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Original Work

Interaction analysis of hemin with antimalaria artemisinin groups through *in-silico* and *in-vitro* approach

Surya Dwira M.Si, Fadilah[ψ] S.Si, M.Si and Aryo Tedjo S.Si, M.Si

Department of Medical Chemistry, Faculty of Medicine, University of Indonesia, Indonesia

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ABSTRACT: WHO has recommended the treatment of malaria with Artemisia annua L. in combination with another drug called Artemisinin Combination based Therapy (ACT), to overcome multiple drug resistance malaria. Artemisinin is very effective against *P falciparum*. Artemisinin has problems like short plasma half-life, limitation of bioavailability, less solubility in both oil and water and it is obtained in small amounts from natural sources. This research aims to study the effectiveness of a drug *in-silico* with docking approach and *in-vitro* antimalarial test against hemin bioavailibilitas. Due to the chemical structures, physical-chemical properties, chemical reactivity and the ability of drugs to interact with the receptor depends on the electronic structure, composition and interactions of all electrons with molecules. From this study, interaction between hemin with antimalarial *in-silico* by docking approach indicates that artemisinin derivatives group artesunat (ARTS) has the lowest binding energy compared with other derivatives, and also the level of hydrogen bonding. The interaction of hemin with antimalarial through UV-Vis test showed that at a wavelength of 400 nm, ARTS has a lower free energy of interaction compared with other ligands artemether (ARTE) and artemisinin (ARTM). Analysis interactions of artemisinin compounds or their derivatives with hemin in *in-vitro* by spectrophotometric method are consistent with molecular mechanical calculations using molecular docking. This indicates that the interaction artesunat has a lower free energy compared with other ligands artemether and artemisinin (Arte and ARTM). The existence of a significant interaction between hemin with antimalarial artemisinin derivatives showed no different between *in-silico* and UV-Vis identification.

KEY WORDS: ***Artemisinin; In-silico (docking); Hemin; Antimalarial; In-vitro***

INTRODUCTION

Malaria is very dangerous and 1.2% of total human deaths are caused by this disease.[1] Outbreaks of malaria in Indonesia increase, on the other hand, the malaria parasite *Plasmodium falciparums* resistant to commonly used malaria drugs, so it is necessary to develop new antimalarial drugs. WHO has recommended treatment of malaria with Artemisia annua L. in combination with another drug called Artemisinin-based Combination Therapy (ACT) to overcome multiple drug resistance malaria.[2-4]Artemisinin is a product of secondary metabolites from the plant of Artemisia and is highly effective against *P falciparum*. Preparation of artemisinin by synthesis is difficult and not economical. The easy and cheap way is to extract it from the plant Artemisia. The existence of plants as medicine indicate that it contains bioactive compounds. This compound in Indonesia has been recommended by Department of Health as a drug combination malaria, but artemisinin has problems, like a short plasma half-life, limited bioavailability, less solubility in oil and water and low production of artemisinin from natural sources, and this has urged scientists to develop new synthesis of artemisinin derivatives.[5] One way of research for antimalarial drug design and to investigate the effectiveness of new drugs of artemisinin derivatives is to use interaction analysis of hemin on the antimalarial compound artemisinin

[ψ]**Correspondence at:** Department of Medical Chemistry, Faculty of Medicine, University of Indonesia, Salemba Raya Street no 6 Jakarta 10430, Indonesia; Email: fadilah81@gmail.com

derivatives.[6] Drug design and effectiveness of a drug can be examined *in-silico* and *in-vitro*, this is because the chemical structures,physical-chemical properties, chemical reactivity and the ability of drugs to interact with the receptor depends on the electronic structure, composition and interactions of all electrons with a molecule.[7]

The development of computational chemistry and bioinformatics allows for the calculation of quantum mechanics and the calculation of free energy of a compound,[8-9] so we can obtain from the compound parameters such as solubility, acceptor donor, inhibition concentration and the degree of IC50 of a drug.[10] In addition to *in-silico* test, *in-vitro* test is needed because the process of healing or drug treatment, can be viewed as a process of molecular interaction between micromolecule compounds with biological molecules from the source or cause of disease. This interaction is not static but constantly developing in accordance with the conditions and situation such as solvent conditions and environment. Likewise, the interaction between hemin and artemisinin, in the presence of hemin in a large number of malaria parasites cause hemoglobin digestion[11] and it need prevention of parasitemia in hemoglobin. For optimal function of Antimalarial drug in bioavailibilitas conditions, the solvent must be considered. In previous research, it has been conducted interaction between hemin and antimalarial in aqueous solution[6]. In this medium, the solubility of antimalarial is small, antimalarial drug soluble in alkaline solution and the stable hemin in neutral or slightly acidic solution can not be achieved due to a dimer.[12]

METHODOLOGY

Materials used in the study *in-silico*: MOE software, ACD lab, NCBI web site, web site PDB, 2 GB Ram Computer specifications Intel Pentium core two duo, software Mozilla Firefox 10.Materials research *in-vitro*: DMSO, Hemin, Tris Buffer, Artemisinin, Artesunat, artemether, HCl, Tris hidroksimetil methylamine, aquades.

Preparation of *in-silico* test

HeminPreparation

PDBdata isthree-dimensional structure of hemin can be downloaded from the PDB databasein the Research Collaboratory for Structural Bioinformatics Protein Data Bank through http://www.rcsb.org/pdb/site address with a computer connected to the internet. The operating system used is Microsoft Windows XP with Mozilla Firefox2.0. Optimization and minimization of three-dimensional structure of the enzyme were employed using the software of MOE 2009.10. with addition of hydrogen atoms. Protonation was employed with protonate 3D programs. Furthermore, partial charges and force field was employed with MMFF94x. Solvation of enzymes was performed in the form of a gas phase with a fixed charge, RMS gradient of 0.05 kcal / A^0mol, and other parameters using the standard in MOE 2009.10 software.

LigandPreparation

Artemisinin derivatives consisting of artesunat, arte ether and artemisinin model into three-dimensional structure. This modeling is performed using ACD Lab software. Three-dimensional shape was obtained by storing in the 3D viewer in ACD Labs. Furthermore, the output format was changed into Molfile MDL Mol format using software Vegazz to conform for the docking process. Ligand was in the wash with compute program, adjustments were made with the ligand partial charge and partial charge optimization using MMFF94x forcefield. The conformation structure energy of ligands was minimized using the RMS gradient energy with 0,001 Kcal / A^0mol. Other parameters were in accordance with existing default in the software.

Docking of Hemin with Artemisininandits derivatives

The docking process was begun with the docking preparation, and it was employed using a docking program from MOE 2009.10 software. Hydrogen polar and Gasteiger charge is added to both Both artemisinin molecule (for later called ligands) and hemin, while the hydrogen nonpolar is merged. Ligand and hemin files are saved in mol for later use in preparation parameters. Dimensional grid box used was 60x60x60 with gridspacing 0.375Å. Docking calculation algorithmis run with parameters Lamarckian Genetic Algorithm (LGA) with population size150, as many as 10 million energy evaluations and repetition (search runs) as much as100times. This parameter is saved in MOE as a file that will be used to run the docking process. Docking processes run using software MOE.

Docking simulations were performed by the Compute-Simulation dock program. Placement method was conducted using a triangle matcher with 1.000.000 repetition energy readings each position and other parameters were in accordance with existing default in the MOE software. Furthermore, scoring functions used london DG, refinement of the configuration repetition forcefield with 1.000 populations. The first repetition of 100 times and the second setting was shown only one of the best results.

Preparation of *in-vitro* test

Preparation of Hemin and Artemisinin Solution

Hemin stock solution of 40% aqueous DMSO, 306 µmol / L was prepared with 10 mg dissolved in 20 mL DMSO, then it is added with 30 mL of tris buffer. Tris buffer was prepared by mixing 0.2 mol/L tris (hidroksimetil)-methylamin and 0.2 mol /L HCl to give pH atmosphere. Then 0.1 mol / L Tris-HCL buffer used in all-aqueous solution of 40% DMSO.

Measurement of pH and Absorption

pH measurement is done by using a pH meter and it is previously performed calibration with standard aqueous buffers. Then absorbance measurements were taken using UV-Vis spectrophotometer. Measurements with spectrophotometer to identify the chromophore group of artemisinin and its derivatives which consist of artesunat, artemether. Artemisinin group absorbed weakly at short wavelengths and more difficult for quantification. Then, with titration, characteristics of hemin will appear at a wavelength of 402 nm by mixing a volume of 0.1 mL solution of hemin with the variation of the volume drug solution (artemisinin, artesunat and artemether) and then dissolved in 10 mL with the addition of Tris-DMSO mixture. Thus the constant of hemin concentration is 3µmol / L with a range of drug concentrations between 0-200 µmol / L. Before each absorbance is measured, the solution was incubated at 37C. UV-Vis spectrophotometer can read the spectrum after the incubation period of 10 to 24 hours. The spectrum reading is in the wavelength of 200-450 nm. Results will be detected at wavelenght 412 nm caused by a group of chromophore from porpirin modifications.

RESULT

Interaction of Hemin with Artemisinin derivatives in the *In-silico*

The docking results showed that the ligand of artemisinin derivatives can inhibit hemin better and has a lower Gibbs energy. The results of docking of artemisinin derivatives are shown in **Table 1**.

Ligand conformation against Hemin

Hemin has an active site of N, Fe and Cl that can be inhibited by the presence of active compounds. If the active compounds enter and interact with hemin then these compounds can bind hemin. Based on the spectrum of electrostatic potential, the ligand position or ligand conformation in a hole is observed by using the software MOE as (**Figure 1, 2 and 3).**

Table 1. Results and characterization of ligands docking with hemin

Ligands	Comp. 1 (ARTM)	Comp. 2 (ARTE)	Comp. 3 (ARTS)
MR	282.336	312.406	383.417
Log P	2.361	2.870	0.907
TPSA	152.990	145.150	202.350
H Don	2	2	2
H Acc	8	9	11
Energi (kcal/mol)	-3.317	-3.7001	-4.8922
PKi	6.049	7.089	8.152

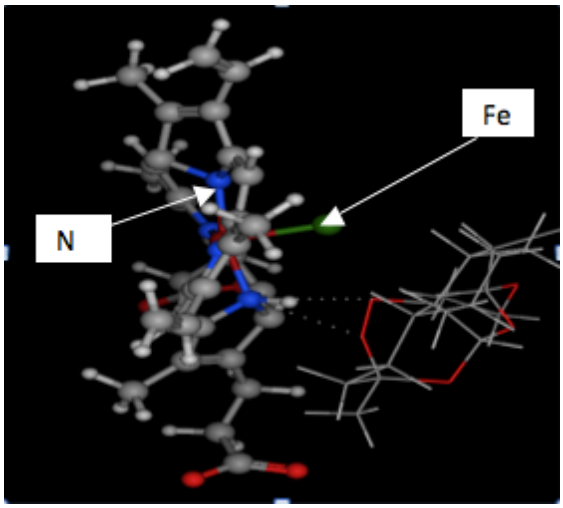


Figure 1: Hydrogen bond between hemin and ARTM ligand

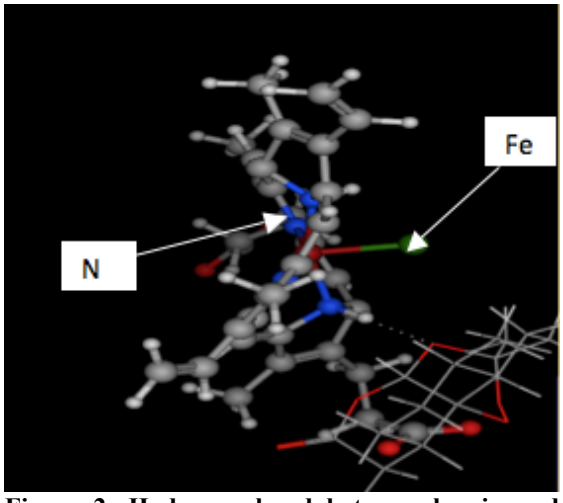


Figure 2: Hydrogen bond between hemin and ARTE ligand

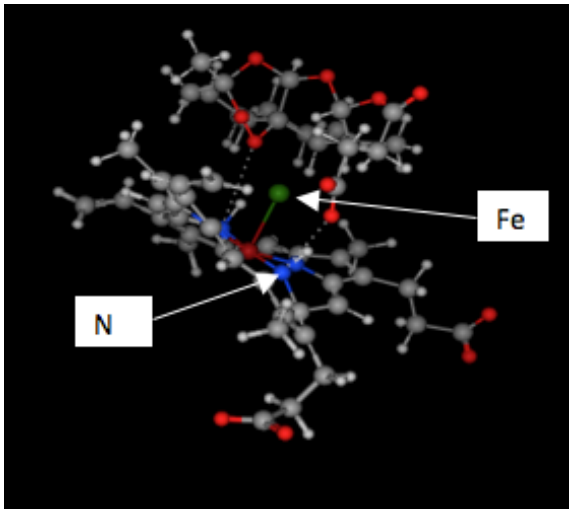


Figure 3: Hydrogen bond between hemin and ARTS ligand

Hydrogen bond

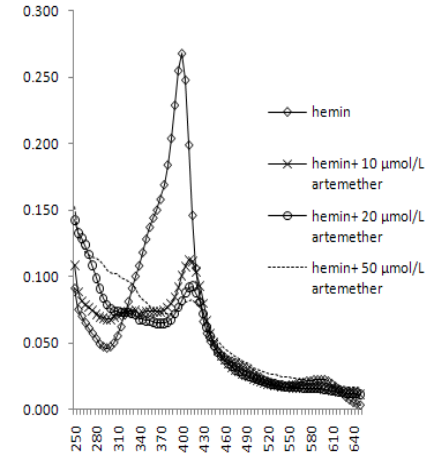
Hydrogen bonding occurs in hemin-ligand complex, hemin-binding ligand identified using hydrogen bond program. Criteria for the occurrence of hydrogen bond is that if the distance between hydrogen and electronegative atoms in the range from 2.5 to 3.5 Å (Bashan and Fletterick, 1989). Hemin, which forms hydrogen bonds with the ligand, can be seen in **Table 2.**

Table 2. Hydrogen bonding between Hemin with ligand

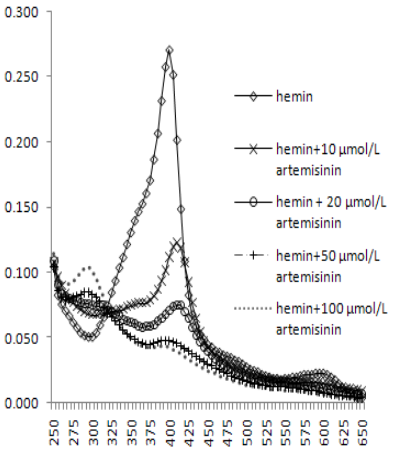
Ligand	Hemin Compound	Number H bond
Compound (ARTM)	Repetition I: Repetition II: N-O Repetition III: N-O	2
Compound (ARTE)	RepetitionI: Repetition II: N-O, N-O RepetitionIII: N-O, N-O	4
Compound (ARTS)	Repetition I: RepetitionII: N-O, N-O Repetition III: N-O, N-O	4

Interaction of Hemin with Artemisinin derivatives in the *In-vitro*

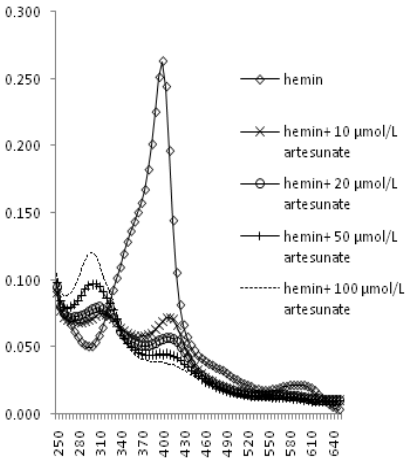
Hemin binding reaction with artemisinin, artesunat, and artemether in DMSO-water mixture can be observed from changes in the typical spectrum as shown in **Figure 4**.
The change of peak absorbance value of absorption spectrum of hemin after addition artemisinin, artesunat, and artemether can be seen in **Table 3**.



4a. Hemin in artemeter (ARTM)



4b. Hemin in arteeter(ARTE)



4c. Hemin in artesunat (ARTS)

Figure 4a, b and c: Hemin solution spectrum changes as a function of changes in the concentration of artemisinin, artemether and artesunat

Table 3: The change of peak absorbance value of absorption spectrum of hemin after addition artemisinin, artesunat and artemether

ADD (µmol/L)	% change absorbance artemisinin addition	% change absorbance artesunat addition	% change absorbance artemether addition
10	55	73	58
20	72	78	65
50	82	83	69
100	84	85	65

DISCUSSION

Stability and interaction strength non covalent on hemin-ligand complexes can be understood from the formation large bond energy. Bond energy derived from free energy released during the interaction of hemin-ligand complex. From Table 1 there are three ligand-ligand 1, ligand 2 and ligand 3, which have a low S value. Ligand 3 (ARTS) has a lowest S value. From the results of binding energy level, then the compound ARTS has a higher stability compared with other artemisinin. It is one of the factors that cause ARTS ligand has a smaller bond energy (-4.8922 kcal / mol) compared with standard ARTM ligand (-3317 kcal / mol). This hydrogen bonding contributes ligand affinity to hemin because the electrostatic interaction between oxygen or nitrogen atom of ligand with a hydrogen or Oxygen atom of hemin or vice versa. Hydrogen bonding occurs between hemin with ligand as Figure 1, 2, and 3. The top-ranked compounds were selected based on the low ΔG binding energy, the high pKi affinity, the number of hydrogen acceptor/hydrogen donor (hydrogen bonding interaction) to the catalytic site of protein target.

In Figure 1, 2 and 3, the ligand is shown by sticks colored gray and red, hemin is shown with ball and stick gray, red, green and hydrogen bonds formed is shown by dashed lines broken gray. ARTS ligands form hydrogen bonds with the active site hemin and its conformation towards hemin, so ARTS ligands can act as potential inhibitors that can bind to hemin more effective than the ARTM and ARTE ligands.

The docking results indicate that both ATRE and ARTS ligands have appropriate shape and conformation structure to bind N present in hemin. The active site of hemin in the form of cavities can be bound by the active compounds of artemisinin and its derivatives. Based on the spectrum of electrostatic potential, figures 2 and 3 indicate that ARTS ligand forms conformation in accordance with ARTE ligand which can bind N of hemin. The difference charge of binding site with the ligand allows the interaction between the binding sites with groups N of hemin with -OH group or -O group of ligand that would increase the affinity and stability complex of hemin-ligand. Stability and affinity complex of the active side with ligand is influenced by the bond distance. This indicates that the ARTS ligand form conformation that can change the conformation or in other words can inhibit hemin.

Hemin binding reaction with artemisinin, artesunat, and artemether in DMSO-water mixture can be observed from changes in the typical spectrum as shown in figure. In figure, it is shown the spectral changes resulting from drug interactions- hemin artemisinin shows the effect of ribbon soret hipokromik hemin at a wavelength of 400 nm which is accompanied by a shift batokromik about 5-10 nm. Decrease in absorbance of the Soret band of hemin could be due to the addition of artemisinin or its derivatives. This addition will induce the aggregation of hemin. In other words, the change reflects the interaction between artemisinin and its derivatives with hemin[1] Generally, changes in the porphyrin iron spectrum of visible light will vary depending on the conditions of pH and type of solvent and drug-specific interaction with hemin. Present study and the study conducted by Mpiana et al demonstrate that the decrease in absorbance of hemin is influenced by changes in drug concentration.[6] Thus, the most plausible explanation for the changes in this spectrum is not due to aggregation of hemin but due to the interaction between artemisinin and its derivatives with hemin.

From the above explanation, we can conclude that the strength and stability of the interaction between artemisinin molecules or its derivatives against hemin can be seen from the change in absorbance value of the hemin spectrum after addition of these compounds. The change of peak absorbance value of artesunat has a lower free energy compared with other ligands artemether and artemisinin (ARTE and ARTM). hemin spectrum of after addition of artemisinin , artesunat, and artemether can be seen in Table 3.

Results of interaction analysis of artemisinin compounds or their derivatives with hemin *in vitro* by spectrophotometric method are consistent with molecular mechanical calculations using molecular docking. This indicates that the interaction artesunat has a lower free energy compared with other ligands (ARTE and ARTM). In other word, artesunat will be more effective when used as an antimalarial.

CONCLUSION

Results of interaction analysis of artemisinin compounds or their derivatives with hemin *in-vitro*

by spectrophotometric method are consistent with molecular mechanical calculations using molecular docking. This indicates that the interaction artesunat has a lower free energy compared with other ligands artemether and artemisinin (Arte and ARTM).

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