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Effect of long term use of PPI on high sensitive C-reactive protein, pilot study

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Antenatal and Perinatal Predictors of Hypoxic Ischemic Encephalopathy in Term Neonates Delivered in Al Zahraa Teaching Hospital in Al Najaf City

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In Vitro study on Antidiabetic Potential of Crude Plant Extracts by Attenuating Oxidative- Stress and Advanced Glycation End-Product

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Determinants of Birth Preparedness and Complication Readiness among Pregnant Women Attending Antenatal Services in Indonesia

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Cesium CS137 ,Coblit CO60 radiosource emit Beta, Gamma rays effected on Staphylococcus aureus

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, RURI PUTRI MARISKA, ABDUL KARIM ZULKARNAIN, RINA KUSWAHYUNING

Anticariogenic And Anticarcinogenic Effects Of Garcinia Mangostana Pericarp Extracts On Cariogenic Bacteria And On Cancer Cell Lines With Molecular Docking Study

DR.SUNITHA JANARDHANAN, DR.JAIDEEP MAHENDRA, DR. REVATHI KASTHURI, DR. LITTLE MAHENDRA, DR.SRUTHI SRINIVASAN, DR. SAJID. T. HUSSAIN



Title of the article: Effect of chronic periodontitis on systemic inflammation – A cross sectional study

DR. SELVAKUMAR JAYARAMAN, DR. JAIDEEP MAHENDRA, DR. LITTLE MAHENDRA, DR.SRUTHI SRINIVASAN



Viral Etiology of Pneumonia in Children with Congenital Heart Disease

RETNO ASIH SETYONIGRUM, MD, PHD, WIGIT KRISTIANO, IRWINA RAHMA ANDRIANI, PRAPTI DEWI SUHIRMAN, I CAHYADI, ALDISE MARETA NASTRI, KAZUFUMI SHIMIZU



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A Comparative Study of Absorbance Value by Heating at Different Temperature During Preparation of Staphysagria Mother Tincture with Coconut Oil By UV-Visible Spectrophotometer

DR. AARTI YASHWANT KHATAL, DR.MRS.GAJENDRAGADKAR MANISH.PRASHANT, DR.JADHAV ARUN BHARGAV

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Inflammation mediated wound healing efficacy of Chloroxylon swietenia DC. fruit phytocompounds.

, RAVISHANKARA B, RIAZ MAHMOOD, V KRISHNA, VINAYKUMAR NM, RAVIKUMAR S



Antidiabetic Activity of Lansau Herb Extract in Glucose Induced Diabetic Male Rats

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A Rare Case: Spontaneous Omental Bleeding After Tenckhoff Catheter Placement

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IL-18, Adiponectin and Metabolic Parameters in Obese Adolescents

ROEDI IRAWAN, NUR AISIYAH WIDJAJA, META HERDIANA HANINDITA, RENDI AJI PRIHANINGTYAS

The Effect of Guava (Psidium guajava L) Fruit Extract on Pulmonary Tissue and Malondialdehyde Levels of Rat (Rattus norvegicus) Exposed to Cigarette Smoke

DEWA KETUT MELES, WURLINA WURLINA, IMAM MUSTOFA, MENTARI RACHMAWATI, NILUH SUWASANTI, DESAK KETUT SEKAR CEMPAKA PUTRI

Synthesis and Evaluation of Some New Quinoline Based 1, 3, 4-Oxadiazoles as Potential Antimicrobial and Antimalarial Agents

HEMALATHA GADEGONI, SARANGAPANI MANDA, SHIVAPRASAD RANGU, SYEDA NISHAT FATHIMA

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Research Article

In-silico Studies and Synthesis of 1,3-benzoxazine Derivatives as Antimalarial Agent through PfATP4 Receptor Inhibition

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ABSTRACT:

The aims of this research is to study in-silico and synthesize of 1,3-benzoxazine derivatives as an antimalarial agent. These compound was predicted to inhibit the growth of Plasmodium sp. by inhibiting the PfATP4 receptor. The PfATP4 receptor is a receptor from Plasmodium sp. which regulates the process of hemostasis from sodium ions. Molecular docking study was performed using Molegro Virtual Docker (MVD) version 5.5 on the active site of PfATP4 receptor (PDB ID 2DQS) compared to the rerank score of its native ligand. The synthesis method of 1,3-benzoxazine derivatives has been obtained more effective and efficient by microwave irradiation. Some of 1,3-benzoxazine derivatives had ranges of rerank score from -91.22 to -74.55 kcal/mol which were lower than its native ligand. To continue on the antimalarial activity study, it is necessary to synthesize the compounds. From the results of the in-silico study and the method development of synthesis of 1,3-benzoxazine derivatives, it is expected that further research can be carried out in vitro study on erythrocyte cells infected with Plasmodium sp.

Keywords : antimalarial, in-silico, microwave; synthesis, 1,3-benzoxazine, 2DQS..

INTRODUCTION:

Infection is a disease caused by pathogenic microorganisms like bacteria, viruses, parasites, or fungi. According to Dipiro et al (2007), malaria is an infectious disease caused by parasites-like- protozoa in the human body which are transmitted through female Anopheles mosquitoes infected with Plasmodium sp. (*P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*)¹. In the tropical and subtropical regions, around 300-500 million individuals are infected with malaria and 1-2 million die annually. According to the data, the death rate of this disease for children aged <5 years is very high. For example, death rates have been controlled in the United States, however, Asia and Africa have not been able to do likewise. Over 90% of deaths caused by malaria in the world occur in Sub-Saharan Africa^{1,2,3}. The main causes of death from malaria are the failure of chemoprophylaxis, errors in the administration of chemoprophylaxis, delays and diagnostic errors¹.

According to Batista in 2008, malaria is a serious problem because the culprit, *P. falciparum*, is

increasingly resisting antimalarial drugs, especially with chloroquine. This phenomenon has been a major problem in treatment therapy since the 1960. Aside that, there are currently multi-drug resistant cases in the treatment of Artemisinin-Based Combination according to the Guidelines for the Treatment of Malaria 2015 by the World Health Organization⁴.

Various research have been carried out to develop new compounds from extracts of natural /semisynthetic materials between 1993 and 2018. The outcome of all the research which were in solving this resistance of antimalarial drugs produced compounds like alkaloids⁵, sesquiterpene, triterpenoids, flavonoids³⁻⁶, and xanthones⁷. These have the potential to be developed into new antimalarial compounds. According to Basco et al in 1994 and Batista et al in 2009, there are five categories of IC₅₀ values-based-antimalarial potential compounds, Excellent / potent activity (<1 μM), good (1-20 μM), moderate (20-100 μM), low (100-200 μM) and inactive (IC₅₀> 200 μM)^{7,8}.

According to Rudyanto et al in 2014; Putra et al in 2016; and Sharma et al., a recent research in 2018, showed that 1,3-benzoxazine derivatives, namely 6-allyl-3- (furan-2-ylmethyl) -8-methoxy-3,4 -dihydro-2H-benzo[e][1,3]oxazine, obtained from the semisynthetic process of clove oil has good antimalarial activity ($17.54 \mu\text{M}$)^{9,10,11}. Also, Sharma et al. in 2018, reported of the ability of the compound as an antimalarial agent considering the fact that it is being produced with nanomaterial graphene oxide drug delivery system with an IC50 value of $0.49 \mu\text{M}$ ¹¹.

According to Sharma et al in 2018, the derivatives of 1,3-benzoxazine compounds interfere with the process of sodium ion homeostasis in erythrocytes. This process in turn induces changes in the intracellular Na⁺ levels and mitochondrial depolarization in intraerythrocytic *P. falciparum*, hence, killing the parasites. Also, Sharma et al. in 2018 reported that it induces the changes in intracellular Na⁺ levels by inhibiting P-type cation ATPase PfATP4 receptors (figure 1). which regulate the process of sodium homeostasis. According to Rudyanto et al in 2014 and Putra et al in 2016, some derivatives of 1,3-benzoxazine have been synthesized but have not been tested for in-vitro activity in

erythrocyte cells infected with *P. falciparum*. This study developed other derivatives of 1,3-benzoxazine with antimalarial activity through in-silico study and docked to the PfATP4 receptor with PDB ID 2DQS before the synthesis and activity testing process were carried out by in vitro study.

The derivatives of 1,3-benzoxazine with a lower rerank score than the compound, 6-allyl-3-furan-2-ylmethyl)-8-methoxy-3,4-dihydro-2H-benzo[e][1,3]oxazine, were screened through the in-silico study, before discovering the compounds that were tested in-vitro. In a previous study, according to Rudyanto et al in 2014 and Putra et al in 2016, several derivatives of 1,3-benzoxazine compounds were successfully synthesized with clove oil which could react with the base of several amine derivatives through reflux heating. However in this study, we synthesized the derivative compounds of 6-allyl-8-methoxy-3-phenyl-3,4-dihydro-2H-benzo[e][1,3]oxazine using the microwave irradiation method in line with another research¹². The microwave irradiation method used which is rapid and efficient resulting in reduced reaction times and increased yields compared to conventional method such as reflux¹³⁻¹⁵.

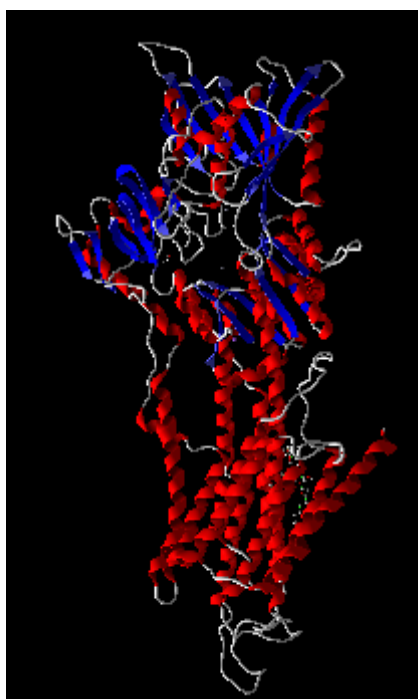


Fig.1: The Ribbon Structure of PfATP4 Receptor

MATERIAL AND METHODS:

In-silico study :

Receptor Preparation

Receptors obtained from Protein Data Banks (PDB) were repared using the Molegro Virtual Docker (MVD) Ver.5.5 software. The P-type cation

ATPase (PfATP4 receptors) were downloaded with PDB ID code 2DQS¹⁷. Furthermore, the comparative ligands from the P-type cation ATPase receptor, TG1 from the x-ray crystallography PDB download files are stored in the form of mol.files for re-docking in order to

validate the MVD Ver.5.5. used to dock the derivatives of 1,3-benzoxazine compounds. The 1,3-benzoxazine derivatives was docked on the

active site of the PfATP4 receptors. PfATP4 receptors has the active site occupied by the native ligand TG1 as shown in Figure 2.

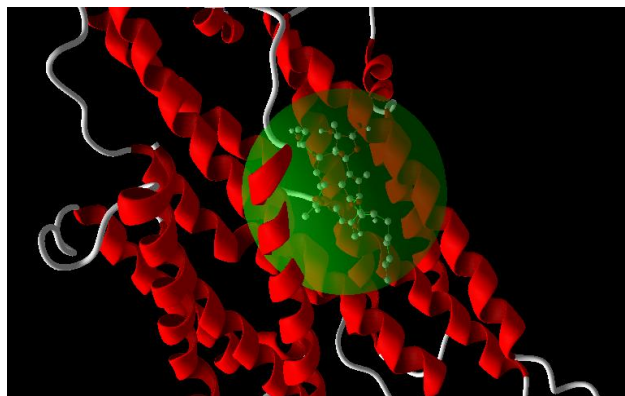


Fig.2: Active site of PfATP4 Receptor

Ligand Receptor Docking Study and Visualization of Docking Results

The docking was carried out with native ligand and 1,3-benzoxazine derivatives using MVD Ver.5.5. Before docking, Compounds 1-9 structures were built with ChemBioDraw Ultra 11.0 and their geometry optimization were performed using MMFF94¹⁸. The results obtained docking score was evaluated using rerank score as scores interpreted as predictions of bond interactions between ligand and receptors. The smaller rerank score is an indication of high level of interaction between the ligand and the receptor. The docking results can also be visualized and interpreted to provide an overview of ligand bond interactions with receptors which include hydrogen bond, steric, and electronic interactions¹⁹.

Receptor Validation and Docking study

The receptor validation is conducted by redocking the native ligand TG1 with the PfATP4 receptor. The redocking is aimed at validating the downloaded PDB file, as well as the software used for the virtual screening of the examined compounds. The redocking acceptance parameter is the Root Mean Square Deviation (RMSD) value $\leq 2.0 \text{ \AA}$ ^{11,19}. This RMSD value shows the suitability of the ligand coordinates of the crystallographic results compared to the ligand coordinates which are redocked with MVD Ver.5.5.

The RMSD value of the TG1 ligand in the redocking process with the PfATP4 receptor is 2,009 \AA as shown in Figure 3. The redocked RMSD scores prove that the downloaded PDB file and MVD Ver.5.5 are capable to be used as a virtual screening method for the derivatives of 1,3-benzoxazine.

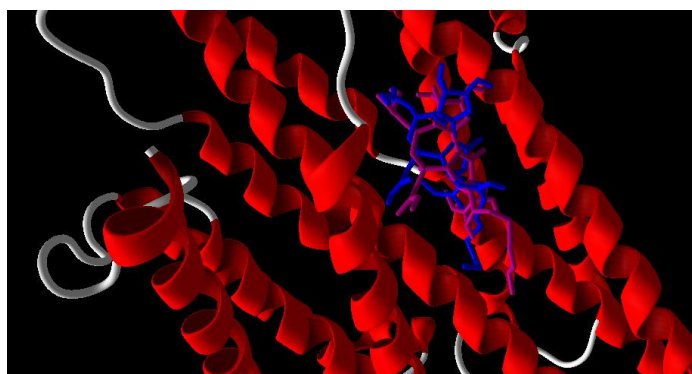


Fig.3 : Conformation Comparison of Ligand X-ray Crystallographic Structure (Purple) and the Redocking Result with Molegro Virtual Docker (MVD) Ver.5.5 (Blue).

General synthesis

Materials

The reagents used in this research, such as eugenol, formalin, aniline as well as the solvents

like methanol, n-hexane, and ethyl acetate were purchased from standard commercial suppliers. The reactions were monitored with TLC using pre-coated aluminum sheets with GF254 silica

gel, 0.2 mm layer thickness (E.Merck). The mobile phase used for TLC were n-hexane: ethyl acetate (10:1) and the spots were visualized under UV light (254 nm). Furthermore, purification of products was carried out with a column chromatography on silica gel using n-hexane: ethyl acetate (5:2). Also, the UV spectra were obtained using Shimadzu UV-1800 spectrophotometer while the FT-IR spectra were obtained using a Perkin Elmer Spectrum spectrophotometer with KBr disks. Then, the ¹H-NMR spectra were obtained on JEOL JNM-ECS400 (¹H-NMR: 400MHz) and a deuterated Chloroform was used as solvent for the analysis.

Method of Synthesis

The process commenced with the reaction between 10 mol formaldehyde with 2.5 mol

aniline, refluxed for 1 hour at 65°C and then cooled at room temperature. The deposits are filtered and recrystallized with 96% ethanol to obtain a yellow powder (base shift).

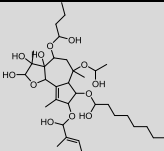
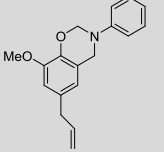
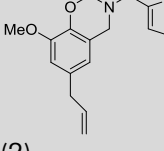
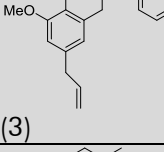
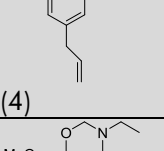
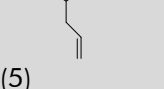
Afterwards, 10 mmol eugenol is reacted with 3.5 mmol base shift, plus 20 mmol formaldehyde, all dissolved in 5 ml of methanol. The solution is irradiated with 360 watts microwave for 5 minutes and then evaporated forming a thick liquid. This is then chromatographed with the mobile phase of n-hexane: ethyl acetate (10: 1).

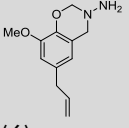
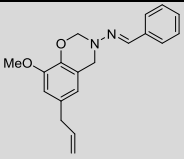
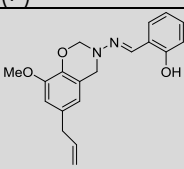
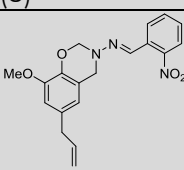
RESULTS :

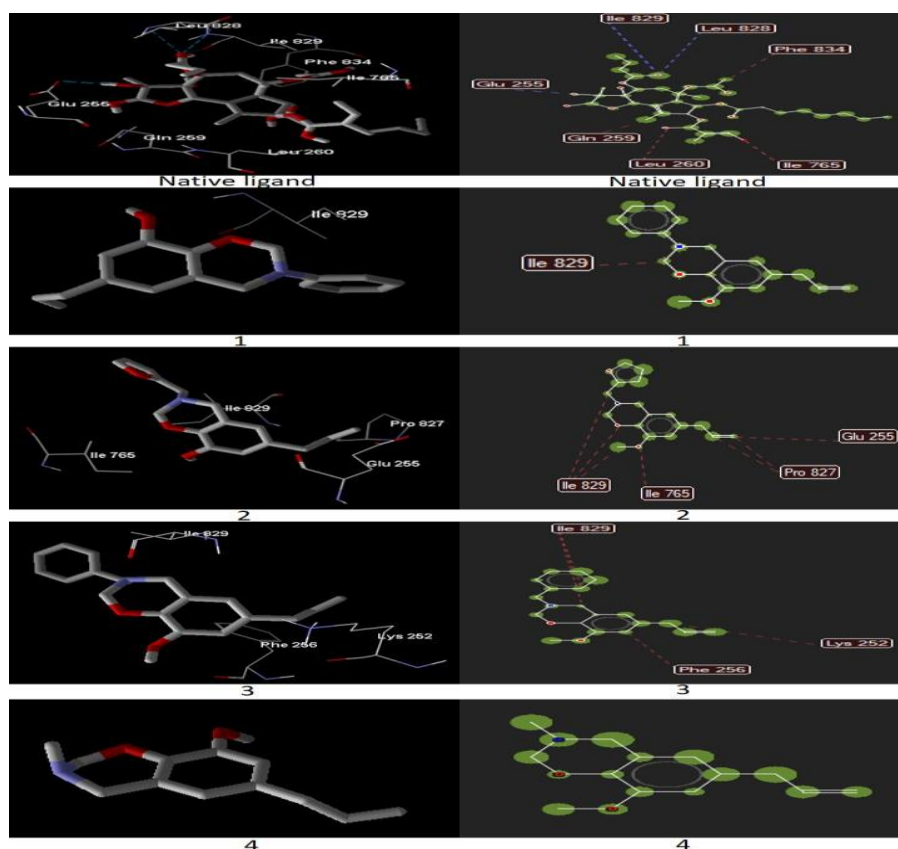
In-silico Study

The docking of 1,3-benzoxazine derivative compounds with PfATP4 receptors using MVD Ver.5.5 produced the data presented in Table 1 and Figure 4

Table 1.: Molecular Docking result of compounds and its native ligand on active site PfATP4 receptors

Compound	Re-rank score (kcal/mol)	Docked Pose	Hydrogen bond	Residues involved	Steric interaction	Residues involved
 Native Ligand	-73.13	√	3	Glu 255 Leu 828 Ile 829	3	Gln 259 Leu 260 Ile 765
 (1)	-79.27	√	-	-	1	Ile 829
 (2)	-80.60	√	-	-	4	Glu 255 Ile 765 Pro 827 Ile 829
 (3)	-74.55	√	-	-	3	Lys 252 Phe 256 Ile 829
 (4)	-64.38	√	-	-	-	-
 (5)	-63.55	√	-	-	1	Gln 259

 <p>(6)</p>	-63.98	√	-	-	-	-
 <p>(7)</p>	-81.70	√	-	-	2	Glu 255 Tyr 837
 <p>(8)</p>	-87.84	√	-	-	3	Glu 255 Tyr 827 Met 838
 <p>(9)</p>	-91.22	√	-	-	6	Gln 259 Val 769 Ile 829 Phe 834 Met 838



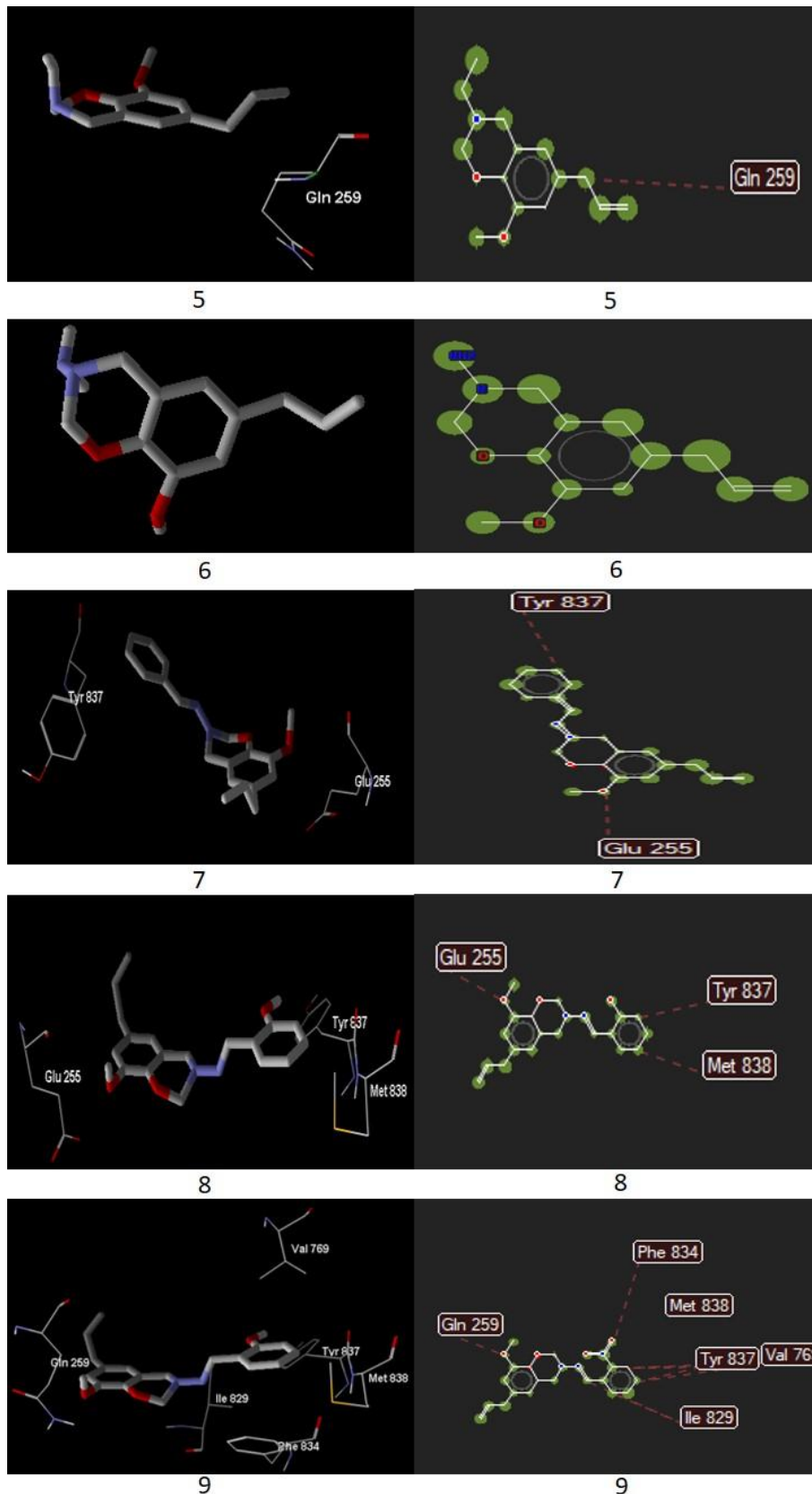


Fig.4 : The Interaction between Native Ligand (TG1) and Compounds 1-9 into Active Site of PfATP4 Receptor

Synthesis

Based on the results using the microwave irradiation, the synthesis of Compound 1 was declared successful with 66% and 5 minutes

efficiency. The UV, IR, H-NMR characteristics of Compound 1, as shown in Figure 5 and table 2-4, are as follows:.

Table 2: Interpretation FTIR Spectrum of 6-alil-8-metoksi-3-fenil-3,4-dihidro-2H-benzo[e][1,3]oksazin

Wavenumbers (cm ⁻¹)	Peak Intensity	Teoritical Wavenumbers (cm ⁻¹)[20]	Type of vibration
3060	weak	3100-3020	-CH (sp ²)
2902	strong	3000-2850	-CH (sp ³) stretch
1639	medium	1680-1600	-C=C alifatis
1651 and 1455	medium	1600 and 1475	-C=C aromatis
1265	strong	1300-1000	-C-O
1083	medium	1350-1000	-C-N

Tabel 3. Interpretation 1H-NMR spectrum of 6-alil-8-metoksi-3-fenil-3,4-dihidro-2H-benzo[e][1,3]oksazin

Hydrogen atom position (label)	Chemical Shift (ppm)	Multiplicity	Σ Hydrogen atoms
a	3.28	doblet (J=6,8 Hz)	2
b	3.83	singlet	3
c	4.55	singlet	2
d	5.04-5.09	multiplet	2
e	5.40	singlet	2
f	5.87-5.97	multiplet	1
g	6.45	singlet	1
h	6.55	singlet	1
i	6.90	triplet (J=7.3 Hz)	1
j	7.09-7.10	multiplet	2
k	7.22-7.26	multiplet	2
Total of Hydrogen Atoms			19

Table 4. Summary spectra data 1D NMR, FTIR and UV of 6-alil-8-metoksi-3-fenil-3,4-dihidro-2H-benzo[e][1,3]oksazin

Kinds of Spectra	Characteristics
¹ H-NMR Spectrum (400 MHz, CDCl ₃)	δ 3.28 (2H, d, J=6.8 Hz); δ 3.83 (3H, s); δ 4.55 (2H, s); δ 5.04-5.09 (1H, m); δ 5.40 (2H, s); δ 5.87-5.97 (1H, m); δ 6.45 (1H, s); δ 6.55 (1H, s); δ 6.90 (1H, t, J=7.3 Hz); δ 7.09-7.10 (2H, m); δ 7.22-7.26 (2H, m). There are 19 atoms of Hydrogen
FT-IR Spectrum (KBr, ν max, cm ⁻¹)	1083 (C-N), 1267 (C-O), 1651 and 1455 (C=C aromatic), 1640 (aliph C=C), 2907 (C-H sp ³), 3060 (C-H sp ²)
Ultraviolet Spectrum	λ max (nm) 282, in ethanol 70 % solution with 50.0 ppm concentration

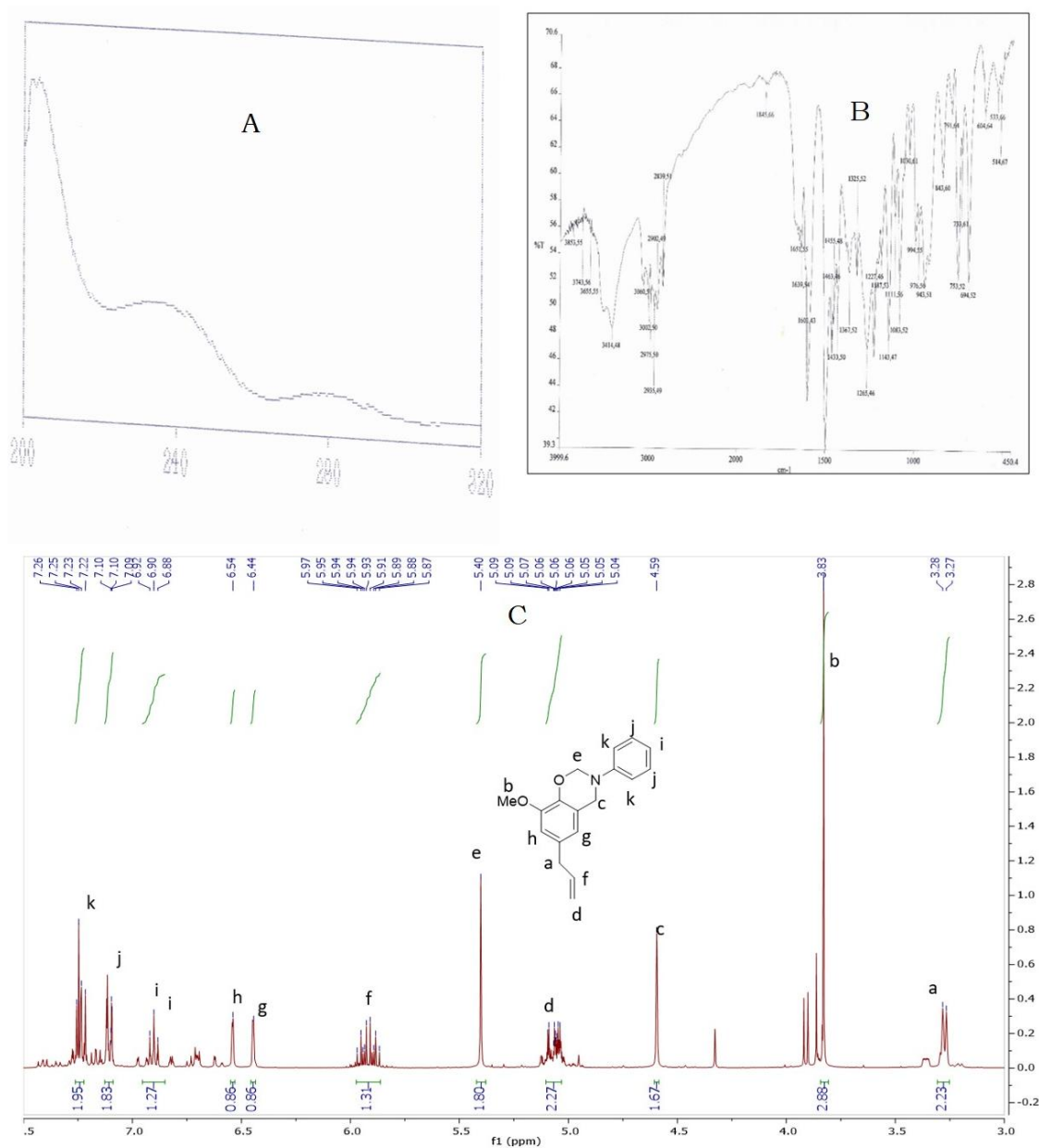


Fig.5 : A. Ultraviolet Spectrum in ethanol 70 % solution (50.0 ppm concentration). B. FT-IR spectrum in KBr pellet. C.1H-NMR spectrum in CDCl3

DISCUSSION :

Based on molecular docking result Compound 2 has a lower rerank score than its native ligand and this was proven in a study conducted by Sharma et al in 2018. According to the study, the Compound 2 has antimalarial activity with good criteria at $IC_{50} = 17.54 \mu M^{11}$. Also, the visualization of the molecular docking of Compound 2 showed some steric interactions on Glu amino acid residues 255; Ile 765; Pro 827; and Ile 829 which are also found in the native ligand. Compound 9 has the potential to be

synthesized as the antimalarial agent because it has a benzohydrazide group which makes its rerank score lower than that of Compound 2 which was believed to be more potent in antimalarial activity.

Based on synthesis result, 6-allyl-8-methoxy-3-phenyl-3,4-dihydro2H-benzo-[1.3]oxazine was successfully obtained with good yield. This was obtained in 66% yield in the form of orange oil. UV (λ_{max}) nm: 282. FT-IR (KBr) cm^{-1} : 1083 (C-N), 1267 (C-O), 1651 and 1455 (C=C aromatic), 1640 (aliph C=C), 2907 (C-H sp^3),

3060 (C-H sp²). ¹H-NMR (CDCl₃, δ, ppm): δ 3.28 (2H, d, J=6.8 Hz); δ 3.83 (3H, s); δ 4.55 (2H, s); δ 5.04-5.09 (1H, m); δ 5.40 (2H, s); δ 5.87-5.97 (1H, m); δ 6.45 (1H, s); δ 6.55 (1H, s); δ 6.90 (1H, t, J=7.3 Hz); δ 7.09-7.10 (2H, m); δ 7.22-7.26 (2H, m).

CONCLUSIONS :

2 The 1,3-benzoxazine derivatives with aromatic substituents are more suitable to be used as antimalarial agents with the PfATP4 receptor. They possess this ability because they have a re-rank score ranging from -91.22 to -74.55 kcal/mol, which is lower than the native ligand. Also, the microwave irradiation method could be used to synthesize of 1,3-benzoxazine derivatives in a more effective and efficient manner compared with the reflux method.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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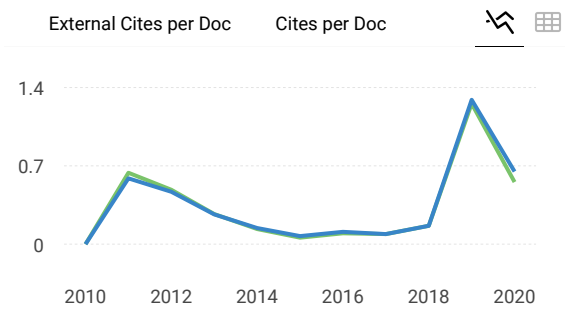
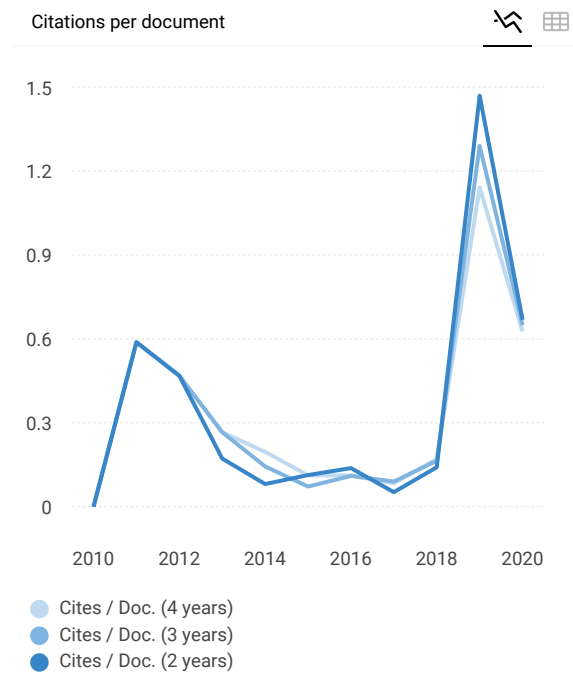
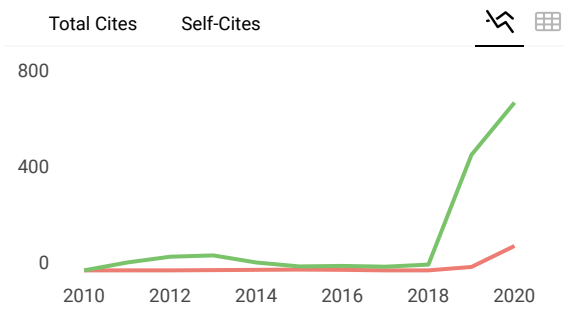
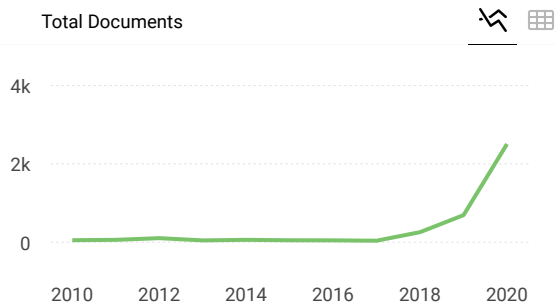
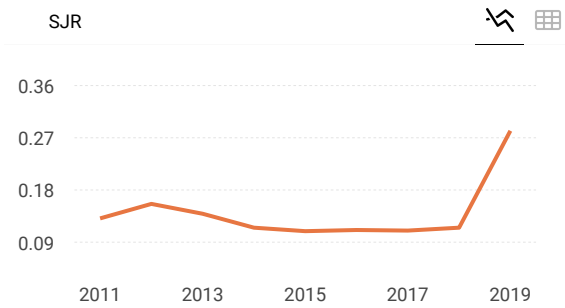
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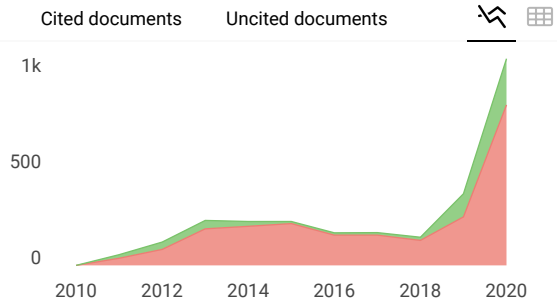
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