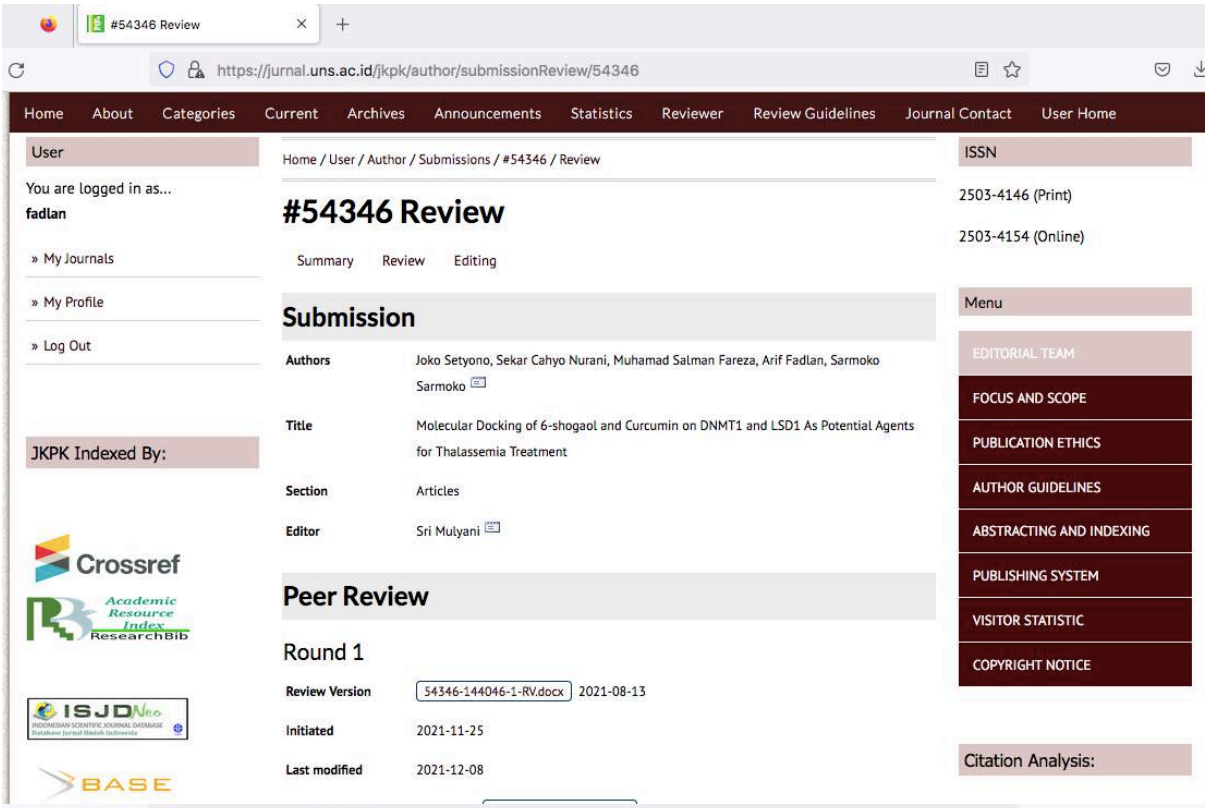
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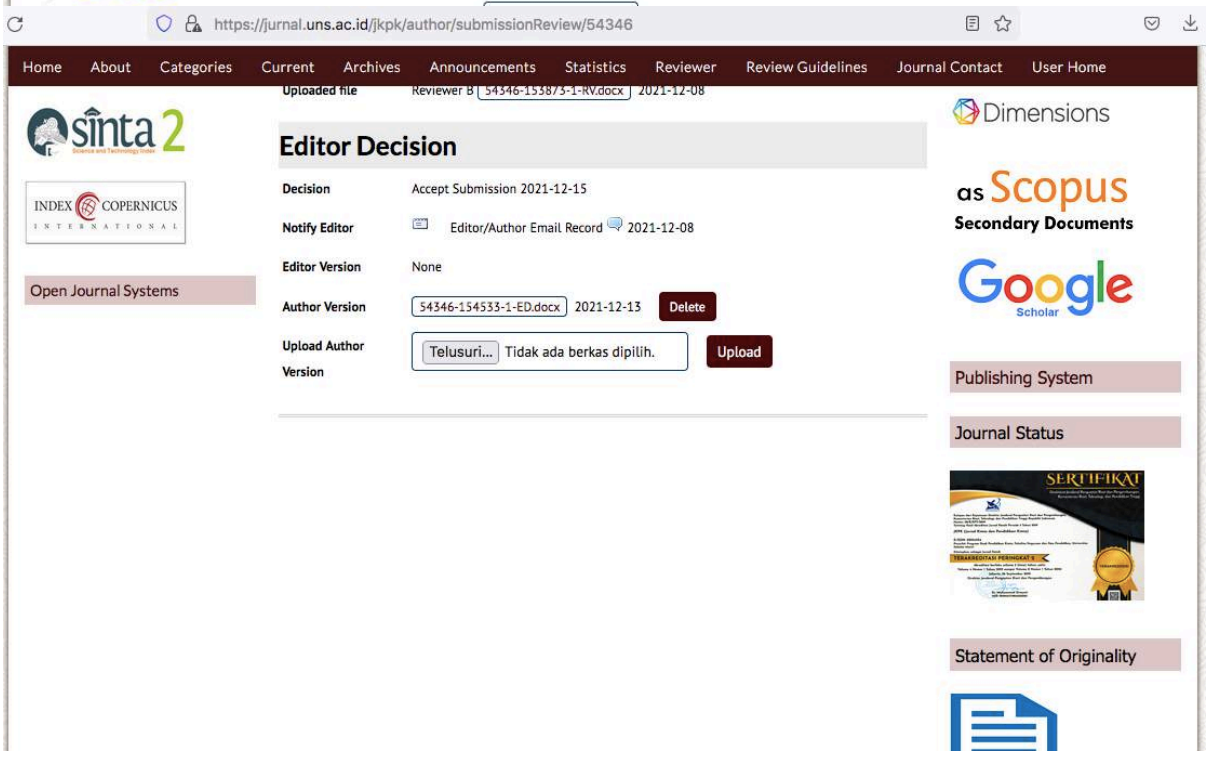
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Molecular docking of 6-shogaol and curcumin on DNMT1 and LSD1 as potential agents for thalassemia treatment

ABSTRACT

Beta-thalassemia therapy is developed by increasing γ-globin production, which binds to α-globin to form haemoglobin fetal (HbF). Meanwhile, DNMT1 and LSD1 play an important role in silencing the *HbF* gene by inhibiting the production of HbF and inducing HbA expression. 6-shogaol and curcumin induce HbF by inhibiting STAT3 expression. Therefore, this study predicts the interaction between 6-shogaol and curcumin on DNMT1 and LSD1. The protein structure was prepared by removing the water molecules, while the validation step was performed by separating protein from native ligands in new PDB files. Furthermore, the protein was docked with a native ligand to obtain grid box coordinates, while RMSD was calculated from the conformation results of the validation process. 6-shogaol and curcumin were docked with coordinates of the validation results, and the best conformation was visualized with Discovery Studio. The validation step results in the RMSD value of 0.861Å and 1.410Å for DNMT1 and LSD, respectively. The binding affinity of 6-shogaol and curcumin on DNMT1 was -6.5 kcal/mol and -8.0 kcal/mol, respectively. Furthermore, the binding affinity of 6-shogaol and curcumin on LSD was -8.2 kcal/mol and -10.1 kcal/mol, respectively. Amino acid residues found in DNMT1 interaction include Gly1147, Phe1145, Glu1168, Asn1278, Pro1225, Leu1151, Val1580, Ala1579, Asn1578, Trp1170, and Ala1579; meanwhile, Val288, Ser289, Arg310, Gly285, Thr624, Leu659, Lys661, Arg316, Leu625, Tyr761, Trp751, Gly330, and Leu659 were found in LSD1. This study showed that curcumin has a greater potential to inhibit DNMT1 as well as LSD1 proven by lower bonding energy and stronger bond types.

Key word: *beta-thalassemia, DNMT1, LSD1, molecular docking*

INTRODUCTION

Beta-thalassemia is a hereditary disease in which haemoglobin production is inhibited, causing anaemia. Haemoglobin is not formed due to insufficient production of β-globin [1]. In general, Indonesia has a high thalassemia carrier frequency of 5% [2], meanwhile, in Banyumas Regency, the frequency is 8% [3]. Currently, lifelong blood transfusion is the mainstay of treatment for β-thalassemia. However, this treatment increases the risk of infection and causes chills, anaphylaxis, accumulation of iron ions in the heart, liver, and endocrine glands [1].

Beta-thalassemia therapy is developed by increasing γ-globin production which binds to α-globin to form haemoglobin fetal (HbF, α2γ2), restoring normal erythrocyte function and lowering the disease severity [4]. Meanwhile, DNMT1 and LSD1 play an important role in silencing *HbF* gene by inhibiting the production of HbF and inducing HbA expression. Inhibition of DNMT1 and LSD1 increase HbF expression [5]. In addition, 6-shogaol and curcumin induce the production of HbF by inhibiting mRNA STAT3 expression in K562 cells [6]. Therefore, this study aims to perform molecular docking to explore the interaction of 6-shogaol and curcumin with DNMT1 and LSD1.

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METHODS

Ligands and Proteins Preparation

Docking study was performed on Windows computer Intel® Celeron® CPU N3060 @1,60GHz (2CPUs) 4096MB RAM using Intel® HD Graphics. Meanwhile, 6-shogaol and curcumin structures were obtained from Pubchem (https://pubchem.ncbi.nlm.nih.gov/) and were optimized with Avogadro 1.2.0 (http://avogadro.cc/) [7]. The proteins DNMT1 (3SWR) and LSD1 (6KGP) were retrieved from Protein Data Bank (PDB) (https://www.rcsb.org/). Furthermore, ligands and proteins were prepared with AutoDockTools 1.5.7 through the removal of water molecules, the addition of polar hydrogen atoms and Gasteiger changes as well as rotatable bonds. The obtained PDB files were converted to PDBQT format files.

Validation

The redocking procedure was executed with native ligands of each protein (sinefungin for 3SWR and FAD for 6KGP) using AutoDock Vina [8]. The position of each native ligand in the active protein site was utilized for grid center and box size parameter determination with a spacing of 1 Å. Furthermore, the native ligand was flexible, while the protein was in a rigid condition. The exhaustiveness value was set to eight and the configuration file was run with AutoDock Vina at Command Prompt (CMD), while the validation result was expressed as RMSD (Root Mean Square Deviation) with PyMol 2.4.0.

Molecular Docking

A similar process of redocking was adapted for 6-shogaol and curcumin using Autodock Vina. The conformation and interactions of docking results were visualized using Discovery Studio 2020 (https://www.3ds.com/).

RESULTS AND DISCUSSION

Ligands and Proteins Preparation

6-Shogaol and curcumin structure was retrieved from PubChem followed by optimization using Avogadro 1.2.0. The ligands were prepared by the addition of all hydrogen atoms, Gasteiger changes, and rotatable bonds. The obtained PDB files were then converted into PDBQT files and prepared for the docking process. Furthermore, ligands preparation also showed that both 6-shogaol and curcumin have 10 torsions.

The crystal structure of DNMT1 (3SWR) and LSD1 (6KGP) proteins at a resolution of 2.49Å and 2.25Å was retrieved from PDB and used in this study. Both proteins have good resolution and native ligand, meanwhile, 3SWR (601-1600) and 6KGP (172-832;901-909) crystallographic data indicated that both contain sinefungin and FAD native ligand, respectively. Furthermore, 3SWR and 6KGP were prepared via the removal of water molecules and native ligands extraction followed by optimization of the prepared proteins using MMFF94 in Avogadro which is commonly applied for organic compounds. Sinefungin and FAD co-crystallized ligands extraction from the binding site showed that the center coordinates and grid box size of 3SWR were x=-5.041, y=-0.949, z=31.815 and 14 × 18 × 20 Å while for 3KGP were x=25.776, y=43.519, z=-19.881 and 22 × 26 × 16 Å.

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Validation

Validation through the redocking procedure was performed to ascertain the docking process. Sinefungin and FAD redocking to the active site in the 3SWR and 6KGP crystals was successful and indicated that the native ligands were docked back onto the binding sites. In addition, the acceptability of the validation was proven by overlaying the docked sinefungin and FAD with similar co-crystallized structures (**Figure 1 and 2**). This step produced RMSD values of 0.861 Å and 1.410 Å for 3SWR and 6KGP, respectively.

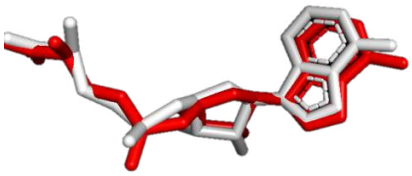

Figure 1 Overlays of redocking ligand (white) with reference ligand from crystallography (red) at 3SWR.

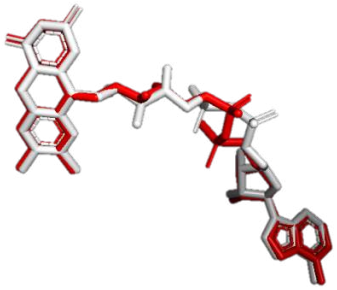

Figure 2 Overlays of redocking ligand (white) with reference ligand from crystallography (red) at 6KGP.

Interaction analysis using Discovery Studio 2020 indicated that sinefungin interacted with 3SWR through eight hydrogen bonding and five hydrophobic interactions, while FAD had 26 hydrogen bonds, one electrostatic interaction and 20 hydrophobic interactions with 6KGP. Sinefungin was bound to Val1580, Leu1151, Glu1168, Met1169, Phe1145, Gly1149, Gly1150 residues of 3SWR by hydrogen bonding interaction (**Figure 3**). A similar interaction of FAD to 6KGP was formed through Gly28, Val288, Ser289, Arg310, Arg316, Arg316, Arg316, Met332, Val333, Val590, Thr624, Val811, Glu308, Val333, Glu308, Val590, Gly285, Gly287, Gly315, Gly800, Val811, Ala809, Glu308, Thr624, Thr624, and Trp756 residues (**Figure 4**).

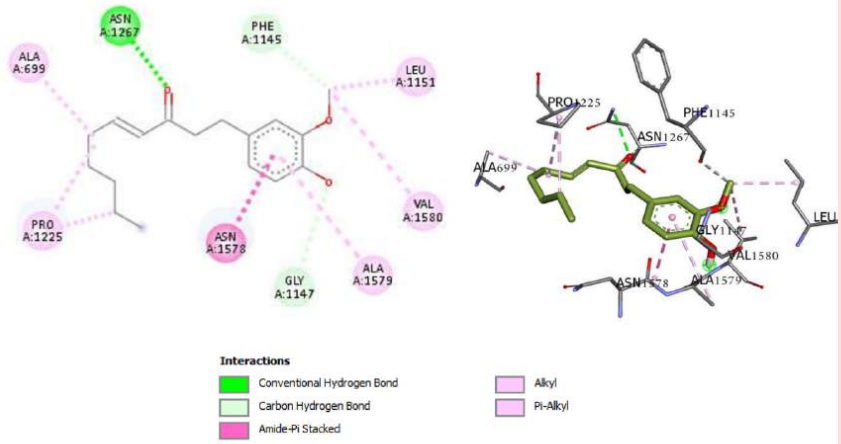

Figure 3 Visualization of validation results 3SWR with sinefungin in 2D and 3D

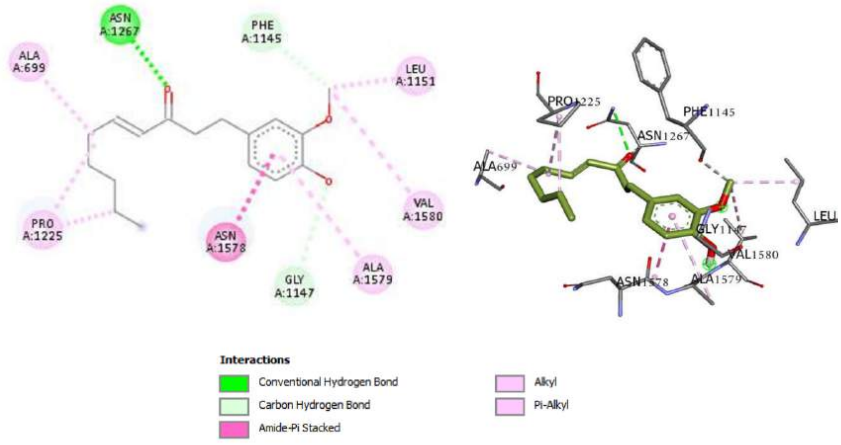

Figure 4 Visualization of docking results 3SWR with 6-shogaol in 2D and 3D

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The hydrophobic interactions occurred between the docked sinefungin and 3SWR via Pro1225, Leu1247, Cys1191, Met1169, Phe1145 residues. Meanwhile, for FAD, the hydrophobic interactions were formed by Ala309, Ala331, Ala331, Trp751, Trp751, Trp756, Tyr761, Tyr761, Gly330, Ala331, Gly330, Ala331, Leu659, Arg316, Trp751, Trp751, Val590, Leu625, Ala309, Leu625, Val629, Ala331 residues of 6KGP. Furthermore, one electrostatic interaction was found between the docked FAD with Arg316 residue of 6KPG. These results produced a good RMSD value (<2.0 Å) [9], while corresponding interactions of the co-crystallized docked ligands indicated that the redocking is appropriate and the protocol is applicable for the next docking process.

Molecular Docking

The docking process was performed using Autodock Vina by flexible docking, given that the ligands were flexible, and the proteins were rigid. The results expressed as binding affinity represent the best configuration of the ligands in the protein binding sites. Meanwhile, the negative value of binding energy indicates stronger interaction of ligand-protein and better prediction [10]. The native ligand, sinefungin of 3SWR had binding energy of -9.0 kcal/mol, while 6-shogaol and curcumin produced interaction with binding energy values of -6.5 and -8.0 kcal/mol respectively. A previous study showed that caffeic acid, a hydroxycinnamic acid and polyphenols derivate has an IC_{50} value of 3.0 µM [11], and binding energy of -5.32 kcal/mol [12]. Furthermore, genistein inhibited DNMT1 in male rats [13] and produced a binding energy value of -6.39 kcal/mol [12]. Meanwhile, parthenolide, a sesquiterpene lactone of the germacranolide class, showed DNMT1 inhibitory activity with an IC_{50} value of 3.5 µM [14] and binding energy of -6.34 kcal/mol [12].

The binding energy value of 6-shogaol and curcumin indicated the potent activity of both compounds as DNMT1 inhibitors. Furthermore, the binding mode analysis of 3SWR as shown in **Figure 5** and **6** indicated that the interaction of 6-shogaol through hydrogen bonding occurred via Asn1267, Phe1145 and Gly1147 residues. It also formed hydrophobic interactions with Asn1578, Ala1579, Val1580, Leu1151, Ala699, and Pro1225 residues. This result is in line with Xie et al. (2019) which stated that the interaction of 6-shogaol with DNMT1 (3SWR) occurred through Phe1145, Gly1147, Asn1578, Ala1759, Val1580, and Pro1225 residues. Meanwhile, curcumin is bound to Asp700, Glu1168, Ala699, and Phe1145 residues through hydrogen bonding. Hydrophobic interactions of curcumin with 3SWR also occurred via Pro1225, Trp1170, Ala1173, Asn1578, Ala1579, Val1580 and Leu1151 residues. Besides, some important amino acid residues involved in the interaction of curcumin with DNMT1 include Glu1168, Phe1145, Pro1225, Trp1170, Asn1578, Ala1579 and Val1580 (Xie et al. 2019).

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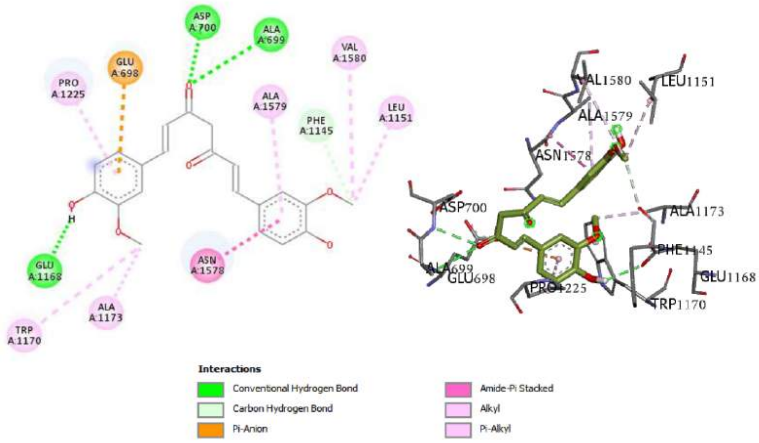

Figure 5 Visualization of docking results 3SWR with curcumin in 2D and 3D

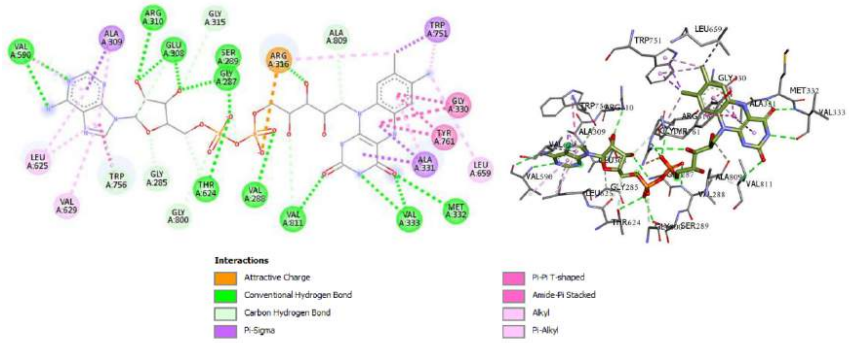

Figure 6 Visualization of validation results 6KGP with FAD in 2D and 3D

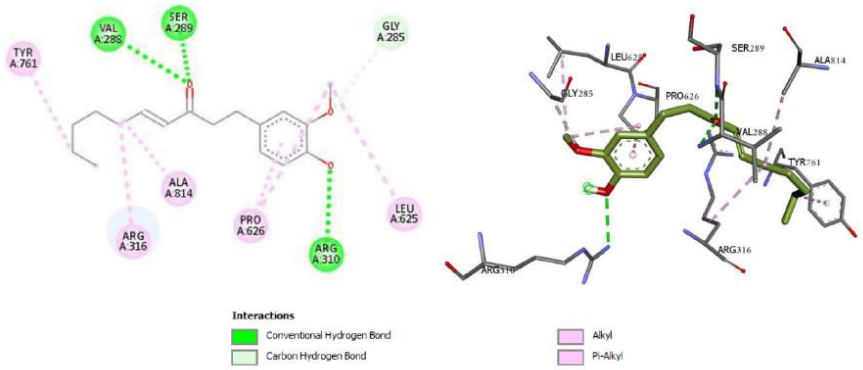

Figure 7 Visualization of docking results 6KGP with 6-shogaol in 2D and 3D

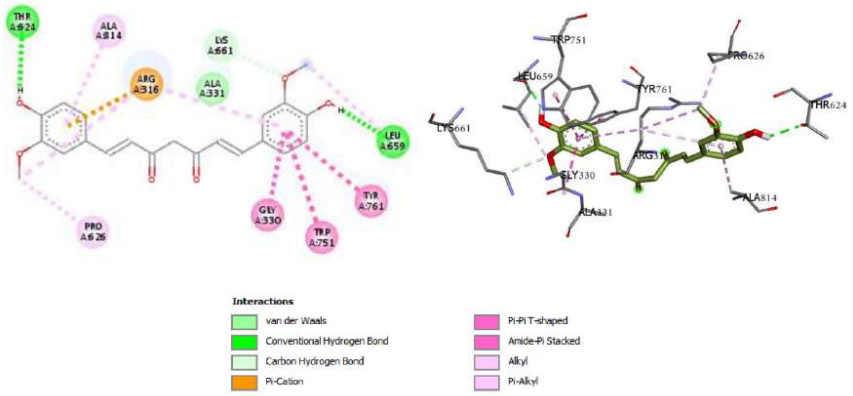

Figure 8 Visualization of docking results 6KGP with curcumin in 2D and 3D

The binding energy of FAD as a native ligand of LSD1 was -16.2 kcal/mol while 6-shogaol and curcumin were -8.2 and -10.1 kcal/mol, respectively. Oleacin, a phenolic LSD1 inhibitor showed inhibitory activity with an IC_{50} value of 2.5 µmol/L and binding energy of -9.33 kcal/mol [15]. Furthermore, a selective LSD1 inhibitor namely iadademstat produced binding energy of -9.09 kcal/mol [16].

The interactions analysis of 6-shogaol with LSD1 indicated hydrogen bonding through Val288, Ser289, Arg310 and Gly285 residues (**Figure 7**). This result correlated with the amino acids responsible for inhibitory activity namely Val288, Ser289, Gly314, Tyr624 and Lys661 [17]. Meanwhile, curcumin in LSD1 exhibits hydrogen bonding interaction with Thr624, Leu659, and Lys661 residues (**Figure 8**). The Thr624 and Lys661 residues are two important amino acids responsible for increasing the inhibitory activity. In addition, Leu659 is also a pivotal residue for the interaction of diaryene, a stilbene derivative, with LSD1. This compound showed an inhibitory activity with pIC_{50} value of 4,991 [17]. DNMT1 and LSD1 bind to BCL11A, hence, when both proteins are inhibited, the mechanism of silencing off fetal haemoglobin performed by BCL11A is averted

CONCLUSION

Based on this study, curcumin has greater interaction to both DNMT1 and LSD1. Therefore, inhibition of both proteins by curcumin and 6-shogaol compounds may increase the production of HbF in the body and reduce, thalassemia severity and the need for blood transfusions. Further study is required to examine this potential effect of curcumin as a compound for beta-thalasemia therapy.

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Molecular docking of 6-shogaol and curcumin on DNMT1 and LSD1 as potential agents for thalassemia treatment

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Molecular docking of 6-shogaol and curcumin on DNMT1 and LSD1 as potential agents for thalassemia treatment

ABSTRACT

Beta-thalassemia therapy is developed by increasing γ-globin production which binds to α-globin to form haemoglobin fetal (HbF). Meanwhile, DNA methyltransferase 1 (DNMT1) and lysine specific demethylase 1 (LSD1) play an important role in silencing the *HbF* gene by inhibiting the production of HbF and inducing haemoglobin subunit alpha (HbA) expression. 6-Shogaol and curcumin induce HbF by inhibiting signal transducer and activator of transcription 3 (STAT3) expression. Therefore, this study predicts the interaction between 6-shogaol and curcumin on DNMT1 and LSD1. The protein structure of DNMT1 (3SWR) and LSD1 (6KGP) was prepared by removing the water molecules, while the validation step was performed by separating protein from native ligands (sinefungin for 3SWR and flavine-adenine dinucleotide (FAD) for 6KGP) in new protein data bank files. Furthermore, the protein was docked with a native ligand to obtain grid box coordinates, while root mean standard deviation (RMSD) was calculated from the conformation results of the validation process. 6-Shogaol and curcumin were docked with coordinates of the validation results, and the best conformation was visualized with Discovery Studio. The validation step results in the RMSD value of 0.861Å and 1.410Å for DNMT1 and LSD1, respectively. The binding affinity of 6-shogaol and curcumin on DNMT1 was -6.5 kcal/mol and -8.0 kcal/mol, respectively. Furthermore, the binding affinity of 6-shogaol and curcumin on LSD1 was -8.2 kcal/mol and -10.1 kcal/mol, respectively. Amino acid residues found in DNMT1 interaction include Gly1147, Phe1145, Glu1168, Asn1278, Pro1225, Leu1151, Val1580, Ala1579, Asn1578, Trp1170, and Ala1579; meanwhile, Val288, Ser289, Arg310, Gly285, Thr624, Leu659, Lys661, Arg316, Leu625, Tyr761, Trp751, Gly330, and Leu659 were found in LSD1. This study showed that curcumin has a potential to inhibit DNMT1 as well as LSD1 proven by lower bonding energy and stronger bond types compared to sinefungin and FAD native ligands, and other DNMT1 and LSD1 inhibitors.

***Key word:** beta-thalassemia, DNMT1, LSD1, molecular docking*

INTRODUCTION

Beta-thalassemia is a hereditary disease in which haemoglobin production is inhibited, causing anaemia. Haemoglobin is not formed due to insufficient production of β-globin [1]. In general, Indonesia has a high thalassemia carrier frequency of 5% [2], distributed in West Java, Jakarta, and Central Java and Yogyakarta provinces [3]. The prevalent treatments for β-thalassemia

include iron chelation therapy, surgery for removing the gallbladder, transplantation of bone marrow, and regular blood transfusion. The three former methods display several drawbacks making the latter the mainstay of β-thalassemia treatment. However, this treatment also increase the risk of infection and causes chills, anaphylaxis, and iron ions accumulation in the heart, liver, and endocrine glands [1].

Beta-thalassemia therapy developed by increasing γ-globin quantity which binds to α-globin to form haemoglobin fetal (HbF, α2γ2) is an alternative for β-thalassemia treatment. The α-globin is able to pairs and restoring normal erythrocyte function, so the disease severity could be suppressed [4]. However, the HbF production could be inhibited by DNA methyltransferase 1 (DNMT1) and lysine specific demethylase 1 (LSD1) which play an important role in silencing HbF gene and inducing haemoglobin subunit alpha (HbA) expression. Thus, inhibition of DNMT1 and LSD1 could increase the HbF expression [5].

6-Shogaol, discovered in the rhizome of *Zingiber officinale*, has anti-inflammatory, antioxidant, and anti-cancer activities. 6-Shogaol induces apoptosis in cancer cells and influences the formation of reactive oxygen species. Meanwhile, curcumin, an active compound of *Curcuma longa*, is a polyphenol that displays anti-inflammatory, anticarcinogenic, antiproliferation, and antioxidant properties. Curcumin inhibits catalase and superoxide dismutase activity while increasing proapoptotic protein activity. 6-Shogaol and curcumin have also been found to increase HbF production by inhibiting mRNA signal transducer and activator of transcription 3 (STAT3) expression in K562 cells [6]. Therefore, this study aims to perform *in silico* molecular docking to explore the interaction of 6-shogaol and curcumin with DNMT1 and LSD1.

METHODS

Ligands and Proteins Preparation

Docking study was performed on Windows computer Intel® Celeron® CPU N3060 @1,60GHz (2CPUs) 4096MB RAM using Intel® HD Graphics. Meanwhile, 6-shogaol and curcumin structures were obtained from Pubchem (https://pubchem.ncbi.nlm.nih.gov/) and were optimized with Avogadro 1.2.0 (http://avogadro.cc/) [7]. The proteins DNMT1 (3SWR) and LSD1 (6KGP) were retrieved from Protein Data Bank (PDB) (https://www.rcsb.org/). Furthermore, ligands and proteins were prepared with AutoDockTools 1.5.7 through the removal of water molecules, the addition of polar hydrogen atoms and Gasteiger changes as well as rotatable bonds. The obtained PDB files were converted to partial charge (Q) and atom type (T) (PDBQT) format files.

Validation

The redocking procedure was executed with native ligands of each protein (sinefungin for 3SWR and flavine-adenine dinucleotide (FAD) for 6KGP) using AutoDock Vina [8]. The position of each native ligand in the active protein site was utilized for grid center and box size parameter determination with a spacing of 1

Å. Furthermore, the native ligand was flexible, while the protein was in a rigid condition. The exhaustiveness value was set to eight as default [8] and the configuration file was run with AutoDock Vina at Command Prompt (CMD), while the validation result was expressed as RMSD (Root Mean Square Deviation) with PyMol 2.4.0.

Molecular Docking

A similar process of redocking was adapted for 6-shogaol and curcumin using Autodock Vina. The conformation and interactions of docking results were visualized using Discovery Studio 2020 (https://www.3ds.com/).

RESULTS AND DISCUSSION

Ligands and Proteins Preparation

6-Shogaol and curcumin structure was retrieved from PubChem followed by optimization using Avogadro 1.2.0. The ligands were prepared by the addition of all hydrogen atoms, Gasteiger changes, and rotatable bonds. The obtained PDB files were then converted into PDBQT files and prepared for the docking process. Furthermore, ligands preparation also showed that both 6-shogaol and curcumin have 10 torsions including the best conformation with the lowest energy.

The crystal structure of DNMT1 (3SWR) and LSD1 (6KGP) proteins at a resolution of 2.49Å and 2.25Å was retrieved from PDB and used in this study. Both proteins have good resolution [9] and native ligand, meanwhile, 3SWR (601-1600) and 6KGP (172-832;901-909) crystallographic data indicated that both contain sinefungin and FAD native ligand, respectively [10,11]. Furthermore, 3SWR and 6KGP were prepared via the removal of water molecules and native ligands extraction followed by optimization of the prepared proteins using MMFF94 in Avogadro which is commonly applied for organic compounds. Sinefungin and FAD co-crystallized ligands extraction from the binding site showed that the center coordinates and grid box size of active sites of 3SWR were x=-5.041, y=-0.949, z=31.815 and 14 × 18 × 20 Å while for 3KGP were x=25.776, y=43.519, z=-19.881 and 22 × 26 × 16 Å.

Validation

Validation through the redocking procedure was performed to ascertain the docking process. Sinefungin and FAD redocking to the active site in the 3SWR and 6KGP crystals was successful and indicated that the native ligands were docked back onto the binding sites. In addition, the acceptability of the validation was proven by overlaying the docked sinefungin and FAD with similar co-crystallized structures (**Figure 1 and 2**). This step produced RMSD values of 0.861 Å and 1.410 Å for 3SWR and 6KGP, respectively.

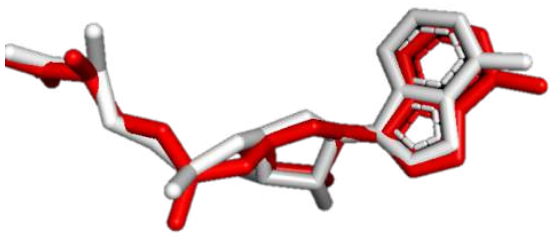

Figure 1 Overlays of redocking ligand (white) with reference ligand from crystallography (red) at 3SWR.

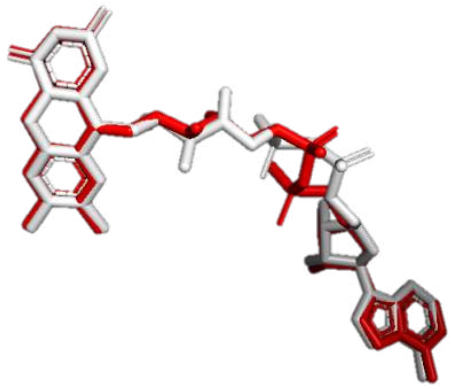

Figure 2 Overlays of redocking ligand (white) with reference ligand from crystallography (red) at 6KGP.

Interaction analysis using Discovery Studio 2020 indicated that sinefungin interacted with 3SWR through eight hydrogen bonding and five hydrophobic interactions, while FAD had 26 hydrogen bonds, one electrostatic interaction and 20 hydrophobic interactions with 6KGP. Sinefungin was bound to Val1580, Leu1151, Glu1168, Met1169, Phe1145, Gly1149, Gly1150 residues of 3SWR by hydrogen bonding interaction (**Figure 3**). A similar interaction of FAD to 6KGP was formed through Gly28, Val288, Ser289, Arg310, Arg316, Arg316, Arg316, Met332, Val333, Val590, Thr624, Val811, Glu308, Val333, Glu308, Val590, Gly285, Gly287, Gly315, Gly800, Val811, Ala809, Glu308, Thr624, Thr624, and Trp756 residues (**Figure 4**).

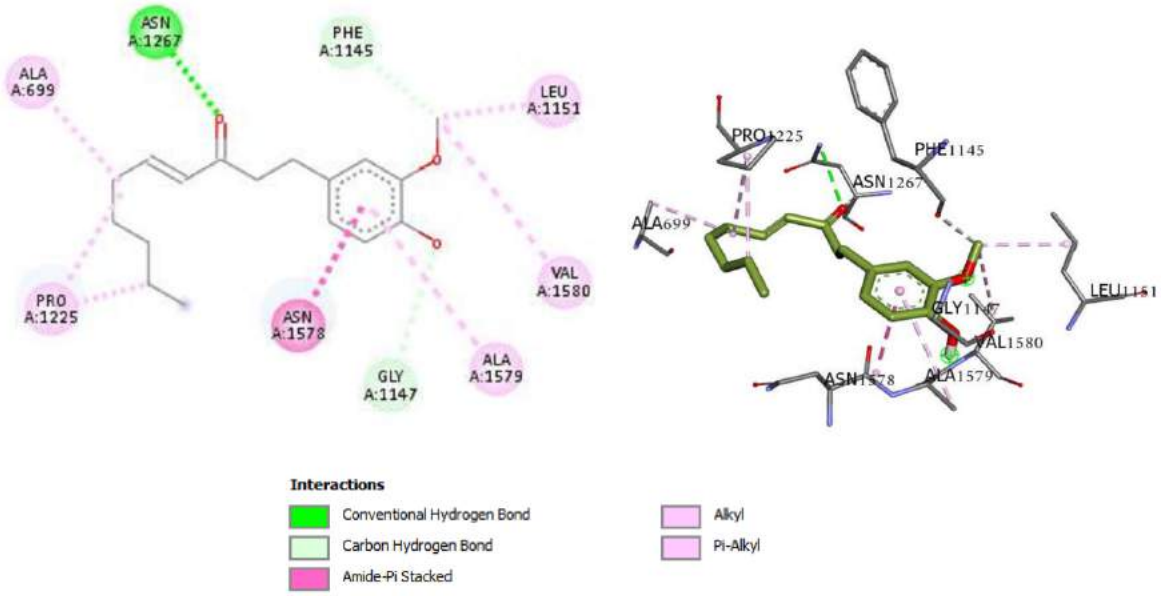

Figure 3 Visualization of validation results 3SWR with sinefungin in 2D and 3D

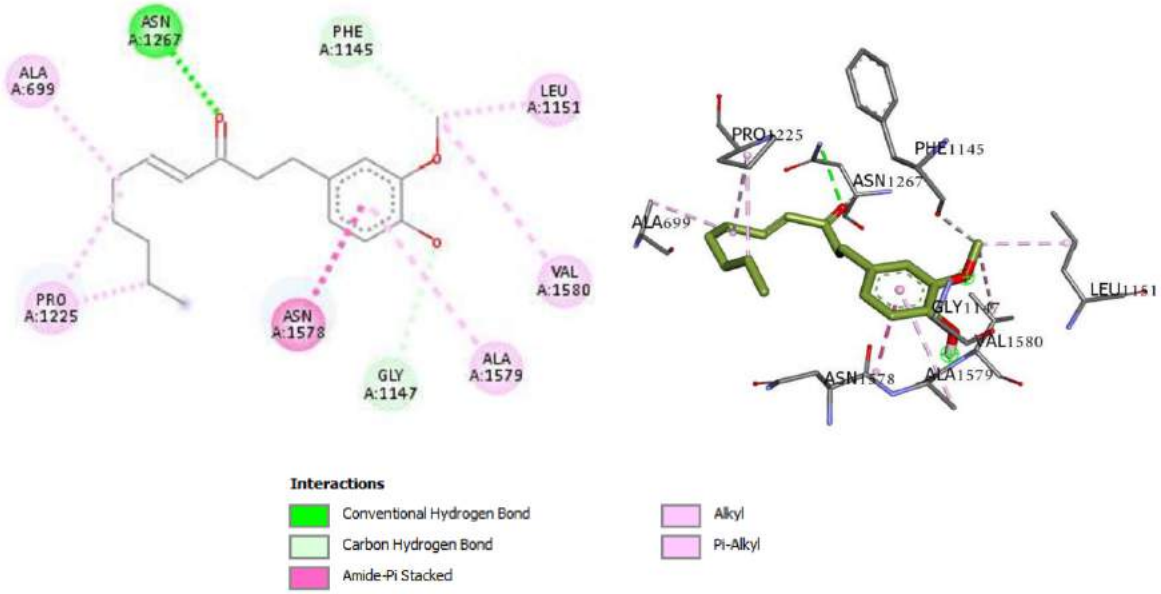

Figure 4 Visualization of docking results 3SWR with 6-shogaol in 2D and 3D

The hydrophobic interactions occurred between the docked sinefungin and 3SWR via Pro1225, Leu1247, Cys1191, Met1169, Phe1145 residues. Meanwhile, for FAD, the hydrophobic interactions were formed by Ala309, Ala331, Ala331, Trp751, Trp751, Trp756, Tyr761, Tyr761, Gly330, Ala331, Gly330, Ala331, Leu659, Arg316, Trp751, Trp751, Val590, Leu625, Ala309, Leu625, Val629, Ala331 residues of 6KGP. Furthermore, one electrostatic interaction was found between the docked FAD with Arg316 residue of 6KPG. These results produced a good RMSD value (<2.0 Å) [12], while corresponding interactions of the co-crystallized docked ligands indicated that the redocking is appropriate and the protocol is applicable for the next docking process.

Molecular Docking

The docking process was performed using Autodock Vina by flexible docking, given that the ligands were flexible, and the proteins were rigid. The results expressed as binding affinity represent the best configuration of the ligands in the protein binding sites. Meanwhile, the negative value of binding energy indicates stronger interaction of ligand-protein and better prediction [13]. The native ligand, sinefungin of 3SWR had binding energy of -9.0 kcal/mol, while 6-shogaol and curcumin produced interaction with binding energy values of -6.5 and -8.0 kcal/mol respectively. A previous study showed that caffeic acid, a hydroxycinnamic acid and polyphenols derivate has an IC_{50} value of 3.0 µM [14], and binding energy of -5.32 kcal/mol [15]. Furthermore, genistein inhibited DNMT1 in male rats [16] and produced a binding energy value of -6.39 kcal/mol [15]. Meanwhile, parthenolide, a sesquiterpene lactone of the germacranolide class, showed DNMT1 inhibitory activity with an IC_{50} value of 3.5 µM [17] and binding energy of -6.34 kcal/mol [15].

The binding energy value of 6-shogaol and curcumin indicated the potent activity of both compounds as DNMT1 inhibitors. Furthermore, the binding mode analysis of 3SWR as shown in **Figure 5** and **6** indicated that the interaction of 6-shogaol through hydrogen bonding occurred via Asn1267, Phe1145 and Gly1147 residues. It also formed hydrophobic interactions with Asn1578, Ala1579, Val1580, Leu1151, Ala699, and Pro1225 residues. This result is in line with Xie et al. (2019) which stated that the interaction of 6-shogaol with DNMT1 (3SWR) occurred through Phe1145, Gly1147, Asn1578, Ala1759, Val1580, and Pro1225 residues. Meanwhile, curcumin is bound to Asp700, Glu1168, Ala699, and Phe1145 residues through hydrogen bonding. Hydrophobic interactions of curcumin with 3SWR also occurred via Pro1225, Trp1170, Ala1173, Asn1578, Ala1579, Val1580 and Leu1151 residues. Besides, some important amino acid residues involved in the interaction of curcumin with DNMT1 include Glu1168, Phe1145, Pro1225, Trp1170, Asn1578, Ala1579 and Val1580 [18].

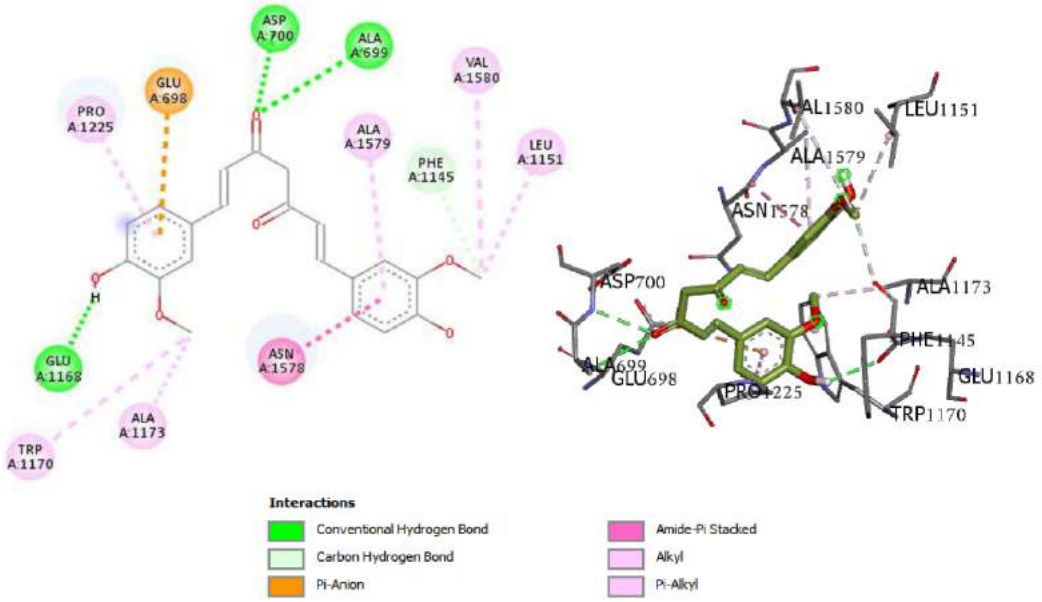

Figure 5 Visualization of docking results 3SWR with curcumin in 2D and 3D

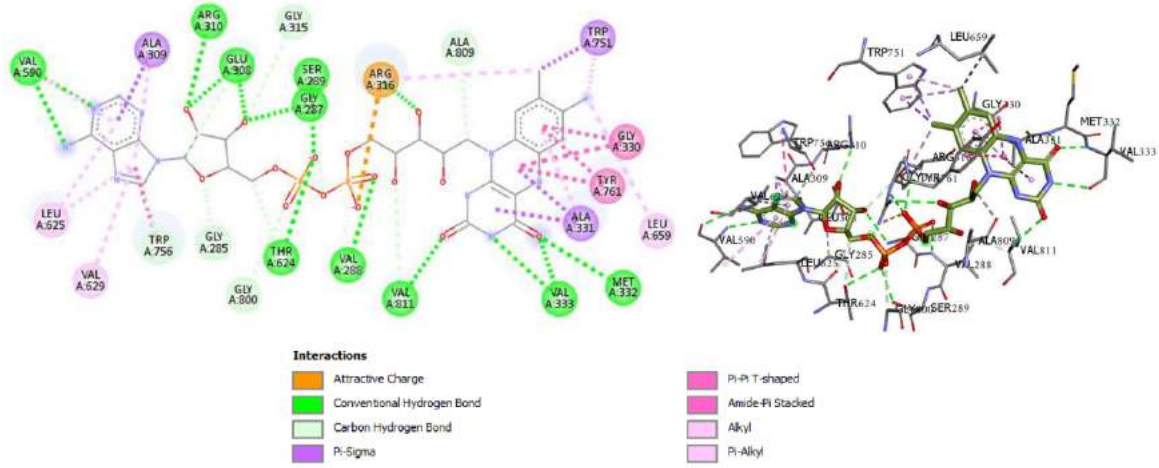

Figure 6 Visualization of validation results 6KGP with FAD in 2D and 3D

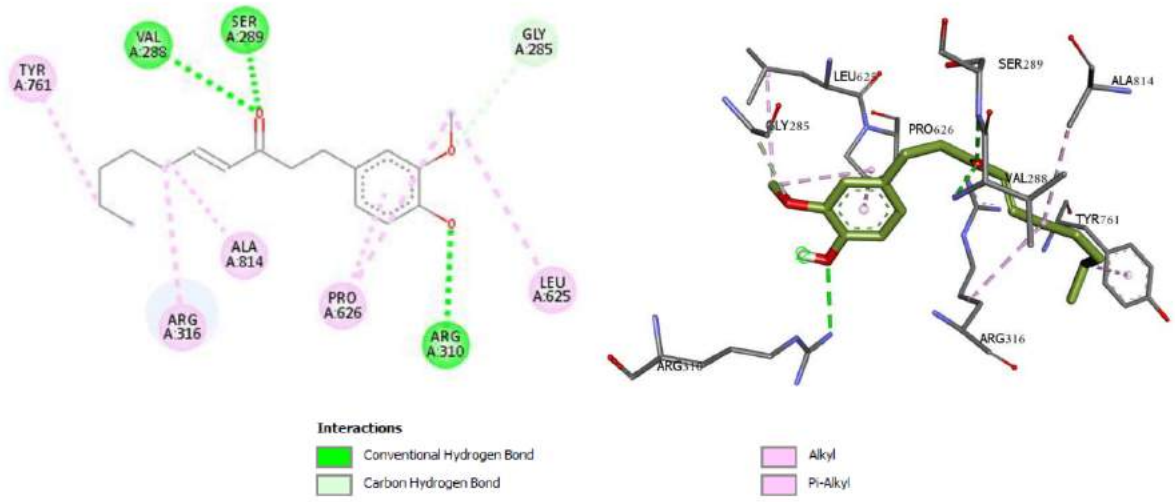

Figure 7 Visualization of docking results 6KGP with 6-shogaol in 2D and 3D

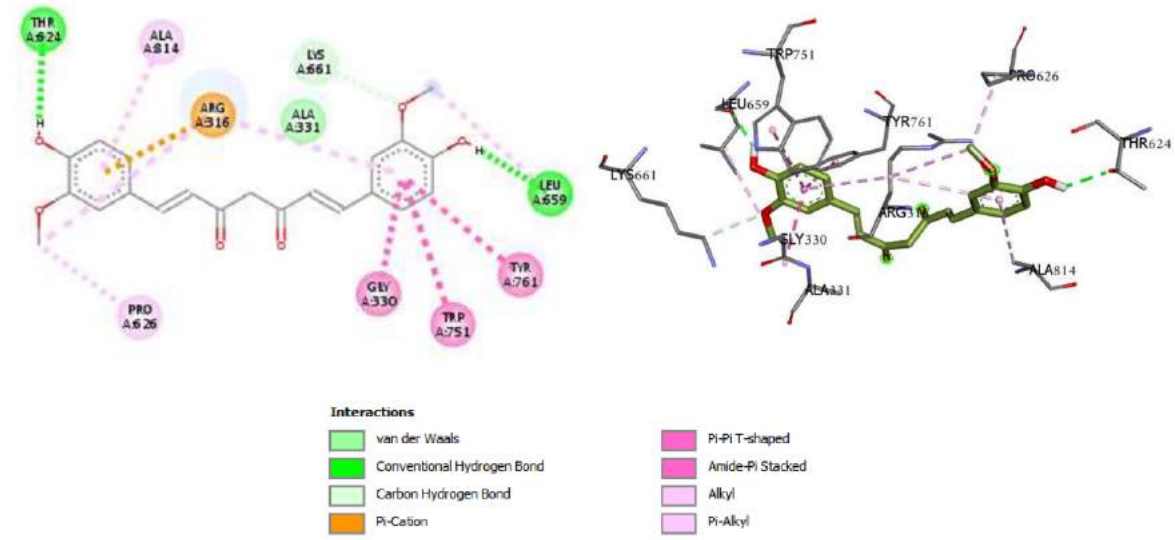

Figure 8 Visualization of docking results 6KGP with curcumin in 2D and 3D

The binding energy of FAD as a native ligand of LSD1 was -16.2 kcal/mol while 6-shogaol and curcumin were -8.2 and -10.1 kcal/mol, respectively. Oleacin, a phenolic LSD1 inhibitor showed inhibitory activity with an IC_{50} value of 2.5 µmol/L and binding energy of -9.33 kcal/mol [19]. Furthermore, a selective LSD1 inhibitor namely iadademstat produced binding energy of -9.09 kcal/mol [20].

The interactions analysis of 6-shogaol with LSD1 indicated hydrogen bonding through Val288, Ser289, Arg310 and Gly285 residues (**Figure 7**). This result correlated with the amino acids responsible for inhibitory activity namely Val288, Ser289, Gly314, Tyr624 and Lys661 [18]. Meanwhile, curcumin in LSD1 exhibits hydrogen bonding interaction with Thr624, Leu659, and Lys661 residues (**Figure 8**). The Thr624 and Lys661 residues are two important amino acids responsible for increasing the inhibitory activity. In addition, Leu659 is also a pivotal residue for the interaction of diaryene, a stilbene derivative, with LSD1. This compound showed an inhibitory activity with pIC_{50} value of 4,991 [18]. DNMT1 and LSD1 bind to BCL11A, hence, when both proteins are inhibited, the mechanism of silencing off fetal haemoglobin performed by BCL11A is averted

CONCLUSION

Based on this study, curcumin has greater interaction to both DNMT1 and LSD1. Therefore, inhibition of both proteins by curcumin and 6-shogaol compounds may increase the production of HbF in the body and reduces thalassemia severity and the need for blood transfusions. Further study is required to examine this potential effect of curcumin as a compound for beta-thalasemia therapy.

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