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THE POTENTIAL ANTIMALARIAL DRUG OF ARYL AMINO ALCOHOL DERIVATIVES FROM EUGENOL: SYNTHESIS, *in-vitro* AND *in-silico* ANALYSIS OF BIOACTIVITY

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ABSTRACT

Malaria is a significant cause of death in numerous countries, and the discovery of effective drugs is crucial. In this research, a new method was employed to synthesize a derivative of aryl amino alcohol using eugenol. Initially, an epoxide compound called 2-methoxy-4-(oxiran-2-ylmethyl) phenol was synthesized from eugenol by reacting it with peroxyacetic acid in dichloromethane. Subsequently, the epoxide compound was subjected to a reaction with various amine compounds, including aniline, naphthylamine, and diphenylamine for 10 minutes under 100 W microwave conditions, resulting in the production of three derivative compounds of aryl amino alcohol. The antimalarial activity of these synthesized compounds was assessed *in-vitro* through an assay called heme polymerization inhibition activity (HPIA). Additionally, an *in-silico* analysis was conducted to evaluate the bioactivity of the three aryl amino alcohol derivatives by performing molecular docking with the Plasmepsin II enzyme. The identification of the products were determined using Fourier Transform Infrared (FTIR) spectrophotometry, proton nuclear magnetic resonance (^{1}H-NMR) spectrometry, and Electrospray ionization-Mass Spectrometry (ESI-MS). The research successfully obtained three aryl amino alcohol derivatives: 2-hydroxy-3-(phenylamino) propyl)-2-methoxyphenol, 4-(2-hydroxy-3-(naphthalene-1-ylamino) propyl)-2-methoxyphenol, and 4-(2-hydroxy-3-(diphenylamine)-2-methoxy phenol. All the synthesized compounds demonstrated lower IC_{50} values compared to chloroquine, indicating their potential as antimalarial drugs. Molecular docking studies revealed that these aryl amino alcohol derivatives effectively inhibited the activity of the Plasmepsin II enzyme.

Keywords: Aryl Amino Alcohol, Antimalarial Drug, *in-vitro*, *in-silico*, Plasmepsin II.

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INTRODUCTION

Malaria, a disease caused by a protozoan parasite, is a significant global cause of death. The World Malaria Report 2020, compiled by the World Health Organization, revealed that approximately 229 million malaria cases occurred in 2019, resulting in an average of 400,000 fatalities. The majority of those affected are children under the age of five. Africa bears the highest burden of malaria cases (around 90%), followed by Southeast Asia, South America, and Sub-Saharan Africa.[1] The malaria parasite is transmitted to humans through female mosquitoes of the Anopheles genus. Anopheles mosquito larvae thrive in stagnant water, such as swamps. Malaria is a disease that occurs due to an infection caused by a tiny organism called a parasite. This parasite belongs to the Plasmodium genus. Humans can be infected by six distinct types of Plasmodium species, namely Plasmodium falciparum, Plasmodium malaria, Plasmodium ovale curtisi, Plasmodium vivax, Plasmodium ovale wallikeri, and Plasmodium knowlesi. Out of these species, Plasmodium falciparum is the most dangerous one. It spreads quickly and widely across the globe, which has motivated researchers to look for new ways to treat malaria. There are several well-known antimalarial chemotypes that show promise as potential antimalarial drugs. These include quinine, quinidine, mefloquine, lumefantrine, and halofantrine. These compounds belong to a class called aryl amino alcohol derivatives, specifically beta- or gamma-amino alcohols. Numerous studies have demonstrated that these classic antimalarial drugs derived from amino alcohols can inhibit heme polymerization, as the antimalarial drugs target.[3-6] Inside the digestive vacuole, hemoglobin is broken down into globin and heme. The heme molecules then combine to form hemozoin, while the globin is hydrolyzed into amino acids.

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The drug of antimalarial works by inhibiting hemozoin formation, generating free radicals within the digestive vacuoles, or inhibiting globin hydrolysis.[7] The hydrolysis of globin is facilitated by various classes of proteases, including vacuole aspartate (Plasmepsin), cysteine (Falcipain), and metalloprotease (Falcilysin), as well as cytosolic metalloaminopeptidases. All of these enzymes represent potential targets for malaria treatment. Four types of Plasmepsin are believed to be involved in the hydrolysis of hemoglobin within the digestive vacuoles, with Plasmepsin II and possibly others playing a role in the initial cleavage.[8] A compound's chemical structure must meet certain criteria to exhibit antimalarial activity, such as the presence of amino alcohol and aromatic groups connected by carbon chains with a length of 2 or 3 carbon atoms.[9] Therefore, it is crucial to conduct research focused on synthesizing new aryl amino alcohol derivative compounds with antimalarial bioactivity. Numerous studies have been conducted on the synthesis of amino alcohol derivatives, with notable contributions from Robin et al. The researchers conducted a study where they created different types of 1-aminopropan-2-ol products by performing reactions on various alkene compounds, specifically by opening the epoxide and using microwave assistance. All the resulting derivatives of 1-aminopropan-2-ol showed effectiveness against malaria, and seven of them exhibited activity against the Plasmodium falciparum strain, with IC_{50} values ranging from 1 to 10 micromolar. Another group of researchers led by Perez synthesized multiple 1-aryl-3-propanol compounds and evaluated their effectiveness against the Plasmodium falciparum strain 3D7. Among these compounds, twelve demonstrated an IC_{50} value below one micromolar. Additionally, Jain and Bele developed a derivative called 2-alkoxy/phenoxy-3-amino-3-arylpropan-1-ol through the reductive ring-opening of various azetidine-2-on compounds. All the synthesized compounds showed activity in inhibiting the growth of Plasmodium falciparum parasites.[12] An aryl amino alcohol compound derived from fluoro naphthyl exhibited an IC_{50} value of 0.5 micromolar, equivalent to chloroquine.[13] Tacon and colleagues developed a semi-synthetic derivative of beta-amino alcohol from totarol. The antiplasmodial activity of all the beta-amino alcohol derivatives derived from totarol was found to be superior when compared to totarol itself.[14] The analog compound 1-(1-aminopropan-2-ol) carbazole showed significant activity against Plasmodium falciparum K1 strains. Further research on structure-activity relationships (SAR) and optimization of early absorption, distribution, metabolism, and excretion (ADME) properties demonstrated good efficacy When given orally to mice infected with Plasmodium berghei, doses of 11.4 and 34.5 mg/Kg/day showed ED_{50} values and reduced the parasites in Plasmodium berghei-infected rats by 99.97% and 99.93%, respectively. Administration of the highest dose of 100mg/kg/day resulted in the same reduction percentage.[15] Aryl amino alcohol compounds can be synthesized from olefin compounds, which are first converted into epoxides and then reacted with amines through epoxide ring-opening reactions.[10] Eugenol, a major chemical component of clove oil, is a readily available source of olefin compounds found in nature. Clove oil contains 45-90% eugenol.[16] In this study, the amine and aryl groups for the aryl amino alcohol framework were obtained from various amine compounds, including aniline, naphthylamine, and diphenylamine. The aim of this research is to synthesize new aryl amino alcohol derivative compounds with antimalarial properties and perform molecular docking studies to analyze the interactions between aryl amino alcohol complexes and the Plasmepsin II enzyme.

EXPERIMENTAL

Materials and Methods

In this study, the synthesis was carried out using several tools including analytic balance, glassware, a Hot plate-magnetic stirrer (Barnstead Thermolyne Cimarec), and a Microwave-assisted reactor. Identification and characterization using TLC (Thin Layer Chromatography) of Silica gel 60 F254 (Merck), TLC Chamber, silica gel 60 F254 with gypsum powder for preparative thin layer chromatography, Gas Chromatography-Mass Spectrometer (Shimadzu QP2010SP), FTIR (Fourier Transform Infrared) Spectrophotometer (UATR Perkin Elmer), ^{1}H-Nuclear Magnetic Resonance Spectrometer (Jeol 500 MHz), Positive Electrospray Ionization-Mass Spectrometer (Thermo Scientific TSQ QUANTUM ACCESS MAX Triple Quadrupole). Equipment used to test antimalarial activity through inhibition of heme polymerization including micropipette (Eppendorf), microcentrifuge tube (Eppendorf), microplate 96 wheels (Iwaki), conical tube (Iwaki), Incubator (Memmert), Microplate reader (xMarkTM) and centrifuge (Thermo Scientific). Molecular tethering uses a computer with a Pentium Core i5 RAM 8 gigabyte specification. The program used is Gaussian 09W, the SPSS release 23.0 statistical program, Chimera 1.10., and Pymol 2.2.

The materials used in this study were eugenol (PT. Natura Aromatic, Karanganyar, Central Java, Indonesia) sulfuric acid (Merck), 30% hydrogen peroxide (Merck), glacial acetic acid (Merck), dichloromethane (Merck), ethanol (Merck), ethyl acetate (Merck), n-hexane (Merck), aniline (Sigma-Aldrich), naphthylamine (Sigma-Aldrich) diphenylamine (Sigma-Aldrich), sodium hydroxide (Merck), acetone (Merck), dimethylsulfoxide/DMSO (Merck), hematin (Sigma-Aldrich), and chloroquine (Konimex).

Synthesis of Epoxide Compound from Eugenol

To start the process, completely dissolved 0.1 mol of eugenol in dichloromethane. Then, slowly add a 0.1 mol solution of peracetic acid to the eugenol solution and stir overnight. Next, the solution is washed with distilled water until the pH reaches 6. Anhydrous sodium sulfate is added to eliminate any remaining water, and the solution is evaporated. This results in a coarse, dense dark brown substance. To isolate 2-methoxy-4-(oxiran-2-ylmethyl) phenol (EE), TLC is performed using a mixture of n-hexane and ethyl acetate (in a ratio of 8:1). The resulting compound is then analyzed for percent yield using FTIR spectrophotometer and ^{1}H-NMR (proton nuclear magnetic resonance) spectrometer.

Synthesis of Aryl Amino Alcohol Derivatives

A solution was prepared by dissolving amine compounds (aniline, naphthyl amine, and diphenylamine) in ethanol, with a maximum of 0.00405 moles. Afterward, the solution was supplemented with a quantity of eugenol epoxide (EE) equal to 0.00270 moles and stirred until a uniform mixture was obtained. The reaction mixture was then subjected to microwave conditions at 100 W for 10 minutes. The resulting reaction mixture was evaporated using a rotary evaporator until a crude product was obtained. To separate and obtain aryl amino alcohol derivatives, preparative TLC was employed using a blend of n-hexane and ethyl acetate in a ratio of 8:1. The resulting compounds were identified using thin-layer chromatography, as well as techniques such as infrared spectroscopy, nuclear magnetic resonance proton spectrometry (^{1}H-NMR), and ESI-MS.

***In-vitro* Antimalarial Activity Test through Heme Polymerization Inhibition Assay**

The heme polymerization inhibition assay is a commonly utilized *in-vitro* method for assessing the effectiveness of compounds in treating malaria. In this study, the assay followed the methodology outlined by Basilico *et al.,* with slight adjustments in the concentrations of the sample and hematin.[17] The experimental group for heme polymerization inhibition consisted of positive controls (chloroquine), negative controls (10% DMSO), and a blank solution (aqua dest). The heme polymerization reaction was initiated by combining 100 microliters of hematin (1000 μM) dissolved in 0.1 M NaOH with 50 microliters of the test material at various concentrations (5.00, 2.50, 1.25, and 0.63 mg/mL). Three replicates were conducted for each concentration. The resulting mixture was incubated at 37°C for 24 hours. Following incubation, the microcentrifuge tubes were centrifuged at 8000 rpm for 10 minutes, and the resulting precipitate was washed three times with 200 microliters of DMSO. The precipitate was then homogenized in 200 microliters of 0.1 M NaOH, and 100 microliters of the resulting solution were transferred to a microplate for measuring the absorbance at 405 nm using a microplate reader. IC50 values were calculated for each test material. The heme polymerization inhibition expressed as IC50 as compared to the negative control using probit analysis. The relationship between the percentage inhibition probit value and the logarithm of the concentrations was determined using a linear regression equation. The percentage of heme polymerization inhibition for each test material was assessed by comparing the IC50 values with the blank using probit analysis and the linear regression equation that correlated the percentage inhibition probit value with the logarithm of the concentrations.

***In-silico* Analysis of Bioactivity through Molecular Docking of Plasmepsin II**

Geometry Optimation

The structural arrangement of all ligands was optimized using Gaussian software through the semi-empirical geometry optimization (AM1) method.

Preparation

The Plasmepsin II enzyme, known for its interaction with R36 inhibitors, is obtained in its three-dimensional form from the Protein Data Bank (PDB) database using the website www.rcsb.org. The specific PDB ID used is 1LEE. The obtained 3D structure is then processed using the UCSF Chimera program with

default settings to ensure compatibility between the protein macromolecule structure and the R36 inhibitors. Each structure is saved in the .pdb format for further analysis.

Molecular Docking

For the molecular docking analysis, the Pyrx 0.8 program was utilized, which integrates Autodock4 and Autodock Vina. The optimized molecular structures of the Plasmepsin II enzyme, R36 inhibitor ligand, and three aryl amino alcohol compounds were subjected to molecular docking simulations. In the docking process, a grid box was defined around the active site of the Plasmepsin II enzyme, utilizing the spatial dimensions occupied by the R36 inhibitor ligand. The same dimensions of 10 cm x 15 cm x 10 cm were employed for the molecular docking of the three aryl amino alcohol compounds. The outcome of this process yielded the binding energy value for each ligand, providing insights into their potential interactions.

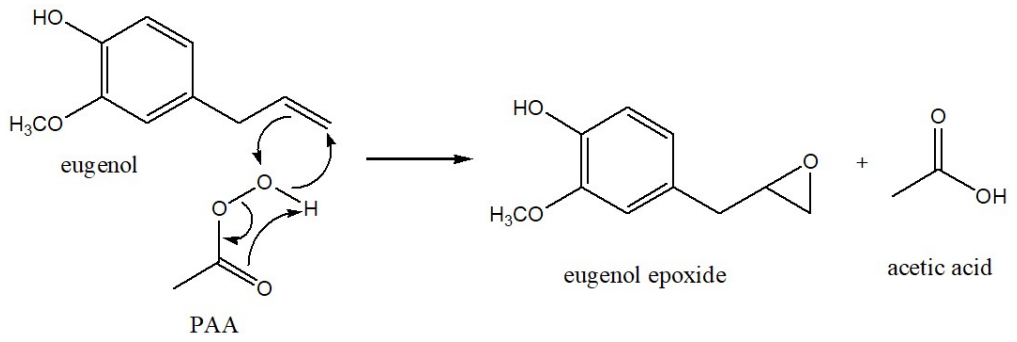
Visualization

Pymol software version 2.2 was employed to visualize and analyze the interaction between the Plasmepsin II enzyme and the ligands.

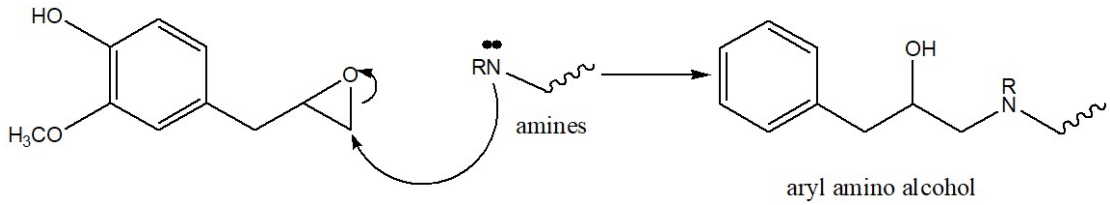
RESULTS AND DISCUSSION

Typically, the synthesis of various aryl amino alcohol derivatives starts with an epoxidation reaction of eugenol (Step-1). This reaction produces a compound known as 2-methoxy-4-(oxiran-2-ylmethyl) phenol (EE). Subsequently, the epoxide product reacts with different amine compounds, such as aniline, naphthylamine, and diphenylamine, to yield aryl amino alcohol compounds (Step-2).

Step-1



Step-2

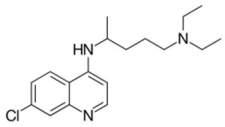
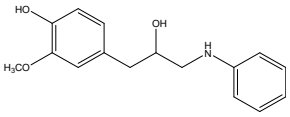
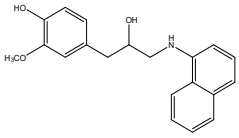
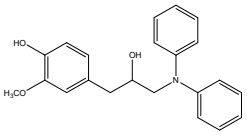


The aryl amino alcohol derivatives derived from eugenol consist of 4-(2-hydroxy-3-(phenylamino) propyl)-2-methoxyphenol (EE-Aniline), 4-(2-hydroxy-3-(naphthalene-1-ylamino) propyl)-2-methoxyphenol (EE-Naph), and 4-(2-hydroxy-3-(diphenylamine) propyl)-2-methoxyphenol (EE-DPA). Chromatographic and spectroscopic identification confirmed the successful synthesis of the expected aryl amino alcohol compounds.

Heme Polymerization Inhibitory Assay

Aryl amino alcohol derivatives combined with aminoquinoline belong to a group of antimalarial compounds that exhibit inhibitory activity in the formation of hemozoin.[18] The findings of the heme polymerization inhibitory assay conducted on the compounds synthesized from aryl amino alcohol derivatives in this research are presented in Table-1.

Table-1: The IC_{50} Values of the Aryl Amino Alcohol Derivative Compounds Synthesized for the Heme Polymerization Inhibition Activity Test

Compounds	Chemical Structure	Concentrations (mg/mL)	average % inhibition	IC_{50} (mg/mL)
Chloroquine		5.00	47.06	4.63
		2.50	41.45	
		1.25	32.81	
		0.62	11.65	
		0.31	-15.11	
EE-Anilin		1.00	83.57	0.17
		0.50	75.04	
		0.25	65.49	
		0.12	57.92	
		0.06	8.95	
EE-Naph		1.20	70.38	0.32
		0.60	58.85	
		0.30	56.13	
		0.15	47.92	
		0.07	39.29	
EE-DPA		2.00	69.67	0.54
		1.00	60.80	
		0.50	51.15	
		0.25	34.18	
		0.12	18.36	

In this research, the IC_{50} values of all the synthesized compounds were found to be higher than those of chloroquine, indicating their strong potential as antimalarial drugs. The presence of amino and hydroxyl groups connected by a few carbon atoms is a key factor contributing to their antimalarial activity. Similar to classic antimalarial compounds like quinine, mefloquine, lumefantrine, and halofantrine, which belong to the beta- or gamma-amino alcohol derivative class, the aryl amino alcohol derivatives synthesized in this study inhibit heme polymerization. The inhibition mechanism involves the formation of Fe(III)-PPIX-aryl amino alcohol complexes. These complexes are formed through a covalent bond between the Fe(III)-PPIX atom (hematin) with an empty d-orbital and the nitrogen atom of the amine group and the oxygen atom of the hydroxyl group within the chemical structure of the aryl amino alcohol. This complex formation between hematin and the active site of the amino alcohol prevents the binding of hematin to other hematin molecules, thereby inhibiting hemozoin polymerization. The synthesized aryl amino alcohol derivatives in this study are classified as beta-amino alcohols with an aryl group attached to the amines, and they exhibit a similar mechanism of heme polymerization inhibition as reported in previous studies. The relationship between the inhibition of heme polymerization by the aryl amino alcohol compounds is illustrated in Fig.-1.

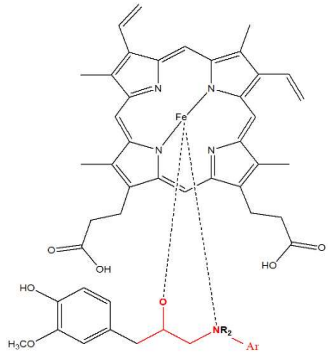


Fig.-1: The Hypothesis of the Mechanism of Heme Polymerization Inhibition by Derivatives of Amino Alcohols Derived from Eugenol

The obtained IC_{50} value suggests that the presence of aryl groups has a significant effect on the inhibitory activity of heme polymerization. The electron delocalization in the phenyl group may cause the electron cloud to be attracted to the nitrogen atom, resulting in decreased electron density. This, in turn, affects the nitrogen atom's ability to bind to the Fe hematin atom. The higher the delocalization ability of the aryl groups directly bonded to the amine's nitrogen atom, the lower the inhibitory capacity of heme polymerization. On the other hand, the substitution of hydroxyl and aryl groups derived from eugenol does not appear to have a substantial impact on heme polymerization inhibition.

Molecular Docking Study on the Inhibitor of Plasmepsin II

In the erythrocytic stage, the parasite depends on the breakdown of hemoglobin within red blood cells as a source of nutrients for its growth. This degradation occurs within parasitic food vacuoles, where a significant portion, ranging from 25% to 75% of hemoglobin, is broken down, releasing amino acids necessary for the synthesis of parasitic proteins.[19] The process of hemoglobin degradation involves various types of protease enzymes. These enzymes can be categorized into different groups based on their roles in erythrocyte invasion, erythrocyte breakdown, and hemoglobin hydrolysis. Within the parasitic digestive vacuoles, three main types of protease enzymes are present: Plasmepsin (aspartate protease), Falcipain (cysteine protease), and Falcilisin (metalloprotease). Inhibiting the mechanism of hemoglobin hydrolysis prevents the parasites from obtaining amino acids as nutrients, leading to their demise.[20] Initially, hemoglobin is hydrolyzed by aspartate protease enzymes (Plasmepsin I and II), followed by the action of cysteine protease enzymes (Falcipain) and other proteases. Inhibitors of cysteine proteases cause osmotic swelling of the digestive vacuoles, eventually resulting in the death of the parasites.[21] Among the various types of Plasmepsin enzymes, Plasmepsin II plays a crucial role in the initial stage of hemoglobin hydrolysis and is the primary target for intervention. In this research, the molecular tethering in silico study focuses on the Plasmepsin II protease enzyme. The crystal structure of Plasmepsin II with PDB ID: 1Lee was obtained from the Protein Data Bank website (http://www.rcsb.org). The Plasmepsin II enzyme interacts with the R36 inhibitor, which serves as the reference ligand. The specific R36 inhibitor used in this study is 4-amino-N-(4-[2-(2,6-dimethyl-phenoxy)-acetylamino]-3-hydroxy-1-isobutyl-5-phenyl-pentyl)-benzamide, as depicted in Fig.-2. By examining its chemical structure, the R36 inhibitor can be categorized as an aryl amino alcohol derivative, similar to the compounds synthesized in this research.

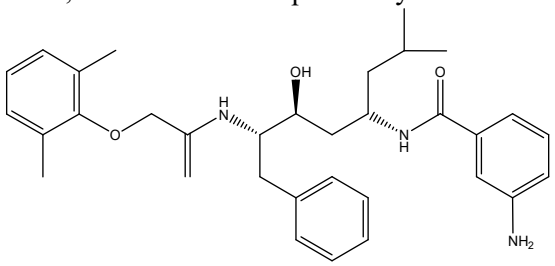


Fig.-2: Chemical Structure of R36 Inhibitor

This study involved the molecular docking analysis of the active ligand R36, as well as chloroquine and three aryl amino alcohol derivatives synthesized in the research (EE-Aniline, EE-Naph, and EE-DPA). The focus of the analysis was on studying binding energy, the Interaction of Van der Waals, and hydrogen bonds using *in-silico* methods. The Autodock Vina program, in conjunction with the Pyrx program, was employed to conduct the analysis, yielding binding energy data for each compound. The binding energy of active ligand and aryl amino alcohol derivatives are shown in Table-2.

Table-2: Binding Energy of Active Ligand R36 and Aryl Amino Alcohol Derivatives

No.	protein-ligand complexes	Binding Energy (kcal/mol)
1	1Lee protein-active ligand (R36)	-7.9
2	1Lee protein-chloroquine	-6.5
3	1Lee-protein-EE-Anilin	-7.1
4	1Lee-protein-EE-Naph	-7.2
5	1Lee-protein-EE-DPA	-7.8

The data presented in Table-2 indicates that the binding energy values for the three aryl amino alcohol derivatives range from -7.1 kcal/mol to -7.8 kcal/mol, which is slightly lower than the active binding energy of the R36 ligand (-7.9 kcal/mol) to the Plasmepsin II enzyme. However, these values are still in close proximity to the active binding energy of the R36 ligand. Therefore, the three aryl amino alcohol derivatives show great potential as inhibitors of the Plasmepsin II enzyme. The interaction between the Plasmepsin II enzyme and all ligands was visualized and analyzed using Pymol software version 2.2.

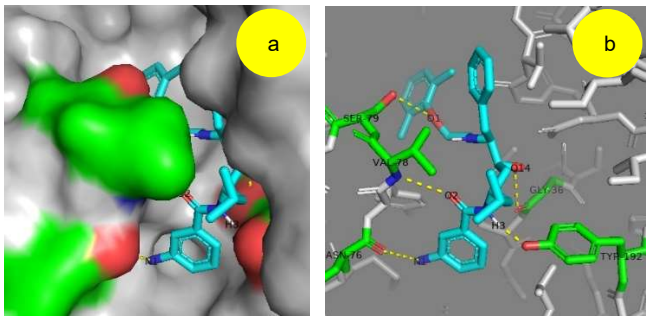


Fig.-3: (a.) R36 in the Active Site of the Plasmepsin II Enzyme, (b.) the Interaction Between R36 and Amino Acids the Plasmepsin II Enzyme

The information presented in Fig.-3 illustrates the interaction between the active ligand R36 and the Plasmepsin II enzyme, which involves the formation of five hydrogen bonds. These hydrogen bonds occur between the N1 atom of R36 and the amino acid ASN-76, the O2 atom of R36 and the amino acid VAL-78, the O1 atom of R36 and the amino acid SER-79, the O14 atoms of R36 and the amino acid GLY-36, and the H3 atom of R36 and the amino acid TYR-192. The presence of these hydrogen bonds contributes to the high binding energy of R36, which is measured at -7.9 kcal/mol. EE-Aniline forms two hydrogen bonds with amino acids in the Plasmepsin II enzyme. The first hydrogen bond is between the hydrogen atom of the amine group and the amino acid ASP-34, and the second hydrogen bond is between the hydrogen atom of the hydroxyl group and the amino acid GLY-36 (Fig.-4).

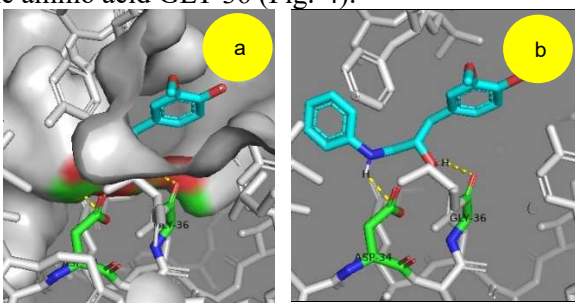


Fig.-4: (a.) EE-Aniline in the Active Site of the Plasmepsin II Enzyme; (b.) The Interaction Between EE-Aniline and Amino Acids of Plasmepsin II Enzyme

The number of hydrogen bonds formed is relatively lower compared to R36, resulting in a binding energy value of -7.1 kcal/mol for EE-Aniline. On the other hand, EE-Naph forms only one hydrogen bond with an amino acid in the Plasmepsin II enzyme, specifically between the amine group's hydrogen atom and the amino acid ASP-214 (Fig.-5).

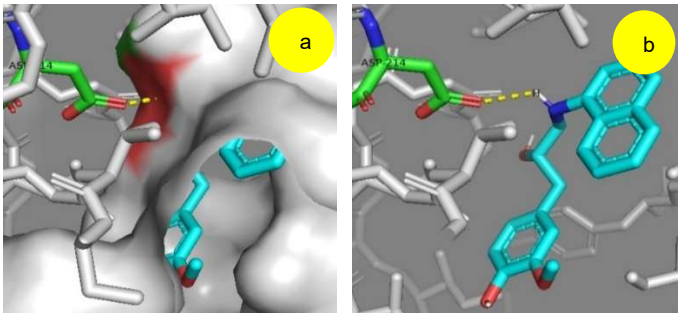


Fig.-5: (a.) EE-Naph in the Active Site of the Plasmepsin II Enzyme; (b.) Interaction Between EE-Naph with Amino Acids Plasmepsin II Enzyme

The slightly higher binding energy value of -7.8 kcal/mol for EE-Naph is mainly influenced by the interaction between EE-Naph and non-polar amino acids. Regarding EE-DPA (Fig.-6), it is assumed that the compound does not interact through hydrogen bonds but rather through the interaction of Van der Waals with non-polar amino acids such as phenylalanine (PHEN-294), serine (SER-37 and SER-79), and valine (VAL-78). The binding energy value of EE-DPA is -7.8 kcal/mol. In Fig.-7, chloroquine forms a single hydrogen bond with the Plasmepsin II enzyme, specifically between the amine group's hydrogen atom and the amino acid GLY-216.

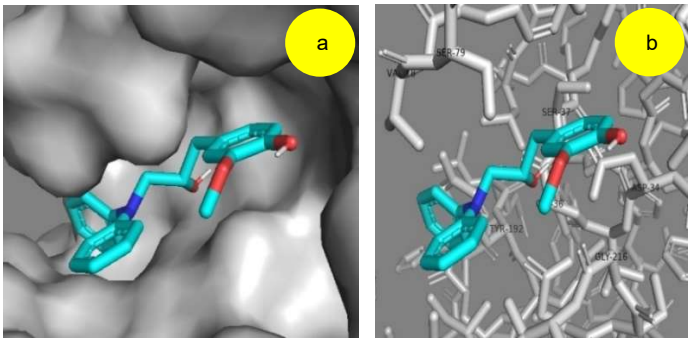


Fig.-6: (a.) EE-DPA in the active site of the Plasmepsin II enzyme; (b.) the interaction between EE-DPA and the amino acids Plasmepsin II enzyme

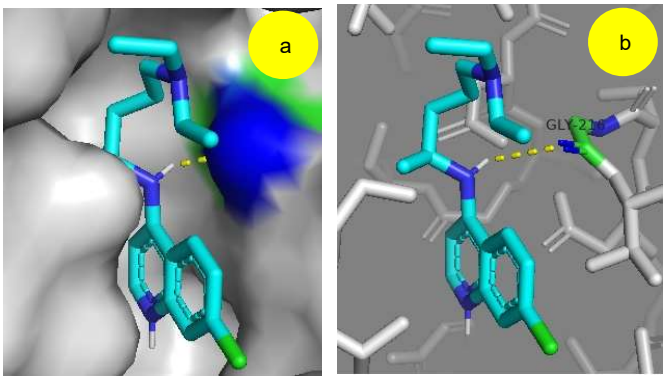


Fig.-7: (a.) Chloroquine in the active site of the Plasmepsin II enzyme; (b.) the interaction between chloroquine and amino acids in the Plasmepsin II enzyme

Data of Chromatographic and Spectrometric Analysis

Synthesis of 2-methoxy-4-(oxiran-2-ylmethyl) phenol (EE)

Brown oil (41.16% yield); ^{1}H NMR ($CDCl_3$) δ (ppm): 6.821- 6.837 (Ph), 5.078 (s, 1H, Ph-OH); 3.878 (s, 3H, -OCH_3); 3.328 (t, 1H, oxirane); 2.900 (d, 2H, oxirane); 3.250 (d, 2H, -CH_2-Ph); Spectra has been confirmed using ^{1}H NMR prediction include in ChemDraw software. FTIR ν (cm^{-1}): 3436.30 (-OH); 3076.30 (weak), 1605.05 and 1511.10 (sharp) as an aromatic alkene; 2938.18 and 2841.75 (stretch. C-H of methylene); 1430.68 (bend. C-H of methylene); 1365.78 stretch. C-H of –OCH_3); 912 (C-O epoxide).

Synthesis of 4-(2-hydroxy-3-(phenylamino) propyl)-2-methoxyphenol (EE-Anilin)

yellow oil (0.81% yield); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.308 – 7.480 (Ph); 5.100 (s, 1H, Ph-OH); 3.800 (s, 3H, -OCH_3); 3.667 – 3.646 (m, 1H, tert. CH); 2.800 (d, 2H, -CH_2-N); 2.300 (d, 2H, -CH_2-Ph); 4.000 (s, 1H, -NH-); 2.000 (s, 1H, -OH). Spectra have been confirmed using ^{1}H NMR prediction include in ChemDraw software. FTIR ν (cm^{-1}): 3379.31 (-OH); 1637.12 (N-H of secondary amine); 1265.69 (stretch. C-N); 2937.34 and 2841.05 (sharp, stretch. C-H of methylene); 1430.56 (bend. C-H of methylene); 1377.67 (stretch. C-H of –OCH_3); 3074.71 (weak, stretch. C-H of alkene); 1602.38 and 1511.49 (C-C aromatic alkene). ESI+ (m/z): 274.178 (M+H), conf. m/z = 273.178 (theoretic m/z = 273.17).

Synthesis of 4-(2-hydroxy-3-(naphthalene-1-ylamino) propyl)-2-methoxyphenol (EE-Naph)

Yellow oil (0,51% yield); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.758 – 7.262 (Ph); 5.000 (s, 1H, Ph-OH); 4.200 (s, 1H, -NH-); 3.700 (s, 3H, -OCH_3); 3.900 (m, 1H, tert. CH); 2.450 (d, 2H, -CH_2-Ph); 2.750 (d, 2H, -CH_2-N);

2.000 (s, 1H, -OH); FTIR ν (cm^{-1}): 2937.18 – 2840.65 (stretch. CH); 1462.45 (bend. CH; $-CH_2-$); 1365.71 (bend. CH, $-CH_3$); 3059.24 (stretch. CH oh alkene); 1603.93 and 1511.08 (stretch C-C Ph); 1637.29 (sec. NH); 1268.18 (stretch. C-N); 3409.65 (-OH); ESI+ (m/z): 362.300 (M+K); 685.500 (2M+K); conf. m/z = 323.400 (theoretic m/z = 323.15).

Synthesis of 4-(2-hydroxy-3- (diphenylamine) propyl)-2-methoxyphenol (EE-DPA)

Yellow heavy oil (0,64% yield); ^1H NMR ($CDCl_3$) δ (ppm): 8.308 – 7.480 (Ph); 5.000 (s, 1H, Ph-OH); 3.800 (s, 3H, $-OCH_3$); 3.667 (m, 1H, tert. CH); 3.000 (d, 2H, $-CH_2$-Ph); 2.800 (d, 2H, $-CH_2$-N); 4.000 (s 1H, -NH-); 2.000 (s, 1H); FTIR ν (cm^{-1}): 3392.95 (-OH); 1265.64 (stretch. C-N); 2937.07 – 2841.29 (stretch. CH); 1461.81 (bend. CH, $-CH_2-$); 1365.99 (bend. CH, $-CH_3$); 3044 (weak, stretch CH of alkene); 1591.25 and 1510.50 (stretch. C-C Ph). ESI+ (m/z): 426.200 (M+2K+H); conf. m/z = 349.300 (theoretic m/z = 349.17).

CONCLUSION

Based on the research findings and discussion presented, it can be concluded that the compounds EE-Aniline, EE-Naph, and EE-DPA can be successfully synthesized from aniline, naphthylamine, and diphenylamine, respectively, through the reaction with the epoxide product of eugenol, 2-methoxy-4-(oxiran-2-ylmethyl) phenol (EE). In this study, all the aryl amino alcohol derivatives that were synthesized demonstrated activity in inhibiting heme polymerization. The IC_{50} values ranged from 0.176 mg/mL to 0.730 mg/mL, which was lower than the IC_{50} value of the standard antimalarial drug chloroquine. Furthermore, the molecular docking study indicated that all the synthesized aryl amino alcohol derivatives have the potential to act as inhibitors of the Plasmepsin II enzyme.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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