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# Synthesis, biological evaluation and molecular docking of novel nereistoxin derivatives containing phosphonates as insecticidal/AChE inhibitory agents†

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In continuation of our program aimed at the discovery and development of natural product-based insecticidal agents, a series of novel nereistoxin derivatives containing phosphonate were synthesized and characterized by <sup>31</sup>P, <sup>1</sup>H, <sup>13</sup>C NMR and HRMS. The bioactivities of the derivatives were evaluated for the acetylcholinesterase (AChE) inhibition potency and insecticidal activity. The AChE inhibitory effects of the derivatives were investigated using the *in vitro* Ellman method. Half of the compounds exhibited excellent inhibition of AChE. All the compounds were assessed for insecticidal activities against *Mythimna separate* (Walker) and *Rhopalosiphum padi* *in vivo*. Some derivatives displayed promising insecticidal activity against *Rhopalosiphum padi*. Compounds **5b** and **6a** displayed the highest activity against *R. padi*, showing LC<sub>50</sub> values of 17.14 and 18.28 μg mL<sup>-1</sup>, respectively, close to that of commercial insecticide flucicotamid (LC<sub>50</sub> = 17.13 μg mL<sup>-1</sup>). Compound **9g** also showed notable insecticidal activity, with an LC<sub>50</sub> value of 23.98 μg mL<sup>-1</sup>. Additionally, the binding modes of the active compounds **5b**, **6a** and **9g** with AChE were analyzed in-depth through molecular docking and the intrinsic reasons for the differences in the strength of the compound's activities were elucidated. In summary, our findings demonstrate the potential of these nereistoxin derivatives as promising candidates for the development of novel pesticides.

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## 1 Introduction

Nowadays, pesticides continue to be extensively employed to enhance crop yields and protect crops from pests.<sup>1</sup> The increasing application of synthetic agrochemicals is progressively leading to the emergence of resistance in various pest populations, posing significant challenges in pest management and crop protection.<sup>2-4</sup> In view of the increasing pesticide resistance and the difficulties in rapidly producing new pesticides,<sup>5,6</sup> it is a prominent way to search for original insecticidal compounds from natural products and their secondary metabolites, as well as utilize these compounds as lead structures for further modification. Nereistoxin (NTX, Fig. 1), a natural neurotoxin with insecticidal activity, was initially isolated from *Lumbriconereis heteropoda*, an organism inhabiting marine sediments,<sup>7</sup> which marked a significant milestone as the first successful development and application of an animal-derived insecticide in the history of pesticide

development. Currently, only five commercially available insecticides are synthesized with NTX as the primary compound, as depicted in Fig. 1. These NTX analogs have demonstrated effective control over lepidopteran, hemipteran, and coleopteran pests.<sup>8,9</sup> Furthermore, they are considered safe for humans and domestic animals since they can be broken down and excreted. However, the long-term, repeated, and extensive use of these insecticides has inevitably led to the development of pesticide resistance.<sup>10,11</sup> Therefore, there is

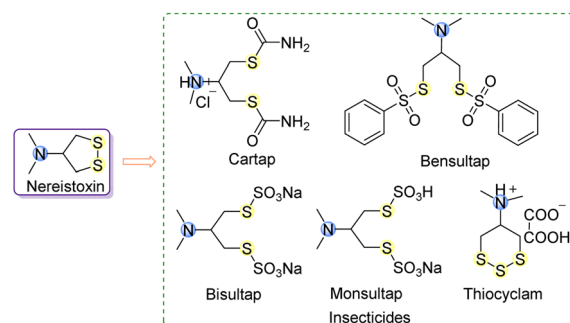


Fig. 1 Nereistoxin and its commercial products.

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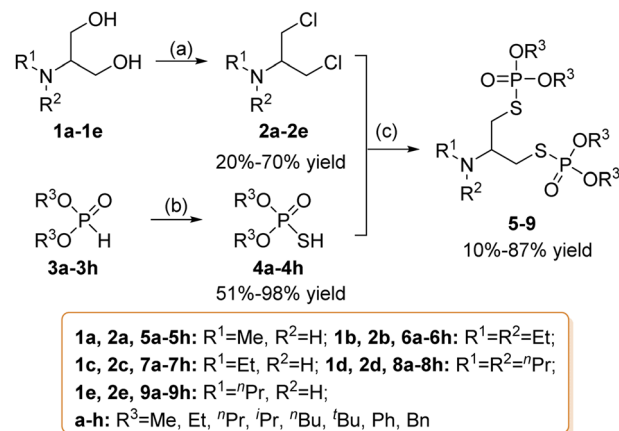
a pressing need to synthesize new compounds to enrich and advance the development of nereistoxin-derived insecticides.

Organophosphorus insecticides are known for their broad-spectrum insecticides, rapid action and significant effects.<sup>12</sup> Despite their numerous benefits, they cause serious reverberations on humans and animals, including organ failure and damage to the nervous system.<sup>13,14</sup> Consequently, researchers are actively exploring innovative strategies to mitigate its major adverse effect on humans. The modification of organophosphorus insecticides aims to retain their efficient insecticidal activity while reducing the environmental impact. This endeavor has led to the development of new pesticides that are relatively safe for mammals and environmentally friendly. By combining organophosphorus components with the natural product nereistoxin, our objective is to explore new compounds that strike a balance between excellent insecticidal activity and environmentally sound characteristics. Our prior research focused on synthesizing *N,N*-dimethyl nereistoxin derivatives containing phosphonates and their bioactivities. We have screened compounds with insecticidal potential.<sup>15</sup> To enhance the activities of the new S-P bond nereistoxin derivatives associated with phosphonates, we conducted a detailed study of derivatives with varied substituents on N atom (Fig. 2, the alkyl substituents on the N atom are changed) and the synthesis, characterization and biological activities (acetylcholinesterase inhibitory activity and insecticidal activity against *Mythimna separata* Walker and *Rhopalosiphum padi*) were fully studied. Our findings revealed that the introduction of various alkyl substituents on the N atom resulted in differing degrees of acetylcholinesterase inhibition and insecticidal activity. These results highlight the potential of these S-P bond nereistoxin derivatives as insecticidal agents and offer valuable insights for further development in the field of insect pest control. Moreover, molecular docking was conducted to illustrate the binding modes of active compounds with AChE, aiming to provide insights into the variations in their activity strengths.

## 2 Results and discussion

### 2.1 Chemistry

The general synthetic route of compounds 5–9 is shown in Scheme 1. The key intermediate 2a–2e was conveniently synthesized from 1a–1e. The hydroxyl of 1a–1e was chlorinated with SOCl<sub>2</sub> to obtain 2a–2e. Other crucial intermediates, such as S-hydrogen phosphorothioates 4a–4h, were obtained from diphosphite 3a–3h. Some of them were commercially available and others were obtained *via* reaction of the appropriate alcohol and PCl<sub>3</sub>, with pyridine as an acid-binding agent. Diphosphite 3a–3h



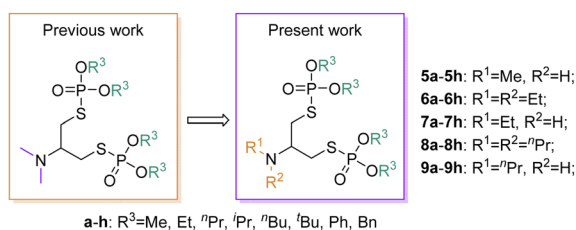
**Scheme 1** The synthesis process of target compounds 5a–5h, 6a–6h, 7a–7h, 8a–8h and 9a–9h. Reagents and conditions: (a) SOCl<sub>2</sub>, reflux, 0.5–1 h, 20–45% for 2a–2e; (b) S<sub>8</sub>, Et<sub>3</sub>N, Et<sub>2</sub>O, rt, 12 h, 51–98% for 4a–4h; (c) CH<sub>3</sub>CN, NaH, 50 °C, 0.5–5 h, 10–87% for 5–9.

was reacted with S<sub>8</sub> and triethylamine to obtain 4a–4h. The end-products of 5–9 were synthesized *via* a substitution reaction between intermediates 2a–2e and various substituted S-hydrogen phosphorothioates 4a–4h. The target compounds 5–9 were characterized *via* <sup>1</sup>H NMR, <sup>13</sup>C NMR, <sup>31</sup>P NMR and HRMS.

### 2.2 The stability of the products

The polarity of the P=O bond and the formation of p-dπ conjugation between the O/S atoms connected to it, the electron cloud density on the C connected to O in alkoxy groups and P is lower, making P or C atoms more susceptible to be attacked by nucleophilic reagent attack and then hydrolysis. For –OR, as the electron-donating ability of alkyl groups follows the order CH<sub>3</sub> < C<sub>2</sub>H<sub>5</sub> < C<sub>3</sub>H<sub>7</sub>, the electron-withdrawing ability is OCH<sub>3</sub> > OC<sub>2</sub>H<sub>5</sub> > OC<sub>3</sub>H<sub>7</sub>. Therefore, the electron cloud density of the P atom connected to OCH<sub>3</sub> is lower than that of the P atom connected to other alkoxy groups, making it more susceptible to hydrolysis through nucleophilic substitution reaction. That is, methyl phosphate is less stable than other alkyl phosphates. The N atoms of compounds 5a, 7a, and 9a are a secondary amine substituted by two alkyl groups, making 5a, 7a, and 9a excellent nucleophilic reagents themselves. In terms of electron-donating ability, H < CH<sub>3</sub> < C<sub>2</sub>H<sub>5</sub>, so the electron cloud density on the C atom attached to O in –OR, CH<sub>3</sub> < C<sub>2</sub>H<sub>5</sub> < C<sub>3</sub>H<sub>7</sub>, so compared with other alkyl S-hydrogen phosphorothioates, C atoms attached to O in dimethyl phosphorothioate are easier to be attacked by nucleophilic reagents. During the synthesis of 5a, 7a, and 9a, there is an excess of dimethyl S-hydrogen phosphorothioates, and the compound is prone to react with the excess dimethyl thiophosphate during purification to be converted into products with one more methyl substitution on the N atom, and we have monitored the corresponding products by LC-MS during purification. Therefore, 5a, 7a, and 9a are unstable during the purification process (see ESI Fig. S140–S160†).

Compounds containing *tert*-butyl groups (5f, 6f, 7f, 8f, and 9f) are unstable due to the high steric hindrance of *tert*-butyl, making them more prone to hydrolyzing –OC(CH<sub>3</sub>)<sub>3</sub> into –OH.



**Fig. 2** The structures of target compounds.



Since the benzyl group forms a carbonium ion and enters displacement reactions much more readily than aliphatic groups do, the benzyl group are theoretically more likely to hydrolyze than aliphatic phosphates.<sup>16</sup> Therefore, **5h**, **7h**, and **9h** are unstable and more likely to hydrolyze.

### 2.3 Biological activities

In this study, in accordance with the guidelines for antipesticide laboratory biological activity tests, the insecticidal activities of all synthesized derivatives **5a–5h**, **6a–6h**, **7a–7h**, **8a–8h** and **9a–9h** against common agricultural pests (*Mythimna separate* Walker and *Rhopalosiphum padi*) were evaluated in the laboratory, and the representative insecticides chlorpyrifos and fluniconamid were used as the positive controls, respectively. In addition, the target compounds **5a–5h**, **6a–6h**, **7a–7h**, **8a–8h** and **9a–9h** were evaluated for acetylcholinesterase inhibitory activities *in vitro*.

**2.3.1 Acetylcholinesterase (AChE) assay (in vitro).** It was known that organophosphate compounds have an inhibitory effect on AChE.<sup>17</sup> The anticholinesterase activity of the synthesized derivatives **5–9** were measured spectrophotometrically against AChE (human) *in vitro* using Ellman's method.<sup>18</sup> In addition, the median inhibitory concentration (IC<sub>50</sub>) was determined using GraphPad Prism 8.3 software (Table 1).

To assess the mode of action of the nereistoxin derivatives, the activity of human AChE was assayed for each compound. All compounds were evaluated for their inhibitory activity on AChE using Ellman's method, except for certain compounds characterized that are easy hydrolysis, poor solubility and instability. It is difficult to calculate the IC<sub>50</sub> value of nereistoxin because it is such a weak AChE inhibitor.

The compounds exhibited varying levels of inhibition on human AChE with IC<sub>50</sub> values ranging from 0.3506 μmol L<sup>-1</sup> to 871 μmol L<sup>-1</sup> (Table 1). The results demonstrated that these derivatives, in general, exhibited exceptional inhibitory potency against human AChE compared with the similar phosphorothioates reported previously.<sup>19</sup> Notably, compounds **5c**, **5e**, **5b**, **6a**, and **9c** showed significant AChE inhibitory activity, with IC<sub>50</sub> values less than 1 μmol L<sup>-1</sup>. Among them, compound **5c** displayed the most potent inhibition with the lowest IC<sub>50</sub> value of 0.3506 μmol L<sup>-1</sup>. It's worth noting that the *N*-methyl-substituted compounds showed higher inhibitory activity against AChE compared to other *N*-alkyl-substituted compounds with the same phosphate ester substituents. Additionally, for *N,N*-dialkyl compounds, the AChE inhibition decreases as the number of C atoms increases when the substituents of the phosphate ester are straight-chain alkyl groups, such as the inhibitory activity of **6a** < **6b** < **6c** < **6e**, **8a** < **8b** < **8c** < **8e**.

**2.3.2 Insecticidal activities against *M. separata*.** The insecticidal activity of nereistoxin and its derivatives **5–9** against the pre-third-instar larvae of *M. separata* was assessed using the topical application method at a concentration of 2 mg mL<sup>-1</sup>.<sup>20</sup> Chlorpyrifos was used as the positive control at a concentration of 50 μg mL<sup>-1</sup>. The results showed that the corrected mortality rate of the synthesized compounds against *M. separata* was extremely low at 24 h, with most compounds less than 10%, and

Table 1 Experimental IC<sub>50</sub> and K<sub>i</sub> values of all synthesized compounds pertinent to AChE

Comp.	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	IC <sub>50</sub> (μM)	K <sub>i</sub> (mM <sup>-1</sup> min <sup>-1</sup> )
<b>5a</b>	Me	H	Me	— <sup>a</sup>	—
<b>5b</b>	Me	H	Et	0.5243	81 338.64 ± 6948.86
<b>5c</b>	Me	H	<sup>n</sup> Pr	0.3506	108 215.90 ± 6501.13
<b>5d</b>	Me	H	<sup>i</sup> Pr	23.43	2254.33 ± 321.25
<b>5e</b>	Me	H	<sup>n</sup> Bu	0.4041	66 859.10 ± 3840.74
<b>5f</b>	Me	H	<sup>t</sup> Bu	— <sup>b</sup>	—
<b>5g</b>	Me	H	Ph	— <sup>a</sup>	—
<b>5h</b>	Me	H	Bn	— <sup>a</sup>	—
<b>6a</b>	Et	Et	Me	0.6813	56 184.77 ± 13 731.42
<b>6b</b>	Et	Et	Et	3.133	24 341.50 ± 1855.40
<b>6c</b>	Et	Et	<sup>n</sup> Pr	8.494	6503.80 ± 486.92
<b>6d</b>	Et	Et	<sup>i</sup> Pr	99.77	792.54 ± 46.93
<b>6e</b>	Et	Et	<sup>n</sup> Bu	17.25	4203.51 ± 878.03
<b>6f</b>	Et	Et	<sup>t</sup> Bu	— <sup>b</sup>	—
<b>6g</b>	Et	Et	Ph	— <sup>c</sup>	—
<b>6h</b>	Et	Et	Bn	— <sup>c</sup>	—
<b>7a</b>	Et	H	Me	— <sup>a</sup>	—
<b>7b</b>	Et	H	Et	1.383	202.01 ± 59.01
<b>7c</b>	Et	H	<sup>n</sup> Pr	19.33	7.01 ± 5.12
<b>7d</b>	Et	H	<sup>i</sup> Pr	28.77	9.60 ± 0.44
<b>7e</b>	Et	H	<sup>n</sup> Bu	36.73	2.26 ± 0.12
<b>7f</b>	Et	H	<sup>t</sup> Bu	— <sup>a</sup>	—
<b>7g</b>	Et	H	Ph	— <sup>a</sup>	—
<b>7h</b>	Et	H	Bn	— <sup>a</sup>	—
<b>8a</b>	<sup>n</sup> Pr	<sup>n</sup> Pr	Me	7.039	6531.33 ± 210.66
<b>8b</b>	<sup>n</sup> Pr	<sup>n</sup> Pr	Et	12.86	2723.50 ± 183.68
<b>8c</b>	<sup>n</sup> Pr	<sup>n</sup> Pr	<sup>n</sup> Pr	45.03	1066.84 ± 98.27
<b>8d</b>	<sup>n</sup> Pr	<sup>n</sup> Pr	<sup>i</sup> Pr	871.00	80.87 ± 1.00
<b>8e</b>	<sup>n</sup> Pr	<sup>n</sup> Pr	<sup>n</sup> Bu	57.98	792.57 ± 8.06
<b>8f</b>	<sup>n</sup> Pr	<sup>n</sup> Pr	<sup>t</sup> Bu	— <sup>a</sup>	—
<b>8g</b>	<sup>n</sup> Pr	<sup>n</sup> Pr	Ph	— <sup>a</sup>	—
<b>8h</b>	<sup>n</sup> Pr	<sup>n</sup> Pr	Bn	— <sup>a</sup>	—
<b>9a</b>	<sup>n</sup> Pr	H	Me	— <sup>a</sup>	—
<b>9b</b>	<sup>n</sup> Pr	H	Et	8.641	21.62 ± 4.64
<b>9c</b>	<sup>n</sup> Pr	H	<sup>n</sup> Pr	0.9667	79.47 ± 18.53
<b>9d</b>	<sup>n</sup> Pr	H	<sup>i</sup> Pr	269.7	0.67 ± 0.07
<b>9e</b>	<sup>n</sup> Pr	H	<sup>n</sup> Bu	5.42	26.75 ± 0.59
<b>9f</b>	<sup>n</sup> Pr	H	<sup>t</sup> Bu	— <sup>a</sup>	—
<b>9g</b>	<sup>n</sup> Pr	H	Ph	— <sup>a</sup>	—
<b>9h</b>	<sup>n</sup> Pr	H	Bn	— <sup>a</sup>	—

<sup>a</sup> Compounds are unstable in purification. <sup>b</sup> Hydrolyzed easily. <sup>c</sup> Poor solubility.

only **10a** achieving 58%. As the time extended to 48 h, only **10a**, **12b**, **12d**, and **12e** exceeded 50%. While the nereistoxin and the positive control chlorpyrifos were 100% at both 24 h and 48 h, indicating that the insecticidal activity of nereistoxin against *M. separata* was much higher than that of its derivatives.

Based on the preliminary results (Table 2), compounds with excellent bioactivity were selected for further bioassays against *M. separata* at different concentrations (the different concentrations of **10a**, **12b**, **12d** and **12e** were 125, 250, 500, 1000, 2000 μg mL<sup>-1</sup>). The results of these bioassays are expressed in Table 3 as half of the lethal concentration (LC<sub>50</sub>, μg mL<sup>-1</sup>).

Table 3 reveals that among the 29 new compounds, only **6a**, **8b**, **8d** and **8e** attained the criteria for further assays (48 h corrected mortality >50%). Notably, compounds **8b**, **8d** and **8e** belong to the *N*-propyl substituted class, suggesting that these



Table 2 Corrected mortality rate of compounds 7a–7h, nereistoxin and positive controls (chlorpyrifos) against *M. separate* at 2 mg mL<sup>-1</sup>

Comp.	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	Corrected mortality rate ±SD (%)	
				24 h	48 h
5a	Me	H	Me	— <sup>a</sup>	—
5b	Me	H	Et	8.33 ± 2.35	25 ± 2.37
5c	Me	H	<sup>n</sup> Pr	8.33 ± 4.11	25 ± 0
5d	Me	H	<sup>i</sup> Pr	0	8.33 ± 1.21
5e	Me	H	<sup>n</sup> Bu	0	16.67 ± 2.74
5f	Me	H	<sup>t</sup> Bu	— <sup>b</sup>	—
5g	Me	H	Ph	0	25 ± 3.50
5h	Me	H	Bn	— <sup>a</sup>	—
6a	Et	Et	Me	58.33 ± 1.98	66.67 ± 2.26
6b	Et	Et	Et	8.33 ± 0.91	25 ± 3.51
6c	Et	Et	<sup>n</sup> Pr	8.33 ± 1.63	8.33 ± 1.47
6d	Et	Et	<sup>i</sup> Pr	0	25 ± 2.28
6e	Et	Et	<sup>n</sup> Bu	0	8.33 ± 0.69
6f	Et	Et	<sup>t</sup> Bu	— <sup>b</sup>	—
6g	Et	Et	Ph	0	33.33 ± 0.62
6h	Et	Et	Bn	16.67 ± 1.59	16.67 ± 1.94
7a	Et	H	Me	— <sup>a</sup>	—
7b	Et	H	Et	0	0
7c	Et	H	<sup>n</sup> Pr	0	0
7d	Et	H	<sup>i</sup> Pr	0	0
7e	Et	H	<sup>n</sup> Bu	20 ± 1.49	0
7f	Et	H	<sup>t</sup> Bu	— <sup>b</sup>	—
7g	Et	H	Ph	8.33 ± 1.57	33.33 ± 1.82
7h	Et	H	Bn	— <sup>a</sup>	—
8a	<sup>n</sup> Pr	<sup>n</sup> Pr	Me	8.33 ± 1.38	16.67 ± 0.75
8b	<sup>n</sup> Pr	<sup>n</sup> Pr	Et	16.67 ± 1.24	58.33 ± 2.17
8c	<sup>n</sup> Pr	<sup>n</sup> Pr	<sup>n</sup> Pr	16.67 ± 1.38	16.67 ± 0.99
8d	<sup>n</sup> Pr	<sup>n</sup> Pr	<sup>i</sup> Pr	8.33 ± 2.21	50 ± 2.88
8e	<sup>n</sup> Pr	<sup>n</sup> Pr	<sup>n</sup> Bu	16.67 ± 2.29	58.33 ± 2.28
8f	<sup>n</sup> Pr	<sup>n</sup> Pr	<sup>t</sup> Bu	— <sup>b</sup>	—
8g	<sup>n</sup> Pr	<sup>n</sup> Pr	Ph	0	16.67 ± 1.73
8h	<sup>n</sup> Pr	<sup>n</sup> Pr	Bn	0	8.33 ± 1.27
9a	<sup>n</sup> Pr	H	Me	— <sup>a</sup>	—
9b	<sup>n</sup> Pr	H	Et	0	0
9c	<sup>n</sup> Pr	H	<sup>n</sup> Pr	0	0
9d	<sup>n</sup> Pr	H	<sup>i</sup> Pr	0	0
9e	<sup>n</sup> Pr	H	<sup>n</sup> Bu	0	0
9f	<sup>n</sup> Pr	H	<sup>t</sup> Bu	— <sup>b</sup>	—
9g	<sup>n</sup> Pr	H	Ph	8.33 ± 1.96	16.67 ± 3.25
9h	<sup>n</sup> Pr	H	Bn	— <sup>a</sup>	—
Nereistoxin	—	—	—	100 ± 0	100 ± 0
Chlorpyrifos	—	—	—	100 ± 0	100 ± 0

<sup>a</sup> Compounds are unstable in purification. <sup>b</sup> Hydrolyzed easily.

compounds possess more significant insecticidal potential than other classes in the experimental group. However, the LC<sub>50</sub> of the synthesized compounds ranged from 649 µg mL<sup>-1</sup> to 882 µg

mL<sup>-1</sup>, which is much higher than the LC<sub>50</sub> of the nereistoxin (150.71 µg mL<sup>-1</sup>). Correspondingly, the LC<sub>50</sub> of the positive control chlorpyrifos is only 16.31 µg mL<sup>-1</sup>, which means that the insecticidal activity of the synthesized compounds against *M. separate* is much weaker compared to that of chlorpyrifos. Compared with the previous research,<sup>15</sup> the LC<sub>50</sub> values of *N,N*-dimethyl compounds against *M. separate* ranged from 136.86 to 836.34 µg mL<sup>-1</sup>, that is, compared to analogues, the lead compound nereistoxin displayed more potent effect on the third instar larvae of *M. separata*. The structure–activity relationship (SAR) analysis revealed that the insecticidal activity against *M. separate* was greatly influenced by the type of the substituents on N and P atoms. The mortality rates of *N,N*-dialkyl compounds were generally higher than those of *N*-alkyl compounds, such as the mortality rates of *N,N*-dimethyl substituents > *N*-methyl substituents, *N,N*-diethyl substituents (6) > *N*-ethyl substituents (7), *N,N*-dipropyl substituents (8) > *N*-propyl substituents (9), indicating that dialkyl substituent on N atom has higher insecticidal activity against *M. separate*. Moreover, for *N,N*-dialkyl compounds, the mortality rate of branched substituents on the P atom exceeded that of straight-chain substituents, e.g. mortality rate 6d > 6c, 8d > 8c, indicating that the branched alkane substituent has higher activity than that of straight-chain alkane substituents.

**2.3.3 Insecticidal activities against *R. padi*.** The insecticidal activities of nereistoxin derivatives 5–9 against *R. padi* were tested by the leaf-dipping method at a concentration of 0.2 mg mL<sup>-1</sup>.<sup>21</sup> Flonicotamid was used as the positive control at a concentration of 40 µg mL<sup>-1</sup>. As shown in Table 4, the corresponding mortality rates caused by these compounds after 48 h were generally higher than those observed after 24 h.

The corrected mortality rates of all compounds at 24 h were lower than that of the positive control, flonicotamid (75.11%). Half of the compounds had a mortality rate of less than 10% at 24 h, while the mortality rate of nereistoxin was 12.71%. Only six compounds had a higher corrected mortality rate than nereistoxin at 24 h. With the time extension to 48 h, the corrected mortality rates of 5b, 6a, 7c, 7g, 8b, 9b, 9c and 9g increased from 1.23–9.87% to 80–90%. The corrected mortality rate of the positive control, flonicotamid, at 48 h was 84%, indicating that the compounds (0.2 mg L<sup>-1</sup>) could achieve the same insecticidal effect as the positive control (0.04 mg L<sup>-1</sup>) after 48 h.

It can also be seen from Table 4 that the corrected mortality rate of nereistoxin at 48 h increased only from 12.71% at 12 h to 40.22%. In comparison, 9b had the highest corrected mortality rate of 90.21%. The corrected mortality rate for 9g increased from 1.23% at 24 h to 84.08% at 48 h, a 68-fold enhancement.

Table 3 LC<sub>50</sub> values of some potent compounds, nereistoxin and chlorpyrifos against *M. separate* in the laboratory

Comp.	$y = ax + b$	LC <sub>50</sub> (µg mL <sup>-1</sup> )	R <sup>2</sup>	CI (95%)
6a	$y = 1.5794x + 0.5582$	649	0.9433	383.76–1097.72
8b	$y = 1.1530x + 1.6038$	882	0.9903	404.33–1924.88
8d	$y = 1.2229x + 1.4573$	789	0.9880	391.17–1591.31
8e	$y = 1.2229x + 1.4132$	857	0.9705	413.00–1779.19
Nereistoxin	$y = 1.8644x + 0.9391$	150.71	0.9784	92.25–246.21
Chlorpyrifos	$y = 1.6690x + 2.9764$	16.31	0.9829	9.44–28.20



Table 4 Corrected mortality rate of compounds 5–9, nereistoxin and positive controls flunicotamid against *R. padi* at 0.2 mg mL<sup>-1</sup>

Comp.	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	Corrected mortality rate ±SD (%)	
				24 h	48 h
5a	Me	H	Me	— <sup>a</sup>	—
5b	Me	H	Et	12.44 ± 1.62	87.74 ± 2.08
5c	Me	H	<sup>n</sup> Pr	5.19 ± 0.91	60.61 ± 3.01
5d	Me	H	<sup>i</sup> Pr	6.76 ± 1.91	59.28 ± 4.11
5e	Me	H	<sup>n</sup> Bu	7.09 ± 2.70	47.96 ± 3.65
5f	Me	H	<sup>t</sup> Bu	— <sup>b</sup>	—
5g	Me	H	Ph	4.62 ± 3.90	44.74 ± 1.05
5h	Me	H	Bn	— <sup>a</sup>	—
6a	Et	Et	Me	6.65 ± 5.32	84.21 ± 4.68
6b	Et	Et	Et	14.22 ± 2.87	49.45 ± 1.48
6c	Et	Et	<sup>n</sup> Pr	5.65 ± 2.18	21.92 ± 5.70
6d	Et	Et	<sup>i</sup> Pr	10.42 ± 2.34	16.78 ± 3.12
6e	Et	Et	<sup>n</sup> Bu	1.59 ± 2.24	33.56 ± 3.26
6f	Et	Et	<sup>t</sup> Bu	— <sup>b</sup>	—
6g	Et	Et	Ph	10.17 ± 4.91	12.88 ± 3.73
6h	Et	Et	Bn	1.23 ± 1.74	46.32 ± 2.42
7a	Et	H	Me	— <sup>a</sup>	—
7b	Et	H	Et	10.92 ± 4.91	21.82 ± 1.19
7c	Et	H	<sup>n</sup> Pr	29.28 ± 3.43	85.08 ± 1.69
7d	Et	H	<sup>i</sup> Pr	9.04 ± 4.17	43.20 ± 4.86
7e	Et	H	<sup>n</sup> Bu	9.48 ± 4.28	23.80 ± 5.52
7f	Et	H	<sup>t</sup> Bu	— <sup>b</sup>	—
7g	Et	H	Ph	2.62 ± 1.86	82.50 ± 3.92
7h	Et	H	Bn	— <sup>a</sup>	—
8a	<sup>n</sup> Pr	<sup>n</sup> Pr	Me	8.35 ± 3.95	26.32 ± 2.22
8b	<sup>n</sup> Pr	<sup>n</sup> Pr	Et	10.35 ± 2.55	89.07 ± 2.66
8c	<sup>n</sup> Pr	<sup>n</sup> Pr	<sup>n</sup> Pr	11.64 ± 1.98	15.55 ± 2.13
8d	<sup>n</sup> Pr	<sup>n</sup> Pr	<sup>i</sup> Pr	10.10 ± 0.71	45.37 ± 3.89
8e	<sup>n</sup> Pr	<sup>n</sup> Pr	<sup>n</sup> Bu	4.46 ± 1.99	24.82 ± 3.38
8f	<sup>n</sup> Pr	<sup>n</sup> Pr	<sup>t</sup> Bu	— <sup>b</sup>	—
8g	<sup>n</sup> Pr	<sup>n</sup> Pr	Ph	6.58 ± 1.83	17.36 ± 4.56
8h	<sup>n</sup> Pr	<sup>n</sup> Pr	Bn	1.08 ± 1.52	22.95 ± 0.58
9a	<sup>n</sup> Pr	H	Me	— <sup>a</sup>	—
9b	<sup>n</sup> Pr	H	Et	36.98 ± 1.79	90.21 ± 0.80
9c	<sup>n</sup> Pr	H	<sup>n</sup> Pr	49.87 ± 1.13	84.00 ± 1.28
9d	<sup>n</sup> Pr	H	<sup>i</sup> Pr	31.32 ± 0.91	31.43 ± 0.83
9e	<sup>n</sup> Pr	H	<sup>n</sup> Bu	23.51 ± 3.83	39.06 ± 2.25
9f	<sup>n</sup> Pr	H	<sup>t</sup> Bu	— <sup>b</sup>	—
9g	<sup>n</sup> Pr	H	Ph	1.23 ± 1.75	84.08 ± 2.99
9h	<sup>n</sup> Pr	H	Bn	— <sup>a</sup>	—
Nereistoxin	—	—	—	12.71 ± 1.61	40.22 ± 0.12
Flunicotamid	—	—	—	75.11 ± 1.96	84.00 ± 2.78

<sup>a</sup> Compounds are unstable in purification. <sup>b</sup> Hydrolyzed easily.

This indicates that such compounds can exert efficient insecticidal activity in a short period of time, which may be similar to the mode of action of organophosphorus insecticides.

Based on these preliminary results (Table 4), some of the compounds with corrected mortality rates more than 80% at 48 h were chosen for further bioassays against *R. padi* at different concentrations (12.5, 25, 50, 100, and 200 µg mL<sup>-1</sup>); the results of these bioassays are presented as half of the lethal concentration (LC<sub>50</sub>; µg mL<sup>-1</sup>) in Table 5.

The tested compounds, including **5b**, **6a**, **7c**, **7g**, **8b**, **9b**, **9c** and **9g**, displayed excellent biological activity against *R. padi* in the laboratory, with LC<sub>50</sub> values range from 17.14 µg mL<sup>-1</sup> to

89.35 µg mL<sup>-1</sup> (Table 5), all of which were less than 90 µg mL<sup>-1</sup>. These results indicated that the compound exhibited outstanding insecticidal activity against *R. padi*. It is worth mentioning that *R. padi* is one of the most destructive wheat pests. They feed on the sap of flowers, trees and crops, thus the affected leaves roll longitudinally to the back of the leaves, and then wither and fall off. The honeydew it secretes affects the photosynthesis of wheat leaves,<sup>22</sup> reducing the yield and quality of wheat. Additionally, this aphid is also a transmission vector of wheat yellows virus.<sup>23,24</sup> Chemical control remains the primary method for managing wheat aphids. However, due to their short life cycle, large and rapid reproduction, frequent control measures, and high drug dosages, drug resistance develops rapidly.<sup>25–27</sup> Thus, it is imperative to search for novel, alternative, effective pesticides.

We therefore concluded that compounds **5b** (LC<sub>50</sub> = 17.13 µg mL<sup>-1</sup>) and **6a** (LC<sub>50</sub> = 18.28 µg mL<sup>-1</sup>) showed comparable bioactivities to the positive control flunicotamid (LC<sub>50</sub> = 17.13 µg mL<sup>-1</sup>).

Similarly, the structure–activity relationship (SAR) analysis revealed that the insecticidal activity against *R. padi* was influenced by the substituents on the N and P atoms. For *N*-alkyl-substituted compounds with identical substituents on the N atom, the LC<sub>50</sub> values showed that **7g** < **7c** and **9g** < **9c**, indicating that compounds with aryl substituents on phosphate esters exhibited higher activity than those with straight-chain substituents. For *N*-alkyl-substituted compounds with the same substituents on phosphate ester, it was observed that the shorter the carbon chain of the substituent on the N atom, the lower the activity of the compound. Comparing LC<sub>50</sub> and mortality rates, the insecticidal activity shows that **9c** > **7c** > **5c**, and **9g** > **7g** > **5g**. For *N,N*-dialkyl substitution, the mortality rates of alkyl-substituted compounds on phosphate esters exceeded those of aryl-substituted compounds. For example, the mortality rate of **6a** is greater than that of other *N,N*-dialkyl substituted compounds, and the mortality rate of **8b** surpassed that of other *N,N*-dipropyl substituted compounds, indicating that alkyl substituted compounds on the phosphate ester have high activity among *N,N*-dialkyl substituted compounds. In addition, the activity of the compounds against *R. padi* is correlated with their AChE inhibition activity. In general, compounds with strong insecticidal activity also have potent AChE inhibition ability.

## 2.4 Molecular docking

Docking simulation was performed to illustrate the differences in binding sites and binding modes between different active compounds with AChE (PDB: 6WVO). Compounds **5b**, **6a**, and **9g** were singled out as the most promising active compounds, based on a comprehensive evaluation of AChE inhibition, anti-aphid activity and compound substituents. The results of the docking simulations are presented in Table 6, and the binding sites and binding modes of these compounds to AChE are visually depicted in Fig. 3–5.

The information extracted from the docking simulation indicates that compound **5b** and **6a** bind to the same active



Table 5 LC<sub>50</sub> values of some potent compounds and flunicotamid against *R. padi* in the laboratory

Comp.	$y = ax + b$	LC <sub>50</sub> (μg mL <sup>-1</sup> )	R <sup>2</sup>	CI (95%)
<b>5b</b>	$y = 1.2105x + 3.5061$	17.14	0.9637	11.53–25.48
<b>6a</b>	$y = 1.2805x + 0.9847$	18.28	0.9695	12.72–26.26
<b>7c</b>	$y = 1.0586x + 2.9346$	89.35	0.9899	70.59–113.09
<b>7g</b>	$y = 2.3099x + 1.5743$	30.41	0.9579	25.39–36.43
<b>8b</b>	$y = 1.2102x + 3.1332$	34.88	0.9727	26.47–45.96
<b>9b</b>	$y = 1.2496x + 2.6866$	71.02	0.9779	58.22–86.62
<b>9c</b>	$y = 1.5811x + 2.1970$	59.27	0.9832	51.44–68.29
<b>9g</b>	$y = 1.3763x + 3.1009$	23.98	0.9701	17.65–32.58
Flunicotamid	$y = 1.0967x + 3.6471$	17.13	0.9720	11.25–26.07

pocket of AChE but adopt distinct binding modes. Each of them forms a hydrogen bond with Ser125 and Tyr124 respectively within the AChE active pocket through the oxygen atom of P=O group on the phosphate moiety. The length of hydrogen bond between **5b** and Ser125 is predicted to be shorter than that of **6a** and Tyr124 (2.9 Å vs. 3.2 Å). Furthermore, in comparison to **6a**, **5b** interacts with a greater number of amino acid residues in the AChE active pocket, which caused that the binding energy of **5b** with AChE is slightly lower (−7.0 kcal mol<sup>-1</sup>) than that of **6a** with AChE (−6.5 kcal mol<sup>-1</sup>), indicating that **5b** has a stronger affinity with AChE and exhibits more potent AChE inhibitory activity than **6a**. Experimental AChE inhibitory activity assays corroborate these findings by revealing that the IC<sub>50</sub> value of **5b** (0.5243 μM) is slightly lower than that of **6a** (0.6813 μM), thus affirming the superior AChE inhibitory activity of **5b**. Additionally, regarding anti-aphid activity, **5b** has an LC<sub>50</sub> of 17.14 μg mL<sup>-1</sup>, slightly lower than that of **6a** (LC<sub>50</sub> = 18.28 μg mL<sup>-1</sup>). This suggests a close relationship between AChE inhibitory activity and insecticidal effectiveness, where a stronger AChE inhibitory effect corresponds to more potent insecticidal activity.

Compound **9g** is a phosphate with four phenyl substituents, significantly differing in structure from compounds **5b** and **6a**. Molecular docking results revealed that in comparison to **5b** and **6a**, **9g** binds to a distinct active pocket of AChE, and forms different types of interactions with amino acid residues at the active site. The oxygen atoms of P=O and P-O on the phosphate in **9g** form hydrogen bonds with Tyr510, His381, Arg525 and Ala528. Additionally, the presence of phenyl substituents on the phosphate ester of **9g** leads to π-π stacking, cation-π, and anion-π interactions with HIS381, Arg525 and Asp400. Table 6 shows that the binding energy of **9g** with AChE is −8.5 kcal mol<sup>-1</sup>, which is lower than that of **5b** and **6a**. This indicates that, compared to **5b** and **6a**, **9g** exhibits stronger binding affinity with AChE. Theoretically speaking, **9g** should have stronger AChE inhibitory activity and, consequently, more

potent anti-aphid activity. However, because the AChE inhibitory activity experiment was conducted in a PBS buffer, the hydrophobicity of the benzene ring in **9g** limits the solubility in this solution, making it challenging to determine an exact IC<sub>50</sub> value. As a result, it was not possible to accurately compare the IC<sub>50</sub> values of **5b**, **6a** and **9g**, thereby hindering a direct comparison of their AChE inhibitory activities. Regarding the anti-aphid activity, the LC<sub>50</sub> value of **9g** anti-aphid was 23.98 μg mL<sup>-1</sup>, slightly higher than the LC<sub>50</sub> values for **5b** and **6a**. This difference may be attributed to the presence of four phenyl substituents in compound **9g**, which reduces its solubility in the physiological environment of aphids and may hinder metabolism, resulting in lower insecticidal activity.

Molecular docking analysis reveals that the phosphate moiety of the compound significantly influences AChE inhibitory activity. The binding of compounds to AChE primarily relies on the formation of hydrogen bonds between phosphate esters (P=O and P-O bonds) and amino acid residues of AChE. Different phosphate ester substituents resulted in varied interactions with amino acid residues. Alkyl groups, being flexible, share the same active pocket probably with different binding modes to AChE. In contrast, aryl (phenyl)-substituted phosphate esters have a higher molecular weight and greater spatial hindrance, causing them to bind to different active sites in comparison to alkyl-substituted compounds. Moreover, the presence of the aryl group allows for the formation of π-π stacking, cation-π, and anion-π interactions between the compounds and AChE amino acid residues. Consequently, aryl-substituted compounds are expected to exert a stronger AChE inhibitory effect.

## 3 Experimental section

### 3.1 Chemistry

**3.1.1 General procedures.** All reagents and anhydrous solvents were generally used as received from the commercial

Table 6 Docking of compounds **5b**, **6a** and **9g**

Comp.	Binding energy (kcal mol <sup>-1</sup> )	H-bond residues	No. of H-bond	π-π/cation-π/anion-π stacking residues
<b>5b</b>	−7.0	Ser125	1	—
<b>6a</b>	−6.5	Tyr124	1	—
<b>9g</b>	−8.5	Tyr510, His381, Arg525, Ala528	4	HIS381, Arg525, Asp400



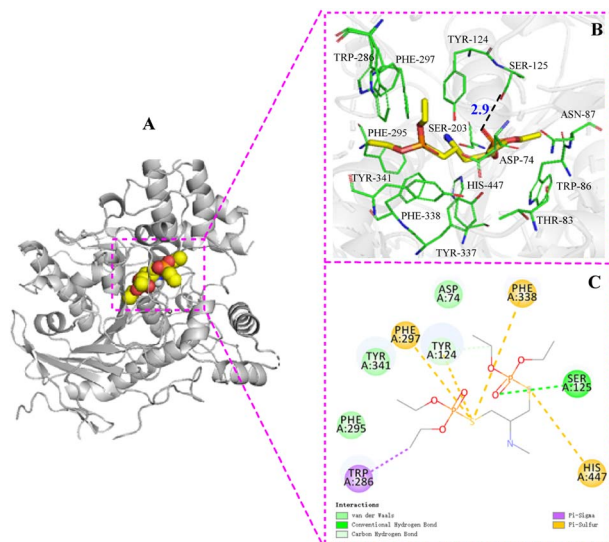


Fig. 3 The binding mode of AChE with 5b. (A) The 3D structure of the complex, (B) the hydrogen bond donor receptor network of the complex, (C) the 2D binding mode of the complex.

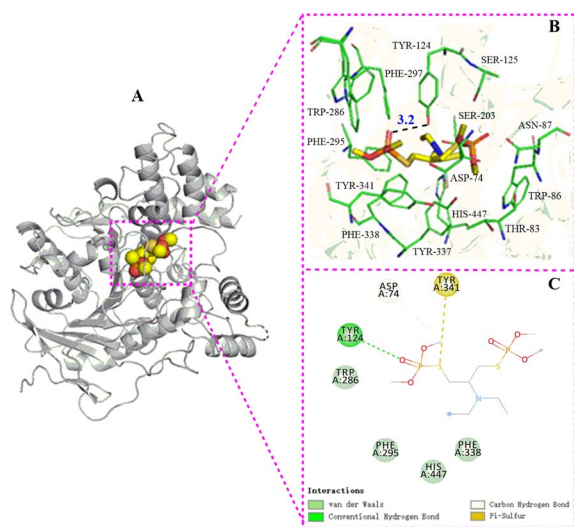


Fig. 4 The binding mode of AChE with 6a. (A) The 3D structure of the complex, (B) the hydrogen bond donor receptor network of the complex, (C) the 2D binding mode of the complex.

supplier. Melting points were determined with an electrothermal melting point apparatus (Campbell Electronic, Mumbai, India). The homogeneity of all newly synthesized compounds was checked by thin layer chromatography (TLC) on silica gel-G coated plates. Eluent was a mixture of petroleum ether/ethyl acetate or ethyl acetate/methanol in different proportions, and spots were visualized in potassium permanganate chromogenic agent. High-resolution mass spectra (HRMS) were obtained with Agilent 1290/6545 UHPLC-QTOF/MS. With tetramethylsilane as the internal standard, a Bruker 300 spectrometer was used to record  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{31}\text{P}$  nuclear magnetic resonance (NMR) spectra in chloroform ( $\text{CDCl}_3$ ) and

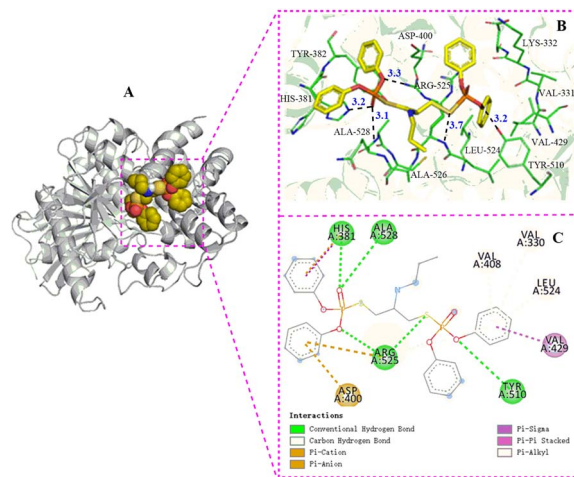


Fig. 5 The binding mode of AChE with 9g. (A) The 3D structure of the complex, (B) the hydrogen bond donor receptor network of the complex, (C) the 2D binding mode of the complex.

deuterioxide ( $\text{D}_2\text{O}$ ). Melting points were determined using METTLER TOLEDO MP90 Melting Point System.

### 3.1.2 General procedure for the synthesis of 2a–2e, 4a–4h and 5–9

**3.1.2.1 Synthesis of *N*-alkyl-1,3-dichloropropane and *N,N*-dialkyl-1,3-dichloropropane (2a–2e).** Compounds 2a–2e were synthesized by refluxing 1 mmol (1 eq.) *N*-alkyl-1,3-propanediol or *N,N*-dialkyl-1,3-propanediol with 6 mmol (6 eq.) of  $\text{SOCl}_2$  for 0.5 h–1 h.

**3.1.2.2 Synthesis of *S*-hydrogen phosphorothioates (4a–4h).** *S*-hydrogen phosphorothioates 4a–4h were prepared by the reaction of 1.1 mmol (1.1 eq.) of  $\text{S}_8$  and 1 mmol (1 eq.) of corresponding diphosphate 3a–3h in 30 mL of dry diethyl ether, then 1.1 mmol (1.1 eq.)  $\text{Et}_3\text{N}$  was added dropwise and stirred overnight at room temperature.

**3.1.2.3 Synthesis of phosphorothioic acid, *S,S'*-[alkyl amino-1,3-propanediyl]O,O',O'-tetra ester (5–9).** Novel synthesized compounds 5–9 were prepared by the reaction of 3 mmol (3 eq.) *S*-hydrogen phosphorothioates 4a–4h, NaH 3 mmol (3 eq.) and 1 mmol (1 eq.) *N*-alkyl-1,3-dichloropropane and *N,N*-dialkyl-1,3-dichloropropane 2a–2e in 20 mL of dry acetonitrile at 50 °C. Then the precipitate was filtered, and the filtrate was concentrated and extracted with dichloromethane. The organic phase was dried with anhydrous  $\text{MgSO}_4$  and then concentrated, and finally purified using silica gel column. All the synthesized compounds were characterized by  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{31}\text{P}$  NMR and HRMS. The purity of all compounds is over 95% (see ESI†).

## 3.2 Biological assays

All of the bioassays were performed on representative test organisms reared in the laboratory. The bioassay was repeated in triplicate. Insecticidal assessments were made on a dead/alive basis, and mortality rates were corrected using Abbott's formula.

**3.2.1 Inhibitory activities of acetylcholinesterase (AChE).** *In vitro* AChE inhibitory activity of the synthesized derivatives was determined spectrophotometrically against AChE using



Ellman's method. The solutions and concentrations involved in the assay are as follows: phosphate buffer (0.1 M, Na<sub>2</sub>HPO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub>, pH = 7.4), the enzyme (0.4 μg mL<sup>-1</sup> in phosphate buffer, pH = 7.4), DTNB (5,5-dithiobis (2-nitrobenzoic acid)) (1.5 mmol L<sup>-1</sup> concentration) and ATCh (acetylthiocholine iodide) (2.25 mmol L<sup>-1</sup> concentration). The compounds were dissolved in dimethyl sulfoxide (DMSO, 99%, Macklin) and configured as a stock solution with a concentration of 200 mmol L<sup>-1</sup>. The solutions were then added to the buffer. The maximum concentration of DMSO in the assays is 2%, which has no effect on inhibitory activity in independent experiments without the inhibitor. The absorbance at 412 nm was determined at 37 °C, continuously measured for 30 min, each measurement interval was 1 min, and the linear reaction part was taken for kinetic calculation. The same amount of PB buffer solution replaced the test sample as the blank control. It was considered that the absorbance change was proportional to the enzyme concentrations in principle. The IC<sub>50</sub> values were determined by probit analysis using the GraphPad Prism 8.3 software.

The formulas involved in the experiment are as follows:

$$\text{Inhibitory mortality rate (\%)} = (B_{412} - T_{412}) \times 100/B_{412}$$

where  $B_{412}$  is the absorbance at 412 nm of blank control, and  $T_{412}$  is the absorbance at 412 nm of the treated group.

$$K_i = \ln[v_0/v_t]/[IN]t$$

where [IN] is the initial concentration of the tested compound,  $v_0$  and  $v_t$  are the reaction rates at time zero and time  $t$ , respectively.<sup>28</sup>

**3.2.2 Insecticidal activities against *Mythimna separata* Walker.** The insecticidal activities of the synthesized compounds were evaluated in the laboratory using the topical application method.<sup>29</sup> The fresh tender leaves of corn were cut into square leaves with a side length of about 0.5 cm. A 1 μL micro dropper was used to drip the sample solution of different concentrations twice on each leaf and dried them in the air, then put them into the insect-rearing box. Ten pre-third-instar larvae of *M. separata* were raised on the insect box, and ten leaves were placed in each box. Repeat 4 times, with acetone as the blank control and 97% chlorpyrifos as the positive control. The experiment was carried out at 25 ± 2 °C and relative humidity (RH) of 65–80% and on 12 h/12 h (light/dark) photoperiod. The number of survivors and deaths in 24 h and 48 h were recorded. The insecticidal activity of the tested compounds against the pre-third-instar larvae of *M. separate* was calculated by the formula:

$$\text{Corrected mortality rate (\%)} = (T - C) \times 100/(1 - C)$$

where  $T$  is the mortality rate in the treated group expressed as a percentage and  $C$  is the mortality rate in the untreated group expressed as a percentage.

**3.2.3 Insecticidal activities against *Rhopalosiphum padi*.** The insecticidal activities of the synthesized compounds against *R. padi* were evaluated in the laboratory using the leaf-dipping method.<sup>30</sup> The compounds were dissolved in acetone

and then diluted in 0.1% Tween-80 aqueous solution to final concentrations ranging from 12.5 to 200 μg mL; the diluent (0.1% Tween-80) alone served as a blank control and 96% flunicotamid as the positive control. The wheat leaves were cut into 2–5 cm (3–5 leaves for each concentration), immersed in different concentrations of sample solution for 3–5 s, and then taken out. The excess liquid on the leaves was sucked off with filter paper and dried the leaves in the air. 20–50 aphids were raised on each leaf at room temperature. Repeat 4 times for each concentration. The mortality and survival rates at 24 h and 48 h were calculated and recorded, respectively.

### 3.3 Molecular docking

The potential binding modes and the main interactions of compounds **5b**, **6a** and **9g** with the AChE were determined using AutoDock Vina (version 1.1.2). The crystal structure of AChE (PDB ID: 6WVO) was obtained from Protein Data Bank (<https://www.rcsb.org>). After water and other irrelevant small molecules were removed by Pymol 2.1 software, the AChE structure was pretreated and converted into pdbqt format using AutoDockTools v.1.5.6. The size of the grid box was set to 60 × 70 × 80 Å with a grid spacing 0.375 Å, and the grid was centered at -17.222, -38.784, and 28.591 (in  $x$ ,  $y$ , and  $z$  dimensions, respectively). The optimized **5b**, **6a** and **9g** at the DFT/B3LYP/6-31G(d) level was also pretreated and converted into pdbqt format using AutoDockTools v.1.5.6.

## 4 Conclusions

In this study, a series of novel nereistoxin derivatives were designed, synthesized and evaluated for their biological activity, specifically involving acetylcholinesterase inhibitory activity and insecticidal activity. The AChE assay demonstrated that the studied compounds effectively inhibited AChE activity. However, the insecticidal activity of these compounds against *M. separate* indicated that the *in vivo* insecticidal activity was not only related to the AChE inhibition. This suggests that AChE may not be the unique active site for the compounds to exert insecticidal effect against *M. separate in vivo*. Whether it will be related to the active site AChR, where nereistoxin derivatives act, *etc.*, remains to be further confirmed. In addition, the preliminary bioassay showed that 8 target compounds had significant insecticidal activity against *R. padi* compared to the positive control. Among them, compound **5b** exhibited the highest activity against *R. padi*, with an LC<sub>50</sub> of 17.14 μg mL<sup>-1</sup>. Simultaneously, **5b** also showed strong AChE inhibitory activity, suggesting that compounds may exert their insecticidal activity against *R. padi* as an acetylcholinesterase inhibitor. The SAR analysis provided some valuable insights into the discovery and development of new insecticidal agents against *M. separate* and *R. padi*. In addition, molecular docking revealed significant differences in the binding sites and binding modes of the more active compounds **5b**, **6a**, and **9g** with AChE. These differences were attributed to the larger steric hindrance of the aryl group and the fact that compared to alkyl substituents, increasing in  $\pi$ - $\pi$  stacking/cation- $\pi$ /anion- $\pi$  interactions of aryl



substituents with the active site of AChE could easily cause more inhibition potent in AChE. Thus, the aryl-substituted compounds theoretically have higher AChE inhibitory activity and more potent anti-aphid activity. However, their lower water solubility prevented us from obtaining the IC<sub>50</sub> values. The docking results inspired us that incorporating hydrophilic groups into aryl-substituted compounds may offer potential avenues for exploring novel acetylcholinesterase inhibitors and nereistoxin pesticides. This work advances the development and deployment of nereistoxin-based insecticides and organophosphorus pesticides while also identifying viable candidate insecticides to solve current pesticide resistance challenges.

## Conflicts of interest

There are no conflicts to declare.

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