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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 14 (2004) 4135–4139

Synthesis and biological evaluation of novel 4″-alkoxy avermectin derivatives

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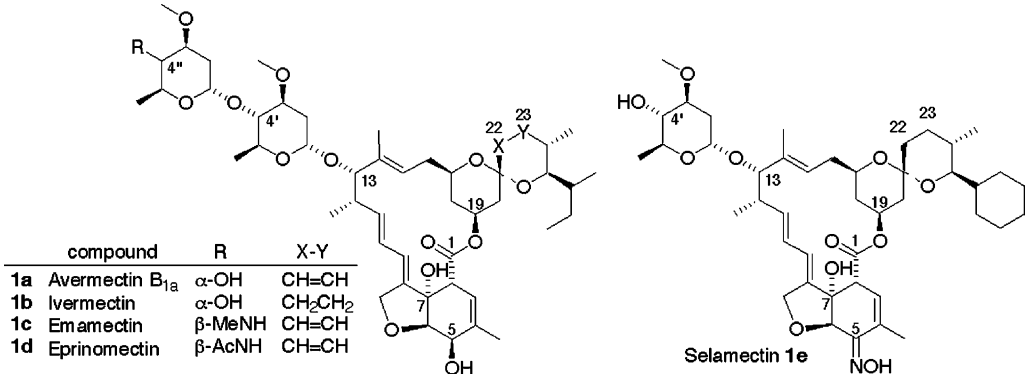
Received 28 May 2004; revised 9 June 2004; accepted 10 June 2004
Available online 2 July 2004

Abstract—Novel 4″-alkoxy avermectin derivatives were synthesized via rhodium carbenoid mediated O–H insertion reaction and tested for antiparasite activity against *Artemia salina* and *Caenorhabditis elegans*.

Avermectins are a series of unique 16-membered ring macrolides fused to spiroketal and hexahydrobenzofuran units with a disaccharide attached to the C13 position, and have been known to exhibit exceptionally potent anthelmintic, acaricidal, and insecticidal activities.[1] Avermectins have fascinated academic and pharmaceutical scientists over the last two decades,[1] and their complex structures have made them attractive synthetic targets for chemists.[2] Avermectin B_1 (**1a**, Fig. 1), the major product from the culture broth of *Streptomyces avermitilis* and the most effective avermectin against insects and mites, has been commercialized for agricultural use.[3,4] Chemical modifications of **1a** resulted in the more potent and safer derivative ivermectin **1b**, which has been widely used in treating livestock.[5] In addition, ivermectin is also used in human for the treatment of onchocerciasis,[6] strongyloidiasis,[7] and lymphatic filariasis.[8] Following the success of ivermecitin, various avermectin derivatives have been synthesized in order to develop second-generation avermectins



	compound	R	X-Y
1a	Avermectin B_{1a}	α-OH	CH=CH
1b	Ivermectin	α-OH	CH_2CH_2
1c	Emamectin	β-MeNH	CH=CH
1d	Eprinomectin	β-AcNH	CH=CH

Figure 1. Structure of avermectins.

Keywords: Avermectins; Anthelmintics; Insecticides; O–H insertion reaction.
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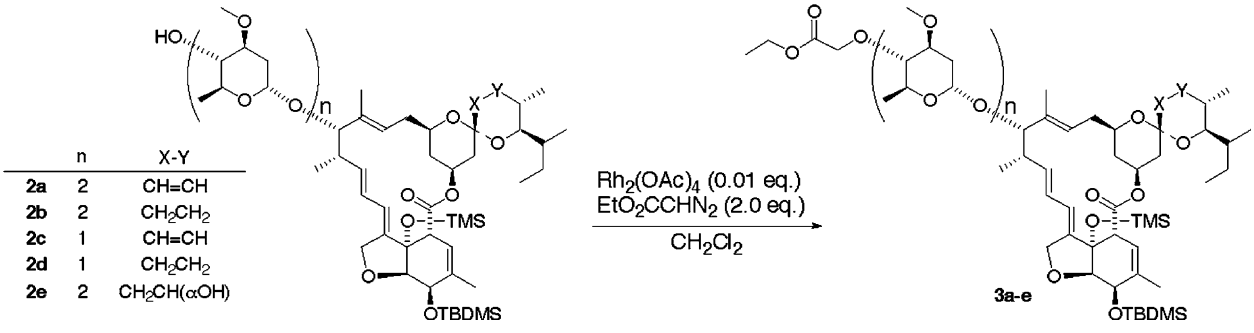
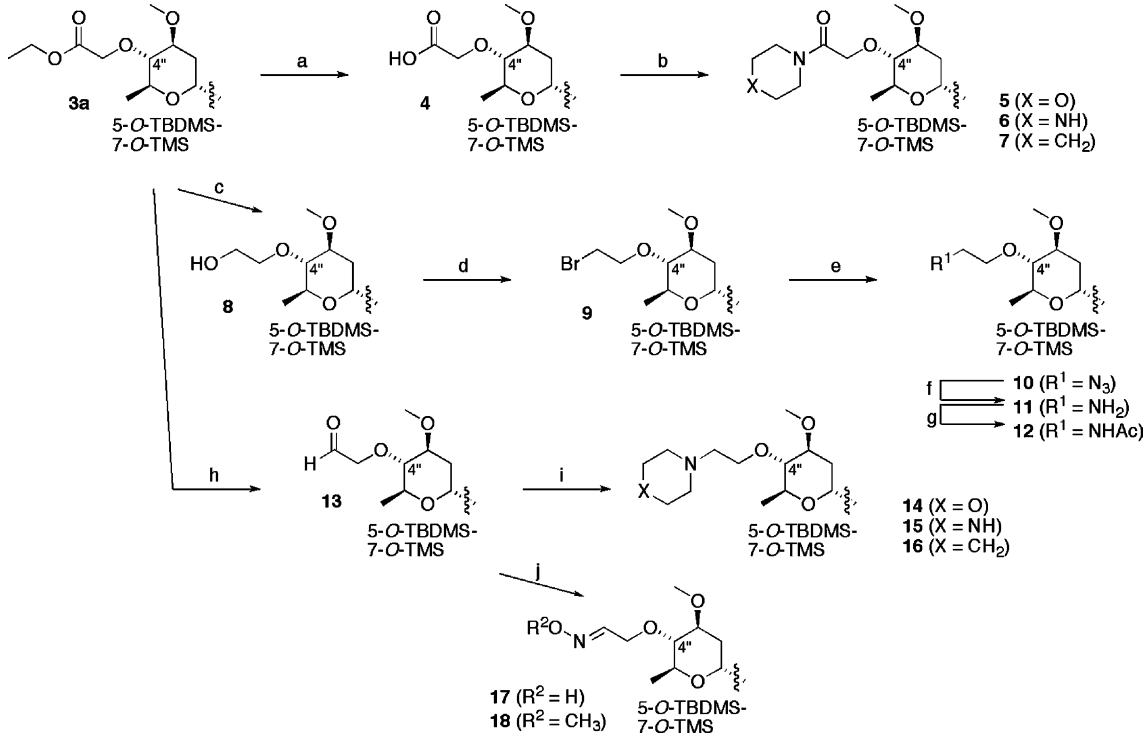
doi:10.1016/j.bmcl.2004.06.027

with higher potency and broader spectra of activities.[9] Among them, emamectin **1c**, which has an *epi* methylamino group at the 4″-position, was developed as an agricultural insecticide.[10] Eprinomectin **1d**, in which the 4″-hydroxy group is replaced with an *epi* acetylamino group exhibits potent endectocidal activity with minimal residues in milk, and is used for treating parasites in lactating dairy cattle.[11] During the course of searching for avermectin derivatives with antiflea activity, selamectin **1e**, with a C5 oxime, was developed to treat in endectocide companion animals.[12] In our research into novel synthetic avermectins, we focused on chemical modification of the 4″-position on the L-oleandrose moiety in order to improve the activities and pharmacokinetic profiles. Introduction of acyloxy, amino, and thio groups at this moiety was found to generally induce preferable characteristics in their solubilities, distributions, chemical stabilities, and activity spectra.[9,13,14] We recently reported efficient preparation of 4″-alkoxy avermectin derivatives by O–H insertion reaction with ethyl diazoacetate in the presence of $Rh_2(OAc)_4$ as a catalyst (Scheme 1).[15] In this letter, we describe the synthesis of 4″-alkoxy avermectin derivatives and their biological activities.

We hypothesized that the introduction of polar substitutes on the sugar could further enhance potency, as was the case with eprinomectin and emamectin. Scheme 2 illustrates the procedures leading to a variety of derivatives in order to examine the roles of this moiety. Our synthesis commenced with hydrolysis of ethyl ester **3a** to give carboxylic acid **4**, which was converted into amides **5**, **6**, and **7** under EDCI–HOBt conditions. Reduction of **3a** with super-H® at 0 °C gave the corresponding alcohol



Scheme 1. Rhodium carbenoid mediated O–H insertion reaction of avermectins.



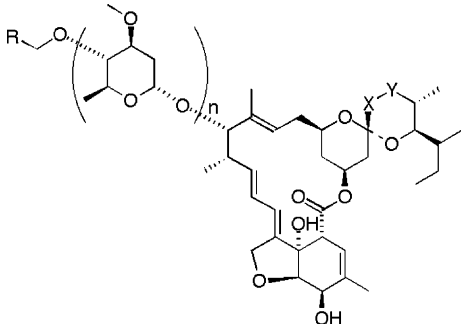
Scheme 2. Reagents and conditions: (a) 1 N KOH, THF–EtOH, rt, 89%; (b) amine, EDCI, HOBt, CH_2Cl_2, 68–84%; (c) super-H®, THF, −60–0 °C, 85%; (d) Ph_3P, CBr_4, Et_3N, CH_2Cl_2, rt, 91%; (e) NaN_3, DMSO, 40 °C, quant.; (f) Ph_3P, NH_4OH, THF, 40 °C, 67%; (g) Ac_2O, CH_2Cl_2, rt, quant.; (h) super-H®, THF, −78 °C, 82%; (i) $NaBH(OAc)_3$, $ZnCl_2$, amine, CH_2Cl_2, 0 °C, 66–91%; (j) NH_2OR_3, pyridine, EtOH, rt, 26–36%.

8 in 85% yield without decomposition of the lactone moiety. When the reaction was carried out at −78 °C, aldehyde **13** was obtained in 82% yield. The use of DIBAL or $LiAlH_4$ instead of super-H® afforded a complex mixture. Bromination of **8**, followed by nucleophilic substitution of the resulting bromide **9** with NaN_3 gave azide **10**. Staudinger reaction of **10** with Ph_3P–NH_4OH afforded amine **11a**, which was treated with acetic anhydride to give acetamide **12**.

Attempts to use **8** as a starting material for the preparation of amines **14**, **15**, and **16** were unsuccessful, because the basic or heated conditions tended to give 2,3-conjugated products. However, it was possible to convert **13** into the desired amines (**14**–**16**) using a combination of $NaBH(OAc)_3$ and $ZnCl_2$.[16] Treatment of **13** with hydroxylamine or methoxylamine afforded oxime derivatives **17** and **18**. Ivermectin and monosaccharide derivatives **19**, **20**, and **21** were also prepared from alkoxy derivatives (**3a**–**d**) according to the same method. 23-Keto- and oxime derivatives (**22**, **23**) were obtained through avermectin B_{2a} derivative **3e** using the previously reported strategy.[17] These synthetic derivatives were subjected to biological assays, after desilylation of 5-*O*-TBDMS and 7-*O*-TMS by HF-pyridine solution.[18,19]

Synthetic avermectin derivatives were evaluated by microplate assay for insecticidal and nematocidal activities against the free living-nematode *Caenorhabditis elegans*, and the brine shrimp *Artemia salina*.[20] Most of avermectin derivatives exhibited good to moderate growth inhibition of *C. elegans* and *A. salina* (Table 1). Among them, carboxy **4** showed the most potent activity against *A. salina* (0.1 ng/mL) and *C. elegans* (0.02 ng/mL). Carboxy ivermectin **19** also displayed higher efficacy than avermectin **1a** and ivermectin **1b**. The polarity of the carboxy group was important for the in vitro activity. Although we expected amine compounds (**11**, **12**, **14**, **15**, **16**) to enhance the activity, similarly to eprinomectin

Table 1. In vitro activities of the alkylidene derivatives against *C. elegans* and *A. salina*



No.	R	*n*	X–Y	MIC (ng/mL)	
				C. elegans	*A. salina*
1a	—	2	CH=CH	2	0.5
1b	—	2	CH_2CH_2	2	2
3a	CO_2Et	2	CH=CH	2	0.5
4	CO_2H	2	CH=CH	0.1	0.02
5	CO morphorine	2	CH=CH	0.5	0.5
6	CO piperazine	2	CH=CH	—	—
7	CO piperidine	2	CH=CH	10	10
8	CH_2OH	2	CH=CH	10	2
9	CH_2Br	2	CH=CH	10	2
10	CH_2N_3	2	CH=CH	10	0.5
11	CH_2NH_2	2	CH=CH	50	10
12	CH_2NHAc	2	CH=CH	10	10
13	CHO	2	CH=CH	—	—
14	CH_2 morphorine	2	CH=CH	10	2
15	CH_2 piperazine	2	CH=CH	50	10
16	CH_2 piperidine	2	CH=CH	50	10
17	CH=NHOH	2	CH=CH	2	2
18	CH=NHOMe	2	CH=CH	2	0.5
3b	CO_2Et	2	CH_2CH_2	2	0.5
19	CO_2H	2	CH_2CH_2	0.5	0.1
3c	CO_2Et	1	CH=CH	2	0.5
20	CO_2H	1	CH=CH	10	2
3d	CO_2Et	1	CH_2CH_2	2	0.5
21	CO_2H	1	CH_2CH_2	10	10
3e	CO_2Et	2	$CH_2CH(\alpha OH)$	2	0.5
22	CO_2Et	2	$CH_2C{=}O$	10	2
23	CO_2Et	2	$CH_2C{=}NOMe$	2	2

and emamectin, increased activity was not observed. Oxime derivatives (**17**, **18**) retained these activities. Hydroxyl, bromo, and azido substitutions slightly reduced the growth inhibition activity (**8**, **9**, **10**). Monosaccharides (**3c**, **3d**, **20**, **21**) and avermectin B_{2a} derivatives (**3e**, **22**, **23**) showed moderate activity.

Preliminary studies on the avermectin derivatives indicated good efficacy in a mouse *Heterakis spumosa* infection model (op). Compounds **3** and **4** exhibited more potent activity than ivermectin at a dosage of 0.025 mg/kg. Further in vitro and in vivo studies on the novel avermectin derivatives are in progress.

Acknowledgements

We would like to thank Dr. K. Shirahata of Kyowa Hakko Kogyo Co., Ltd, for furnishing us with a large quantity of avermectin B_{1a}. This study was supported in part by the Grant of the 21st Century COE Program, Ministry of Education, Culture, Sports, Science, and Technology.

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19. Selected data for deprotected carboxylic acid **4**, HRFABMS: calcd for $C_{50}H_{73}O_{16}Na_2$ $[M+2Na-H]^+$ 975.4694 found 975.4738, ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 5.85 (m, 1H), 5.77–5.70 (m, 3H), 5.54 (dd, $J = 9.5$, 2.5 Hz, 1H), 5.40 (s, 1H), 5.39 (d, $J = 3.0$ Hz, 1H), 5.36 (m, 1H), 4.97 (m, 1H), 4.76 (d, $J = 3.0$ Hz, 1H), 4.70 (dd, $J = 14.5$, 2.5 Hz, 1H), 4.64 (dd, $J = 14.5$, 2.5 Hz, 1H), 4.39 (d, $J = 17.5$ Hz, 1H), 4.28 (d, $J = 6.0$ Hz, 1H), 4.10 (d, $J = 17.5$ Hz, 1H), 3.95 (d, $J = 6.0$ Hz, 1H), 3.92 (br s, 1H), 3.90–3.75 (m, 3H), 3.72–3.58 (m, 2H), 3.47 (s, 3H), 3.46 (d, $J = 9.0$ Hz, 1H), 3.42 (s, 3H), 3.28 (q, $J = 2.5$ Hz, 1H), 3.20 (t, $J = 9.0$ Hz, 1H), 2.93 (t, $J = 9.0$ Hz, 1H), 2.51 (m, 1H), 2.41 (m, 1H), 2.35–2.20 (m, 4H), 2.01 (ddd, $J = 12.0$, 4.0, 2.0 Hz, 1H), 1.86 (s, 3H), 1.76 (m, 1H), 1.64–1.41 (m, 9H), 1.27 (d, $J = 7.0$ Hz, 3H), 1.22 (t, $J = 6.0$ Hz, 3H), 1.15 (d, $J = 7.0$ Hz, 3H), 0.92 (t, $J = 7.5$ Hz, 3H), 0.90 (d, $J = 6.0$ Hz, 3H), 0.89 (d, $J = 7.0$ Hz, 3H), 0.84 (m, 1H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ (ppm): 173.7, 172.0, 139.6, 137.9 (×2), 136.2, 135.1, 127.7, 124.7, 120.3, 118.2, 117.9, 97.9, 95.7, 94.8, 86.5, 81.9, 80.5, 80.3, 79.2, 79.1, 77.1, 74.8, 70.3, 68.3 (×4), 67.6, 67.0, 56.4, 55.9, 45.6, 40.4, 39.7, 36.5, 35.1, 34.4 (×2), 34.2, 30.5, 27.4, 20.2, 19.9, 18.3, 17.7, 16.3, 15.1, 12.9, 12.0. Morpholine **14**, HRFABMS: calcd for $C_{54}H_{84}O_{15}N$ $[M+H]^+$ 986.5841 found 986.5848, ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 5.84 (m, 1H), 5.74 (dd, $J = 9.5$, 1.5 Hz, 1H), 5.73–5.70 (m, 2H), 5.53 (dd, $J = 10.0$, 2.5 Hz, 1H), 5.40 (s, 1H), 5.38 (m, 1H), 5.28 (d, $J = 3.0$ Hz, 1H), 4.96 (m, 1H), 4.75 (d, $J = 3.5$ Hz, 1H), 4.69 (dd, $J = 14.5$, 2.5 Hz, 1H), 4.64 (dd, $J = 14.5$, 2.5 Hz, 1H), 4.27 (d, $J = 5.5$ Hz, 1H), 4.01 (s, 1H), 3.94 (d, $J = 5.5$ Hz, 1H), 3.92 (br s, 1H), 3.91 (m, 2H), 3.84 (m,

1H), 3.81–3.65 (m, 6H), 3.62–3.49 (m, 2H), 3.46 (d, $J = 10.0$ Hz, 1H), 3.41 (s, 3H), 3.40 (s, 3H), 3.27 (q, $J = 2.5$ Hz, 1H), 3.18 (t, $J = 9.0$ Hz, 1H), 2.81 (t, $J = 9.0$ Hz, 1H), 2.55 (t, $J = 5.0$ Hz, 2H), 2.48 (m, 4H), 2.30–2.16 (m, 5H), 2.00 (ddd, $J = 12.0$, 5.0, 2.0 Hz, 1H), 1.85 (s, 3H), 1.76 (m, 1H), 1.62–1.40 (m, 9H), 1.23 (d, $J = 6.5$ Hz, 3H), 1.20 (t, $J = 6.0$ Hz, 3H), 1.14 (d, $J = 7.0$ Hz, 3H), 0.92 (t, $J = 7.0$ Hz, 3H), 0.90 (d, $J = 6.0$ Hz, 3H), 0.89 (d, $J = 7.0$ Hz, 3H), 0.84 (m, 1H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ (ppm): 173.7, 139.6, 138.0, 137.9, 136.2, 135.1, 127.7, 124.7, 120.3, 118.2, 117.9, 98.5, 95.7, 94.8, 84.8, 81.7, 80.9, 80.3, 79.2, 79.1, 78.3, 74.8, 69.8, 68.4, 68.3 (×3), 67.6, 67.5, 67.2, 66.8 (×2), 58.7, 57.1, 56.6, 53.8 (×2), 45.6, 40.4, 39.7, 36.6, 35.3, 35.1, 34.4, 34.2, 30.5, 27.4, 20.1, 19.9, 18.3, 17.9, 16.3, 15.0, 12.9, 12.0.

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