



Research paper

Synthesis, biological activities and structure–activity relationships for new avermectin analogues



Jian Zhang ^{a,1}, Xiang Nan ^{a,1}, Hai-Tao Yu ^c, Pi-Le Cheng ^a, Yan Zhang ^a, Ying-Qian Liu ^{a,*}, Shao-Yong Zhang ^b, Guan-Fang Hu ^c, Huanxiang Liu ^{a,**}, An-Liang Chen ^b

^a School of Pharmacy, Lanzhou University, Lanzhou, 730000, China

^b Provincial Engineering Laboratory of Biopesticide Preparation, Zhejiang A&F University, Lin'an, 311300, Zhejiang Province, China

^c Institute of Plant Protection, Gansu Academy of Agricultural Sciences, Lanzhou, 730070, China

ARTICLE INFO

Article history:

Received 22 August 2014

Received in revised form

23 May 2016

Accepted 25 May 2016

Available online 27 May 2016

Keywords:

Avermectin

Structural modifications

Insecticidal activities

QSAR analysis

ABSTRACT

In an effort to discover new molecules with good insecticidal activities, more than 40 new avermectin derivatives were synthesized and evaluated for their biological activities against three species of arachnids, insects and nematodes, namely, *Tetranychus Cinnabarinus*, *Aphis craccivora* and *Bursaphelenchus xylophilus*. All the tested compounds showed potent inhibitory activities against three insect species. Notably, the majority of compounds exhibited high selectivity against *T. cinnabarinus*, some of which were much better in comparison with avermectin. Especially compounds **9j** (LC₅₀: 0.005 μM) and **16d** (LC₅₀: 0.002 μM) were 2.5- and 4.7-fold more active than avermectin (LC₅₀: 0.013 μM), respectively, against *T. cinnabarinus*. Moreover, compounds **9b**, **9d–f**, **9h**, **9j**, **9l**, **9n**, **9p**, **9r**, **9v** and **17d** showed superior activities with LC₅₀ values of 2.959–5.013 μM compared to that of **1** (LC₅₀: 6.746 μM) against *B. xylophilus*. Meanwhile, the insecticidal activities of compounds **9f**, **9g**, **9h**, and **9m** against *A. craccivora* were 7–8 times better than that of avermectin, with LC₅₀ values of 7.744, 5.634, 6.809, 7.939 and 52.234 μM, respectively. Furthermore, QSAR analysis showed that the molecular shape, size, connectivity degree and electronic distribution of avermectin analogues had substantial effects on insecticidal potency. These preliminary results provided useful insight in guiding further modifications of avermectin in the development of potential new insecticides.

© 2016 Elsevier Masson SAS. All rights reserved.

1. Introduction

Avermectins are a family of 16-membered ring macrocyclic lactones isolated from the fermentation broth of *Streptomyces avermitilis*, which are known to possess exceptionally potent anthelmintic, acaricidal, and insecticidal activities [1,2]. A major fermentation product, avermectin B1a (**1**, Fig. 1), is the most effective avermectin against insects and mites, and has widely been commercialized for agricultural use in China now [3]. Its semi-synthetic derivative with the generic name ivermectin, the 22,23-dihydroavermectin B1a (**2**), has been introduced as a broad spectrum antiparasitic agent for veterinary uses [4,5]. These compounds selectively act on the γ -aminobutyric acid (GABA)-related chloride

ion channels unique to nematodes, insects, ticks, and arachnids, with relatively low or no mammalian toxicity [6–8]. Their remarkable biological activities and intriguing mechanism of action have stimulated considerable interest in the scientific community. In this vein, a number of publications and patents in an attempt to obtain compounds with higher potency and broader spectra of activities have appeared describing intensive modification of avermectin at different positions [9–22].

Among the many reports on SAR investigation, the compounds which were modified at the 4''-position appeared to be the most efficient approach to increase the insecticidal potency, as a result of which most structural modifications of avermectins have focused on position 4''. In particular, various substituents, such as alkylamino, oxyiminoalkyl, alkylsilyl, and alkylthio, etc were introduced at the 4''-position of **1** to afford highly potent analogs [23–32]. Following these efforts, some 4''-substituted analogs, particularly 4''-N-linked congeners were found to exhibit improved activities and pharmacokinetic profiles compared to **1** [33–37]. Among them,

* Corresponding author.

** Corresponding author.

E-mail address: yqliu@lzu.edu.cn (Y.-Q. Liu).

¹ These authors contributed equally to this work.

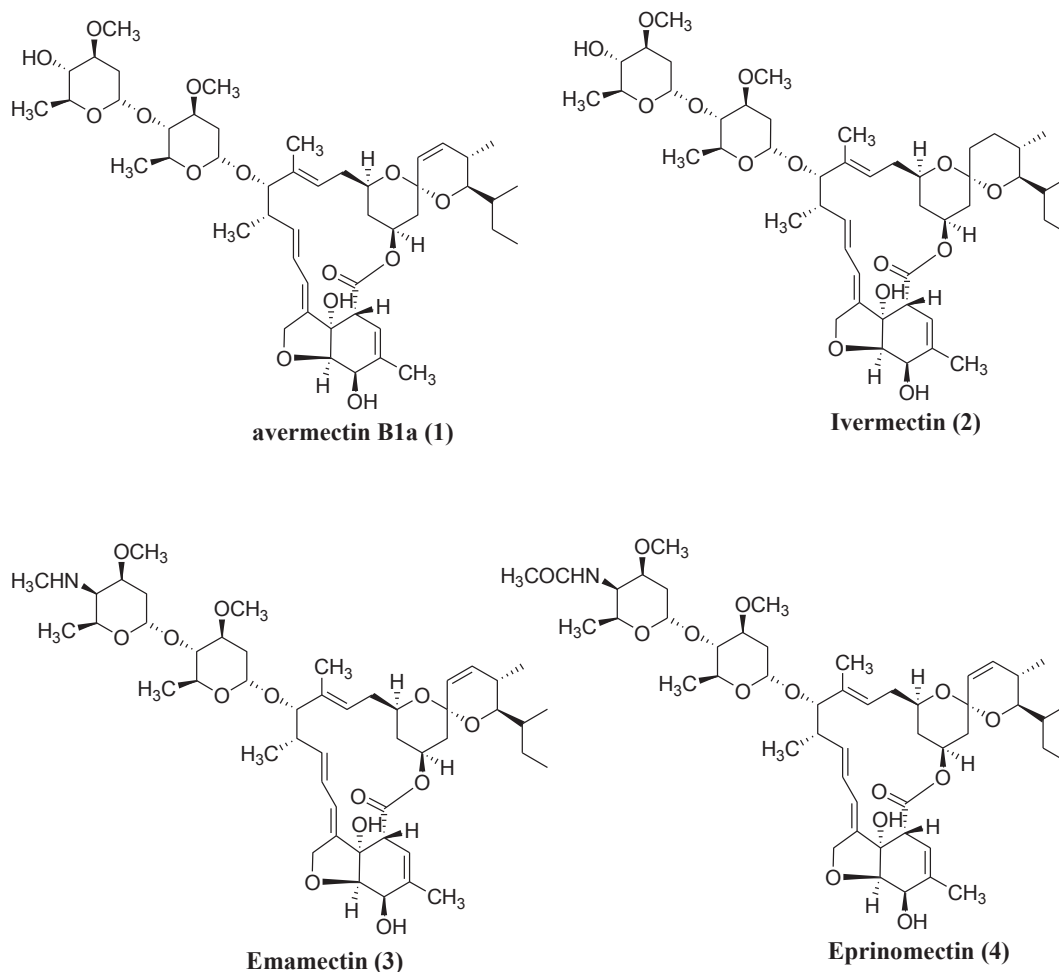


Fig. 1. Chemical structures of avermectin B1a (1), ivermectin (2), emamectin (3) and eprinomectin (4).

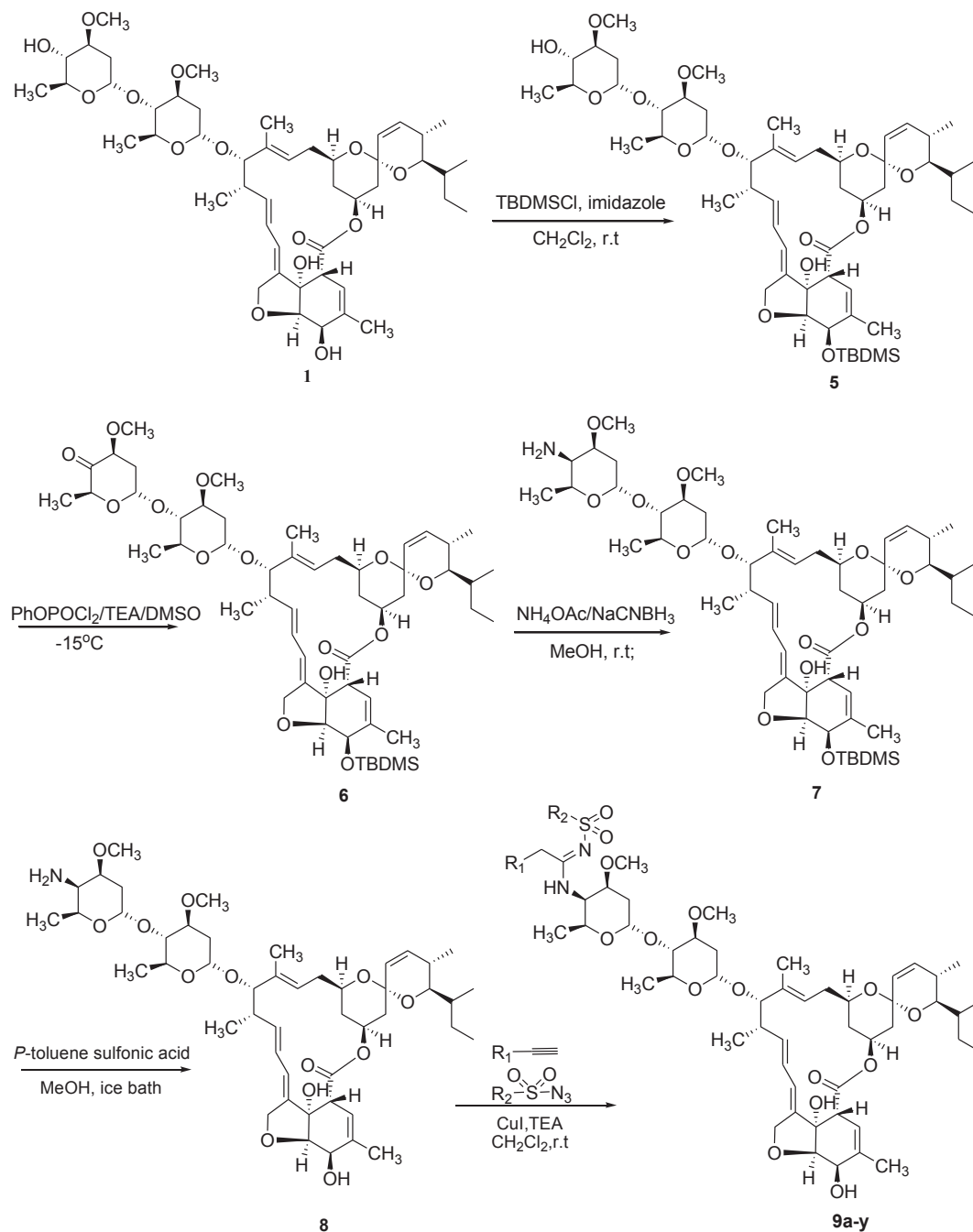
a major breakthrough came from the discovery of 4''-amino-avermectins. For instance, emamectin (3) having a methylamino group in epi-orientation at the 4''-position was one of the most effective compounds reported, with a 1500-fold increase in potency vs avermectin B1 in insecticidal activity, which has achieved commercial success. Eprinomectin (4) in which the 4''-hydroxyl group was replaced by an *epi* acetylamino group exhibited potent endectocidal activity with minimal residues in milk, and is used for treatment of lactating dairy cattle against parasites. Overall, these variants displayed preferable characteristics in their solubility, distribution, chemical stability as well as activity spectra, suggesting the possibility of further optimizing avermectins through rational C-4'' modifications.

Based on these critical clues, in continuation of our program aimed at the discovery and development of natural-product-based pesticidal agents, more than 40 new avermectin derivatives were synthesized. Three types of sulfonyl groups (i.e. sulfonylamidine, sulfonylurea and sulfonylamine) were chosen based on the facts that these groups are commonly found in various drugs and introduction of a sulfonyl group could usually potentiate the biochemical or pharmacological properties of the original molecule [38,39]. In addition, to the best of our knowledge, there is no report on the synthesis of avermectin analogs with *N*-sulfonylamidino group at the C-4'' position of 1 using the copper-catalyzed three-component coupling reaction as the key reaction. For further insight into SAR investigation, the synthesis of the *N*-

sulfonylamidino derivatives of avermectin monosaccharide and avermectin aglycone was described, respectively. The activities of all target compounds against *Tetranychus cinnabarinus*, *Aphis craccivora* and *Bursaphelenchus xylophilus* were evaluated accordingly. Furthermore, the structure-activity relationship (SAR) of these analogs is also discussed. Quantitative structure-activity relationships (QSARs) models were built to understand the relationship between the biological activity and molecular structure of avermectin analogues.

2. Chemistry

The synthesis of intermediate and target compounds were performed as illustrated in Schemes 1–3. Initially, the 5-hydroxyl group of avermectin B1 (1) was selectively protected with *tert*-butyldimethylsilyl chloride (TBDMSCl) in acetonitrile to give 5-*O*-*tert*-butyldimethylsilylavermectin B1 (5) in 82% yield. Subsequent oxidation of 5 using PhOPOCl₂/Et₃N reagent system in dried DMSO afforded 4''-oxo-5-*O*-*tert*-butyl dimethylsilyl avermectin B1 (6) in 60% yield. Reductive amination of 6 using ammonium acetate/NaBH₃CN to give 4''-*epi*-NH₂-5-*O*-TBDMS-4''-deoxyavermectin B1 (7) in 40% yield. Subsequent removal of the *t*-butyldimethylsilyl protecting group of 7 with *p*-toluenesulfonic acid in methanol (1:1) formed the key intermediate 4''-*epi*-Amino-4''-deoxyavermectinB1 (8) [33,36], which was successfully employed as an efficient reacting partner in the Cu-catalyzed three-component reaction



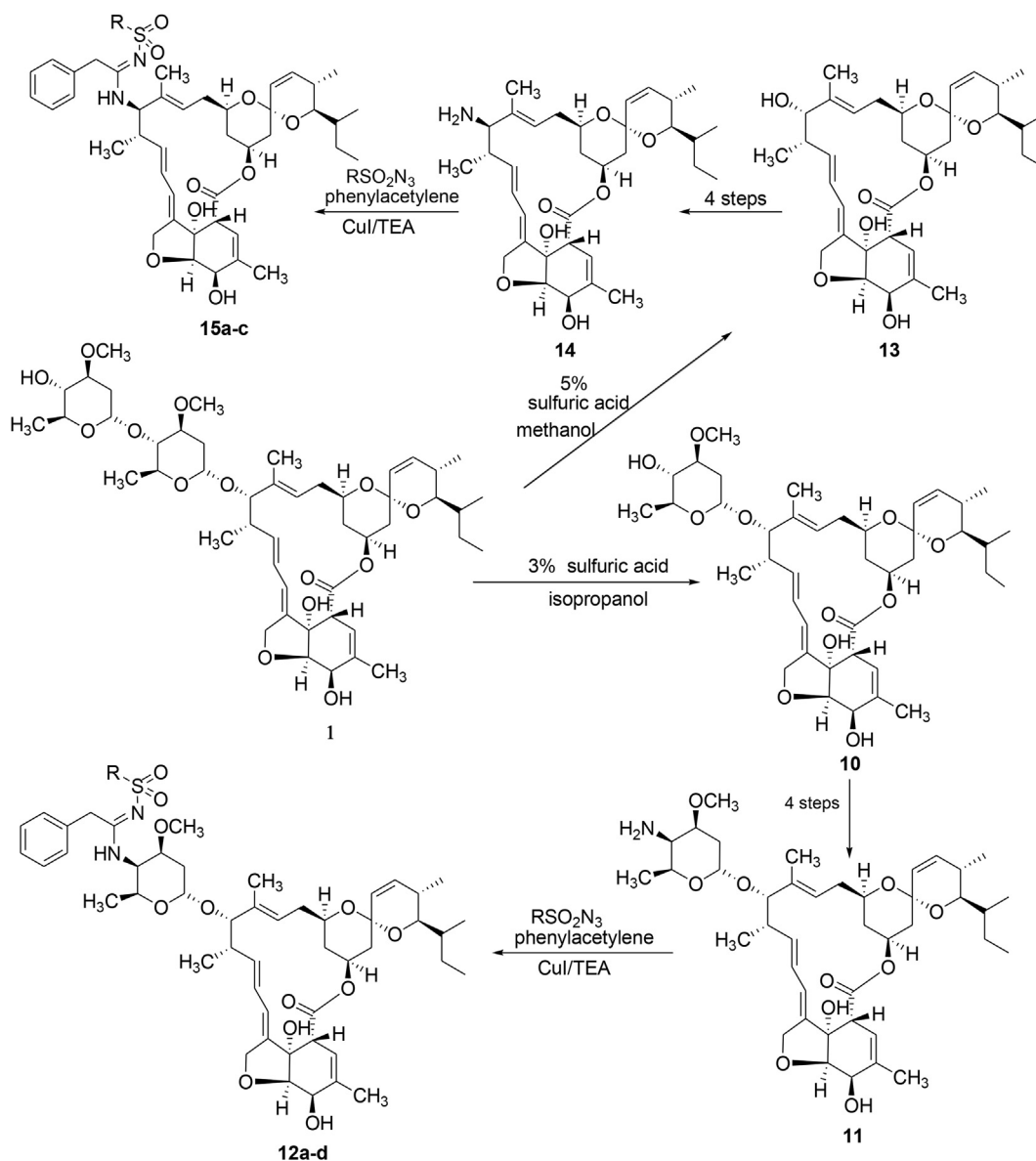
Scheme 1. General synthetic routes for the target compounds **9a–y**.

with sulfonyl azides and alkynes to afford the desired 4''-epi-(sulfonyl amidino)-4''-deoxyavermectin B1 (**9a–y**) in moderate yield. The coupling reaction has a wide substrate scope, a high tolerance to various functional groups, and very mild reaction condition. The reaction proceeds through a ketenimine intermediate, which is generated in situ from the triazole cycloadduct upon release of N₂ gas [49].

Based on the methodology in **Scheme 1**, the synthesis of target compounds **12a–d** and **15a–c** was conducted according to the following procedures (**Scheme 2**). Firstly, avermectin B1 (**1**) was desugared by 3% concentrated sulfuric acid in isopropanol as solvent to afford avermectin monosaccharide (**10**) in 82% yield. Avermectin aglycone (**13**) was prepared by a reaction between

avermectin and 5% concentrated sulfuric acid in methanol as solvent using the same method [50,51]. Similarly, their corresponding amines (**11** and **14**) were prepared through protection, oxidation, reduction and deprotection *via* a similar procedure to that described above for **8** in good yield, respectively. Finally, using similar methods to those for **9a–y**, target compounds **12a–d** and **15a–c** were obtained from **11** and **14** in yields ranging from 36% to 52%.

The target compounds **16a–f** and **17a–d** were synthesized from **8** as shown in **Scheme 3**. Briefly, intermediate **8** was reacted with commercially available sulfonyl chloride using triethylamine and DMAP as acid acceptor and catalyst to furnish the target compounds **16a–f** in excellent yields. In addition, intermediate **8** was



Scheme 2. General synthetic routes for the target compounds **12a–d** and **15a–c**.

coupled with newly prepared sulfonylcarbamates in dry acetonitrile to afford another series of 4 β -sulfonylurea **17a–d** in 30–40% yields.

All newly synthesized compounds were purified by column chromatography and their structures were characterized by ^1H NMR, ^{13}C NMR, EI-MS and elemental analysis.

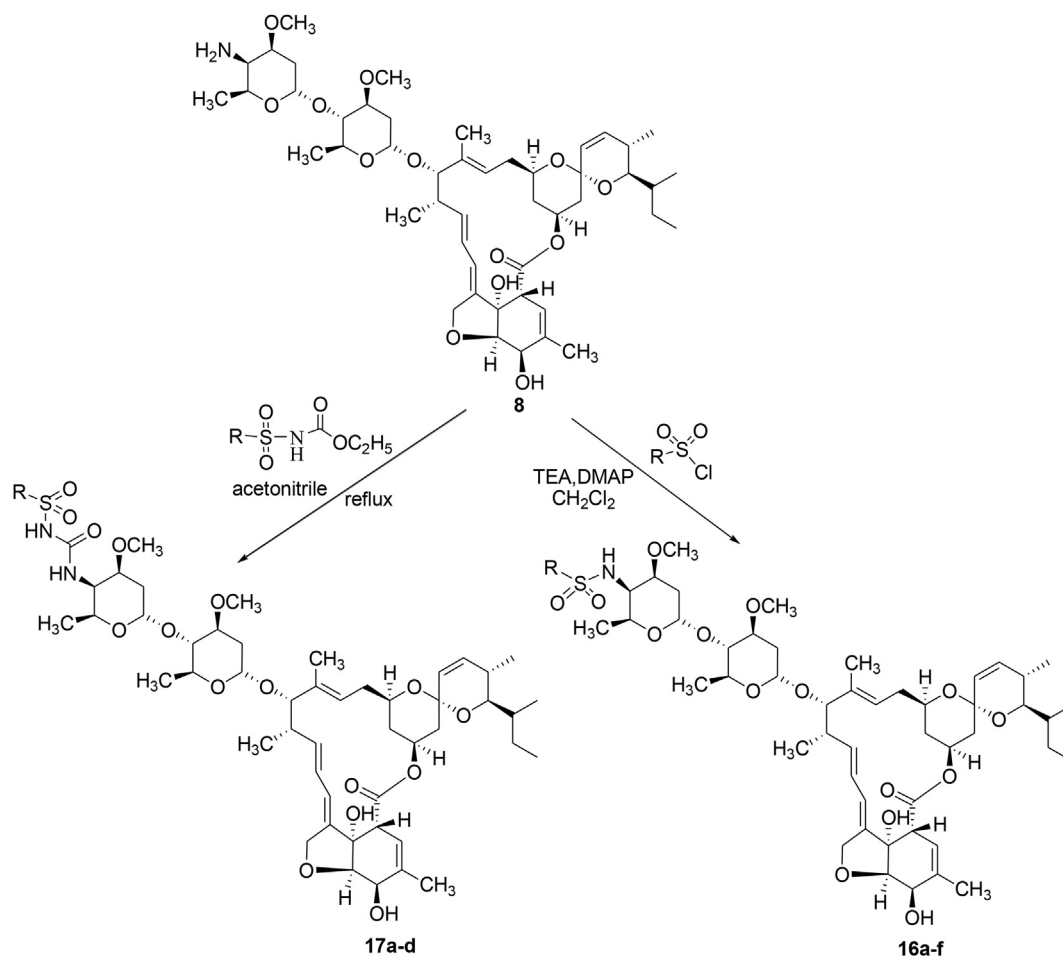
3. Results and discussion

3.1. Biological activity

Results from the assays on the three representative organisms showed that some of the new compounds displayed excellent inhibitory activity, and some important structural features for potency were observed, and a preliminary structure-activity relationship (SAR) in this work was also generated based on calculated LC_{50} . The data presented in **Tables 1–3** suggest that the relationship between the substituted groups and biological activities could be summarized as follows.

3.1.1. Lethal activity against *B. xylophilus*

As shown in **Tables 1–3**, most of the target compounds exhibited moderate to potent nematocidal activity against *B. xylophilus*, and were as or more potent than **1**. Notably, compounds **9b**, **9d–f**, **9h**, **9j**, **9l**, **9n**, **9p**, **9r**, **9v** and **17d** showed superior activity with LC_{50} values of 2.959–5.013 μM compared to that of **1** (LC_{50} : 6.746 μM) against *B. xylophilus*. Furthermore, to investigate whether the disaccharide moiety can influence activity, the corresponding monosaccharide (**12a–d**) and aglycone (**15a–c**) sulfonylamidine congeners were subsequently prepared. From **Tables 1–2**, the avermectin sulfonylamidine derivatives (**9e–h**) were more potent than their corresponding monosaccharide (**12a–d**) and aglycone (**15a–c**) sulfonylamidine analogues, respectively. While **9h** showed significant activity with LC_{50} value of 4.458 μM , its corresponding **12b** and **15a** displayed only marginal activities with LC_{50} values of 157.285 and 75.093 μM , respectively. In particular, compound **12c** (LC_{50} : >200 μM) led to complete loss of activity in the corresponding **9f** (LC_{50} : 4.127). These results highlight the critical role of the disaccharide functionality in sulfonylamidino substituted



Scheme 3. General synthetic routes for the target compounds **16a-f** and **17a-d**.

Table 1
Inhibitory activity against *T. cinnabarinus*, *A. craccivora* and *B. xylophilus* of compounds **9a-y** and avermectin.

Compd	R ₁	R ₂	LC ₅₀ (μM)		
			<i>T. cinnabarinus</i>	<i>A. craccivora</i>	<i>B. xylophilus</i>
9a	Ph	(4-Ome)Ph	0.166 ± 0.065	13.816 ± 0.147	46.191 ± 1.891
9b	Ph	2-Naphth	0.523 ± 0.119	85.738 ± 0.269	3.539 ± 0.899
9c	Ph	(4-Me)Ph	0.529 ± 0.165	53.368 ± 0.159	68.082 ± 3.397
9d	Ph	Butyl	0.422 ± 0.096	18.034 ± 0.171	3.817 ± 0.713
9e	Ph	2-thienyl	0.393 ± 0.115	29.075 ± 0.173	4.347 ± 1.860
9f	Ph	3-pyridyl	0.060 ± 0.024	7.744 ± 0.131	4.127 ± 1.045
9g	Ph	Me	0.010 ± 0.008	5.634 ± 0.195	7.535 ± 1.050
9h	Ph	(4-F)Ph	0.519 ± 0.126	6.809 ± 0.127	4.458 ± 0.514
9i	Ph	(2,4-F)Ph	0.542 ± 0.204	20.601 ± 0.130	>200
9j	Ph	(CH ₃) ₂ N	0.005 ± 0.005	9.061 ± 0.123	5.013 ± 0.694
9k	(4-Ome)Ph	Butyl	0.127 ± 0.056	65.847 ± 0.182	134.657 ± 7.715
9l	(4-Ome)Ph	(2,4-F)Ph	0.010 ± 0.007	49.372 ± 0.135	3.006 ± 0.157
9m	(4-Ome)Ph	Me	0.024 ± 0.009	7.939 ± 0.148	9.562 ± 1.049
9n	(4-Ome)Ph	(4-F)Ph	0.123 ± 0.033	39.507 ± 0.128	2.959 ± 0.416
9o	(4-Ome)Ph	2-thienyl	0.457 ± 0.098	50.640 ± 0.167	17.079 ± 4.043
9p	(4-Ome)Ph	3-pyridyl	0.267 ± 0.061	61.366 ± 0.161	3.140 ± 0.957
9q	(4-Ome)Ph	2-Naphth	1.499 ± 0.547	12.667 ± 0.210	100.397 ± 3.298
9r	(4-Ome)Ph	(4-Ome)Ph	0.038 ± 0.010	46.844 ± 0.111	4.276 ± 0.984
9s	(4-Ome)Ph	(4-Me)Ph	0.024 ± 0.013	49.030 ± 0.188	82.184 ± 2.945
9t	(4-Ome)Ph	(CH ₃) ₂ N	0.023 ± 0.011	11.560 ± 0.146	6.746 ± 1.875
9u	Ph	Et	0.027 ± 0.014	72.150 ± 0.201	29.518 ± 1.231
9v	(2-OH)Et	Et	0.020 ± 0.010	14.660 ± 0.131	4.313 ± 0.377
9w	Ph	(4-Cl)Ph	0.090 ± 0.028	14.961 ± 0.148	196.135 ± 4.027
9x	Ph	Ph	0.085 ± 0.028	21.231 ± 0.165	>200
9y	(4-Ome)Ph	Ph	0.221 ± 0.079	11.063 ± 0.140	>200
Avermectin			0.013 ± 0.009	52.234 ± 0.134	6.746 ± 1.168

Table 2Inhibitory activity against *T. cinnabarinus*, *A. craccivora* and *B. xylophilus* of compounds **12a–d** and **15a–c**.

Compd	R ₁	R ₂	LC ₅₀ (μM)		
			<i>T.cinnabarinus</i>	<i>A.craccivora</i>	<i>B.xylophilus</i>
12a	Ph	Me	0.032 ± 0.014	20.778 ± 0.138	16.952 ± 1.572
12b	Ph	(4-F)Ph	0.083 ± 0.017	19.675 ± 0.201	157.285 ± 4.810
12c	Ph	3-pyridyl	0.058 ± 0.026	25.668 ± 0.136	>200
12d	Ph	2-thienyl	0.092 ± 0.026	13.616 ± 0.123	137.262 ± 5.616
15a	Ph	(4-F)Ph	0.278 ± 0.101	53.555 ± 0.203	75.093 ± 3.118
15b	Ph	Me	0.289 ± 0.061	25.640 ± 0.131	43.611 ± 2.570
15c	Ph	2-thienyl	0.189 ± 0.063	47.332 ± 0.164	62.931 ± 3.817

Table 3Inhibitory activity against *T. cinnabarinus*, *A. craccivora* and *B. xylophilus* of compounds **16a–f** and **17a–d**.

Compound	R	LC ₅₀ (μM)		
		<i>T.cinnabarinus</i>	<i>A.craccivora</i>	<i>B.xylophilus</i>
16a	Me	0.030 ± 0.017	7.416 ± 0.184	13.140 ± 2.592
16b	3-pyridyl	0.042 ± 0.020	14.739 ± 0.130	5.701 ± 1.106
16c	Ph	0.065 ± 0.027	20.314 ± 0.174	37.279 ± 1.043
16d	(4-Me)Ph	0.002 ± 0.001	16.157 ± 0.121	9.239 ± 1.112
16e	(4-Cl)Ph	0.108 ± 0.027	72.955 ± 0.225	70.312 ± 4.048
16f	2-Naphth	0.115 ± 0.033	25.438 ± 0.132	>200
17a	Ph	0.042 ± 0.017	10.463 ± 0.142	8.477 ± 2.315
17b	(4-Me)Ph	0.250 ± 0.085	161.656 ± 0.234	>200
17c	(4-Cl)Ph	0.011 ± 0.007	15.779 ± 0.139	10.303 ± 1.048
17d	2-Naphthyl	2.468 ± 0.958	12.103 ± 0.171	3.813 ± 0.174

avermectin derivatives.

To assist in further identification of more efficient sulfonylated **1**-derivatives, preliminary SAR correlations were formulated. Firstly, within the sulfonylamidine series (**9a–y**), the effects of different substituent groups in the sulfonylamidine side chains were investigated. As shown in Table 1, when the R₁ group was fixed as phenyl and the R₂ group in the sulfonylamidine side chains was varied, the general rank of potency based on the R₂ group was **9b** (2-naphthyl) > **9d** (butyl) > **9f** (3-pyridinyl) > **9e** (2-thienyl) > **9h** (*p*-fluorophenyl) ≥ **9j** (dimethylamino) > **9g** (methyl) > **9a** (*p*-methoxyphenyl) > **9c** (*p*-methylphenyl). Thus the R₂ substituent was important for potency, and 2-naphthyl (**9b**) gave the best result compared with other synthetic derivatives. Interestingly, when the R₁ group was changed from phenyl to *p*-methoxyphenyl, the order of potency was somehow changed, and further investigation is needed. For example, compound **9b** bearing a phenyl R₁ group displayed greater activity than **9q** with a *p*-methoxyphenyl R₁ group. In addition, compound **9v** (LC₅₀: 4.313 μM) bearing a hydroxymethyl R₁ group displayed greater activity as compared to **9u** (LC₅₀: 29.518 μM) with a phenyl R₂ group. These findings suggested that their nematocidal potency was dual-controlled by both the R₁ and R₂ groups in the sulfonylamidine side chain.

In addition to sulfonyl amidines, another two series of sulfonylamine and sulfonylurea-substituted **1**-derivatives were investigated as well. In contrast, the activity of compounds with sulfonylamidino groups were predominantly higher than those of sulfonylamine or sulfonylurea-substituted **1**-derivatives, indicating that the electron distribution and substituents on disaccharide side chain play an important role in the derivatives' activity. As seen from Table 3, most of compounds were less potent than **1**. Significantly, the introduction of pyridinyl (**16b**) group into the sulfonylamine side chain resulted in obviously improved activity. Excitingly, compound **17d** with a 2-naphthylsulfonylurea group displayed the most potent activity among the tested compounds against *B. xylophilus*, and better than the positive control

avermectin. The results further underlined the nematocidal differences could be ascribed to a combination of factors, like the nature of the substituents (which may depend on the size of substituents, electronic characteristics of substituents, or other factors) or a different interaction at the site.

3.1.2. Lethal activity against *T. cinnabarinus*

As listed in Tables 1–3, the LC₅₀ rates of the target compounds against *T. cinnabarinus* formed a sharp contrast to that of the activities against other two tested organisms, implying that mite pests were especially susceptible to these derivatives. The biological selectivity against *T. cinnabarinus* may be the most important characteristic property of these synthetic derivatives. Remarkably, all of the target compounds exhibited significant acaricidal activity against *T. cinnabarinus*, with LC₅₀ values ranging from 0.002 to 2.468 μM. Among all the tested derivatives, most compounds were found to be equally potent or possess superior acaricidal activities to avermectin. In particular, compounds **9g**, **9j**, **9l**, **16d** and **17c** showed pronounced acaricidal activities with LC₅₀ values of 0.010, 0.005, 0.010, 0.002 and 0.011 μM, respectively, higher than that of avermectin B1a (0.013 μM). Within the sulfonylamidine series (**9a–y**), for compounds containing phenyl R₁ group (**9a–9j**), the analogues bearing the smaller R₂ groups (**9g** and **9j**) showed relatively better acaricidal potency in each series. For example, compound **9g** (LC₅₀: 0.010 μM) with methyl R₂ group showed 40-fold more potent activity than **9d** (LC₅₀: 0.422 μM) with a butyl R₂ group, indicating that the substituent's size is critical. Moreover, compounds with substituted phenyl groups did not display a significant improvement in activities relative to **1**, regardless of the presence of the electron donating methyl (**9c**) or electron withdrawing fluoro (**9h**) substituents at the 4-position. Meanwhile, changing the substituted phenyl R₂ group in sulfonyl amidine side chain into aromatic heterocycle (**9e** and **9f**) or condensed ring group (**9q**) was not effective. Among them, compound **9f** with pyridinyl R₂ group showed potent activity with LC₅₀ value of 0.060 μM. Therefore, small aliphatic chains with/without polar functionality appear to be the best substituents for R₂. Similar results were seen in compounds containing methoxyphenyl R₁ group (**9a–9j**), and highlight the critical role of the length and flexibility of the substituent R₂ in sulfonylamidine side chain of these derivatives. Similarly, monosaccharide analogues **12a–d** and aglycone analogues **15a–c** displayed less potent acaricidal activities against *T. cinnabarinus* than their corresponding avermectin analogues. Overall, considering the discussion above, it was further revealed that the acaricidal potencies of our designed sulfonylamidine analogues were dual controlled by altering the length of the sulfonylamidine arm and the size of the substituent group. Only when a molecule can keep a good balance between the flexibility and the size will it attain the best acaricidal activity, such as the analogues **9g** and **9j**. As shown in Table 3 that compounds **16a–f** and **17a–d** displayed similar or more potent activity than **1** against *T. cinnabarinus*. The acaricidal activities of compounds **16d** and **17c** against *T. cinnabarinus* were 10–100 times better than that of other compounds. In particular, compound **16d** (LC₅₀: 0.002 μM) exhibited much better acaricidal activity against *T. cinnabarinus* than avermectin (LC₅₀: 0.013 μM).

3.1.3. Lethal activity against *A. craccivora*

As shown in Tables 1–3 that all of target compounds displayed similar SAR against *A. craccivora*. The activities of these compounds in Table 1–3 varied drastically, depending upon the types and patterns of substitution on the side chains of avermectin skeleton. Except for compounds **9b**, **9c**, **9u**, **9p**, **9k**, **15a**, **16e** and **17b**, all other compounds were found to be equally potent or to possess superior insecticidal activities to avermectin. In particular, the insecticidal

activities of compounds **9f**, **9g**, **9h** and **9m** against *A. craccivora* were 7–8 times better than that of avermectin, as the LC₅₀ values of compounds **9f**, **9g**, **9h**, **9m**, and avermectin against *A. craccivora* were 7.744, 5.634, 6.809, 7.939 and 52.234 μM, respectively. It is unexpected to note that compound **17b** (LC₅₀:161.656 μM) displayed approximately 4-fold decreased activity in comparison with avermectin B1a, although some activity still remained. More interestingly, most of the monosaccharide analogues **12a–d** and aglycone analogues **15a–c** displayed comparable or higher insecticidal activities against *A. craccivora* than avermectin. As we envisioned, the introduction of sulfonyl groups into the avermectin molecule potentiated the insecticidal activity. Thus, the design and synthesis of these compounds have provided valuable information to potentially increase the biological value of avermectin.

3.2. QSAR analysis

To select the most relevant descriptors to pLC₅₀ of the compounds, GA was used to do the feature selection based on the training samples. The optimum number of variables (Vn) was selected when adding new descriptors did not improve the performance of the model significantly. In the present work, the optimum Vn was 6. The best model and corresponding statistical parameters are given below:

$$\text{pLC}_{50} = -0.610\text{nDB} - 6.82\text{EEig06x} + 22.7\text{BELm4} \\ + 1.02\text{Mor14v} - 1.99\text{Mor30v} - 61.0\text{R1v} + -2.44$$

$$N_{\text{tr}} = 35, R_{\text{tr}}^2 = 0.806, Q_{\text{loo}}^2 = 0.714, \text{RMSE}_{\text{tr}} = 0.300$$

$$N_{\text{tst}} = 8, Q_{\text{ext}}^2 = 0.720, \text{RMSE}_{\text{ext}} = 0.362$$

These statistical parameters indicated that the built model is stable and predictive. The experimental and predicted pLC₅₀ are given in Table 4. The pairwise correlations of the selected descriptors are given in Table 5. The regression plot of predicted values vs experimental ones is shown in Fig. 2. Fig. 3 shows the Williams plot of the built model. All these results indicate that the built model is robust, reliable, stable and predictive to all compounds. From Fig. 2 and Table 4, it can be seen that the predicted activity of compound **17b** had a difference of 0.99 from the experimental one. Moreover, Williams plot (Fig. 3) also showed compound **17b** had a large standard error, suggesting that compound **17b** may be a Y outlier. From Fig. 3, it can be also seen that the hat value (h* = 0.600), indicating that avermectin is an X outlier. Actually, relative to other compounds, avermectin is a little different with minimum structure. Even though avermectin is out of the application domain, the built model can predict the activity of the compound accurately with the predicted error of 0.14.

According to the regression model, the standardized regression coefficients (Std. Coeff.) of 6 descriptors are -0.526 (nDB), -0.498 (EEig06x), 0.480 (BELm4), 0.859 (Mor14v), -0.658 (Mor30v), and -0.316 (R1v+), respectively. The Std. Coeff. determines the relative importance of the descriptors. The most important two descriptors are Mor14v and Mor30v. These two descriptors are 3D-MorSE (3D-Molecule Representation of Structures based on Electron diffraction) descriptors weighted by the van der Waals volume, reflecting the close correlations between the molecular size and the activities of the studied compounds. The third important descriptor is nDB, which is the number of double bonds in a molecule. The descriptor has a negative correlation with the biological activity, suggesting that the smaller the number of double bonds in a molecule is, the higher is its inhibitory activity of one compound. For example,

Table 4

The experimental and predicted activities against *T. cinnabarinus* in the QSAR Model (The compounds were sorted according to the increased order of experimental activities).

No.	Compd	Exp pLC ₅₀	Pred pLC ₅₀	Error
1	17d	5.61	5.77	0.16
2	9q	5.82	5.98	0.16
3	9i	6.27	6.26	-0.01
4	9b	6.28	6.25	-0.03
5	9c	6.28	6.46	0.18
6	9h	6.28	6.89	0.61
7	9o^a	6.34	6.5	0.16
8	9d	6.37	6.29	-0.08
9	9e	6.41	6.57	0.16
10	15b	6.54	6.72	0.18
11	15a	6.56	6.58	0.02
12	9p	6.57	6.78	0.21
13	17b	6.6	7.59	0.99
14	9y	6.65	6.54	-0.11
15	15c^a	6.72	6.82	0.1
16	9a	6.78	6.23	-0.55
17	9k	6.89	7.02	0.13
18	9n^a	6.91	6.38	-0.53
19	16f	6.94	6.75	-0.19
20	16e	6.97	7.43	0.46
21	12d	7.03	7.02	-0.01
22	9w	7.04	6.76	-0.28
23	9x^a	7.07	6.98	-0.09
24	12b^a	7.08	7.15	0.07
25	16c^a	7.19	7.54	0.35
26	9f	7.22	6.83	-0.39
27	12c	7.23	7.34	0.11
28	16b	7.37	7.08	-0.29
29	17a	7.37	7.25	-0.12
30	9r^a	7.42	6.91	-0.51
31	12a	7.49	7.22	-0.27
32	16a	7.52	7.55	0.03
33	9u	7.56	7.72	0.16
34	9m	7.61	7.56	-0.05
35	9s	7.61	7.43	-0.18
36	9t	7.63	7.89	0.26
37	9v	7.69	7.55	-0.14
38	avermectin	7.87	8.01	0.14
39	17c	7.92	7.59	-0.33
40	9l	7.97	7.66	-0.31
41	9g^a	8	7.42	-0.58
42	9j	8.27	8.08	-0.19
43	16d	8.54	8.07	-0.47

^a Compounds in test set.

Table 5

Pair correlations (R²) between the selected descriptors.

Descriptors	nDB	EEig06x	BELm4	Mor14v	Mor30v	R1v+
nDB	1					
EEig06x	0.333	1				
BELm4	0.535	0.550	1			
Mor14v	0.0756	0.470	0.0570	1		
Mor30v	-0.160	0.596	0.393	0.495	1	
R1v+	0.217	-0.313	0.0695	-0.171	-0.195	1

avermectin with the smallest value of nDB has good inhibitory activity. The descriptor EEig06x is calculated from edge adjacency matrix weighted by edge degrees. The edge adjacency matrix can encode the connectivity between graph edges and thus the descriptor can reflect the molecular complexity and branching. Another descriptor with a positive regression coefficient is BELm4, which is the lowest eigenvalue n. 4 of Burden matrix weighted by atomic masses. The descriptor can reflect the overall topology features and the size of molecule. R1v+ is a R-GETAWAY (GEometry, Topology, and Atom-Weights Assembly) descriptor, which is

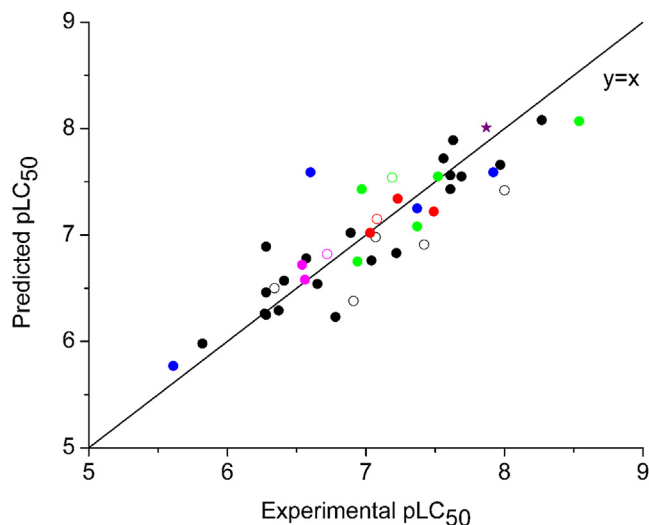


Fig. 2. The experimental and predicted activity in the training and test sets. The test set shown in open symbols for the test set and the training set shown in closed symbols with compounds **9a–y**, **12a–d**, **15a–c**, **16a–f** and **17a–d** and avermectin shown in black, red, magenta, green, blue and violet, respectively. (For interpretation of the references to colour in this figure caption, the reader is referred to the web version of this article.)

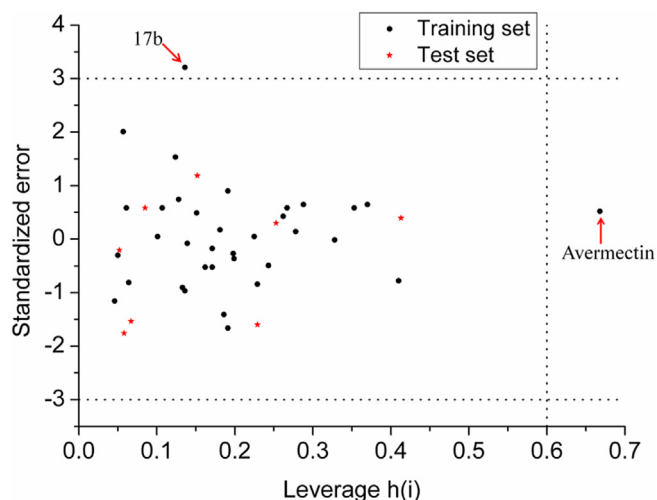


Fig. 3. Williams plot of the training and test sets. The dashed lines are the warning value of hat ($h^* = 0.600$) and the 3σ limit, respectively.

calculated by using the molecular influence matrix (MIM) and interatomic distance matrix. The descriptor can encode information about molecular size and shape [52]. The negative correlation of this descriptor with the inhibitory activity indicates the increasing of the value of $R1v +$ will decrease the inhibitory activity of the compound.

Overall, by explaining the involved descriptors in the built QSAR model, it can be seen that the molecule size, shape, branching degree and the number of double bonds will influence the inhibitory activity of the studied avermectin analogues. Even though the presented QSAR model consisting of six descriptors is quite complex and not easy to interpret in detail, it proved to have reasonable predictive power and may hence serve as a valuable tool to predict the activity of yet untested derivatives.

4. Conclusions

In summary, on the basis of commercial insecticides emamectin and eprinomectin, more than 40 new avermectin derivatives were synthesized and their structures were identified by ^1H NMR, ^{13}C NMR, EI-MS and elemental analysis. The biological activities against *T. cinnabarinus*, *A. craccivora* and *B. xylophilus* were evaluated. Most of the target compounds possessed good-to-excellent activities against three insect species, some of which were much better in comparison with the commercial avermectin. As we envisioned, SARs demonstrated that the disaccharide functionality may be essential in increasing biological activity in the **1**-derived compounds, and the size, electron density, and distribution of the substituents within the sulfonyl side chain are critical to the derivatives' activity. Furthermore, the built QSAR model has given some important data that are consistent with the SAR analysis, indicating that the insecticidal potency was mainly influenced by several factors such as the molecular shape, size, branching degree and the number of double bonds of avermectin analogues. With a concise synthesis and potent biological profiles, these findings support our further optimization of **1** to develop potential avermectin-derived pesticides. Continuing studies to substantiate and improve activity profiles are underway in our laboratory and will be reported in due course.

5. Experimental protocols

5.1. General

All reagents and solvents were purchased from commercial sources and were used as received. 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.). Melting points were determined in Kofler apparatus and were uncorrected. Mass spectra were recorded on a Bruker Daltonics APEXII49e spectrometer (Bruker Company, USA) with ESI source as ionization. NMR spectra were recorded on a Bruker AM-400 spectrometer (Bruker Company, USA) at 400 MHz using TMS as the reference. The starting avermectin B1a was purchased from Shanghai DEMO Medical Tech Co., Ltd, Shanghai, China. Sulfonylcarbamates were prepared according to the procedure reported previously [25,26]. Intermediates **5**, **6**, **7**, **8**, **10**, **11**, **13** and **14** were synthesized from avermectin B1a (see Supporting Information).

5.2. General procedure for the preparation of target compounds **9a–y**, **12a–d** and **15a–c**

To a stirred mixture of CuI (0.021 mmol), sulfonyl azide (0.24 mmol), and alkyne (0.26 mmol) in CH_2Cl_2 (10 mL), amine nucleophile (0.2 mmol) was added slowly at room temperature under an N_2 atmosphere. Triethylamine (0.26 mmol) was added prior to the addition of nucleophiles, if necessary. After the reaction was completed, as monitored with TLC, the reaction mixture was diluted by adding CHCl_3 (20 mL) and aqueous NH_4Cl solution (3 mL). The mixture was stirred for an additional 30 min and two layers were separated. The aqueous layer was extracted with CHCl_3 (30 mL \times 3). The combined organic layers were dried over anhydrous magnesium sulfate, filtered, and concentrated *in vacuo*. The resulting residue was purified by flash chromatography on silica gel using a mixture of petroleum ether and ethyl acetate (10:1–20:1 by volume) as the eluent to afford target compounds.

5.2.1. Data for compound **9a**

Yield = 45%; white solid; mp: 138–140 °C; ^1H NMR (400 MHz,

CDCl₃) δ : 7.87 (d, 2H, R₂-H₂, H₆, J = 8.4 Hz), 7.40–7.19 (m, 5H, R₁-H₂, H₃, H₄, H₅, H₆), 6.93 (d, 2H, R₂-H₃, H₅, J = 8.4 Hz), 5.84 (m, 1H, H₉), 5.78–5.70 (m, 3H, H₂₃, H₁₀, H₁₁), 5.54 (d, 1H, H₂₂, J = 8.4 Hz), 5.42–5.39 (m, 3H, H₁₉, H_{1'}, H₃), 5.06 (m, 1H, H₁₅), 4.96 (br. s, 1H, –NH–), 4.73–4.68 (m, 3H, H_{1'}, H_{8a}), 4.51 (d, 1H, –CH₂C=N–, J = 8.0 Hz), 4.35–4.25 (m, 2H, H₅, –CH₂C=N–), 4.03 (s, 1H, 7-OH), 3.98–3.75 (m, 8H, H₆, H₁₃, H_{5'}, H₁₇, H_{5''}, R₂-OCH₃), 3.59–3.44 (m, 3H, H_{3'}, H₂₅, H_{3''}), 3.38 (s, 3H, 3'-OCH₃), 3.28 (s, 3H, 3'-OCH₃), 3.07 (t, 1H, H_{4'}, J = 9.2 Hz), 2.50 (m, 1H, H₁₂), 2.36–2.17 (m, 6H, 5-OH, H₁₆, H₂₄, H_{2'}), 2.01 (d, 1H, H₂₀, J = 8.8 Hz), 1.87 (s, 3H, 4-Me), 1.76 (d, 1H, H₁₈, J = 11.2 Hz), 1.59–1.43 (m, 9H, H₂₀, H₂₆, H₂₇, 14-Me, H_{2''}), 1.25–0.86 (m, 19H, 6'-Me, 6''-Me, 12-Me, 27-Me, 24-Me, 26-Me, H₁₈). ¹³C NMR (100 MHz, CDCl₃) δ : 173.6, 167.6, 162.0, 139.6, 137.9, 137.7, 136.2, 135.0, 129.9, 129.3 (2C), 128.6 (2C), 128.3 (2C), 128.1, 127.6, 124.7, 120.2, 118.2, 117.9 (2C), 113.6, 113.5, 98.3, 95.7, 94.8, 81.9, 81.1, 80.3, 79.1, 77.3, 77.0, 76.6, 74.8, 73.4, 68.2, 67.6, 66.8, 64.8, 56.4, 55.4, 50.3, 45.6, 40.3, 39.7, 39.6, 36.5, 35.0, 34.3, 34.1, 31.6, 30.4, 29.6, 27.4, 20.0, 19.8, 18.0, 16.9, 16.2, 15.0, 12.8, 11.9. Anal. Calcd. For C₆₃H₈₆N₂O₁₆S: C, 65.26; H, 7.48; N, 2.42. Found: C, 65.24; H, 7.48; N, 2.40. ESI-MS m/z : 1159.40 [M+H]⁺.

5.2.2. Data for compound 12a

Yield = 36%; white solid; mp: 148–150 °C; ¹H NMR (400 MHz, CDCl₃) δ : 7.42–7.27 (m, 5H, Ar-H), 5.84 (m, 1H, H₉), 5.82–5.67 (m, 3H, H₂₃, H₁₀, H₁₁), 5.54 (d, 1H, H₂₂, J = 9.2 Hz), 5.42 (s, 1H, H₃), 5.38–5.32 (m, 1H, H₁₉), 4.93 (d, 1H, H₁₅, J = 7.2 Hz), 4.66 (m, 2H, H_{8a}), 4.50 (t, 1H, –CH₂C=N–), 4.52–4.48 (m, 1H, H_{4'}), 4.37 (m, 1H, H₅), 4.29 (m, 1H, –CH₂C=N–), 4.13–4.10 (m, 1H, –NH–), 4.03 (s, 1H, 7-OH), 3.97–3.81 (m, 3H, H_{5'}, H₆, H₁₃), 3.70–3.67 (m, 2H, H₁₇, H_{3'}), 3.48 (s, 3H, 3'-OCH₃), 3.04 (s, 3H, R₂-CH₃), 3.11 (m, 2H, H₂₅, H₂), 2.48 (m, 1H, H₁₂), 2.35–1.99 (m, 7H, 5-OH, H₁₆, H₂₄, H_{2'}, H₂₀), 1.87–1.71 (m, 4H, 4-Me, H₁₈), 1.60–1.26 (m, 7H, H₂₀, H₂₆, H₂₇, 14-Me), 1.12–0.95 (m, 6H, 6'-Me, 12-Me), 0.91–0.81 (m, 10H, 27-Me, 24-Me, 26-Me, H₁₈). ¹³C NMR (100 MHz, CDCl₃) δ : 173.5, 167.5, 139.8, 137.8, 137.3, 136.3, 134.8, 132.7, 130.0, 129.3 (2C), 128.9 (2C), 128.2, 127.5, 124.9, 120.1, 118.4, 117.9, 95.6, 94.9, 82.3, 80.2, 78.9, 77.3, 76.6, 74.7, 73.5, 70.2, 68.3, 67.5, 64.8, 59.0, 56.5, 50.3, 45.5, 43.1, 40.3, 39.4, 36.4, 35.0, 34.1, 31.3, 30.4, 27.4, 19.8, 17.0, 16.3, 14.9, 12.9, 11.9. Anal. Calcd For C₅₀H₇₀N₂O₁₂S: C, 65.05; H, 7.64; N, 3.03. Found: C, 65.06; H, 7.64, N, 3.02. ESI-MS m/z : 945.43 [M+Na]⁺.

5.2.3. Data for compound 15a

Yield = 48%; pale yellow solid; mp: 159–161 °C; ¹H NMR (400 MHz, CDCl₃) δ : 7.92 (d, 2H, R₂-H₂, H₆, J = 6.4 Hz), 7.53 (m, 3H, R₂-H₂, H₅, R₁-H₄), 7.27–7.08 (m, 4H, R₁-H₂, H₃, H₅, H₆), 5.73 (m, 1H, H₉), 5.70–5.66 (m, 3H, H₂₃, H₁₀, H₁₁), 5.53 (d, 1H, H₂₂, J = 11.2 Hz), 5.41 (s, 1H, H₃), 5.30–5.23 (m, 2H, H₁₉, H₁₅), 4.64–4.56 (m, 3H, H_{8a}, –NH–), 4.51–4.49 (m, 1H, –CH₂C=N–), 4.44–4.35 (m, 2H, H₅, –CH₂C=N–), 4.03 (s, 1H, 7-OH), 4.00–3.60 (m, 4H, H₆, H₁₃, H₁₇, H₂₅), 3.23 (m, 1H, H₂), 2.62 (m, 1H, H₁₂), 2.37–2.13 (m, 4H, 5-OH, H₁₆, H₂₄), 2.05 (s, 1H, H₂₀), 1.87 (s, 3H, 4-Me), 1.60–1.21 (m, 7H, H₂₀, H₂₆, H₂₇, 14-Me), 0.95–0.78 (m, 10H, 27-Me, 24-Me, 26-Me, H₁₈). ¹³C NMR (100 MHz, CDCl₃) δ : 173.3, 165.4, 141.5, 137.8, 134.7, 133.8, 132.6, 129.9 (2C), 129.7 (2C), 128.8 (2C), 126.2, 119.3, 117.9 (2C), 117.8, 115.6, 97.6, 80.2, 78.9, 77.3, 77.0, 76.6, 71.6, 68.2, 67.5, 67.0, 59.9, 56.5, 45.3, 41.4, 40.0, 38.3, 38.1, 36.6, 36.2, 35.5, 34.1, 29.6, 27.2, 19.8, 18.7, 15.4, 13.1, 12.5, 11.4. Anal. Calcd For C₄₈H₅₉FN₂O₉S: C, 67.11; H, 6.92; N, 3.26. Found: C, 67.10; H, 6.93; N, 3.26. ESI-MS m/z : 881.19 [M+Na]⁺.

5.3. General procedure for the preparation of target compounds 16a–f

A solution of sulfonyl chloride (0.50 mmol) in dried dichloromethane (5 mL) at 0 °C was added dropwise to a solution of

intermediate **8** (0.21 g, 0.25 mmol), triethylamine (0.50 mmol), and DMAP (0.01 mmol) in dichloromethane (8 mL). The mixture was stirred at room temperature for 8 h. The reaction mixture was poured into water and extracted with dichloromethane (3 × 10 mL). The organic layer was washed with 5% dilute hydrochloric acid (3 × 10 mL), 5% aqueous sodium bicarbonate (3 × 10 mL), and saturated sodium chloride solution (3 × 10 mL), dried over anhydrous sodium sulfate, filtered. The organic phase was evaporated under reduced pressure, and the residue was subjected to flash chromatography on silica gel, eluting with petroleum ether/ethyl acetate (10:1–20:1) to afford target products **16a–f**.

5.3.1. Data for compound 16a

Yield = 45%; white solid; mp: 163–165 °C; ¹H NMR (400 MHz, CDCl₃) δ : 5.86 (m, 1H, H₉), 5.79–5.72 (m, 3H, H₂₃, H₁₀, H₁₁), 5.55 (d, 1H, H₂₂, J = 9.2 Hz), 5.43–5.36 (m, 3H, H₁₉, H_{1'}, H₃), 4.98 (m, 1H, H₁₅), 4.77–4.67 (m, 3H, H_{1'}, H_{8a}), 4.51 (d, 1H, H₅), 4.07 (s, 1H, 7-OH), 3.99–3.81 (m, 5H, H₆, H₁₃, H_{5'}, H₁₇, H_{5''}), 3.62–3.44 (m, 3H, H_{3'}, H₂₅, H_{3''}), 3.43 (s, 6H, 3''-OCH₃, 3'-OCH₃), 3.30 (s, 1H, H₂), 3.22 (t, 1H, H_{4'}, J = 8.8 Hz), 3.10 (s, 3H, R-CH₃), 2.53 (m, 1H, H₁₂), 2.36–2.22 (m, 6H, 5-OH, H₁₆, H₂₄, H_{2'}), 2.18 (s, 1H, H₂₀), 1.88 (s, 3H, 4-Me), 1.77 (d, 1H, H₁₈, J = 9.2 Hz), 1.55–1.43 (m, 9H, H₂₀, H₂₆, H₂₇, 14-Me, H_{2''}), 1.26–1.15 (m, 6H, 6'-Me, 6''-Me), 0.96–0.87 (m, 13H, 12-Me, 27-Me, 24-Me, 26-Me, H_{18ax}). ¹³C NMR (100 MHz, CDCl₃) δ : 173.7, 139.6, 137.9, 136.2, 135.1, 127.6, 124.7, 120.3, 118.2, 117.9, 98.5, 95.7, 94.9, 81.9, 80.9, 80.3, 79.1, 77.3, 77.0, 76.6, 74.8, 74.5, 68.2, 67.6, 67.0, 65.7, 56.5, 56.0, 54.7, 45.6, 42.9, 40.3, 39.6, 36.5, 35.1, 34.3, 34.1, 30.8, 30.5, 29.6, 27.4, 20.1, 19.9, 18.2, 17.1, 16.3, 15.0, 12.9, 12.0. Anal. Calcd For C₄₉H₇₅NO₁₅S: C, 61.94; H, 7.96; N, 1.47. Found: C, 61.95; H, 7.95; N, 1.47. ESI-MS m/z : 972.45 [M+Na]⁺.

5.4. General procedure for the preparation of target compounds 17a–d

Intermediate **8** (0.2 mmol) was added dropwise to a solution of 0.4 mmol of sulfonylcarbamate in dry toluene (20 mL). The reaction mixture was refluxed for 2–4 h and then concentrated. The residue was purified by chromatography on silica gel using petroleum ether/ethyl acetate (10:1–20:1) as eluant to give target products **17a–d**.

5.4.1. Data for compound 17a

Yield = 30%; white solid; mp: 145–147 °C; ¹H NMR (400 MHz, CDCl₃) δ : 8.02 (dd, 2H, R-H₂, H₆, J = 8.0 Hz, 14.4 Hz), 7.66–7.54 (m, 3H, R-H₃, H₄, H₅), 5.84–5.68 (m, 4H, H₉, H₂₃, H₁₀, H₁₁), 5.56 (d, 1H, H₂₂, J = 8.0 Hz), 5.43–5.39 (m, 3H, H₃, H₁₉, H_{1'}), 4.99 (m, 1H, H₁₅), 4.77 (s, 1H, H_{1'}), 4.58–4.47 (m, 2H, H_{8a}), 4.21 (d, 1H, H₅), 4.07 (s, 1H, 7-OH), 3.98–3.50 (m, 8H, H₆, H₁₃, H_{5'}, H₁₇, H_{5''}, H_{3'}, H₂₅, H_{3''}), 3.38 (s, 3H, 3''-OCH₃), 3.30 (s, 3H, 3'-OCH₃), 2.52 (m, 1H, H₁₂), 2.29–2.01 (m, 7H, 5-OH, H₁₆, H₂₄, H_{2'}, H₂₀), 1.88 (s, 3H, 4-Me), 1.75 (m, 1H, H₁₈), 1.68–1.49 (m, 9H, H₂₀, H₂₆, H₂₇, 14-Me, H_{2''}), 1.25–1.05 (m, 9H, 6'-Me, 6''-Me, 12-Me), 0.96–0.87 (m, 10H, 27-Me, 24-Me, 26-Me, H₁₈). ¹³C NMR (100 MHz, CDCl₃) δ : 173.0, 139.6, 138.6, 138.1, 136.2, 135.1, 133.6, 132.5, 128.8 (2C), 128.2, 127.5 (2C), 127.0, 124.0, 121.7, 120.5, 118.2, 98.5, 95.7, 94.9, 81.0, 80.6, 79.1, 77.3, 77.0, 76.6, 74.8, 73.3, 72.3, 68.6, 68.2, 67.0, 65.2, 56.6, 55.8, 50.8, 49.9, 45.5, 40.4, 39.6, 35.1, 34.1, 31.6, 30.9, 30.5, 29.6, 27.4, 20.1, 19.4, 18.2, 16.9, 16.3, 15.0, 12.9, 12.0. Anal. Calcd For C₅₅H₇₈N₂O₁₆S: C, 62.60; H, 7.45; N, 2.65. Found: C, 62.62; H, 7.44; N, 2.65. ESI-MS m/z : 1055.19 [M+H]⁺.

5.5. Biological assay

Bioassays were conducted with three insect species, *Tetranychus*

cinnabarinus and *Aphis craccivora* (Gansu Pesticides Research Center, Gansu Academy of Agricultural Sciences, China). *Bursaphelenchus xylophilus* (Provincial Engineering Laboratory of Biopesticide Preparation, Zhejiang A&F University, China). All bioassays were performed on representative test organisms reared in the laboratory. The bioassay was repeated at 25 ± 2 °C and $70 \pm 10\%$ relative humidity. Each test sample was prepared in acetone and diluted to the required concentration with distilled water containing TW-80 (0.1 mL L^{-1}) for bioassays. Each concentration was tested three times in parallel. Concentrations of 5.0, 2.50, 1.0, 0.50, 0.10, 0.050 and 0.010 mg L^{-1} for *Tetranychus cinnabarinus*, 100.0, 50.0, 25.0, 10.0, and 5.0 mg L^{-1} for *Bursaphelenchus xylophilus*, and 250.0, 100.0, 50.0, 10.0 and 1.0 mg L^{-1} for *Brevicoryne brassicae* were used. For comparative purpose, avermectin was tested under the same conditions. Assessments were made on a dead/alive basis, and mortality rates were corrected using Abbott's formula [40]. Evaluations are based on a percentage scale of 0–100, in which 0 = no activity and 100 = total kill. The deviation of values was $\pm 5\%$. Standard probit analysis was used to determine lethal concentrations of 50% (LC_{50}) by using the SPSS program, version 13.0. All bioassay results are summarized in Tables 1–3

5.5.1. Lethal activity against carmine spider mite (*Tetranychus cinnabarinus*)

The acaricidal activity of compounds **9a–y**, **12a–d**, **15a–c**, **16a–f**, **17a–d** and avermectin (positive control) was evaluated using the slide immersion method recommended by FAO [41]. Thirty adult spider mites were fixed dorsally to a strip of double-sided tape attached to the slide by using a small brush. The slide was immersed and shaken for 3 s in the diluted solution of the test compound. After the excess solution was removed, the treated slides with the mites were kept at 25 ± 2 °C in a Petri dish with moist filter paper. Percentage mortalities were determined 24 h after treatment. Each treatment was replicated with triplicate experiments and each replicate involved 30 adult mites. Control groups were tested with acetone only.

5.5.2. Lethal activity against cowpea aphid (*Aphis craccivora*)

The insecticidal activity of compounds **9a–y**, **12a–d**, **15a–c**, **16a–f**, **17a–d** and avermectin (positive control) against *A. craccivora* was evaluated according to the reported procedure [42]. Thirty healthy adult aphids were dipped into the diluted solutions of tested compound for 5 s, superfluous fluid was removed, and aphids were placed in an air-conditioned room. Percentage mortalities were determined 24 h after treatment. Each treatment was performed in triplicate. Control groups were tested with acetone only.

5.5.3. Lethal activity against pine wood nematode (*Bursaphelenchus xylophilus*)

[43] Acetone solutions of compounds **9a–y**, **12a–d**, **15a–c**, **16a–f**, **17a–d** and avermectin (positive control) were first prepared at different concentrations. Then $10 \mu\text{L}$ of the above solutions was added to the aqueous suspension ($90 \mu\text{L}$) containing approximately 2500 living nematodes (third-instar and fourth-instar larvae of *B. xylophilus*) per milliliter. The blank control group was prepared in the same way but lacked the tested compound. Three replicates in each trial were made and kept at 25 °C for 24 h. Finally, the activities of five concentrations of the tested compounds were monitored under a microscope by recording the death rate of the tested nematodes. Nematodes that did not move when prodded with a needle were considered to be dead. Percentage mortalities were evaluated 24 h after treatment. The LC_{50} values of tested compounds were calculated using the probit method.

5.6. Quantitative structure–activity relationships analysis

The initial structures of the compounds were sketched in Chem-Draw. Then, the geometry optimization of these compounds were performed in the HyperChem 7.0 [44] using molecular mechanics force field (MM+) with the convergence criterion of 0.01 kcal/mol, and the minimized geometry was further refined by means of the more precise semi-empirical quantum chemical method (AM3). The molecular descriptors of refined compounds were calculated in Dragon 5.4 software [45] and 1664 descriptors were obtained. They are respectively constitutional descriptors (0D molecular descriptors), functional groups counts, atom-centered fragments (1D molecular descriptors), topological descriptors, walk and path counts, connectivity indices, information indices, 2D autocorrelations, edge adjacency indices, Burden eigenvalues, topological charge index, eigenvalue-based index (2D molecular descriptors), Randic molecular profiles, geometrical descriptors, RDF descriptors, 3D-MoRSE descriptors, WHIM descriptors, GETAWAY descriptors (3D molecular descriptors) and other molecular descriptors including charge descriptors and molecular properties. To reduce the nonuseful and redundant information, constant variables, near-constant variables and one of any two descriptors with a correlation coefficient of 0.99 or higher were excluded by using the program Dragon 5.4 and thus 685 descriptors were used for further analysis.

To build and test the QSAR model, 43 compounds were randomly split into training set and the test set. The insecticidal activities of these compounds against *T. Cinnabarinus* expressed as pLC_{50} values were defined as dependent variable in the following analysis.

After calculation of the molecular descriptors and dataset splitting, genetic algorithm (GA) approach [46,47] was used to search the feature space and select descriptors correlated with to the insecticidal activities. In the study, QSAR model of avermectin analogues was built using multiple linear regression (GA-MLR) in MobyDigs [48]. Leave-one-out (LOO) cross validation correlation coefficient (Q^2_{100}) was used as the fitness function to evaluate performance of the developed models. The population size was set to 100 and maximum allowed variables in a model was 6. Other corresponding parameters were defined as default.

The built model here were validated using several statistic terms such as correlation coefficient (R^2), leave-one-out (LOO) cross-validated correlation coefficient Q^2_{100} and root-mean-square error (RMSE). Moreover, to evaluate the predictive ability of the QSAR model, the compounds from the test set were used to validate the model.

The value of the leverage matrix $h(i)$ was used to evaluate the applicability domain (AD) of the model, defined as follows:

$$h_i = x_i (X^T X)^{-1} x_i^T \quad (i = 1, \dots, m)$$

x_i is row vector of the descriptors of the query compound i , m is the number of query compounds and X is the $n \times k$ matrix of the descriptors of the training set (n and k is the number of compounds in training set and the number of descriptors of the model, respectively). The plot of cross-validated standardized errors and $h(i)$ values (the Williams plot) visually shows Y outliers and X outliers in a model. The horizontal and vertical dashed lines display the limits of normal values in the plot. For example, compounds whose cross-validated standardized errors are more than 3.0 standard deviation units are Y outliers in a model. A compound with the $h(i)$ value greater than h^* is considered as an X outlier. The warning $h(i)$ (h^*) is defined by $3k/n$. Here, n and k are the number of compounds in training set and the number of descriptors of the model plus 1, respectively.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (30800720, 31371975); the Post-Doctor Research Foundation (20090450142); the Fundamental Research Funds for the Central Universities (Izujbky-2013-69), as well as the Foundation of Priority forestry disciplines in Zhejiang A&F University (KF201325).

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejmech.2016.05.056>.

References

- [1] F.E. Dayan, C.L. Cantrell, S.O. Duke, Natural products in crop protection, *Bioorg. Med. Chem.* 17 (2009) 4022–4034.
- [2] A. Awasthi, M. Razzak, R. Al-Kassas, J. Harvey, S. Garg, An overview on chemical derivatization and stability aspects of selected avermectin derivatives, *Chem. Pharm. Bull.* 60 (2012) 931–944.
- [3] P. Sun, Q.F. Zhao, F.T. Yu, H. Zhang, Z.H. Wu, Y.Y. Wang, Q.L. Zhang, W. Liu, Spiroketol formation and modification in avermectin biosynthesis involves a dual activity of AveC, *J. Am. Chem. Soc.* 135 (2013) 1540–1548.
- [4] W.C. Campbell, History of avermectin and ivermectin, with notes on the history of other macrocyclic lactone antiparasitic agents, *Curr. Pharm. Biotechnol.* 13 (2012) 853–865.
- [5] J.C. Chabala, H. Mrozik, R.L. Tolman, P. Eskola, A. Lusi, L.H. Peterson, M.F. Woods, M.H. Fisher, Ivermectin, a new broad-spectrum antiparasitic agent, *J. Med. Chem.* 23 (1980) 1136–1139.
- [6] S.P. Rohrer, J.P. Arena, Ivermectin interactions with invertebrate ion channels, in: J. Marshall Clark (Ed.), *Molecular Action of Insecticides on Ion Channels*, vol. 591, Merck Research Laboratories, Rahway, New Jersey, 1995, pp. 264–283.
- [7] J.P. Arena, K.K. Liu, P.S. Paress, E.G. Frazier, D.F. Cully, H. Mrozik, J. Schaeffer, The mechanism of action of avermectin in *Caenorhabditis elegans*: correlation between activation of glutamate-sensitive chloride current, membrane binding and biological activity, *J. Parasitol.* 81 (1995) 286–294.
- [8] A.J. Wolstenholme, Recent progress in understanding the interaction between avermectins and ligand-gated ion channels: putting the pests to sleep, *Invert. Neurosci.* 10 (2010) 5–10.
- [9] M.H. Fisher, Structure-activity relationships of the avermectins and milbemycins, in: A.H. Paul, M.H. Robert, P.M. Edward, M. Junshi (Eds.), *Phytochemicals for Pest Control*, vol. 658, Merck Research Laboratories, Rahway, New Jersey, 1997, pp. 220–238.
- [10] W.C. Campbell, R.W. Burg, M.H. Fisher, R.A. Dybas, The discovery of ivermectin and other avermectins, in: P.S. Magee, G.K. Kohn, J.J. Menn (Eds.), *Pesticide Synthesis through Rational Approaches*, vol. 255, Merck Institute for Therapeutic Research, Rahway, NJ, 1984, pp. 5–20.
- [11] H.G. Davies, R.H. Green, Avermectins and milbemycins part I, *Chem. Soc. Rev.* 20 (1991) 211–269.
- [12] H.G. Davies, R.H. Green, Avermectins and milbemycins part II, *Chem. Soc. Rev.* 20 (1991) 271–339.
- [13] M.H. Fisher, Recent advances in avermectin research, *Pure Appl. Chem.* 62 (1990) 1231–1240.
- [14] W.L. Shoop, H. Mrozik, M.H. Fisher, Structure and activity of avermectins and milbemycins in animal health, *Veterinary Parasitol.* 59 (1995) 139–156.
- [15] R.J. Cvetovich, C.H. Senanayake, J.S. Amato, L.M. DiMichele, T.J. Bill, R.D. Larsen, R.F. Shuman, T.R. Verhoeven, E.J.J. Grabowski, Practical syntheses of 13-O-[(2-methoxyethoxy)-methyl]-22,23-dihydroavermectin B1 aglycon [dimecetin-*n*-isopropanol, MK-324] and 13-epi-O-(methoxymethyl)-22,23-dihydroavermectin B1 aglycon [L-694,554], flea active ivermectin analogues, *J. Org. Chem.* 62 (1997) 3989–3993.
- [16] B.O. Linn, H.H. Mrozik, Substituted and Unsubstituted 13-(alkoxy)methoxy Derivatives of the Avermectin Aglycones, Compositions and Use, US Patent 4587247 (1986).
- [17] R.C. Newbold, T.L. Shih, Avermectin Derivatives, US Patent 5411946 (1995).
- [18] L.A. Liao, Z.M. Li, H.Y. Fang, W.G. Zhao, Z.J. Fan, G.L. Liu, Synthesis and bioactivity of 5-ivermectin bil esters, *Acta Chim. Sin.* 60 (2002) 468–474.
- [19] H. Mrozik, P. Eskola, M.H. Fisher, Avermectin acyl derivatives with anthelmintic active, *J. Med. Chem.* 25 (1982) 658–663.
- [20] T.A. Blizzard, G.M. Margiatta, H. Mrozik, Syntheses and biological activities of 13-epi-avermectins: potent anthelmintic genes with an increased margin of safety, *J. Med. Chem.* 35 (1992) 3873–3878.
- [21] H. Michale, R. Fisher, M. Helmut, 5-Oxime Avermectin Derivatives, US Patent 5015630 (1991).
- [22] P. Jeschke, G. Karig, R. Velten, R. Fischer, O. Malsam, U. Goergens, C. Arnold, E. Sanwald, A. Harder, A. Turberg, Avermectin Derivatives, WO 2008019784 (2008).
- [23] T. Pitterna, J. Cassayre, O.F. Hüter, P.M.J. Jung, P. Maienfisch, F.M. Kessabi, L. Quaranta, H. Tobler, New ventures in the chemistry of avermectins, *Bioorg. Med. Chem.* 17 (2009) 4085–4095.
- [24] K. Nagai, K. Shiomi, T. Sunazuka, A. Harder, A. Turberg, S. Omura, Synthesis and biological evaluation of novel 4'-alkoxy avermectin derivatives, *Bioorg. Med. Chem. Lett.* 14 (2004) 4135–4139.
- [25] P. Maienfisch, K.F. Murphy, J. Cassayre, L. Quaranta, T. Pitterna, O.F. Hueter, P. Jung, Preparation of Avermectin B1 and Avermectin B1 Monosaccharide Derivatives Having an Alkoxyethyl Substituent in the 4'- or 4'-position as Pesticides, WO 2004056844, 2004.
- [26] C.S. Zhang, C. Albermann, X. Fu, J.S. Thorson, The in vitro characterization of the iterative avermectin glycosyltransferase AveB1 reveals reaction reversibility and sugar nucleotide flexibility, *J. Am. Chem. Soc.* 128 (2006) 16420–16421.
- [27] J.H. Zhao, M.H. Ji, X.H. Xu, J.L. Cheng, G.N. Zhu, Synthesis analogues of milbemycin and their bioactivity evaluation, *Chin. Chem. Lett.* 21 (2010) 1391–1394.
- [28] E. Lamy, P. Luthi, C. Paturel, T. Winkler, P.M.J. Jung, Synthesis and reactivity of 4'-phenylsulfonimine-avermectin B1 and 4'-phenylsulfonimine-avermectin B1 monosaccharide derivative, *Tetrahedron Lett.* 47 (2006) 5657–5660.
- [29] T.K. Jones, L. Chu, H. Mrozik, L. Slayton, B. Rafalko, M.L. Goldmann, W.L. Shoop, G. Margiatta, M.H. Fisher, Synthesis and biological activity of 4a,4'-Disubstituted avermectins, *J. Agric. Food Chem.* 42 (1994) 1786–1790.
- [30] P.T. Meinke, S.P. O'Connor, D.A. Ostlund, W.L. Shoop, M.H. Fisher, H. Mrozik, New, potent anthelmintic and Acaricidal agents: 4'-Deoxy-C'-Thio-Substituted avermectin derivatives, *Bioorg. Med. Chem. Lett.* 13 (1993) 2675–2680.
- [31] G.H. Wei, Y.G. Dua, R.J. Linhardt, New potent insecticidal agent: 4'-fucosyl avermectin derivative, *Tetrahedron Lett.* 45 (2004) 6895–6898.
- [32] K. Nagai, T. Sunazuka, K. Shiomi, A. Harder, A. Turberg, S. Omura, Synthesis and biological activities of novel 4'-Alkylidene avermectin derivatives, *Bioorg. Med. Chem. Lett.* 13 (2003) 3943–3946.
- [33] M.K. Fu, X. Wu, J. Ning, J.Z. Li, Solid phase synthesis of 4''-epi-Methylamino-4''-deoxyavermectin B1 benzoate, *Chin. J. Chem.* 23 (2005) 901–904.
- [34] Y.M. Jia, X.M. Liang, X.Q. Fang, J.P. Wu, D.Q. Wang, C.H. Rui, X.L. Fan, H.Y. Zhao, Y.X. Wang, Synthesis of 4''-benzyloxymino-4''-deoxyavermectin B1a derivatives, *Chin. Chem. Lett.* 18 (2007) 895–898.
- [35] H. Mrozik, P. Eskola, B.H. Arison, B.O. Linn, A. Lusi, A. Matzuk, T.L. Shih, M. Tischler, F.S. Waksmunski, M.J. Wyvratt, T.A. Blizzard, G.M. Margiatta, M.H. Fisher, W.L. Shoop, J.R. Egerton, 4''-Deoxy-4''-aminoavermectins with potent broad spectrum antiparasitic activities, *Bioorg. Med. Chem. Lett.* 5 (1995) 2435–2440.
- [36] R.J. Cvetovich, D.H. Kelly, L.M. DiMichele, R.F. Shuman, E.J.J. Grabowski, Syntheses of 4'-epi-Amino-4'-deoxyavermectin B1, *J. Org. Chem.* 59 (1994) 7704–7708.
- [37] M.F. Loewe, R.J. Cvetovich, L.M. DiMichele, R.F. Shuman, E.J.J. Grabowski, Glycosidation route to 4'-epi-(Methylamino)-4'-Deoxyavermectin B, (MK-244E, mamectin benzoate, *J. Org. Chem.* 59 (1994) 7870–7875.
- [38] A. Casini, A. Scozzafava, A. Mastrolorenzo, C.T. Supuran, Sulfonamides and sulfonylated derivatives as anticancer agents, *Curr. Cancer Drug Targ.* 2 (2002) 55–75.
- [39] C. Lamberth, Sulfur chemistry in crop protection, *J. Sulfur Chem.* 25 (2004) 39–62.
- [40] W.S. Abbott, A method of computing the effectiveness of an insecticide, *J. Econ. Entomol.* 18 (1925) 265–267.
- [41] Y.J. Wang, X.M. Ou, H. Pei, X.M. Lin, K. Yu, Toxicities of novel insecticide chlorfenpyr against several insects in lab, *Agrochem. Res. Appl.* 10 (2006) 20–23.
- [42] Q.Q. Zhao, Y.Q. Li, L.X. Xiong, Q.M. Wang, Design, synthesis and insecticidal activity of novel phenylpyrazoles containing a 2,2,2-trichloro-1-alkoxyethyl moiety, *J. Agric. Food Chem.* 58 (2010) 4992–4998.
- [43] T. Kohno, K. Togashi, N. Fukamiya, The nematocidal activity and the structure-activity relationships of stilbenes, *Nat. Prod. Res.* 21 (2007) 606–615.
- [44] R. Hyperchem, 7.0, Hypercube, Inc. Gainesville, 2002.
- [45] S. Talet, Dragon for Windows (Software for Molecular Descriptor Calculations), Version 5.4. Talet, Srl, Milano, Italy, 2006. <http://www.talet.mi.it>.
- [46] D.E. Goldberg, J.H. Holland, Genetic algorithms and machine learning, *Mach. Learn.* 3 (1988) 95–99.
- [47] E. Papa, F. Villa, P. Gramatica, Statistically validated QSARs, based on theoretical descriptors, for modeling aquatic toxicity of organic chemicals in Pimephales p. romelas (fathead Minnow), *J. Chem. Inf. Model.* 45 (2005) 1256–1266.
- [48] R. Todeschini, V. Consonni, M. Pavan, MOBYDIGS Software (Version 1.2) for Windows, Talet Srl, Milan, Italy, 2002.
- [49] E.J. Yoo, M. Ahlquist, I. Bae, K.B. Sharpless, V.V. Fokin, S. Chang, Mechanistic studies on the Cu-catalyzed three-component reactions of sulfonyl azides, 1-alkynes and amines, alcohols, or water: dichotomy via a common pathway, *J. Org. Chem.* 73 (2008) 5520–5528.
- [50] H. Mrozik, P. Eskola, B.H. Arison, A.S. George, M.H. Fisher, Avermectin aglycones, *J. Org. Chem.* 47 (1982) 489–492.
- [51] Q.A. Wu, Z.Y. Xu, P. Zheng, D.Q. Xu, Z.H. Chen, Y.C. Shen, Preparation of avermectin B1 monosaccharide and avermectin B1 aglycone, *Chin. J. Pestic.* 43 (2004) 28–30.
- [52] V. Consonni, R. Todeschini, M. Pavan, P. Gramatica, Structure/response correlations and similarity/diversity analysis by GETAWAY descriptors. 2. Application of the novel 3D molecular descriptors to QSAR/QSPR studies, *J. Chem. Inf. Comput. Sci.* 42 (2002) 693–705.