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Evaluation of andrographolide-based analogs derived from *Andrographis paniculata* against *Mythimna separata* Walker and *Tetranychus cinnabarinus* Boisduval

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ABSTRACT

To discover new natural-product-based pesticides, we structurally modified andrographolide, a labdane diterpenoid isolated from *Andrographis paniculata*, and stereoselectively prepared a series of 12α-(substituted) benzylamino-14-deoxyandrographolide derivatives (**I**–**V**). Three-dimensional structures of compounds **3c**, **3d**, **IIIa** and **IIIb** were further determined by single-crystal X-ray diffraction. Compounds **IIa** (R^1 = *n*-C_3H_7, R^2 = $PhCH_2$) exhibited more promising insecticidal activity against *Mythimna separata* than toosendanin. Compounds **3a** (R^1 = H), **Ib** (R^1 = H, R^2 = 4-ClPhCH$_2$), and **IVa** (R^1 = 4-ClPh, R^2 = $PhCH_2$) showed potent acaricidal activity against *Tetranychus cinnabarinus*.

1. Introduction

Andrographis paniculata, an erect annually medicinal herb, is widely distributed in India, Sri Lanka, Malay Peninsula, China and Thailand [1], and its plant extracts are known to contain diterpenes, flavonoids and stigmasterols [2]. Andrographolide (**1**, Fig. 1), a labdane diterpenoid, is extracted and isolated from the plants of *Andrographis paniculata*. Andrographolide and its derivatives showed lots of interesting biological properties including antiviral and anti-inflammatory activities [3], antioxidant activity [4], antitumor activity [5,6], anti-angiogenic activity [7], anti-influenza virus activity [8], and insecticidal activity [9]. On the other hand, the infestations of *Tetranychus cinnabarinus* Boisduval and *Mythimna separata* Walker are very serious to a wide range of citrus/other fruit trees and crops, and they are usually difficult to control and manage by the chemical pesticides [10,11]. Currently, new potential alternatives to effectively and selectively control pests should be urgently developed [12–14].

Although a variety of plant secondary metabolites are extracted and isolated, their activities are usually too low to develop as pesticidal agents [15,16]. Structural modification of those plant secondary metabolites is an imperative need to improve their activities [17]. Previously, some 19-alkyl(aryl)carbonyloxyandrographolide derivatives exhibited more potent insecticidal activity than toosendanin, a commercial botanical insecticide isolated from *Melia azedarach* (Fig. 1) [9]. Therefore, in this article, we further prepared a series of 12-(substituted)benzylamino-14-deoxyandrographolide derivatives (**I**–**V**, Fig. 1), and evaluated for their pesticidal activities against *M. separata* and *T. cinnabarinus*.

2. Experimental section

2.1. Preparation of 12-(substituted)benzylamino-14-deoxyandrographolides (I(a–c)–V(a–c))

A mixture of compounds **3a**–**e** (0.25 mmol), different benzylamines (0.45 mmol), and Et_3N (0.75 mmol) in dry dichloromethane (10 mL) was stirred at room temperature. After 16–24 h, the mixture was washed with saturated aq. $NaHCO_3$ (10 mL), brine (10 mL) and water (10 mL), dried over anhydrous Na_2SO_4, concentrated in *vacuo*, and purified by silica gel chromatography to afford target products **I**(**a**–**c**)–**V**(**a**–**c**). Exemplary data for compound **Ia**: Yield 68%, white solid, m.p. 62–64 °C; $[\alpha]_D^{20} = 7$ (c 3.0 mg/mL, $CHCl_3$); IR cm^{-1} (KBr): 3409, 3077, 2942, 1749, 1642, 1452, 1200, 1023, 744; ^1H NMR (500 MHz, $CDCl_3$) δ: 7.32–7.27 (m, 4H), 7.25–7.22 (m, 1H), 7.18 (s, 1H), 4.91 (d, J = 6.5 Hz, 1H), 4.83–4.81 (m, 3H), 4.79 (d, J = 6.0 Hz, 1H), 4.62 (s, 1H), 3.96 (d, J = 11.5 Hz, 1H), 3.74 (d, J = 13.0 Hz, 1H), 3.59–3.56

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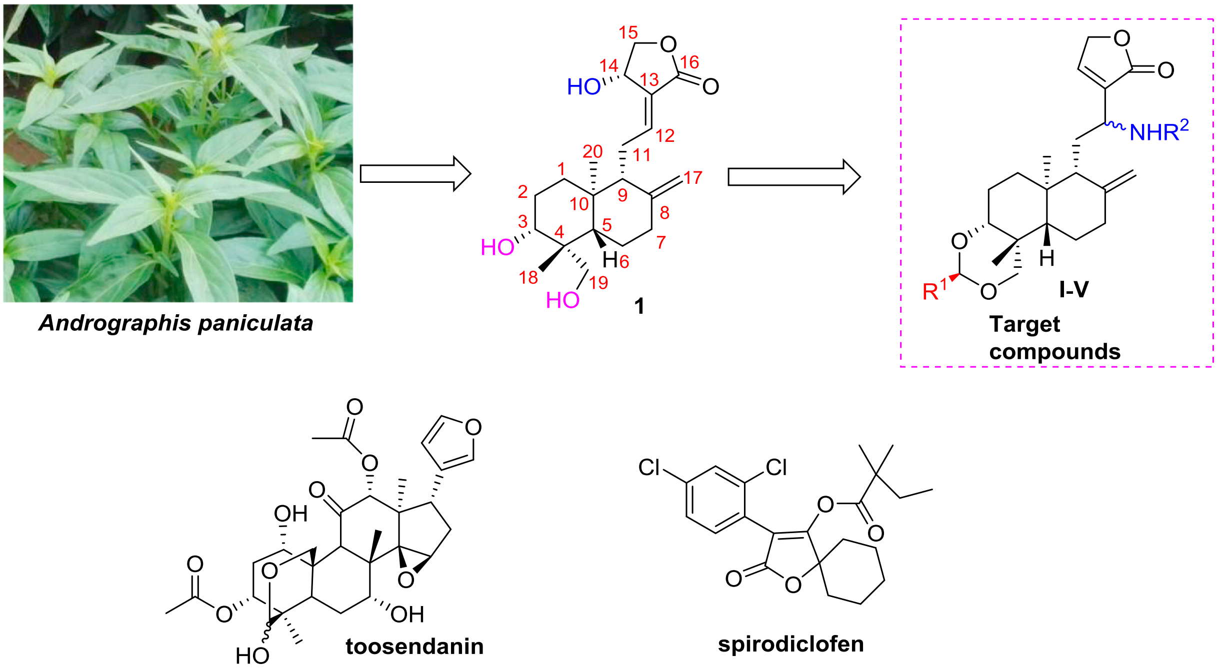
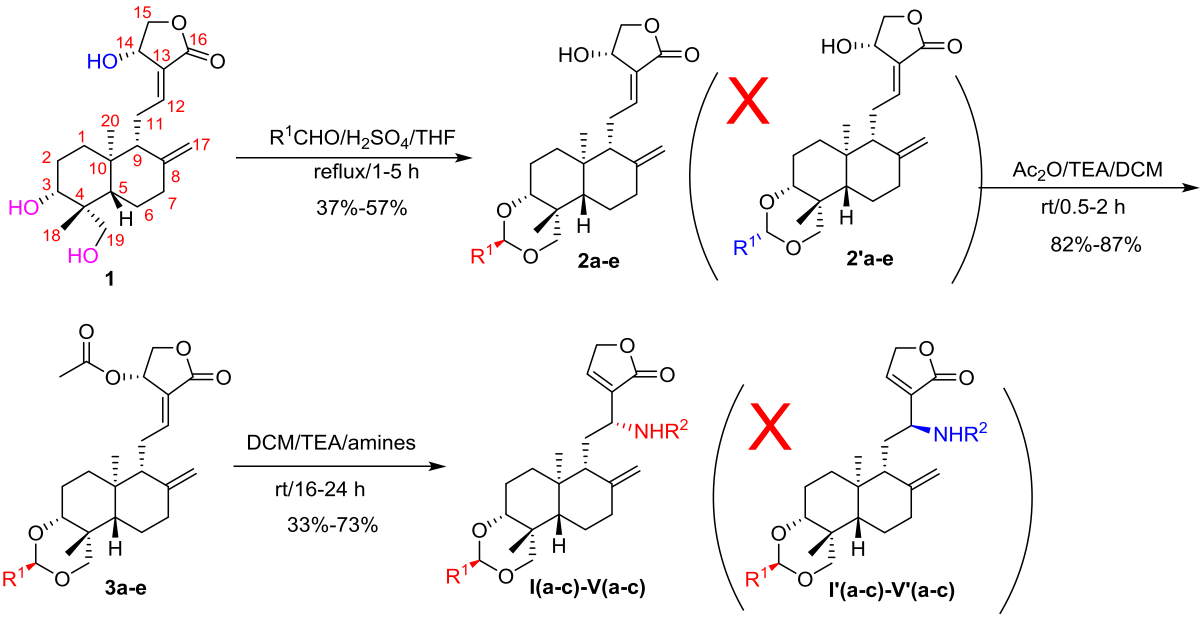


Fig. 1. Chemical structures of toosendanin, spirodiclofen, andrographolide (**1**), and its target compounds (**I**–**V**).



2a-e, and **3a-e**

R^1 =
a: H
b: n-C_3H_7
c: Ph
d: 4-ClPh
e: 4-OCH_3Ph

I(a-c)-V(a-c)

Ia: R^1 = H, R^2 = $PhCH_2$
Ib: R^1 = H, R^2 = 4-$ClPhCH_2$
Ic: R^1 = H, R^2 = 4-OCH_3PhCH_2
IIa: R^1 = n-C_3H_7, R^2 = $PhCH_2$
IIb: R^1 = n-C_3H_7, R^2 = 4-$ClPhCH_2$
IIc: R^1 = n-C_3H_7, R^2 = 4-OCH_3PhCH_2
IIIa: R^1 = Ph, R^2 = $PhCH_2$
IIIb: R^1 = Ph, R^2 = 4-$ClPhCH_2$
IIIc: R^1 = Ph, R^2 = 4-OCH_3PhCH_2
IVa: R^1 = 4-ClPh, R^2 = $PhCH_2$
IVb: R^1 = 4-ClPh, R^2 = 4-$ClPhCH_2$
IVc: R^1 = 4-ClPh, R^2 = 4-OCH_3PhCH_2
Va: R^1 = 4-OCH_3Ph, R^2 = $PhCH_2$
Vb: R^1 = 4-OCH_3Ph, R^2 = 4-$ClPhCH_2$
Vc: R^1 = 4-OCH_3Ph, R^2 = 4-OCH_3PhCH_2

Scheme 1. Semisynthesis of 12α-(substituted)benzylamino-14-deoxyandrographolides (**I**(**a**–**c**) –**V**(**a**–**c**)).

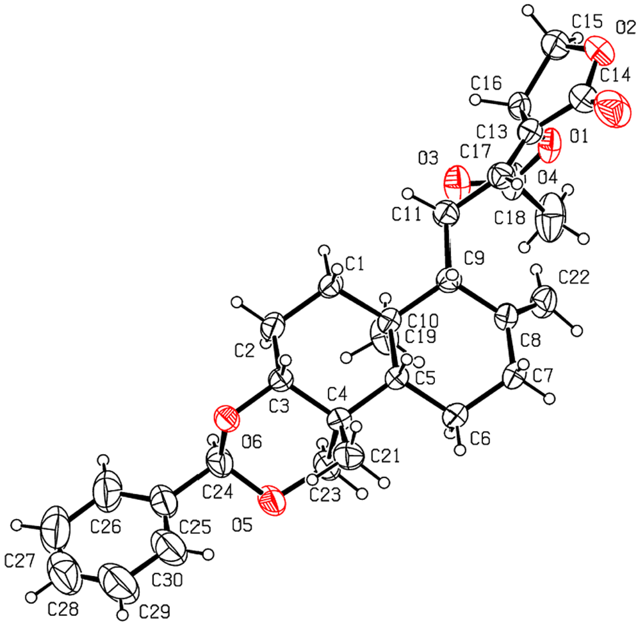


Fig. 2. X-ray crystal structure of compound **3c**.

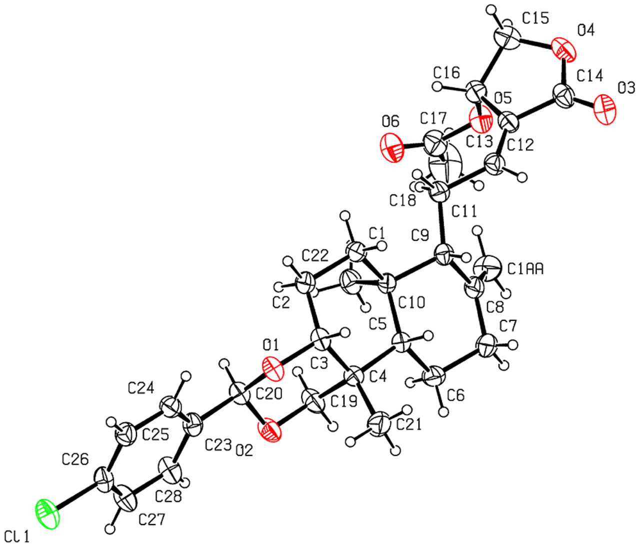


Fig. 3. X-ray crystal structure of compound **3d**.

(m, 2H), 3.42–3.37 (m, 2H), 2.38 (d, $J = 11.5$ Hz, 1H), 2.24–2.21 (m, 1H), 2.03–1.99 (m, 1H), 1.87–1.80 (m, 3H), 1.75–1.73 (m, 1H), 1.63–1.60 (m, 1H), 1.35 (s, 3H), 1.34–1.33 (m, 1H), 1.19–1.16 (m, 1H), 1.07–1.05 (m, 1H), 0.94–0.91 (m, 1H), 0.74 (s, 3H); HRMS (ESI): Calcd for $C_{28}H_{38}NO_4$ ($[M + H]^+$) 452.2795; found, 452.2812.

2.2. Biological assay

The growth inhibitory and acaricidal activities of compounds **1**, **2a–e**, **3a–e** and **I**(**a–c**)–**V**(**a–c**) were tested against early 3rd-instar larvae of *M. separata* and female adults of *T. cinnabarinus*, respectively [11,18].

3. Results and discussion

3.1. Chemistry

As shown in Scheme 1, to our delight, only intermediates **2a–e** (not **2′a–e**) were stereoselectively obtained by reaction of andrographolide (**1**) with different aldehydes R^1CHO in the presence of conc. H_2SO_4. Then compounds **2a–e** reacted with acetic anhydride in the presence of Et_3N to give compounds **3a–e**. Finally, only target products **I(a–c)–V (a–c)** (not **I'(a–c)–V'(a–c)**) were stereoselectively produced by reaction of compounds **3a–e** with different benzylamines [19]. Besides their structures characterized by melting points, optical rotation, IR, ^1H NMR and HRMS of target compounds **3c** (Fig. 2, CCDC 1886158), **3d** (Fig. 3, CCDC 1886163), **IIIa** (Fig. 4, CCDC 1848412), and **IIIb** (Fig. 5, CCDC 1886159) were further determined by X-ray crystallography. Notably, the substituents R^1 and C14-acetyloxy of compounds **3c** and **3d** were at β and α configuration, respectively (Figs. 2 and 3). Moreover, the substituents R^1 and R^2NH of compounds **IIIa** and **IIIb** were at β and α configuration, respectively (Figs. 4 and 5). It further demonstrated that compound **1** reacting with different aldehydes R^1CHO catalyzed by conc. H_2SO_4, and compounds **3a–e** reacting with different benzylamines in the presence of Et_3N were stereoselective. Because of the steric hindrance of the C-3α-OH and C-4α-CH_2OH of compound **1**, R^1 as β configuration was beneficial. Due to the steric hindrance of hydrogens at the C-9 and C-11 positions of compounds **3a–e**, the Michael addition of benzylamines to their C-12 position from the back of the double bond was more easily than that from the front.

3.2. Pesticidal activities

3.2.1. Growth inhibitory activity of compounds 1, 2a–e, 3a–e and I (a–c)–V(a–c) against M. Separata

As described in Table 1, *against M. separata*, compounds **2b**, **2c**, **3a**, **3d**, **IIa–c**, **IIIb**, and **IVb** displayed more potent insecticidal activity than toosendanin. The final mortality rates (FMRs) of **2b**, **2c**, **3a**, **3d**, **IIa–c**, **IIIb**, and **IVb** were 58.6%, 58.6%, 58.6%, 48.3%, 62.1%, 51.7%, 55.2%, 51.7%, and 48.3%, respectively; whereas the FMRs of their precusor and toosendanin were 37.9%, and 44.8%, respectively. Among all derivatives, compound **IIa** ($R^1 = n\text{-}C_3H_7$, R^2 = benzyl) showed the most potent insecticidal activity with the FMR of 62.1%. Moreover, the toxic symptoms for the treated *M. separata* during the larval, pupation and adult periods were recorded and observed as the same in our previous papers [18,20]. The percentages of FMRs at three different growth stages of compounds **2b**, **2c**, **3a**, **IIa**, **IIc** and toosendanin were shown in Fig. 6. More than 50.0% (the largest partion) of FMRs for compounds **2c**, **3a**, **IIa**, and **IIc** was at the larval stage, which was the same as that for esters of fraxinellone C4/10-oxime [11], matrine ethers [21], and toosendanin. It suggested that, when compared with

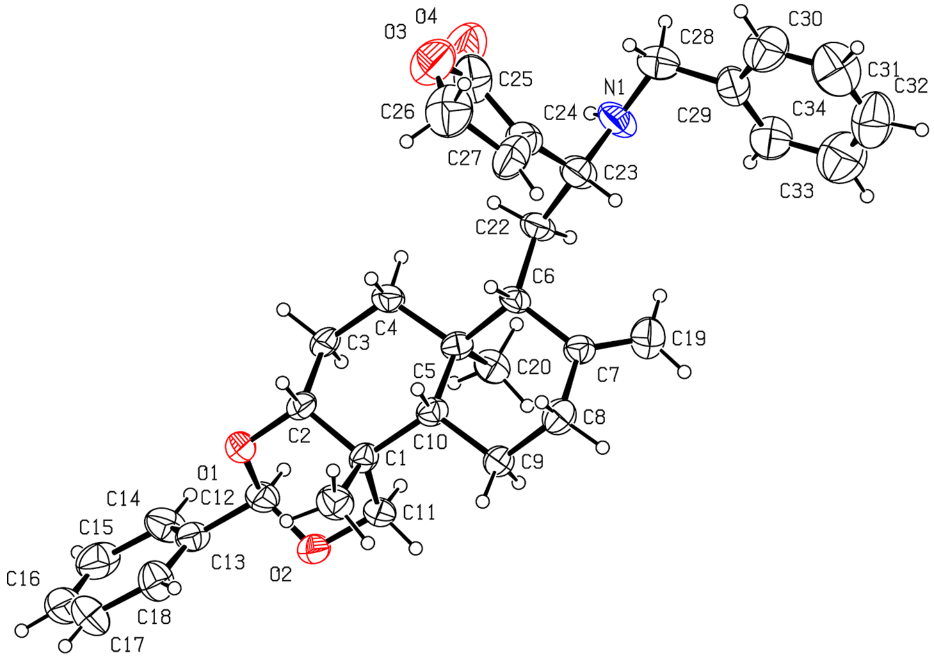


Fig. 4. X-ray crystal structure of compound **IIIa**.

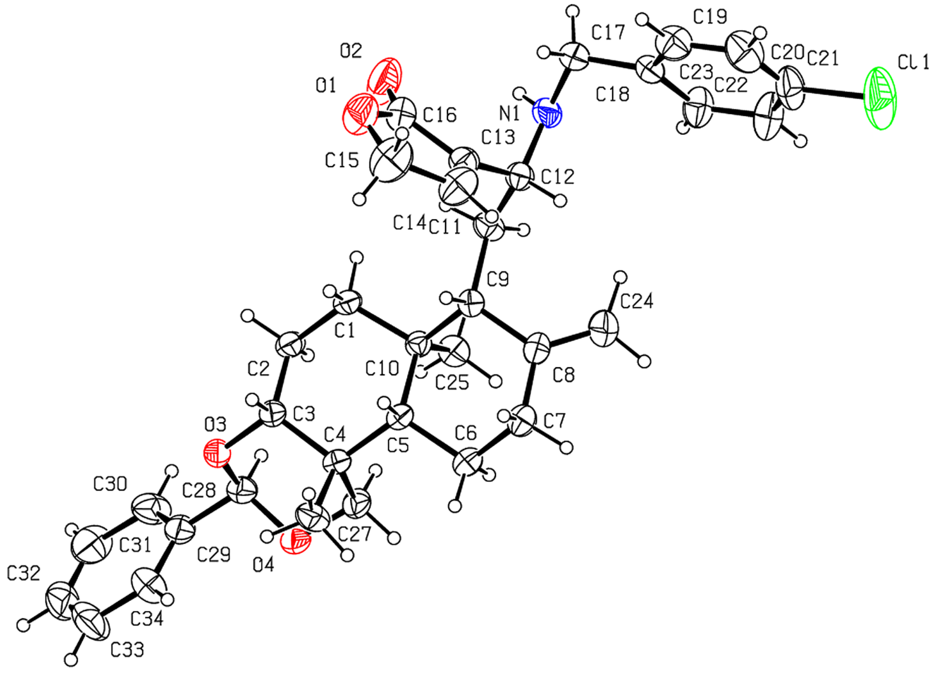


Fig. 5. X-ray crystal structure of compound **IIIb**.

toosendanin, fraxinellone C4/10-oxime esters and matrine ethers, these andrographolide derivatives may exhibit the same mechanism of action against early 3rd-instar larvae of *M. separata*. To compounds **2a**–**e**, R^1 as *n*-C_3H_7 or phenyl could lead to potent compounds **2b** and **2c**; whereas introduction of the chlorine atom or the methoxy on the phenyl of **2c** resulted in less active compounds **2d** and **2e**. To compounds **3a**–**e**, interestingly, when the acetyloxy was introduced at the C-14 position of compound **2a**, the FMR of the corresponding compound **3a** was increased from 44.8% to 58.6%. To compounds **I**(**a**–**c**)–**V**(**a**–**c**), R^1 as *n*-C_3H_7 was important for the insecticidal activity. For example, FMRs of **IIa**–**c** were 62.1%, 51.7%, and 55.2%, respectively. It demonstrated that the insecticidal activity of different compounds was probably based on the nature and size of their substituents.

Table 1
Growth inhibitory activity of compounds **1**, **2a**–**e**, **3a**–**e** and **I**(**a**–**c**)–**V**(**a**–**c**) against *M. separata* at 1 mg/mL.[a]

Compound	Corrected mortality rate (%)		
	10 days	20 days	35 days
1	6.7 ± 3.3	17.2 ± 6.0	37.9 ± 6.0
2a	10.0 ± 5.8	34.5 ± 6.9	44.8 ± 3.4
2b	13.3 ± 3.3	24.1 ± 9.1	58.6 ± 6.0
2c	23.3 ± 3.3	44.8 ± 3.4	58.6 ± 6.0
2d	10.0 ± 5.8	20.7 ± 3.4	34.5 ± 3.4
2e	20.0 ± 5.8	27.6 ± 6.0	34.5 ± 3.4
3a	16.7 ± 3.3	41.4 ± 6.9	58.6 ± 6.0
3b	13.3 ± 3.3	17.2 ± 6.0	34.5 ± 3.4
3c	10.0 ± 5.8	20.7 ± 6.9	27.6 ± 6.0
3d	13.3 ± 3.3	31.0 ± 3.4	48.3 ± 6.0
3e	20.0 ± 5.8	31.0 ± 3.4	37.9 ± 6.0
Ia	10.0 ± 5.8	24.1 ± 6.9	34.5 ± 3.4
Ib	16.7 ± 6.7	20.7 ± 6.9	44.8 ± 3.4
Ic	16.7 ± 6.7	27.6 ± 6.0	41.4 ± 9.1
IIa	36.7 ± 3.3	41.4 ± 3.4	62.1 ± 3.4
IIb	10.0 ± 5.8	31.0 ± 3.4	51.7 ± 3.4
IIc	6.7 ± 6.7	24.1 ± 3.4	55.2 ± 3.4
IIIa	16.7 ± 6.7	34.5 ± 3.4	44.8 ± 3.4
IIIb	3.3 ± 3.3	20.7 ± 3.4	51.7 ± 3.4
IIIc	10.0 ± 5.8	34.5 ± 3.4	44.8 ± 3.4
IVa	10.0 ± 5.8	20.7 ± 3.4	31.0 ± 3.4
IVb	23.3 ± 3.3	31.0 ± 3.4	48.3 ± 6.0
IVc	16.7 ± 3.3	27.6 ± 6.0	41.4 ± 6.9
Va	10.0 ± 5.8	20.7 ± 6.9	41.4 ± 3.4
Vb	6.7 ± 3.3	13.8 ± 6.9	20.7 ± 3.4
Vc	13.3 ± 6.7	20.7 ± 9.1	34.5 ± 3.4
toosendanin	26.7 ± 6.7	37.9 ± 6.0	44.8 ± 3.4

[a] Values are the mean ± SE of three replicates.

3.2.2. *Acaricidal activity of compounds 1, 2a–e, 3a–e and I(a–c)–V(a–c) against T. Cinnabarinus*

As shown in Table 2, after structural modifications of andrographolide (**1**), all derivatives (except **2c**, **2e**, and **IVb**) showed more potent acaricidal activity than compound **1** (spirodiclofen (Fig. 1) was used as a positive control). Among them, 72 h MRs of compounds **2b**, **3a**–**c**, **Ib**, **Ic**, **IIb**, **IIIc**, **IVa**, and **IVc** were greater than 30%. Especially 72 h MRs of compounds **3a**, **Ib**, and **IVa** were 38.3%, 41.1%, and 39.2%, respectively; whereas 72 h MR of compound **1** was only 14.4%. To compounds **2a**–**e**, R^1 as *n*-C_3H_7 was important for the acaricidal activity, and led to potent compound **2b**. Notably, when the acetyloxy was introduced at the C-14 position of compounds **2a**–**e**, the corresponding compounds **3a**–**e** all showed potent activity. It suggested that the OH at the C-14 position of compounds **2a**–**e** was not vital for the acaricidal activity. For example, 72 h MRs of compounds **2a**–**e** were 23.2%, 34.3%, 11.6%, 16.8%, and 7.8%, respectively; whereas 72 h MRs of compounds **3a**–**e** were 38.3%, 31.4%, 32.2%, 29.6%, and 29.1%, respectively. To compounds **I**(**a**–**c**)–**V**(**a**–**c**), R^1 as H and R^2 as 4-$ClPhCH_2$ or R^1 as 4-ClPh and R^2 as $PhCH_2$ was necessary for the acaricidal activity. For example, 72 h MRs of compounds **Ib** and **IVa** were 41.1%, and 39.2%, respectively.

4. Conclusion

In this study, 12α-(substituted)benzylamino-14-deoxyandrographolides (**I**–**V**) were prepared by structural modifications of andrographolide. Especially compounds **2b**, **3a** and **IIb** displayed promising pesticidal activities against *M. separata* and *T. cinnabarinus*. It suggested that R^1 as *n*-C_3H_7 was important for the insecticidal activity, R^1 as H and R^2 as 4-$ClPhCH_2$ or R^1 as 4-ClPh and R^2 as $PhCH_2$ was necessary for the acaricidal activity; and the pesticidal activity of different compounds was probably determined by the nature and size of their substituents.

Conflicts of interest

The authors declare no competing financial interest.

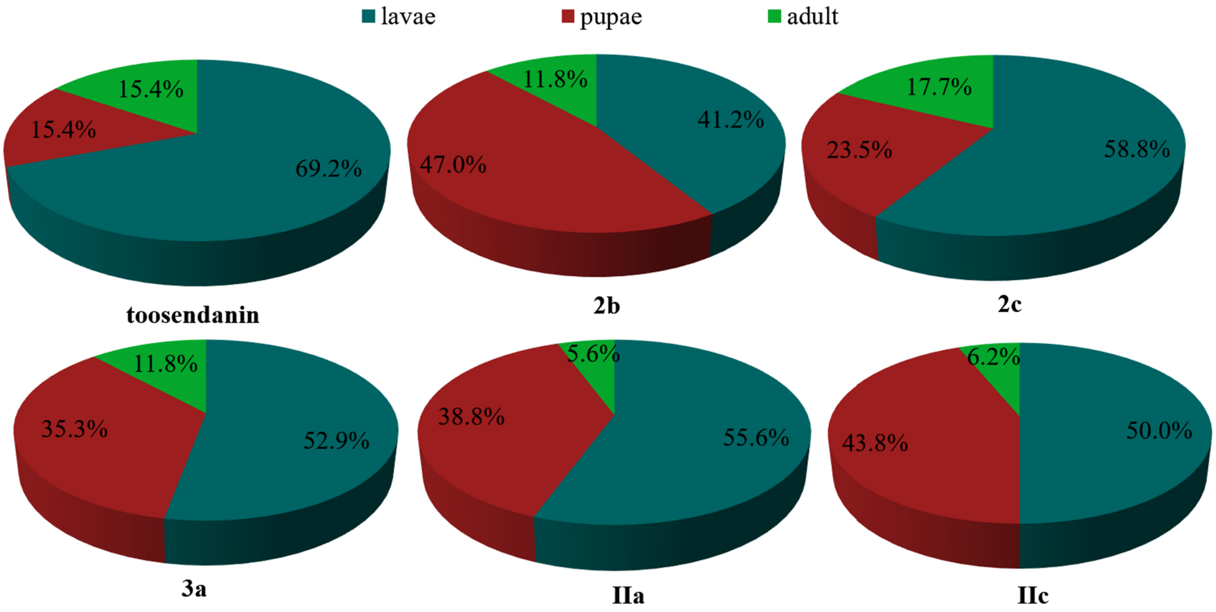


Fig. 6. The percentages of FMRs at three different growth stages of compounds **2b**, **2c**, **3a**, **IIa**, **IIc** and toosendanin.

Table 2
Acaricidal activity of compounds **1**, **2a–e**, **3a–e** and **I**(**a**–**c**)–**V**(**a**–**c**) against *T. cinnabarinus* at 0.5 mg/mL.[a]

Compound	Corrected mortality rate (%)	
	48 h	72 h
1	5.9 ± 0.8	14.4 ± 1.7
2a	3.5 ± 1.2	23.2 ± 4.8
2b	7.8 ± 1.5	34.3 ± 4.8
2c	9.2 ± 3.0	11.6 ± 3.2
2d	12.1 ± 1.2	16.8 ± 0.5
2e	5.0 ± 2.2	7.8 ± 2.2
3a	6.0 ± 2.0	38.3 ± 6.0
3b	11.0 ± 2.8	31.4 ± 3.9
3c	7.1 ± 0.8	32.2 ± 4.2
3d	14.5 ± 2.6	29.6 ± 3.5
3e	7.9 ± 2.2	29.1 ± 4.5
Ia	20.7 ± 1.8	25.6 ± 1.4
Ib	26.5 ± 5.8	41.1 ± 4.6
Ic	22.0 ± 4.0	32.9 ± 5.7
IIa	15.4 ± 2.4	23.1 ± 0.5
IIb	19.4 ± 4.0	32.1 ± 1.9
IIc	19.0 ± 3.5	21.6 ± 3.5
IIIa	15.9 ± 0.9	21.0 ± 3.6
IIIb	14.5 ± 5.4	16.7 ± 4.3
IIIc	8.5 ± 0.8	30.1 ± 6.0
IVa	25.1 ± 2.8	39.2 ± 1.8
IVb	6.7 ± 1.2	11.4 ± 1.6
IVc	20.1 ± 1.4	33.0 ± 2.5
Va	13.7 ± 4.7	26.1 ± 1.7
Vb	16.9 ± 1.7	18.0 ± 3.0
Vc	16.1 ± 1.5	24.7 ± 3.7
spirodiclofen	70.3 ± 4.6	95.2 ± 3.0

[a] Values are the mean ± SE of three replicates.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.01.020.

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