Research Article

The interaction and mechanism of monoterpenes with tyramine receptor (SoTyrR) of rice weevil (*Sitophilus oryzae*)

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Abstract
Rice weevils (*Sitophilus oryzae)* are pests that feed on grain products. One strategy employed in the safe pest management is the use of essential oils from plant materials as biopesticide. Monoterpene compounds, present in essential oils, are generally less acutely toxic than other conventional insecticides and are known to possess biopesticide activity against octopaminergic receptors (OAR). Tyramine receptor (TyrR) is a desired biopesticide target due to its absence in vertebrates and its role in insect's physiological and cellular response. In this study, the biochemical basis of monoterpenes and SoTyrR interactions were determined using in silico methods: ensemble docking, 3DQSAR analysis, and toxicity prediction. Ensemble docking results showed that the lead compounds has binding affinity of − 4.2 to − 6.8 kcal/mol. Four monoterpene compounds: terpinolene, carvacrol, carene, and pulegone were considered top hits based on their favorable binding affinity. Furthermore, hydrophobic interactions of monoterpenes with residues Asp114, Val404, Lys189, Leu190, Tyr196, Phe397, and Tyr401 stabilized the observed docking poses. Upon consolidation of docking and 3DQSAR results, we functionalized top hit ligands and showed significant increase in the average binding affinity of candidate compounds, ranging from − 4.7 to − 8.3 kcal/mol. A carene derivative exhibited the highest binding energy of − 8.3 kcal/mol with a calculated K_i of 0.547 µM which surpassed the known activators of OAR. The top hit modified ligands were also clear of toxicity risks as predicted by Osiris Property Explorer. This work could provide insights in the development of effective biopesticides for rice weevils that is less toxic than conventional pesticides.

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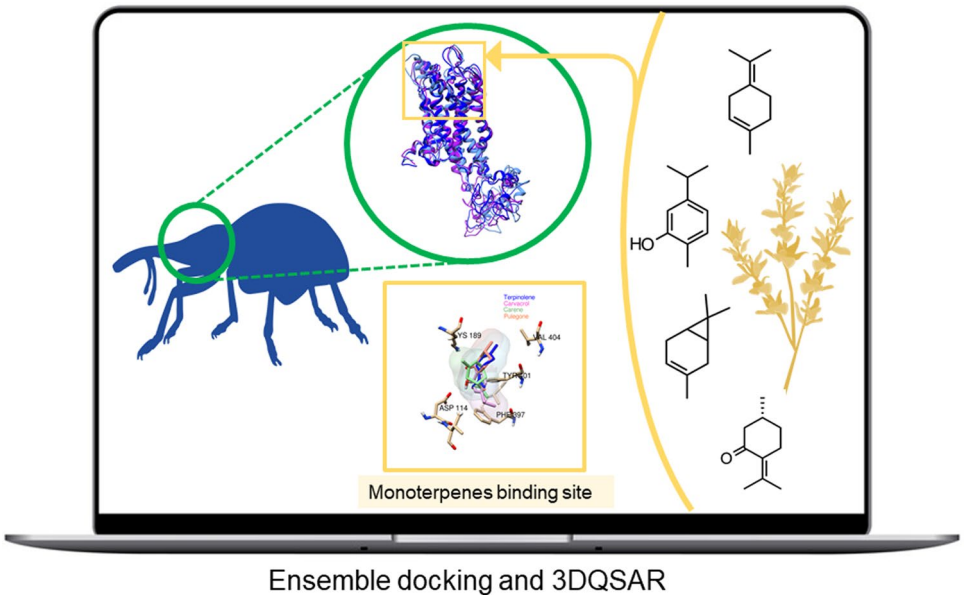
Graphic abstract



Keywords Tyramine receptors · Monoterpenes · Biopesticide · Rice weevil · Ensemble docking · and 3DQSAR

1 Introduction

Rice weevils (*Sitophilus oryzae*) are one of the most destructive pests of cereal products [1]. They cause significant losses especially at conditions that are favorable to their development [2]. They attack rice, grains, and other crops by feeding and multiplying in stored grain [3]. These creatures attack fields in vast number and destroyed enormous amount of crops in the past [4–7]. One strategy used as safe pest management is the use of essential oils from plant materials as biopesticide [8–10]. The neuromodulatory activity and neurotoxicity of monoterpene compounds were observed to be caused by its ability to interact to protein targets and selectively inhibit specific protein [e.g., acetylcholinesterase (AChE) and octopamine receptors (OAR)] [11]. Despite of their usability as pesticide, the basis of monoterpene interaction with insect receptors remain unknown.

Insect's octopaminergic receptors (OAR) are potential pesticide target of several essential oil products, including monoterpenes [12–16]. Octopamine is the invertebrate counterpart of norepinephrine in vertebrates that functions as neurotransmitter, neuromodulator, and neurohormone [17, 18]. This compound performs its function when it binds to the rhodopsin-like G protein-coupled receptor (GPCR) located in neuronal cellular membrane in insects [19–22]. GPCRs are known to be involved in extracellular signal sensing. These signals result in some physiological and cellular response which makes GPCRs an interesting target in drug discovery [23].

OARs are grouped into three, namely: (1) α-adrenergic-like octopamine receptors (OAR1); (2) β-adrenergic-like octopamine receptor (OAR2); and (3) tyraminergic receptors (TyrR) [24, 25]. Tyramine has been recently shown to be biologically active independent from octopamine [26, 27]. Tyramine receptor (TyrR) is associated with many important physiological functions in insect locomotion, reproduction, and pheromone response [28]. Also, the abundance of octopamine receptors in insects and its absence in vertebrates make it an interesting target in the development of bioactives for insects [26]. Recent work suggests that monoterpenes, are highly selective to insects because the primary target, octopamine receptor, is a non-mammalian target [29]. Monoterpenes are widely distributed in plant essential oils. These secondary metabolites are generally less toxic than the other natural and conventional insecticides [30, 31]. It is suggested that the repellent activity of several monoterpene compounds depends on the positioning of functional groups and the molecular configuration rather than the volatility and molecular size [32]. Enan and coworkers demonstrated that eugenol, α-terpineol, and several other monoterpenoids bound to OARs can be either antagonists or agonists based on the in vitro competitive binding assay [15]. In

another study by Gross et al. [33] several monoterpenoids were shown to significantly alter the growth rate of yeast cells by interacting with the α-adrenergic-like OAR from *Periplaneta americana*. Furthermore, a recent study demonstrated the positive allosteric modulation of monoterpenes against *Drosophila suzukii* type 1 tyramine receptor (DsTAR1) [34].

Moreover, the identification of key amino acid residues is important for the pesticide development. Virtual screening techniques have been successfully applied in drug discovery and has also been validated in agrochemical research [35, 36]. In the absence of crystal structures of protein receptors, computational methods are employed such as homology modeling, molecular docking, and 3D quantitative structure-activity relationships, 3DQSAR analysis to understand interactions between ligands and target receptors [37–39]. Here, the use of ensemble docking and 3DQSAR provided a general description for interactions between the *S. oryzae* tyramine receptor (SoTyrR) and monoterpenes. Furthermore, target monoterpene compounds were functionalized to improve their interaction while keeping their toxicity low. Understanding the pharmacological features of SoTyrR-monoterpene interaction can provide insight on receptor-specific biopesticides.

2 Methods

2.1 Ensemble docking and ligand functionalization

In this study, key amino acid residues for SoTyrR-monoterpene binding interaction were determined. Ensemble docking in AutoDock Vina was done to evaluate the molecular basis of monoterpene binding [40]. A total of 100 protein conformations (ensemble) of *S. oryzae* tyramine receptor (SoTyrR) were used as previously done [28]. In the absence of SoTyrR crystal structure, homology modeling of the target protein was generated in the online server I-TASSER [41, 42]. SoTyrR was embedded in POPC lipid bilayer and an all-atom MD simulations of SoTyrR were performed. Clustered SoTyrR structures from MD trajectories was analyzed using cpptraj [43]. The 3D structures of monoterpene lead compounds were obtained from the ZINC database online [44]. In AutoDock Tools, a grid box of $20 \times 20 \times 20$ points in x, y, and z-axis direction was used with a grid spacing of 1 Å while centering search box in the extracellular loops of the SoTyrR. Ligands' average binding energy were reported in kcal/mol. AutoDock Tools and Chimera were used to visualize docking results [45, 46]. Ligands were functionalized using MarvinSketch [47].

2.2 3DQSAR analysis and in silico toxicity evaluation

Both binding affinities and docking poses were used to assess 3DQSAR in Open3D Align [48] and in Open3DQSAR [49]. The van der Waals and electrostatic fields were calculated using the Merck force field [49]. Using the generated text files from the Open3DQSAR step, the calculated molecular fields were visualized using PyMOL [50]. Contour levels for van der Waals and electrostatic interactions isosurface were adjusted to 0.03 and 0.1, respectively. The Fractional Factorial Design (FFDSEL) were used to generate van der Waals contour plots. Then, plots generated from Uninformative Variable Elimination by Partial Least Squares (UVEPLS) were used to visualize the electrostatic interactions. The generated plots were used as guide for iterative ligand functionalization. The modified structures were redocked to SoTyrR. On the other hand, OSIRIS Property Explorer [51] was used to assess toxicity of monoterpene compounds. To assess the statistical significant difference between functionalized ligands and the top hit, paired-*t* test was employed. The *t* value was calculated using the formula: $t_{calculated} = \bar{d}/(s_d/\sqrt{n})$ where $\bar{d}$ is the mean difference between the binding energies of the functionalized ligand to the top hit, s_d is the standard error of differences, and $n = 100$ [52]. From the *t* value, GraphPad Software was used to compute for the *p* value [53]. The *p* value is the probability that the observed difference is random. A very low *p* value (usually below 0.10) means the difference is statistically significant [54, 55].

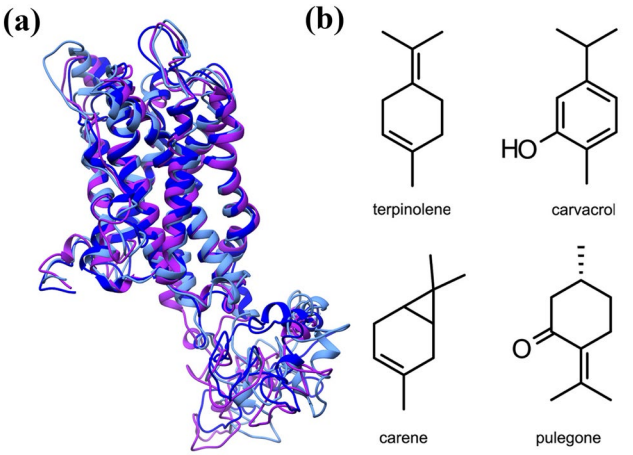


Fig. 1 **a** Molecular dynamics derived representative structures of *Sitophilus oryzae* tyramine receptor (SoTyrR); and **b** structures of top hit monoterpene ligands

Table 1 Binding energies of lead monoterpene compounds to target SoTyrR

Ligand	ZINC ID [44]	Binding energy (kcal/mol)
Nerol	12405252	− 5.31 ± 0.32
L-menthol	1482164	− 5.59 ± 0.35
Citral	1529208	− 5.38 ± 0.30
cis-geraniol	1529210	− 5.38 ± 0.29
Linalool	1529819	− 5.30 ± 0.29
Myrcene	1530331	− 5.18 ± 0.35
(+)-citronellal	1531600	− 5.22 ± 0.29
(−)-citronellal	1532245	− 5.18 ± 0.29
(+)-citronellol	1531601	− 5.23 ± 0.30
(−)-citronellol	1532246	− 5.20 ± 0.29
Ocimene	1531618	− 5.40 ± 0.33
(+)-sabinene	1599725	− 5.49 ± 0.32
(−)-sabinene	1599726	− 5.56 ± 0.32
Camphene	1673034	− 5.38 ± 0.36
(+)-α-thujene	2035755	− 5.52 ± 0.34
(−)-α-thujene	2035757	− 5.55 ± 0.34
(+)-3-bromocamphor	507383	− 5.50 ± 0.48
Pulegone	5735752	− 5.86 ± 0.39
D-limonene	967513	− 5.49 ± 0.32
(1R)-camphor	967520	− 5.35 ± 0.42
Carene	967562	− 5.68 ± 0.44
Carvacrol	967563	− 5.88 ± 0.33
Eucalyptol	967566	− 5.43 ± 0.36
(1S)-(−)-α-pinene	967580	− 5.58 ± 0.37
β-pinene	967582	− 5.51 ± 0.41
Thymol	967597	− 5.75 ± 0.31
(+)-3-carene	967794	− 5.67 ± 0.34
(+)-borneol	968100	− 5.21 ± 0.44
(−)-borneol	968099	− 5.27 ± 0.43
Terpinolene	968225	− 5.76 ± 0.37
Camphene	968230	− 5.40 ± 0.37
p-cymene	968246	− 5.64 ± 0.34

3 Results

3.1 Ensemble docking

Representative structures of SoTyrR and top hit ligand structures are reported in Fig. 1. Complete results from ensemble docking of monoterpenes are presented in Table 1 reported as the binding energy (kcal/mol) ± standard deviation (SD) for all 100 protein conformations. Also, the heat map showing the frequency of residues that are closely interacting with the ligands is shown in Fig. 2.

A close interacting residue is determined if the distance between atoms of SoTyrR residues and ligand is ≤ 8 Å. Most frequent interacting residues are defined as those residues that closely-interacted with ≥ 50 % of overall SoTyrR conformations. These residues are reported in Table 2. See SI for a complete list of closely interacting residues and representative protein-ligand diagrams done in Ligplot+ [56]. For instance, terpinolene is found to be interacting with residues found in the space enclosed by α-helices structures as displayed in Fig. 3. Top hits were found to share the same binding pocket as shown in Fig. 4. Highest binding affinity of the ligands were recorded from the proximate interactions with Asp114, Lys189, Phe397, Tyr401, and Val404. These residues are the most frequent closely interacting residues that are common to pulegone, carene, carvacrol, and terpinolene.

3.2 3DQSAR analysis

The docking poses from the ensemble docking were used to assess 3DQSAR of the top hits. Ligands initially chosen for 3DQSAR were the docking poses from all four top hits. This approach gave an R^2 of 0.0769 at PC = 5 and low values of q^2. Due to the low score correlation and unsatisfactory $q^2 (q^2 > 2)$, docking results from the top hits that exhibited the top 10 highest docking score were used instead. This approach gave a higher score correlation of $R^2 = 1$ at PC = 21 and acceptable q^2 values [57]. Figure 5 shows the 3DQSAR model generated.

3.3 Ligand modification

Ligands' 3DQSAR analysis is presented in Fig. 5. Using our findings from ensemble docking and 3DQSAR analysis, top hit monoterpenes were functionalized. Most of the top-hit ligands were modified by adding hydrophobic alkyl and hydroxyl groups at different chain lengths and positions. Figure 6 shows functionalized ligands. The subsequent ensemble docking results are summarized in Table 2.

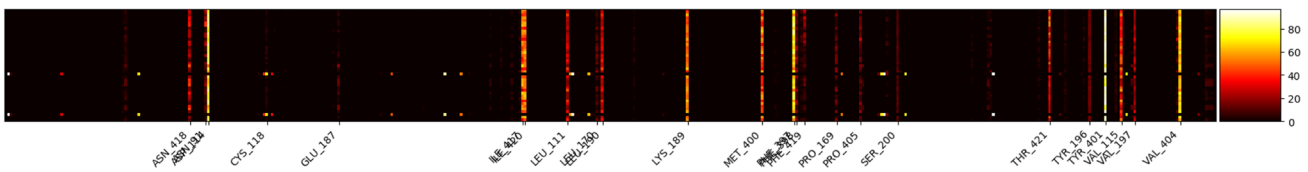


Fig. 2 Heat map showing the frequency of each SoTyrR amino acid residue closely interacting with the docked top-hits ligands. A contact is counted when the distance between ligand and receptor atoms is ≤ 8 Å

Table 2 SoTyrR close-interacting residues with the top hit ligands

Ligand	ZINC ID [44]	Close-interacting residues
Terpinolene	968225	Asp114, Lys189, Phe397, Tyr401, Val404
Carvacrol	967563	Asp114, Val115, Lys189, Phe397, Tyr401, Val404
Carene	967562	Asp114, Lys189, Phe397, Met400, Tyr401, Val404, Ile420
Pulegone	5735752	Asp114, Val115, Lys189, Phe397, Tyr401, Val404

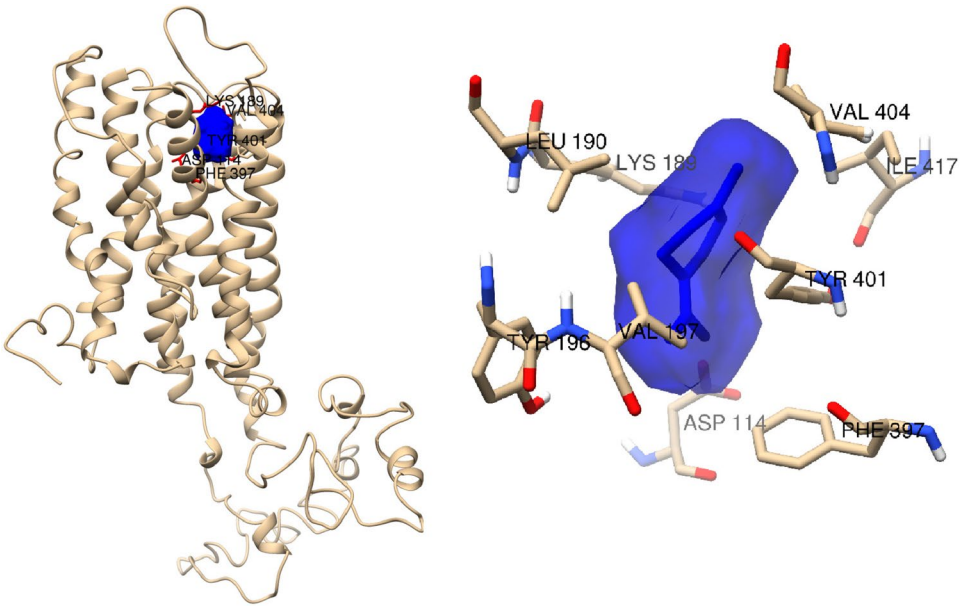


Fig. 3 Terpinolene binding site and SoTyrR close interacting residues

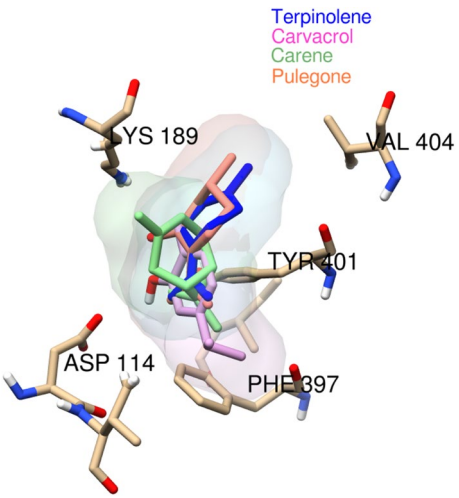


Fig. 4 Top hit ligands occupy similar pose in the SoTyrR binding pocket

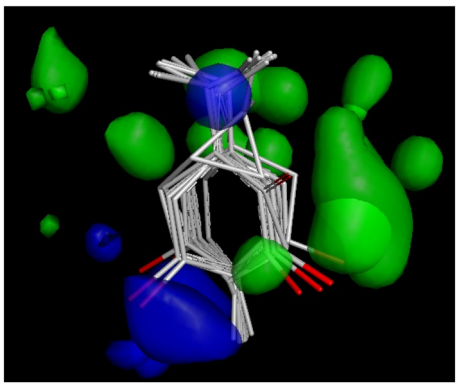


Fig. 5 3DQSAR model for the top 10 of the top hits (R^2 = 1.00). Green: areas where steric groups should be added; yellow: areas to avoid adding steric groups; blue: sites for positively charged functional groups, red: areas for negatively charged functional groups

Results from ensemble docking of the functionalized ligands are shown in Table 3. Docking scores of each ligand are reported as the average docking score ± SD for all 100 protein conformations. Modified compounds were also subjected to toxicity predictions using Osiris Property Explorer as summarized in Table 4.

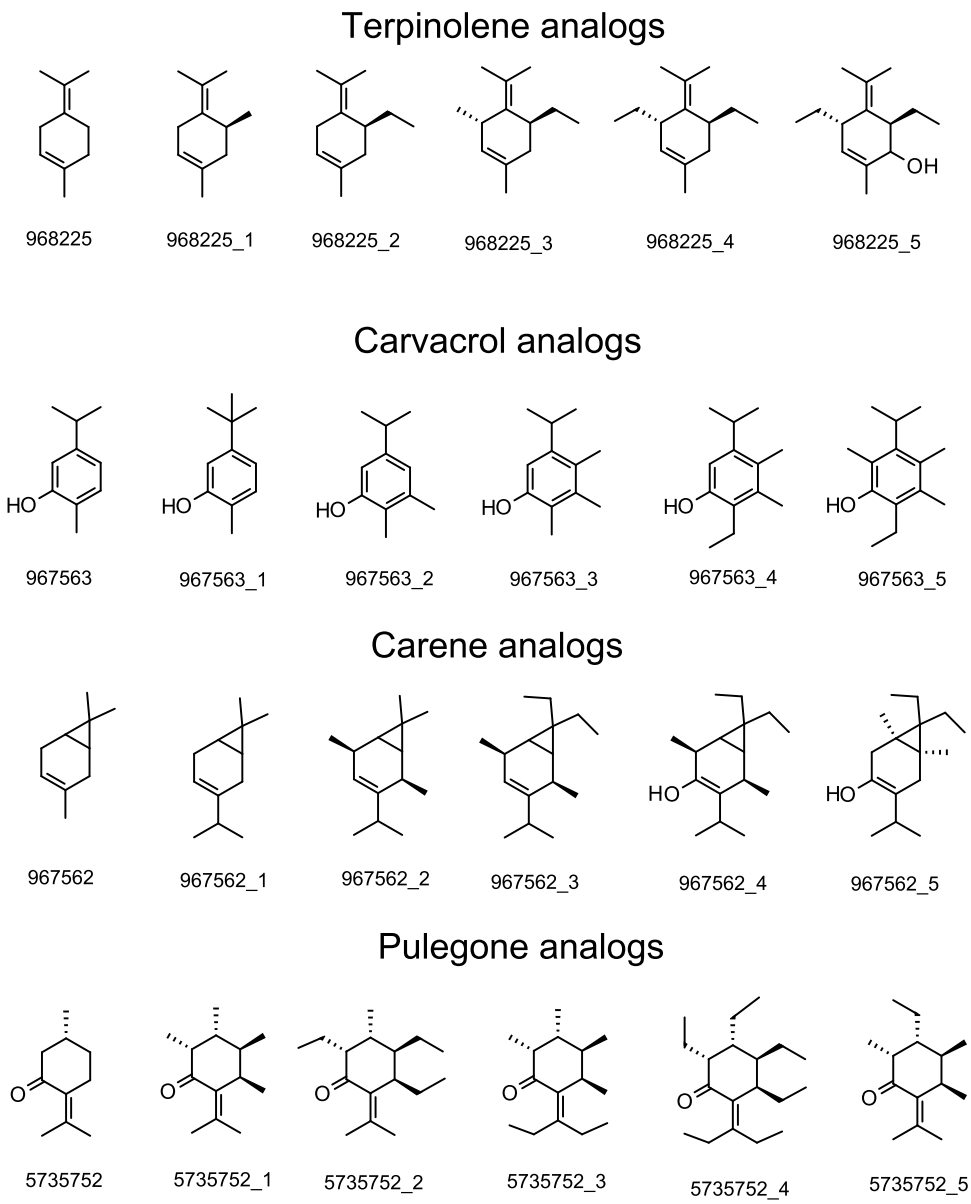


Fig. 6 Chemical structures of functionalized ligands

4 Discussion

Sitophilus oryzae is one of the pests that have caused significant damages to crops like rice and grains. The identification of protein targets of less toxic monoterpenes found in plant essential oils is crucial for formulating species-specific biopesticide. OARs have gained attention as a target for these essential oils. Furthermore, understanding the molecular features of ligand binding to SoTyrR is important to design safer and more effective biopesticide.

Exploring the binding modes of candidate monoterpenes to protein can be done by ensemble docking. This method can give the ligand's binding energy and 3D pose to a protein conformation. This can address the limitation of crystal structures that represents single conformation of proteins and none of its dynamic behavior [58].

Our results show that a hydrophobic motif induces favorable binding due to its interactions with the hydrophobic fragments in the α-helices residues in SoTyrR. There were no $\pi-\pi$, π-cation, cation-π, or t-shaped interactions observed. However, the aromatic residue of Tyr410 is crucial in the binding of terpinolene, carvacrol, carene, and pulegone. Hydrophobic interactions were also found in these 4 monoterpenes with residues Lys189, Val197, and Phe397. In addition, carvacrol has distinct H-bonding interaction with Asp114. These four top hits were also seen to have insecticidal activities to rice weevil. Terpinolene was seen to be effective against rice weevil adults as fumigant [59]. Carvacrol achieved 96.4% mortality to

Table 3 Binding energies of modified top hits compounds to SoTyrR

Ligand	Binding energy (kcal/mol)	Calculated *t* value	*p* value
968225_1	− 5.979 ± 0.42	3.77	0.000
968225_2	− 6.174 ± 0.48	6.73	0.000
968225_3	− 6.145 ± 0.47	6.31	0.000
968225_4	− 6.18 ± 0.51	6.51	0.000
968225_5	− 6.264 ± 0.58	7.20	0.000
967563_1	− 5.973 ± 0.39	1.67	0.098
967563_2	− 6.045 ± 0.31	3.46	0.000
967563_3	− 5.942 ± 0.43	1.07	0.287
967563_4	− 5.947 ± 0.43	1.21	0.229
967563_5	− 5.838 ± 0.51	− 0.70	0.486
967562_1	− 6.177 ± 0.43	7.88	0.000
967562_2	− 6.433 ± 0.51	11.36	0.000
967562_3	− 6.372 ± 0.52	10.21	0.000
967562_4	− 6.358 ± 0.58	9.46	0.000
967562_5	− 6.217 ± 0.57	7.52	0.000
5735752_1	− 6.188 ± 0.53	5.31	0.000
5735752_2	− 6.243 ± 0.56	5.77	0.000
5735752_3	− 6.144 ± 0.54	4.51	0.000
5735752_4	− 6.223 ± 0.65	5.05	0.000
5735752_5	− 6.322 ± 0.51	7.60	0.000

Table 4 Results from toxicity prediction using Osiris Property Explorer

Ligand	Mutagenic	Tumorigenic	Irritant	Reproductive effective
968225_1				
968225_2				
968225_3				
968225_4				
968225_5				
967563_1				
967563_2				
967563_3				
967563_4				
967563_5				
967562_1				
967562_2				
967562_3				
967562_4				
967562_5				
5735752_1				
5735752_2				
5735752_3				
5735752_4				
5735752_5				

(Red: toxicity risk is highly present; orange: toxicity risk is moderately present; green: toxicity risk is not present)

rice weevils when used as fumigant at 46.2 mg/L air for 96 h [60]. Carene is seen as a major constituent of *Pinus longifolia* oil that effectively reduced the population of rice weevils in stored grain [61]. Pulegone at 50 mg/mL air also caused 100% mortality to rice weevils tested [62]. Terpinolene, carvacrol, carene, and pulegone were also seen to have repellent potentials to other stored grain insects [62–65].

Based on these key features, the 3DQSAR model confirmed the docking results that most of the interactions were attributed to hydrophobicity. It also showed that the carboxyl group of Asp114 acts as the hydrogen bond donor. The model suggests that the similarity between the top hits' activity were mostly due to their hydrophobic motifs.

Furthermore, ligand functionalizations were based on the combined information from docking and 3DQSAR analyses. Since both analyses suggested that the interaction is mostly hydrophobic in nature, different hydrophobic groups were added in different positions.

The majority of the observed binding affinity of the functionalized ligands were improved. The highest binding affinity was − 8.3 kcal/mol recorded from ligand 967562_5 compared to the base top hit molecule (− 6.8 kcal/mol). The carene analog ligand 967562_5 is bound in the same binding pocket as its base molecule. Its improved binding affinity is due to the interaction of the added hydrophobic functional groups to Phe397 and Leu190 and the interaction of the added hydroxyl group to Asp114.

The binding site of tyramine is found in the core of hydrophobic transmembrane bundles. This hydrophobic region putatively form the transmembrane domains of G protein-coupled receptors that mediate signal transduction via an intracellular heterotrimeric G protein. This makes highly hydrophobic ligand binding as one of the most common features due to the nature of the transmembrane helices bundle [28].

The inhibition constant, K_i, was calculated using the equation $K_i = exp[\Delta G R^{-1} T^{-1}]$ where ΔG is the binding energy in kcal/mol, R is equal to 1.9187 cal/mol K and T is the temperature equal to 300 K. There is an inverse relationship between the binding affinity and inhibition constant where the higher the binding affinity, the lower is the concentration requirement to inhibit SoTyrR [12]. The calculated K_i for ligand 967562_5 is 0.547 µM which surpassed the known activators of octopaminergic receptors tyramine and amitraz which has K_i of 85.4 µM and 1.59 µM, respectively [28].

Upon subjecting the modified ligands to toxicity test using Osiris Property Explorer, it was seen that majority of the top modified ligands are safe for humans. Ligand 967562_5 exhibited no risks of toxicity. These results support the non-toxic nature of monoterpenes and their analogs in general. Despite these findings, careful examinations should still be necessary as some functionalized monoterpenes appear to be highly toxic.

5 Summary

Tyramine receptors (TyrR) are important in an insect's regulation of physiological functions. Here, an attempt in understanding the key binding features in SoTyrR-monoterpene binding was done using ensemble docking and 3DQSAR. Findings from ensemble docking suggest that binding interactions are mostly hydrophobic and are mediated by residues Asp114, Lys189, Leu190, Tyr196, Phe397, Val404, and Tyr401. This was confirmed through 3DQSAR analysis of the top hit docking poses. This work also suggests the carene analog (ligand 967562_5) is a potential inhibitor of SoTyrR with $K_i = 0.547\ \mu M$. It was observed that adding hydrophobic alkyl groups and hydrogen bond acceptor to carene can improve the binding affinity of this compound to SoTyrR. This computational approach could aid in further understanding of the mechanisms and the effectivity of monoterpenes as safe biopesticide for *S. oryzae* and related pest insects.

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Author contributions R.B.N. designed the experiment. M.K.E.B ran MD simulations and homology modeling. A.B.O. and M.K.E.B performed ensemble docking and analyses. A.B.O. did 3DQSAR analyses and toxicity prediction. All authors reviewed the results and wrote the manuscript.

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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