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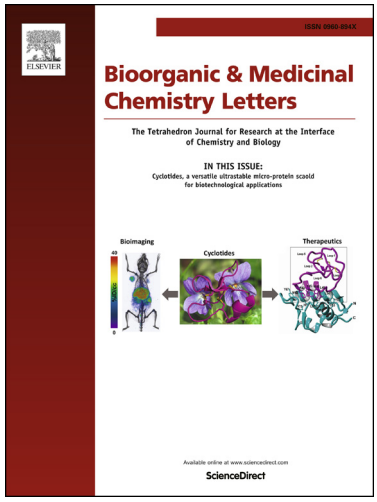

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Synthesis of novel isoxazoline-containing podophyllotoxin/2′(2′,6′)-(di)halogenopodophyllotoxin derivatives and their insecticidal/acaricidal activities

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Abstract:

In continuation of our program aimed at the development of natural product-based pesticidal agents, a series of isoxazoline-containing podophyllotoxin/2′(2′,6′)-(di)halogenopodophyllotoxin derivatives were prepared, and their structures were well characterized by ^{1}H NMR, IR, optical rotation, HRMS and mp. Especially the structure of compound **Ia** was further confirmed by ^{1}H-^{1}H COSY and NOESY spectrum. Among them, two compounds showed good insecticidal and acaricidal activities against *Mythimna separata* and *Tetranychus cinnabarinus*. Their structure-activity relationships were also observed.

Keywords: Podophyllotoxin; Isoxazoline; Natural-product-based; Insecticidal activity; Acaricidal activity

Podophyllotoxin (**1**, Fig. 1), isolated from the roots and rhizomes of *Podophyllum hexandrum* and *Juniperus Sabina*, contains four almost planar fused rings (labeled A–D) and four consecutive chiral centers (labeled C-1–C-4).[1,2] Compound **1** is usually used as a lead compound for preparation of potent bioactive derivatives, which displayed a variety of interesting properties including anticancer activity, insecticidal activity, antifungal activity, antiviral activity, anti-inflammatory activity, and antirheumatic activity.[1-5]

Recently, we have investigated halogenation of E-ring of podophyllotoxins,[6] and found some esters of 2′(2′,6′)-(di)halogenopodophyllotoxins (**2**, Fig. 1),[7] 2α-chloro-2′(2′,6′)-(di)halogenopicropodophyllotoxins (**3**, Fig. 1),[8,9] and 2′-chloro-4′-demethoxyepipodophyllotoxin (**4**, Fig. 1)[10] exhibited more potent insecticidal activity than toosendanin.[6-9] On the other hand, to our delight, it demonstrated that once a chlorine atom was introduced at the C-2' position of podophyllotoxin derivatives, the corresponding compounds showed no significant cytotoxicity.[11] Isoxazolines (Fig. 1) also showed some interesting biological activities including antimicrobial activity, fungicidal activity, mosquitocidal activity, anti-Alzheimer activity, anticancer activity, and anti-inflammatory activity.[12-17] Moreover, we further prepared a series of isoxazolopodophyllic acid-based esters (**5**, Fig. 1), isoxazolopodophyllol-based esters (**6**, Fig. 1) and isoxazolopodophyllal-based hydrazones (**7**, Fig. 1), and some derivatives showed more promising insecticidal activity than their precusors.[18,19]

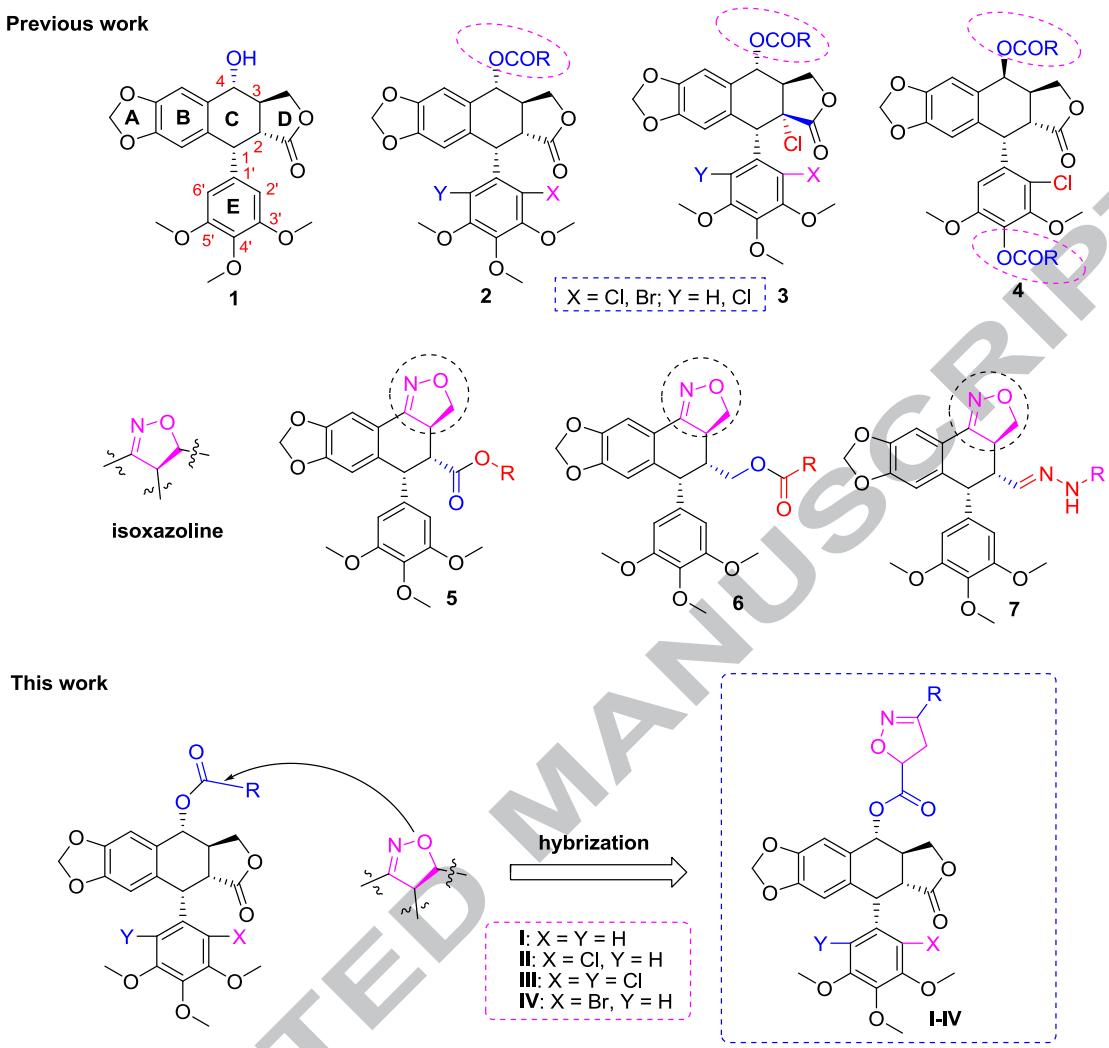
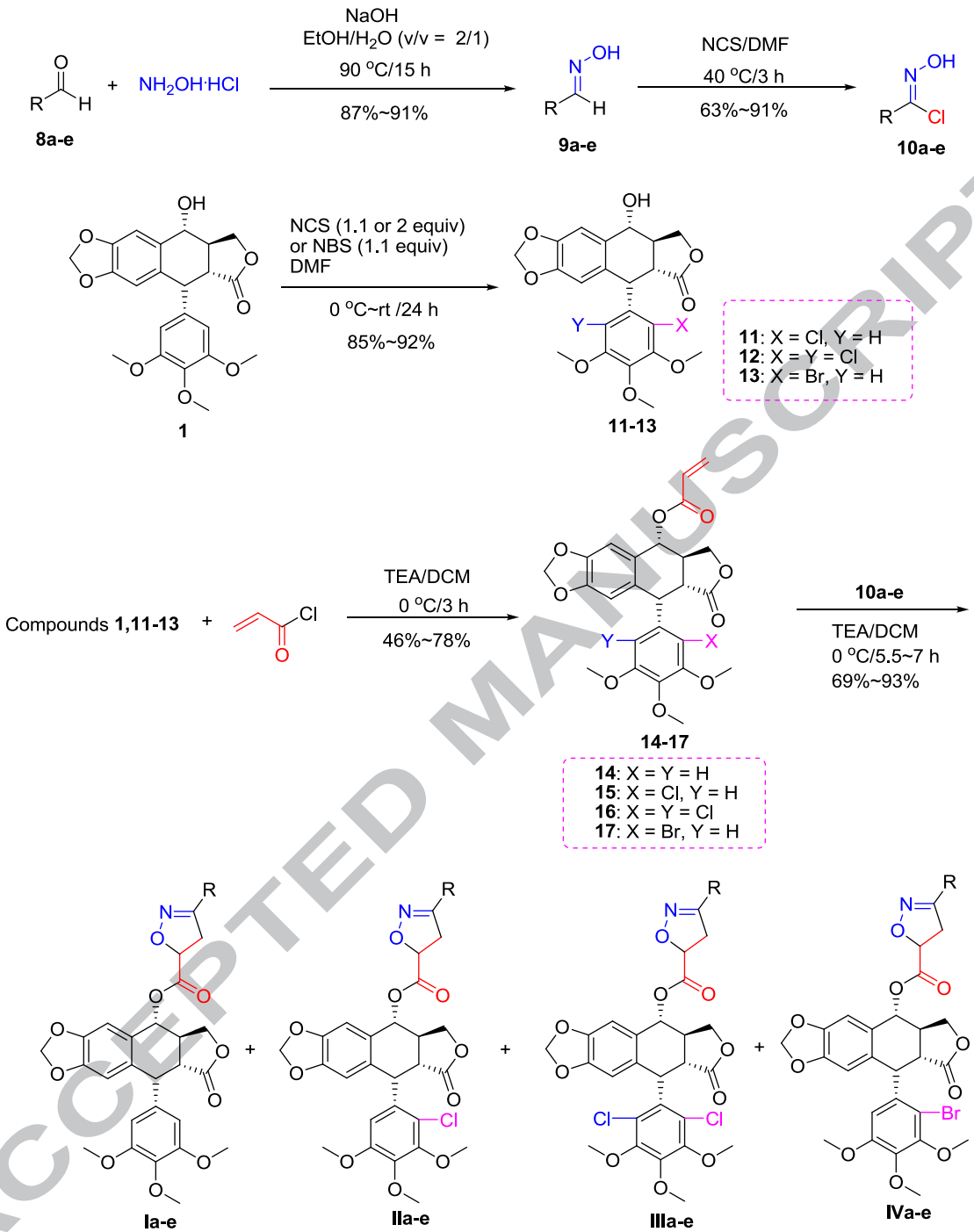


Fig. 1. The chemical structures of podophyllotoxin (**1**), podophyllotoxin esters (**2**–**4**), isoxazoline, isoxazoline derivatives of podophyllotoxin (**5**–**7**), and target compounds (**I**–**IV**).

In the meantime, *Mythimna separata* Walker (oriental armyworm) and *Tetranychus cinnabarinus* Boisduval (spider mite) are two typical and crop-threatening insect pests, and hard to control.[20] Because of extensive and unreasonable applications of synthetic pesticides for controlling pests, resistance in pest populations and negative impacts on human health and environment have already emerged.[21,22] Nowadays, discovery of the potential alternatives from natural products has received much research attention.[23-30] Based on the above results, and in

continuation of our program aimed at the development of new potentially pesticidal agents,[31-33] therefore, in this Letter we prepared a series of isoxazoline-containing podophyllotoxin/2′(2′,6′)-(di)halogenopodophyllotoxin derivatives (**Ia**–**e**, **IIa**–**e**, **IIIa**–**e**, and **IVa**–**e**, Fig. 1) by introduction of the isoxazoline fragment into esters of podophyllotoxin/2′(2′,6′)-(di)halogenopodophyllotoxins. Their insecticidal and acaricidal activities were evaluated against *Mythimna separata* and *Tetranychus cinnabarinus.*

As described in Scheme 1, first, a series of aromatic aldehydes (**8a**–**e**) were condensed with hydroxylamine to afford oximes (**9a**–**e**), which were chlorined with *N*-chlorosuccinimide (NCS) to give hydroximoyl chlorides (**10a**–**e**) in 63%–91% yields.[34] Three 2′(2′,6′)-(di)halogenopodophyllotoxins (**11**–**13**) were then smoothly prepared by reaction of compound **1** with *N*-chlorosuccinimide (NCS, 1.1 or 2 equiv) or *N*-bromosuccinimide (NBS, 1.1 equiv).[6,7] Subsequently, compounds **1** and **11**–**13** reacting with acryloyl chloride in the presence of NEt_3 gave 4α-acryloyloxy-2′(2′,6′)-(di)halogenopodophyllotoxins (**14**–**17**) in 46%–78% yields.[34] Finally, isoxazoline-containing podophyllotoxin/2′(2′,6′)-(di)halogenopodophyllotoxin derivatives (**Ia**–**e**, **IIa**–**e**, **IIIa**–**e**, and **IVa**–**e**) were efficiently obtained by 1,3-dipolar cycloaddition of compounds **14**–**17** with the nitrile oxide dipoles, which were generated from compounds **10a**–**e** in the presence of NEt_3.[35] Their structures were well characterized by ^{1}H NMR, IR, optical rotation, HRMS and mp (see Supplementary data).[36]



Scheme 1. Synthesis of isoxazoline-containing podophyllotoxin/2′(2′,6′)-(di)halogenopodophyllotoxin derivatives (**Ia**–**e**, **IIa**–**e**, **IIIa**–**e**, and **IVa**–**e**).

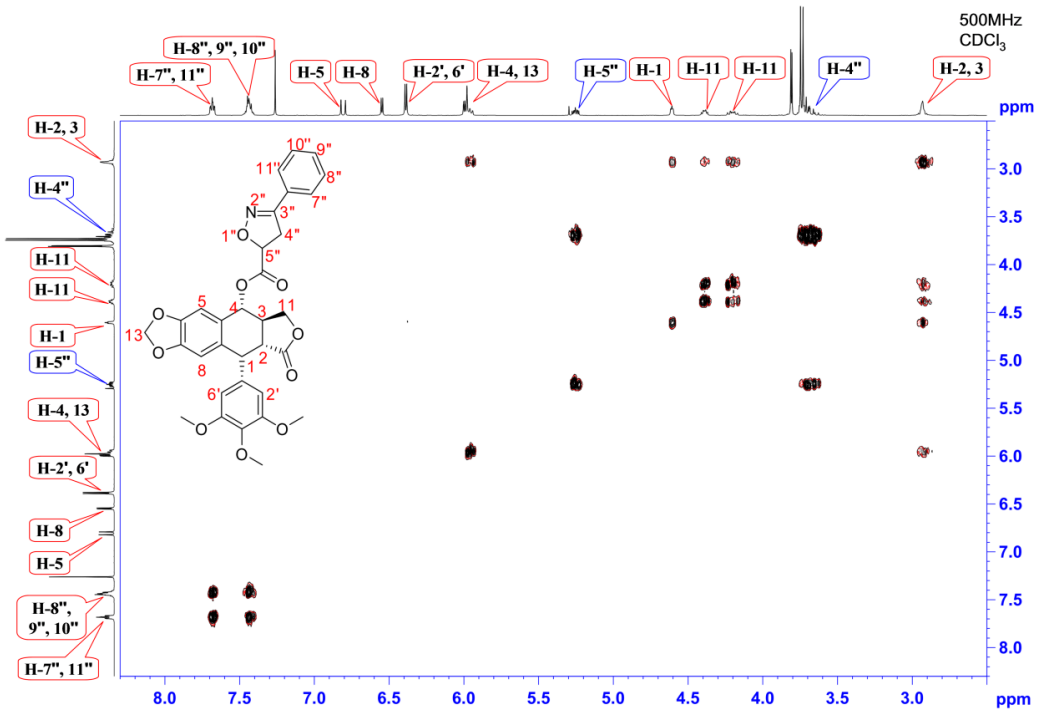


Fig. 2. ^{1}H-^{1}H COSY spectrum of compound **Ia**.

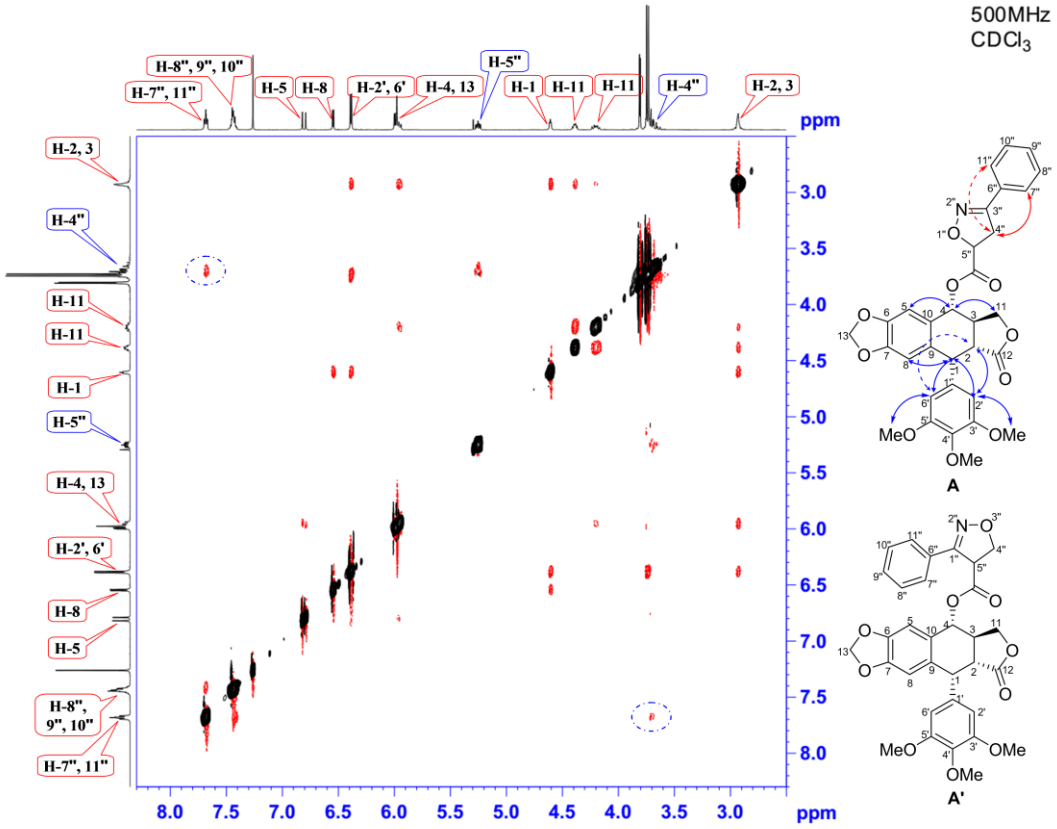


Fig. 3. NOESY spectrum of compound **Ia**.

The assignments of the chemical shifts for the protons of compound **Ia** were further determined by ^{1}H-^{1}H COSY spectrum. For example, as shown in Fig. 2, the ^{1}H-^{1}H COSY correlations of H-4″ and H-5″, H-3 and H-4, H-3 and H-11, and H-1 and H-2 were observed. Moreover, based on the NOESY correlation of H-4″ and H-7″ (or H-11″), the phenyl of the isoxazoline fragment was close to H-4″, therefore, the configuration of compound **Ia** should be as **A** (Fig. 3). Obviously, it revealed that 1,3-dipolar cycloaddition of the nitrile oxides to the carbon-carbon double bond of compounds **14**–**17** was regioselective.

Table 1

Acaricidal activity of compounds **1**, **11-17**, **Ia-e**, **IIa-e**, **IIIa-e**, and **IVa-e** against the female adults of *T. cinnabarinus* at a concentration of 0.5 mg/mL.

Compound	Corrected mortality rate (%) [a]	
	48 hours	72 hours
1	1.3 ± 0.7	1.9 ± 1.0
11	6.8 ± 0.5	6.0 ± 0.4
12	7.1 ± 0.6	6.6 ± 0.4
13	5.6 ± 0.7	5.6 ± 0.9
14	6.9 ± 0.3	13.3 ± 0.5
15	9.9 ± 0.7	11.4 ± 0.7
16	14.0 ± 1.6	17.8 ± 1.4
17	8.6 ± 1.2	11.7 ± 1.3
Ia	10.7 ± 1.4	19.9 ± 0.4
Ib	13.3 ± 1.2	22.9 ± 1.8
Ic	14.0 ± 1.3	23.4 ± 1.3
Id	10.6 ± 1.1	20.2 ± 1.5
Ie	7.7 ± 0.8	15.2 ± 1.3
IIa	12.9 ± 1.0	12.2 ± 1.2
IIb	9.0 ± 1.0	21.8 ± 1.0
IIc	3.8 ± 0.4	29.4 ± 0.9
IId	9.6 ± 2.3	22.5 ± 1.3
IIe	8.4 ± 1.0	15.3 ± 1.1
IIIa	9.9 ± 1.7	20.7 ± 1.5
IIIb	14.2 ± 1.6	24.2 ± 1.6
IIIc	11.5 ± 1.4	34.0 ± 1.2
IIId	5.4 ± 0.6	25.0 ± 1.2

IIIe	7.2 ± 0.8	18.4 ± 0.7
IVa	10.1 ± 0.5	19.0 ± 0.8
IVb	9.2 ± 1.5	13.3 ± 0.8
IVc	9.8 ± 1.7	22.4 ± 1.0
IVd	12.2 ± 1.3	20.1 ± 0.8
IVe	10.1 ± 0.8	15.5 ± 1.3
spirodiclofen	29.8 ± 2.6	65.7 ± 0.9

[a]Values are means ± SE of three replicates.

The acaricidal activity of compounds **1**, **11-17**, **Ia-e**, **IIa-e**, **IIIa-e**, and **IVa-e** against the female adults of *Tetranychus cinnabarinus* was tested by slide-dipping method.[31,37] Spirodiclofen was used as a positive control. The mortality rates (MRs) at 24 h and 72 h of the tested compounds were shown in Table 1. The MR at 72 h of compound **1** was only 1.9%, so compound **1** almost had no acaricidal activity against *T. cinnabarinus*. Meanwhile, the MRs at 72 h of three 2′(2′,6′)-(di)halogenopodophyllotoxins (**11**–**13**) were less than 7%; however, after structural modifications of compound **1**, all ester derivatives showed more potent acaricidal activity than compound **1** and their precusors. Especially compounds **IIc** and **IIIc** showed the most promising acaricidal activity. For example, the MRs at 72 h of compounds **IIc** and **IIIc** were 29.4% and 34.0%, respectively; that is, the MRs at 72 h of compounds **IIc** and **IIIc** were larger than 18-fold of that of **1**. It was noteworthy that generally introduction of a chlorine atom at the C-4 position on the phenyl of the isoxazoline fragment of compound **Ia**, **IIa**, **IIIa** or **IVa**, could give the most potent compounds **Ic**, **IIc**, **IIIc** and **IVc** of their own series. For example, the MRs at 72 h of compounds **Ic**, **IIc**, **IIIc** and **IVc** were 23.4%, 29.4%, 34.0%, 22.4%, respectively.

Table 2
Insecticidal activity of compounds **1**, **14-17**, **Ia-e**, **IIa-e**, **IIIa-e**, and **IVa-e** against *M. separata* on leaves treated at a concentration of 1 mg/mL.

Compound	Corrected mortality rate (%) [a]		
	10 days	20 days	35 days
14	16.7 ± 2.7	20.7 ± 2.7	31.0 ± 2.7
15	23.3 ± 2.7	31.0 ± 2.7	37.9 ± 0
16	23.3 ± 2.7	37.9 ± 0	44.8 ± 2.7
17	20.0 ± 0	34.5 ± 2.7	37.9 ± 0
Ia	23.3 ± 2.7	31.0 ± 2.7	34.5 ± 2.7
Ib	33.3 ± 2.7	41.4 ± 2.7	44.8 ± 2.7
Ic	40.0 ± 4.7	48.3 ± 0	55.2 ± 2.7
Id	36.7 ± 2.7	41.4 ± 2.7	48.3 ± 0
Ie	33.3 ± 2.7	34.5 ± 2.7	41.4 ± 2.7
IIa	26.7 ± 2.7	31.0 ± 2.7	37.9 ± 0
IIb	50.0 ± 0	58.6 ± 0	65.5 ± 2.7
IIc	40.0 ± 0	55.2 ± 2.7	62.1 ± 2.7
IId	46.7 ± 2.7	55.2 ± 2.7	58.6 ± 0
IIe	33.3 ± 2.7	37.9 ± 4.7	48.3 ± 0
IIIa	26.7 ± 2.7	27.6 ± 0	41.4 ± 2.7
IIIb	36.7 ± 2.7	41.4 ± 2.7	48.3 ± 0
IIIc	53.3 ± 2.7	65.5 ± 2.7	72.4 ± 2.7
IIId	30.0 ± 4.7	44.8 ± 2.7	58.6 ± 0
IIIe	23.3 ± 2.7	27.6 ± 0	44.8 ± 2.7
IVa	20.0 ± 0	41.4 ± 2.7	44.8 ± 2.7
IVb	30.0 ± 4.7	34.5 ± 5.4	41.4 ± 2.7
IVc	43.3 ± 2.7	55.2 ± 2.7	62.1 ± 2.7
IVd	33.3 ± 2.7	37.9 ± 4.7	51.7 ± 2.7
IVe	33.3 ± 2.7	37.9 ± 0	41.4 ± 2.7
1	20.0 ± 0	31.0 ± 2.7	34.5 ± 2.7
toosendanin	26.7 ± 2.7	37.9 ± 0	55.2 ± 2.7

[a]Values are means ± SE of three replicates.

The insecticidal activity of compounds **1**, **14-17**, **Ia-e**, **IIa-e**, **IIIa-e**, and **IVa-e** against the pre-third-instar larvae of *Mythimna separata* was tested by leaf-dipping method.[18,31] Toosendanin was used as a positive control. Leaves treated with acetone alone were used as a blank control group. The symptoms of

the treated *M. separata* were found in the same way as in our previous reports.[7,8] For example, some larvae with the wrinkled and slim bodies died at the larval stage (Fig. 4); as shown in Fig. 5, some larvae molted to malformed pupae and died during the pupation period; finally, some malformed moth appeared at the adult emergence stage (Fig. 6).

Fig. 4. Representative abnormal larvae of *M. separata* produced by compounds **Id** (YRG-173), **IIb** (YRG-181), **IId** (YRG-183), **IIIc** (YRG-187), **IIId** (YRG-188), **IVc** (YRG-192) and **IVe** (YRG-194) at 1 mg/mL during the larval period (**CK**: blank control group).

Fig. 5. Representative malformed pupae of *M. separata* produced by compounds **IIb** (YRG-181), **IId** (YRG-183), **IIIc** (YRG-187) and **IVc** (YRG-192) at 1 mg/mL during the pupation period (**CK**: blank control group).

Fig. 6. Representative malformed moth of *M. separata* produced by compounds **Id** (YRG-173), **IId** (YRG-183), **IIId** (YRG-188) and **IVe** (YRG-194) at 1 mg/mL during the stage of adult emergence (**CK**: blank control group).

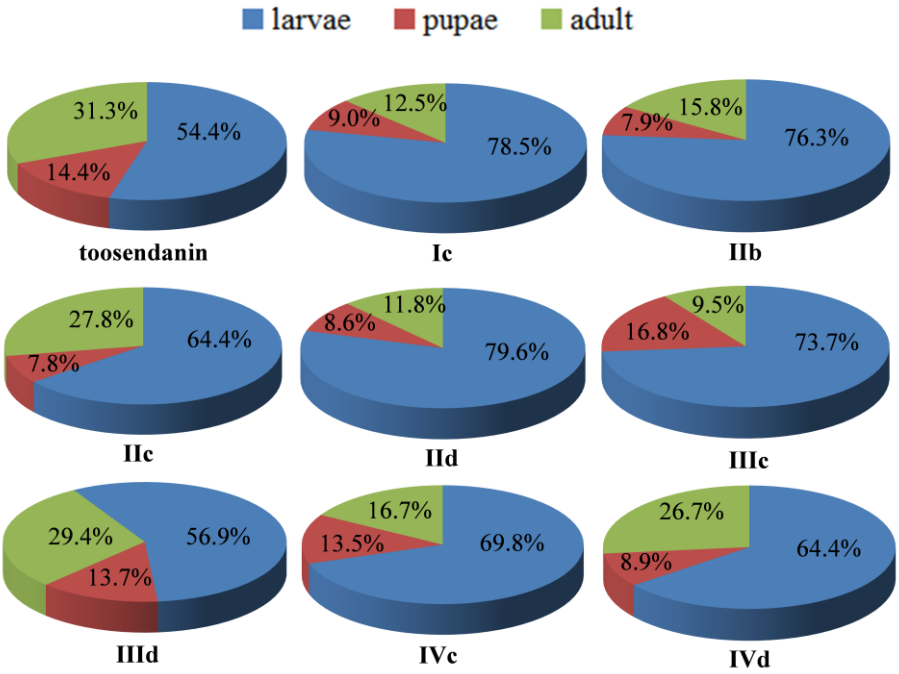


Fig. 7. The percentages of the final mortality rates at three different growth stages of compounds **Ic**, **IIb-d**, **IIIc**, **IIId**, **IVc**, **IVd** and toosendanin against of *Mythimna separata*.

As depicted in Table 2, among all derivatives, compounds **Ic**, **IIb-d**, **IIIc**, **IIId**, **IVc**, and **IVd** showed the potent insecticidal activity with the final mortality rates (FMRs) greater than 50%; For example, the FMRs of compounds **Ic**, **IIb-d**, **IIIc**, **IIId**, **IVc**, and **IVd** were 55.2%, 65.5%, 62.1%, 58.6%, 72.4%, 58.6%, 62.1% and 51.7%, respectively; whereas the FMR of their precusor (compound **1**) was only 34.5%. Especially compound **IIIc** exhibited the most potent pesticidal

activity with the FMR of 72.4%. As shown in Fig. 7, the percentages of FMRs at the larval stage of compounds **Ic**, **IIb-d**, **IIIc**, **IIId**, **IVc**, and **IVd** were all greater than 56%; especially the percentages of FMRs at the larval stage of compounds **Ic**, **IIb** and **IId** were 78.5%, 76.3%, and 79.6%, respectively. On the contrary, the percentages of FMRs at the pupation stage of compounds **Ic**, **IIb-d**, **IIIc**, **IIId**, **IVc**, and **IVd** were less than 17%, and the percentages of FMRs at the adult stage of compounds **Ic**, **IIb-d**, **IIIc**, **IIId**, **IVc**, and **IVd** were less than 30%, These results were different with those of other 2′(2′,6′)-(di)chloropicropodophyllotoxins derivatives, and more than half of their FMRs were generally at the pupation stage.[38]

Generally, as shown in Table 2, introduction of the halogen atom/atoms at the C-2′/C-2′,6′ position of isoxazoline-containing podophyllotoxins (**Ia-e**) could afford more potent 2′(2′,6′)-(di)halogenopodophyllotoxin derivatives (**IIa**–**e**, **IIIa**–**e**, and **IVa**–**e**). Interestingly, especially compounds **Ic**, **IIc**, **IIIc** and **IVc** (all containing 4-Cl on the phenyl of their isoxazoline fragment), exhibited more potent insecticidal activity than those (except **IIb**) containing other substituents (e.g., **Ic** vs **Ia,b,d,e**; **IIc** vs **Ia,d,e**; **IIIc** vs **IIIa,b,d,e**; **IVc** vs **IVa,b,d,e**). For example, once the chlorine atom of compound **Ic**, **IIc**, **IIIc** or **IVc** was substituted by other electron-withdrawing groups (such as the fluorine atom or the nitro group), the insecticidal activity of the corresponding compounds **Id**,**e**; **IId**,**e**; **IIId**,**e**; and **IVd**,**e** was sharply decreased.

In conclusion, a series of isoxazoline-containing

podophyllotoxin/2′(2′,6′)-(di)halogenopodophyllotoxin derivatives (**Ia**–**e**, **IIa**–**e**, **IIIa**–**e**, and **IVa**–**e**) were prepared, and evaluated for their pesticidal activities against *Mythimna separata* and *Tetranychus cinnabarinus*. Among all derivatives, compounds **IIc** and **IIIc** exhibited good insecticidal and acaricidal activities. It demonstrated that introduction of the halogen atom/atoms at the C-2′/C-2′,6′ position of podophyllotoxin, and introduction of a chlorine atom at the C-4 position on the phenyl of their isoxazoline fragment were very important for their insecticidal and acaricidal activities. These results will lay the foundation for further structural modifications of podophyllotoxin as potentially botanical acaricidal and insecticidal agents in crop protection.

Acknowledgements

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Supplementary data

Supplementary data (spectral data, and the protocol used for insecticidal and acaricidal studies) associated with this article can be found, in the online version.

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36. Representative spectral data for **Ia**, **IIa**, **IIIa**, and **IVa**: Compound **Ia**: Yield: 92%, white solid, mp = 96-98 °C; $[\alpha]^{20}_{D}$ = -24 (*c* 2.0 mg/mL, $CHCl_3$); IR cm^{-1} (KBr): 3004, 2924, 2852, 1775, 1588, 1504, 1483, 1460, 1238, 1125, 998, 764, 692; ^1H NMR (500 MHz, $CDCl_3$) δ: 7.66-7.69 (m, 2H), 7.40-7.46 (m, 3H), 6.82 (s, 0.5H, H-5), 6.79 (s, 0.5H, H-5), 6.55 (s, 0.5H, H-8), 6.54 (s, 0.5H, H-8), 6.39 (s, 1H, H-2′, H-6′), 6.38 (s, 1H, H-2′, 6′), 5.94-6.00 (m, 3H, H-4, OCH_2O), 5.23-5.27 (m, 1H, H-5″ (isoxazoline)), 4.59-4.61 (m, 1H, H-1), 4.36-4.40 (m, 1H, H-11), 4.16-4.23 (m, 1H, H-11), 3.81 (s, 1.5H, 3′-OCH_3), 3.80 (s, 1.5H, 3′-OCH_3), 3.74 (s, 3H, 5′-OCH_3), 3.72 (s, 3H, 4′-OCH_3), 3.58-3.70 (m, 2H, H-4″ (isoxazoline)), 2.87-2.98 (m, 2H, H-2, H-3). HRMS (ESI): Calcd for $C_{32}H_{29}NO_{10}Na$ ($[M+Na]^+$), 610.1689; found, 610.1691. Compound **IIa**: Yield: 85%, white solid, mp = 128-130 °C; $[\alpha]^{20}_{D}$ = -23 (*c* 3.7 mg/mL, $CHCl_3$); IR cm^{-1} (KBr): 3000, 2935, 2858, 1785, 1741, 1485, 1397, 1238, 1200, 1109, 1037, 1003, 864, 692; ^1H NMR (500 MHz, $CDCl_3$) δ: 7.68-7.71 (m, 2H), 7.43-7.47 (m, 2H), 6.78 (s, 0.5H, H-5), 6.77 (s, 0.5H, H-5), 6.43 (s, 0.5H, H-8), 6.40 (s, 0.5H, H-8), 6.22 (s, 1H, H-6′), 5.93-6.01 (m, 3H, H-4, OCH_2O), 5.27-5.31 (m, 1H, H-5″ (isoxazoline)), 5.24-5.25 (m, 1H, H-1), 4.39-4.46 (m, 1H, H-11), 4.15-4.22 (m, 1H, H-11), 3.915 (s, 1.5H, 3′-OCH_3), 3.910 (s, 1.5H, 3′-OCH_3), 3.870 (s, 3H, 5′-OCH_3), 3.866 (s, 3H, 5′-OCH_3), 3.69-3.78 (m, 2H, H-4″ (isoxazoline)), 3.63 (s, 3H, 4′-OCH_3), 3.14-3.23 (m, 1H, H-3), 3.00-3.05 (m, 1H, H-2). HRMS (ESI): Calcd for $C_{32}H_{28}ClNO_{10}Na$ ($[M+Na]^+$), 644.1299; found, 644.1291. Compound **IIIa**: Yield: 91%, white solid, mp = 129-131 °C; $[\alpha]^{20}_{D}$ = -6 (*c* 2.2 mg/mL, $CHCl_3$); IR

cm^{-1} (KBr): 3005, 2929, 2853, 1786, 1504, 1484, 1411, 1396, 1240, 1190, 1086, 1013, 935, 762, 692; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.70-7.72 (m, 2H), 7.43-7.46 (m, 3H), 6.85 (s, 0.5H, H-5), 6.74 (s, 0.5H, H-5), 6.35 (s, 0.5H, H-8), 6.33 (s, 0.5H, H-8), 6.07 (d, J = 10.0 Hz, 0.5H, H-4), 6.02 (d, J = 10.0 Hz, 0.5H, H-4), 5.94-5.95 (m, 2H, OCH_2O), 5.55 (d, J = 2.0 Hz, 0.5H, H-1), 5.34 (d, J = 2.0 Hz, 0.5H, H-1), 5.28-5.32 (m, 1H, H-5″ (isoxazoline)), 4.36-4.43 (m, 1H, H-11), 4.11-4.19 (m, 1H, H-11), 3.95 (s, 3H, 3′-OCH_3), 3.94 (s, 3H, 5′-OCH_3), 3.805 (s, 1.5H, 4′-OCH_3), 3.800 (s, 1.5H, 4′-OCH_3), 3.65-3.75 (m, 3H, H-3, H-4″ (isoxazoline)), 3.06-3.13 (m, 1H, H-2). HRMS (ESI): Calcd for $C_{32}H_{27}Cl_2NO_{10}Na$ ($[M+Na]^+$), 678.0909; found, 678.0933. Compound **IVa**: Yield: 78%, white solid, mp = 126-128 ^oC; $[\alpha]^{20}_D$ = -22 (c 2.0 mg/mL, $CHCl_3$); IR cm^{-1} (KBr): 3000, 2936, 2853, 1786, 1744, 1484, 1391, 1237, 1199, 1106, 1038, 1002, 929, 692; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.68-7.71 (m, 2H), 7.42-7.48 (m, 3H), 6.78 (s, 0.5H, H-5), 6.76 (s, 0.5H, H-5), 6.43 (s, 0.5H, H-8), 6.41 (s, 0.5H, H-8), 6.24 (s, 1H, H-6′), 5.93-6.00 (m, 3H, H-4, OCH_2O), 5.27-5.31 (m, 2H, H-1, H-5″ (isoxazoline)), 4.40-4.46 (m, 1H, H-11), 4.14-4.22 (m, 1H, H-11), 3.906 (s, 1.5H, 3′-OCH_3), 3.901 (s, 1.5H, 3′-OCH_3), 3.865 (s, 1.5H, 5′-OCH_3), 3.861 (s, 1.5H, 5′-OCH_3), 3.68-3.80 (m, 2H, H-4″ (isoxazoline)), 3.63 (s, 3H, 4′-OCH_3), 3.15-3.25 (m, 1H, H-3) 2.99-3.04 (m, 1H, H-2). HRMS (ESI): Calcd for $C_{32}H_{28}BrNO_{10}Na$ ($[M+Na]^+$), 688.0794; found, 688.0796.

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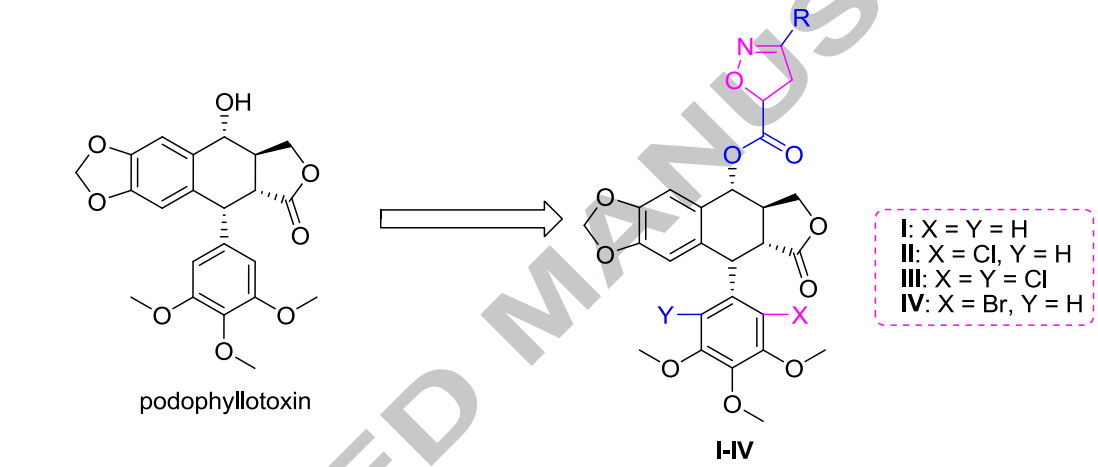
Graphic abstract

Synthesis of novel isoxazoline-containing podophyllotoxin/2′(2′,6′)-(di)halogenopodophyllotoxin derivatives and their insecticidal/acaricidal activities

Ruige Yang, Yuanyuan Zhang, and Hui Xu*



Tetranychus cinnabarinus

Against *Mythimna separata*