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Nematicidal effect against *Bursaphelenchus xylophilus* of harmine quaternary ammonium derivatives, inhibitory activity and molecular docking studies on acetylcholinesterase

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Abstract In the present study, we have investigated nematicidal effects against *Bursaphelenchus xylophilus* and inhibition potential, molecular docking of 43 harmine derivatives on acetylcholinesterase in vitro and in vivo. Among them, harmine quaternary ammonium derivatives **10**, **11**, **12** and **13** displayed promising nematicidal effects with 48 h LC_{50} values of 1.63, 1.63, 1.75 and 1.44 μg/mL, respectively and remarkable inhibition effects on acetylcholinesterase (IC_{50} values are 0.92, 0.90, 0.82, 0.07 μg/mL in vitro and 17.16, 14.56, 13.63, 3.06 μg/mL in vivo, respectively). The structure–activity analysis indicated that the presence of the methyl group in 1–position, the electron–donating substituents in 2–and 9–positions, bromine in 6–position, and the electron–withdrawing substituents in 7–position of carboline ring, could enhance the nematicidal effect and inhibition of acetylcholinesterase. Moreover, a molecular model was provided for the binding between compound **13** and the active site of acetylcholinesterase based on the computational docking results and helps us to optimize these new leading compounds.

Keywords Harmine derivatives · Natural product – Based nematicide · Acetylcholinesterase · Structure – Activity relationships · Molecular docking

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Introduction

The pinewood nematode, *Bursaphelenchus xylophilus*, is the causal agent of pine wilt disease (Kiyohara and Tokushige 1971; Mamiya 1983). The nematode is transmitted by oviposition of vector insects, such as *Monochamus alternatus*, in dead or dying pine trees (Kobayashi et al. 1984). Control of the disease depends primarily on fumigation of disease–infected trees, aerial application of synthetic pesticides against *M. alternatus* or injection of synthetic nematicides against *B. xylophilus* (Lee et al. 2003; Yoshida 2006). However, the discovery and development of new nematicides is getting more expensive (Chitwood 2002). For this reason, several new chemical products are in the process of being registered, and only a few commercial nematicides remain in use. Among these pesticides, organophosphates (OPs) and carbamates (CBs) pesticides are mainly used in the *B. xylophilus* control programme. Acetylcholinesterase (AChE), the target for the action of OPs and CBs pesticides that terminates nerve

impulses by hydrolyzing the neurotransmitter acetylcholine (ACh) to acetic acid and choline at the synapses and neuromuscular junction in most vertebrates, insects and nematodes (Kang et al. 2011; Massoulie et al. 1993; Opperman and Chang 1990; Selkirk et al. 2005). Thus, the inhibition of AChE leads to the dysfunction of the nervous system and death (Kang et al. 2012). However, OPs and CBs pesticides are known to have negative environmental side effects, including high toxicity (Kang et al. 2013). To avoid hazards to the environment and humans caused by the use of traditional synthetic nematicides, it is necessary to find safer alternatives, that are environmentally benign, cheap and effective with a design based on naturally occurring compounds in plants.

Harmine is a beta–carboline alkaloid of *Peganum harmala* that possesses various types of pharmaceutical properties in vitro and in vivo (Cao et al. 2007; Lin et al. 2002), and extensive spectrum of biological activities such as antitumoral (Chen et al. 2005), antiprotozoal (Mirzaie et al. 2007), antimicrobial (Behidj-Benyounes et al. 2014; Benzekri et al. 2016), insecticidal (Chermenskaya et al. 2010; Nenaah 2011; Rharrabe et al. 2007), and nematicidal (Jakobsen et al. 2013; Ntalli and Caboni 2012) activities. A variety of mechanisms were proposed for harmine, including intercalation into DNA (Sharma et al. 2016), inhibition of cyclooxygenase (Hamsa and Kuttan 2010), monoamine oxidase (Herraiz et al. 2010), AChE (He et al. 2015; Zhang et al. 2013; Zheng et al. 2009), and interaction with L–type Ca^{2+} channel, opioid receptor, dopamine receptor, γ–aminobutyric acid receptor, 5–hydroxytryptamine receptor, benzodiazepine receptor, imidazoline receptor, and peroxisome proliferator–activated receptor gamma (PPARγ) in the nervous system (Glennon et al. 2000; Khorana et al. 2003; Moloudizargari et al. 2013; Waki et al. 2007).

In our earlier work, we have evaluated the antitumor activity of a series of harmine derivatives by substitution of 1–, 2–, 7– and 9–position of beta–carboline nucleus through C1, N2, N9–alkylation, and C7–oxyalkylation (Guo et al. 2014; Wu et al. 2014). However, 7–position as alkoxy group substituted harmine derivatives showed more potent neurotoxicity than harmine in our experiment (Cao et al. 2013). Here we investigate the nematicidal effect against *B. xylophilus* and inhibitory activity of these derivatives on AChE in vitro and in vivo.

Materials and methods

Harmine derivatives structure

Harmine derivatives were provided by Rihui Cao (Sun Yat–sen University). The structures of compounds are shown in Table 1

Chemicals and instruments

Harmine hydrochloride (>98%) was obtained from AMRESCO. The following chemicals were obtained from Sigma–Aldrich (USA): dimethyl sulfoxide, AChE from electric eel (*Electrophorus electricus*), acetylthiocholine iodide, 5,5–dithiobisbis–nitrobenzoic acid (DTNB), L–glutathione (L–GSH), chloroform (AR), isopropanol (AR), alcohol absolute (AR).

Instruments used in the study were microplate reader (Bio–Tek Instruments, USA), Precise pH instrument (Lab850, SCHOTT insruments, Germany), 5804R Refrigerated centrifuge (Eppendorf, Germany), and ND1000 Nucleic acid protein quantitative instrument (Eppendorf, Germany).

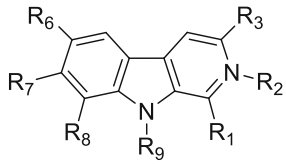
Nematodes

Pinewood nematodes, *B. xylophilus*, were provided by the Research Center of Nematodes of Plant Quarantine of South China Agricultural University and cultured on fungal mats of *Pestalotia* sp. grown on potato dextrose agar (PDA) plates at 25 °C for about ten days. Nematodes were extracted from fungal cultures with sterile distilled water in shallow pans. They were collected after 4–8 h and concentrated. The nematodes were rinsed from the filter disks with sterile distilled water and collected (Park et al. 2005).

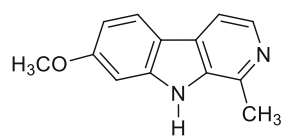
Nematicidal effect

Solutions of compounds were prepared by serial dilution with distilled water containing 2% DMSO (dimethyl sulfoxide). The nematicidal effect assay was conducted, according to the method described by Kim et al. (2008) with minor modifications. Test solutions were introduced into wells of 24–well plates. In each well, the concentration of nematodes was between 150 and 200 specimens (mixtures of juveniles and adults) per 500 μl of water. Controls received distilled water containing

Table 1 Structure of harmine derivatives



Structure of harmine derivatives



Harmine

Compound	R_1	R_2	R_3	R_6	R_7	R_8	R_9
Harmine hydrochloride	CH_3	$\cdot Cl^-$	H	H	OCH_3	H	H
1	H	$CH_2C_6H_5 \cdot Br^-$	$COOCH_2CH_3$	H	H	H	$CH_2C_6H_5$
2	CH_3	$CH_2C_6H_5 \cdot Br^-$	H	H	H	H	$CH_2C_6H_5$
3	CH_3	–	H	H	$OCH_2C_6H_5$	H	C_4H_9
4	CH_3	–	H	H	OC_6H_{13}	H	C_4H_9
5	H	–	H	H	H	H	C_3H_7
6	CH_3	–	H	H	$OCH(CH_2)_2$	H	C_4H_9
7	CH_3	–	H	H	$OCH(CH_2CH_3)_2$	H	C_4H_9
8	H	–	H	H	H	H	C_4H_9
9	CH_3	–	H	H	$O(CH_2)_3C_6H_5$	H	C_2H_5
10	CH_3	$CH_2C_6H_5 \cdot Br^-$	H	H	$OCH_2CH(CH_3)_2$	H	C_4H_9
11	CH_3	$-CH_2C_6H_5$	H	H	$O(CH_2)_3C_6H_5$	H	$(CH_2)_3C_6H_5$
12	CH_3	$-CH_2C_6H_5$	H	H	OC_4H_9	H	$CH_2CH(CH_3)_2$
13	CH_3	$CH_2C_6H_5 \cdot Br^-$	H	H	$OCH_2CH_2C_6H_5$	H	C_2H_5
14	$C_6H_2(OCH_3)_3$	$\cdot Cl^-$	$CONH(CH_2)_2OH$	H	H	H	H
15	H	$\cdot Cl^-$	$CONH(CH_2)_2OH$	H	H	H	C_4H_9
16	H	$\cdot Cl^-$	$CONH(CH_2)_2OH$	H	H	H	$CH_2C_6H_5$
17	CH_3	–	CH_2OH	H	H	H	C_4H_9
18	$COOCH_2CH_3$	$\cdot Cl^-$	H	H	H	H	CH_3
19	H	$\cdot Cl^-$	$CONH(CH_2)_2OH$	H	H	H	$(CH_2)_3C_6H_5$

20	H	– $CH_2C_6H_5 \cdot Br^-$	$CONH(CH_2)_2OH$	H	H	H	$(CH_2)_3C_6H_5$
21	CH_3	–	H	H	H	H	$CH_2C_5NH_4$
22	CH_3	–	H	H	H	H	$CH_2C_5NH_4$
23	H	–	H	Br	H	H	H
24	H	–	H	H	H	Br	H
25	CH_3	$\cdot Cl^-$	Br	Br	H	H	H
26	H	–	H	Br	H	H	CH_3
27	H	$-CH_3 \cdot I^-$	H	Br	H	H	CH_3
28	H	–	H	H	H	H	$CH_2C_6H_4OCH_3$
29	H	$\cdot Cl^-$	$COOCH_2CH_3$	H	H	H	$CH_2C_6H_5$
30	H	$\cdot Cl^-$	$COOCH_2CH_3$	H	H	H	CH_3
31	H	$\cdot Cl^-$	$COOC_4H_9$	H	H	H	H
32	$CH(CH_3)_2$	$\cdot Cl^-$	$COOCH_2CH_3$	H	H	H	CH_2CH_3
33	CH_3	$\cdot Cl^-$	H	H	H	H	$CH_2C_6F_5$
34	CH_3	$\cdot Cl^-$	H	H	H	H	H
35	CH_3	$\cdot Cl^-$	H	H	OCH_3	H	$CH_2C_6H_5$
36	H	– $CH_2C_6H_5 \cdot Br^-$	H	H	H	H	$(CH_2)_3C_6H_5$
37	H	–	$CH{=}NNHC{=}SNH_2$	H	H	H	CH_3
38	H	–	$CH{=}NNHC{=}SNH_2$	H	H	H	$(CH_2)_3C_6H_5$
39	CH_3	–	$CH{=}NNHC{=}SNH_2$	H	H	H	$CH_2C_6H_4F$
40	H	$\cdot Cl^-$	$COOCH_2CH_3$	H	H	H	H
41	H	$\cdot Cl^-$	$COOCH_2CH_3$	H	H	H	CH_3
42	H	$\cdot Cl^-$	$COOCH_2CH_2Cl$	H	H	H	H
43	H	$\cdot Cl^-$	$COOCH_2CH_2Cl$	H	H	H	CH_3

2% DMSO solution. The active compounds were diluted successively to a series of concentrations for nematicidal assay. Treated and control nematodes were kept at 25 °C. Dead and active nematodes in each well were recorded after incubation for 48 h under a stereomicroscope. Nematodes were defined as dead if their bodies were motionless and straightened. The mortality in the assays was corrected by eliminating natural death in the water containing 2% DMSO solution according to the formula: corrected mortality (%) = (mortality of treatment- mortality of control) / (1- mortality of control) × 100. Each treatment repeated three times.

Inhibitory effect on acetylcholinesterase in vitro

The inhibitory effect on AChE were measured using the spectrophotometric method, according to the method described previously (Choi et al. 2015; Ellman et al. 1961). The median inhibitory concentration (IC_{50}) was calculated using an enzyme inhibition dose response

curve, with harmine hydrochloride as a positive control. The assays were conducted in triplicate.

Inhibitory effect on acetylcholinesterase in vivo

The inhibitory effect on AChE in vivo was measured using the spectrophotometric method as described previously (Revathi et al. 2013). *B. xylophilus* was treated by 20 μg/mL active compounds for 48 h. The treated and control nematodes were washed with double distilled water and centrifuged to remove compounds. The nematodes were separately homogenized in centrifuge tubes (1.5 mL) using a TIANGEN hand homogenizer in 500 μL of ice–cold sodium phosphate buffer (20 mM, pH 7.2) to estimate the AChE enzyme activity. The homogenates were centrifuged (10,000 g at 4 °C) for 20 min and supernatants were used for the further analyses. The final homogenates were held in −20 °C until used in assays. Two hundred microlitre supernatants were mixed with 100 μL sodium phosphate buffer (20 mM, pH 7.2) and 100 μL 5 mM substrate solution incubated for 30 min at 25 °C. Then 100 μL 5 mM DTNB−ethanol reagent was added and the absorption was read immediately at 412 nm on a microplate reader. The median inhibitory concentration (IC_{50}) was calculated using an enzyme inhibition dose response curve, with harmine hydrochloride as a positive control. All experiments were performed in triplicate.

Docking study

Docking studies were performed using BioSolveIT LeadIT Version: 2.1.8 (Germany). For this purpose, crystal structure of AChE (PDB codes: 1C2O) of electric eel (*Electrophorus electricus*) was obtained from the Protein Data Bank in order to prepare the protein for docking studies (Bourne et al. 1999). The docking procedure was followed using the standard protocol implemented in LeadIT. The amino acid sequence of the subunit was edited to remove the extracellular region and residues. The initial structures of these compounds were built and energetically minimized. The active site of the receptor for docking studies was identified as the amino acid residues in 6.5 Å pocket.

Statistical analysis

All data were expressed as means ± standard deviations of triplicate measurements. Standard deviations (SD) did not exceed 5% for the majority of the values obtained. The treatment means were subjected to a one–way ANOVA and Tukey's multiple. The median lethal concentration (LC_{50}) against *B. xylophilus* and IC_{50} on AChE were obtained according to probit analysis.

Results

Nematicidal effect of harmine derivatives

Nematicidal effects of harmine hydrochloride and harmine derivatives are shown in Table 2. Compounds **10**, **11**, **12**, **13** and **26** showed 100% nematicidal effect against *B. xylophilus*; compounds **20**, **23**, **24**, **25** and **35** showed 40–90% nematicidal effect, and slightly higher than the parent compound, harmine hydrochloride (39%), 15 compounds had an effect ranging between 3 and 40%, the other 18 compounds have no activity, at a concentration of 20 μg/mL. In our experiment, the dead nematodes treated with harmine derivatives **10**, **11**, **12** and **13** usually had straight bodies, whereas nematodes treated with harmine derivatives **20**, **23**, **24**, **25, 26** and **35**, usually had semicircular and coiling shapes.

To gain further insight into potency of these compounds, harmine hydrochloride and the compounds **10**, **11**, **12**, **13, 20**, **23**, **24**, **25**, **26** and **35** were investigated further in a serial concentration gradient to determine their LC_{50} values (Table 3). The LC_{50} values of harmine hydrochloride and the compounds **10**, **11**, **12**, **13, 20**, **23**, **24**, **25**, **26** and **35** were 27.50, 1.63, 1.63, 1.75, 1.44, 25.59, 18.31, 10.22, 12.72, 5.84 and 13.32 μg/mL, respectively.

Inhibitory effect of harmine derivatives against AChE in vitro

The inhibitory effects of harmine hydrochloride and harmine derivatives on AChE from electric eel in vitro were evaluated (Table 4). Compound **13** showed the greatest potential to inhibit AChE activity. Compounds **10**, **11**, **12 and 13** had a prominent inhibitory effect on AChE, with IC_{50} values of 0.92, 0.90, 0.82 and 0.07 μg/mL, respectively. Compounds **20**, **23**, **24**, **25** and **35** had low to moderate effect on AChE activity with IC_{50} values of 11.65, 28.26, 13.26, 14.40 and 5.00 μg/mL, respectively. The IC_{50} values of harmine hydrochloride was 5.50 μg/mL. However, compound **26** showed no inhibitory effect.

Table 2 Preliminary nematicidal effects of harmine hydrochloride and harmine derivatives against *Bursaphelenchus xylophilus* at a concentration of 20 μg/mL

Compound	Mortality ±SD (%)	Compound	Mortality ±SD (%)	Compound	Mortality ±SD (%)
Harmine hydrochloride	39 ± 1.5 e*	**15**	NA	**30**	NA
1	11 ± 0.7 h	**16**	5 ± 1.0 k	**31**	NA
2	29 ± 1.4 e	**17**	9 ± 0.9 i	**32**	NA
3	6 ± 0.4 ij	**18**	6 ± 0.9 ij	**33**	NA
4	NA**	**19**	NA	**34**	NA
5	5 ± 0.2 k	**20**	40 ± 1.0 e	**35**	67 ± 1.7 d
6	7 ± 0.2 ij	**21**	3 ± 0.5 m	**36**	NA
7	7 ± 0.5 ij	**22**	6 ± 0.6 jk	**37**	NA
8	3 ± 0.2 m	**23**	66 ± 2.2 d	**38**	NA
9	19 ± 0.1 f	**24**	87 ± 1.2 b	**39**	NA
10	100 ± 0.0 a	**25**	76 ± 1.5 c	**40**	NA
11	100 ± 0.0 a	**26**	100 ± 0.0 a	**41**	NA
12	100 ± 0.0 a	**27**	16 ± 0.9 g	**42**	NA
13	100 ± 0.0 a	**28**	NA	**43**	NA
14	NA	**29**	4 ± 0.7 l		

*Means followed by the same letters are not significantly different based on one–way ANOVA at $P = 0.05$ (Tukey's test); **NA, not active

Inhibitory effect of harmine derivatives against AChE in vivo

Because these compounds showed good inhibitory effect on AChE in vitro, we further evaluated the inhibitory effect of harmine hydrochloride and harmine derivatives on AChE of *B. xylophilus* in vivo. Similar results were obtained (Table 5). Compound **13** proved to be the most active. IC_{50} values of the compounds **10**, **11**, **12** and **13** were 17.16, 14.56, 13.63 and 3.06 μg/mL, respectively, significantly lower than that of harmine hydrochloride, 578.44 μg/mL. Compound **20**, **23**, **24**, **25, 26** and **35** had no effect.

Study on structure–activity relationship (SAR)

In our study, 2,7,9–trisubstituted harmine derivative **10**, **11**, **12**, **13, 20**, **23**, **24**, **25**, **26** and **35** showed good nematicidal effect (Table 1). Among the 2,9–positions together as the electron–donating substituted and the 7–

Table 3 Nematicidal effects of harmine hydrochloride and harmine derivatives against *Bursaphelenchus xylophilus*

Compound	LC_{50}(μg/mL)	Linear equation	Confidence interval(μg/mL)	R
Harmine hydrochloride	27.50	Y = 2.8947 + 1.4628X	21.37–35.38	0.9796
10	1.63	Y = 4.3611 + 3.0060X	1.45–1.83	0.9993
11	1.63	Y = 4.2081 + 3.7193X	1.44–1.85	0.9308
12	1.75	Y = 4.2978 + 2.8946X	1.55–1.98	0.9938
13	1.44	Y = 4.5374 + 2.9248X	1.28–1.62	0.9616
20	25.59	Y = 2.1589 + 2.0177X	21.01–31.18	0.9162
23	18.31	Y = 1.9513 + 2.4145X	15.84–21.16	0.9198
24	10.22	Y = 2.3001 + 2.6749X	9.11–11.46	0.9283
25	12.72	Y = 2.2752 + 2.4667X	11.24–14.40	0.9911
26	5.84	Y = 2.5876 + 3.1481X	5.24–6.50	0.9996
35	13.32	Y = 1.5363 + 3.0805X	11.94–14.86	0.9933

Table 4 Inhibition of acetylcholinesterase by harmine hydrochloride and harmine derivatives in vitro

Compound	IC_{50}(μg/mL)	Linear equation	95%Confidence interval(μg/mL)	R
Harmine hydrochloride	5.50	Y = 4.0704 + 1.2558X	4.33–6.98	0.9970
10	0.92	Y = 5.0627 + 1.7379X	0.76–1.12	0.9880
11	0.90	Y = 5.0906 + 1.9143X	0.77–1.04	0.9756
12	0.82	Y = 5.1087 + 1.2952X	0.67–1.01	0.9969
13	0.07	Y = 7.1565 + 1.8382X	0.05–0.08	0.9778
20	11.65	Y = 3.4692 + 1.4726X	9.15–14.84	0.9878
23	28.26	Y = 3.6456 + 0.9334X	15.76–50.66	0.9531
24	13.26	Y = 2.9817 + 1.7978X	10.40–16.90	0.9965
25	14.40	Y = 4.0892 + 0.7862X	9.01–23.02	0.9218
26	>50	–	–	–
35	5.00	Y = 3.5868 + 2.0224X	4.33–5.76	0.9823

"–" = Absent

position as electron–withdrawing substituted harmine derivatives **10**, **11**, **12** and **13**, showed 100% mortality at a concentration of 20 μg/mL, with LC_{50} values of 1.63, 1.63, 1.75 and 1.44 μg/mL, respectively (Tables 2 and 3), and had also a remarkable inhibitory effect on AChE activity, with IC_{50} values of 0.92, 0.90, 0.82 and 0.07 μg/mL in vitro and 17.16, 14.56, 13.63 and 3.06 μg/mL in vivo, respectively (Tables 4 and 5). In the case of compound **20** with 2,9–positions together as the electron–donating substituent groups, compounds **23** and **25** with 6–position as bromine, compound **24** with 8–position as bromine and compound **35** with 7–position as electron–withdrawing substituent groups, showed 40–87% mortality at a concentration of 20 μg/mL, with LC_{50} values of 25.59, 18.31, 12.72, 10.22 and 13.32 μg/mL, respectively (Tables 2 and 3). These compounds had a moderate inhibitory effect on AChE activity in vitro, with IC_{50} values of 11.65, 28.26, 14.40, 13.26 and 5.00 μg/mL, respectively (Table 4), but had no inhibitory effect in vivo (Table 5). In addition, the harmine derivative **26**, with 9–position as the electron–donating and 6–position as bromine substituted, showed also 100% mortality at a concentration of 20 μg/mL, with LC_{50} values of 5.84 μg/mL (Tables 2 and 3); but showed no inhibitory effect on AChE activity in vitro nor in vivo in our experiment (Table 5).

Table 5 Inhibition of acetylcholinesterase by harmine hydrochloride and harmine derivatives in vivo

Compound	IC_{50}(μg/mL)	Linear equation	Confidence interval(μg/mL)	R
Harmine hydrochloride	578.44	Y = 3.8723 + 0.4083X	61.57–11,693.53	0.9123
10	17.16	Y = 4.0378 + 0.7794X	9.88–29.81	0.9842
11	14.56	Y = 4.0952 + 0.7778X	6.99–30.33	0.9955
12	13.63	Y = 3.9227 + 0.9495X	7.47–24.86	0.9939
13	3.06	Y = 4.6811 + 0.6568X	2.05–4.56	0.9269
20	>600	–	–	–
23	>600	–	–	–
24	>600	–	–	–
25	>600	–	–	–
26	>600	–	–	–
35	>600	–	–	–

"–" = Absent

Docking study

To clarify the binding mode of our synthesized compounds, compound **13** with high AChE inhibitory activity was docked into the active site of AChE by FlexX™ Docking within BioSolveIT LeadIT Version: 2.1.8 software package. The result revealed that compound **13** was flexibly docked automatically in the active site of AChE. Firstly, the beta–carboline ring and the ethyl group in 9- position of harmine derivatives are oriented into substrate access AChE in the middle and forms hydrophobic and Van der Waals interactions with surrounding hydrophobic residues (yellow). Secondly, the phenyl group attached to the beta–carboline of the side chain interacts with the phenyl group of aromatic amino acids through the formation of π-π face-to-edge interaction and N^+ cation in 2–position interacts with the anionic site in the peripheral anionic site (PAS) (green). Furthermore, the phenethoxy group in 7- position of beta–carboline can bind to the catalytic active site (CAS) (orange) of AChE through the former binding mode (Fig. 1).

Discussion

The naturally and synthetic beta–carboline derivatives provide a valuable scaffold in medicinal chemistry as well as agrochemical applications with herbicidal, fungicidal, insecticidal and nematicidal activities (Bloomquist et al. 1997; El Hassan et al. 2013; Larson et al. 1988; Li et al. 2015; Liu et al. 2014; Saeed and Shawkat 2014; Sodaeizadeh et al. 2010; Song et al. 2014). In the current study, we report the 2,7,9–trisubstituted harmine derivatives against *B. xylophilus* and their inhibitory effects on AChE in vitro and in vivo and detailed studies of SAR. This work clearly shows that compounds **10**, **11**, **12** and **13** with 2,9–positions together as electron–donating substituent groups and the 7–position as electron–withdrawing substituent group caused 100% mortality at a concentration of 20 μg/mL and had remarkable inhibitory effect on AChE activity in vitro and in vivo. This result agrees with previous findings that the substituent at the 7–position of the beta–carboline alkaloids and 3,9–positions of quinoline alkaloids played a critical role in AChE or BChE inhibition (Yang et al. 2015; Zhao et al. 2013;

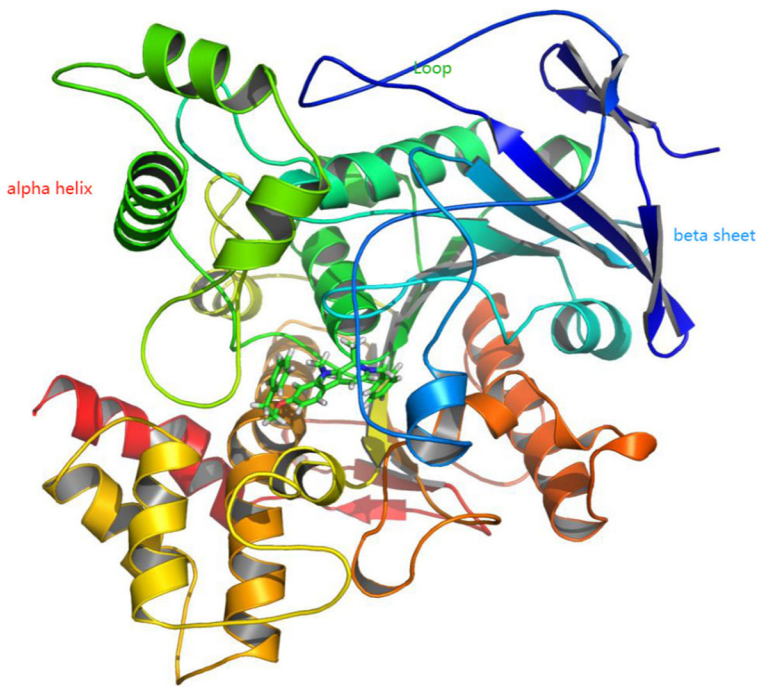


Fig. 1 Simulated binding site interactions of compound **13** with the acetylcholinesterase (AChE) structure of electric eel (*Electrophorus electricus*) depicted as spectrum-colored cartoon (containing α-helix, β-sheet and flexible loop) and the arrow shows the AChE core (yellow) colored in spectrum with the N-terminus beginning in blue and the C terminus finishing in red. The carbon atoms of compound **13** are green. Simulated docking shows docking compound **13** in the same site formed $\pi - \pi$ stacking interactions and hydrogen bonding

Zheng et al. 2009). This also agrees with previous findings that 2,9–positions together as the electron–donating substituted and 7–position as electron–withdrawing substituted harmine derivatives had remarkable neurotoxic effects including tremor, twitch and jumping in experimental animal models and cytotoxic properties in vitro (Shi et al. 2013). Due to the different residues in the PAS, the three AChEs (BxACE-1, BxACE-2, BxACE-3) of pinewood nematode showed differential inhibition properties by the plant essential oils, organophosphates (OPs) and carbamates (CBs) (Kang et al. 2011, 2012, 2013). The differential inhibition activities against AChE in vitro and in vivo of harmine derivatives can be helpful in developing an efficient anti-AChE nematicidal agent for control of pinewood nematode.

On the other hand, compound **26** with 9–position as electron–donating substituent groups and 6–position as bromine, compound **20** with 2,9–positions together as the electron–donating substituent groups, compounds **23** and **25** with 6–position as bromine, compound **24** with 8–position as bromine and compound **35** with 7–position as electron–withdrawing substituent groups caused 40–100% mortality at a concentration of 20 μg/mL, but proved to be ineffective in inhibiting AChE in vitro and in vivo, and so their exact mode of action against pinewood nematode is unclear. Kong et al. (2006) reported that pinewood nematode bodies treated with the muscle activity blockers levamisole hydrochloride and morantal tatrate usually exhibited semicircular and coiling shapes, respectively. Because the symptoms resemble nerve intoxication, we speculate that the compounds might act on the nervous system.

These results suggest that the nematicidal mode of action among the different substituent groups of harmine derivatives might be different. As compared with previous work, the binding conformation of compound **13** in this docking simulation showed the similar binding mode of alkylene-linked tacrine dimers (Chen et al. 2014; Lu et al. 2011; Rydberg et al. 2006). It helps us to optimize these harmine derivatives to inhibit AChE. To some extent, these findings, combined with the SARs results, could explain the novel mechanism of the nematicidal effect by these new derivatives.

Overall, harmine derivatives **10**, **11**, **12** and **13**, appear to be useful as natural product–based nematicides for *B. xylophilus* through inhibition of AChE. In addition, the docking results revealed that the structural uniqueness of our newly designed harmine derivatives led to a unique molecular recognition and binding mode and were in good agreement with their high nematicidal potential, which also explained the SARs observed in vitro and in vivo. These results suggest that beta–carboline nucleus is the primary pharmacophore of the nematicidal activity of beta–carboline derivatives and may be helpful in structure–guided design and development of novel nematicidal harmine derivatives based on AChE inhibitors. Since harmine derivatives **20**, **23**, **24**, **25, 26** and **35**, have potential as natural product–based nematicides for *B. xylophilus*, the mechanism of their nematicidal activity deserves further study. The active derivatives could be considered as potential alternatives to currently used pinewood nematode control agents or as lead compounds for the development of synthetic environment friendly nematicides with enhanced activity.

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Compliance with ethical standards

Conflict of interest All authors certify that 1) they do not have any actual or potential conflict of interest, 2) the study described is original and has not been published previously, and is not under consideration for publication elsewhere, 3) all prevailing local, national and international regulations and conventions, and normal scientific ethical practices, have been respected. We also certify that all authors have reviewed the manuscript and approved the final version of manuscript before submission.

Human and animal subjects This article does not contain any studies with human or animal subjects.

Informed consent All the authors certify that the work carried out in this research followed the principles of ethical and professional conduct have been followed. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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