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In Silico Analysis of Chalcone Derivatives as Potential Antibacterial Agents against DHPS Enzyme

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***Abstract*—Chalcone and its derivatives have been reported to perform as antibacterial agents. With the increasing threat of antibacterial resistance in pharmaceutical sector today, the discovery of new antibacterial agents is essential to accomplish good health and well-being in supporting Sustainable Development Goals (SDGs) point 4. *In silico* analysis is a method used to evaluate some candidates of active compounds before the synthesis process is conducted. This study aims to investigate three chalcone derivatives as potential antibacterial agents using *in silico* method of molecular docking. The three chalcone derivatives, 3-(4-methoxyphenyl)1-phenylprop-2-en-1-one** (1)**, 1-(4-aminophenyl)-3-(4- methoxyphenyl) prop-2-en-1-one** (2) **and 1-(4-bromophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one** (3), **were designed as *p*ABA competitive inhibitor on DHPS and analyzed against *Escherichia coli*. This inhibitory mechanism was folate synthesis inhibition as precursor to DNA and RNA synthesis. Molecular docking of three chalcone derivatives with DHPS was generated using *Autodock4*. The results of this study showed that free energy binding (kcal/mol) of compounds (1), (2) and (3) were -6.27, -5.35 and -5.77, respectively. Besides, the Ki constant for three compounds in order were 25.50 µM, 120.32 µM and 58.84 µM, respectively. In fact, the molecular docking positions illustrated that three chalcone derivatives occupied the active site cleft. Specifically, compound (1) indicated the best outcome among the two other candidates. Meanwhile, sulfadiazine molecular docking as positive control showed lower free binding energy (-0.86 kcal/mol) and Ki constant (233.19 mM) compared to three other candidates. Therefore, three chalcone derivatives analyzed in this study demonstrated a role as potential antibacterial agents.**

***Keywords*—chalcone derivatives, *in silico*, molecular docking, antibacterial, good health and well-being.**

I. INTRODUCTION

Antimicrobial resistant (AMR) is a condition in which antimicrobials including antibacterial, antiviral, antifungal, etc are no longer effective in curing infection and diseases. It poses a serious threat as it is estimated to cause more than 1 million deaths and more than 4 million infection-related diseases in 2019 [1],[2]. This issue becomes one of United Nation concerns as it relates to the Sustainable Development Goals (SDGs) on health problem which leads to point 4, good health and well-being. Therefore, it is important for current researchers to discover new potential antimicrobial agents.

Sulfonamides are the first commercial antibiotics which targets dihydropteroate synthase (DHPS). DHPS is an enzyme involved in the synthesis of folate which is essential for bacterial growth by catalyzing *para*-aminobenzoic acid (*p*ABA) and 6-hydroxymethyl-7,8-dihydropterin pyrophosphate (DHPP) to generate 7,8-dihydropteroic acid (7,8-DHP) (Figure 1). The further transformation will generate tetrahydrofolate for DNA and RNA synthesis. DHPS has two cleft for its substrates, *p*ABA and DHPP [1], [3]–[5]. Thus, some inhibitors could mimic the structure of the substrates and act as competitive inhibitor.

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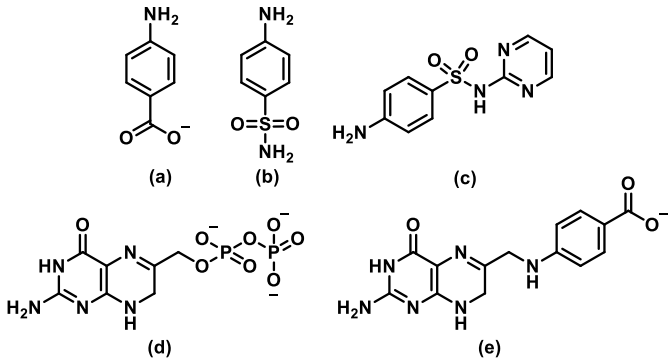


Figure 1. The structure of (a) *p*-ABA, (b) sulphonamide, (c) sulfadiazine, (d) DHPP and (e) 7,8-DHP.

Chalcone and its derivatives have been proven to perform as antibacterial, antifungal, antimalarial, anti-inflammatory, antitumor and anticancer, which can be synthesized by using the Claisen-Schmidt reaction of acetophenone or its derivatives, and also benzaldehyde or its derivatives in the presence of base [6], [7]. Suwito, et. al [8], [9] have investigated the methoxy-4'-amino chalcone derivatives which have antibacterial, anticancer and antimalarial activities. For the antibacterial agent, the methoxy-4'-amino chalcone derivatives were designed as a competitive inhibitor of *p*ABA [10]. However, this study only covered 4-amino chalcone derivatives against the DHPS enzyme. Therefore, in this study three chalcone derivatives: 3-(4-methoxyphenyl)1-phenylprop-2-en-1-one **(1)**, 1-(4-aminophenyl)-3-(4- methoxyphenyl) prop-2-en-1-one **(2)** and 1-(4-bromophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **(3)** (Figure 2) were designed and analyzed using *in silico* method to predict and investigate their efficacy against *Escherichia coli*. These chalcone derivatives could be synthesized by reacting acetophenone derivatives and 4-methoxy benzaldehyde in the presence of base (Figure 2). With the use of *in silico* of molecular docking, potential antibacterial agents could be screened prior the synthesis process, in order to overcome antibacterial resistance.

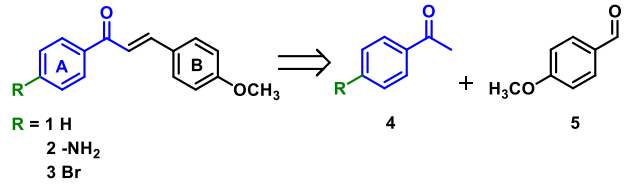


Figure 2. The structure of chalcone derivatives **(1)**, **(2)** and **(3)**.

II. METHODS

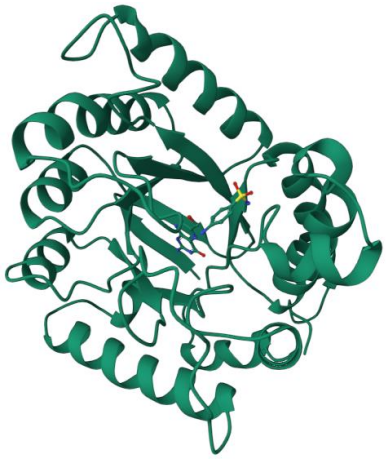
The use of the methods involving preparation and docking validation was followed by docking of chalcone derivatives with the DHPS enzyme [10]. The 3-dimensional structure of *E.coli* DHPS (ecDHPS) with code 1AJ0 was obtained from Protein Data Bank (PDB) as an enzyme receptor with its ligands of 2-amino-6-hydroxymethyl-7,8-dihydro-3H-pteridine-4-one, sulfate ion and sulfonamide [4]. Then, an enzyme and three chalcone derivatives as ligands were prepared, visualized and docked by using the following softwares: *ChemBio Office* 2008 11.0, *PyMOL Delano Scientific*, *Discovery Studio Visualizer* 2.5, *Phyton* 2.5.2, *Autodock Tools* 1.5.6 and *Autodock4* 4.2.

The first process to perform *in silico* analysis was to minimize ligands and DHPS energy structures at the preparation stage. Next, the second stage involved docking validation (redocking) by using DHPS native ligand from PDB structure, in this case was sulfonamide, to obtain the grid box position of binding pocket. In this process, the native ligand of DHPS was released and redocked based on the determined grid box position. The following stage was stacking or overlapping the redocking result with the position of the native DHPS ligand. Once this overlaping native ligands were superimposed, the docking process of chalcone derivatives could be proceed using the grid box coordinate that had been determined and visualized.

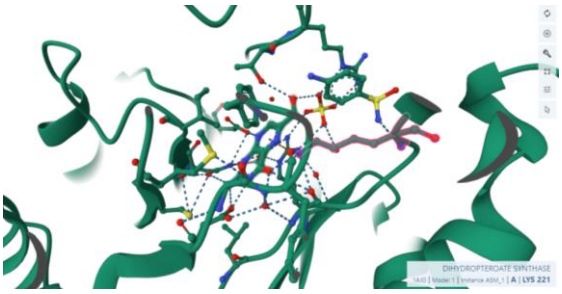
III. RESULT AND DISCUSSION

A. Preparation and Docking Validation

The DHPS has two binding clefts for 7,8-dihydropterin pyrophosphate binding located in the deep cleft, and the *p*ABA binding which are closer to the surface [4], [10]. The ecDHPS with code 1AJ0 X-Ray crystal structure was obtained from PDB with three ligands: 2-amino-6-hydroxymethyl-7,8-dihydro-3H-pteridine-4-one, sulfate ion and sulfonamide as reported by Achari et. al (1997) [4]. In the study, Achari et. al. (1997) discussed that the DHPS structure has 8 α-helices and β-strands. In addition, the 1AJ0 DHPS X-Ray crystal structure used sulfonamide substrate as *p*ABA analogue structure (Figure 3) [4]. Based on the results of macromolecules preparation that was conducted in this study, the crystal structure of DHPS 1AJ0 showed several active sites residues for sulfonamide binding as follows: Asn22, Thr62, Arg63, Asp96, Asn115, Ile117, Asp185, Phe190, Leu215, Gly217, Lys221, Arg255 and His257.



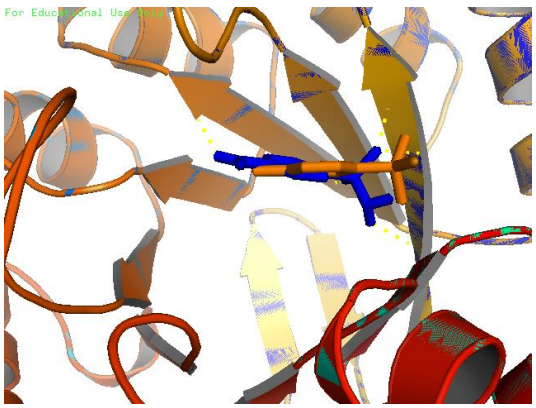
(a)



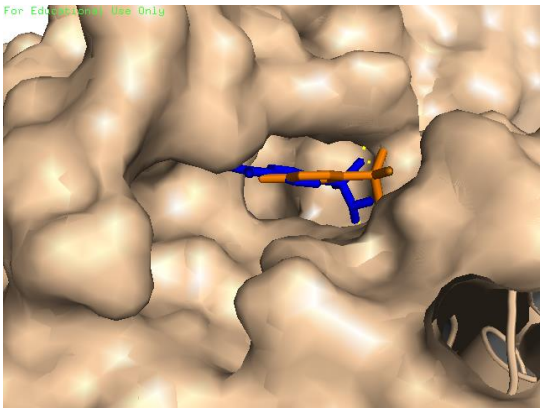
(b)

Figure 3. (a) EcDHPS (green) with its ligands (ball and stick structure) retrieved from PDB website showed (b) a complex interaction.

In this *in silico* study analysis, a sulfonamide binding pocket was used to perform docking with three chalcone derivatives. Prior to chalcone derivatives and DHPS docking process, docking validation (redocking) was conducted to confirm the binding pocket attachment and to determine the grid box coordinate. The process was initiated by the detachment of the sulfonamide from DHPS and then both ligand and enzyme were docking according to the correct grid box position obtained (1 Å RMSD). The following process was performed by superimposed of sulfonamide 1AJ0 native ligand (orange) with the redocking ligand (blue) to confirm that the grid box coordinate was validated and located inside the active cleft. As a result, the sulfonamide native ligand position of DHPS 1AJ0 from PDB was superimposed with the redocking position were illustrated at Figure 4a and 4b. From this redocking stage, the free binding energy of sulfonamide as native ligand of DHPS 1AJ0 was -4.48 kcal/mol with three hydrogen bonds noted at Thr62, Arg63 and Arg255; and also for 6 amino acid residues of van der Waals-electrostatic interactions noted at Thr62, Arg63, Pro64, Ser219, Arg255 and His257 (Figure 4c). Thus, the coordinate of grid box was then used for the docking process between three chalcones derivatives and DHPS.



(a)



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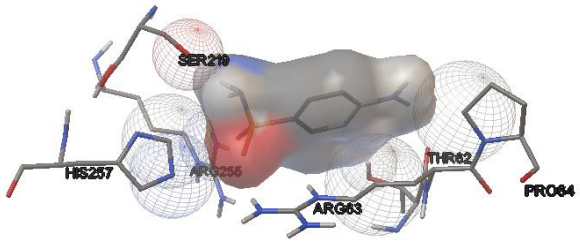


(c)

Figure 4. Docking validation by superimposed both of 1AJ0 DHPS native sulfonamide ligand (orange) and redocking result (blue): (a) the superimposed, (b) superimposed on active site cleft, (c) the van der Waals and electrostatic interactions between ligand and DHPS from the redocking results.

B. Docking of DHPS with Chalcone Derivatives

The docking of the three chalcone derivatives **(1)**, **(2)** and **(3)** with DHPS was performed using the grid box coordinate resulting from the docking validation stage. From the docking of these three ligands with DHPS, several parameters were obtained, including the complex free binding energy (Δ*G*), inhibition constant value and interactions binding between amino acid residues that formed hydrogen bond and van der Waals-electrostatic interactions (Table 1). In fact, the free energy binding (kcal/mol) resulting from the chalcone derivatives **(1)**, **(2)** and **(3)** were -6.27, -5.35 and -5.77, respectively. As a control positive, a sulfo group antibiotic (sulfadiazine) was also presented (-0.86 kcal/mol). It is noted that the negative free energy binding value indicated that the complex formation process between DHPS and four ligands occurred spontaneously. In addition, it was also an indication of an equilibrium state of binding affinity and stable formation between the complexes [11].

Table 1. Molecular docking analysis of ecDHPS 1AJ0 with chalcone derivatives and sulfodiazine

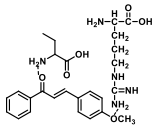
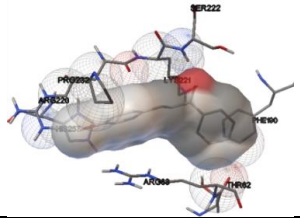
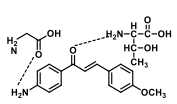
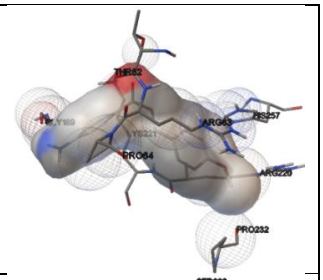
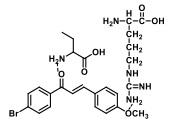
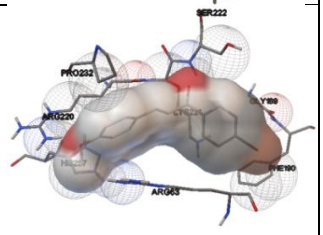
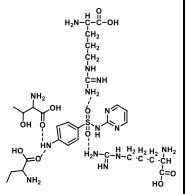
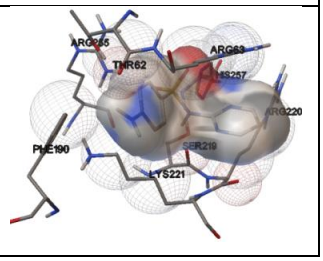
Compound	Free Binding Energy (kcal/mol)	Ki	Hydrogen Bond	Van der Waals-electrostatic Interactions
(1)	-6.27	25.50 µM	Arg220, Ser222	Thr62, Arg63, Phe190,

				Arg220, Lys221, Ser222, Pro232, His257
(2)	-5.35	120.32 µM	Thr62, Gly189	Thr62, Arg63, Pro64, Gly189, Arg220, Lys221, Pro232, His257
(3)	-5.77	58.84 µM	Arg220, Ser222	Arg63, Gly189, Phe190, Arg220, Lys221,Ser222, Pro232, His257
Sulfa-diazine	-0.86	233.19 mM	Thr62, Arg63, Ser219, Arg255	Thr62, Arg63, Phe190, Ser219, Arg220, Lys221, Arg255, His257

From the analysis, it is highlighted that chalcone derivatives acted as competitive inhibitor by mimicking the structure of its substrate, *p*ABA, on the ring A (Figure 2). This inhibition value was described as the inhibition constant (Ki) on Table 1 for compounds of **(1)**, **(2)** and **(3)** as follows: 25.50 µM, 120.32 µM and 58.84 µM, respectively.

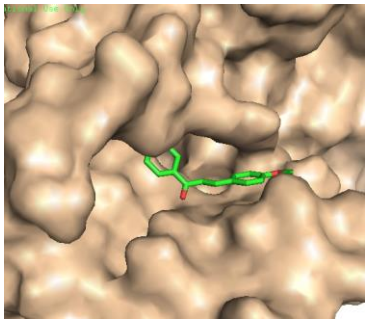
As a positive control, sulfadiazine gave the free binding energy that higher than the three chalcone derivatives. Moreover, it was found that Ki constant of sulfadiazine also showed the highest value (233.19 mM) among three candidates. These two parameters indicated that three chalcone derivatives have potential as antibacterial due to the free energy binding value and Ki constant were lower. It is believed that the lower the Ki constant, the higher the efficacy of the compound. Then, it was also found that of the three chalcone derivatives, compound **(1)** have had the lowest free energy binding and Ki constant compared to the other compounds.

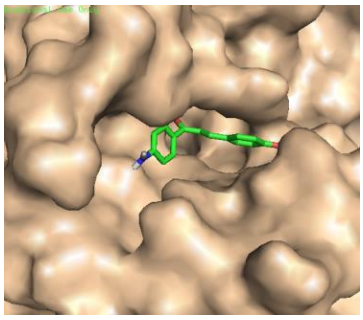
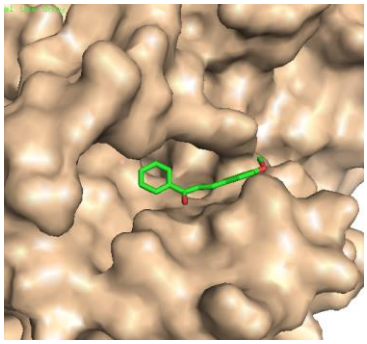
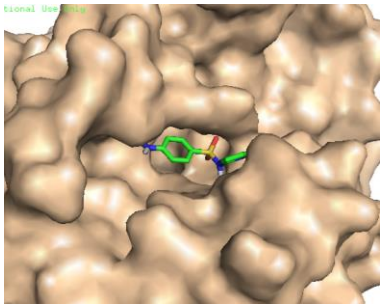
Table 2. Visualization of molecular docking analysis of DHPS 1AJ0 with three chalcone derivatives located in the active site (hydrogen bond and van der Waals-electrostatic interactions)

Compound	Hydrogen Bond	Van der Waals-electrostatic interactions
(1)		
(2)		
(3)		
Sulfa-diazine		

Compound **(1)** and **(3)** have 2 hydrogen bonds at Arg220 and Ser222, which were formed between the O carbonyl of chalcone derivative compound and H of NH_2 from Ser222. The second hydrogen bonding was formed between the O methoxy of chalcone derivative and H of NH_2 from Arg220. Meanwhile, compound **(2)** has 2 hydrogen bonds at Thr62 and Gly189 which were formed between the NH_2 of chalcone derivative and the carbonyl of Gy189; and also O carbonyl to NH_2 of Thr62, respectively. It is important to note that all the van der Waals-electrostatic interactions of compounds **(1)**, **(2)** and **(3)** occurred due to 8 amino acid from the active site residues. Meanwhile, molecular docking of sulfadiazine presented 4 hydrogen bonds which were formed by NH_2 to O carbonyl of Thr62 and Ser219, and also O carbonyl to NH_2 of Arg63 and Arg255. In total, there were 8 van der Waals-electrostatic interactions occurred between sulfadiazine-DHPS complex as presented in Table 2.

Table 3. Surface visualization of molecular docking analysis of DHPS 1AJ0 with three chalcone derivatives located in the active site

Compound	Surface
(1)	

(2)	
(3)	
Sulfadiazine	

To be more specific, Table 3 presents a visualization of the molecular docking results from the complex DHPS with all three chalcone derivatives and sulfadiazine. It is assumed that the binding affinity of the chalcone derivatives-DHPS complex could be affected by the attachment of the 3D structure of the ligands [10]. Table 3 illustrates that the Ring A of chalcone derivatives was located deeper in the pocket compared to the position of ring B which was located closer to the surface. Moreover, compound **(1)** showed that its ring A structure occupied deeper to the cleft compared to compound **(2)** and **(3)**. This finding was used as evidence to conclude that this compound has lower free energy binding and Ki constant which leads to adequate potential efficacy as antibacterial agent. In addition, the carbonyl position of compound **(1)** and **(3)** faced downward while ring A of compound **(2)** faced upward indicating that the substituent on ring A affected the ligand position in the cleft.

Research conducted by Patel et. al. (2010) reported that aryl methoxy and halogen substituents could improve the antibacterial activity [12]. Into the bargain, halogen that functions as electron-withdrawing substituent play a role in enhancing the antibacterial efficacy [13], [14]. Therefore, compound **(1)** bearing methoxy substituent and compound **(3)** bearing methoxy (Ring B) and –Br substituent (Ring A) were shown to have low binding energy values and Ki constants. On the other hand, according to the result of molecular docking analysis, compound **(2)** bearing $–NH_2$ substituent (Ring A) and methoxy (Ring B) performed to have the lowest efficacy as antibacterial agent against the DHPS. This result turned out to be in line with the results of the antibacterial assay on *E.coli* which had a lower inhibition zone (8.5 mm) among other amino and methoxy chalcone derivatives [10] and gave an inhibition zone of 9.38 mm at 500 µg/mL [8]. As a result, it is worth to say the chalcone derivatives **(1)** and **(3)** in this study highlight the potential activity value as the potential antibacterial agents.

IV. Conclusion

It can be concluded that *in silico* analysis through molecular docking method has a role in predicting the potential active compounds as antibacterial agents. By predicting the efficacy of these active compounds, the interactions of ligand-enzyme complex could be indicated to design and to screen of thousands candidates before the synthesis process in the future. This study also conclude that chalcone derivatives bearing methoxy and halogen substituent had proven to have potential activity as antibacterial agents. Therefore, these chalcone derivatives could be synthesized and further investigated on enzymatic assay, as well as, a research on the effects of electron-withdrawing and electron–donating groups as substituents should be conducted in the future. Moreover, these three candidate compounds can be proposed to be active compounds and be applied in nanotechnology, for example as bioactive compounds in drug delivery, or functionalized with nanomaterials to enhance the bioactivity in medical applications.

Acknowledgment

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