Research Article

Received: 2 February 2022 Revised: 28 September 2022 Accepted article published: 19 October 2022 Published online in Wiley Online Library: 3 November 2022

(wileyonlinelibrary.com) DOI 10.1002/ps.7245

Aphid-repellent, ladybug-attraction activities, and binding mechanism of methyl salicylate derivatives containing geraniol moiety

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Abstract

BACKGROUND: Aphids have been mainly controlled by traditional chemical insecticides, resulting in unamiable risk to the environment over the last decades. Push–pull strategy is regarded as a promising eco-friendly approach for aphid management through repelling aphid away and attracting their natural enemy. Methyl salicylate (MeSA), one of typical HIPVs (herbivore-induced plant volatiles), can repel aphids and attract ladybugs. Our previous studies discovered a new lead compound 3e**, a salicylate-substituted carboxyl (*E*)-*β*-farnesene derivative that had effective aphid-repellent activity. However, whether** 3e **has attractive activity to ladybug like MeSA is unknown. Meanwhile, to discover a new derivative for both deterring aphid and recruiting ladybug is meaningful for green control of aphids.**

RESULTS: Through the structural optimization of 3e**, 14 new derivatives were designed and synthesized. Among them, compounds** 4e **and** 4i **had good aphid (*Acyrthosiphon pisum*) repellent activity, and compounds** 3e, 4e **and** 4i **had significant ladybug (*Harmonia axyridis*) attractive activity to males. Particularly,** 4i **exhibited manifest attractive effect on the females as well. Binding mechanism showed that** 4i **not only bound effectively with the aphid (*Acyrthosiphon pisum*) target *Apis*OBP9 thanks to its multiple hydrophobic interactions and hydrogen-bond, but also had strong binding affinity with ladybug target *Haxy*OBP15 due to the suitable steric space. Additionally,** 4i **displayed low toxicity to bee *Apis mellifera*.**

CONCLUSION: Compound 3e **does exhibit attractive activity to male ladybug as MeSA. However, the new derivative** 4i**, with both pleasant aphid-repellent and ladybug-attraction activities, can be considered as a novel potential push–pull candidate for aphid control in sustainable agriculture.**

Supporting information may be found in the online version of this article.

Keywords: methyl salicylate; HIPV derivatives; odorant-binding proteins; aphids repellent; ladybugs attraction; push–pull strategy

1 INTRODUCTION

Aphids are one of the most serious and abundant pests in agriculture because they breed rapidly, suck plants directly and transmit viruses efficiently, resulting in significant damage to the yield and quality of crops.[1,2] A variety of traditional chemical pesticides have been developed to control aphids. The aphicidal activity of these chemicals is excellent, but their toxicity to the non-target organisms is also a noticeable issue.[3,4] Consequently, some neonicotinoid insecticides, such as imidacloprid and thiamethoxam have been forbidden because of their toxicity to bees in Europe.[5,6] Under these conditions, controlling aphids with an eco-friendly approach is quite important in sustainable agriculture.[7,8]

The push–pull strategy, which aims at manipulating insect behavior by combination of attractive and repulsive stimuli using either plant-derived volatile organic compounds or insect host plant preferences, has interested scientists in recent years owing to its favorable features such as benign to the environment.[9–13] 'Push' means repelling the pests away and 'pull' means attracting the natural enemies to prey on the pests.[14] The strategy has been effectively applied for pest management in some regions, such as sub-Saharan Africa.[15] The key role of push–pull technology, which involves tritrophic interactions of plants, herbivores and their natural enemies, is herbivore-induced plant volatiles (HIPVs).[16]

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HIPVs are volatile organic compounds that plants release when they are invaded by pests or herbivores.[16] HIPVs are signaling molecules in agricultural ecological systems and exhibit multiple biological activities. For example, they can not only repel feeding pests but also recruit natural enemies of herbivores, even triggering plant defense responses.[16] Many recent studies have provided a wealth of information on inducible plant volatiles as well as their application potential.[17] As a tool of the push–pull strategy, HIPVs are helpful for controlling aphids and integrated pest management (IPM).[10,16]

Methyl salicylate (MeSA), a natural repellent for aphids, is an HIPV.[18,19] For example, when *Vicia faba* was invaded by black bean aphid (*Aphis fabae*), it released MeSA to drive aphids away.[18] Similarly, *Prunus padus* released MeSA in winter to inhibit the overwintering and spring settlement of oat aphids.[19] In addition to deterring aphids, MeSA can also attract aphid natural predators, including ladybugs (*Coccinella septempunctata*) and lacewing (*Chrysopa nigricornis*).[20,21] The Asian multicolored ladybug, *Harmonia axyridis* (Coleoptera: Coccinellidae), is an important natural predator of small pests such as aphids and can be attracted by MeSA.[22] Geraniol (GA) is another plant volatile that repels aphids and released by many plants, such as *Pelargonium graveolens*.[23–25] Fewer corn leaf aphids (*Rhopalosiphum maidis*) landed on leaves treated with GA.[23] In a behavioral response test for green peach aphid (*Myzus persicae)*, GA was also found to significantly inhibit aphid settling.[25] These plant volatiles regulate insect behavior through odorant binding proteins (OBPs).[26,27]

OBPs are soluble binding proteins and are generally known to transmit external semiochemicals to olfactory receptors to mediate behavioral responses.[28] In the push–pull strategy for aphid control, OBPs of both aphid and natural enemies were responsible for the detection and perception of HIPVs.[28] For example, OBP3 and OBP7 from *Acyrthosiphon pisum*, *Sitobion avenae* and *Rhopalosiphum padi* were found to recognize HIPVs.[29–32] Another aphid OBP, OBP9 from *Acyrthosiphon pisum*, *S. avenae* and *Myzus persicae* also strongly bound to HIPVs, but with a higher binding property than that of OBP3 and OBP7.[33–36] The OBPs from natural enemies of aphids were also crucial targets in the recognition of HIPVs. For instance, three OBPs from the aphid predator *Chrysopa pallens*, CpalOBP3, CpalOBP6 and CpalOBP10, showed good binding affinity to HIPVs and regulated the behavioral response of *Chrysopa pallens*.[37] Recently, HaxyOBP15 was identified as an important OBP from *H. axyridis* with strong and broad binding affinity to HIPVs.[38]

MeSA and GA have activity to repel aphids, but their aphid-repellent activities were not significant and persistent.[18,19,23,25] Therefore, the active substructure splicing method was used to develop new eco-friendly agrochemicals. In our previous work, compound **3e** was found to have effective repellent activity against aphids after splicing the substructures of MeSA and GA with a linker of ester group (-COO).[33] The hydrophobic nature of the analog was responsible for the repellent activity, in which the hydrophobic ester group was especially favorable for compounds binding with aphid target OBPs. Thus, **3e** with outstanding aphid-repellent activity could be a new lead compound for aphid control agents.[33]

As mentioned earlier, MeSA can both deter aphids and attract their natural predators.[18–21] However, as an analog of MeSA, whether **3e** has attractive activity to ladybugs is unknown. In addition, based on compound **3e**, discovering new derivatives that both deter aphids and recruit ladybugs is meaningful for controlling aphid. Given that the hydrophobic ester group plays a crucial role in the discovery of highly active agrochemicals,[39–41] we hypothesized that introducing the hydrophobic ester group into the structure of **3e** would result in the discovery of novel agrochemicals with both aphid-repellent and ladybug-attractive activity. Therefore, in the present work, the structural optimization of **3e** was performed by introducing a hydrophobic ester group (R_2-COO-) to replace the hydrophilic hydroxyl group (2-OH) on the salicylic ring, and 14 new compounds were designed and synthesized (Fig. 1). Their aphid-repellent activity against *Acyrthosiphon pisum* and ladybug-attractive activity to *H. axyridis* were determined in the laboratory. Their binding affinities and mechanisms to target OBPs in aphids and ladybugs were also studied through fluorescent competitive binding assays and molecular docking method, respectively.

2 MATERIALS AND METHODS

2.1 Chemicals and instruments

All utilized laboratory reagents (analytical grade) were acquired from HEOWNS Corporation (Tianjin, China), and used without additional purification. Silica gel (200–300 mesh, Puke Corporation, Qingdao, China) was used for column chromatographic purification. The proton nuclear magnetic resonance (^{1}H-NMR) spectra at 500 MHz and carbon-13 (^{13}C) NMR spectra at 125 MHz of the target compounds were determined on an AVANCE NEO spectrometer (Bruker, Bremen, Germany) using chloroform-*d* ($CDCl_3$) or dimethyl sulfoxide-d_6 (DMSO-d_6) as the solvent, and tetramethylsilane (TMS) as the internal standard. High-resolution mass spectrometry (HRMS) data of the target compounds were obtained on a 7.0 T FTICR-MS instrument (Varian, Palo Alto, CA, USA). The fluorescence binding assay data was obtained with Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies, Santa Clara, CA, USA).

2.2 General synthesis procedure for target compounds

The key intermediates **3a** and **3e** were prepared according to the reported procedure and described in the Supporting Information (see the chemical synthesis data in the supporting information, contents 11 and 12).[33] The synthesis of target compounds **4a**–**4n** (Scheme 1) began by stirring intermediate **3a** or **3e** (6.6 mmol) with

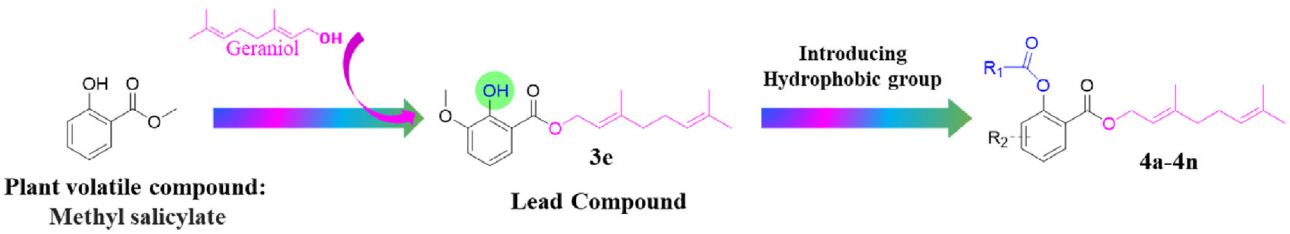
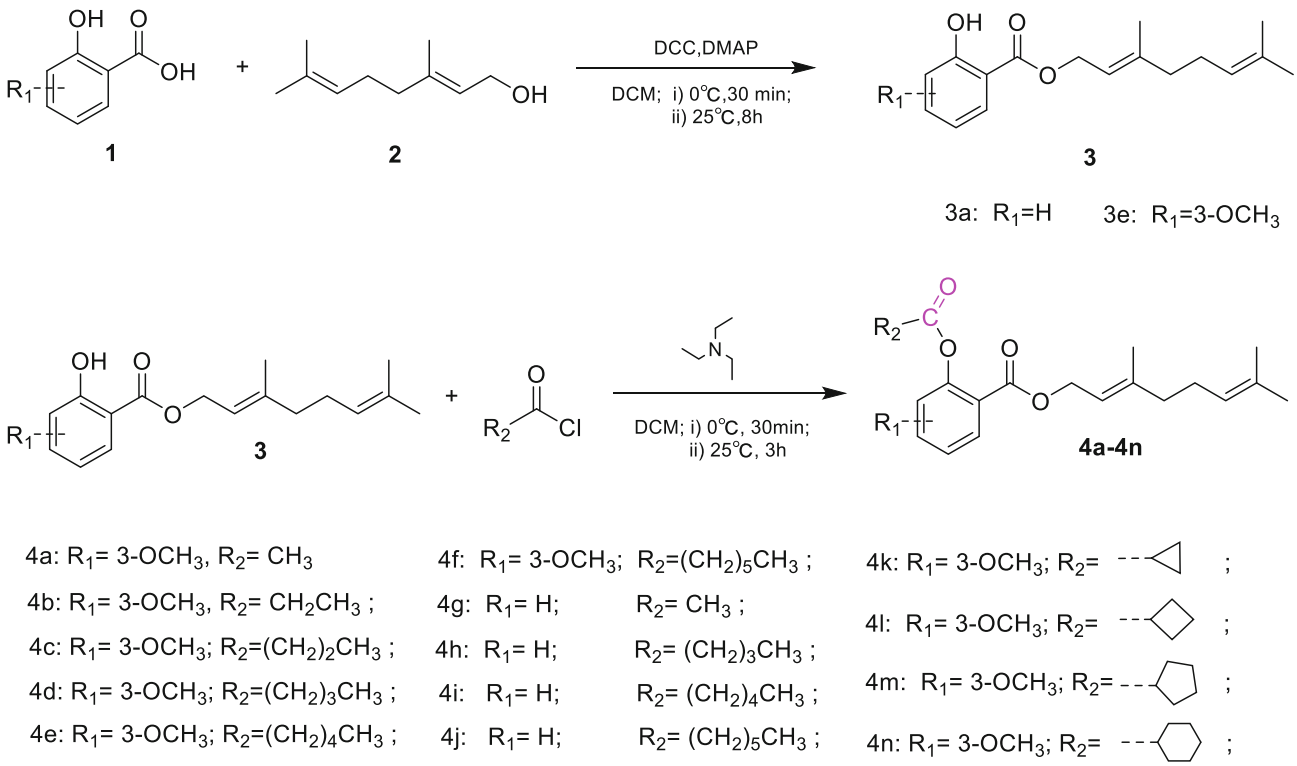


Figure 1. The design strategy of target compounds.



Scheme 1. General synthetic routes for MeSA derivatives **4a**–**4n**.

triethylamine (13.2 mmol) in dichloromethane (DCM, 30 mL) in an ice bath. After that, substituted acyl chloride (R-COCl, 13.2 mmol) in 20 mL anhydrous DCM was added dropwise to the solution. Then the mixture was stirred at 40 °C for 3 h, washed with water three times, extracted with DCM, dried with anhydrous sodium sulfate, and filtered. The organic phase was concentrated under reduced pressure and purified by column chromatography to obtain **4a–4n**. The structures of the target compounds were confirmed by ^{1}H-NMR, ^{13}C-NMR and HRMS (see the chemical synthesis data in the Supporting Information contents 11 and 12).

2.3 Fluorescence binding assays

After expression and purification were performed according to our previous procedure, the ApisOBP9[33] and HaxyOBP15[38] proteins were used to determine the binding affinity with synthetic derivatives using fluorescence competitive binding assays. The detailed processes were performed as our previously reported method[33,38] and are described in the Supporting Information. Emission fluorescence spectra were recorded on a Cary eclipse fluorescence spectrophotometer at room temperature in a right-angle configuration, with a 1 cm light path quartz cuvette and 5 nm slits for both excitation and emission. The affinity of the fluorescent probe *N*-phenyl-1-naphthylamine (1-NPN) to each protein was measured by titrating a 2 μmol L^{-1} solution of the protein in 50 mmol L^{-1} Tris–HCl buffer, pH 8.0 with aliquots of 1 mmol L^{-1} 1-NPN in methanol to final concentrations of 2–20 μmol L^{-1}. The solutions were excited at 337 nm and emission spectra were recorded from 350 to 500 nm. The comparative binding of derivatives was measured in a solution of the protein and 1-NPN, both at the concentration of 2 μmol L^{-1}, which was titrated with 1 mmol L^{-1} methanol solutions of each competitor to final concentrations of 2–20 μmol L^{-1}. The dissociation constant (Ki) of each ligand binding to ApisOBP9 or HaxyOBP15 was calculated from the corresponding half maximal inhibitory concentration (IC_{50}), according to the equation $Ki = [IC_{50}]/(1 + [1\text{-NPN}]/K_{1\text{-NPN}})$, where [1-NPN] is the free concentration of 1-NPN, and $K_{1\text{-NPN}}$ is the dissociation constant of the complex protein/1-NPN. Therefore, 1/Ki was used to represent the bound constant.

2.4 Behavioral bioassays in the laboratory

2.4.1 *Behavioral bioassays of aphids*

The aphids (*Acyrthosiphon pisum*) for our colony were provided by the Laboratory of Creation and Application of Biological Insecticides, Institute of Plant Protection, CAAS. They were maintained on *Pisum sativum* in the Laboratory of Pesticide Molecule Design & Discovery at China Agricultural University and reared in a growth chamber (RXZ-300B, Ningbo, China) at 23 ± 1 °C, with a 16 h:8 h light/dark photoperiod and 70% relative humidity.

The behavioral response of *Acyrthosiphon pisum* to each derivative was carried out with a two-way olfactometer according to the published method[33] and the process is described in detail in the Supporting Information. Twenty apterous adult aphids were introduced into the center of the olfactometer and allowed to move freely for 15 min. Then the numbers of aphids in each arm were recorded. Each measurement was repeated four times.

The repellent activity was evaluated using the repellency proportion (RP) as the formula $RP = (C - T)/(C + T) \times 100\%$, where C indicates the number of aphids in the control arm and T indicates the number of aphids in the treatment arm. The RP values of the derivatives were analyzed statistically with SPSS 23.0 (SPSS Inc., Chicago, IL, USA) using a one-way analysis of variance (ANOVA) followed by Duncan's test for significant differences at $P < 0.05$. Analysis of the significant difference between two compounds was adopted by the independent-sample *t*-test.

2.4.2 Behavioral bioassays of ladybugs

The behavioral response of ladybugs (*H. axyridis*) to each derivative was observed with a behavior olfactometer. The olfactometer consisted of three spherical bottles interconnected by a tube between two adjacent bottles. The ladybugs (*H. axyridis*) were purchased from a commercial company (Henan Jiyuan Baiyun Industry Co., Ltd, Henan, China).

The bioassay was carried out using a two-way choice assay according to the reported method.[42] The tested compound was dissolved in paraffin oil to a concentration of 10 μg μL^{-1}. For each measurement, 10 μL of solution or 10 μL of paraffin oil was applied to a filter paper, and placed into treatment and control bottles, respectively. An airflow (0.5 L min^{-1}) was introduced into the olfactometer after drying and purification by passing activated granular carbon and washing in distilled water. For each assay, 40 adult female ladybugs were introduced into the middle of the box and allowed to move freely to any bottle. After 30 min, the number of ladybugs in the treatment and control bottles was recorded. Three replications were performed for each test. Under the same conditions and procedures, the behavioral bioassay of male ladybugs was performed. The attractive activity was calculated by the preference index (PI),[43] $PI = (T - C)/(T + C) \times 100\%$, where T and C indicate the number of ladybugs in the treatment and control bottles, respectively. The choice values in the treatment and control bottles were compared using the x^2 goodness-of-fit test. A one-way ANOVA was performed followed by Duncan's test ($P < 0.05$) of different compounds. Analysis of significant difference between two compounds was adopted by the independent-sample t-test. All statistical comparisons were analyzed with SPSS 23.0.

2.5 Molecular docking

The structures of ApisOBP9 and HaxyOBP15 were first modeled with a deep residual neural network named trRosetta (https://yanglab.nankai.edu.cn/trRosetta),[44] which is an algorithm to quickly and accurately predict protein structures. The final three-dimensional (3D) model was then assessed using methods on the online Structure Analysis and Verification Server (http://services.mbi.ucla.edu/SAVES/),[45] including Procheck, Verify_3D and ERRAT.

Molecular docking studies between the ligands and ApisOBP9 or HaxyOBP15 were carried out using the Surflex-Dock algorithm in Sybyl 7.3 software.[46] Two representative compounds (**4e** and **4i**), lead compound **3e** and two plant volatiles (MeSA and GA) were selected as ligands. A suitable putative conformation, named protomol, of the ligand was generated rapidly under the Hammerhead scoring function with a surface-based molecular similarity mode.

2.6 The toxicity assessment of the derivatives toward mammals and honeybee

2.6.1 Toxicity prediction of the derivatives

To further explore the toxicity of the derivatives toward mammals and beneficial insects, their carcinogenicity, honeybee toxicity, and rat oral toxicity were predicted by the ProTox-II,[47] admetSAR,[48] and CompTox Chemicals Dashboard,[49] respectively. The toxicity grade was analyzed based on the US Environmental Protection Agency (EPA) pesticide toxicity classification standards.

2.6.2 Bee toxicity test

To evaluate the effects of representative derivative **4i** on beneficial insects, *Apis mellifera* was selected for the bee toxicity test. Two toxicities to *Apis mellifera* including the oral and contact toxicity of **4i** were evaluated. The test was carried out according to the reported method.[50] Sucrose water and the solvent were used as the blank control and negative control, respectively. Each experiment was performed in three replicates and each replicate contained ten bees. Fisher's exact test was selected for significance judgment. The test process is described in detail in the Supporting Information, content 9.

3 RESULTS AND DISCUSSION

3.1 Design and synthesis of target compounds

In this process (Scheme 1), target compounds **4a–4n** were obtained by introducing a carbon chain containing an ester group (R_2-COO-) to replace 2-OH on the salicylic ring. For **4a–4f**, substitution R_2, which was linked with the ester group, was a straight chain with one to six carbon atoms, in **4k–4n**, R_2 was a cricoid chain containing three to six carbon atoms, and R_1 in all of them was 3-methoxy (3-OCH_3). For compounds **4g–4j**, R_2 was the straight chain with one, four, five or six carbon atoms, and R_1 was H atom. The target compounds were synthesized via a nucleophilic substitution reaction between intermediate **3a** or **3e** and the substituted acyl chloride (R_2-COCl) in the presence of triethylamine according to a reported method.[51]

3.2 Binding affinity to ApisOBP9 and HaxyOBP15

3.2.1 The binding affinity to ApisOBP9

Given the fact that the lead **3e** had a stronger binding affinity with ApisOBP9 than ApisOBP3 and ApisOBP7,[33] the binding capacities of its derivatives **4a**–**4n** to ApisOBP9 were evaluated in the present work. The binding constant (1/Ki) values were determined by a competitive fluorescence binding experiment. The results are shown in Fig. 2.

Previous research did not compare the binding difference between MeSA, GA and **3e** with ApisOBP9. In this research, we found that the protein binding affinity of **3e** (1.51 μmol L^{-1}) was stronger than that of two volatiles MeSA (0.13 μmol L^{-1}) and GA (0.12 μmol L^{-1}) (Supporting Information Table Table S1). In addition, almost all of the new derivatives showed better binding affinity than MeSA and GA, but lower than lead **3e** except **4i**, which showed a similar level to this lead.

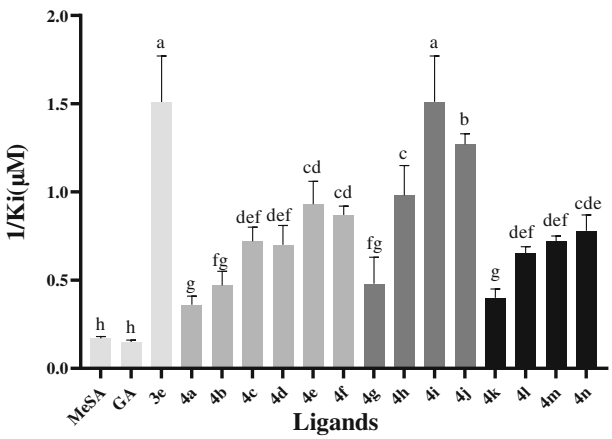


Figure 2. Binding properties of the target compounds to ApisOBP9 from *Acyrthosiphon pisum*. A one-way ANOVA followed by Duncan's test was used for significant differences at $P < 0.05$, marked with lowercase letters. Different lowercase letters indicate significant differences.

Obviously, the R_2 group influenced the binding affinity to ApisOBP9. In ligands **4a–4f** and **4g–4j**, the binding affinity increased generally with the elongation of the R_2 carbon chain (**4e** ≥ **4d** ≈ **4c** > **4b** ≥ **4a**, and **4i** > **4h** > **4g**), but decreased slightly (**4f** ≈ **4e** and **4j** < **4i**). When the number of carbon atoms in R_2 group was five, the binding affinity reached the peak (**4e** and **4i**) regardless the R_1 group was 3-OCH_3 or H. For the ligands with an ester group and R_2 as a cricoid carbon (**4k–4 n**), the protein binding affinity increased generally with the ring enlarging (**4n** ≥ **4m** ≈ **4l** > **4k**).

The influence of the R_1 group on the binding capacity to ApisOBP9 was also noticeable. Interestingly, when replacing 3-OCH_3 with hydrogen (H), the binding affinity of the derivative with ApisOBP9 was generally improved according to the fact that the binding affinities of **4h–4j** were obviously higher than that of **4d–4f** (Table Table S1). In our previous work, for the binding affinity with the target ApisOBP9, the R_1 group as 3-OCH_3 was more favorable than H when there was a OH on the 2-position of salicylic ring.[33] However, in the current study, when 2-OH was replaced with an ester group R_2-COO-, the R_1 group as H appears more considerable than that of 3-OCH_3.

Our previous study found ApisOBP9 can be bound with aphid alarm pheromone E-β-farnesene (EβF) and its derivatives.[33] The current work also showed ApisOBP9 had binding affinities with plant volatiles and their derivatives. This phenomenon can be found in OBP9 of other aphids. For example, OBP9 of *S. avenae* was proved to have broad and high binding affinities with most of the wheat volatiles.[34] OBP9 of *Myzus persicae* also has binding affinities with plant volatiles, aphid alarm pheromone and sex pheromones.[36] In addition, the OBP9 of *Acyrthosiphon pisum* was found to bind with alcohols and aldehydes of 16 carbon atoms as well.[35] Therefore, OBP9 is a key odorant binding protein target for aphids, that may contribute mainly to the behavioral response of aphid to plant volatiles and the aphid pheromones.

3.2.2 *The binding affinity to HaxyOBP15*

Harmonia axyridis is an important natural enemy against many agricultural pests including aphids, relying on sensitive olfactory sensory systems to detect plant hosts. OBPs are involved in the first step of the olfactory signal transduction pathway. The study on the ladybug OBP was fewer than that on aphid OBP. HaxyOBP15 from *H. axyridis*, initially characterized by Qu *et al*., has a significantly high expression level in the adult stage.[52] Our latest results indicate that HaxyOBP15 has a broad binding profile with EβF and plant volatiles β-ionone, α-ionone, geranyl acetate, dihydro-β-ionone, nonyl aldehyde, and linalyl acetate with the Ki values from 4.33 to 40.02 μmol L^{-1}.[38] For instance, it can be bound with EβF (Ki = 4.33 μmol L^{-1}) and geranyl acetate (Ki = 23.38 μmol L^{-1}). Therefore, HaxyOBP15 was suggested to be a potential target in the detection of HIPVs and regulation of *H. axyridis* behavior.[38]

In the current work, HaxyOBP15 was chosen for the binding ability determination on the target compounds. As shown in Fig. 3 and Table Table S1, the protein binding affinities of the derivatives **4c–4e**, **4 h**, **4i** and **4n** were higher than that of lead **3e**, indicating that introducing the hydrophobic group of the C3-C5 straight chain or the C6 cricoid chain into R_2 is beneficial for binding affinity. The plant volatiles MeSA and GA both bound to HaxyOBP15 with a low affinity. Interestingly, the diester derivative **4i** (6.07 μmol L^{-1}), a salicylate derivative containing GA moiety discovered in this work, showed stronger binding affinity than that of the plant volatiles MeSA (0.09 μmol L^{-1}) and GA (0.08 μmol L^{-1}).

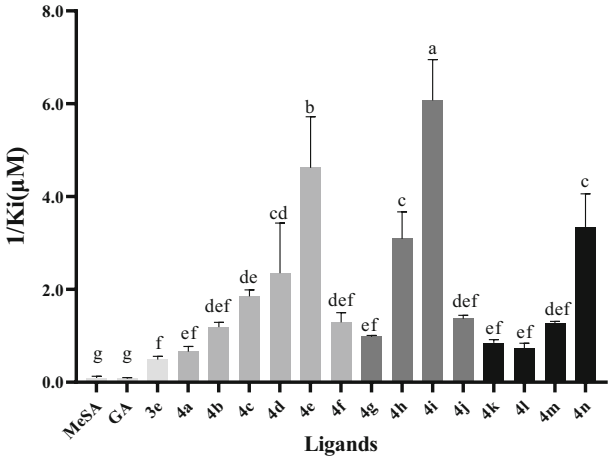


Figure 3. Binding properties of the target compounds to HaxyOBP15 from *Harmonia axyridis*. A one-way ANOVA followed by Duncan's test was used for significant differences at $P < 0.05$, marked with lowercase letters. Different lowercase letters indicate significant differences.

In **4a–4f** and **4g–4j**, it can be found that the binding affinity first increased and then decreased with the elongation of the straight carbon chain, and it reached the highest value (**4e** and **4i**) when the number of carbon atoms was five. Compared with **4e** (4.62 μmol L^{-1}), the binding affinity of **4i** (6.07 μmol L^{-1}) was improved significantly after replacing 3-OCH_3 of the R_1 site with H atom, indicating that when R_2 was an ester group with C5 alkyl chain, H atom in R_1 site was beneficial for the ligand to bind with HaxyOBP15. When the R_2 group was a cricoid carbon chain, the binding affinity increased generally with the extension of the ring (**4n** > **4m** ≥ **4l** ≈ **4k**), and that when R_2 was a cyclohexyl substituent, the ligand had a good binding affinity with HaxyOBP15 (**4n**, 3.34 μmol L^{-1}).

A comparative analysis between the binding affinity of target compounds to ApisOBP9 and HaxyOBP15 exhibited some interesting results. For ApisOBP9, lead **3e** generally showed higher activity than derivatives **4a–4n**. However, for HaxyOBP15, **3e** generally displayed lower affinity than **4a–4n**. In other words, these novel compounds with ester groups in the R_2 substituent increased the binding affinity with HaxyOBP15 but decreased the binding affinity with ApisOBP9 except for **4i**. Thus, the ester group introduced in R_2 was detrimental for binding with ApisOBP9 but beneficial for HaxyOBP15 when there was 3-OCH_3. However, compound **4i**, with a C5 straight chain in R_2 and without 3-OCH_3 in the salicylic ring, displayed good binding affinity to both ApisOBP9 and HaxyOBP15. The chemical structure features of **4i** provide valuable clues for the rational design of push–pull agents for eco-friendly aphid control. The reason why **4i** has a dual effect on both ApisOBP9 and HaxyOBP15 will be analyzed in Section 3.4.

3.3 Behavior activity

3.3.1 *Aphid repellent activity of compounds*

The repellent activity of compounds MeSA, GA, **3e** and newly synthesized derivatives was tested against *Acyrthosiphon pisum*, an important agricultural aphid. As shown in Fig. 4 and Table S2, the RP of the derivatives varied from 18.7% to 57.8%. The lead **3e** and derivative **4i** exhibited superior repellent activity to MeSA

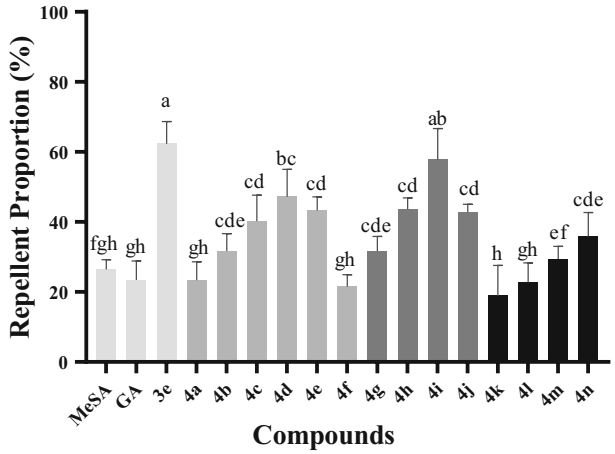


Figure 4. Repellent activity of target compounds against aphids *Acyrthosiphon pisum*. A one-way ANOVA followed by Duncan's test was used for significant differences at $P < 0.05$, marked with lowercase letters. Different lowercase letters indicate significant differences.

and GA. Generally, after introducing hydrophobic ester groups (R_2-COO-) to replace 2-OH on the salicylic ring, the aphid-repellent activity of the derivatives became lower than that of **3e**. Comparing the repellent activity of derivatives **4a–4n** with the lead **3e**, the RP value decreased significantly. For instance, **4a** ($P = 0.003$) and **4k** ($P = 0.001$) exhibited the significant lower repellent activity than **3e**. Therefore, the di-ester substituent compounds did not show better aphid-repellent activity than that of **3e**.

The repellent activity was influenced by substitutes R_1 and R_2. For derivatives with R_2 as straight carbon chain (**4a**–**4f**, and **4g**–**4j**), repellent activity generally increased with carbon chain elongation (**4d** ≥ **4c** ≥ **4b** > **4a**, and **4i** > **4h** ≥ **4g**), achieved the highest activity when the number of carbon atoms was five (**4i**, 57.8%), and then subsequently decreased (**4f** < **4e** ≤ **4d**, and **4j** < **4i**). For derivatives with R_2 as cricoid carbon (**4k–4n**), repellent activity generally increased with ring enlargement (**4n** ≥ **4m** > **4l** ≥ **4k**). The R_1 substituent also influenced the repellent activity. When R_1 (3-OCH_3) in the salicylic ring was replaced with H, the aphid-repellent activities of **4g** (31.7%), **4i** (57.8%) and **4j** (42.8%) became higher than those of **4a** (23.3%), **4e** (43.3%) and **4f** (21.5%), respectively. In our previous work, the R_1 group as 3-OCH_3 on the salicylic ring was more favorable than H when a hydroxyl substitute was on the 2-position of the salicylic ring.[33] However, in the present study, after we introduced an ester group (R_2-COO-) to replace 2-OH, the R_1 group as H compared to 3-OCH_3 was more considerable for repellent activity.

Comparing the aphid repellent activity and binding affinity with ApisOBP9 of some target compounds, these two activities are consistent with each other. For example, in compounds **4a–4d**, and **4k–4n**, with the elongation of the straight carbon chain or the enlargement of the cricoid carbons in the R_2 substituent, both of these two activities generally increased (repellent proportion: **4d** ≥ **4c** ≥ **4b** > **4a**, **4n** ≥ **4m** > **4l** ≥ **4k**; ApisOBP9 affinity: **4d** ≈ **4c** ≥ **4b** ≥ **4a**, **4n** ≥ **4m** ≈ **4l** > **4k**). More importantly, among the test compounds, **3e** and **4i** not only had the best repellent activity against *Acyrthosiphon pisum* but also showed the highest binding affinity with ApisOBP9.

As a natural repellent against aphids, MeSA exhibites effective repellent activity against the black bean aphid *Aphis fabae*[18] and oat aphid *R. padi*.[19] To explore the access to improve the aphid-repellent activities based on **3e**, 14 new MeSA derivatives were synthesized in the current work. All the aphid-repellent activities of these derivatives were higher than MeSA, but lower than **3e**. The structure activity relationship study in this work was quite meaningful to find a candidate for controlling aphid.

Furthermore, EβF was also able to repel aphids.[33,53,54] In recent research, slow release of EβF and MeSA actually enhance biocontrol efficacy against the wheat aphids (*Sitobion miscanthi*, *S. avenae*, *R. padi* and *Metopolopium dirhodum*) and pea aphid (*Acyrthosiphon pisum*) in the field.[53–55] Moreover, a formulation of the mixture of EβF and MeSA had a significant reduction effect on apterous *S. miscanthi* abundance, better than EβF or MeSA alone.[53] Interestingly, the lead compound **3e**, designed by merging the structures of EβF and MeSA, showed lower repellent activity but more stable than EβF in our previous work.[33] Furthermore, the target compound **4i** containing moieties of MeSA and GA, acquired by optimizing the structure of **3e** through introducing a hydrophobic ester group (R_2-COO-) to replace the hydrophilic hydroxyl group (2-OH) on the salicylic ring, displayed higher repellent activity than MeSA and GA in the current work. These indoor experiment results indicated the importance of the molecular designing and the structural optimization. However, to confirm the rationality in merging the structures of two plant volatiles EβF (or GA) and MeSA into one molecule, further evaluation of the control effect of the compounds **3e** and **4i** against aphids will be conducted in the field in the future, using MeSA, EβFa, or even MeSA + EβF as positive control.

3.3.2 *Attractive activity to* Harmonia axyridi

Given that the plant volatile MeSA has an attractive effect on ladybugs, whether the synthesized MeSA derivatives maintain this property was evaluated in the current work. The compound **3e** and highly active derivatives **4e** and **4i**, and low-activity derivative **4g** were selected as representative compounds to determine the *in vivo* attractive activity to *H. axyridis* using a behavioral olfactometer.

As shown in Fig. 5(A) and Table S3, MeSA had an attractive effect on male ladybugs at 30 min, preferring to pull males (attracted 61.0%, $P = 0.0359$) than females (attracted 52.7%, $P = 0.6714$). Furthermore, like MeSA, its derivatives **3e**, **4e**, **4g** and **4i** also exhibited attractive activity toward *H. axyridis*. Interestingly, these derivatives showed higher attractive activity to males than females, similar to MeSA. In particular, regardless of whether R_1 substituent was H or 3-OCH_3, **4e** and **4i**, containing C5 straight chain for R_2, displayed the best attractiveness to male ladybugs. Furthermore, when R_1 substituent was H and R_2 was C5 straight chain, compound **4i** also exhibited the highest attractive percentage to females.

As shown in Fig. 5(B) and Table S3, for males, the attractive activity of compound **4i** was obviously higher than that of MeSA and **4g**, and slightly better than **3e** and **4e**. For females, compound **4i** displayed significantly higher attractive activity than MeSA ($P = 0.005$), **3e** ($P = 0.002$), **4e** ($P = 0.006$) and **4g** ($P = 0.018$).

Harmonia axyridis is a widely used predacious biological control agent for many agricultural and forestry pests. However, few individuals have tried to modify the plant volatiles to obtain a ladybug attractant. In this work, it was proved that **4i** can attract *H. axyridis* especially male. The derivative **4i** was supposed to enhance the effectiveness of attracting *H. axyridis* as a biological control agent. And introducing hydrophobic ester groups (R_2-COO-) to replace 2-OH on the salicylic ring of **3e**, derivative with 3-OCH_3 in R_1

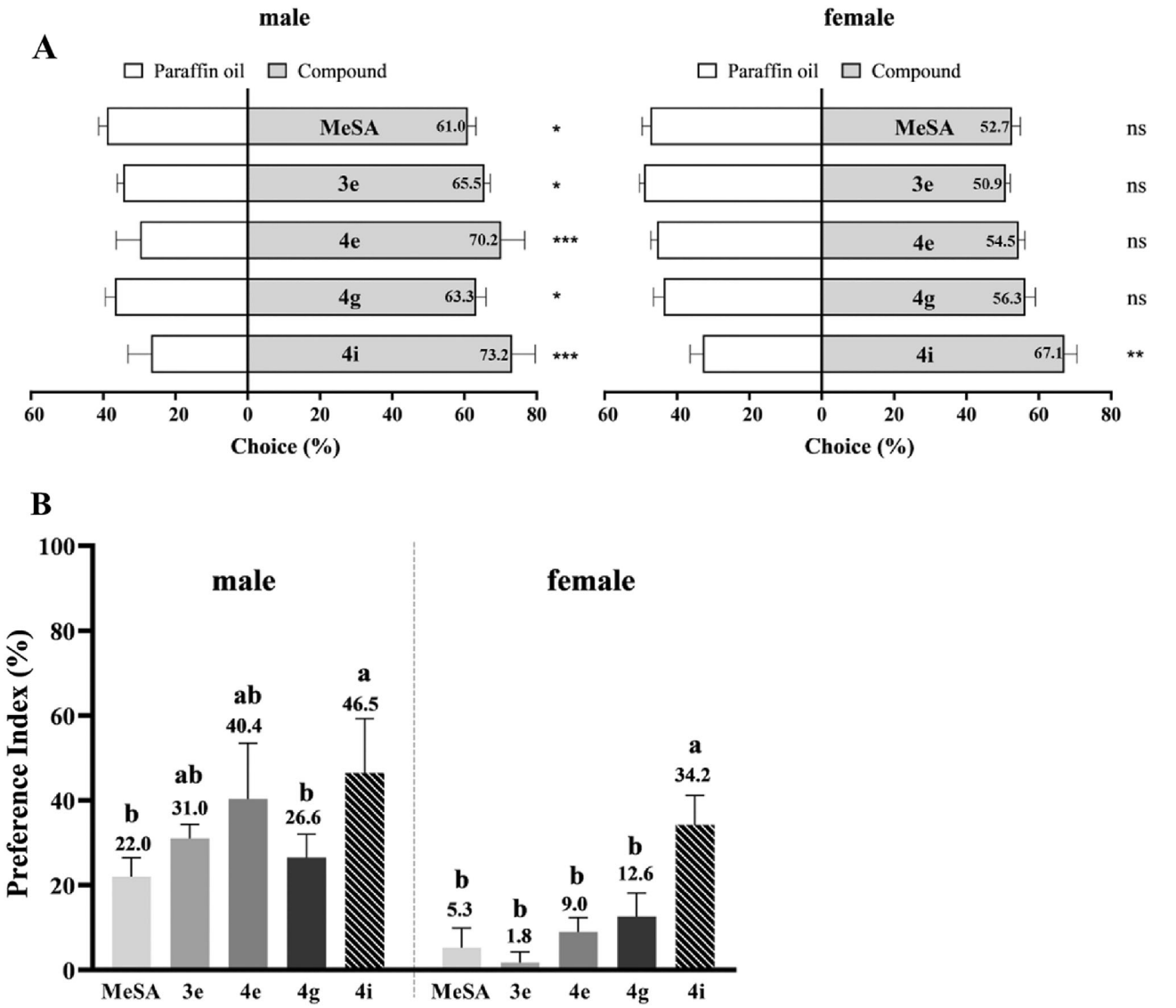


Figure 5. Attractive activity of MeSA, **3e**, **4e**, **4g** and **4i** to *Harmonia axyridis*. (A) Choice of behavior response (ns = no significance, $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$); (B) Preference index of behavior response. A one-way ANOVA followed by Duncan's test was used for significant differences at $P < 0.05$, marked with lowercase letters. Different lowercase letters indicate significant differences.

(**4e**), still maintained male ladybug-attractive activity, which was higher than MeSA or **3e**. Particularly, elongating the straight carbon chain in R_2 can improve the attractive activity for both male and female (**4i** > **4g**).

Plant volatiles MeSA and EβF can attract aphid natural enemies, such as ladybugs, hoverflies and parasitoids.[20,53–57] For example, EβF was an effective kairomone to the ladybug *Adalia bipunctata* in the laboratory,[56] and it attracted ladybugs significantly higher than control and MeSA in the field.[54] Meanwhile, traps with MeSA captured greater numbers of ladybugs than control.[57] The mixture of these two semiochemicals can also attract the natural enemies of aphids.[53] Interestingly, **4i** had better attractive activity to ladybug (*H. axyridis*) than MeSA. However, whether the compound **4i** has the attractive effect on ladybugs in the field needs to be evaluated in the future, using MeSA, EβF, or even MeSA + EβF as positive control. At the same time, these results shed light on potential utilization of the compounds with attractive activity to *H. axyridis* in aphid control.

3.4 Molecular docking

3.4.1 *Molecular docking of ligands to ApisOBP9*

To further study the molecular mechanisms of ligands binding with the target ApisOBP9, five typical compounds (GA, MeSA, **4e**, **4i** and **3e**), possessing binding affinity from low to high, were selected as representative ligands for molecular docking work.

In our previous work, the molecular docking between **3e** and ApisOBP9 was studied on the basis of homologous modeling.[33] Along with the development of protein prediction methods in recent years, a more accurate method (named trRosetta) for protein structure prediction was used to build the ApisOBP9 model.[44] Molecular docking between five ligands and ApisOBP9 was carried out to generate more meaningful results.

As shown in Fig. 6(A), all ligands were located in the central area of the ApisOBP9 binding pocket, and divided the pocket into two domains (S1 and S2) according to the docking conformation of the ligands. Most of the binding surface in the binding pocket was hydrophobic for ApisOBP9 (brown area in Fig. 6(A)). In Fig. 6

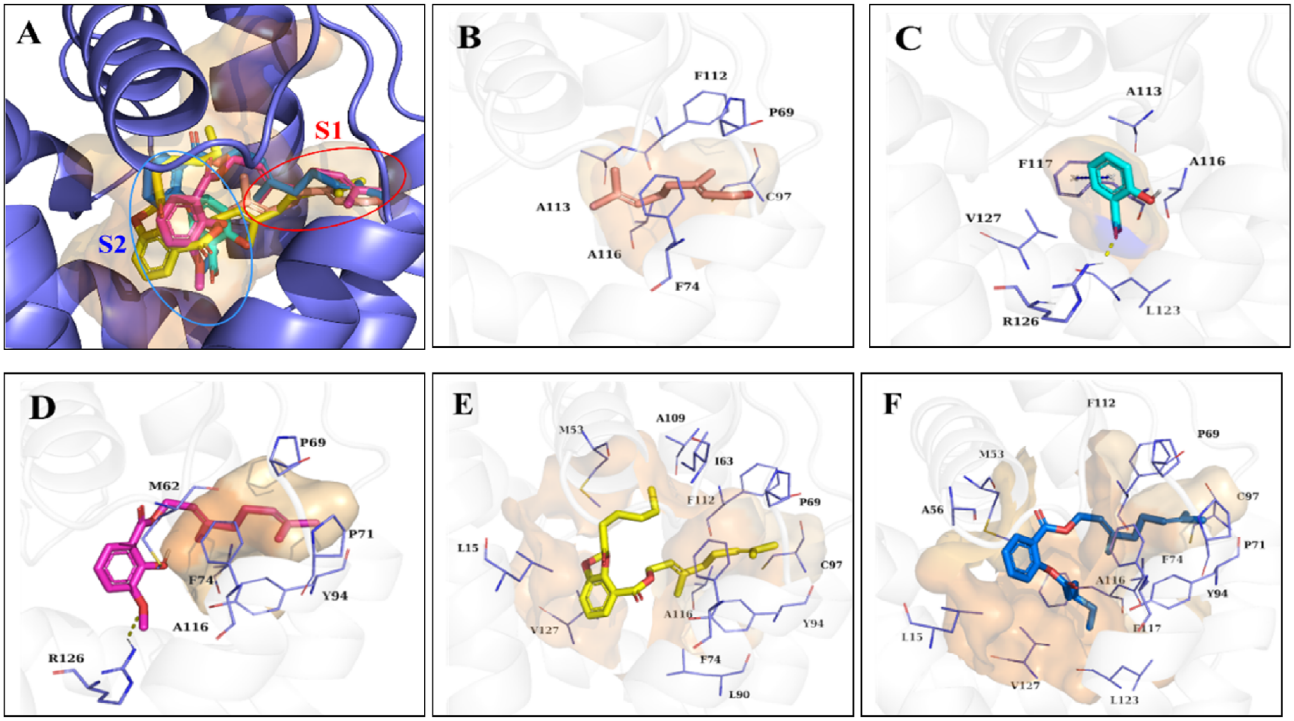


Figure 6. Molecular docking of representative ligands with ApisOBP9 (superimposed conformations of the molecular docking ligands (A) and the docking conformation of GA (B), MeSA (C), **3e** (D), **4e** (E) and **4i** (F), respectively). Brown and blue surface represents the hydrophobic and hydrophilicity area of docking pocket, respectively, in all images.

(B), GA is located in the S1 domain and forms hydrophobic interactions with Pro69, Phe74, Cys97, Phe112, Ala113 and Ala116. Figure 6(C) shows that MeSA is located in the S2 domain and formed an H-bond through Arg126 and hydrophobic interaction with Ala113, Ala116, Phe117, Leu123 and Val127. GA and MeSA only occupied the S1 or S2 domain, resulting in binding with fewer hydrophobic residues.

In Fig. 6(D), the **3e** is located in the 'S1 + S2' domain. Similar to MeSA and GA, the salicylic ring of **3e** is located in the S2 domain and the straight carbon chain occupies the S1 domain. It also formed a H-bond with Arg126 and hydrophobic interactions with Met62, Pro69, Pro71, Phe74, Tyr94 and Ala116. Therefore, the binding mechanism of molecule **3e** can be thought as a combination of MeSA and GA. In Fig. 6(E,F), due to C5 carbon chain in R_2, **4e** and **4i** did not form H-bonds with Arg126 inferring the reason why these two ligands showed the decreased binding affinity. However, the hydrophobic interaction increased due to the introduction of a carbon chain in R_2. Particular, **4i** had the largest binding surface and the most potent hydrophobic interaction with ApisOBP9 and kept a preferred conformation. The interactions in the model are suggestive of the good binding affinity of **4i** with ApisOBP9 evident in our experiment. However, the '3-OCH_3' of **4e** hindered the formation of preferred conformation, which speculated why the binding affinity of **4e** was much lower than that of **3e**.

In a previous publication, only the docking mode between **3e** and ApisOBP9 was studied.[33] In this work, more ligands including MeSA, GA, **4e** and **4i** were used to elucidate the binding mechanism between ligands and target ApisOBP9 through molecular docking, indicating hydrophobic interaction and H-bond were vital for the derivatives binding with ApisOBP9. This result was consistent with that observed in our previous docking study for ApisOBP9.[33,58] The Arg126 was key residue for H-bond receptor. Compound **3e** had both hydrophobic interactions and H-bonds with ApisOBP9 and **4i** had the most potent hydrophobic interactions suggesting why these two ligands showed the best binding affinity in our experiments.

3.4.2 *Molecular docking of ligands to HaxyOBP15*

To explore the mechanism between the ligands and HaxyOBP15, the modeled 3D structure of HaxyOBP15 was used for molecular docking.[38] Five ligands (MeSA, GA, **3e**, **4e**, and **4i**) were selected as representative compounds, owing to **4e** and **4i** displayed the best binding affinity with HaxyOBP15 and **3e** was lead compound for **4e** and **4i**.

The binding modes of the five ligands are shown in Fig. 7(A). Clearly, most of the binding surface in the binding pocket was hydrophobic (brown area in Fig. 7(A)). As shown in Fig. 7(B,C), GA and MeSA are located in the right domain of the pocket. The binding space of the two ligands was insufficient, which led to low hydrophobic interactions and van der Waals interaction deducing why these two ligands exhibited so weak binding affinity to HaxyOBP15 in our experiments.

Figure 7(D) shows that **3e** occupied a significantly larger steric space in the binding pocket of HaxyOBP15 than MeSA and GA. This means there are higher hydrophobic interaction and van der Waals interaction between **3e** and HaxyOBP15. The interactions in the model are inferential of the higher binding affinity of **3e** than MeSA and GA in our experiments.

After introducing a C5 straight chain with an ester group into **3e** to obtain **4e**, the binding conformation of **4e** was reversed and it occupied larger steric space than **3e**. The aromatic ring of **4e** interacted with His53 and Phe65 by π-π interaction. At the same time, due to the larger steric space, **4e** had more potent hydrophobic

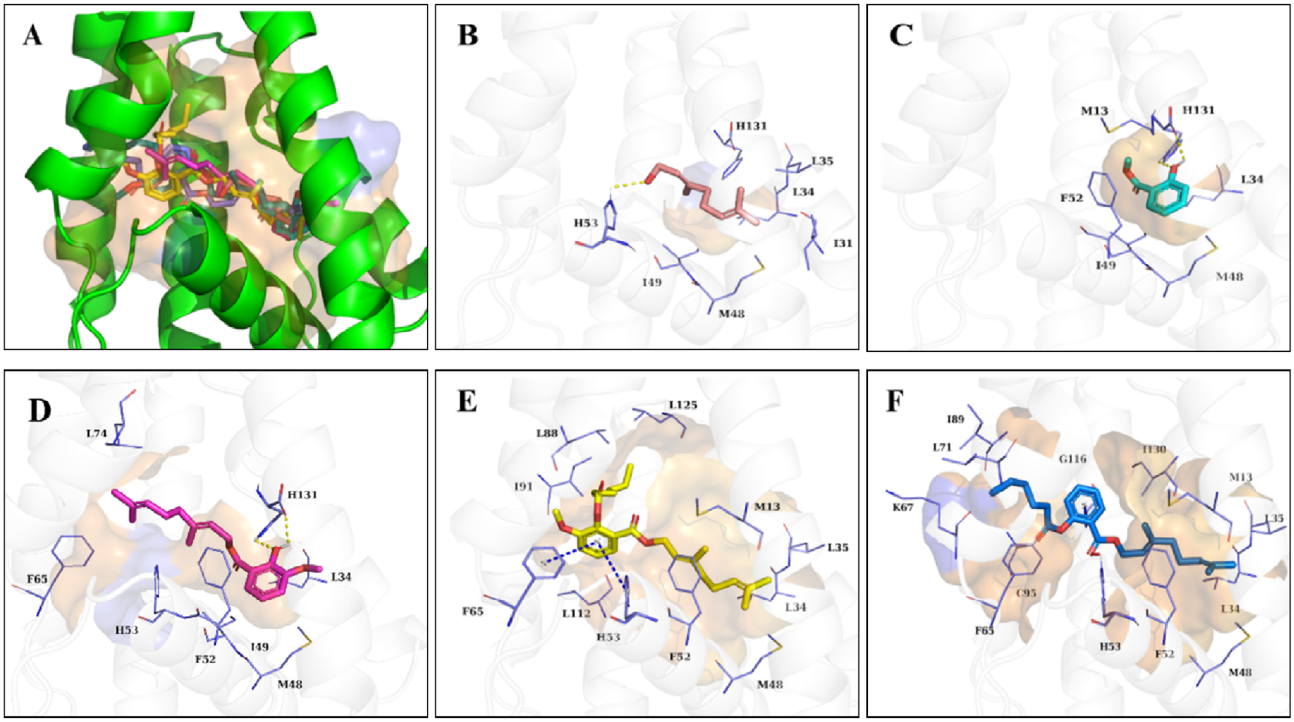


Figure 7. Molecular docking of representative ligands with HaxyOBP15 (superimposed conformations of the molecular docking ligands (A) and the docking conformation of GA (B), MeSA (C), **3e** (D), **4e** (E) and **4i** (F), respectively). Brown and blue surface represents the hydrophobic and hydrophilicity area of docking pocket, respectively, in all images.

interaction with more amino acids, including Met13, Leu34, Leu35, Met48, Phe52, Leu88, Ile91, Leu112 and Leu125. This conjectured the reason why **4e** exhibited a higher binding affinity than that of **3e**.

As shown in Fig. 7(F), after replacing 3-OCH_3 with H atom, **4i** had a preferred binding conformation, larger binding surface and higher steric space effect than **4e**. The C5 straight chain could interact with more residues if there was no 3-OCH_3 in the salicylic ring. This suggests that **4i** showed the best binding affinity with HaxyOBP15. In other words, if the 3-OCH_3 in the salicylic ring and C5 carbon chain at R_2 coexisted, it was unfavorable for the compound to bind with HaxyOBP15.

The steric space effect was significant for the derivatives binding with HaxyOBP15. Ligands with small steric space effects means poor binding affinity, similar to MeSA and GA. On the contrary, the larger the steric space effect of the ligands is, the higher the binding affinity indicates, similar to **3e**, **4e** and **4i**.

In previous research, eight ligands (β-ionone, dihydro-β-ionone, α-ionone, EβF, geranyl acetate, linalyl acetate, nonyl aldehyde) have been used to conduct molecular docking studies to speculate the mode of action between ligand and target HaxyOBP15.[38] Compared with these eight natural products, the synthesized derivatives including **3e**, **4e** and **4i** bound with HaxyOBP15 occupied larger space in binding pocket. A H bonding interaction existed between the key residue His53 in HaxyOBP15 and two ligands geranyl acetate and linalyl acetate.[38] Similarly, a H bonding interaction also existed between the key residue His53 in HaxyOBP15 and two ligands **4i** and GA. It was clear that the His53 was speculated as a key amino residue for HaxyOBP15 to bind with **4i**.

Above all, compounds such as **4i** with potent hydrophobic interaction with ApisOBP9 and high steric space effect with HaxyOBP15 have dual-activity to both *Acyrthosiphon pisum* and *H. axyridis*, and can potentially be used as a push–pull tool for aphid control.

3.5 The toxicity of the derivatives toward mammals and honeybee

The predicted toxicity results (shown in Table S4) indicated that all the derivatives were inactive for carcinogenicity, had a low toxicity grade for oral rats and had a low level for honeybee toxicity.

The tested toxicity results (shown in Table S5) of **4i** exhibited that both oral ($P = 0.423$) and contact ($P = 0.725$) toxicities to honeybee *Apis mellifera* were not significant ($P > 0.05$) compared with the solvent control. Therefore, the new derivative **4i** can be regarded as a bee-friendly compound.

4 CONCLUSION

In summary, by introducing an active hydrophobic group (R_2-COO-) to replace the hydroxyl on the salicylic ring, a new series of MeSA derivatives were designed and synthesized through modifying the structure of **3e**, a lead compound containing MeSA and GA moiety. Bioassay indicated that lead compound **3e** had a similar attractive activity to male ladybugs as MeSA. Furthermore, the derivative **4i** not only displayed effective aphid-repellent activity against *Acyrthosiphon pisum*, but also exhibited strong attractive activity to aphid natural enemy, ladybug *H. axyridis*. Compound **4i** can be considered as a potential push–pull candidate for aphid control. The binding mechanism study indicated that the MeSA derivatives with a high aphid-repellent activity exhibited a high binding affinity to the aphid target ApisOBP9, and compounds with strong attractive activity had a strong binding affinity to ladybug target HaxyOBP15. Further molecular docking studies revealed that hydrophobic interaction and H-bonds

were vital for the derivatives binding with ApisOBP9, and the steric space was necessary for the derivatives binding with HaxyOBP15. This work provides valuable clues to discover novel push–pull candidates for aphid control in sustainable agriculture.

ACKNOWLEDGEMENTS

This study was supported by the National Natural Science Foundation of China (No. 31801739) and the National Key Research and Development Plan (No. 2017YFD0200504). The authors thank Prof. Li Xuesheng from Guangxi University for his kind help in the honeybee toxicity experiment.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Xinling Yang at https://orcid.org/0000-0002-0118-402X.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Karina W and Agnieszka BN, Invasive aphids of the tribe Siphini: a model of potentially suitable ecological niches. *Agric For Entomol* **16**:434–443 (2015).
2 Dedryver CA, Ralec AL and Fabre F, The conflicting relationships between aphids and men: a review of aphid damage and control strategies. *C R Biol* **333**:539–553 (2010).
3 Woodcock BA, Bullock JM, Shore RF, Heard MS, Pereira MG, Redhead J *et al*., Country-specific effects of neonicotinoid pesticides on honey bees and wild bees. *Science* **356**:1393–1395 (2017).
4 Yang Y, Ma SL, Liu F, Wang Q, Wang X, Hou CS *et al*., Acute and chronic toxicity of acetamiprid, carbaryl, cypermethrin and deltamethrin to *Apis mellifera* larvae reared in vitro. *Pest Manag Sci* **76**:978–985 (2019).
5 Butler D, Scientists hail European ban on bee-harming pesticides. *Nature* **557**:14 (2018). https://doi.org/10.1038/d41586-018-04987-4.
6 Erik S, European Union expands ban of three neonicotinoid pesticides. *Science* **360**:468–469 (2018). https://doi.org/10.1126/science.aau0152.
7 Zhang CL, Li XL, Song DL, Ling Y, Zhou YL and Yang XL, Synthesis, aphicidal activity and conformation of novel insect kinin analogues as potential eco-friendly insecticides. *Pest Manag Sci* **76**:3432–3439 (2020).
8 Deng XL, Zhao PY, Zhou XM and Bai LY, Excellent sustained-release efficacy of herbicide quinclorac with cationic covalent organic frameworks. *Chem Eng J* **405**:126979 (2021).
9 Lamy F, Dugravot S, Cortesero AM, Chaminade V, Faloya V and Poinsot D, One more step toward a push-pull strategy combining both a trap crop and plant volatile organic compounds against the cabbage root fly *Delia radicum*. *Environ Sci Pollut Res* **25**:29868–29879 (2018).
10 Pickett JA and Khan ZR, Plant volatile-mediated signalling and its application in agriculture: successes and challenges. *New Phytol* **212**:856–870 (2016).
11 Khan ZR, Midega CAO, Pittchar JO, Murage AW, Birkett MA and Bruce TJA, Achieving food security for one million sub-saharan african poor through push–pull innovation by 2020. *Philos Trans R Soc Lond B Biol Sci* **369**:20120284 (2014).
12 Cusumano A, Harvey JA, Bourne ME, Poelman EH and de Boer JG, Exploiting chemical ecology to manage hyperparasitoids in biological control of arthropod pests. *Pest Manag Sci* **76**:432–443 (2020).
13 Dembilio O and Jaques JA, Biology and Management of red palm weevil, in *Sustainability in Plant and Crop Protection*, ed. by Wakil W, Faleiro JR and Miller TA. Springer International Publishers, Switzerland, pp. 13–36 (2015).
14 Pickett JA, Woodcock CM, Midega CAO and Khan ZR, Push-pull farming systems. *Curr Opin Biotechnol* **26**:125–132 (2014).
15 Khan ZR, Midega CAO, Wadhams LJ, Pickett JA and Mumuni A, Evaluation of Napier grass (*Pennisetum purpureum*) varieties for use as trap plants for the management of African stemborer (*Busseola fusca*) in a push-pull strategy. *Entomol Exp Appl* **124**:201–211 (2007).
16 Turlings TCJ and Matthias E, Tritrophic interactions mediated by herbivore-induced plant volatiles: mechanisms, ecological relevance, and application potential. *Annu Rev Entomol* **63**:433–452 (2018).
17 Dudareva N, Negre F, Nagegowda DA and Irina O, Plant volatiles: recent advances and future perspectives. *Crit Rev Plant Sci* **25**:417–440 (2011).
18 Hardie J, Isaacs R, Pickett JA, Wadhams LJ and Woodcock CM, Methyl salicylate and (-) -(1R, 5 S)-myrtenal are plant-derived repellents for black bean aphid, *Aphis fabae Scop*. (Homoptera: Aphididae). *J Chem Ecol* **20**:2847–2855 (1994).
19 Pettersson J, Pickett JA, Pye BJ, Quiroz A, Smart LE and Wadhams LJ, Winter host component reduces colonization by bird-cherry-oat aphid, *Rhopalosiphum padi* (L.) (homoptera, aphididae), and other aphids in cereal fields. *J Chem Ecol* **20**:2565–2574 (1994).
20 Zhu J and Park KC, Methyl salicylate, a soybean aphid-induced plant volatile attractive to the predator *Coccinella septempunctata*. *J Chem Ecol* **31**:1733–1746 (2005).
21 Katerina VG, Man-Yeon C and Jana CL, Attracting *Chrysopidae* with plant volatiles for lace bug (*Hemiptera: Tingidae*) control in rhododendrons and azaleas. *J Insect Sci* **20**:1–10 (2020).
22 Zhao JH, Wang ZY, Li ZS, Shi JY, Meng L, Wang GR *et al*., Development of lady beetle attractants from floral volatiles and other semiochemicals for the biological control of aphids. *J Asia Pac Entomol* **23**:1023–1029 (2020).
23 Halbert SE, Corsini D, Wiebe M and Vaughn SF, Plant-derived compounds and extracts with potential as aphid repellents. *Ann Appl Biol* **154**:303–307 (2010).
24 Gupta R, Mallavarapu GR, Banerjee S and Kumar S, Characteristics of an isomenthone-rich somaclonal mutant isolated in a geraniol-rich rose-scented geranium accession of *Pelargonium graveolens*. *Flavour Fragrance J* **16**:319–324 (2001).
25 Gutierrez G, Fereres A, Reina M, Cabrera R and Gonzalez-Coloma A, Behavioral and sublethal effects of structurally related lower terpenes on *Myzus persicae*. *J Chem Ecol* **23**:1641–1650 (1997).
26 Pelosi P, Iovinella I, Zhu J, Wang GR and Dani FR, Beyond chemoreception: diverse tasks of soluble olfactory proteins in insects. *Biol Rev* **93**:184–200 (2018).
27 Loreto F and Auria SD, How do plants sense volatiles sent by other plants? *Trends Plant Sci* **27**:29–38 (2021).
28 Vandermoten S, Francis F, Haubruge E and Leal WS, Conserved odorant-binding proteins from aphids and eavesdropping predators. *PLoS One* **6**:e23608 (2011).
29 Zhong T, Yin J, Deng SS, Kebin L and Cao YZ, Fluorescence competition assay for the assessment of green leaf volatiles and trans-β-farnesene bound to three odorant-binding proteins in the wheat aphid *Sitobion avenae* (Fabricius). *J Insect Physiol* **58**:771–781 (2012).
30 Fan J, Xue WX, Duan HX, Jiang X, Zhang Y, Yu WJ *et al*., Identification of an intraspecific alarm pheromone and two conserved odorant-binding proteins associated with (E)-β-farnesene perception in aphid *Rhopalosiphum padi*. *J Insect Physiol* **101**:151–160 (2017).
31 Zhang RB, Wang B, Grossi G, Falabella P, Liu Y, Yan SC *et al*., Molecular basis of alarm pheromone detection in aphids. *Curr Biol* **27**:55–61 (2017).
32 Sun YF, Biasio FD, Qiao HL, Iovinella I, Yang SX, Ling Y *et al*., Two odorant-binding proteins mediate the behavioural response of aphids to the alarm pheromone (E)-β-farnesene and structural analogues. *PLoS One* **7**:e32759 (2012).
33 Qing YG, Yang ZK, Song DL, Wang Q, Gu SH, Li WH *et al*., Bioactivities of synthetic salicylate-substituted carboxyl (E)-β-Farnesene derivatives as ecofriendly agrochemicals and their binding mechanism with potential targets in aphid olfactory system. *Pest Manag Sci* **76**:2465–2472 (2020).
34 Ullah RMK, Quershi SR, Adeel MM, Abdelnabby H and Wang MQ, An odorant binding protein (SaveOBP9) involved in chemoreception of the wheat aphid *Sitobion avenae*. *Int J Mol Sci* **21**:8331–8349 (2020).
35 Chiara DO, Wolfgang K and Paolo P, Aphid odorant-binding protein 9 is narrowly tuned to linear alcohols and aldehydes of sixteen carbon atoms. *Insects* **12**:741–751 (2021).

36 Wang Q, Liu JT, Zhang YJ, Chen JL, Li XC, Liang P *et al*., Coordinative mediation of the response to alarm pheromones by three odorant binding proteins in the green peach aphid *Myzus persicae*. *Insect Biochem Mol Biol* **130**:103528 (2021).

37 Li ZQ, Zhang S, Cai XM, Luo JY, Dong SL, Cui JJ *et al*., Three odorant binding proteins may regulate the behavioural response of *Chrysopa pallens* to plant volatiles and the aphid alarm pheromone (E)-β-farnesene. *Insect Mol Biol* **26**:255–265 (2017).

38 Qu C, Yang ZK, Wang R, Li FQ, Yang XL and Luo C, Binding affinity characterization of four antennae-enriched odorant binding proteins from *Harmonia axyridis* (Coleoptera: Coccinellidae). *Front Physiol* **13**:829766 (2022).

39 Kandasamy R, London D, Stam L, Deyn W, Zhao XL, Salgado VL *et al*., Afidopyropen: new and potent modulator of insect transient receptor potential channels. *Insect Biochem Mol Biol* **84**:32–39 (2017).

40 Wood PM and Hollomon DW, A critical evaluation of the role of alternative oxidase in the performance of strobilurin and related fungicides acting at the Qo site of complex III. *Pest Manag Sci* **59**:499–511 (2003).

41 Natarajan K, Singh S, Burke TR, Grunberger D and Aggarwal BB, Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-KB. *Proc Natl Acad Sci U S A* **93**:9090–9095 (1996).

42 Xiu CL, Zhang W, Xu B, Wyckhuys KAG, Cai XM, Su HH *et al*., Volatiles from aphid-infested plants attract adults of the multicolored Asian lady beetle *Harmonia axyridis*. *Biol Control* **129**:1–11 (2019).

43 Goelen T, Vuts J, Sobhy IS, Wackers F, Caulfield JC, Birkett MA *et al*., Identification and application of bacterial volatiles to attract a generalist aphid parasitoid: from laboratory to greenhouse assays. *Pest Manag Sci* **77**:930–938 (2021).

44 Yang JY, Anishchenko I, Park H, Peng ZL, Ovchinnikov S and Baker D, Improved protein structure prediction using predicted interresidue orientations. *Proc Natl Acad Sci U S A* **117**:1496–1503 (2020).

45 Colovos C and Yeates TO, Verification of protein structures: patterns of nonbonded atomic interactions. *Protein Sci* **2**:1511–1519 (1993).

46 Du SQ, Yang ZK, Qin YG, Wang SS, Duan HX and Yang XL, Computational investigation of the molecular conformation-dependent binding mode of (E)-β-farnesene analogs with a heterocycle to aphid odorant-binding proteins. *J Mol Model* **24**:70 (2018).

47 Banerjee P, Eckert OA, Schrey AK and Preissner R, ProTox-II: a webserver for the prediction of toxicity of chemicals. *Nucleic Acids Res* **46**: W257–W263 (2018).

48 Cheng FX, Li WH, Zhou YD, Shen J, Wu ZR, Liu GX *et al*., AdmetSAR: a comprehensive source and free tool for evaluating chemical ADMET properties. *J Chem Inf Model* **52**:3099–3105 (2012).

49 Williams AJ, Grulke CM, Edwards J, McEachran AD, Mansouri K, Baker NC *et al*., The CompTox chemistry dashboard: a community data resource for environmental chemistry. *J Chem* **9**:61 (2017).

50 Zhou YL, Li XL, Zhang YM, Shi Y, Li HH, Zhang Z *et al*., A novel bee-friendly peptidomimetic insecticide: synthesis, aphicidal activity and 3D-QSAR study of insect kinin analogs at Phe^2 modification. *Pest Manag Sci* **78**:2952–2963 (2022).

51 Liu YY, Qi JM, Bai LS, Xu YL, Ma N and Sun FF, Graphite oxide-catalyzed acetylation of alcohols and phenols. *Chin Chem Lett* **5**:726–730 (2016).

52 Qu C, Wang R, Che WN, Li FQ, Zhao HP, Wei YY *et al*., Identification and tissue distribution of odorant binding protein genes in *Harmonia axyridis* (Coleoptera: Coccinellidae). *J Integr Agric* **20**:2204–2213 (2021).

53 Liu J, Zhao X, Zhan Y, Wang K, Francis F and Liu Y, New slow release mixture of (E)-β-farnesene with methyl salicylate to enhance aphid biocontrol efficacy in wheat ecosystem. *Pest Manag Sci* **77**:3341–3348 (2021).

54 Xu Q, Hatt S, Lopes T, Zhang Y, Bodson B, Chen J *et al*., A push-pull strategy to control aphids combines intercropping with semiochemical releases. *J Pest Sci* **91**:105 (2018).

55 Wang K, Liu J, Zhan Y and Liu Y, A new slow-release formulation of methyl salicylate optimizes the alternative control of *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae) in wheat fields. *Pest Manag Sci* **75**:676–682 (2019).

56 Francis F, Lognay G and Haubruge E, Olfactory responses to aphid and host plant volatile releases: (*E*)-β-farnesene an effective kairomone for the predator *Adalia bipunctata*. *J Chem Ecol* **30**:741–755 (2004).

57 Rodriguez-Saona C, Kaplan I, Braasch J, Chinnasamy D and Williams L, Field responses of predaceous arthropods to methyl salicylate: a meta-analysis and case study in cranberries. *Biol Control* **59**:294–303 (2011).

58 Qin YG, Yang ZK, Zhou JJ, Zhang SY, Pan SX, Liu Y *et al*., Effects of carboxyl and acylamino linkers in synthetic derivatives of aphid alarm pheromone (E)-β-farnesene on repellent, binding and aphicidal activity. *J Mol Struct* **1268**:133658 (2022).