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Targeting larvae and odorant-binding proteins for mosquito control: Experimental and in-silico investigations of Schiff bases containing aminophenol moiety

Felicia Ndidi Ejiah[1]*, Fouad Abidemi Adetoro[2], Oluwabusola Folake Lawal[2], Mujeeb Olarewaju Rofiu[1]

Abstract

Background: Controlling mosquitoes is crucial for public health globally. Presently, the emphasis is on transitioning from conventional insecticides to viable alternatives such as larvicide options. This study aims to determine the effectiveness of two Schiff base compounds as larvicides and repellents for mosquito control.

Methods: The stock solution of two Schiff base compounds (Z)-2-((2-hydroxybenzylidene)amino)phenol (MO) and (Z)-4-bromo-2-(((2-hydroxyphenyl)imino)methyl)-phenol (MY), was prepared in dimethyl sulfoxide and water in a 1:40 ratio. A total of ten 3rd-4th instar larvae of *Anopheles* larvae in duplicates were exposed to varying concentrations of the test compounds, mortality rate and lethal toxicity were evaluated per unit of time for data analysis. The compounds were also modelled as a repellent to Odorant Binding Protein (OBP) using molecular docking studies.

Results: The results showed that both compounds were highly effective in controlling the larvae in the laboratory, with LC_{50} values ranging (24 to 96 hrs) from 36.922 mg/ml to 0.001 mg/L. Molecular docking experiment confirmed the potential of inhibiting the olfactory odorant protein binding role in the human host target. Computed data showed that both compounds were found to be lethal to the *Anopheles* mosquito larvae species. However, the 5-bromosalicylaldehyde was more active on the larvae when compared to 2-aminophenol with salicylaldehyde.

Conclusion: These findings indicate that the tested compounds have promising larvicidal and repellent activity, suggesting an integrative pest management strategy that first focuses on killing the larvae.

Keywords: Aminophenol; anopheles mosquito; larvicides; repellent; Schiff base.

*Correspondence: Tel.: 2349060002998; E-mail address: fejiah@unilag.edu.ng; ORCID: https://orcid.org/0000-0001-7727-522X (Felicia Ndidi Ejiah)

[1]Chemistry Department, Faculty of Earth and Physical Sciences, University of Lagos, Akoka, Lagos, Nigeria; [2]Applied Entomology Unit, Department of Zoology, Faculty of Life Sciences, University of Lagos, Nigeria

Other authors
Email: fadetoro@unilag.edu.ng, ORCID: https://orcid.org/0000-0002-8750-4986 (Fouad Abidemi Adetoro); Email: Oluwabusola154@gmail.com (Oluwabusola Folake Lawal); Email: mujeebrofiu@gmail.com; ORCID: https://orcid.org/0009-0008-1744-4034 (Mujeeb Olarewaju Rofiu)

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Background

Mosquitoes, belonging to the Culicidae family, are an important group of hematophagous insects that target various vertebrates, including humans [1]. In Nigeria, they pose a substantial threat as vectors of diseases, with malaria, yellow fever, dengue fever, and filariasis being the most prominent. Malaria has been a major public health concern, contributing to both health issues and economic challenges in the country [2, 3, 4]. With about 60 species found worldwide, mosquitoes, particularly those belonging to the Anopheles, Culex, Aedes, Haemagogus, and Mansonia complexes, play a significant role as pests [5, 6, 7]. Their breeding occurs in water pools formed during rainy season storms and permanent swamps in wetlands. Additionally, septic tanks become breeding sites for mosquitoes such as *Aedes aegypti*, *Anopheles vitatus*, Culex horridus, *Culex cinereus*, *Culex pipiens quinquefasciatus*, and *Culex tigripes* [8]. Mosquitoes, beyond causing biting discomfort, engage in hematophagy by sucking human blood and transmitting pathogens. The hematophagous behavior, particularly in female mosquitoes, holds public health significance, as various parasitic and viral diseases have been successfully transmitted through mosquito bites. Members of the Aedes, Culex, and Mansonia complexes transmit *Wuchereria bancrofti* and *Brugia malayi*, causing lymphatic filariasis in humans [9]. Furthermore, mosquitoes in these complexes are responsible for transmitting yellow fever and dengue viruses [10]. Controlling mosquitoes is of utmost importance due to their significant threats to public health.

In developed regions, government-supported agencies implement organized strategies involving water management, biological control agents, and insecticide use to target mosquito larvae and adults. In Africa, the focus has shifted from traditional larvicide and environmental management to area-wide and domestic adulticide since the discovery of dichlorodiphenyltrichloroethane (DDT). However, this chemo-centric approach has had adverse effects on the environment, including humans [11, 12]. Synthetic larvicides, particularly pyrethroids, have also contributed to mosquito population resurgence and the emergence of new strains due to cross-resistance. Despite concerted efforts, new cases of mosquito-borne diseases, especially among under-five children and pregnant women, persist. The juvenile stages of mosquitoes, especially the larval stage, have been identified as the most vulnerable, due to their confined aquatic environments, making control practices effective and sustainable [13, 14, 15]. Targeting mosquito larvae can break the transmission chain, contributing to a more sustainable control program involving insecticides with less toxicity to the environment. One of such compounds that have shown less toxicity to the environment is Schiff bases [16, 17, 18]. Schiff bases are organic compounds that contain the azomethine group. In the last three decades, attention has been drawn to the chemistry of Schiff bases and their metal complexes [19]. Hugo Schiff in 1864 was the first to synthesize Schiff base under azeotropic distillation by using aldehyde or ketone and primary amine [20].

Schiff bases are excellent coordinating ligands that have shown various biological activities, including antimicrobial [21], antifungal [22], anticancer [23], and antioxidant properties [24].

Mishra and Gupta have reported metal complexes of Schiff bases derived from 2-thiophene carboxaldehyde with 2,6-dichloro-4-nitroaniline (TDNA) and 4-anisidine as active insecticidal agents [25]. The insecticidal activity of Schiff base derived from anthranilic acid and acetoacetanilide and its copper complex on *Spodoptera litura* has been reported by Raman et al., 2008 [26]. Recently, the toxicity test results of some Schiff bases showed insecticidal activity against the second instar larvae of the strain of polyphagous pest, *S. frugiperda*, at different concentrations. The report showed that the Schiff base derivatives exhibited high activity on the second-instar larvae and on the fourth-instar larvae. The results showed that the mortality percentages directly increase with increasing concentration and days post-treatment. The Schiff bases were compared with methomyl as a reference insecticide, in which the mortality showed harmful effects [27].

Furthermore, another successful way for controlling widespread malaria transmission involves the reduction of the contact frequency of the mosquito vectors with human body. Research has identified olfactory proteins including odorant binding proteins (OBPs) and olfactory receptors (ORs) as targets using manipulation of mosquito larval habitats and olfactory-mediated behavioral responses [28]. The olfactory sensing in mosquitoes and other insects presents important roles including mating, feeding, and host-seeking. In the case of female Anopheles gambiae mosquitoes, olfactory sensing is used to identify and locate probable human targets [28, 29]. The wide characterization and structural studies of OBP macromolecules make them appealing targets for the discovery of inhibitors that disrupt the olfactory behavioral role in host detection amongst other olfactory macromolecular targets [30, 31].

Liggri et al. reported AgamOBP5, out of more than 70 OBPs expressed by the Anopheles gambiae genome is the most highly expressed OBP in female Anopheles gambiae antennae mRNA which greatly decreases three hours after a blood meal [32]. The in-vitro screening through a fluorescence assay and Differential Scanning Calorimetry (DSC) experiments revealed Carvacrol and Thymol binding to AgamOBP5 with enhanced affinity, gives detailed structural information on AgamOBP5 targets for the discovery of effective, safe, and eco-friendly mosquito control agents.

Considering these insecticidal activities, the present study intends to control mosquitoes by employing Schiff bases and their derivatives, introducing them to the mosquito larvae, and studying their larvicidal efficacy. In line with this, two Schiff bases *2-((2-hydroxy benzylidene)amino)phenol* (MO) and *4-bromo-2-(((2-hydroxyphenyl)imino)methyl)-phenol* (MY) have been investigated to determine the potential of the compounds as bioactive agents (larvicides) on mosquito larvae. This research also modelled the Schiff bases as AgamOBP5 targets repellent or protein restructuring agents in female *Anopheles gambiae* larvae and adults. This would help to understand the importance of controlling mosquitoes at their juvenile stage since the larvae are usually isolated in aquatic environments and as target repellent.

Methods

Materials

Dimethyl sulfoxide (DMSO) was purchased from Sigma Aldrich Chemical Co. Ltd. Schiff bases 2-((2-hydroxybenzylidene)amino)phenol (MO), 4-bromo-2-(((2-hydroxyphenyl)imino)methyl)-phenol (MY) have been previously synthesized and fully characterized. *Anopheles Gambiae* specie, mosquito larvae were obtained from the Insectary of Department of Zoology, University of Lagos Nigeria, while the holding cups were purchased from the University of Lagos Campus mall. Distilled water was obtained from the Department of Chemistry, University of Lagos.

Synthesis and characterization of Schiff bases

Schiff bases synthesized from 2-aminophenol and salicylaldehyde *2-((2-hydroxy benzylidene)amino)phenol,* MO) and 2-aminophenol and 5-bromosalicylaldehyde *4-bromo-2--(((2-hydroxyphenyl)imino)methyl)-phenol*, MY) have been reported by Ejiah et al., 2023 [33].

Mosquito larvae collection

Mosquito larvae (1st-2nd in-star) were collected by the standard dipper method. Collected larvae were then transferred into a larger holding container and transported to the laboratory acclimatized for 2 days prior and sorted into species *Anopheles gambiae* species.

Laboratory bioassay

Five different concentrations of the two test compounds in DMSO:water, 1:40 ratio (i.e. 0.5 mg/ml, 0.75 mg/ml, 1.0 mg/ml, 2.0 mg/ml, and 2.5 mg/ml) were introduced as diluents into holding cups containing 100 ml of distilled water. The solution was contained in 24 transparent holding cups and control. The cups were arranged in triplicates, and each cup was filled with 100 ml of distilled water and a predetermined concentration of the two chemical compounds. A total of ten 3rd-4th in-star larvae of *Anopheles gambiae* in triplicates were introduced to the experimental cups, to determine the larvicidal efficacy of the synthesized Schiff base compounds. Larval mortality numbers were computed for a period of five days following the exposure period. The morphological changes observed were also recorded.
After the procedures above were carried out, the mortality rate was measured and recorded after 24 hrs. The larvae were considered dead when they were moribund or immotile. The dead larvae were counted, and the average percentage mortality was calculated. Data were adjusted to control mortality using Abott's formula. Table 2 was calculated using Abbott's formula to obtain a reliable and meaningful result.

$$\text{Corrected mortality (\%)} = \frac{A}{100-B} \times 100$$

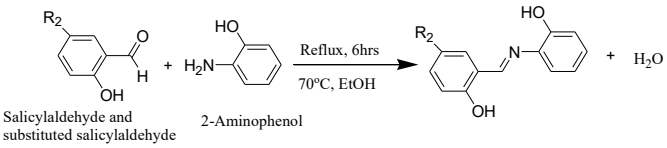
Where, A = % of survival in the control larva population
B = % of survival in the treated larva population

Molecular Docking

The Schiff bases' geometry optimizations were performed using the Gaussian 09 software program [34, 35]. The molecular docking studies were performed with the minimum optimized geometry of Schiff bases MO and MY from the semi-empirical quantum mechanical approach using the PM6 Hamiltonian method [36]. The studies use the X-ray crystal structure of Anopheles gambiae's Odorant Binding Protein 5 (PDB ID: 8BXW) obtained from the Protein Data Bank (https://www.rcsb.org) [32]. Before the docking exercise, the addition of polar hydrogen atoms to the protein molecules and the removal of water molecules, other atoms, and complexes apart from the protein residue were performed using the Biovia Discovery Studio 4.5 Client. The docking simulation includes the grid center (x = 4.5378, y = -2.5380, z = 15.5837), grid size of (x = 49.3427, y = 50.1916, z = 53.9483), and 1.000 Å spacing were set up on Pyrx-virtual screening tool (Open babel, Autodock Vina). The docking simulation and computations were conducted using AutoDock Vina [37], (MGL tools- 1.5.6). The visualization of the docking results and other structural interaction analyses were done using Biovia 2019 Discovery Studio 4.5 and PyMOL (Console Edu).

Results

Synthesis and characterization of Schiff bases
The general synthesis for 2-((2-hydroxy benzylidene)amino)phenol (MO) and 4-bromo-2-(((2-hydroxyphenyl)imino)methyl)-phenol (MY) is provided in Scheme 1 below.



MO= R2, H
MY= R2, Br

Scheme 1. General scheme for synthesis of *2-((2-hydroxy benzylidene)amino)phenol* (MO) and *4-bromo-2-(((2-hydroxyphenyl)imino)methyl)-phenol* (MY)

The characterization using NMR, FTIR, Mass spectrometry, and DFT calculations confirm the compounds.

Larvicidal activity

The efficacy of the two chemical compounds namely, *2-((2-hydroxy benzylidene)amino)phenol* (MO) and *4-bromo-2-(((2-hydroxyphenyl)imino)methyl)-phenol* (MY) on *Anopheles gambiae* larvae was evaluated using laboratory bioassay. As a result of natural mortality in control, Abbott's formula was implemented in order to accurately assess the efficacy of the treatment used, which is represented in Table 1. The represented data showed that the percentage mortality depicts a random pattern of mortality in each concentration of MO and MY. Also, the mortality percentage had a direct correlation with concentrations of the two test compounds. Mortality rate of MO and MY against Anopheles larvae for 24, 48, 72 and 96 hours is represented in Figures S1 and S2, while the mortality response of both Schiff base compounds is listed in Tables S1 and S2 (supplementary materials). Tables 2 and 3 show the LC_{50} Dose-response relationship of MO and MY against *Anopheles gambiae* larvae.

Molecular Docking Studies

Schiff base (MO) from Figure 2 forms a conventional hydrogen bond (2.372 Å) with THR 109 of orange-α6 helix (HG1) using the hydroxyl group from aminophenol moiety and a carbon-hydrogen bond (3.772 Å) with THR 68 of yellow-α4 helix (OG1) through its condensed carbon from the salicylaldehyde moiety. Also, there are established solvent-accessible hydrophobic interactions (pi-alkyl) including ALA51;CB and ALA54;CB (blue-α3 helix, 4.24 Å and 5.00 Å), ILE63; (4.97 Å), VAL65;CG1 (yellow-α4 helix, 5.23 Å), ILE88;CG2 (magenta-α5 helix, 4.51 Å) and LEU112;CD1 (orange-α6 helix, 4.93 Å). Figure 1 revealed the occupation of the central pocket through van der Waal interactions of MO with various other amino acid residues within the cavity. These residues include SER10, MET11, and MET14 (red: α1 helix), CYS91 (magenta: α5 helix), LEU121, and others as indicated in Figures 1B and 1C.

The illustration from Figure 4 also showed a strong hydrogen bonding contact between MY through the hydrogen from the hydroxyl group on the salicylaldehyde moiety to the AgamOBP5 THR56;OG1 (2.04 Å).

The hydrogen, hydrophobic and Van der Waal contacts as shown in Figures 3 and 4 practically indicate the location of the two Schiff bases MO and MY on the same binding site on the AgamOBP5 but with a slightly different conformation.

Bioactivity parameters such as ligand efficiency (LE), Fit quality (FQ), and ligand-efficiency dependent lipophilicity of Schiff bases MO and MY with AgamOBP5 target (8BXW) is listed in Table 4.

Discussion

Schiff bases synthesized from 2-aminophenol and salicylaldehyde *2-((2-hydroxy benzylidene)amino)phenol,* MO) and 2-aminophenol and 5-bromosalicylaldehyde *4-bromo-2-(((2-hydroxyphenyl)imino)methyl)-phenol*, MY) have been reported by Ejiah et al., 2023 [33]. This paper reports on the larvicidal activity of both Schiff bases.

The laboratory bioassay investigation evaluated the larvicidal efficacy of synthesized Schiff bases (MO) and (MY) intended for control of *Anopheles gambiae* mosquito larvae. The results of the bioassay showed that MY exhibited stronger larvicidal activity in comparison with MO after four days of exposure. This could be attributed to the effect of bromo substituted group as this has been reported to increase biological activity [38-41].

For treating *Anopheles gambiae* larvae in MO, the LC_{50} values range (24 to 96 hours) from 36.922 mg/ml to 0.001 mg/L, and for treating *Anopheles gambiae* larvae in MY, LC_{50} ranges (24 to 96 hours) from 22.065 mg/ml to 0.254 mg/ml, suggesting larvicidal activity for both compounds. Following a 4-day period, the target chemicals tested against *Anopheles gambiae* larvae showed MY had relatively lower values compared to MO, corresponding to higher LC_{50} values.

Generally, several approaches have been used in mosquito control and allied diseases. These approaches either abort the development of the parasite within the mosquito body or suppress the mosquito vector itself. Effective transmission of mosquito-borne disease requires successful contact between female mosquitoes and their hosts [42, 43]. In the United States, the Environmental Protection Agency (EPA) and the Centre for Disease Control and Prevention (CDCP) advocate for integrative pest management strategies that first focus on killing the larvae. Use of larvicides is less controversial than use of adulticides, although use of larvicides may lead to public concern about their effects on untargeted beneficial aquatic arthropods and vertebrates. However, the use of larvicide should be targeted at the most productive larval habitats which might have been difficult to eliminate through habitat modification.

Understanding the effect of substituents on biological activity using Schiff base is key in developing antimicrobial, anti-fungicidal and insecticidal agents. Earlier reports showed that biological activity of compounds is mainly dependent on their molecular structure [44]. The study revealed that substituted salicylaldehyde were more active at inhibiting Gram-positive bacteria and that such compounds can be employed in formulation of narrow spectrum antibiotics for treatment of infections caused by gram positive bacteria particularly *S. aureus*.

Consequently, from our study, 4-bromo-2-(((2-hydroxyphenyl)imino)methyl)-phenol (MY) with bromo-substituent was more effective on the larvae of *Anopheles gambiae* mosquito when compared to 2-((2-hydroxy benzylidene)amino)phenol (MO) which had no substituent group. MY exhibited a faster activity and higher mortality percentage than MO. In addition, biological activity can be related to electrochemical behavior which influences a molecule's ability to interact with biological targets. Bromo substituted Schiff bases have demonstrated excellent electro-activeness [33, 45].

Molecular docking study was evaluated to validate the importance of controlling mosquito at their larvae stage since they are usually isolated in aquatic environments by employing AgamOBP5 target repellent which is a protein de-structuring agent in female Anopheles gambiae larvae and adults.

According to Liggri et. al [32], the structure of AgamOBP5 has three stabilizing disulfide bridges Cys18-Cys49, Cys45-Cys102, and Cys91– Cys111. It also presents a solvent-accessible hydrophobic pocket through an opening lined by the residues Ser10 and Met14 (red: α1 helix), Ala54 (blue: α3 helix), and Gln71 and Met75 (yellow: α4 helix). The work also investigated the central pocket of AgamOBP5 structure, which was revealed by three MPD and include cavities around Thr109, Ser10;OG (red: α1 helix) and Ala54;O (blue: α3 helix), Leu121 belonging to the C terminal segment and established polar contacts with these protein residues.

A notable interaction is a hydrophobic pi-alkyl interaction of MY with one of the disulfides through CYS91;SG [32] (magenta-α6 helix, 5.66 Å) which may destabilize the protein structure and its functional role in human sensing. Other hydrophobic bonding contacts includes ALA51;CB and ALA54;CB (blue-α3 helix, 5.13 Å and 5.28 Å), ILE63; (5.06 Å), VAL65;CG2 (yellow-α4 helix, 5.17 Å), ILE88;CG1 (magenta-α5 helix, 4.53 Å), LEU112;CG (orange-α6 helix, 5.00 Å), PHE122;pi (4.59 Å) and PRO123;pi (4.70 Å). Hydrophobic-hydrophobic contacts in protein folding have been observed to be distance dependent, as a distance lower than or equal to 3.8 Å (very short-range interactions), host interacting hydrophobic residues contacts are almost absent whereas several short-range interactions are detectable between 3.8 and 6.5 Å [46].

The results showed that OBP5 was a promising target for the Schiff bases against the *Anopheles gambiae* larvae and adults' olfactory behavioral role in host detection. Based on this study, MO and MY are good inhibitors or disruptors of the olfactory protein functional role played by AgamOP5.

The binding affinity (ΔG), kcal/mol of the Schiff base ligands MO and MY for AgamOBP5 was determined as -7.9 and -8.0 respectively. The AgamOBP5 complexed with the compounds

MO and MY and gave a binding constant (Ki) of 1.6 µM and 1.35 µM respectively. The larger negative value of binding energy and lower binding constant for MO and MY depicts the higher affinity of the compounds for the AgamOBP5 target. This can be attributed to the presence of hydrogen bond and various hydrophobic interactions between the ligands and the protein. The binding constant gives information on the distribution of the drug in the plasma membrane of the antenna of *Anopheles Gambiae* larvae as high value of the binding constant shows a weak binding and a short lifetime while strong binding is shown in the low value of the binding constant. For other bioactivity metrics, such as ligand efficiency (LE), Fit quality (FQ), and ligand-efficiency dependent lipophilicity (LELP), the thresholds for a hit are ≥0.3≥0.8 and -10 to 10 respectively.

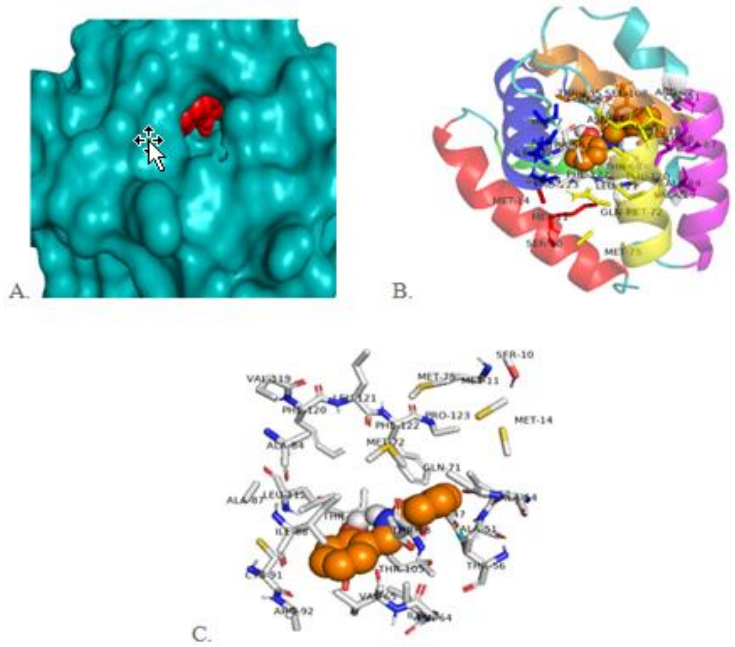


Figure 1. A. The surface structure of AgamOBP5 with MO ligand embedded in the active site, B. and C. The amino acid residues that surround the binding site occupied by the MO ligand

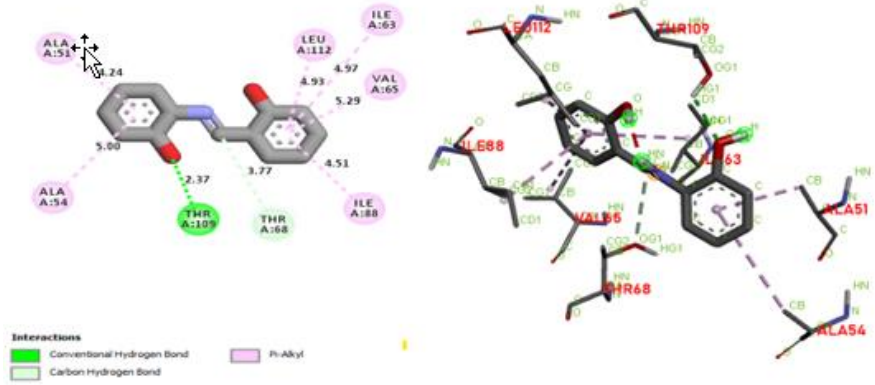


Figure 2. The various binding interactions of MO with AgamOBP5

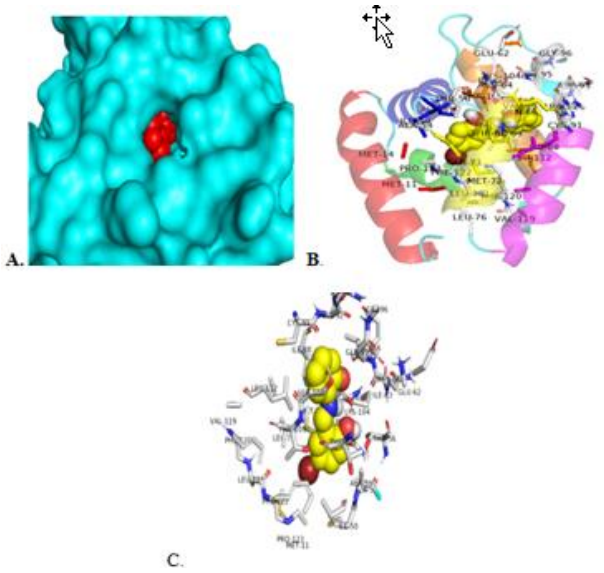


Figure 3. A. The surface structure of AgamOBP5 with MY ligand embedded in the active site, B. and C. The amino acid residues that surround the binding site occupied by MY ligand.

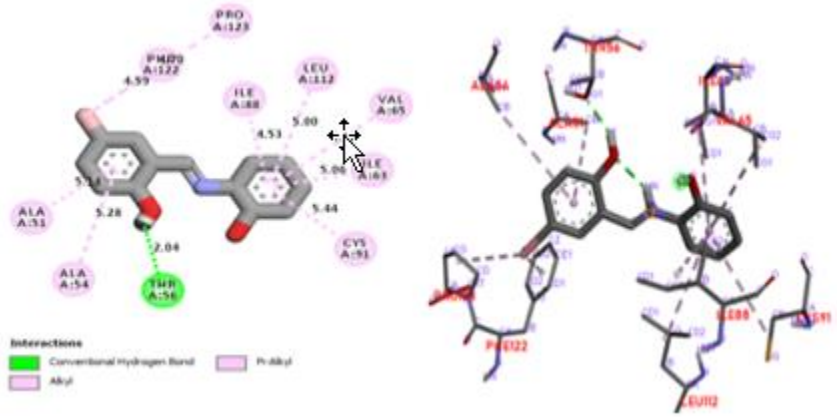


Figure 4. The various binding interactions of MY with AgamOBP5

Table 1. Percentage mortality rate and the corrected mortality at different concentrations of MO and MY

Compound	%Mortality rate			%Corrected mortality	
	Conc. (mg/ml)		A - B = Y	Y/(100-B)=Z	Z*100
MO	Control	5			
	0.5	70	65	0.684	68.4
	0.75	60	55	0.579	57.9
	1.0	75	70	0.736	73.6
	2.0	70	65	0.684	68.4
	2.5	70	65	0.684	68.4
MY	Control	15			
	0.5	65	50	0.588	58.5
	0.75	70	55	0.647	64.7
	1.0	75	60	0.705	70.5
	2.0	65	50	0.588	58.8
	2.5	80	65	0.765	76.5

MO= 2-aminophenol with salicylaldehyde; MY= 2-aminophenol with 5-bromosalicylaldehyde
A= percentage mortality of larvae in treatment; B= percentage mortality of larvae in control

Table 2. LC_{50} Dose-Response Relationship of MO against *Anopheles gambiae* larvae

Time	LC_{50}	Probit equation line	DF
24 hours	36.922	-1.22+0.44x	3
48 hours	41.784	-0.9+0.55x	3
72 hours	1.676	0.07+0.31x	3
96 hours	0.001	0.46+0.14x	3

Table 3. LC_{50} Dose-Response Relationship of MY against *Anopheles gambiae* larvae

Time	LC_{50}	Probit equation line	DF
24 hours	22.065	-1.11+0.74x	3
48 hours	8.941	-0.63+0.66x	3
72 hours	0.960	0.01+0.7x	3
96 hours	0.254	0.44+0.75x	3

LC_{50}= Lethal Concentration for ≥50% mortality; DF= Degree of Freedom

Table 4. Bioactivity parameters of Schiff bases MO and MY with AgamOBP5 target (8BXW)

Schiff bases-protein	Binding Energy (ΔG), kcal/mol	Binding Constant (Ki), μM	Number of Heavy Atom	Log P	Ligand efficiency (LE) kcal/mol/heavy atom)	LE-scale	Fit quality (FQ)	Ligand-efficiency-dependent lipophilicity (LELP)
MO-8bxw	-7.9	1.6	16	3.14	0.493	0.512	0.963	6.37
MY-8bxw	-8	1.35	17	4.2	0.47	0.497	0.947	8.94

Conclusion

This research indicated promising larvicidal activity of 2-aminophenol Schiff base derivatives. The larvicidal activity of the synthesized compounds MO and MY were found to depend on the substituent group on the Schiff base. Molecular docking experiment confirmed the potential of MO and MY in inhibiting the olfactory odorant protein binding role in human host target identification through hydrogen bonding and hydrophobic binding affinity to the active site. Further work on the efficacy of other insect pests, and the development of other series of Schiff base derivatives that can be tested as larvicides or insecticides. Overall, our findings contribute to a better understanding of the use of Schiff bases as alternative larvicides and open avenues for future research in this field for the development of innovative, easy preparation and environmentally friendly strategies in control of mosquito larvae.

Additional file

Supplementary file.PDF: available at https://www.investchempharma.com/supplementary-information_imcp122_ndidi-ejiah-et-al/

Abbreviations

AgamOBP: Anopheles gambiae odorant binding protein
CDCP: Centre for Disease Control and Prevention
DDT: Dichlorodiphenyltrichloroethane
DF: Degree of freedom
DFT: Density functional theory
DMSO: Dimethyl sulfoxide
EPA: Environmental Protection Agency
FQ: Fit quality
LE: Ligand efficiency
LELP: Ligand-efficiency dependent lipophilicity
MPD: 2-methyl-2,4-pentanediol
OBP: Odorant binding protein
OR: Olfactory receptors
PDB: Protein data bank

Authors' Contribution

FNE, FAA, OFL and MOR carried out the study; FNE and FAA supervised the study; All authors read and approved the final version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest

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