Tyrosinase-mediated synthesis of larvicidal active 1,5-diphenylpent-4-en-1-one derivatives against *Culex quinquefasciatus* and investigation of their ichthyotoxicity

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1,5-diphenylpent-4-en-1-one derivatives were synthesised using the grindstone method with Cu(II)-tyrosinase used as a catalyst. This method showed a high yield under mild reaction conditions. The synthesised compounds were identified by FTIR, ¹H NMR, ¹³C NMR, mass spectrometry, and elemental analysis. In this study, a total of 17 compounds (1a–1q) were synthesised, and their larvicidal and antifeedant activities were evaluated. Compound 1i (1-(5-oxo-1,5-diphenylpent-1-en-3-yl)-3-(3-phenylallylidene)thiourea) was notably more active (LD₅₀: 28.5 µM) against *Culex quinquefasciatus* than permethrin(54.6 µM) and temephos(37.9 µM), whereas compound 1i at 100 µM caused 0% mortality in *Oreochromis mossambicus* within 24 h in an antifeedant screening, with ichthyotoxicity determined as the death ratio (%) at 24 h. Compounds 1a, 1e, 1f, 1j, and 1k were found to be highly toxic, whereas 1i was not toxic in antifeedant screening. Compound 1i was found to possess a high larvicidal activity against *C. quinquefasciatus* and was non-toxic to non-target aquatic species. Molecular docking studies also supported the finding that 1i is a potent larvicide with higher binding energy than the control (~ 10.0 vs. ~ 7.6 kcal/mol) in the 3OGN protein. Lead molecules are important for their larvicidal properties and application as insecticides.

In the broadest sense, human beings are part of nature; however, our activity is often understood and interpreted as a category that is unique and separate from the rest of the natural phenomena. It is both the legal and moral obligation of every human to protect planet Earth by undertaking activities that would prevent contamination of our planet and thereby protect it for future generations. For instance, as a scientist in chemical industries or academia, one could focus on protecting nature by employing green chemistry to produce various chemical and pharmaceutical active ingredients. Of the several green chemistry methodologies, the grindstone chemistry technique is a simple practice for the preparation of chemical compounds. Toda et al. developed a range of chemical reactions carried out by simply grinding or triturating the solids together. We will now focus on Mannich reactions, which are a widely studied type of reaction in the organic and medicinal chemistry domains.

Mushroom tyrosinase, which has a dinuclear copper active centre, catalyses the hydroxylation and subsequent oxidation reactions that convert phenol to the related ortho-quinone as well as the oxidation of catechol to quinone. Tyrosinase, alongside catechol oxidase and hemocyanin, belongs to the type 3 copper protein class. The dicopper core of this type-3 copper protein takes three redox forms. The active core of the deoxy type [Cu(I)–Cu(I)] contains two cuprous ions, which attach dioxygen to produce the oxy form. Dioxygen bonds as a peroxide ion in the oxy form in the µ-ŋ2:ŋ2 side-on bridging mode [Cu(II)–O 2–Cu(II)]. The met type [Cu(II)–Cu(II)] signifies a condition wherein copper atoms only at the active site have been oxidised but have not...
not been bound by dioxygen. The met type of tyrosinase is an enzymatic form wherein two cupric ions are bridged by one or two tiny ligands, along with water molecules or hydroxide ions, while the enzyme is at rest and acting as a catalyst.

Mannich-type reactions face significant challenges in terms of reaction time, reaction conditions, toxicity, catalyst requirements, and separation and determination of the purity of final product(s). Other challenges include synthetic methodologies such as ultrasound or microwave irradiation, the use of Lewis acids or bases, and the use of solubilizing agents or surfactant-type catalysts. In addition, some of the known green trends in Mannich reactions consist of ball milling without solvents, using ionic liquid mediums, using ionic liquids reinforced with nanoparticles, or applying enzymes under bio-catalytic conditions. However, the present study focused on the grindstone green chemistry method in order to overcome the abovementioned challenges in the preparation of Mannich base derivatives.

Mosquitoes are an important transmission vector for several diseases, particularly malaria. These types of diseases have economic and social impacts worldwide. Among the mosquito species, Culex quinquefasciatus is particularly associated with various vector-spread diseases in several regions. Larvicides are insecticides designed to kill insects during their larval stage. Methoprene is an insect growth controller that prevents larvae from developing significantly beyond the pupa stage by interrupting their growth period. Methoprene is mildly toxic to a variety of crabs, shrimp, lobster, and crayfish and is extremely toxic to a variety of fish and aquatic herbivores; it tends to accumulate in fish tissues. Olfaction plays an important role in many species and is linked to host-seeking, replication, predator recognition, and food detection. Odorant-binding proteins (OBPs) aid signal transduction by transporting odorants to olfactory receptors. Some example, consider previous reports, the ligand (5R,6S)-6-acetoxy-5-hexadecanolide was bound to OBP of the C. quinquefasciatus mosquito (PDB ID: 3OGN), it is best model for selection 1,5-diphenyl pent-4-en-1-one targets and molecular docking in this study.

The control of mosquitos presents a substantial challenge, and currently inhibitors such as permethrin, organophosphates, fenthion, chlorpyrifos, temephos, diflubenzuron, and methoprene are used; Fig. 1 details the compositions of these commercial insecticides. However, the use of chemical insecticides pose bigger challenges and various potential environmental problems, such as the widespread development of resistance and disruption of natural biological control systems. These problems require overcoming new mosquito larvae inhibitors and improving green methodologies, which can be achieved through Mannich base condensation reactions.
Mannich base synthesis is one of the best tools for green synthesis, in this way preparation of target compound based on cinnamylacetophenone (1,5-diphenylpent-4-en-1-one) comparable to cinnamylphenone (1,3-diphenylprop-2-en-1-one or chalcone, Fig. 1), basically chalcone derivatives have mosquito larvicidal properties. Some publications have investigated the environmental study of chalcones and 1,5-diphenylpent-4-en-1-one (cinnamylacetophenone). In general, chemical insecticides are the main agents used to reduce populations of vector mosquitoes, even though their accessibility and use are limited by their toxicity to the environment and non-target organisms as well as the resistance of some mosquito species to them.

Chemically modified chalcones have been recently used to control insect populations; for instance, chalcone derivatives are toxic to Ae. aegypti first instar larvae and adults, and Aedes albopictus larvae. Some furan-chalcones are toxic to Culex quinquefasciatus larvae in the fourth stage of development. The current work was focused on the presence of alkenyl imine/β-amino ketones, particularly imines, which are frequently used in organic synthesis because of their high reactivity and the synthetic utility of the ensuing products. Furthermore, β-amino ketones and their analogues have shown effective medicinal properties as organocatalysts for enantioselective carbon–carbon bond-forming reactions, thus resulting in a product yield of 81%; an 82% yield was obtained in this study. There is no enzymatic catalysis was involved in the synthesis of compounds 1a–1q, in the literature. In the present study, copper containing the Cu(II)-tyrosinase enzyme was used as a catalyst for producing compounds 1a–1q via grindstone green chemistry methodology that can be used to inhibit the second instar Culex mosquito larvae as a bio-indicator of aquatic pollution.

**Results and discussion**

**Chemistry.** A one-pot multicomponent synthesis of the title compounds was achieved using the grindstone green chemistry method. A mixture of acetophenone, cinnamaldehyde, substituted amine, and a catalytic amount of Cu(II)-tyrosinase enzyme was ground together in a pestle mortar. This was then followed by purification via column chromatography, in order to obtain the title compounds (1a–1q). The synthetic route outline is shown in Scheme 1. The chemical structures of synthesized compounds (1a–1q) were represented in Fig. 2. The active site in hydrolases is often thought to be responsible for promiscuous catalysis. We suggest a mechanism for the Cu(II)-tyrosinase-catalysed Mannich reaction, outlined in Scheme 2, by combining this perspective with our findings, as mentioned above. First, the aldehyde and amine can easily react to form the Schiff base, and the ketone is simultaneously pre-activated by Cu(II)-tyrosinase to produce the enolate anion. Second, with the aid of the His residue of Cu(II)-tyrosinase, the Schiff base may form an intermediate complex. The Mannich adduct is then freed from the oxynion hole after a proton is moved from the Schiff base to the enolate anion to create a new carbon–carbon bond. The core steps in this enzymatic mechanism are the formation of the enolate anion and the intermediate complex. Copper-containing materials such as coppertriflate, copperacetate, copperbromide, and copper nanoparticles play a vital role in Mannich base reactions. The one-pot multicomponent Mannich reaction was catalysed via various enzymes, such as trypsin, lipase, and protease. In the present study, copper containing the Cu(II)-tyrosinase enzyme was used as a catalyst for the synthesis of N-Mannich base (1a–1q) derivatives.

Some of the previously reported compounds, such as compound 1l, were reported by β-acetamido ketones from cinnamaldehyde to react with acetophenone at room temperature, with L-proline used as a catalyst, to result in a yield of 75%. Another method was reported previously where N-substituted β-amino ketone derivatives had been produced by a one-pot multi-component process using copper(II)-phthalocyanine as a catalyst to result in an yield of 51%, which is comparable to the compound produced in the present work, which showed an 84% yield. Compound 1m was also reported previously; an imine derived from an α,β-unsaturated aldehyde was also related to the present high binaphthol-derived monophosphoric acids as organocatalysts for enantioselective carbon–carbon bond-forming reactions, thus resulting in a product yield of 81%; an 82% yield was obtained in this study. There is no enzymatic catalysis was involved in the synthesis of compounds 1l and 1m in the literature. In our study we utilized Cu(II)-tyrosinase as a catalyst for producing compounds 1l and 1m and also the compounds acquired with high yields comparing previous literatures.

The compound 1a was synthesised using the catalysts trypsin, lipase, protease, CuCl2.2H2O, and Cu(II)-tyrosinase with yields of 64%, 72%, 68%, 84%, and 92%, respectively. The use of the Cu(II)-tyrosinase enzyme green catalyst, instead of CuCl2.2H2O, increased the yield of the Mannich derivatives to 92% and reduced the reaction time. The optimisation of the reaction conditions and catalysts is presented in Table 1. The obtained compounds were analysed via FT-IR, 1H, and 13C NMR spectroscopy. The key assignments of the compounds showed significant bands at 3170.23–3176.54, 2595.45–2599.98, and 1710.68–1716.70 cm⁻¹ in the IR spectrum, confirming to the –NH, –C=N, and –C=O groups, respectively. The 1H NMR showed signals at 8.03–9.70, 3.82–4.81 and 2.40–2.98 ppm, indicating –NH, 4-CH, and –CH₂ protons, respectively. The 13C NMR showed
peaks at δ 197.4–197.6, 48.4–59.2, and 48.0–50.6 ppm, which conforms to –C=O, –CH, and –CH₂ atoms, respectively. Mass spectra and elemental analysis were used to determine the conformation of all these compounds.

“...In general, E-alkenyl imines are organized from the corresponding E-alkenyl aldehydes through imine precursors. In this reaction, the carbon–carbon bond formation rate allows the isomerisation of the in situ generated E-alkenyl imine from E-alkenyl aldehydes with secondary amine and acetophenone, in the presence of 5 mol% of Cu(II)-tyrosinase catalysis to afford the corresponding Mannich adducts (1a-1q) in moderate to good yields with high E-selectivity."

NOE NMR data (see Supplementary Material) clearly confirmed the stereochemistry of the E isomers of compounds 1a, and 1i. Thus, based on this study the spectroscopic characteristic downfield shift is observed for this pent-4-en-1-one proton in the E-isomer than in the Z-isomer.

Catalyst recovery studies. The recovered catalyst was recycled for at least 10 run times with a small defeat in catalytic action (Fig. 3). The decrease in catalytic action perceived through the reinforced catalyst on recycling might be owing to limited loss of basic locates or loss of catalyst surface area during regeneration/reaction. The values are displayed in Table 2.

Biological activity. A total of 17 compounds (1a–1q) were tested against second instar C. quinquefasciatus larvae, and the toxicity of the title compounds was assessed in the marine fish Oreochromis mossambicus. Toxicity was defined as the ratio of deaths (%) at 24 h. Structure–activity relationships showed that the final compounds contained 1,5-diphenylpent-4-en-1-one with different types of amines, thus exerting larvicidal and toxic effects based on the formation of the specific chemical composition.

Compound 1i showed a higher larvicidal activity than other compounds, with an LD₅₀ of 28.5 µM, which was better than that of the controls temephos (LD₅₀ of 37.9 µM) and permethrin (LD₅₀ of 54.6 µM). The antifeedant induced 0% mortality even at LD₅₀ > 100 µM, which was represented by no toxicity in water.

Compound 1a induced 80% mortality at 100 µM and its LD₅₀ value was 223.0 µM, whereas the antifeedant induced 100% mortality at 100 µM and had a LD₅₀ value of 49.5 µM. This suggests that the presence of the hydrazine group may be the reason for the observed antifeedant-induced 100% mortality, as evident from toxicity against O. mossambicus fingerlings within 15 min of screening.

Compounds 1f and 1j induced a mortality rate of 80% with LD₅₀ values of 177.4 µM and 154.9 µM, respectively, in larvicidal screening whereas they induced 100% mortality in antifeedant screening. This suggests that the presence of aniline and naphthalen-2-amine groups may be the reason for the observed biological effects, respectively.
Compounds \textbf{1m} and \textbf{1n} induced a mortality rate of 80\% with LD$_{50}$ values of 159.8 µM and 190.9 µM, respectively, in larvicidal screening whereas they induced 0\% mortality in antifeedant screening. This suggests that the presence of the benzamide and urea groups could be the reason for the respective observed biological effects.
Compounds 1d and 1o induced 0% mortality at 100 µM in both the larvicidal and antifeedant screening. This suggests that the presence of the 5-hydrazonopentanal and 1-benzylideneurea groups may be the reason for the observed biological effect as they exhibited no active or toxic behaviour.

The above analysis therefore indicates that compound 1i was significantly active in larvicidal screening and displayed low toxicity in antifeedant screening. The percentages of mortality and LD50 values are presented in Tables 3 and 4.

*Culex quinquefasciatus* larval growth regulation. To explore the impact of 1,5-diphenylpent-4-en-1-one formulations on *C. quinquefasciatus* larvae growth, metamorphosis, and production, we exposed the larvae to compound 1i for 72 h. Table 5 summarizes the effects of compound 1i impact on larval weight and growth inhibition. When subjected to 10 µM of compound 1i, the eclosion rate and time of the pupal and adult periods of administered *C. quinquefasciatus* is calculated, and the findings are seen in Table 6. Compound 1i had a growth-inhibition score of 41.36% and suppressed larval weight development. Furthermore, compound 1i had little effect on the duration of the adult and pupal periods, but it did result in a 55 percent eclosion rate. Compound 1i hindered the production and growth of *C. quinquefasciatus* larvae, according to these findings.

**Docking results.** The Autodock Vina program was used to assess the docking behavior between compounds 1i, permethrin and temephos with the mosquito odorant binding protein (PDB ID: 3OGN). Compound 1i displayed more binding affinity (~10.0 kcal/mol) than other compounds and permethrin (~9.7 kcal/mol) and temephos (~7.6 kcal/mol) with the mosquito odorant binding protein (PDB ID: 3OGN). Residues of the amino acids Leu19, Leu73, Leu76, His77, Ala78, Trp114, and Leu124 were tangled in hydrophobic connections. The interaction of compound 1i with mosquito odorant binding protein (PDB ID: 3OGN) is shown in Fig. 4. In the control permethrin, residues of the amino acids Leu15, Leu19, Phe59, Leu73, Leu76, His77, Leu80, Ala88, Met89, Gly92, His111, Trp114, Phe123, and Leu124 were tangled in hydrophobic connections.

The positive control permethrin connected in the mosquito odorant binding protein (PDB ID: 3OGN) protein is shown in Fig. 5. The control temephos displayed three hydrogen bond interactions with the receptor mosquito odorant binding protein (PDB ID: 3OGN). The amino acid residue Ser79 showed two hydrogen bonds with temephos, with the bond lengths of 3.32 and 2.26 Å, and the amino acid residue Ala88 showed one hydrogen bond with temephos, with the bond length of 3.25 Å. Residues of the amino acids Leu19, Ala62, Leu76, Met91, Trp114, and Tyr122 were involved in hydrophobic contacts with the receptor. The interaction of the control temephos with the mosquito odorant binding protein (PDB ID: 3OGN) protein is shown in Fig. 6. The helix representation of inhibitor molecule docked into the receptor was shown in Figs. 4a, 5a, and 6a. The inhibitor...
Table 3. Larvicidal activity of compounds (1a–1q). Larvicidal activity model is used for the activity assays (second instar *C. quinquefasciatus*), one-day-old larvae were considered as 2nd instar. a Values are mean ± SD (n = 3). LD$_{50}$: the LD$_{50}$ is one way to measure the short-term poisoning potential (acute toxicity) of a material.

| Compounds | % of Mortality at 25 µM | % of Mortality at 50 µM | % of Mortality at 100 µM | LD$_{50}$ (µM)$^a$ |
|-----------|------------------------|------------------------|--------------------------|------------------|
| 1a        | 24.1 ± 0.2             | 43.2 ± 0.1             | 80.2 ± 0.2               | 223.0 ± 0.0      |
| 1b        | 11.2 ± 0.2             | 27.1 ± 0.2             | 40.1 ± 0.1               | 282.1 ± 0.0      |
| 1c        | 19.1 ± 0.4             | 26.3 ± 0.4             | 40.2 ± 0.6               | 262.8 ± 0.0      |
| 1d        | 0 ± 0.0                | 0 ± 0.0                | 0 ± 0.0                  | 286.9 ± 0.0      |
| 1e        | 33.3 ± 0.1             | 48.3 ± 0.2             | 60.4 ± 0.2               | 193.3 ± 0.3      |
| 1f        | 25.0 ± 0.2             | 44.1 ± 0.2             | 80.0 ± 0.2               | 177.4 ± 0.2      |
| 1g        | 22.1 ± 0.2             | 34.2 ± 0.2             | 40.1 ± 0.3               | 322.1 ± 0.0      |
| 1h        | 34.5 ± 0.2             | 47.9 ± 0.3             | 60.1 ± 0.2               | 165.1 ± 0.2      |
| 1i        | 68.2 ± 0.4             | 88.2 ± 0.6             | 100 ± 0.0                | 285 ± 0.2        |
| 1j        | 26.1 ± 0.2             | 44.5 ± 0.2             | 80.4 ± 0.3               | 154.9 ± 0.2      |
| 1k        | 0 ± 0.0                | 0 ± 0.0                | 0 ± 0.0                  | 292.8 ± 0.0      |
| 1l        | 20.8 ± 0.1             | 20.8 ± 0.1             | 20.8 ± 0.1               | 340.8 ± 0.0      |
| 1m        | 29.9 ± 0.3             | 42.3 ± 0.3             | 80.9 ± 0.3               | 159.8 ± 0.2      |
| 1n        | 29.9 ± 0.2             | 43.6 ± 0.2             | 81.0 ± 0.2               | 190.9 ± 0.0      |
| 1o        | 0 ± 0.0                | 0 ± 0.0                | 0 ± 0.0                  | 261 ± 0.0        |
| 1p        | 34.5 ± 0.2             | 47.9 ± 0.3             | 60.1 ± 0.2               | 165.1 ± 0.2      |
| 1q        | 68.2 ± 0.4             | 88.2 ± 0.6             | 100 ± 0.0                | 285 ± 0.2        |

Table 4. Antifeedant activity of compounds (1a–1q). Antifeedant activity for the toxicity measurement against marine fish *Oreochromis*. a Values are mean ± SD (n = 3). LD$_{50}$: the LD$_{50}$ is one way to measure the short-term poisoning potential (acute toxicity) of a material.

| Compounds | % of Mortality at 10 µM | % of Mortality at 25 µM | % of Mortality at 50 µM | % of Mortality at 100 µM | LD$_{50}$ (µM)$^a$ |
|-----------|------------------------|------------------------|--------------------------|--------------------------|------------------|
| 1a        | 33.3 ± 0.2             | 66.2 ± 0.0             | 88.2 ± 0.0               | 100 ± 0.0                | 49.5 ± 0.7       |
| 1b        | 20.2 ± 0.3             | 20.2 ± 0.3             | 20.2 ± 0.3               | 20.2 ± 0.3               | 282.1 ± 0.0      |
| 1c        | 0 ± 0.0                | 0 ± 0.0                | 0 ± 0.0                  | 0 ± 0.0                  | 262.8 ± 0.0      |
| 1d        | 0 ± 0.0                | 0 ± 0.0                | 0 ± 0.0                  | 0 ± 0.0                  | 286.9 ± 0.0      |
| 1e        | 31.3 ± 0.0             | 66.1 ± 0.0             | 82.2 ± 0.0               | 100 ± 0.0                | 47.8 ± 0.0       |
| 1f        | 41.2 ± 0.0             | 51.3 ± 0.0             | 72.2 ± 0.0               | 100 ± 0.0                | 64.4 ± 0.4       |
| 1g        | 5.2 ± 0.1              | 10.3 ± 0.1             | 20.6 ± 0.2               | 322.1 ± 0.0             |
| 1h        | 5.3 ± 0.1              | 20.2 ± 0.1             | 49.4 ± 0.1               | 60.4 ± 0.1              | 131.2 ± 0.8      |
| 1i        | 0 ± 0.0                | 0 ± 0.0                | 0 ± 0.0                  | 0 ± 0.0                  | 235.5 ± 0.0      |
| 1j        | 42.2 ± 0.4             | 59.2 ± 0.3             | 88.2 ± 0.0               | 100 ± 0.0                | 26.7 ± 0.2       |
| 1k        | 33.1 ± 0.0             | 67.1 ± 0.74            | 87.9 ± 0.0               | 100 ± 0.0                | 40.4 ± 0.6       |
| 1l        | 5.2 ± 0.1              | 10.3 ± 0.1             | 20.2 ± 0.1               | 340.8 ± 0.0             |
| 1m        | 0 ± 0.0                | 0 ± 0.0                | 0 ± 0.0                  | 0 ± 0.0                  | 281.3 ± 0.0      |
| 1n        | 0 ± 0.0                | 0 ± 0.0                | 0 ± 0.0                  | 0 ± 0.0                  | 339.7 ± 0.0      |
| 1o        | 0 ± 0.0                | 0 ± 0.0                | 0 ± 0.0                  | 0 ± 0.0                  | 261.4 ± 0.0      |
| 1p        | 0 ± 0.0                | 0 ± 0.0                | 0 ± 0.0                  | 0 ± 0.0                  | 244.8 ± 0.0      |
| 1q        | 5.2 ± 0.1              | 10.3 ± 0.1             | 20.2 ± 1.0               | 376.8 ± 0.0             |

Table 5. Compound 1i on the growth of *Culex quinquefasciatus*. a The concentration of 1i was 10 µM. b Control is not containing the compounds.

| Compound | Weight of larvae (mg) | Weight gain (mg) | Inhibition (%) |
|----------|-----------------------|------------------|----------------|
| 1i       | 100.3 ± 1.9           | 3.9 ± 0.9        | 41.4 ± 2.8     |
| Control  | 100.16 ± 0.3          | 6.6 ± 1.4        | –              |
molecule docked into the binding pocket of the receptor was shown in Figs. 4b, 5b, and 6b. The 3D representation of inhibitor molecule docked into the receptor was shown in Figs. 4c, 5c, and 6c. The 2D representation molecule docked with receptor was shown in Figs. 4d, 5d, and 6d. The results show that compound 1i possesses comparable inhibition abilities relative to the controls permethrin and temephos. The results are listed in Table 7.

**Table 6.** Analysis of progress of *Culex quinquefasciatus* growth. a The concentration of 1i was 10 µM. b Control is not containing the compounds.

| Compound | Duration of pupae (h) | Duration of adult (h) | Rate of eclosion (%) |
|----------|-----------------------|-----------------------|-----------------------|
| 1i       | 68.1 ± 0.64           | 23.1 ± 1.36           | 55 ± 1.7              |
| Control  | 65.5 ± 1.21           | 24.2 ± 0.82           | 80 ± 1.0              |

**Figure 4.** Molecular docking representation of ligand 1i within the active site of mosquito odorant binding protein (PDB ID: 3OGN). Chemical structures were drawn by ChemDraw Ultra 12.0 Suite (PerkinElmer, USA) and analyzed by the Discovery studio visualizer (BIOVIA Discovery studio 2019 Client).

molecule docked into the binding pocket of the receptor was shown in Figs. 4b, 5b, and 6b. The 3D representation of inhibitor molecule docked into the receptor was shown in Figs. 4c, 5c, and 6c. The 2D representation molecule docked with receptor was shown in Figs. 4d, 5d, and 6d. The results show that compound 1i possesses comparable inhibition abilities relative to the controls permethrin and temephos. The results are listed in Table 7.

**MD simulation analysis.** The protein–ligand complex structure of ligand 1i with 3OGN stability was carried out by Molecular Dynamics (MD) simulation method using Gromacs. Root Mean Square Deviation (RMSD) plot is an important to know the stability of the complex structure. From the analysis of values of RMSD plot, the values from 4.5 to 10 ns shows that the structure was stable because Cα backbone of protein was not fluctuated more (Fig. 7).
Root Mean Square Fluctuation (RMSF) is an important analysis to characterize the protein residues throughout the simulation time period. From the RMSF analysis, the protein residues other than C terminal were not fluctuated more, especially the residues which were interacted by the ligand Leu 73, Leu 76, His 77, Ala 88, Trp 114 and Leu 124 were within the range of 0.3 nm (Fig. 8).

The hydrogen bond interaction between the protein 3OGN and ligand 1i was formed during the period of simulation. 3 hydrogen bonds and pi–pi interaction were formed between the docked complex structures during different nano seconds of simulation system (Fig. 9).

The radius of gyration value of complex structure of protein 3OGN bounded with the ligand 1i shows that the ligand causes an alteration of the protein microenvironment. The radius started with 1.36 nm and it is decreased upto 1.33 nm at 6 ns and finally it is increased to 1.34 nm at the 10 ns (Fig. 10).

From this MD simulation analysis, the compound ligand 1i is stable with the respective of protein and it has good interaction with the important residues of protein. Hence, this compound may suggest to good inhibitor against the 3OGN protein.

Materials and methods
Chemistry. Thermo scientific Nicolet iS5 FTIR (4000–400 cm⁻¹) was used for analysis of all compounds. Bruker DRX-300 MHz, 75 MHz was used for the analysis of ¹H and ¹³C NMR spectra. An elemental analyzer (model Vario EL III) was used to analyze elements (C, H, N, and S) percentage (%). Mass spectra were recorded by Perkin Elmer GCMS model Clarus SQ8 (EI).
General procedure for the synthesis of compounds (1a–1q). A reaction mixture made up of cinnamaldehyde (0.01 mol, 1.32 mL), acetophenone (0.01 mol, 1.20 mL), substituted amine (0.01 mol) and Cu(II)-tyrosinase enzyme (0.5 g) was mixed in a mortar and ground at RT. Then 2 mL of 50 mM potassium phosphate buffer (pH 6.0) was added and filtered to recover the catalyst. The final filtered solid material was separated using column chromatography (Ethyl acetate4:hexane6). The same method was followed when mixing compounds 1b–1q.

3-Hydrazinyl-1,5-diphenylpent-4-en-1-one (1a). White solid; mp: 110–112 °C; Yield: 92%; Water solubility: 0.11 mM/mL; IR(KBr) ν: 3171.48, 3065.51, 3041.02, 1715.02, 1624.53 cm⁻¹; ¹H NMR (300 MHz): δ 9.20 (s, 1H), 8.84 (s, 2H, NH₂), 7.97–7.96 (dd, J = 7.33 Hz, J = 7.37 Hz, 2H, Ar-ring), 7.63–7.60 (d, J = 6.21 Hz, 1H, Ar-ring), 7.53–7.51 (dd, J = 7.30 Hz, J = 7.34 Hz, 2H, Ar-ring), 7.41–7.37 (dd, J = 7.33 Hz, J = 7.37 Hz, 2H, Ar-ring), 7.34 (d, J = 6.22 Hz, 1H, Ar-ring), 7.21 (dd, J = 7.30 Hz, J = 7.35 Hz, 2H, Ar-ring), 6.56–6.51 (d, J = 6.22 Hz, 1H, CH), 6.19–6.14 (d, J = 6.22 Hz, 1H), 3.84–3.80 (m, 1H), 2.94–2.91 (d, J = 6.21 Hz, 1H), 3.25 (d, J = 6.22 Hz, 1H, CH), 59.2 (1C), 48.0 (1C); EIMS (m/z): 267.15 (M⁺,18%); Anal. Calcd. for C₁₇H₁₈N₂O: C, 76.66; H, 6.81; N, 10.52%; found: C, 76.68; H, 6.80; N, 10.51%.

3-(2-Benzylidenehydrazinyl)-1,5-diphenylpent-4-en-1-one (1b). Greenish solid; mp: 145–148 °C; Yield: 86%; Water solubility: 0.06 mM/mL; IR(KBr) ν: 3176.51 (NH), 3072.50, 3032.32, 2596.43, 1716.08, 1623.43;
1H NMR (300 MHz): δ 9.21 (s, 1H), 8.36 (s, 1H, –CH), 7.97–9.94 (dd, \( J = 7.33 \) Hz, \( J = 7.37 \) Hz), 7.86–7.81 (dd, \( J = 7.33 \) Hz, \( J = 7.37 \) Hz), 7.63–7.60 (d, \( J = 6.21 \) Hz, 1H, Ph), 7.55–7.53 (dd, \( J = 7.31 \) Hz, \( J = 7.34 \) Hz), 7.50–7.47 (m, 3H, Ar ring), 7.24–7.21 (dd, \( J = 7.31 \) Hz, \( J = 7.35 \) Hz, 2H, Ar ring), 6.54–6.52 (d, \( J = 6.21 \) Hz, 2H, CH), 6.17–6.12 (d, \( J = 6.21 \) Hz, 2H, CH), 3.80–3.76 (m, 1H, CH), 2.95–2.92 (d, \( J = 6.21 \) Hz, 2H, CH2); 13C NMR (75 MHz): 197.6 (1C), 143.3 (1C), 136.6, 133.0, 128.7, 128.5 (6C, Ph ring), 136.5, 128.7, 128.6, 128.0 (6C, Ar ring), 134.4 (1C), 133.7, 131.0, 129.2, 128.8 (6C, Ph ring), 128.5 (1C), 55.1 (1C), 48.5 (1C); EIMS (m/z) 355.18 (M+, 26%); Anal. Calcd. for C24H22N2O: C, 81.33; H, 6.26; N, 7.90%; found: C, 81.31; H, 6.27; N, 7.91%.

1,5-Diphenyl-3-(2-(3-phenylallylidene)hydrazinyl)pent-4-en-1-one (1c). Light green powder; mp: 148–150 °C; Yield: 88%; Water solubility: 0.14 mM/mL; IR (KBr) ν 3176.50, 3073.51, 3031.30, 2595.45, 1714.08, 1624.40 cm⁻¹; 1H NMR (300 MHz): δ 9.26 (s, 1H, NH), 7.95–7.91 (dd, \( J = 7.33 \) Hz, \( J = 7.37 \) Hz), 7.60–7.57 (dd, \( J = 6.21 \) Hz, 1H, Ph), 7.55–7.53 (dd, \( J = 7.31 \) Hz, \( J = 7.34 \) Hz, 2H, Ar ring), 7.34–7.31 (d, \( J = 6.21 \) Hz, 1H, Ar ring), 7.20–7.17 (dd, \( J = 7.31 \) Hz, \( J = 7.35 \) Hz, 2H, Ar ring), 6.58–6.54 (d, 1H, \( J = 6.21 \) Hz, CH), 6.18–6.14 (d, \( J = 6.21 \) Hz, 1H, CH), 3.80–3.76 (m, 1H, CH), 2.95–2.92 (d, \( J = 6.21 \) Hz, 2H, CH2); 13C NMR (75 MHz): 197.6 (1C), 143.3 (1C), 136.6, 133.0, 128.7, 128.5 (6C, Ph ring), 136.5, 128.7, 128.6, 128.0 (6C, Ar ring), 134.4 (1C), 133.7, 131.0, 129.2, 128.8 (6C, Ph ring), 128.5 (1C), 55.1 (1C), 48.5 (1C); EIMS (m/z) 355.18 (M+, 26%); Anal. Calcd. for C24H22N2O: C, 81.33; H, 6.26; N, 7.90%; found: C, 81.31; H, 6.27; N, 7.91%.

Table 7. Molecular docking interaction of compounds (1a–1q) and control Temephos, Permethrin.

| Compounds | Mosquito odorant-binding protein 3OGN | Binding affinity (kcal/mol) | No. of H-bonds | H-bonding residues |
|-----------|-----------------------------------|-----------------------------|----------------|-------------------|
| 1a        | −9.0                              | 2                           | 1              | His121, Phe123    |
| 1b        | −9.7                              | 0                           | –              | –                 |
| 1c        | −9.0                              | 0                           | –              | –                 |
| 1d        | −8.8                              | 0                           | –              | –                 |
| 1e        | −9.7                              | 1                           | Phe123         |
| 1f        | −9.6                              | 0                           | –              | –                 |
| 1g        | −8.3                              | 0                           | –              | –                 |
| 1h        | −9.3                              | 0                           | –              | –                 |
| 1i        | −10.0                             | 0                           | –              | –                 |
| 1j        | −9.8                              | 0                           | –              | –                 |
| 1k        | −9.8                              | 0                           | –              | –                 |
| 1l        | −8.9                              | 0                           | –              | –                 |
| 1m        | −9.8                              | 0                           | –              | –                 |
| 1n        | −8.8                              | 0                           | –              | –                 |
| 1o        | −9.5                              | 0                           | –              | –                 |
| 1p        | −9.2                              | 0                           | –              | –                 |
| 1q        | −8.3                              | 0                           | –              | –                 |
| Temephos  | −7.6                              | 3                           | Ser79, Ala88   |
| Permethrin| −9.7                              | 0                           | –              | –                 |

Figure 7. Graphical representation of Time vs. RMSD map for Protein after ligand fit to the protein during molecular dynamics simulation. XMGrace (Version 5.1.19) tool was used to prepare the graphs (Turner, Land-Margin Research, & Technology, 2005).
5-(2-(5-Oxo-1,5-diphenylpent-1-en-3-yl)hydrazono)pentanal (1d). White powder; mp: 126–129 °C; Yield: 85%; Water solubility: 0.08 mM/mL; IR(KBr) ν: 3176.54, 3073.50, 3031.28, 1714.10, 1624.38 cm⁻¹; ¹H NMR (300 MHz): δ 9.70(s, 1H, CH), 9.24(s, 1H), 7.97–7.94(dd, J = 7.33 Hz, J = 7.37 Hz, 2H), 7.60–7.57(dd, J = 6.21 Hz, 1H), 7.53–7.50(dd, J = 7.31 Hz, J = 7.33 Hz, 2H), 7.42–7.37(dd, J = 7.33 Hz, J = 7.37 Hz, 2H, Ar ring), 7.34–7.31(d, J = 6.21 Hz, 1H, Ar ring), 7.21(dd, J = 7.31 Hz, J = 7.35 Hz, 2H, Ar ring), 6.97(s, 1H, CH), 6.56–6.51(d, J = 6.21 Hz, 1H), 6.16–6.13(1H, d, J = 6.21 Hz, CH), 3.85–3.82(m, 2H, CH), 2.93–2.88 (d, J = 6.21 Hz, 2H), 2.42–2.36(m, 2H), 1.82–1.74(m, 2H), 1.53–1.49 (m, 2H); ¹³C NMR(75 MHz): 349.19(M⁺, 24%); Anal.Calcd. for C₂₆H₂₄N₂O: C, 82.07; H, 6.36; N, 7.36%; found: C, 82.05; H, 6.37; N, 7.37%.

1,5-Diphenyl-3-(2-phenylhydrazinyl)pent-4-en-1-one (1e). White powder; mp: 143–145 °C; Yield: 88%; Water solubility: 0.20 mM/mL; IR(KBr) ν: 3176.52, 3073.50, 3031.28, 1714.10, 1624.38 cm⁻¹; ¹H NMR(300 MHz): δ 9.22 (s, 1H), 9.16(s, 1H), 7.97(dd, J = 7.34 Hz, J = 7.38 Hz, 2H, Ph), 7.65(d, J = 6.21 Hz, 1H), 7.55–7.53(dd,
\[ J = 7.31 \text{ Hz}, J = 7.35 \text{ Hz}, 2H), 7.38-7.34(dd, J = 7.33 \text{ Hz}, J = 7.37 \text{ Hz}, 2H, Ar ring), 7.35-7.32(dd, J = 7.31 \text{ Hz}, J = 7.35 \text{ Hz}, Ph), 7.32-7.30(d, J = 6.21 \text{ Hz}, 1H, Ar-ring), 7.21-7.19(dd, J = 7.31 \text{ Hz}, J = 7.35 \text{ Hz}, 2H, Ar-ring), 7.02-6.98(dd, J = 7.31 \text{ Hz}, J = 7.35 \text{ Hz}, 2H, Ph), 6.88-6.86 (d, J = 6.21 \text{ Hz}, 1H, Ar-ring), 6.56-6.54(d, J = 6.22 \text{ Hz}, 1H), 6.17-6.15(d, J = 6.21 \text{ Hz}, 1H), 3.84-3.79(m, 1H, -CH), 2.95-2.92(d, J = 6.21 \text{ Hz}, 2H); 13C NMR (75 MHz): 197.4(1C), 136.7, 133.1, 128.8, 128.6, (6C, Ph ring), 136.4, 128.6, 128.5, 127.8 (6C, Ar ring), 151.0, 129.2, 122.8, 113.2 (6C, Ph ring), 134.2, 127.9, 56.6, 48.3; EIMS(m/z): 343.18 (M+, 25%); Anal. Calcd. for C23H22N2O: C, 80.67; H, 6.48; N, 8.18%; found: C, 80.65; H, 6.47; N, 8.19%.

1,5-Diphenyl-3-(phenylamino)pent-4-en-1-one (1f). Yellow powder; mp: 101–103 °C; Yield: 86%; Water solubility: 0.16 mM/mL; IR(KBr) \( \nu \): 3176.53, 3072.50, 3030.28, 1715.10, 1623.38; 1H NMR (300 MHz,): \( \delta \) 9.26(s, 1H, NH), 7.97–7.95(dd, \( J = 7.33 \text{ Hz}, J = 7.37 \text{ Hz}, 2H), 7.67–7.63(d, J = 6.21 \text{ Hz}, 1H), 7.53–7.51(dd, J = 7.31 \text{ Hz}, J = 7.35 \text{ Hz}, 2H, Ph), 7.32–7.30(d, J = 6.21 \text{ Hz}, 1H), Ar-ring), 6.56–6.54(d, J = 6.22 \text{ Hz}, 1H, CH), 6.19–6.17(d, J = 6.21 \text{ Hz}, 1H), 3.84–3.79(m, 1H, -CH), 2.90–2.87(d, J = 6.21 \text{ Hz}, 2H); 13C NMR(75 MHz,): 197.4(1C), 182.0(1C), 136.7, 133.1, 128.8, 128.6, (6C, Ph ring), 136.4, 128.6, 128.5, 127.9 (6C, Ar ring), 147.6, 129.5, 120.8, 119.7 (6C, Ph ring), 133.1, 127.7, 57.2, 50.5; EIMS(m/z): 311.12 (M+, 19%); Anal. Calcd. for C18H18N2OS: C, 69.65; H, 5.84; N, 9.02%; found: C, 69.68; H, 5.85; N, 9.06%.

1-benzylidene-3-(5-oxo-1,5-diphenylpent-1-en-3-yl)thiourea (1h). Brown powder; mp: 111–114 °C; Yield: 80%; Water solubility: 0.40 mM/mL; IR(KBr)v: 3175.53, 3072.45, 1623.53, 1510.45; 1H NMR (300 MHz,): \( \delta \) 9.22(1H, s, NH), 9.47(s, 1H, NH), 8.52(s, 2H, NH), 7.97-7.94(dd, J = 7.33 Hz, J = 7.37 Hz, 2H), 7.67–7.63(d, J = 6.21 Hz, 1H), 7.53–7.51(dd, J = 7.31 Hz, J = 7.35 Hz, 2H, Ph), 7.23–7.19(dd, J = 7.31 Hz, J = 7.35 Hz, 2H, Ph), 6.74–6.71(d, J = 6.21 Hz, 1H, Ar ring), 6.67–6.64 (d, J = 6.20 Hz, 1H, CH), 6.19–6.17(d, J = 6.21 Hz, 1H), 3.82–3.79(m, 1H, -CH), 2.98–2.96 (d, J = 6.20 Hz, 2H); 13C NMR(75 MHz,): 197.4(1C), 182.0(1C), 136.7, 133.1, 128.8, 128.6, (6C, Ph ring), 136.4, 128.6, 128.5, 127.9 (6C, Ar ring), 142.4, 128.2, 55.6, 50.6; EIMS(m/z): 311.12 (M+, 19%); Anal. Calcd. for C14H16N2OS: C, 69.65; H, 5.84; N, 9.02%; found: C, 69.68; H, 5.85; N, 9.06%.

Figure 10. Radius of gyration value of complex structure of protein 3OGN bounded with the compound 1i. XMGrace (Version 5.1.19) tool was used to prepare the graphs (Turner, Land-Margin Research, & Technology, 2005).
1-(5-Oxo-1,5-diphenylpent-1-en-3-yl)-3-(3-phenylallylidene)thiourea (1i). Light yellow powder; mp: 276-279 °C; Yield: 87%; Water solubility: 0.20 mM/mL; IR(KBr): v: 3174.23, 3065.30, 3037.68, 1711.70, 1622.38; 1H NMR (300 MHz): δ 9.26 (s, 1H, NH), 7.97–7.94 (dd, J = 7.33 Hz, J = 7.37 Hz, 2H, Ph); 7.88–7.84 (d, J = 6.21 Hz, 1H, Naphthyl), 7.83–7.81 (d, J = 6.21 Hz, 1H, Naphthyl), 7.77–7.74 (d, J = 6.21 Hz, 1H, Naphthyl), 7.49–7.45 (d, J = 6.21 Hz, 1H, Naphthyl), 7.45–7.41 (d, J = 6.21 Hz, 1H, Naphthyl), 7.50 - 7.48 (dd, J = 7.31 Hz, J = 7.35 Hz, 2H, Phenyl), 7.63–7.59 (d, J = 6.23 Hz, 1H, Ph), 7.53–7.50 (d, J = 6.21 Hz, 2H, Arring), 7.50–7.49 (d, J = 7.31 Hz, J = 7.35 Hz, 2H, Arring), 7.47–7.46 (dd, J = 7.33 Hz, J = 7.37 Hz, 2H, Arring), 7.35–7.33 (d, J = 6.21 Hz, 1H, Arring), 7.25–7.21 (dd, J = 7.31 Hz, J = 7.35 Hz, 2H, Arring), 6.56–6.54 (d, J = 6.21 Hz, 1H, CH), 6.19–6.17 (d, J = 6.21 Hz, 1H), 3.84–3.81 (m, 1H), 2.90–2.87 (d, J = 6.21 Hz, 2H), 1.34–1.31 (m, 3H). 13C NMR (75 MHz): 197.4(C1), 189.3(C1), 146.7(C1), 137.1(C1), 131.1(C1), 128.8(C1), 128.6(C6, Ph ring), 136.4(C1), 128.6(C1), 128.5(C1), 127.9(C6, Ar ring), 146.0(C1), 133.7(C1), 129.0(C1), 126.8(C1), 126.5(C1), 125.3(C1), 124.6(C1), 118.1(C1), 104.5(C10, Naphthyl ring), 134.4(C1), 128.1(C1), 57.2(C1), 50.5(C1), 3174.21, 3064.28, 3026.66, 1711.70, 1622.38; 1H NMR (300 MHz): δ 9.28(s, 1H, NH), 7.50(s, 1H, –CH), 7.97–7.96(dd, J = 7.35 Hz, J = 7.39 Hz, 2H, Ph), 7.64(d, J = 6.21 Hz, 1H, Naphthyl), 7.53–7.51 (d, J = 7.31 Hz, J = 7.35 Hz, 2H, Ar ring), 7.35–7.33 (d, J = 6.21 Hz, 1H, Arring), 7.25–7.21 (dd, J = 7.31 Hz, J = 7.35 Hz, 2H, Arring), 6.56–6.54 (d, J = 6.21 Hz, 1H, CH), 6.19–6.17 (d, J = 6.21 Hz, 1H), 3.84–3.81 (m, 1H), 2.90–2.87 (d, J = 6.21 Hz, 2H), 1.34–1.31 (m, 3H). 13C NMR (75 MHz): 197.4(C1), 189.3(C1), 146.7(C1), 137.1(C1), 131.1(C1), 128.8(C1), 128.6(C6, Ph ring), 136.4(C1), 128.6(C1), 128.5(C1), 127.9(C6, Ar ring), 146.0(C1), 133.7(C1), 129.0(C1), 126.8(C1), 126.5(C1), 125.3(C1), 124.6(C1), 118.1(C1), 104.5(C10, Naphthyl ring), 134.4(C1), 128.1(C1), 57.2(C1), 50.5(C1); EI-MS(m/z) 342.18(M⁺, 26%); Anal. Calcd. for C₁₉H₁₉NO₂: C, 84.42; H, 6.53; N, 4.77%; found: C, 84.40; H, 6.49; N, 4.72%.
$J = 7.35$ Hz, 2H), 7.52 (m, 2H, Ph), 7.40–7.37 (dd, $J = 7.35$ Hz, $J = 7.38$ Hz, 1H, Ar ring), 7.35–7.31 (d, $J = 6.21$ Hz, 2H, Ar ring), 7.27–7.23 (dd, $J = 7.31$ Hz, $J = 7.35$ Hz, 2H, Ar ring), 6.56–6.54 (d, $J = 6.21$ Hz, 1H), 6.19–6.16 (d, $J = 6.22$ Hz, 1H), 4.81–4.79 (m, 1H, 2H, d, $J = 6.21$ Hz, 2H); $^{13}$C NMR (75 MHz); 197.4(1C), 164.5(1C), 163.7(1C), 136.7, 133.1, 128.8, 128.6(6C, Ph ring), 136.4, 136.2, 128.5, 127.9 (6C, Ar ring), 133.7, 131.0, 129.2, 128.6(6C, Ph ring), 133.8, 132.7, 130.8, 49.9; EIMS (m/z): 383.17 (M+, 28%); Anal. Calcd. for C$_{25}$H$_{22}$N$_{2}$O$_{2}$: C, 78.51; H, 5.80; N, 7.32%; found: C, 78.50; H, 5.82; N, 7.31%.

1-(5-Oxido-1,5-diphenylpent-1-en-3-yl)-3-(3-phenylallylidene)urea (1p). White greenish powder; mp: 145–148 °C; Yield: 89%; Water solubility: 0.52 mM/mL; IR(KBr): v: 3175.23, 3070.30, 3032.68, 2998.98, 1716.70, 1627.38; H NMR (300 MHz): $\delta$ 8.04(s, 1H), 7.50(s, 1H), 7.96–7.93(dd, $J = 7.33$ Hz, $J = 7.37$ Hz, 2H, Ar ring), 7.60–7.54(dd, $J = 7.31$ Hz, $J = 7.35$ Hz, 2H, Ar ring), 7.64–7.59(d, $J = 6.21$ Hz, 1H, Ph), 7.54–7.51(dd, $J = 7.31$ Hz, $J = 7.35$ Hz, 2H), 7.42–7.39(dd, $J = 7.33$ Hz, $J = 7.37$ Hz, 4H, Ar ring), 7.32–7.28(d, $J = 6.21$ Hz, 2H, Ar ring), 7.28–7.25(dd, $J = 7.31$ Hz, $J = 7.35$ Hz, 2H, Ar ring), 7.24–7.21(d, $J = 6.21$ Hz, 1H, CH), 6.85–6.83 (d, $J = 6.21$ Hz, 1H, CH), 6.54–6.51 (d, $J = 6.21$ Hz, 1H, CH), 6.17–6.13 (d, $J = 6.21$ Hz, 1H, CH), 4.81–4.78 (m, 1H, 2H, d, $J = 6.21$ Hz, 2H); $^{13}$C NMR (75 MHz); 197.4(1C), 136.7, 133.1, 128.8, 128.6(6C, Ph ring), 136.4, 136.2, 128.5, 127.9(6C, Ar ring), 133.2, 132.4, 116.1, 20.6(6C, Ph ring), 134.1, 133.5, 128.5, 119.9, 50.8, 49.9; EI-MS: 409.19 (M+, 29%); Elemental analysis: Anal. Calcd. for C$_{18}$H$_{19}$NO: C, 81.47; H, 7.22; N, 5.28%; found: C, 81.40; H, 7.25; N, 5.32%.

Biological activities. Larvicidal activity. Larvicidal activity assessed to control the breed of mosquitoes at their larval stage by using chemical compounds as larvicides. Test compounds were deviated in various concentrations of 10, 25, 50 and 100 μM according to a method described previously[48]. Mortality caused by the compounds was assessed as ratios (%) of the numbers of dead vs. live larvae. The LD$_{50}$ values were calculated using probit analysis.

Antifeedant activity. Antifeedant activity was evaluated to study the effect of larvicides against non-target aquatic species. The antifeedant activity was screened via 10, 25, 50 and 100 μM concentrations of the tested samples and evaluated for marine fingerlings (O. mossambicus). Mortality caused by the compounds was assessed as ratios (%) of the numbers of dead vs. live fingerlings. Table 4 summarizes the results. The method followed was described previously[48].

Larval growth inhibition and regulation. The regulation and inhibition of larval growth in C. quinquefasciatus by compound 1i (10 μM) were analysed via the water-immersion method[48].

Molecular docking. Preparation of ligands. The ligand molecules (1a–1q) were drawn via Chemdraw 12.0 and energy was minimized by using the MM2 force field in Chem3Dpro software. The ligand molecules were then saved in Protein Data Bank (PDB) format and further used for molecular docking studies.

Preparation of receptor. The 3D crystal structure of mosquito odorant binding protein (PDB ID: 3OGN) was downloaded from Protein Data Bank. The water molecules and inbound co-crystallized ligands were removed from the receptor using the Discovery Studio 2019 program. The receptor was then used for molecular docking evaluation.

Identification of binding pocket. The binding pocket of the target protein was recognized by using inbound co-crystallized ligands via the Discovery Studio 2019 Program. Residues of the amino acids Tyr10, Leu15, Leu19, Leu23, Leu80, Met84, Ile87, Ala88, Met91, His111, Trp114, His121, and Phe123 were situated in the binding pocket. The search grid of the 3OGN protein was stable with the dimensions sizes x: 22, y: 20, and z: 22 with center_x: 18.681, y: 49.66, and z: 11.409, with a spacing of 1.0 Å[65]. The value of exhaustiveness was set to 8 and the interactions were visually examined using the Pymol and Discovery studio 2019 programs.

Molecular dynamics simulations. Gromacs 2020.1 version was used to carry out the Molecular dynamics simulation for docked complex structure of ligand 1i with protein 3OGN to understand the stability of the docked
complexes. Ligand topology was generated using PRODRG server and it is combined with protein topology for making complex topology, the system was generated using force field GROMOS 43a1, solvated using a single point charge (SPC) water model. The system was framed by cubic box with a distance of 2 nm from the box to the surface of the protein.

The necessary ions were further added in order to neutralize the systems. The docked complex energy was minimized by energy minimization process using steepest descent algorithm, for each simulation, 50,000 steps were used for energy minimization. The LINCS algorithm was used to constrain the bond lengths and the electrostatics computed by PME method. NVT and NPT ensembles were used to equilibrate the systems for each 100 ps. The V-rescale thermostat was used for equilibration with a reference temperature of 300 K. Finally, the production MD run was approved for 10 ns with a time-step of 2 fs. Docked complex structure coordinates were hoarded every 10 ps and used for further analysis. The result was analysed through the RMSD, RMSF, gyration, hydrogen bonds plots and Xmgrace software was used for plotting graphs.

**Statistical analysis.** The LD$_{50}$ values were calculated based on at least three independent assessments and the standard deviations (SD) were calculated using Microsoft Excel.

**Conclusions**

In this study, we identified the most effective and easily prepared active larvicalid Mannich base synthesis derivatives using the grindstone method using Cu(II)-tyrosinase as a catalyst, which is economical and leads to good coating and high yield. These compounds were investigated for their use as larvicides against Culex quinquefasciatus and for their toxicity against non-target aquatic species through ichthyotoxic activity. A total of 17 compounds were screened, and compound 1i was found to be the most active (LD$_{50}$ = 12.09 µM) against Culex quinquefasciatus compared to Permethrin (LD$_{50}$ = 54.6 µM). The compound 1i was highly active compared to Permethrin > 10 differences compared with standard permethrin and also compound 1i induced 0% mortality within 24 h against Oreochromis mossambicus in an antifeedant screening. Molecular docking was carried out with all compounds 1a–1q and the controls temephos and permethrin against the 3OGN protein, and the resulting docking score was the best for compound 1i. In conclusion, our results indicate that compound 1i is the most effective insecticide and that the compounds outlined in this paper may serve as a prospective foundation for emerging ecologically significant bioactive compounds as well as eco-friendly pesticides and biopharmaceuticals.

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Received: 27 February 2021; Accepted: 30 August 2021
Published online: 20 October 2021

**References**

1. Toda, E., Tanaka, K. & Sekikawa, A. Host–guest complex formation by a solid–solid reaction. *J. Chem. Soc. Chem. Commun.* 4, 279–280. https://doi.org/10.1039/C3870000279 (1987).
2. Mannich, C. Eine synthese von β-ketonasen. *Arch. Pharm.* 255, 261–276. https://doi.org/10.1002/arpd.19172550217 (1917).
3. Sanchez-Ferrer, A., Rodriguez-Lopez, J., Garcia-Canovas, F. & Garcia-Carmona, F. Tyrosinase: A comprehensive review of its mechanism. *Biochim. Biophys. Acta.* 1247, 1–11. https://doi.org/10.1016/0167-4838(94)00204-T (1995).
4. Solomon, E. I., Sundaram, U. M. & Machonkin, T. E. Multicopper oxidases and oxygenases. *Chem. Rev.* 96, 2563–2606. https://doi.org/10.1021/cr9500460 (1996).
5. Solomon, E. I., Chen, P. M., Metz, M., Lee, S. K. & Palmer, A. Oxygen binding, activation, and reduction to water by copper proteins. *Angew. Chem. Int. Ed. Engl.* 40, 4570–4590. https://doi.org/10.1002/1521-3773(20112117)40:24<4570::AID-ANIE4570>3.0.CO;2-9 (2001).
6. Rolff, M., Schottenhamel, J., Decker, H. & Tuczek, F. Copper-O$_2$ reactivity of tyrosinase models towards external monophenolic substrates: Molecular mechanism and comparison with the enzyme. *Chem. Soc. Rev.* 40, 4077–4088. https://doi.org/10.1039/C9CSR00202I (2011).
7. Quist, D. A., Diaz, D. E., Liu, J. J. & Karlin, K. D. Activation of dioxygen by copper metalloproteins and insights from model complexes. *J. Biol. Inorg. Chem.* 22, 253–288. https://doi.org/10.1007/s00775-016-1415-2 (2017).
8. Hamann, J. D., Herzugk, B., Jurgesleit, R. & Tuczek, F. Small-molecule models of tyrosinase: From ligand hydrolyzation to catalytic monoxygenation of external substrates. *Coord. Chem. Rev.* 334, 54–66. https://doi.org/10.1016/j.ccr.2016.07.009 (2017).
9. Gerdemann, C., Eicken, C. & Krebs, B. The crystal structure of catechol oxidase: New insight into the function of type-3 copper proteins. *Acc. Chem. Res.* 35, 183–191. https://doi.org/10.1021/ar990019a (2002).
10. van Holde, K. E. & Miller, K. I. Hemocyanins. *Adv. Protein Chem.* 47, 1–81. https://doi.org/10.1016/S0065-3233(08)60545-8 (1994).
11. Manabe, K. & Kobayashi, S. Mannich-type reactions of aldehydes, amines, and ketones in a colloidal dispersion system created by a Bronsted Acid-Surfactant-combined catalyst in water. *Org. Lett.* 1, 1965–1967. https://doi.org/10.1021/ol50022s (1999).
12. Jing-Bo, Y., Gang, P., Zhi-Jiang, J., Zi-Kun, H. & Wei-Ke, S. Mechan o-chemical oxidative Mannich reaction: evaluation of chemically and mechanical parameters for the mild and chemoselective coupling of N-tert-butyloxycarbonyl- (hydroxy)quinolines and ketones. *Eur. J. Org. Chem.* 22, 5340–5344. https://doi.org/10.1002/ejoc.201609987 (2016).
13. Khanna, G., Aggarwal, K. & Khurana, J. L. An efficient and conduent approach for the synthesis of novel 3,4-dihydro-2H-naphtho[2,3-e] [1,3 ] oxazine-5,10-dione derivatives by a three component reaction in ionic liquid. *RSC Adv.* 5, 46448–46454. https://doi.org/10.1039/C5RA06196E (2015).
14. Ghomi, J. S. & Zahedi, S. Novel ionic liquid supported on Fe$_3$O$_4$ nanoparticles and its application as a catalyst in Mannich reaction under ultrasonic irradiation. *Ultrason. Sonochem.* 34, 916–923. https://doi.org/10.1016/j.ultsonch.2016.08.003 (2017).
15. Ling-Ling, W., Yang, X., Da-Cheng, Y., Zhi, G. & Yan-Hong, H. Bio-catalytic asymmetric Mannich reaction of ketimines using wheat germ lipase. *Catal. Sci. Technol.* 6, 3963–3970. https://doi.org/10.1039/C5C801923K (2016).
16. Abdel-Fattah Mostafa, A. et al. Synthesis of novel benzoypryan- connected pyrimidine and pyrazole derivatives via a green method using Cu(II)-tyrosinase enzyme catalyst as potential larvicidal, antifeedant activities. *RSC Adv.* 9, 25533–25543. https://doi.org/10.1039/C9RA04496E (2019).
17. Georges, K., Jayaprakasam, B., Dalvoy, S. S. & Nair, M. G. Pest-managing activities of plant extracts and anthraquinones from Cassia nigricans from Burkina Faso. *Bioresour. Technol.* 99, 2037–2045. https://doi.org/10.1016/j.biortech.2007.02.049 (2008).
18. Govindarajan, M. Chemical composition and larvicidal activity of leaf essential oil from *Clausena anisata* (Willd.) Hook. f. ex Benth (Rutaceae) against three mosquito species. *Asian Pac. J. Trop. Med.* 3, 874–887. https://doi.org/10.1016/S1995-7645(10)60210-6 (2010).
58. Xue, Y. et al. Protease-catalysed direct asymmetric Mannich reaction in organic solvent. *Sci. Rep.* 2, 761. https://doi.org/10.1038/srep00761 (2012).

59. Kano, T., Sakamoto, R., Akakura, M. & Maruoka, K. Stereocontrolled synthesis of vicinal diamines by organocatalytic asymmetric mannnich reaction of N-protected aminoacetaldehyde: Formal synthesis of (−)-Agelastatin A. *J. Am. Chem. Soc.* 134, 7516–7520. https://doi.org/10.1021/ja301120z (2012).

60. Artega, F. A. et al. Direct catalytic asymmetric mannnich-type reaction of alkylamides. *Org. Lett.* 18, 2391–2394. https://doi.org/10.1021/acs.orglett.6b00879 (2016).

61. Kanazawa, A. M., Denis, J. N. & Greene, A. E. Highly stereocontrolled and efficient preparation of the protected, esterification-ready docetaxel (taxotere) side chain. *J. Org. Chem.* 59, 1238–1240. https://doi.org/10.1021/jo00085a004 (1994).

62. Chen, C. D. et al. Laboratory bioefficacy of nine commercial formulations of temephos against larvae of *Aedes aegypti* (L.), *Aedes albopictus* Skuse and *Culex quinquefasciatus* Say. *Trop. Biomed.* 26, 360–365 (2009).

63. Song, G. P. et al. Synthesis and larvicidal activity of novel thenoylhydrazide derivatives. *Sci. Rep.* 6, 22977–22990. https://doi.org/10.1038/srep22977 (2016).

64. Trott, O. & Olson, A. J. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multi-threading. *J. Comput. Chem.* 31, 455–461. https://doi.org/10.1002/jcc.21334 (2010).

65. Abreu, R. M. V., Froufe, H. J. C., Queiroz, M. R. P., Isabel, C. F. R. & Ferreira, I. C. F. R. Selective flexibility of side-chain residues improves VEGFR-2 docking score using AutoDock Vina. *Chem. Biol. Drug. Des.* 79, 530–534. https://doi.org/10.1111/j.1747-0285.2011.01313.x (2012).

**Acknowledgements**

This work was funded by Researchers Supporting Project number (RSP-2021/27), King Saud University, Riyadh, Saudi Arabia.

**Author contributions**

C.S. Synthesis of compounds and docking result analysis; D.A. Design the biological experiment; S.A. Methodology of biological activity analysis; G.R. Biological data analysis Molecular dynamics simulation studies; R.S. chemical data analysis; A.I. Investigation total work chemistry and Biology. All authors were contributing through writing—original draft.

**Competing interests**

The authors declare no competing interests.

**Additional information**

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1038/s41598-021-98281-5.

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