Research Article

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Biological evaluation of a series of benzothiazole derivatives as mosquitocidal agents

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Abstract: *Aedes aegypti* is associated with the transmission of numerous human and animal diseases, such as yellow fever, dengue fever, chikungunya, and more recently Zika virus. Emerging insecticide resistance has created a need to develop new mosquitocidal agents for effective control operations. A series of benzothiazole-piperidine derivatives (**1-24**) were investigated for their larvicidal and adulticidal effects on *Ae. aegypti*. It was observed that compounds **2**, **4**, **6**, **7**, **8**, **11** and **13** showed notable larvicidal activity. Furthermore, compounds **6** and **10** showed promising adulticidal activity. Based on the mosquitocidal properties of these compounds, docking studies were also carried out in the active site of the *Ae*SCP2 enzyme to explore any insights into further *in vitro* enzyme studies. Docking results indicated that all these active compounds showed reasonable interactions with critical residues in the active site of this enzyme. This outcome suggested that these compounds might show their larvicidal and adulticidal effects *via* the inhibition of *Ae*SCP2. According to *in vitro* and *in silico* studies, compounds **2**, **4**, **6**, **7**, **8**, **10**, **11** and **13** stand out as candidates for further studies.

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Keywords: mosquito control; *Aedes aegypti*; benzothiazole; piperidine; docking studies.

1 Introduction

Mosquitoes are one of the most dangerous insect vectors and infectious disease carriers in developing countries [1]. Among mosquito species, *Aedes aegypti* L. transmits yellow fever, dengue fever, chikungunya, and more recently, Zika virus [2]. Dengue is an endemic viral disease found mainly in the tropical and subtropical regions across the globe [3,4]. Dengue is characterized by fever, headache, muscle, and joint pain together with nausea and vomiting [4-6]. Zika virus also causes severe brain defects and threatens the lives and health of adults and newborns from infected mothers [7].

The battle against mosquitoes has become a crucial environmental, economic and social health issue. Generally, chemical insecticides are considered as the first option for reducing vector-borne disease but evolving resistance caused by cytochrome P450 monooxygenases (P450s), which are capable of metabolizing many insecticides, as well as decreases in target site sensitivity can limit the success of insecticide treatment [8-10]. In particular, overexpression of P450s such as the CYP9J32 gene in *Ae. aegypti* is associated with pyrethroid resistance [11,12].

Sterol carrier protein-2 (SCP-2), a nonspecific intracellular lipid carrier, is expressed throughout the animal kingdom including insects. Moreover, single SCP-2 domain genes have also been proven to be expanded in mosquitoes [13]. Cholesterol is crucial for insects in order to grow, develop and reproduce, but they are not capable of synthesizing cholesterol *de novo* [14,15]. The mosquito SCP-2, *Ae. aegypti* SCP-2 (*Ae*SCP2), has been reported to be involved in cholesterol and fatty acid uptake in the midgut in both larval and adult mosquitoes [16]. A small number of studies have focused on developing new insecticides that prevent cholesterol biosynthesis targeting *Ae*SCP2

[17-19]. Fifty seven compounds were identified *in silico* as possible inhibitors of the cholesterol-binding capacity of SCP-2 from the library of 16000 compounds [20]. Therefore, targeting this cholesterol transport pathway associated with *Ae*SCP2 could be an alternative target for the development of specific mosquitocidal agents.

Benzothiazole (BT) is a privileged bicyclic ring system present in a wide variety of synthetic and natural products. BT and its derivatives play a distinctive role in medicinal chemistry due to their diverse biological activities such as antiprotozoal, antimicrobial, anticancer, antischizophrenia, antihypertensive, anti-inflammatory, and antiviral activities [21-25]. Venugopala et al. 2013 [26], also screened benzothiazole analogs for their mosquitocidal and repellent properties against *Anopheles arabiensis* by mosquito feeding-probing assay, cone bio-assay and standard World Health Organization (WHO) larvicidal assay. Similarly, piperidine is a strong base mainly found in several natural alkaloid skeletons [27]. Diversely substituted piperidines are the leading heterocycles in the structure of several important pharmaceuticals such as bupivacaine, troxipide, tofacitinib, fexofenadine, astemizole, fentanyl, haloperidol, loperamide, trimeperidine, etc. [28].

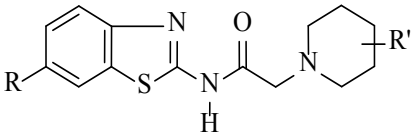
On the basis of these findings, benzothiazole-piperidine derivatives, which had been reported previously for their antimicrobial effects on pathogenic bacteria and *Candida* species by our research group [29], were evaluated for their insecticidal activities against *Ae. aegypti*. In addition, molecular docking studies were performed for the most effective compounds within the active site of *Ae*SCP2 (PDB code: 1PZ4) to provide a mechanistic approach for further studies [30].

2 Experimental

2.1 Chemistry

2-Chloro-*N*-(benzothiazol-2-yl)acetamide derivatives were synthesized *via* the reaction of 2-aminobenzothiazoles with chloroacetyl chloride in the presence of triethylamine. *N*-(Benzothiazol-2-yl)-2-(piperidin-1-yl)acetamide derivatives (**1-24**) (**Table 1**) were obtained by the nucleophilic substitution reaction of 2-chloro-*N*-(benzothiazol-2-yl) acetamides with piperidine derivatives in the presence of potassium carbonate. The synthetic protocol and spectral data of the compounds were reported previously by our research group [29].

Table 1: Structures of compounds **1-24** [29].



Compound	R	R'
1	H	4-methyl
2	H	3-methyl
3	H	2,6-dimethyl
4	H	3,5-dimethyl
5	Cl	4-methyl
6	Cl	3-methyl
7	Cl	2,6-dimethyl
8	Cl	3,5-dimethyl
9	CH_3	4-methyl
10	CH_3	3-methyl
11	CH_3	2,6-dimethyl
12	CH_3	3,5-dimethyl
13	OCH_3	4-methyl
14	OCH_3	3-methyl
15	OCH_3	2,6-dimethyl
16	OCH_3	3,5-dimethyl
17	OC_2H_5	4-methyl
18	OC_2H_5	3-methyl
19	OC_2H_5	2,6-dimethyl
20	OC_2H_5	3,5-dimethyl
21	NO_2	4-methyl
22	NO_2	3-methyl
23	NO_2	2,6-dimethyl
24	NO_2	3,5-dimethyl

2.2 Biological assays

2.2.1 Mosquitoes

The Orlando 1952 strain (ORL1952) of *Ae. aegypti* is a laboratory susceptible colony that has been without wildtype supplementation for over seventy years. The strain was originally collected near Orlando Florida, USA and has been maintained by the USDA ARS Center for Medical, Agricultural and Veterinary Entomology (CMAVE) in Gainesville, Florida (previously the Insects Affecting

Man Laboratory). Insecticide susceptibility of this strain has been characterized for a large number of common pesticides [31,32]. Colony maintenance and organism rearing for bioassays were described previously [31].

2.2.2 Larvicidal activity

The larval bioassay was described in detail previously [31]. Briefly, ORL1952 eggs were hatched overnight in approximately 100 mL of deionized water. Five first instar larvae were transferred into the wells of 96-well flat-bottom tissue culture plate in 188 µL of deionized water. Ten microliters of a 2% solution of finely ground alfalfa powder were added to each well.

Compounds **1**-**24** were dissolved in DMSO to produce a 100 mg/µL stock solution. Dilutions of each stock solution were created by adding 2, 1, 0.5, or 0.2 microliters to the wells containing larva and food media for the initial screening bioassay. Negative control wells were prepared with 2 microliters of DMSO and positive control wells contained permethrin. Bioassay plates were covered and maintained on the benchtop at 22-23°C. Mortality was scored at 24 hours and the assay was repeated on three separate days. Additional dilutions (to 0.01 mg/µL; 0.005 mg/µL for compound **11**) of active compounds (>80% mortality at 0.1 mg/µL) were tested to determine the full dose response curve and allow calculation of LC_{50} values.

2.2.3 Adulticidal activity

Three to seven-day post-emergence female *Ae. aegypti* were sorted into groups of 10 in TK35 plastic cups after one hour of chilling at 4°C. Compound stock solutions in DMSO (100 µg/µL) were diluted in acetone to produce a 10 mg/µL solution. Five hundred nanoliters of the compound solution were applied to the thorax of each mosquito using a repeating syringe with a blunt tip needle (Hamilton PB600 with 7100 syringe). Cups containing treated mosquitoes were capped with tulle mesh and mosquitoes were allowed to recover with access to 10% sucrose saturated cotton balls. Mortality was recorded at 24 hours and the assay was repeated at least three times. Acetone was used as a negative control, whereas permethrin (mixture of 46.1% *cis* and 53.2% *trans* isomers (Chemservice, West Chester, PA, USA)) was used as a positive control. Additional dilutions (from 5 ug/mosq to 0.5 ug/mosq) and assays of active compounds were performed to calculate LD_{50} values. Specific details of the adult topical bioassay were published previously [33].

2.2.4 LD_{50} and LC_{50} calculation

Median lethal doses were calculated using SigmaPlot (v13). Raw mortality counts from each dose of each replicate were converted to percentage mortality and plotted. Curve fitting of dose and mortality data was performed using a four parameter logistic model with constrained minimum and maximum values of 0 and 100, respectively.

2.3 Molecular docking studies

Molecular docking studies were carried out to understand the relationship between protein structures and substrates, which can provide reasonable explanations for substrate specificities and differences in the active site of the target structure. As previously mentioned, inhibition of *Ae*SCP2 can reduce the uptake of cholesterol and lead to death in both larval and adult mosquitoes. For this purpose, compounds **2**, **4**, **6**, **7**, **8**, **10**, **11** and **13** were docked to the active site of *Ae*SCP2 due to their *in vitro* mosquitocidal potencies. Ligands were sketched and cleaned in Maestro molecular modeling workspace followed by energy minimization in ligand preparation program of Schrödinger's Maestro molecular modeling package (Schrödinger Release 2016-2: Schrödinger, LLC, New York, NY, USA) using Optimized Potential Liquid Simulations (OPLS_2005) force field. The X-ray crystallographic structure of the *Ae*SCP2 complex with palmitic acid (PDB code: 1PZ4) [30] was retrieved from the Protein Data Bank (PDB) server and optimized for docking analysis in protein preparation module of Schrödinger software. In molecular docking simulations, Glide/XP docking protocols were applied for the prediction of topologies of active compounds in the active site of *Ae*SCP2.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and Discussion

Compounds **1**-**24** were investigated for their larvicidal and adulticidal activities against *Ae. aegypti*. Initial screening of the first instar larvae was completed at four concentrations (1.0, 0.5, 0.25, 0.1 mg/µL) with seven compounds producing more than 80% mortality at the 0.1 mg/µL dose (**Table 2**). Compounds **6** and **10** produced more than 80% mortality in initial screening of adult *Ae. aegypti* females (Table 2). LC_{50} or LD_{50} values were subsequently determined for compounds that produced

Table 2: Twenty-four hour larval and adult topical mortality of compounds **1-24** against the susceptible Orlando (ORL1952) strain of *Ae. aegypti*.

#	Larvicidal activity against 1st instar *Ae. aegypti**							Adulticidal activity against adult female *Ae. aegypti***			
	1 mg/µL	0.5 mg/µL	0.25 mg/µL	0.1 mg/µL	LC_{50} ± SE (mg/µL)	IC_{50} (µM)	R^2	5 mg/ mosquito	LD_{50} ± SE (mg/mosq.)	LD_{50} (µM)	R^2
1	93 ± 12	87 ± 12	100	73 ± 31	-		-	64 ± 6	-		-
2	100	100	100	100	0.096 ± 0.005	332.2	0.984	47 ± 6	-		-
3	73 ± 12	40 ± 20	0	0	-		-	47 ± 29	-		-
4	100	100	100	87 ± 23	0.065 ± 0.011	214.5	0.794	60 ± 27	-		-
5	100	93 ± 12	87 ± 23	60 ± 53	-		-	77 ± 21	-		-
6	100	100	100	100	0.053 ± 0.011	163.8	0.643	83 ± 12	2.084 ± 0.338	6442.0	0.926
7	100	100	93 ± 12	93 ± 12	0.128 ± 0.019	379.2	0.803	47 ± 29	-		-
8	100	100	100	87 ± 23	0.123 ± 0.015	364.4	0.981	63 ± 12	-		-
9	47 ± 42	47 ± 42	33 ± 31	33 ± 58	-		-	70	-		-
10	100	87 ± 12	80 ± 20	67 ± 12	-		-	83 ± 6	2.962 ± 0.670	9775.6	0.533
11	100	100	100	100	0.034 ± 0.003	107.3	0.647	40 ± 17	-		-
12	100	87 ± 23	73 ± 46	67 ± 58	-		-	57 ± 15	-		-
13	100	100	100	100	0.098 ± 0.001	307.2	0.958	67 ± 25	-		-
14	100	100	100	73 ± 23	-		-	50 ± 36	-		-
15	55 ± 22	40 ± 0	25 ± 7	15 ± 7	-		-	73 ± 12	-		-
16	100	100	73 ± 46	67 ± 58	-		-	53 ± 12	-		-
17	100	73 ± 31	47 ± 50	67 ± 58	-		-	63 ± 15	-		-
18	100	93 ± 12	87 ± 23	47 ± 31	-		-	37 ± 6	-		-
19	100	93 ± 12	80 ± 35	67 ± 31	-		-	67 ± 12	-		-
20	65 ± 21	45 ± 7	30 ± 14	15 ± 7	-		-	50 ± 10	-		-
21	93 ± 12	53 ± 50	33 ± 58	33 ± 58	-		-	50 ± 30	-		-
22	93 ± 12	53 ± 50	33 ± 58	33 ± 58	-		-	63 ± 6	-		-
23	33 ± 31	20 ± 20	27 ± 46	7 ± 12	-		-	73 ± 12	-		-
24	100	100	93 ± 12	67 ± 31	-		-	73 ± 12	-		-

* Positive control permethrin at 3.0 pg/µL resulted in 26.7 ± 23.1 % mortality. Negative control solvent control (DMSO) had 0% mortality.
** The average mortality in the two permethrin positive controls of 0.1935 and 0.4772 ng/mosquito was 63.3 ± 15.3 and 100, respectively. Acetone controls had 0% mortality.

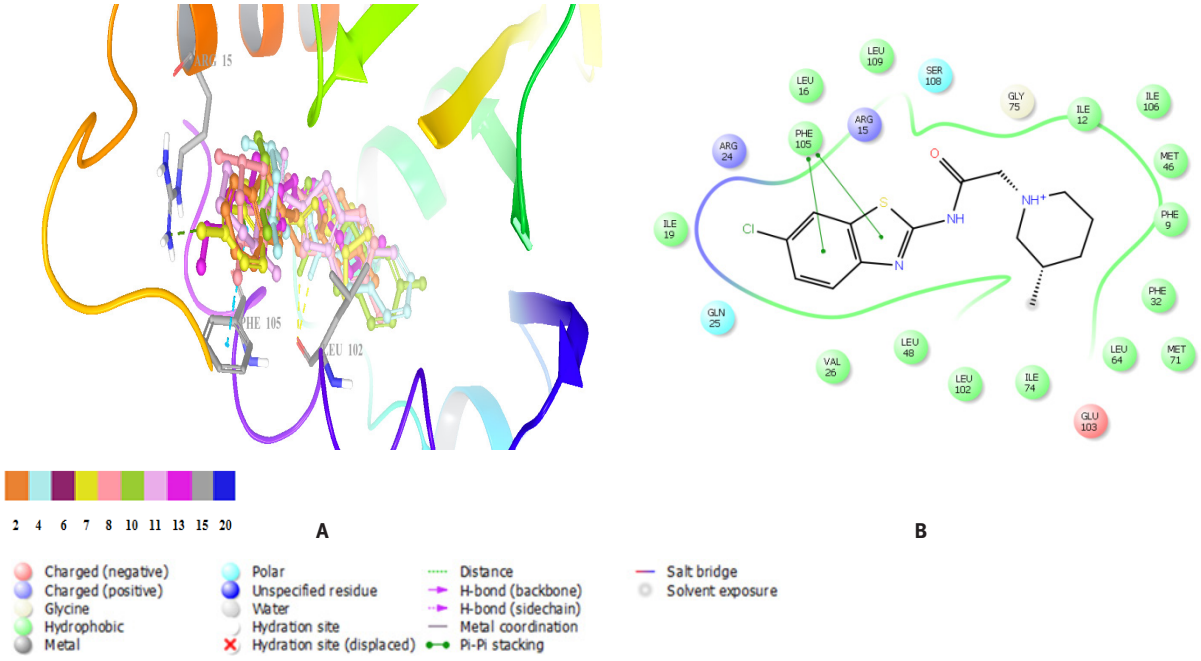


Figure: 1: Docked poses of compounds **2**, **4**, **6**, **7**, **8**, **10**, **11** and **13** (Yellow dashes: Hydrogen bonding; Blue and green dashes: π-π interactions) (**A**) and interactions of compound **6** in the active site of *Ae*SCP2 (**B**).

>80% mortality at the discriminating dose in either or both bioassays by further subdilutions and additional assays (**Table 2**). Compounds **11**, **6**, **4**, **2**, **13**, **8**, and **7** showed the highest larvicidal activity with LC_{50} values of 0.034 (107.3 μM), 0.053 (163.8 μM), 0.065 (214.5 μM), 0.096 (332.2 μM), 0.098 (307.2 μM), 0.123 (364.4 μM), and 0.128 (379.2 μM) μg/μL, respectively. Compounds **6** and **10** exhibited the highest adulticidal activity with LD_{50} values of 2.084 (6442.0 μM) and 2.962 (9775.6 μM) μg/mosquito, respectively. Compounds **1**, **3**, **5**, **9**, **12**, **14-24** did not show strong larvicidal or adulticidal activity.

Generally, 4-methyl substitution on the piperidine ring and 6-methoxy substitution on the benzothiazole ring, apart from compound **13**, caused the loss of larvicidal activity. On the other hand, 6-chloro substituted benzothiazoles (**6**, **7** and **8**) displayed reasonable larvicidal activity; only compound **5** did not possess any antimosquito properties related to 4-methyl substitution on the piperidine ring. This outcome indicated the importance of the chloro substitution at the sixth position of the benzothiazole scaffold. Among nonsubstituted benzothiazoles (**1-4**), only 3-methyl substituted piperidine-based compound **2** and 3,5-dimethyl substituted piperidine-based compound **4** showed significant larvicidal activity, indicating that the methyl substitution at the third position of the piperidine ring enhanced the larvicidal activity. The presence of 3-methyl moiety on piperidine ring also influenced adulticidal activity positively as observed in compounds **6** and **10**. In general, the loss of larvicidal activity was detected with 6-ethoxy and 6-nitro substitutions on the benzothiazole ring as observed in compounds **17-24**.

According to docking results, compounds **2**, **4**, **6**, **7**, **8**, **10**, **11**, and **13** showed high affinity and substrate-specific interactions in the active site of *Ae*SCP2 [30,34] (**Figure: 1**). Benzothiazoles of compounds **2**, **6**, **7**, **10**, **11**, and **13** presented π-π interactions with Arg15 and Phe105 residues of the binding site of *Ae*SCP2. The methoxy substitution on the benzothiazole ring of compound **13** could account for all interactions of this compound. Piperidines of compounds **4**, **6**, **7**, **8**, **10** and **11** displayed cation-π interaction with the Arg15 residue and only 4-methyl substitution on the piperidine ring did not have a positive influence. All interactions of compounds **4** and **8** were related to 3,5-dimethyl substitution on the piperidine ring. Moreover, the acetamido moieties of compounds **2** and **10** served as H-bond donors for the in pocket residue Leu102. The docking score, glide gscore and glide emodel results of compounds **2**, **4**, **6**, **7**, **8**, **10**, **11**, and **13** are also given in **Table 3**. The emodel score is appropriate for the comparison of different conformations of the same ligand, but the docking score is generally used for the comparison of different ligands [35]. The docking scores of the compounds were determined to range between -8.19 and -8.95 kcal/mol as similar to each other.

Table 3: Docking score (kcal/mol), glide gscore (kcal/mol) and glide emodel (kcal/mol) results of compounds **2**, **4**, **6**, **7**, **8**, **10**, **11** and **13** for *Ae*SCP2 (PDB code: 1PZ4).

Compound	Docking score	Glide gscore	Glide emodel
2	-8.30	-8.38	-57.51
4	-8.46	-8.53	-48.20
6	-8.81	-8.85	-53.64
7	-8.19	-8.24	-61.35
8	-8.21	-8.27	-56.70
10	-8.95	-8.98	-67.02
11	-8.92	-8.99	-47.42
13	-8.22	-8.25	-32.66

4 Conclusions

In the present work, compounds carrying benzothiazole and piperidine rings were investigated for first instar larvicidal activity and adulticidal activity against *Ae. aegypti*. The most potent larvicidal and adulticidal compounds in this series were also analyzed for molecular docking interactions in the active site of the *Ae*SCP2 to provide mechanistic insight for further *in vitro* enzyme studies. According to mosquitocidal assays, compounds **2**, **4**, **6**, **7**, **8**, **11** and **13** were found to be the most promising larvicidal agents, whereas compounds **6** and **10** were identified as the most promising adulticidal agents. Based on the results, 3-methyl substitution on the piperidine ring enhanced the larvicidal and adulticidal activity. Besides, 6-chloro substitution on the benzothiazole ring also increased the larvicidal activity. The docking results of compounds **2**, **4**, **6**, **7**, **8**, **10**, **11** and **13** suggested that π-π interactions and hydrogen bonds were responsible for the observed affinity in the active site of the *Ae*SCP2. This outcome supported the hypothesis that the larvicidal and adulticidal activities of these compounds could result from the inhibition of *Ae*SCP2.

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

References

[1] David J.P., Ismail H.M., Chandor-Proust A., Paine M.J., Role of cytochrome P450s in insecticide resistance: impact on the control of mosquito-borne diseases and use of insecticides on Earth, Philos. Trans. R. Soc. Lond. B: Biol. Sci., 2013, 368, 20120429.

[2] Smith L.B., Kasai S., Scott J.G., Voltage-sensitive sodium channel mutations S989P + V1016G in *Aedes aegypti* confer variable resistance to pyrethroids, DDT and oxadiazines, Pest. Manag. Sci., 2018, 74, 737-745.

[3] Shu P.Y., Huang J.H., Current advances in dengue diagnosis, Clin. Diagn. Lab. Immunol., 2004, 11, 642-650.

[4] Chakraborty G., Shin J., Nguyen Q.T., Harikishore A., Baek K., Yoon H.S., Solution structure of FK506-binding protein 12 from *Aedes aegypti*, Proteins, 2012, 80, 2476-2481.

[5] Bhatt S., Gething P.W., Brady O.J., Messina J.P., Farlow A.W., Moyes C.L., et al., The global distribution and burden of dengue, Nature, 2013, 496, 504-507.

[6] WHO Regional Office for the Eastern Mediterranean Integrated vector management, 2017, Strategic framework for the Eastern Mediterranean Region 2016–2020. Cairo: Licence: CC BYNC-SA 3.0 IGO.

[7] Pompon J., Morales-Vargas R., Manuel M., Huat Tan C., Vial T., Hao Tan J., et al., A Zika virus from America is more efficiently transmitted than an Asian virus by *Aedes aegypti* mosquitoes from Asia, Sci. Rep., 2017, 7, 1215.

[8] Schuler M.A., Werck-Reichhart D., Functional genomics of P450s, Annu. Rev. Plant. Biol., 2003, 54, 629-667.

[9] Li X., Schuler M.A., Berenbaum M.R., Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics, Annu. Rev. Entomol., 2007, 52, 231-253.

[10] Liu N., Insecticide resistance in mosquitoes: impact, mechanisms, and research directions, Annu. Rev. Entomol., 2015, 60, 537-559.

[11] Stevenson B.J., Pignatelli P., Nikou D., Paine M.J., Pinpointing P450s associated with pyrethroid metabolism in the dengue vector, *Aedes aegypti*: Developing new tools to combat insecticide resistance, PLoS Negl. Trop. Dis., 2012, 6, e1595.

[12] Chandor-Proust A., Bibby J., Régent-Kloeckner M., Roux J., Guittard-Crilat E., Poupardin R., Riaz M.A., Paine M., Dauphin-Villemant C., Reynaud S., David J.P., The central role of mosquito cytochrome P450 CYP6Zs in insecticide detoxification revealed by functional expression and structural modelling, Biochem. J., 2013, 455, 75-85.

[13] Dyer D.H., Vyazunova I., Lorch J.M., Forest K.T., Lan Q. Characterization of the yellow fever mosquito sterol carrier protein-2 like 3 gene and ligand-bound protein structure, Mol. Cell Biochem., 2009, 326, 67-77.

[14] Li T., Lan Q., Liu N., Larvicidal activity of mosquito sterol carrier protein-2 inhibitors to the insecticide-resistant mosquito *Culex*

quinquefasciatus (Diptera: Culicidae), J. Med. Entomol., 2009, 46, 1430-1435.
[15] Ma H., Ma Y., Liu X., Dyer D.H., Xu P., Liu K., et al., NMR structure and function of *Helicoverpa armigera* sterol carrier protein-2, an important insecticidal target from the cotton bollworm, Sci. Rep., 2015, 5, 18186.
[16] Radek J.T., Dyer D.H., Lan Q., Effects of mutations in *Aedes aegypti* sterol carrier protein-2 on the biological function of the protein, Biochemistry, 2010, 49, 7532-7541.
[17] Kim M.S., Wessely V., Lan Q., Identification of mosquito sterol carrier protein-2 inhibitors, J. Lipid Res., 2005, 46, 650-657.
[18] Kumar R.B., Shanmugapriya B., Thiyagesan K., Kumar S.R., Xavier S.M., A search for mosquito larvicidal compounds by blocking the sterol carrying protein, AeSCP-2, through computational screening and docking strategies, Pharmacognosy Res., 2010, 2, 247-253.
[19] Singarapu K.K., Ahuja A., Potula P.R., Ummanni R., Solution nuclear magnetic resonance studies of sterol carrier protein 2 like 2 (SCP2L2) reveal the insecticide specific structural characteristics of SCP2 proteins in *Aedes aegypti* mosquitoes, Biochemistry, 2016, 55, 4919-4927.
[20] Senthil Raja P., Kathiresan K., Computational selection of mangrove-derived compounds as mosquito larvicides by blocking the sterol carrying protein, AeSCP-2, Res. Bioscientia, 2011, 2, 1-6.
[21] Sharma P.C., Sinhmar A., Sharma A., Rajak H., Pathak D.P., Medicinal significance of benzothiazole scaffold: an insight view, J. Enzyme Inhib. Med. Chem., 2013, 28, 240-266.
[22] Keri R.S., Patil M.R., Patil S.A., Budagumpi S., A comprehensive review in current developments of benzothiazole-based molecules in medicinal Chemistry, Eur. J. Med. Chem., 2015, 89, 207-251.
[23] Rouf A., Tanyeli C., Bioactive thiazole and benzothiazole derivatives, Eur. J. Med. Chem., 2015, 97, 911-927.
[24] Agarwal S., Gandhi D., Kalal P., Benzothiazole: a versatile and multitargeted pharmacophore in the field of medicinal Chemistry, Lett. Org. Chem., 2017, 14, 729-742.
[25] Mao M.Z., Wang H.Y., Wang W., Ning B.K., Li Y.X., Xiong L.X., Li Z.M., Synthesis and biological evaluation of novel *N*-pyridylpyrazolecarboxamides containing benzothiazole, Phosphorus Sulfur Silicon Relat. Elem., 2017, 192, 42-46.
[26] Venugopala K.N., Krishnappa M., Nayak S.K., Subrahmanya B.K., Vaderapura J.P., Chalannavar R.K., Synthesis and antimosquito properties of 2,6-substituted benzo[*d*]thiazole and 2,4-substituted benzo[*d*]thiazole analogues against *Anopheles arabiensis*, Eur. J. Med. Chem., 2013, 65, 295-303.
[27] Rubiralta M., Giralt E., Diez A., Piperidine: structure, preparation, reactivity, and synthetic applications of piperidine and its derivatives, Elsevier Science Publishers, Amsterdam-Oxford- New York- Tokyo, 1991.
[28] Vardanyan R., Piperidine-based drug discovery, Elsevier, Amsterdam, 2017.
[29] Altintop M.D., Özdemir A., Kaplancikli Z.A., Turan-Zitouni G., İşcan G., Akalın Çiftçi G., Synthesis and biological evaluation of some amide derivatives bearing benzothiazole and piperidine moieties as antimicrobial agents, Lett. Drug Des. Discov., 2013, 10, 453-461.
[30] Dyer D.H., Lovell S., Thoden J.B., Holden H.M., Rayment I., Lan Q., The structural determination of an insect sterol carrier protein-2 with a ligand-bound C16 fatty acid at 1.35-Å resolution, J. Biol. Chem., 2003, 278, 39085-39091.
[31] Pridgeon J.W., Becnel J.J., Clark G.G., Linthicum K.J., A high-throughput screening method to identify potential pesticides for mosquito control, J. Med. Entomol., 2009, 46, 335-341.
[32] Estep A.S., Sanscrainte N.D., Waits C.M., Louton J.E., Becnel J.J., Resistance status and resistance mechanisms in a strain of *Aedes aegypti* (Diptera: Culicidae) from Puerto Rico, J. Med. Entomol., 2017, 54, 1643-1648.
[33] Pridgeon J.W., Pereira R.M., Becnel J.J., Allan S.A., Clark G.G., Linthicum K.J., Susceptibility of *Aedes aegypti*, *Culex quinquefasciatus* Say, and *Anopheles quadrimaculatus* Say to 19 pesticides with different modes of action, J. Med. Entomol., 2008, 45, 82-87.
[34] da Silva J.B., Navarro D.M., da Silva A.G., Santos G.K., Dutra K.A., Moreira D.R., et al., Thiosemicarbazones as *Aedes aegypti* larvicidal, Eur. J. Med. Chem., 2015, 100, 162-175.
[35] Van Den Driessche G., Fourches D., Adverse drug reactions triggered by the common HLA-B*57:01 variant: A molecular docking study, J. Cheminform., 2017, 9, 13.