Research Article

Identification of Potential Receptor Targets of Alstonia Spectabilis Using Network Pharmacology Integrated With Gene Ontology and KEGG Pathway Enrichment Analysis

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Abstract

Malaria is an ancient disease that has not been eradicated until now. The antimalarial drug chloroquine is currently ineffective due to resistance to Plasmodium sp. Therefore, the public urgently needs a new antimalarial drug that is both effective and affordable. In the metabolite profile using gas chromatography mass spectrometry (GC-MS) and ultra-performance liquid chromatography quadrupole time of flight mass spectrometry (ULPC-QToF-MS/MS) instruments, the 70% ethanol extract of A. spectabilis contains compounds that are efficacious as antimalarial, anti-inflammatory, antipyretic, and analgesic, such as macralstonidine, pleiocarpamine, villalstonine, and various other compounds. Compounds obtained from metabolite profiling testing are prepared by physicochemical screening based on Lipinski's Law of Five, predicting the toxicity of the compound and predicting the pharmacokinetics of the compound, and then molecular docking is carried out on the receptor. Molecular dynamics was also carried out on compounds resulting from metabolite profiling on a personal computer (PC) Linux Ubuntu 20.04.1 LTS 64-bit system, NVIDIA Quadro P2200 graphics card, 16 GB RAM, and Intel Xeon(R) W-2223 @ 3.60 GHz octa-core processor with Desmond at Maestro Schrödinger 2021–2. The results of this study revealed that there were 5 potential compounds that bind to PfENR receptor and 5 potential compounds to PfCRT receptor.

Keywords: Alstonia spectabilis; Antimalaria; Molecular Dynamics.

INTRODUCTION

Malaria is an ancient disease that has not been eradicated until now. Malaria spreads to around 85 countries in the world, with 241 million new cases in 2022, and causes 627,000 deaths, two-thirds of which are children under 5 years of age[1]. In Indonesia, malaria is the main infectious disease in the Eastern region, which includes Papua, West Papua, and East Nusa Tenggara (NTT)[2]. The antimalarial drug chloroquine is currently ineffective due to resistance to Plasmodium sp. Instead, artemisinin and its derivative (artesunate) are used, which is a compound isolated from Artemisia annua[3]. However, based on several research reports, indications of resistance to Plasmodium falciparum, the most dangerous type of plasmodium and commonly found in Indonesia, were found to one of the artesunate-based combination therapy (ACT) drugs[4]. Therefore, new antimalarial drugs are needed that are effective and affordable for the public.

The Tetun tribe who inhabit East Nusa Tenggara recognize malaria as a disease that causes moras isin manas (heat, fever). The traditional treatment of malaria by the Tetun people has been around for hundreds of years and is a symptomatic treatment to cure heat or fever, chills, swollen spleen, headaches, and muscle and joint pain[5]. This traditional treatment is carried out either herbally using various types of endemic plants or non-herbally[6]. One of the plants that is often used traditionally by the Tetun tribe and has the potential to be developed into a new antimalarial drug is kroti metan, or black pule (Alstonia spectabilis)[6].

In metabolite profiling using gas chromatography mass spectrometry (GC-MS) and ultra-performance liquid chromatography quadrupole time of flight mass spectrometry (ULPC-QToF-MS/MS) instruments, the 70% ethanol extract of A. spectabilis contains compounds that are efficacious as antimalarial, anti-inflammatory, antipyretic, and analgesic, such as macralstonidine, pleiocarpamine, villalstonine, and various other compounds. In vitro antimalarial effect testing also showed that 70% ethanol extract of A. spectabilis could inhibit the growth of Plasmodium sp. with an inhibition concentration value of -50 (IC50 1.23 ug/mL)[3]. Based on the results of previous research, which shows the high potential of A. To develop A. spectabilis as an antimalarial, we need to conduct research on molecular docking and molecular dynamics, which can lead to rational drug design or chemical process modeling by offering profound molecular insights into structure and mechanisms [7].

MATERIALS AND METHODS

Materials

Computational studies were carried out using a personal computer (PC) Linux Ubuntu 20.04.1 LTS 64-bit system, NVIDIA Quadro P2200 graphic card, 16 GB RAM, and Intel Xeon(R) W-2223 @3.60 GHz octa-core processor with Desmond on Maestro Schrödinger 2021–2. Metabolite profiling results from UPLC-QToF-MS/MS and GC-MS were obtained from preliminary research.

Method

Compounds obtained from metabolite profiling testing are prepared by screening physicochemical analysis based on Lima Lipinski's law, predicting the toxicity of the compound and predicting the pharmacokinetics of the compound, and then molecular docking is carried out on the receptors responsible for antimalarial activity such as PfENR (PDB ID: 3AM4), pfDHFR-TS (PDB ID: 3UM8), PfATP4 (PDB ID: 4HQJ), and PfCRT (PDB ID: 6UKJ). Molecular dynamics was also carried out on compounds resulting from metabolite profiling on a personal computer (PC) Linux Ubuntu 20.04.1 LTS 64-bit system, NVIDIA Quadro P2200 graphic card, 16 GB RAM, and Intel Xeon(R) W-2223 @ 3.60 GHz octa-core processor with Desmond on Maestro Schrödinger 2021–2 [8].

RESULTS AND DISCUSSION

Prediction of Compound Toxicity

Toxicity is used to determine whether a compound has the potential to be toxic if it enters the body. Prediction of toxicity properties can be done using a website such as pkCSM. Some of the parameters used in predicting toxicity this time include LD50, Ames toxicity, hepatotoxicity, and skin sensitivity. Enter the compound's SMILES code into the pkCSM site at http://biosig.unimelb.edu.au/pkcsm/ .

Prediction of the pharmacokinetic profile of a compound

Pharmacokinetic predictions include ydrazine, distribution, metabolism, and excretion. There are 29 compounds that have predicted pharmacokinetics (ADME) from compounds resulting from metabolite profiling of A. spectabilis using the pkCSM tool website (http://biosig.unimelb.edu.au/pkcsm/). The procedure is that the Cannonical SMILES compound is input into the site or website, then ADMET is selected. Human Intestinal Absorption (HIA) is used to predict ydrazine absorption, VDSS is used to predict ydrazine distribution, CYPA34 is used to predict ydrazine metabolism, and OCT2 is used to predict ydrazine compound excretion.

Molecular Docking

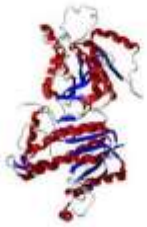


Figure 1. PfENR Receptor

Figure 2. pfDHFR-TS Receptor

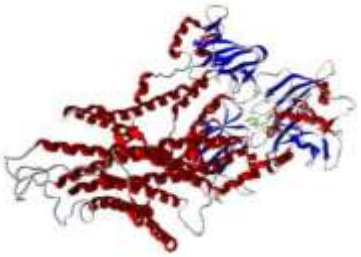


Figure 3. pfATP4 Receptor

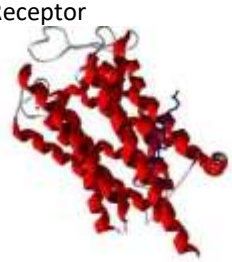


Figure 4. PfCRT Receptor

The receptors used as antimalarials are PfENR (PDB ID: 3AM4), pfDHFR-TS (PDB ID: 3UM8), pfATP4 (PDB ID: 4HQJ), and PfCRT (PDB ID: 6UKJ). The above receptors are downloaded via the Protein Data Bank (PDB) with a download file (PDB Format) and input into Molegro Virtual Docker. Compounds resulting from metabolite profiling of A. Spectabilis were prepared for binding to the receptors PfENR (PDB ID: 3AM4), pfDHFR-TS (PDB ID: 3UM8), pfATP4 (PDB ID: 4HQJ), and PfCRT (PDB ID: 6UKJ). Compounds resulting from GC-MS and UPLC-QToF-MS/MS profiling metabolites were drawn in 2D structure form using ChemDraw Professional 16.0 software. Then the compound is saved in.cdx format. Then the compound is subjected to energy minimization to obtain a stable structural form using MMFF94. Energy minimization with MMFF94 is carried out by pressing Calculation MMFF94 minimization in the Chem 3D 16.0 software and saved in SYBYL2 (*.mol) format.
The prepared compounds, target proteins, and chloroquine ligands were input into the Molegro Virtual Docker software. Receptor preparation is needed to detect the cavity (hole) contained in the receptor, which will later be used as a site for interaction between the ligand and the compound. In the molecular docking validation process, the parameter observed is the RMSD value. If the RMSD value is ≤ 2.5 Å, then the docking method used is valid; conversely, a docking method is said to be invalid if the RMSD value is > 2.5 Å[9]. Then we look at the interactions that occur at each receptor with the metabolite profiling compounds.

Molecular Dynamics

Molecular Dynamics (MD) is a type of atomistic simulation that describes the interactions between atoms and molecules over a certain period of time based on certain basic principles to visualize the movement of the atoms. Complex systems can consist of many atoms that are impossible to resolve analytically, but numerical MD approaches allow treating such systems by observing binding affinity, conformational changes, and overall stability. Therefore, MD simulations bridge between theory and experiment and can be considered virtual experiments [10 11].
The molecular dynamics study was carried out using MOE (Molecular Operating Environment) by carrying out molecular dynamics simulations and setting the time, speed, and number of checkpoints to be detected. Then water is formed in the contact chamber, and Nacl is added. After adding the solvent, molecular dynamics are carried out for each receptor.

Prediction of Physicochemical Properties of Compounds

ADME parameters (Absorption, Distribution, Metabolism, and Excretion) are criteria used in pharmacology to evaluate the pharmacokinetic properties of chemical compounds. To find out whether a compound is able to penetrate biological membranes and has good permeability, the compound must fulfill Lipinski's law of five[12]. Based on the table above, it shows that out of a total of 29 compounds, there are 7 UPLC-QToF-MS/MS compounds contained in A. spectabilis that do not meet the Lipinski Law qualifications. Thus, of the 29

compounds contained in black pule, 7 of these compounds can be predicted to have low absorption. Besides, the ADME properties of one compound, Macralstonidine, remain unpredicted.

Prediction of Compound Toxicity

Based on the prediction of toxicity properties using pkCSM, there are two compounds that are included in class IV (danger if swallowed). These compounds are compounds 12 and 29. Then the remaining compounds are included in class V (possibly dangerous). 5 compounds, namely compounds 8, 9, 20, 23, and 25, which have Ames toxicity, and 2 compounds that have skin sensitization, namely compounds 11 and 29, and there are 19 compounds that have hepatotoxicity properties.

Prediction of Pharmacokinetic Profiles of Compounds

Based on the pharmacokinetic profile test using the pkCSM website (table 1), it can be seen that almost all compounds meet the requirements for a good intestinal absorption profile with an overall percentage above 80%. A percentage above 80% indicates good intestinal absorption. There are 5 compounds that have poor intestinal absorption profiles, namely the compounds 2,3-Dihydroxybenzoic acid, Morphine, Methyl (1-{2-(3-{[2-(3-{[2-(1-acetamidoethyl)-1,3-thiazol-4-yl]methyl}-2-oxo-1-imidazolidinyl)-3methylpentanoyl]amino}-2-hydroxy-4-phenylbutyl)-2-[4-(2 pyridinyl)benzyl]hydrazino}-3,3- dimethyl-1-oxo- 2-butanyl)carbamate, 1,4-Bis[(2-hydroxyethyl)amino]-9,10- anthraquinone, and Hexadecyl 3-O- {(6R)-5-acetamido-3,5-dideoxy-6-[(1R,2R)-1,2,3-trihydroxypropyl]-â-L-threo-hex-2- ulopyranonosyl}- â-D- galactopyranoside, among others, with percentages of 74.644%, 74.354%, 60.497%, 31.822%, and 21.588%. From these 5 compounds, it can be concluded that the intestinal absorption profile is poor because it is less than 80%[13].

Based on the pharmacokinetic profile distribution test using the pkCSM website (table 1), it can be seen that 12 compounds have high VDss. VDss is said to be high if the VDss value is >0.45 log L/kg. The higher the VDss value of a compound, the more perfectly the compound can be distributed in body tissues. Meanwhile, the 4 compounds are (5bS,8R,11R,11aR,12S,13R,13aR)- 13a-[(1R)-1-Acetoxyethyl]-8-hydroxy- 5b,9,11a-trimethyl-8-[(1Z) -2-phenyl-1- propen-1-yl]- 1,3,5a,5b, 6,7,7a,8,11,11a,11b,12,13,13 a-tetradecahydrophenanthro[2,1- c]oxepine-11, 12,13- triyl triacetate , 1-Benzyl-3-{[4'-(4-[4-(hydroxymethyl)phenyl]-6-{[2-(1 pyrrolidinylmethyl)-1-pyrrolidinyl]methyl}-1,3-dioxan-2-yl)-3- biphenylyl] methyl}urea, Methyl (1-{2-(3-{[2-(3-{[2- (1-acetamidoethyl)- 1,3-thiazol-4-yl]methyl}-2-oxo-1-imidazolidinyl)-3- methylpentanoyl]amino}-2-hydroxy-4 phenylbutyl)-2-[4-(2-pyridinyl)benzyl] hydrazino}-3,3-dimethyl-1-oxo-2-butanyl)carbamate, N-{[4-({[4,6-Bis(ethylamino)-1,3,5-triazin-2-yl]amino} methyl)cyclohexyl]methyl}-8 quinolinesulfonamide has a VDss < 0.45 Log L/kg. and there are 13 compounds that have high permeability to the central nervous system. Compounds are said to be able to penetrate the central nervous system if they have a Log PS value > -2 (Wibisino & Martino, 2023).

Based on the pharmacokinetic profile metabolism test using the pkCSM website (table 1), it can be seen that 12 compounds have inhibition of CYP3A4 substrates and CYP3A4 inhibitors. Meanwhile, the remaining 9 compounds did not inhibit CYP3A4 substrates or CYP3A4 inhibitors. And there are 8 compounds that inhibit CYP3A4 substrates and do not inhibit CYP3A4 inhibitors.

Based on the pharmacokinetic profile excretion test using the pkCSM website (table 1), it can be seen that all compounds are not 0CT 2 substrates except for 1 compound, namely Vincadifformine, which is an OCT2 substrate. When a substance is an OCT2 substrate, there is a potential risk of undesirable drug interactions if co-injected with an OCT2 inhibitor[14].

Molecular Docking

PfENR Receptor

Based on the table, the lowest Rerank score value is found for the compound 2,5-Bis(2- ethylhexyl)-3,6-bis (5-phenyl- 2-thienyl)-2,5- dihydropyrrolo[3,4- c]pyrrole -1,4-dione with a value of 173.68; 1-Benzyl-3-{[4'-(4-[4-(hydroxymethyl)phenyl]-6-{[2-(1- pyrrolidinylmethyl)-1- pyrrolidinyl]methyl}-1,3-dioxan-2- yl)-3- biphenylyl] methyl}urea with a value of -164.79; N- {[4-({[4,6-Bis(ethylamino)-1,3,5- triazin-2- yl]amino} methyl)cyclohexyl]methyl}-8- quinolinesulfonamide with a value of -149.06; 1-[3-(Dimethylamino)propyl]-4-[hydroxy(4- isobutoxy-3-methylphenyl)methylene]-5-(3- phenoxyphenyl)-2,3-pyrrolidinedione with values of -146.06 and 1-(4- {2-Isopropyl-6-[4-(3- methylphenyl)-1-piperazinyl]-4- pyrimidinyl}-1- piperazinyl)-3- (4-morpholinyl)-1-propanone with a value of -144.79. Apart from the 5 compounds above, there are 17 other compounds that have a lower rerank score compared to the native ligand and one of the antimalarial drugs,

namely chloroquine. The lower the rerank score value (more negative), the lower the binding free energy, and the better the binding affinity and ligand interaction.
Based on the results of binding the ligand to the PfENR receptor, 3 types of amino acid residues were obtained, namely hydrogen bonds at residue Tyr 277 and steric bonds at residues He 368 and Tyr 267. These results were used as a reference for comparing the amino acids that bind to the native ligand for the compound test. in black pule with pfENR receptors.

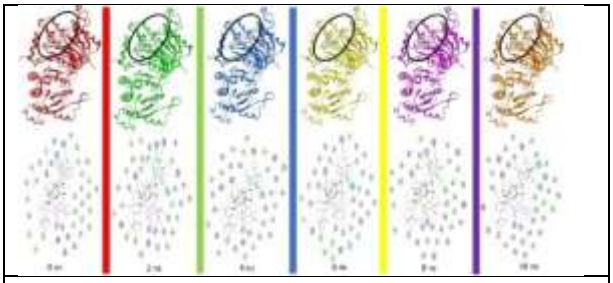
PfATP4 Receptor
Based on the table, it can be seen that not a single compound has a lower rerank value than the native ligand, so there is not a single compound that is more active. However, there are compounds that are almost close to the native ligand value, namely the compound 2,5-Bis(2- ethylhexyl)-3,6-bis (5-phenyl- 2-thienyl)-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione with a value of -115.94. Meanwhile, when compared with one of the malaria drugs, namely chloroquine, the rerank score for this compound is still smaller than the rerank score for chloroquine.
Based on the results of ligand docking with the PfATP4 receptor, 9 types of binding amino acid residues were obtained, namely hydrogen bonds at residues Cys 561, Arg 489, Gln 202, Arg 678, Glu 439, and Lys 515, electrostatic bonds at residues Arg 489, MG_1001 [A], Arg 678, Ag 560, and steric bonds at residues Cys 561, Arg 489, Gln 202, Gly 626, Arg 678, Arg 560, Glu 439, and Lys 515. These results are used as a reference for comparing amino acids that bind to native ligands for compounds in black powder with the pfATP4 receptor.

PfCRT Receptor
The lowest Rerank score value was obtained for the compound 2,5-Bis(2-ethylhexyl)-3,6- bis (5-phenyl- 2-thienyl)-2,5- dihydropyrrolo[3,4- c]pyrrole-1 ,4-dione with a value of -110.44; 1- [3-(Dimethylamino)propyl]-4-[hydroxy(4-isobutoxy-3-methylphenyl)methylene]-5-(3- phenoxyphenyl)-2,3-pyrrolidinedione with a value of -108.96;1-(4-{2-Isopropyl-6-[4-(3- methylphenyl)-1-piperazinyl]-4- pyrimidinyl}-1-piperazinyl)-3-(4- morpholinyl)-1-propanone with a value of - 108.45; Methyl (1-{2-(3-{[2-(3-{[2-(1- acetamidoethyl)-1,3-thiazol-4- yl]methyl}-2-oxo-1-imidazolidinyl)-3-methylpentanoyl]amino}-2-hydroxy-4-phenylbutyl)-2-[4-(2-pyridinyl)benzyl] hydrazino}-3,3- dimethyl-1-oxo- 2-butanyl)carbamate with a value of - 105.04; and Bis(ù-piperidinylacetyl) dibenzofuran with a value of -104.93. Apart from the 5 compounds above, there are 16 other compounds (compounds 22,9,10,12,20,28,13,2,16,24,4,7,15,6, and 3) that have lower rerank values compared to native ligands and one of the antimalarial drugs, namely chloroquine. The lower the rerank score value (more negative), the lower the binding free energy, and the better the binding affinity and ligand interaction. These results indicate that A. spectabilis contains the 21 compounds mentioned above. spectabilis can be used as alternative antimalarial drugs.
Based on the results of ligand docking with the PfCRT receptor, it was obtained that 3 amino acid residues were bonded, namely hydrogen bonds at residues Ser 388 and Arg 392, electrostatic bonds at residue Arg 392, and steric bonds at residues Asn 395, Arg 392, and Ser 388. These results are used as a reference to compare the amino acids that bind to the native ligand of the black pulse compound test with the PfCRT receptor.

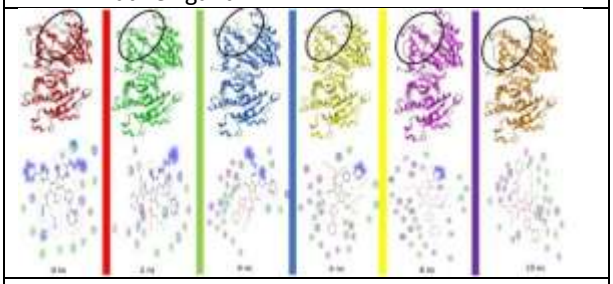
pfDHFR-TS Receptor
It was found that none of the compounds had a lower rerank value than the native ligand, so none of the compounds were more active. However, there are compounds that are almost close to the native ligand value, namely the pleiocarpamine compound with a value of -111,607. Based on the results of ligand docking with the pfDHFR-TS receptor, 6 types of bonded amino acid residues were obtained, namely hydrogen bonds at residues Ile 14, Ile 164, Tyr 170, Cys 15, Asp 54, electrostatic bonds at residues none, and steric bonds at residue Ile 164. These results are used as a reference for comparing the amino acids that bind to the native ligand of the black pule compound test with the pfDHFR-TS receptor.

Molecular Dynamic

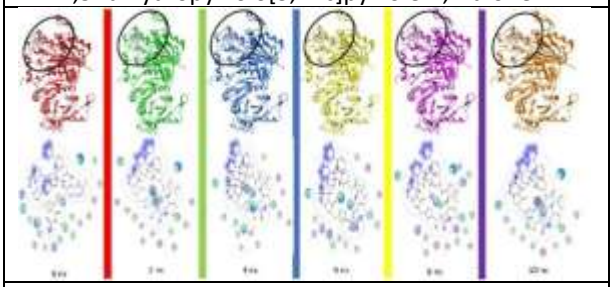
PfENR Receptor



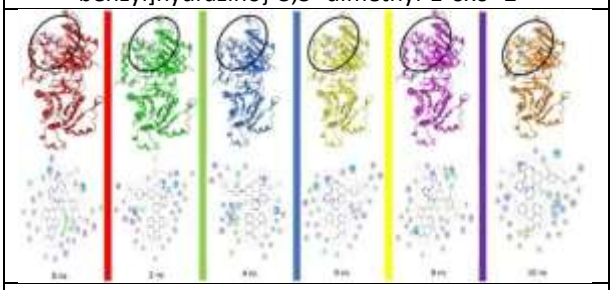
1. Native ligand



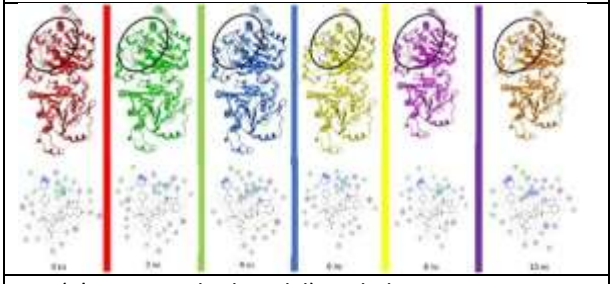
2. 2,5-Bis(2-ethylhexyl)-3,6-bis (5-phenyl- 2-thienyl)-2,5- dihydropyrrolo[3,4- c]pyrrole-1,4-dione



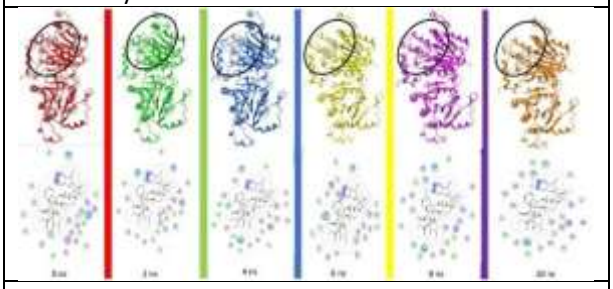
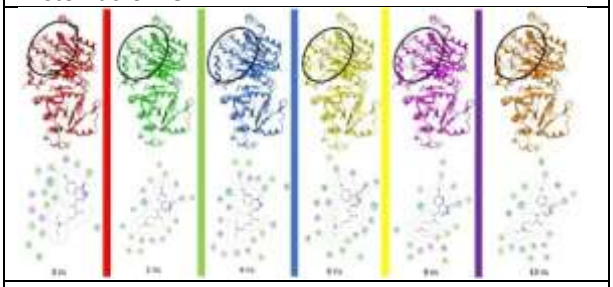
3. Methyl (1-{2-(3-{[2-(3-{[2-(1- acetamidoethyl)-1,3-thiazol-4- yl]methyl}-2-oxo-1-imidazolidinyl)-3-methylpentanoyl]amino}-2-hydroxy-4-phenylbutyl)-2-[4-(2- pyridinyl) benzyl]hydrazino}-3,3- dimethyl-1-oxo- 2



4. 1-Benzyl-3-{[4'-(4-[4-(hydroxymethyl)phenyl]-6-{[2-(1-pyrrolidinylmethyl)-1-pyrrolidinyl]methyl}-1,3-dioxan-2-yl)-3- biphenylyl] methyl}urea

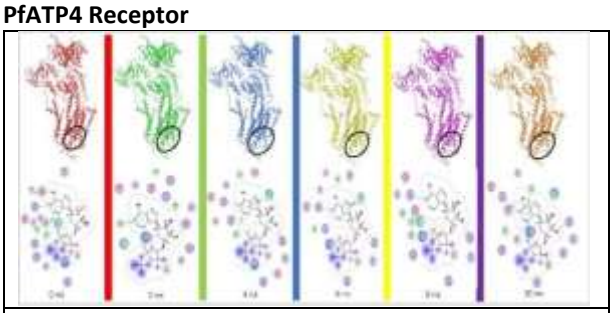


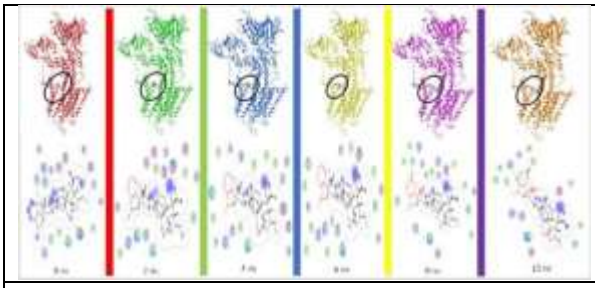
5. (2'-Cyano-4- biphenylyl)methyl (1R,3aS,5aR,5bR,7aR ,8S,13aR,13bR,15aR,15bR)-8-(hydroxymethyl)-1-isopropenyl - 5a,5b,8,13a-

tetramethyl-1,2,3,4,5,5a,5b,6,7, 7a,8,13,13a,13b,14,15,15a,15b-octadecahydro-3aH-cy c

Alstomacroline

7. Chloroquine

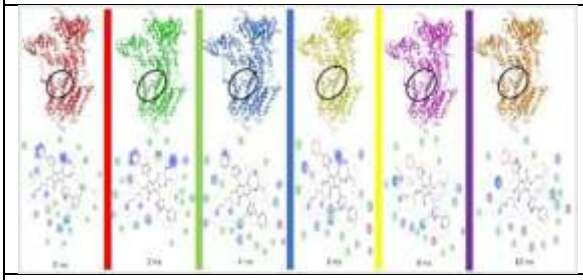
In compound 1, it shows that the residue interacts with the ligand in each track frame. The radius fluctuations show that the compound trajectory shows a stable conformation during the 10 ns MD simulation.
In compound 2, it shows that the residue interacts with the ligand in each track frame. The radius fluctuations show that the compound trajectory is unstable during the MD simulation of 4–8 ns. This can be caused by differences in complex compounds, which can cause differences in the amino acids that bind to each ligand. resulting in complex structural changes. However, after the MD process is complete, the complex compounds still interact around the binding site, as seen[15].
In compound 3, it shows that the residue interacts with the ligand in each track frame. The radius fluctuations show that the compound trajectory shows a stable conformation during the 10 ns MD simulation.
In compound 4, it shows that the residue interacts with the ligand in each track frame. The radius fluctuations show that the compound trajectory shows a stable conformation during the 10 ns MD simulation.
In compound 5, it shows that the residue interacts with the ligand in each track frame. The radius fluctuations show that the compound trajectory shows a stable conformation during the 10 ns MD simulation.
In compound 6, it shows that the residue interacts with the ligand in each track frame. The radius fluctuations show that the compound trajectory shows a stable conformation during the 10 ns MD simulation.
In compound 7, it shows that the residue interacts with the ligand in each track frame. The radius fluctuations show that the compound trajectory shows a stable conformation during the 10 ns MD simulation.

PfATP4 Receptor

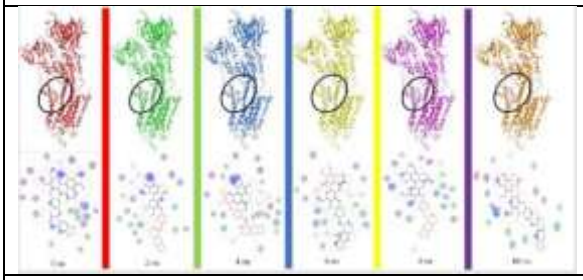

1. Native ligand



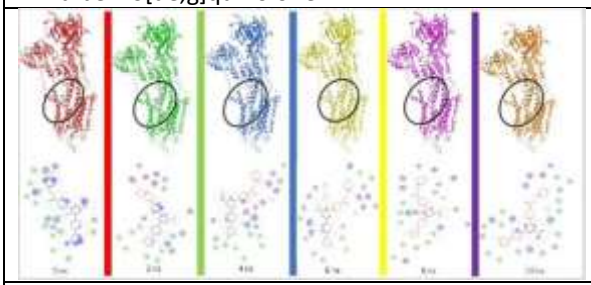
2. Villastonine



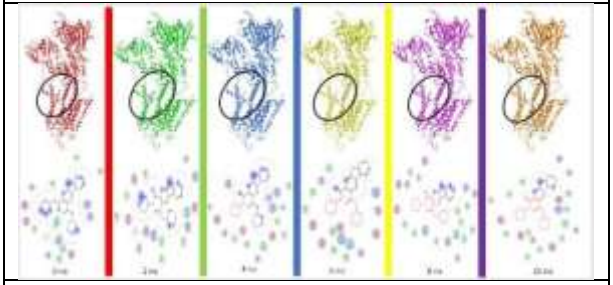
3. 2,5-Bis(2-ethylhexyl)-3,6-bis (5-phenyl- 2-thienyl)-2,5- dihydropyrrolo[3,4- c]pyrrole-1,4-dione



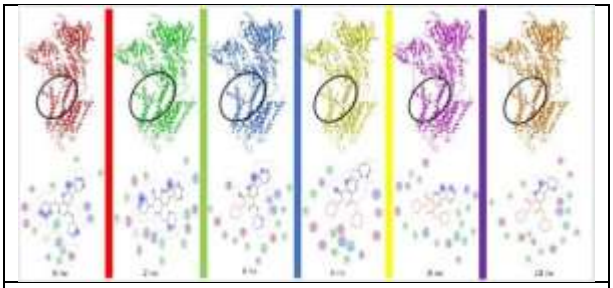
4. (6aS)-1,2,3,9,10-Pentamethoxy- 6- methyl-8- (4-{[(5S)-6-methyl-5,6,7,8-tetrahydro[1,3]dioxolo[4,5- g]isoquinoline-5-yl]methyl} phenoxy)- 5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolone



5. 1-(4-{2-Isopropyl-6-[4-(3- methylphenyl)-1-piperazinyl]-4- pyrimidinyl}-1-piperazinyl)-3- (4-morpholinyl)-1-propanone



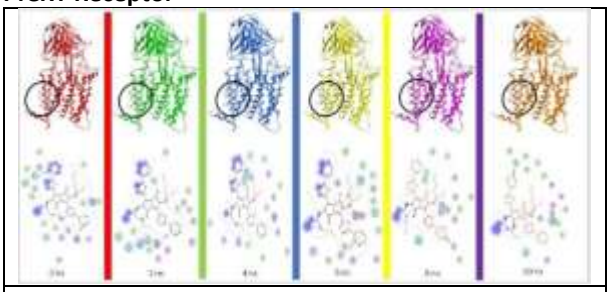
Bis(ù-piperidinylacetyl) dibenzofuran



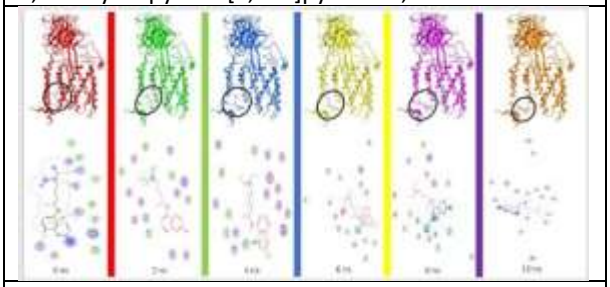
7. Chloroquine

Based on the figure above, all compounds show that the residues interact with the ligand in each pathway frame. The radius fluctuations show that the compound trajectory shows a stable conformation during the 10 ns MD simulation.

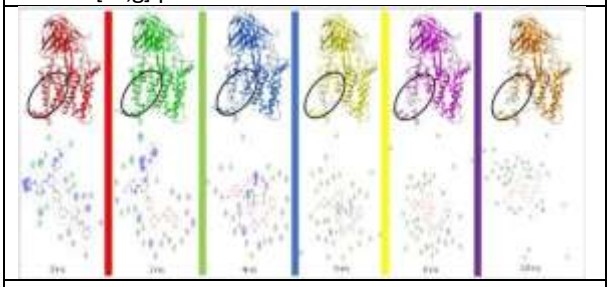
PfCRT Receptor



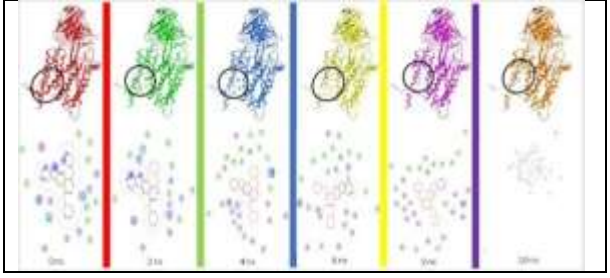
2,5-Bis(2-ethylhexyl)-3,6-bis (5-phenyl- 2-thienyl)-2,5- dihydropyrrolo[3,4- c]pyrrole-1,4-dione



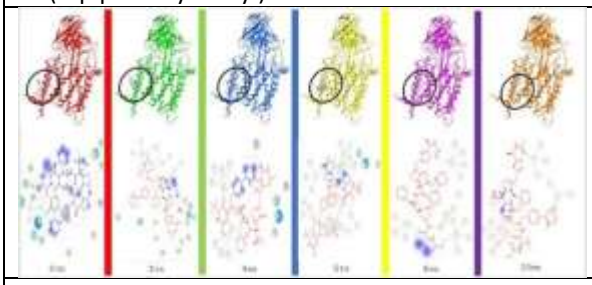
(6aS)-1,2,3,9,10-Pentamethoxy- 6- methyl-8- (4-{[(5S)-6-methyl-5,6,7,8- tetrahydro[1,3]dioxolo[4,5- g]isoquinoline-5- yl]methyl} phenoxy)- 5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinoline



1-(4-{2-Isopropyl-6-[4-(3- methylphenyl)-1-piperazinyl]-4-pyrimidinyl}-1-piperazinyl)-3- (4- morpholinyl)-1-propanone



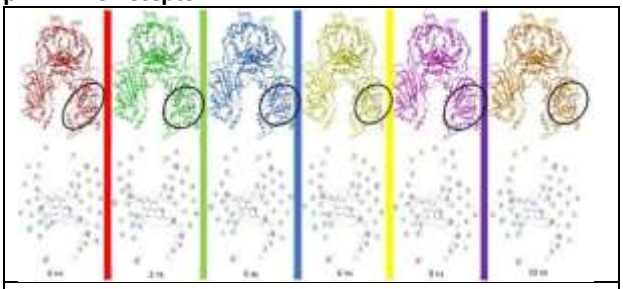
Bis(ù-piperidinylacetyl) dibenzofuran



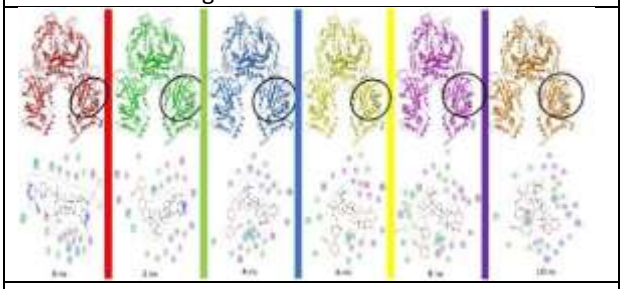
Chloroquin

Based on the figure above, all compounds show that the residues interact with the ligand in each pathway frame. The radius fluctuations show that the compound trajectory shows a stable conformation during the 10 ns MD simulation.

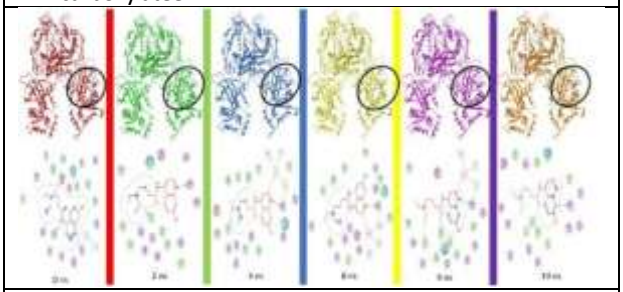
pfDHFR-TS Receptor



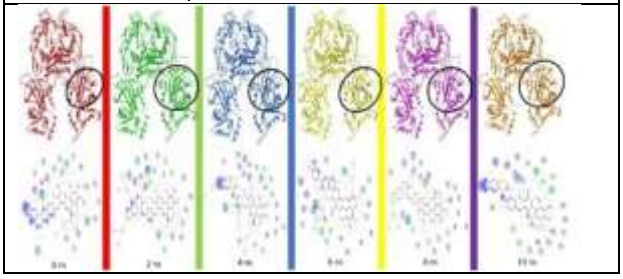
1. Native ligand



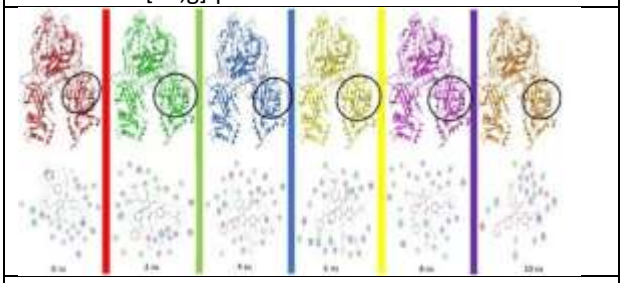
2. (2'-cyano-4-biphenylyl)methyl (1r,3as,5ar,5br,7ar ,8s,13ar,13br,15ar,15br)-8-(hydroxymethyl)-1-isopropenyl13a-tetramethyl-1,2,3,4,5,5a,5b,6,7, 7a,8,13,13a,13b,14,15,15a,15b-octadecahydro-3ah-cyclopenta[7,8]phenanthro [1,2-g]quinoxaline 3a-carboxylatee



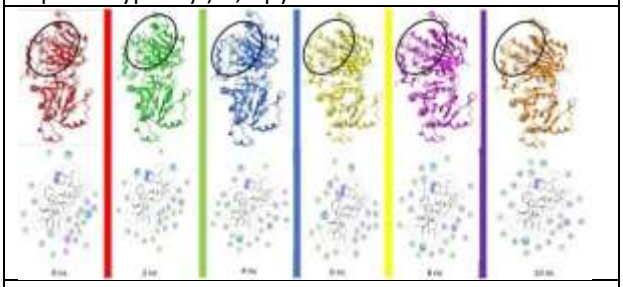
3. Chloroquine



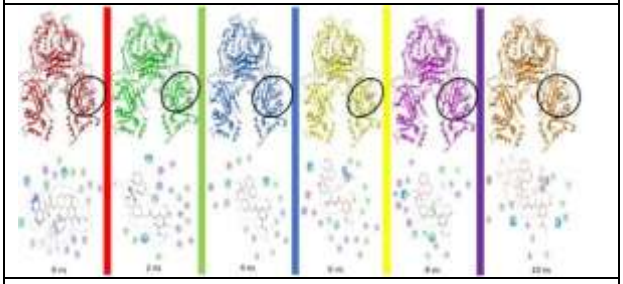
4. (6aS)-1,2,3,9,10-Pentamethoxy- 6- methyl-8- (4-{[(5S)-6-methyl-5,6,7,8-tetrahydro[1,3]dioxolo[4,5- g]isoquinoline-5-yl]methyl} phenoxy)- 5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolone



5. 1-[3-(Dimethylamino)propyl]-4- [hydroxy(4-isobutoxy-3-methylphenyl)methylene]-5-(3-phenoxyphenyl)-2,3-pyrrolidinedione



6. Macralstonidine



7. N-{[4-({[4,6-Bis(ethylamino)-1,3,5- triazin-2-yl]amino} methyl)cyclohexyl]methyl}-8-quinolinesulfonamide

In compound 1, it shows that the residue interacts with the ligand in each track frame. The radius fluctuations show that the compound trajectory shows a stable conformation during the 10 ns MD simulation.
In compound 2, it shows that the residue interacts with the ligand in each track frame. The radius fluctuations show that the compound trajectory is unstable during the MD simulation of 6–8 ns. This can be caused by differences in complex compounds, which can cause differences in the amino acids that bind to each ligand. resulting in complex structural changes. However, after the MD process is complete, the complex compounds still interact around the binding site, as seen[14].
In compound 3, it shows that the residue interacts with the ligand in each track frame. The radius fluctuations show that the compound trajectory shows a stable conformation during the 10 ns MD simulation.
In compound 4, it shows that the residue interacts with the ligand in each track frame. The radius fluctuations show that the compound trajectory shows a stable conformation during the 10 ns MD simulation.
In compound 5, it shows that the residue interacts with the ligand in each track frame. The radius fluctuations show that the compound trajectory shows a stable conformation during the 10 ns MD simulation.
In compound 6, it shows that the residue interacts with the ligand in each track frame. The radius fluctuations show that the compound trajectory shows a stable conformation during the 10 ns MD simulation.

CONCLUSION

Through a series of tests using techniques such as metabolite profiling and molecular docking, it was found that several compounds in A. spectabilis have good physicochemical and pharmacokinetic properties. However, some compounds also show potential toxicity. The docking results showed that A. spectabilis can compete with existing antimalarial drugs, with rerank

scores showing good binding affinity to Plasmodium target receptors in the form of 5 compounds that can bind to the PfENR receptor and 5 compounds to the PfCRT receptor. This study suggests the need for further research to develop these compounds into new and more effective antimalarial drugs, considering the challenges of resistance faced by existing drugs.

ACKNOWLEDGMENT

Thank you to the entire team who helped with this research.

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