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Synthesis and insecticidal activities of 5-deoxyavermectin B2a oxime ester derivatives†

Guoshao Sun,[‡] Jingjing Zhang,[‡] Shuhui Jin and Jianjun Zhang^{*}

Three series of avermectin B2a oxime ester derivatives were synthesized using avermectin B2a as starting material. All of the compounds were characterized by ¹H NMR, ¹³C NMR, and HRMS. Bioassay results indicated that some of the derivatives (**8b**, **8c**, **8d**, **8f**, **11k**, **11l**, **14c**, **14j**) showed potent insecticidal activities against *Myzus persicae*, *Caenorhabditis elegans*, or *Tetranychus cinnabarinus*. As shown by initial insecticidal activity data, compound **8d** showed excellent activities (>90%) against *M. persicae* and *C. elegans*, which were more potent than that of avermectin B2a. Compound **8d** might be a lead compound for designing new avermectin B2a derivatives.

Introduction

Although chemical pesticides remain the most effective method to control pests in agriculture, the increasing and long-term application of chemical pesticides has resulted in pest resistance and negative impacts on human health and environmental safety.^{1–3} Considering that the use of natural pesticides may decelerate resistance development and reduce environmental pollution, greater attention has been placed on the development of nature-product-based agrochemicals.^{4–8} Avermectins, one of the most important classes of natural insecticides, anthelmintics, and acaricides, are a family of 16-membered ring macrocyclic lactones isolated from the culture broth of *Streptomyces avermitilis*.^{9–11} Compared with traditional pesticides, avermectins can interact stereoselectively and with a high affinity with the glutamate-gated chloride channel, leading to a chloride ion flux into neurons, causing paralysis and death.^{12–14} Due to their remarkable biological activity and low mammalian toxicity, avermectins have long been widely commercialized for agricultural use in China.

During the industrial production of avermectin, two end-products are obtained – avermectin B1a (Fig. 1) and avermectin ointment.¹⁵ Avermectin ointment, composed of approximately 30% avermectin B2a (Fig. 1), is the mother liquor retrieved during the purification of avermectin B1a.¹⁶ In 2012, China banned the use of avermectin ointment in the production of avermectin emulsifiable concentrate, which was the primary use of avermectin ointment.¹⁶ However, given the high toxicity of avermectin ointment, its disposal requires special treatment

and high costs, resulting in a waste of resources. Thus, we developed a purification method to retrieve avermectin B2a from avermectin ointment, which allowed for further molecule structure optimization.

Various efforts have been made to obtain compounds with higher potency and broader activity spectra by modifying the avermectin B1a molecule.^{13,17–19} For example, ivermectin (22,23-dihydro-avermectin B1a, Fig. 1), has the same excellent potency and spectrum against nematode parasites as avermectin B1a, albeit with a greater safety profile.²⁰ Indeed, ivermectin is widely used as an anthelmintic agent for human and animal health. Furthermore, emamectin (Fig. 1), which has a 1500-fold increased insecticidal activity compared to avermectin B1a, has achieved commercial success.¹³ However, few studies and patents have been concerned with the structural modification of avermectin B2a (Fig. 1).^{21,22} Avermectin B2a has a slightly lower

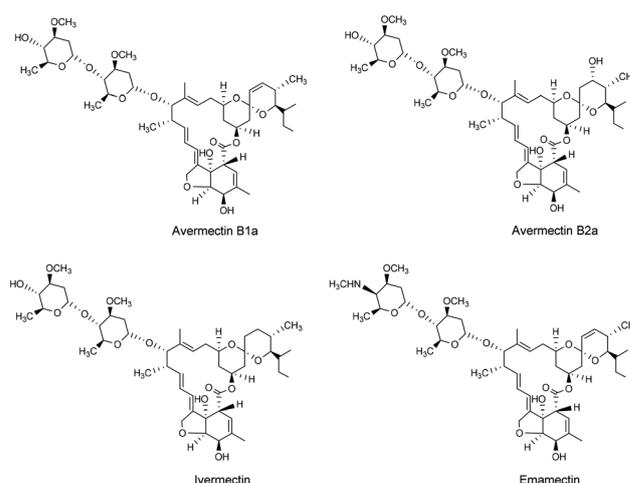


Fig. 1 The chemical structures of avermectin B1a, B2a, ivermectin, and emamectin.

Department of Applied Chemistry, College of Science, China Agricultural University, Beijing, 100193, P. R. China. E-mail: zhangjianjun@cau.edu.cn

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‡ These authors contributed equally to this work.



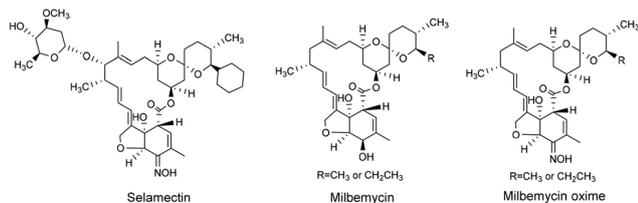


Fig. 2 The chemical structures of selamectin, milbemycin, and milbemycin oxime.

biological activity than avermectin B1a, but with greater safety,²³ making it a favorable candidate for further optimization and development.

Various avermectin and milbemycin (Fig. 2) oximes have been reported to have excellent biological activity.^{18,24,25} For instance, selamectin (avermectin monosaccharide analogue, Fig. 2), and milbemycin oxime (Fig. 2) are well-known broad-spectrum anthelmintics with outstanding efficacy and safety.^{26,27} Further, 5-keto-5-oxime ester derivatives of milbemycin have shown a higher efficacy in microfilariae control than milbemycin itself.²⁵

Based on the previous considerations, we reported herein on the design and synthesis of three series of avermectin B2a oxime ester derivatives. The insecticidal activities of the target compounds against *M. persicae*, *C. elegans*, and *T. cinnabarinus* were evaluated. Additionally, the impact of changes involving carboxylic group substitutions and saccharide functionality were discussed, potentially providing some useful structural insights for further refinement.

Experimental

Chemicals and instruments

All reagents and solvents were of analytical grade and used without further purification. The melting points were measured on a YRT-3 apparatus and were uncorrected. ¹H-NMR and ¹³C-NMR spectra were recorded on a Bruker Avance 300 spectrometer (300 MHz, ¹H; 75 MHz, ¹³C) in CDCl₃ or DMSO-*d*₆ solution with tetramethylsilane as the internal standard. Chemical shifts were reported in ppm (δ). High-resolution mass spectra (ESI-HRMS) were performed on a Bruker Daltonics Bio-TOF-Q III mass spectrometer.

Synthesis of 5-*o*-alloc avermectin B2a (compound 2; Scheme 1)

A solution of allyl chloroformate (AllocCl, 5.3 g, 44.0 mmol) in dried dichloromethane (CH₂Cl₂, 50 mL) was added dropwise over 30 minutes to a solution of avermectin B2a (30.0 g, 33.7 mmol) and *N,N,N',N'*-tetramethylethylene diamine (TEMED, 7.8 g, 67.1 mmol) in CH₂Cl₂ (100 mL) at -25 °C. The mixture was stirred for 1 h at -25 °C. The reaction mixture was poured into water and extracted with CH₂Cl₂ (3 × 100 mL). The organic layer was washed with 5% dilute hydrochloric acid (100 mL), 5% aqueous sodium bicarbonate (100 mL), and saturated sodium chloride solution (100 mL), dried over anhydrous sodium sulfate, filtered, and evaporated. The resulting residue was purified by column chromatography on silica gel using

a mixture of petroleum ether and ethyl acetate (v/v = 3 : 1) as the eluent to afford compound 2 as a pale yellow foamy solid (28.2 g, 86%). Mp 122–125 °C. ¹H NMR (300 MHz, CDCl₃) δ 5.68–6.06 (m, 4H, H9, H10, H11, CH₂=CH–CH₂O), 5.53–5.65 (m, 1H, H3), 5.24–5.46 (m, 5H, H5, H1'', H19, CH₂=CH–CH₂O), 4.91–5.06 (m, 1H, H15), 4.57–4.86 (m, 5H, H1', H8a × 2, CH₂=CH–CH₂O), 4.14 (d, *J* = 6.1 Hz, 1H, H13), 3.71–4.03 (m, 6H, 7-OH, H23, 23-OH, H5'', H5', H6), 3.41–3.71 (m, 10H, H25, H17, H3'', H3', 3''-OCH₃, 3'-OCH₃), 3.36–3.42 (m, 1H, H2), 3.26 (t, *J* = 9.0 Hz, 1H, H4'), 3.18 (t, 1H, *J* = 9.2 Hz, H4''), 2.48–2.79 (m, 2H, H12, H24), 2.18–2.43 (m, 4H, H16 × 2, H2', H2''), 1.76–2.10 (m, 5H, H18, Me4a, H20), 1.45–1.69 (m, 11H, Me14a, H20, H26, H27 × 2, H22 × 2, H2', H2''), 1.24–1.35 (m, 6H, Me6', Me6''), 1.19 (d, *J* = 6.9 Hz, 3H, Me12a), 0.86–1.03 (m, 10H, H28, Me24a, Me26a, H18); ¹³C NMR (75 MHz, CDCl₃) δ 173.19, 154.79, 139.31, 137.98, 135.66, 133.09, 131.46, 124.72, 121.47, 120.40, 118.66, 117.54, 99.61, 98.47, 94.81, 81.64, 80.88, 80.40, 79.30, 78.18, 77.51, 76.06, 73.51, 70.81, 69.83, 68.69, 68.38, 68.29, 68.12, 67.74, 67.24, 56.42, 56.36, 45.71, 41.16, 40.74, 39.71, 36.37, 35.69, 35.11, 34.46, 34.21, 34.09, 27.24, 20.15, 19.58, 18.35, 17.63, 15.09, 13.72, 12.37, 11.76. HRMS (ESI) calcd for C₅₂H₇₉O₁₇ (M + H)⁺ 975.5317, found 975.5328.

Synthesis of 5-*o*-alloc-4'',23-bis-*o*-(*tert*-butyldimethylsilyl) avermectin B2a (compound 3; Scheme 1)

Triethylamine (TEA, 19.6 g, 193.7 mmol) and *tert*-butyldimethylsilyl chloride (TBDMSCl, 25.0 g, 165.9 mmol) were added to a solution of 5-*o*-alloc avermectin B2a (27.0 g, 27.7 mmol) in 150 mL of dry *N,N*-dimethylformamide (DMF). The reaction mixture stirred at -5 °C for 48 h. The reaction mixture was diluted with 300 mL of ether, water (150 mL) was added and the layers were separated. The aqueous layers was extracted twice with ether (2 × 200 mL) and the combined organic layers was dried over magnesium sulfate, filtered, and evaporated to a yellow foam. The foam was purified by column chromatography on silica gel using a mixture of petroleum ether and ethyl acetate (v/v = 10 : 1) as the eluent to afford compound 3 as a yellow foamy solid (26.9 g, 81%). Mp 128–131 °C. ¹H NMR (300 MHz, CDCl₃) δ 5.68–6.05 (m, 4H, H9, H10, H11, CH₂=CH–CH₂O), 5.52–5.63 (m, 1H, H3), 5.22–5.43 (m, 5H, H5, H1'', H19, CH₂=CH–CH₂O), 4.92–5.06 (m, 1H, H15), 4.59–4.86 (m, 5H, H1', H8a × 2, CH₂=CH–CH₂O), 4.09–4.16 (m, 2H, H13, 7-OH), 3.94 (s, 1H, 23-OH), 3.71–4.03 (m, 7H, H23, H5'', H5', H6, H17, H3'', H3'), 3.30–3.48 (m, 8H, H25, 3''-OCH₃, 3'-OCH₃, H2), 3.22 (t, *J* = 8.9 Hz, 1H, H4'), 3.14 (t, 1H, *J* = 8.8 Hz, H4''), 2.45–2.60 (m, 1H, H12), 1.77–2.39 (m, 10H, H16 × 2, H2', H2'', H24, H18, Me4a, H20), 1.45–1.69 (m, 11H, Me14a, H20, H26, H27 × 2, H22 × 2, H2', H2''), 1.13–1.32 (m, 9H, Me6', Me6'', Me12a), 0.84–1.01 (m, 28H, H28, Me24a, Me26a, H18, (CH₃)₃Si(CH₃)₂), 0.04–0.12 (m, 12H, (CH₃)₃Si(CH₃)₂); ¹³C NMR (75 MHz, CDCl₃) δ 173.47, 154.81, 139.12, 138.17, 134.55, 133.00, 131.50, 124.62, 121.63, 120.47, 118.85, 118.65, 98.57, 97.62, 94.88, 81.87, 79.29, 78.55, 77.20, 77.14, 73.58, 69.90, 69.40, 69.01, 68.88, 68.70, 68.45, 67.34, 67.27, 56.57, 56.26, 45.73, 42.48, 41.69, 39.77, 36.55, 36.36, 35.33, 34.64, 34.15, 29.65, 27.34, 26.01, 25.86, 20.18, 19.61, 18.36, 18.30, 18.27,



18.13, 15.06, 13.96, 12.25, 11.95, -3.99, -4.40, -4.77, -4.99. HRMS (ESI) calcd for $C_{64}H_{107}O_{17}Si_2$ ($M + H$)⁺ 1203.7047, found 1203.7083.

Synthesis of 4'',23-bis-*o*-(*tert*-butyldimethylsilyl) avermectin B2a (compound 4; Scheme 1)

Ammonium acetate (33.3 g, 432.0 mmol) was added to a solution of 5-*o*-alloc-4'',23-bis-*o*-(*tert*-butyldimethylsilyl) avermectin B2a (26.0 g, 21.6 mmol) in methanol (MeOH, 40 mL) and tetrahydrofuran (THF, 40 mL) at -10 °C. With vigorous stirring, sodium borohydride (NaBH₄, 0.41 g, 10.8 mmol), tetrakis (triphenylphosphine) palladium (Pd[PPh₃]₄, 1.0 g, 0.9 mmol), and another portion of NaBH₄ (0.41 g, 10.8 mmol) were added sequentially and quickly. The reaction was monitored with TLC until completion. The reaction mixture was concentrated under vacuum. The residue was diluted with CH₂Cl₂ (100 mL) and washed with brine (100 mL), then the organic phase was dried over anhydrous sodium sulfate, filtered, and evaporated to a yellow foam. The foam was purified by column chromatography on silica gel using a mixture of petroleum ether and ethyl acetate (v/v = 6 : 1) as the eluent to afford compound 4 as a yellow foamy solid (21.3 g, 88%). Mp 145–148 °C. ¹H NMR (300 MHz, CDCl₃) δ 5.82–5.93 (m, 1H, H9), 5.68–5.80 (m, 2H, H10, H11), 5.26–5.45 (m, 3H, H3, H1', H19), 4.93–5.09 (m, 1H, H15), 4.79 (d, *J* = 2.8 Hz, 1H, H1'), 4.67 (s, 2H, H8a × 2), 4.29 (t, *J* = 7.1 Hz, 1H, H5), 4.14 (s, 1H, 7-OH), 3.54–4.01 (m, 9H, H13, 23-OH, H23, H5'', H5', H6, H17, H3'', H3'), 3.27–3.48 (m, 8H, H25, 3''-OCH₃, 3'-OCH₃, H2), 3.23 (t, *J* = 9.0 Hz, 1H, H4'), 3.15 (t, 1H, *J* = 8.8 Hz, H4''), 2.46–2.68 (m, 2H, H12, H24), 2.14–2.39 (m, 4H, H16 × 2, H2', H2''), 1.77–1.97 (m, 5H, H18, Me4a, H20), 1.44–1.67 (m, 11H, Me14a, H20, H26, H27 × 2, H22 × 2, H2', H2''), 1.13–1.35 (m, 9H, Me6', Me6'', Me12a), 0.84–1.03 (m, 28H, H28, Me24a, Me26a, H18, (CH₃)₃CSi(CH₃)₂), 0.02–0.17 (m, 12H, (CH₃)₃CSi(CH₃)₂); ¹³C NMR (75 MHz, CDCl₃) δ 173.60, 139.47, 138.06, 137.78, 134.47, 124.58, 120.38, 118.88, 118.04, 98.52, 97.57, 94.87, 81.88, 80.83, 80.32, 79.24, 79.16, 78.50, 77.20, 77.11, 69.85, 69.38, 68.96, 68.64, 68.30, 67.65, 67.31, 67.23, 56.48, 56.14, 45.66, 42.45, 41.69, 39.77, 36.55, 36.33, 35.29, 34.57, 34.11, 29.58, 27.28, 25.97, 25.82, 25.63, 20.11, 19.83, 18.29, 18.23, 18.07, 14.98, 13.90, 12.19, 11.91, -4.04, -4.45, -4.81, -5.03. HRMS (ESI) calcd for $C_{60}H_{103}O_{15}Si_2$ ($M + H$)⁺ 1119.6836, found 1119.6873.

Synthesis of 5-oxo-4'',23-bis-*o*-(*tert*-butyldimethylsilyl) avermectin B2a (compound 5; Scheme 1)

A solution of phenyl dichlorophosphate (4.9 g, 23.2 mmol) in CH₂Cl₂ (30 mL) was added dropwise over 20 minutes to a solution of 4'',23-bis-*o*-(*tert*-butyldimethylsilyl) avermectin B2a (20.0 g, 17.9 mmol), dimethyl sulfoxide (DMSO, 5.6 g, 71.7 mmol) and TEA (9.06 g, 89.5 mmol) in CH₂Cl₂ (100 mL) at -15 °C. The mixture was stirred for 1 h at -15 °C and then poured into 1% H₃PO₄. The aqueous phase was extracted with CH₂Cl₂ (2 × 100 mL), and the combined organic phase was washed with saturated aqueous solution of sodium bicarbonate (100 mL) and saturated sodium chloride solution (100 mL). Then the organic phase was dried over anhydrous sodium

sulfate, filtered, and evaporated to a yellow foam. The foam was purified by column chromatography on silica gel using a mixture of petroleum ether and ethyl acetate (v/v = 10 : 1) as the eluent to afford compound 5 as a yellow foamy solid (16.2 g, 81%). Mp 134–137 °C. ¹H NMR (300 MHz, CDCl₃) δ 6.56–6.64 (m, 1H, H3), 5.89–6.00 (m, 1H, H9), 5.66–5.87 (m, 2H, H10, H11), 5.30–5.48 (m, 2H, H1'', H19), 4.92–5.08 (m, 1H, H15), 4.81 (d, *J* = 3.0 Hz, 1H, H1'), 4.75 (s, 2H, H8a × 2), 4.29 (t, *J* = 7.1 Hz, 1H, H5), 4.06 (s, 1H, 7-OH), 3.97 (s, 1H, 23-OH), 3.54–3.91 (m, 9H, H13, H25, H23, H5'', H5', H6, H17, H3'', H3'), 3.29–3.50 (m, 7H, 3''-OCH₃, 3'-OCH₃, H2), 3.25 (t, *J* = 8.9 Hz, 1H, H4'), 3.16 (t, 1H, *J* = 8.8 Hz, H4''), 2.49–2.66 (m, 1H, H12), 2.19–2.42 (m, 5H, H24, H16 × 2, H2', H2''), 1.81–2.02 (m, 5H, H18, Me4a, H20), 1.42–1.65 (m, 11H, Me14a, H20, H26, H27 × 2, H22 × 2, H2', H2''), 1.19–1.33 (m, 9H, Me6', Me6'', Me12a), 0.87–1.03 (m, 28H, H28, Me24a, Me26a, H18, (CH₃)₃CSi(CH₃)₂), 0.02–0.14 (m, 12H, (CH₃)₃CSi(CH₃)₂); ¹³C NMR (75 MHz, CDCl₃) δ 192.03, 172.18, 139.17, 138.01, 137.69, 136.69, 134.60, 124.53, 121.86, 118.83, 98.53, 97.60, 94.87, 81.91, 81.75, 80.79, 79.26, 78.53, 77.13, 69.91, 69.77, 69.39, 69.00, 67.35, 67.28, 56.51, 56.19, 46.60, 42.46, 41.71, 39.90, 36.53, 36.36, 35.30, 34.60, 34.53, 34.10, 29.62, 27.34, 25.99, 25.85, 20.02, 18.33, 18.27, 18.10, 15.38, 15.03, 13.94, 12.22, 11.94, 0.94, -4.01, -4.42, -4.78, -5.01. HRMS (ESI) calcd for $C_{60}H_{101}O_{15}Si_2$ ($M + H$)⁺ 1117.6679, found 1117.6682.

Synthesis of 5-oxo avermectin B2a (compound 6; Scheme 1)

70% hydrogen fluoride–pyridine complex (50 g, 353.1 mmol) was added to a cold mixture of pyridine (25 mL) and THF (55 mL) at 0 °C. A portion (84 mL) of the resulting reagent solution was added to a cold (0 °C) solution of 5-oxo-4'',23-bis-*o*-(*tert*-butyldimethylsilyl) avermectin B2a (15.0 g, 13.4 mol) in THF (100 mL). The reaction mixture stirred at room temperature for 4 days. The reaction mixture was concentrated under vacuum. The residue was diluted with CH₂Cl₂ and washed with 5% dilute hydrochloric acid (100 mL), 5% aqueous sodium bicarbonate (100 mL), and saturated sodium chloride solution (100 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and evaporated. The resulting residue was purified by column chromatography on silica gel using a mixture of petroleum ether and ethyl acetate (v/v = 4 : 1) as the eluent to afford compound 6 as a pale yellow foamy solid (7.8 g, 66%). Mp 143–145 °C. ¹H NMR (300 MHz, CDCl₃) δ 6.59–6.64 (m, 1H, H3), 5.92–6.01 (m, 1H, H9), 5.69–5.87 (m, 2H, H10, H11), 5.35–5.48 (m, 2H, H1'', H19), 4.91–5.08 (m, 1H, H15), 4.67–4.84 (m, 3H, H8a × 2, H1'), 3.95–4.06 (s, 2H, 7-OH, 23-OH), 3.74–3.89 (m, 4H, H23, H13, H5'', H5'), 3.41–3.68 (m, 11H, H17, H25, H2, H3'', H3', 3''-OCH₃, 3'-OCH₃), 3.27 (t, *J* = 9.0 Hz, 1H, H4'), 3.18 (t, 1H, *J* = 9.2 Hz, H4''), 2.51–2.62 (m, 2H, H12, 4''-OH), 2.21–2.41 (m, 5H, H24, H16 × 2, H2', H2''), 1.84–2.05 (m, 5H, H18, Me4a, H20), 1.40–1.65 (m, 11H, Me14a, H20, H26, H27 × 2, H22 × 2, H2', H2''), 1.15–1.36 (m, 9H, Me6', Me6'', Me12a), 0.84–1.03 (m, 10H, H28, Me24a, Me26a, H18); ¹³C NMR (75 MHz, CDCl₃) δ 191.94, 171.71, 138.88, 138.10, 137.81, 136.55, 135.65, 124.58, 121.78, 117.45, 99.54, 98.37, 94.74, 81.99, 81.47, 80.80, 80.27, 79.21, 78.11, 77.20, 75.93, 70.76, 69.73, 69.63, 68.24, 68.16, 68.07, 67.20, 56.33, 56.28, 46.48, 41.04, 40.69, 39.77, 36.24,



35.60, 35.02, 34.16, 33.98, 27.18, 19.96, 18.28, 17.58, 15.34, 15.01, 13.65, 12.31, 11.70. HRMS (ESI) calcd for $C_{48}H_{73}O_{15}$ ($M + H$)⁺ 889.4949, found 889.4950.

Synthesis of 5-oximino-5-deoxyavermectin B2a (compound 7; Scheme 1)

A solution of $NH_2OH \cdot HCl$ (2.8 g, 40.3 mmol) in water (12 mL) was added dropwise to a solution of 5-oxoavermectin B2a (7.1 g, 8.0 mmol) in methanol (25 mL) and 1,4-dioxane (25 mL). The mixture was stirred at room temperature for 8 h and then evaporated under reduced pressure. The residue was dissolved in dichloromethane (100 mL) and washed with water (50 mL). The organic phase was then dried over anhydrous sodium sulfate, filtered, and evaporated to a yellow foam, which was purified by silica gel column chromatography with petroleum ether–ethyl acetate ($v/v = 1 : 1$) to afford compound **7** as a white solid (6.3 g, 87%). Mp: 164–166 °C. 1H NMR (300 MHz, $CDCl_3$) δ 5.70–6.03 (m, 4H, H3, H9, H10, H11), 5.32–5.49 (m, 2H, H1', H19), 4.90–5.10 (m, 1H, H15), 4.68–4.85 (m, 4H, H1', H8a \times 2, H6), 3.73–4.02 (m, 6H, 7-OH, 23-OH, H23, H13, H5'', H5'), 3.42–3.71 (m, 11H, H2, H25, H17, H3'', H3', 3''-OCH₃, 3'-OCH₃), 3.28 (t, $J = 8.9$ Hz, 1H, H4'), 3.19 (t, 1H, $J = 9.1$ Hz, H4''), 2.48–2.63 (m, 1H, H12), 2.23–2.42 (m, 5H, H24, H16 \times 2, H2', H2''), 1.89–2.08 (m, 5H, H18, Me4a, H20), 1.43–1.75 (m, 11H, Me14a, H20, H26, H27 \times 2, H22 \times 2, H2', H2''), 1.26–1.34 (m, 6H, Me6', Me6''), 1.21 (d, $J = 6.9$ Hz, 3H, Me12a), 0.84–1.04 (m, 10H, H28, Me24a, Me26a, H18); ^{13}C NMR (75 MHz, DMSO) δ 170.20, 150.34, 140.08, 136.64, 135.15, 129.57, 126.29, 125.34, 121.38, 117.92, 99.00, 97.68, 94.63, 81.48, 79.74, 79.08, 78.89, 77.65, 75.59, 72.65, 69.46, 68.52, 68.38, 68.06, 67.43, 66.86, 56.68, 55.90, 45.61, 41.21, 40.95, 35.62, 35.25, 34.77, 34.14, 33.62, 26.72, 19.96, 18.24, 17.80, 17.51, 14.62, 13.65, 12.16, 12.09; HRMS (ESI) calcd for $C_{48}H_{74}NO_{15}$ ($M + H$)⁺ 904.5058, found 904.5063.

Synthesis of 5-oxoavermectin B2a monosaccharide (compound 9; Scheme 2)

5-Oxoavermectin B2a (4.0 g, 4.5 mmol) was added to a solution of concentrated sulfuric acid (1.5 mL) in 2-propanol (23.5 mL) and stirred at -5 °C for 36 h. The reaction mixture was diluted with 100 mL of ethyl ether, and the solution was washed with 5% aqueous sodium bicarbonate and water, dried, and concentrated *in vacuo* to a yellow foam, which was purified by silica gel column chromatography with petroleum ether–ethyl acetate ($v/v = 4 : 1$) to afford compound **9** as a yellow foam solid (2.7 g, 81%). Mp 152–155 °C. 1H NMR (300 MHz, $CDCl_3$) δ 6.34–6.78 (m, 1H, H3), 5.64–5.99 (m, 3H, H9, H10, H11), 5.26–5.49 (m, 1H, H19), 4.96 (t, $J = 7.6$ Hz, 1H, H15), 4.64–4.85 (m, 4H, H1', H8a \times 2, H6), 3.70–4.03 (m, 5H, 7-OH, H23, H13, H5', H17), 3.41–3.68 (m, 6H, H25, H3', H2, 3'-OCH₃), 3.19 (t, 1H, $J = 9.1$ Hz, H4'), 2.46–2.67 (m, 2H, H12, H24), 2.20–2.41 (m, 3H, H16 \times 2, H2'), 1.84–2.09 (m, 5H, Me4a, H18, H20), 1.42–1.73 (m, 10H, Me14a, H20, H2', H26, H27 \times 2, H22 \times 2), 1.29 (d, $J = 6.2$ Hz, 3H, Me6'), 1.17 (d, $J = 6.9$ Hz, 3H, Me12a), 0.83–1.04 (m, 10H, H28, Me24a, Me26a, H18); ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.02, 171.65, 138.90, 138.22, 138.09, 137.78, 131.46, 124.58, 121.85, 118.76, 117.45, 99.57, 94.93, 82.02, 81.41, 80.84, 78.21, 75.93, 70.76, 69.77, 69.63, 68.35, 68.26, 68.10, 56.44, 46.49, 41.03,

40.71, 39.78, 36.25, 35.60, 35.03, 33.98, 27.19, 19.97, 17.61, 15.35, 15.04, 13.67, 12.32, 11.71; HRMS (ESI) calcd for $C_{41}H_{61}O_{12}$ ($M + H$)⁺ 745.4163, found 745.4168.

Synthesis of 5-oximino-5-deoxyavermectin B2a monosaccharide (compound 10; Scheme 2)

A solution of $NH_2OH \cdot HCl$ (1.0 g, 14.4 mmol) in water (5 mL) was added dropwise to a solution of 5-oxoavermectin B2a monosaccharide (2.2 g, 3.0 mmol) in methanol (10 mL) and 1,4-dioxane (10 mL). The mixture was stirred at room temperature for 8 h and then evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (50 mL) and washed with water (25 mL). The organic phase was then dried over anhydrous sodium sulfate, filtered, and evaporated to a yellow foam, which was purified by silica gel column chromatography with petroleum ether–ethyl acetate ($v/v = 1 : 1$) to afford compound **10** as a white solid (1.9 g, 83%). Mp 214–217 °C. 1H NMR (300 MHz, $CDCl_3$) δ 5.66–6.04 (m, 4H, H3, H9, H10, H11), 5.25–5.45 (m, 1H, H19), 5.00 (t, $J = 6.9$ Hz, 1H, H15), 4.58–4.86 (m, 4H, H8a \times 2, H6, H1'), 3.68–4.06 (m, 5H, 7-OH, 23-OH, H23, H13, H5'), 3.37–3.65 (m, 7H, H17, H25, H3', H2, 3'-OCH₃), 3.17 (t, 1H, $J = 9.9$ Hz, H4'), 2.15–2.63 (m, 4H, H12, H24, H16 \times 2), 1.37–2.10 (m, 16H, H2', Me4a, H18, H20, Me14a, H20, H2', H26, H27 \times 2, H22 \times 2), 1.27 (d, $J = 6.2$ Hz, 3H, Me6'), 1.15 (d, $J = 6.9$ Hz, 3H, Me12a), 0.80–1.04 (m, 10H, H28, Me24a, Me26a, H18); ^{13}C NMR (75 MHz, DMSO) δ 170.22, 150.37, 140.03, 136.80, 135.17, 129.61, 126.29, 125.29, 121.45, 117.87, 99.04, 94.66, 81.17, 79.24, 79.10, 77.86, 75.65, 72.70, 69.50, 68.55, 68.44, 68.11, 67.44, 56.64, 45.64, 41.22, 40.98, 35.66, 35.27, 34.80, 34.67, 33.64, 26.75, 20.03, 17.85, 17.53, 14.67, 13.67, 12.17, 12.11. HRMS (ESI) calcd for $C_{41}H_{62}NO_{12}$ ($M + H$)⁺ 760.4272, found 760.4278.

Synthesis of 5-oxoavermectin B2a aglycone (compound 12; Scheme 2)

5-Oxoavermectin B2a (4.0 g, 4.5 mmol) was added to a solution of concentrated sulfuric acid (2.5 mL) in methanol (22.5 mL) and stirred at -5 °C for 24 h. The reaction mixture was diluted with 100 mL of ethyl ether, and the solution was washed with 5% aqueous sodium bicarbonate and water, dried, and concentrated in a vacuum to a yellow foam, which was purified by silica gel column chromatography with petroleum ether–ethyl acetate ($v/v = 6 : 1$) to afford compound **12** as a yellow solid foam (2.3 g, 85%). Mp 180–183 °C. 1H NMR (300 MHz, $CDCl_3$) δ 6.54–6.63 (m, 1H, H3), 5.67–5.95 (m, 3H, H9, H10, H11), 5.23–5.38 (m, 2H, H15, H19), 4.65–4.72 (m, 2H, H8a \times 2), 4.01 (s, 1H, 7-OH), 3.91 (s, 1H, 23-OH), 3.69–3.86 (m, 3H, H23, H13, H6), 3.44–3.62 (m, 3H, H17, H25, H2), 2.45–2.60 (m, 1H, H12), 2.21–2.40 (m, 2H, H24, H20), 1.77–2.01 (m, 6H, H16 \times 2, Me4a, H18), 1.38–1.72 (m, 9H, Me14a, H20, H26, H27 \times 2, H22 \times 2), 1.17 (d, $J = 7.0$ Hz, 3H, Me12a), 0.80–1.03 (m, 10H, H28, Me24a, Me26a, H18); ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.04, 171.46, 139.15, 138.35, 138.22, 137.77, 136.41, 124.48, 121.90, 116.41, 99.48, 81.91, 80.86, 77.25, 71.30, 69.83, 69.66, 68.31, 68.11, 46.44, 41.04, 40.78, 40.08, 36.06, 35.58, 35.06, 34.09, 27.37, 18.96, 15.34, 14.46, 13.70, 12.41, 11.39; HRMS (ESI) calcd for $C_{34}H_{49}O_9$ ($M + H$)⁺ 601.3377, found 601.3381.



Synthesis of 5-oximino-5-deoxyavermectin B2a aglycone (compound 13; Scheme 2)

A solution of $\text{NH}_2\text{OH}\cdot\text{HCl}$ (1.1 g, 15.8 mmol) in water (5 mL) was added dropwise to a solution of 5-oxoavermectin B2a aglycone (2.0 g, 3.3 mmol) in methanol (10 mL) and 1,4-dioxane (10 mL). The mixture was stirred at room temperature for 6 h and then evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (50 mL) and washed with water (25 mL). The organic phase was then dried over anhydrous sodium sulfate, filtered, and evaporated to a yellow foam, which was purified by silica gel column chromatography with petroleum ether–ethyl acetate ($v/v = 2 : 1$) to afford compound 13 as a white solid (1.7 g, 84%). Mp 210–213 °C. ^1H NMR (300 MHz, CDCl_3) δ 5.60–5.98 (m, 4H, H3, H9, H10, H11), 5.24–5.43 (m, 2H, H19, H15), 4.63–4.81 (m, 3H, H8a \times 2, H6), 4.01 (s, 1H, 7-OH), 3.87 (s, 1H, 23-OH), 3.69–3.83 (m, 2H, H23, H13), 3.50–3.66 (m, 2H, H17, H25), 3.32–3.43 (m, 1H, H2), 2.46–2.61 (m, 1H, H12), 2.23–2.42 (m, 2H, H24, H20), 1.87–2.07 (m, 5H, H16 \times 2, Me4a), 1.40–1.78 (m, 10H, H18, Me14a, H20, H26, H27 \times 2, H22 \times 2), 1.18 (d, $J = 6.9$ Hz, 3H, Me12a), 0.83–1.02 (m, 10H, H28, Me24a, Me26a, H18); ^{13}C

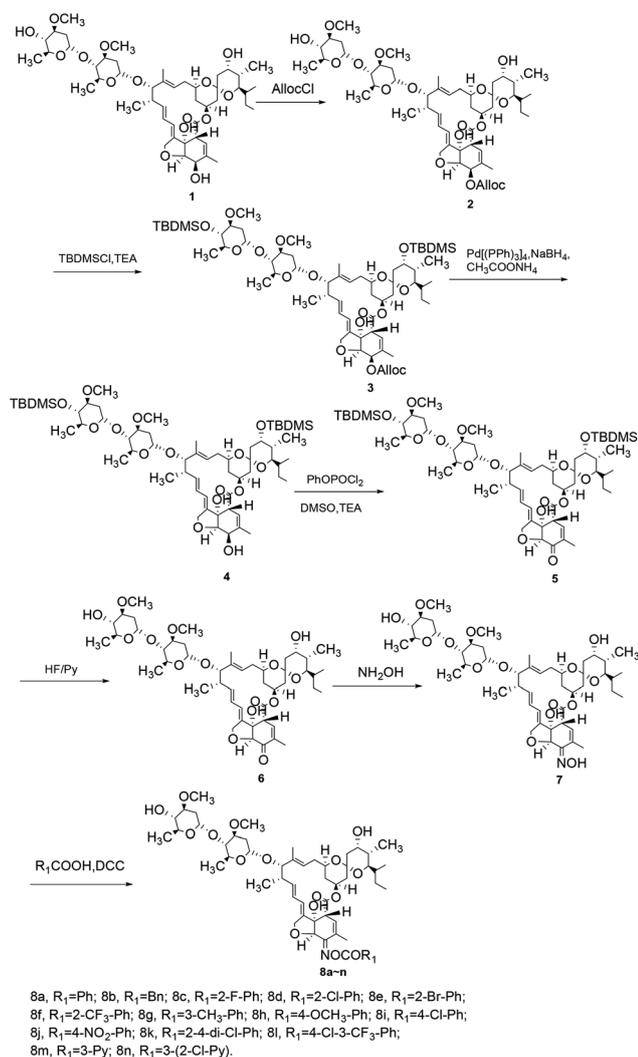
NMR (75 MHz, CDCl_3) δ 172.70, 151.19, 139.18, 138.21, 137.36, 131.88, 125.30, 124.80, 121.42, 116.47, 99.57, 78.58, 77.49, 72.99, 71.35, 70.00, 68.68, 68.39, 67.65, 46.36, 41.08, 40.18, 36.15, 35.65, 35.12, 34.15, 27.41, 19.04, 17.45, 14.53, 13.74, 12.47, 11.45, 0.94; HRMS (ESI) calcd for $\text{C}_{34}\text{H}_{50}\text{NO}_9$ ($\text{M} + \text{H}$) $^+$ 616.3486, found 616.3486.

General synthetic procedure for compounds 8a–n, 11a–n, and 14a–l (Schemes 1 and 2)

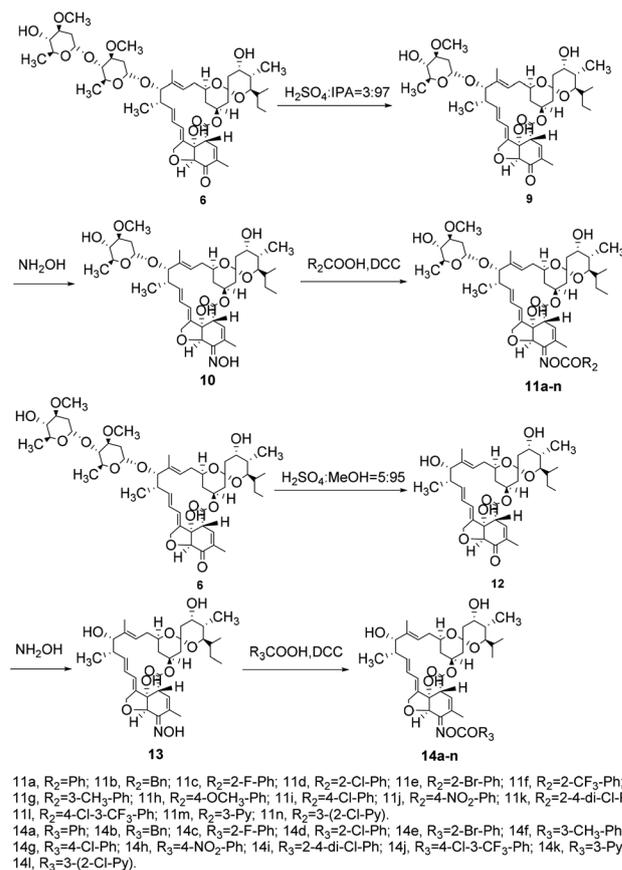
Dicyclohexylcarbodiimide (DCC, 0.4 g, 1.9 mmol) and carboxylic acid (R_1COOH , R_2COOH or R_3COOH , 1.9 mmol) were added to a solution of 5-oxime-5-deoxyavermectin B2a in CH_2Cl_2 (15 mL) and the mixture was stirred at room temperature. After the reaction was completed, as monitored with TLC, the reaction mixture was then filtered and the filtrate was evaporated under reduced pressure to yield a residue. The residue was purified by silica gel column chromatography with petroleum ether–ethyl acetate ($v/v = 6 : 1-1 : 1$) to afford the target compounds. The ^1H -NMR, ^{13}C -NMR and HRMS spectral data of the compounds 8a–n, 11a–n, and 14a–l are supplied as ESI.†

Insecticidal assay

All bioassays were performed on representative test organisms reared in the laboratory. Each test sample was prepared in methanol and diluted to the required concentration with



Scheme 1 Synthesis of target compounds 8a–n.



Scheme 2 Synthesis of the target compounds 11a–n and 14a–l.



distilled water. The insecticidal activities of the target compounds against *M. persicae*, *C. elegans*, and *T. cinnabarinus* were tested according to previously reported procedures.^{24,28,29} Assessments were made on a dead/live basis, and mortality rates were corrected using Abbott's formula.³⁰

Results and discussion

Chemistry

In this research, three series of novel avermectin B2a oxime ester derivatives were synthesized and spectroscopic data was obtained, which agreed well with their expected structures. The

synthesis of the target compounds **8a–n** was shown in Scheme 1. Initially, the 5-hydroxyl group of avermectin B2a (**1**) was selectively protected with AllocCl in CH₂Cl₂ to give compound **2**. The subsequent reaction, using TBDMSCl as a protective agent for the hydroxyl groups at the 4- and 23-positions, yielded compound **3**. Removal of the AllocCl protecting group of compound **3** with NaBH₄ and Pd(PPh₃)₄ in MeOH–THF (1 : 1) formed compound **4**. Oxidation of compound **4** using a PhOPOCl₂, Et₃N, and DMSO reagent system in dried CH₂Cl₂ afforded compound **5**. The *t*-butyldimethylsilyl protecting group was removed using a HF/Py complex to form the important intermediate compound **6**. Compound **6** was reacted with

Table 1 Insecticidal activities of avermectin B2a derivatives (mortality (%) ± SD)

Compound	<i>M. persicae</i>	<i>C. elegans</i>	<i>T. cinnabarinus</i>		
	50 µg mL ⁻¹	1 µg mL ⁻¹	10 µg mL ⁻¹	1 µg mL ⁻¹	0.1 µg mL ⁻¹
8a	85.1 ± 2.4 ^a	59.1 ± 3.1	100	100	63.7 ± 2.7
8b	87.7 ± 3.2	82.3 ± 1.8	100	100	79.5 ± 3.2
8c	90.3 ± 2.4	77.8 ± 3.3	100	100	83.2 ± 3.3
8d	94.3 ± 2.3	90.4 ± 1.8	100	93.3 ± 3.1	62.2 ± 3.5
8e	82.3 ± 2.8	73.6 ± 2.8	100	100	73.1 ± 2.3
8f	88.6 ± 3.2	76.2 ± 2.4	100	100	88.3 ± 3.7
8g	63.1 ± 1.2	43.2 ± 1.5	100	91.1 ± 3.0	
8h	81.7 ± 4.7	61.6 ± 3.1	100	87.2 ± 2.5	
8i	90.6 ± 2.6	46.5 ± 2.4	100	92.6 ± 2.2	
8j	71.7 ± 3.4	54.8 ± 1.9	100	70.8 ± 3.1	
8k	95.1 ± 2.8	61.0 ± 1.3	100	83.2 ± 1.9	
8l	87.0 ± 1.0	63.8 ± 2.2	100	100	55.8 ± 2.4
8m	51.3 ± 2.7	70.9 ± 1.4	100	83.4 ± 3.1	
8n	44.2 ± 4.1	70.3 ± 3.2	100	73.2 ± 2.9	
11a	65.0 ± 2.4	23.0 ± 2.1	93.4 ± 2.1		
11b	70.6 ± 1.7	40.9 ± 3.4	100	72.2 ± 2.1	
11c	60.9 ± 2.8	75.9 ± 2.7	97.1 ± 3.6		
11d	69.4 ± 3.0	83.7 ± 3.0	90.8 ± 2.3		
11e	50.2 ± 1.8	61.6 ± 2.9	94.5 ± 3.1		
11f	67.9 ± 0.6	63.2 ± 2.5	95.4 ± 1.5		
11g	58.8 ± 3.1	34.1 ± 2.1	90.4 ± 2.0		
11h	72.6 ± 2.4	20.9 ± 2.8	75.2 ± 1.9		
11i	71.4 ± 5.7	20.7 ± 1.5	76.7 ± 1.3		
11j	52.1 ± 3.7	70.2 ± 2.1	71.0 ± 2.1		
11k	67.1 ± 2.1	73.7 ± 2.8	86.8 ± 2.7		
11l	68.8 ± 2.4	74.4 ± 2.1	82.2 ± 2.9		
11m	40.8 ± 2.6	71.0 ± 1.8	95.0 ± 1.3		
11n	38.0 ± 3.7	55.7 ± 3.3	86.3 ± 1.6		
14a	33.6 ± 3.1	7.0 ± 1.0	54.1 ± 1.9		
14b	41.6 ± 5.1	28.3 ± 3.0	84.3 ± 3.1		
14c	23.3 ± 3.1	65.9 ± 2.8	94.7 ± 2.7		
14d	52.0 ± 1.0	62.5 ± 1.5	36.3 ± 2.4		
14e	22.6 ± 1.2	60.7 ± 1.5	53.11 ± 1.6		
14f	27.6 ± 1.8	19.6 ± 2.3	62.4 ± 2.8		
14g	36.5 ± 2.9	15.3 ± 2.5	26.1 ± 1.6		
14h	68.7 ± 1.9	66.2 ± 2.0	21.4 ± 3.1		
14i	44.9 ± 3.8	32.7 ± 2.3	43.1 ± 2.5		
14j	20.4 ± 2.7	69.9 ± 1.5	92.9 ± 2.7		
14k	31.8 ± 1.9	32.5 ± 2.7	55.8 ± 2.6		
14l	24.3 ± 2.9	27.4 ± 2.1	41.6 ± 1.7		
7	90.0 ± 3.8	91.9 ± 2.4	100	100	74.8 ± 1.6
10	68.4 ± 1.1	82.7 ± 1.9	94.7 ± 2.6		
13	45.8 ± 2.1	42.0 ± 1.3	57.2 ± 2.5		
B2a	93.5 ± 2.1	87.1 ± 2.7	100	100	78.7 ± 2.9



NH₂OH-HCl to yield intermediate 7, which on reaction with various carboxylic acids, gave the target compounds **8a-n**.

The target compounds **11a-n** and **14a-l** were synthesized from compound **6** as shown in Scheme 2. The intermediate **6** was de-sugared with 3% concentrated H₂SO₄ in isopropanol as a solvent to afford compound **9**, and compound **12** was prepared by a reaction between intermediate **6** and 5% concentrated H₂SO₄ in methanol. Similarly, the target compounds **11a-n** and **14a-l** were prepared through oximate and esterification, respectively, *via* a similar procedure as described above for compounds **8a-n** in good yield.

Insecticidal activity

The insecticidal activities against *M. persicae*, *C. elegans*, and *T. cinnabarinus* of the 5-deoxyavermectin B2a oxime ester derivatives were evaluated using avermectin B2a as the positive control. The results were summarized in Table 1.

Most of the target compounds **8a-n** exhibited moderate to excellent insecticidal activities against the three systems (Table 1). For example, compounds **8b**, **8d**, and **8k** exhibited a 87.7%, 94.3%, and 95.1% insect mortality in *M. persicae*, similarly to the corresponding parent compound avermectin B2a (93.5%). The insecticidal activities of compounds **8b** and **8c** against *T. cinnabarinus* at 0.1 μg mL⁻¹ were 79.5% and 83.2%, respectively, as compared with a 78.7% mortality achieved by avermectin B2a at the same concentration.

In order to investigate whether the disaccharide group can influence insecticidal activity, the corresponding monosaccharide (**11a-n**) and aglycone (**14a-l**) oxime ester congeners were also prepared. The insecticidal activities of avermectin disaccharide derivatives (**8a-n**) were more potent than their corresponding monosaccharide (**11a-n**) and aglycone (**14a-l**) analogues (Table 1). For instance, the insecticidal activity of compound **8b** against *C. elegans* at 1 μg mL⁻¹ was 82.3%, and the corresponding compounds **11b** and **14b** displayed mortalities as high as 40.9% and 28.3%. Thus, the disaccharide moiety played a significant role in the insecticidal activity of these oxime ester analogues.

By changing the position of the phenyl ring substitution and introducing different substituent groups on the phenyl ring in compounds **8a**, **11a**, and **14a**, the monosaccharide (**11a-l**) and aglycone (**14a-l**) oxime ester analogues were shown to have a similar structure-activity relationship as the disaccharide derivatives (**8a-l**). Therefore, compounds **8a-l** were used to investigate structure-activity relationship correlations. Compound **8d**, with a chlorine atom substitution in the 2-position, showed the best insecticidal activities against *M. persicae* and *C. elegans* among compounds **8c-f**. With regards to *T. cinnabarinus*, compounds with trifluoromethyl or fluorine in the 2-position (**8c**, **8f**) showed better insecticidal activity. By introducing a chlorine atom in the 4-position, the activity of compound **8i** against *M. persicae* was slightly increased compared with that of compound **8a**, but with a subsequent detrimental impact on activity against *C. elegans* and *T. cinnabarinus*. The insecticidal activities of compounds **8k** and **8l** were affected by the above effects and other reasons (shape *etc.*)

together. Compound **8b**, with a methylene group inserted between the phenyl and the formoxyl groups, showed slightly higher insecticidal activities against the three insect systems than compound **8a**. In general, introduction of electron donating moieties at the phenyl ring (**8g**, **8h**) resulted in poor control of *M. persicae*, *C. elegans*, and *T. cinnabarinus*. Furthermore, when the substitution group was changed from a phenyl ring to a pyridyl ring, compounds **8m** and **8n** showed much weaker insecticidal activities against *M. persicae* and *T. cinnabarinus* than compound **8a**, but possessed superior insecticidal activity against *C. elegans*. Compounds **11m**, **11n**, **14k**, and **14l** showed similar structure-activity relationship correlations as the corresponding compounds **8m** and **8n**.

Conclusions

In summary, three series of avermectin B2a derivatives bearing oxime ester moieties were synthesized and screened for their insecticidal activity against *M. persicae*, *C. elegans*, and *T. cinnabarinus*. The results showed that lots of the synthesized compounds **8a-n** showed excellent insecticidal activity. In particular, compound **8d** showed higher insecticidal activities against *M. persicae* and *C. elegans* in comparison with avermectin B2a. Significantly, the present study revealed important structure-activity relationship information such as the finding that the disaccharide moiety played a pivotal role in the insecticidal activity of these derivatives or that the position of substitution on the aromatic ring and electronic distribution of substituent groups had substantial effects on insecticidal potency. These results will be of particular value in the further optimization of avermectin B2a.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Notes and references

- 1 K. Etebari, M. J. Furlong and S. Asgari, *Sci. Rep.*, 2015, **5**, 14642.
- 2 J. K. Kim, S. R. Choi, J. Lee, S. Y. Park, S. Y. Song, J. Na, S. W. Kim, S. J. Kim, I. S. Nou and Y. H. Lee, *J. Agric. Food Chem.*, 2013, **61**, 11222-11230.
- 3 J. L. Huang, M. Lv and H. Xu, *RSC Adv.*, 2017, **7**, 15997-16004.
- 4 X. Yu, Z. P. Che and H. Xu, *Chem.-Eur. J.*, 2017, **23**(19), 4467-4526.
- 5 Y. Guo, R. G. Yang and H. Xu, *Sci. Rep.*, 2016, **6**, 35321.
- 6 Y. Guo, R. G. Yang and H. Xu, *RSC Adv.*, 2017, **7**, 28009-28015.
- 7 Q. Li, X. B. Huang, S. C. Li, J. C. Ma, M. Lv and H. Xu, *J. Agric. Food Chem.*, 2016, **64**, 5472-5478.



- 8 R. Wang, X. Y. Zhi, J. Li and H. Xu, *J. Agric. Food Chem.*, 2015, **63**, 6668–6674.
- 9 T. Yamada, Y. Horimatsu, T. Hirose, A. Sugawara, S. Omura and T. Sunazuka, *Tetrahedron Lett.*, 2017, **58**(32), 3119–3124.
- 10 W. X. Guan, W. X. Zhang, L. M. Tang, Y. Wang and H. X. Cui, *J. Agric. Food Chem.*, 2017, DOI: 10.1021/acs.jafc.7b01427.
- 11 P. T. Meinke, S. P. Rohrer, E. C. Hayes, J. M. Schaeffer, M. H. Fisher and H. Mrozik, *J. Med. Chem.*, 1992, **35**(21), 3879–3884.
- 12 J. P. Arena, K. K. Liu, P. S. Paress, E. G. Frazier, D. Cully, H. Mrozik and J. M. Schaeffer, *J. Parasitol.*, 1995, **81**(2), 286–294.
- 13 J. Zhang, X. Nan, H. T. Yu, P. L. Cheng, *et al.*, *Eur. J. Med. Chem.*, 2016, **121**, 422–432.
- 14 J. M. Schaeffer and H. W. Haines, *Biochem. Pharmacol.*, 1989, **38**(14), 2329–2338.
- 15 Z. X. Liu, L. H. Wang, H. M. Nie, J. J. Guo, T. Wang, L. B. Song and S. Lu, WO 20170110520 A1, 2017.
- 16 H. Shan, S. K. Liu and Z. M. Wang, CN 1041176991 A, 2015.
- 17 S. Q. Yan, X. M. Liang, J. J. Zhang and D. Q. Wang, *Chin. J. Org. Chem.*, 2009, **29**(12), 2005–2016.
- 18 X. G. Liu, J. P. Wu, X. M. Liang and D. Q. Wang, *Pest Manage. Sci.*, 2004, **60**(7), 697–702.
- 19 E. Callens, O. Hueter, E. Lamy, P. Luethi, T. Winkler and P. M. J. Jung, *Tetrahedron Lett.*, 2007, **48**(4), 707–709.
- 20 S. Yamashita, D. Hayashi, A. Nakano, Y. Hayashi and M. Hirama, *J. Antibiot.*, 2016, **69**(1), 31–50.
- 21 H. Mrozik, P. Eskola, M. H. Fisher, J. R. Egerton, S. Cifelli and D. A. Ostlind, *J. Med. Chem.*, 1982, **25**(6), 658–663.
- 22 S. Terashima, T. Katoh and E. Saitoh, WO 9945012 A1, 1999.
- 23 W. L. Shoop, H. Mrozik and M. H. Fisher, *Vet. Parasitol.*, 1995, **59**(2), 139–156.
- 24 J. H. Zhao, X. J. Xu, M. H. Ji, J. L. Cheng and G. N. Zhu, *J. Agric. Food Chem.*, 2011, **59**(9), 4836–4850.
- 25 Y. Tsukamoto, K. Sato, S. Mio, S. Sugai, T. Yanai, N. Kitano, S. Muramatsu, Y. Nakada and J. Ide, *Agric. Biol. Chem.*, 1991, **55**(10), 2615–2621.
- 26 S. Moraesda, F. C. V. Mariade, A. Mendesde, *et al.*, *Parasites Vectors*, 2016, **9**, 407.
- 27 C. R. Heredia, N. C. Romero and C. L. Miranda, *Vet. Parasitol.*, 2017, **247**, 77–79.
- 28 L. M. Katiki, J. F. S. Ferreira, A. M. Zajac, *et al.*, *Vet. Parasitol.*, 2011, **182**(2–4), 264–268.
- 29 G. S. Sun, X. Xu, S. H. Jin, L. Lin and J. J. Zhang, *Molecules*, 2017, **22**(6), 958.
- 30 W. S. Abbott, *J. Econ. Entomol.*, 1925, **18**, 265–267.

