DMAP (0.04 mmol) in dry CH_2Cl_2 (2 mL) was stirred at room temperature for 18−26 h. When the reaction was complete according to TLC analysis, the mixture was diluted by DCM (40 mL), washed by water (20 mL), aq. HCl (0.1 mol/L, 20 mL), saturated aq. $NaHCO_3$ (20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4, concentrated in vacuo, and purified by PTLC to give the target compounds **13**–**19** in 36–76% yields.

Data for **13**: Yield: 76%, pale yellow solid, mp 114-116 °C; $[\alpha]^{20}_D$ -25 (c 1.8 mg/mL, $CHCl_3$); ^{1}H NMR (500MHz, $CDCl_3$) δ: 7.74, (s, 1H, NH), 7.42 (s, 1H, H-5), 7.38-7.40 (m, 3H, Ar- H), 7.28 (d, J = 7.5 Hz, 2H, Ar-H), 6.39 (s, 1H, H-8), 6.10 (s, 1H, H-6'), 5.98 (d, J =15.5 Hz, 2H, OCH_2O), 5.22 (d, J = 6.0 Hz, 1H, H-1), 4.85 (t, J = 9.0 Hz, 1H, H-11), 4.21-4.26 (m, 1H, H-11), 3.84 (s, 3H, OCH_3), 3.78-3.83 (m, 1H, H-3), 3.70 (s, 3H, OCH_3), 3.64 (s, 3H, OCH_3), 3.11 (dd, J = 12.5, 6.0 Hz, 1H, H-2); HRMS (ESI): Calcd for $C_{28}H_{26}O_7N_2Cl$ ($[M+H]^+$): 537.1423. Found: 537.1422.

Data for **14**: Yield: 52%, white solid, mp 120-121°C; $[\alpha]^{20}_D$ -92 (c 2.0 mg/mL, $CHCl_3$); ^{1}H NMR (500MHz, $CDCl_3$) δ: 7.78 (s, 1H, NH), 7.42 (s, 1H, H-5), 7.35 (d, J = 9.0Hz, 2H, Ar- H), 7.24 (d, J = 9.5 Hz, 2H, Ar-H), 6.36 (s, 1H, H-8), 6.08 (s, 1H, H-6'), 5.98 (d, J = 16.5 Hz, 2H, OCH_2O), 5.17 (d, J = 5.5 Hz, 1H, H-1), 4.84 (t, J = 9.5 Hz, 1H, H-11), 4.17-4.24 (m, 1H, H-11), 3.84 (s, 3H, OCH_3), 3.78-3.82 (m, 1H, H-3), 3.73 (s, 3H, OCH_3), 3.64 (s, 3H, OCH_3), 3.07 (dd, J = 12.5, 6.0 Hz, 1H, H-2); HRMS (ESI): Calcd for $C_{28}H_{25}O_7N_2Cl_2$ ($[M+H]^+$): 571.1033. Found: 571.1029.

Data for **15**: Yield: 54%, white solid, mp 217-218 °C; $[\alpha]^{20}_D$ -49 (c 2.0 mg/mL, $CHCl_3$); ^{1}H NMR (500MHz, $CDCl_3$) δ: 7.60 (s, 1H, H-5), 7.47-7.53 (m, 3H, Ar- H), 7.30-7.34 (m, 2H, Ar-H), 6.53 (s, 1H, H-8), 6.15 (s, 1H, H-6'), 6.00 (d, J = 9.5 Hz, 2H, OCH_2O), 5.72 (d, J = 5.0 Hz, 1H, H-1), 4.84 (t, J = 8.5 Hz, 1H, H-11), 4.01-4.04 (m, 1H, H-11), 3.87-3.92 (m, 1H, H-3), 3.85 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.64 (s, 3H, OCH_3), 3.47-3.54 (m, 1H, H-2); HRMS (ESI): Calcd for $C_{30}H_{26}O_7N_4ClS$ ($[M+H]^+$): 621.1205. Found: 621.1203.

Data for **16**: Yield: 50%, white solid, mp 127-128 °C; $[\alpha]^{20}_D$ -43 (c 1.6 mg/mL, $CHCl_3$); ^{1}H NMR (500MHz, $CDCl_3$) δ: 7.38 (s, 1H, H-5), 6.34 (s, 1H, H-8), 5.95 (d, J = 8.5 Hz, 2H, OCH_2O), 5.37 (d, J = 7.5 Hz, 1H, H-1), 4.71-4.75 (m, 1H, H-11), 4.05 (d, J = 13.0, 1H, H-11), 3.93 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.58-3.65 (m, 2H), 3.25-3.30 (m, 1H, H-3), 2.63-2.68 (m, 1H, H-2), 1.74-1.80 (m, 2H), 1.52-1.60 (m, 2H), 1.37-1.42 (m, 2H), 0.84-0.88 (m, 2H); HRMS (ESI): Calcd for $C_{27}H_{29}O_7N_2Cl_2$ ($[M+H]^+$): 563.1346. Found: 563.1345.

Data for **17**: Yield: 43%, white solid, mp 138-140 °C; $[\alpha]^{20}_D$ -84 (c 3.0 mg/mL, $CHCl_3$); ^{1}H NMR (500MHz, $CDCl_3$) δ: 7.32 (s, 1H, H-5), 6.32 (s, 1H, H-8), 5.96 (d, J = 11.0 Hz, 2H, OCH_2O), 5.34 (d, J = 7.5 Hz, 1H, H-1), 4.67-4.73 (m, 1H, H-11), 3.95 (d, J = 1.5 Hz, 1H, H-11), 3.94 (s, 3H, OCH_3), 3.89

(s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.72-3.76 (m, 1H, H-3), 3.52-3.66 (m, 8H), 3.05 (dd, *J* = 10.0, 6.0 Hz, 1H, H-2); HRMS (ESI): Calcd for $C_{26}H_{27}O_8N_2Cl_2$ ($[M+H]^+$): 565.1139. Found: 565.1140.

Data for **18**: Yield: 36%, pale yellow solid, mp 117-118 °C; $[\alpha]^{20}_D$ -75 (c 2.2 mg/mL, $CHCl_3$); ^{1}H NMR (500MHz, $CDCl_3$) *δ*: 7.77 (s, 1H, NH), 7.42 (s, 1H, H-5), 7.38-7.39 (m, 3H, Ar- H), 7.26 (d, *J* = 7.5 Hz, 2H, Ar-H), 6.39 (s, 1H, H-8), 6.16 (s, 1H, H-6'), 5.98 (d, *J* = 18.0 Hz, 2H, OCH_2O), 5.26 (d, *J* = 6.0 Hz, 1H, H-1), 4.85 (t, *J* = 9.0 Hz, 1H, H-11), 4.22-4.29 (m, 1H, H-11), 3.84 (s, 3H, OCH_3), 3.78-3.81 (m, 1H, H-3), 3.70 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 3.09 (dd, *J* = 12.5, 6.5 Hz, 1H, H-2); HRMS (ESI): Calcd for $C_{28}H_{26}O_7N_2Br$ ($[M+H]^+$): 581.0918. Found: 581.0918.

Data for **19**: Yield: 38%, white solid, mp 143-144 °C; $[\alpha]^{20}_D$ -84 (c 2.5 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 7.83 (s, 1H, NH), 7.42 (s, 1H, H-5), 7.35 (d, *J* = 8.5 Hz, 2H, Ar- H), 7.23 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.38 (s, 1H, H-8), 6.15 (s, 1H, H-6'), 5.98 (d, *J* = 16.0 Hz, 2H, OCH_2O), 5.24 (d, *J* = 6.0 Hz, 1H, H-1), 4.85 (t, *J* = 9.0 Hz, 1H, H-11), 4.21-4.28 (m, 1H, H-11), 3.84 (s, 3H, OCH_3), 3.78-3.82 (m, 1H, H-3), 3.73 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 3.08 (dd, *J* = 12.5, 6.5 Hz, 1H, H-2); HRMS (ESI): Calcd for $C_{28}H_{25}O_7N_2BrCl$ ($[M+H]^+$): 615.0528. Found: 615.0527.

Biological assay

The acaricidal activity of compounds **1**, **4**–**6** and **10**–**19** against the female adults of *Tetranychus cinnabarinus* was assessed by slide-dipping method as follows:[24-26] Spirodiclofen was used as a positive control. The solutions of **1**, **4**–**6**, **10**–**19** and spirodiclofen were prepared in acetone/deionized water (v/v = 1/1) at 0.5 mg/mL. For each compound, 90–120 healthy and size-consistency female adults of spider mites (30−40 mites per group) were selected. 30–40 spider mites were adfixed dorsally in two lines to a strip of double-coated masking tape on a microscope slide by using a small brush. Then the slides were dipped into the corresponding solution for 5 s, and taken out. Excess solutions on the slides were removed by filter paper. The slides treated with acetone/deionized water (v/v = 1/1) alone were used as a blank control group (CK). The experiment was carried out at 26 ± 1 °C and 60−80% relative humidity (RH), and on 14 h/10 h (light/dark) photoperiod. The results were checked by binocular dissecting microscope. Their mortalities were recorded at 48 h and 72 h after treatment. Their corrected mortality rate values were calculated as follows: corrected mortality rate (%) = (*T* – *C*) × 100/ (100% – *C*); *C* is the mortality rate of CK, and *T* is the mortality rate of the treated *T. cinnabarinus*.

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