SEKOLAH TINGGI ILMU KESEHATAN TELOGOREJO

Science and Community Pharmacy Journal
Volume 1 *Issue* 1 *Year* 2022 *Pages* 1-5
E–ISSN XXXX–XXXX | DOI:
Web https://ojs.stikestelogorejo.ac.id/

Molecular Docking of Betel Leaf (*Piper betle L.*) on Protein Dihydrofolate reductase of *Mycobacterium tuberculosis*

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Abstract: Tuberculosis is an infectious disease of global concern. The treatment challenge of tuberculosis is bacterial resistance to antibiotics. Therefore, it is necessary to search for alternative treatments, one of which is the molecular docking method of Phytol, Chavibetol, Hydroxychavicol compounds contained in betel leaves. The target proteins used were 4KL9 and 4KM2 of the *Dihydrofolate reductase* pathway in *Mycobacterium tuberculosis* bacteria. Dihydrofolate reductase is a therapeutic target in the development of antituberculosis drugs. The molecular docking results showed that the target protein 4KL9 with Phytol, Chavibetol, and Hydroxychavicol compounds had a lower binding affinity when compared to the native ligand. In the 4KM2 target protein, these three compounds have a higher binding affinity value than the native ligand. This value indicates the strength of the ligand when it binds to the target protein, the smaller the value, the stronger the bond. In addition, there are similar residues involved in the target protein 4KL9 between the native ligand and the test compound, but in the target protein 4KM2 there is no similarity in the residues between the native ligand and the test compound. Residual similarity describes the similarity of the test ligand properties to the native ligand. It can be concluded that Phytol, Chavibetol, Hydroxychavicol compounds contained in betel leaf have potential as antituberculosis in the dihydrofolate reductase pathway on the target protein 4KL9 of *Mycobacterium tuberculosis*.

Keywords: betel leaf, antitubeculosis, molecular docking

INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease that is still a public health problem in the world. Tuberculosis has become a global problem that continues to grow along with the increasing number of tuberculosis patients. Tuberculosis infection is still a common thing and is an important factor in morbidity and mortality, especially in underdeveloped and developing countries (Murwaningrum et al., 2016; Pangaribuan et al., 2020). In the world, tuberculosis sufferers are estimated to be around 10 million people in 2017 and are the cause of about 1.3 million deaths in tuberculosis sufferers with HIV (negative) and as many as 300 thousand deaths in tuberculosis patients with HIV infection (positive). From year to year, the mortality rate for tuberculosis infection has decreased by approximately 3%. The fastest decline occurred from 2013 to 2017 in Europe (11%) and Southeast Asia (4%) (WHO, 2017). Tuberculosis is one of the highest infectious diseases in Indonesia (Pratiwi, 2018).

Mycobacterium tuberculosis is the bacteria that causes tuberculosis. 80% of these bacteria infect the lungs, however, these bacteria can also infect other organs of the body. *Mycobacterium tuberculosis* is a gram-positive bacterium that has a rod shape with a bacterial cell wall composed of a glycolipid lipid complex containing a waxy substance so that the bacterial wall will be difficult to penetrate by chemicals. (Kumar et al., 2014).

Treatment to control antituberculosis drug resistance recommended by WHO is the use of drugs through several phases using a combination of antibiotic drugs. However, complex treatment and long duration can cause patients to be less compliant and prone to failure in the desired health targets. This can be a major factor in the transmission of tuberculosis drug resistance in the community (BañUls et al., 2015). In addition, inadequate therapy in patients with resistant tuberculosis bacteria can also lead to amplification and transmission of drug resistant tuberculosis bacteria. This can increase the risk of developing multi-drug resistant tuberculosis (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB). Therefore, to overcome this one of them is to search for natural compound compounds as a choice of antituberculosis.

The use of natural ingredients as an alternative medicine has recently increased, one of which is the use of betel plants. Betel is one of the plants that is widely used by the community for treatment. The betel leaf is the most common part that is often used. Betel leaf extract, essential oil, preparations, and their isolates can inhibit microbial growth and kill various Gram-negative and Gram-positive and fungal species, including those that are resistant to various drugs and cause serious infectious diseases. Compounds that have the potential as antibacterial in betel leaves include Phytol, Chavibetol, Hydroxychavicol (Nayaka et al., 2021).

In the process of discovery and development of new drugs, one of which consists of the synthesis of chemical structures. Technological advances in the field of computation can be used and help in optimizing activity, geometry, and reactivity, before compounds are synthesized experimentally. (Pranowo & Hetadi, 2011). Molecular docking is a simulation with computational methods used to describe or predict the bond between the compound (ligand) and the receptor or target protein (Pratama et al., 2017).

Based on these data, it is expected that the compound content in betel leaf has potential as Anti tuberculosis activity. Therefore, to determine this potential, molecular docking of the compounds contained in betel leaf, namely Phytol, Chavibetol, Hydroxychavicol to the 4KL9 and 4KM2 proteins of the dihydrofolate reductase pathway in *Mycobacterium tuberculosis* was carried out. Dihydrofolate reductase (DHFR) is a potential treatment target as an antituberculosis, which is an important enzyme in the last step of tetrahydrofolate biosynthesis, identified as a potential target for the 776 hit phenotype. The protein has been extensively studied and there are three open conformational crystal structures of the enzyme in complexes with Cycloguanil, Trimethoprim and Pyrimethamine in the European Protein Database (PDBe) (Mugumbate et al., 2015).

The parameters used to determine the antituberculosis potential of phytol, Chavibetol, and Hydroxychavicol compounds were binding affinity and residual similarity between the original ligand and the test ligands (the three compounds). The smaller the value of the binding affinity, the stronger the bond between the ligand and the receptor and the similarity of the residues illustrates the similarity of the test ligand to the original ligand in binding to the protein. (Endriyatno & Santoso, 2018).

METHODS

Materials

Laptop with Intel Core i3 specifications NVIDIA GeForce graphics. Applications used include Discovery Studio 2021, Ms. Excel 2019, Ms.Word 2019, Molegro Molecular Viewer, AutoDockTools-1.5.7, Notepad++, Client, Corel Draw, protein from PDB (4KL9 dan 4KM2), ligand (P33, ATR, phytol, chavibetol, hydroxychavicol).

Ligand Preparation of Phytol, Chavibetol, Hydroxychavicol

The conformation of phytol compounds, chavibetol, hydroxychavicol were obtained from Pubchem with the format (.sdf). the file using molegro is exported to get the format (.pdb).

Docking Method Preparation and Validation

Protein selection was based on data from the Protein Data Bank (GDP) with a resolution of <3Å. obtained 4KL9 protein with P33 ligand 1.39 resolution and 4KM2 with ATR ligand 1.40 resolution. The downloaded protein was prepared using an auto dock which was then continued using Molegro to separate the protein and ligands. The results of the molegro were prepared for ligands in the auto dock. Gridbox settings in the auto dock were adjusted according to the ligands. Running on auto grid and auto dock. Docking results obtained binding affinity and RMSD. The RMSD value <3Å or generally <2Å illustrates that the docking method can be said to be valid (Endriyatno & Santoso, 2018; Farid et al., 2016). Visualization of the docking results of the best ligands and target proteins was carried out with the Discovery Studio Visualizer to see the interactions and residues involved.

Docking on Ligand (Phytol, Chavibetol, Hydroxychavicol)

Docking was using by auto dock so that the binding affinity value of the best ligand was obtained which was then visualized to see interactions and residues and then compared with the original ligand..

RESULT AND DISCUSSION

Ligand Preparation of Phytol, Chavibetol, Hydroxychavicol

Ligand structure of Phytol, Chavibetol, Hydroxychavicol compounds downloaded from Pubchem. The three compounds were formatted initially (.sdf) to (.pdb) so that they could be processed by auto dock. In addition, for ligand visualization using the Discovery Studio Visualizer application, which is shown in Figure 1.

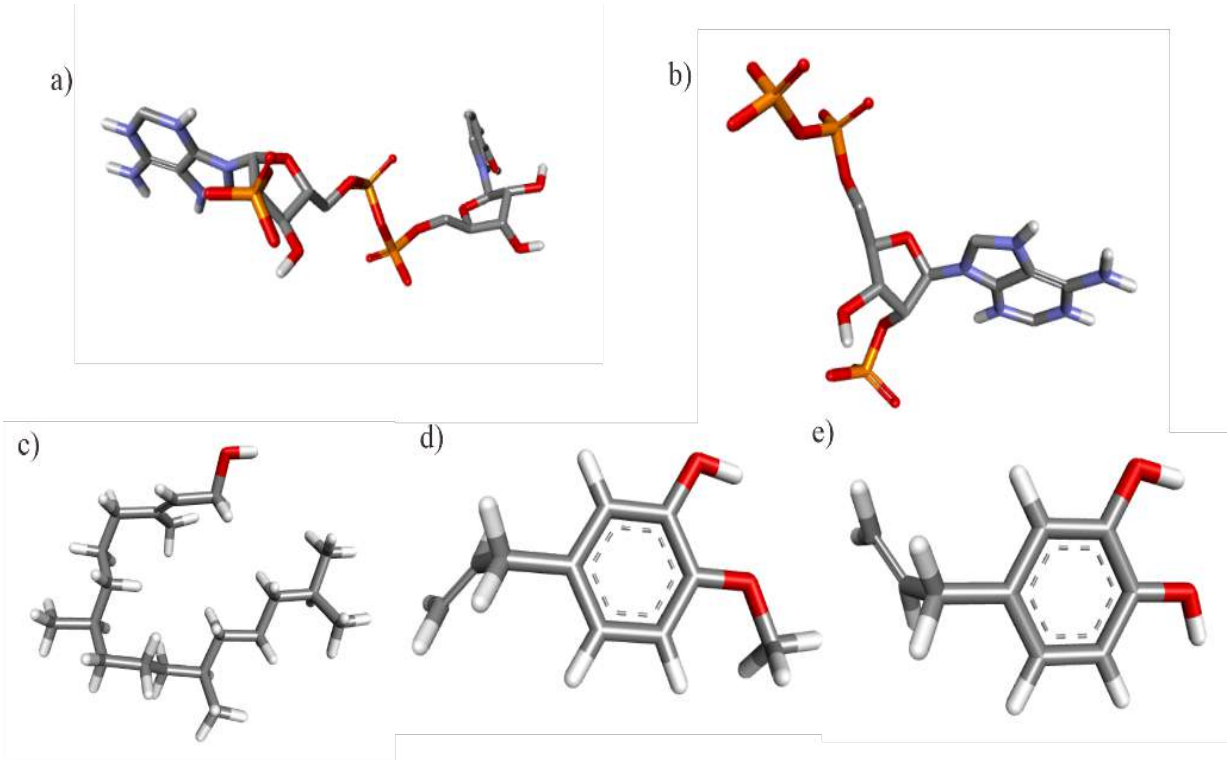

Figure 1. 3D visualization of the ligand a) P33 b) ATR c) Phytol, d) Chavibetol dan e) Hydroxychavicol

Results of Docking Method Preparation and Validation

Dihydrofolate reductase was chosen as the docking pathway with protein targets 4KL9 and 4KM2. Dihydrofolate reductase is a protein that plays an important role in the process of nucleotide biosynthesis, and can even be a target for antibacterial drugs. The target protein was prepared with auto dock for deletion of water. This is done because it is possible that water molecules will be able to bind to the ligand so that hydrogen bonds are formed which can interfere with the docking process. In addition, hydrogen is added which functions in the molecular docking process, especially in hydrogen bonds. The addition of the load (gasteiger) also needs to be done because it is to adjust the docking environment so that calculations can be carried out correctly.

In the molecular docking process according to Manna et al., (2017) through 2 stages: auto grid and autodock. Auto grid is a process to determine the size of the gridbox box, which is a location box that is selected and arranged in such a way for docking experiments to be carried out and the interaction energy of the atoms in the gridbox can be calculated. The center of mass, resolution, and gridbox dimensions of the 4KL9 and 4KM2 ligands are listed in **Table 1.** Then, auto dock is a program for the molecular docking process so that ligand conformations in the target protein will be produced. The data obtained are binding affinity and RMSD. The binding affinity values are listed in **Table 2**. Binding affinity values are negative. The smaller value interprets the complex formed very strongly between the ligand and the receptor. While the RMSD generated is 2.69 in 4KL9-P33 and 1.17 in 4KM2-ATR. The RMSD shows the docking method has high validity as evidenced by the RMSD value <3, meaning that the original ligand that was re-docking is similar to the original ligand.

Table 1. Center of mass, resolution, and gridbox dimensions of 4KL9 and 4KM2 ligands

Ligand	Center of mass (Å)			Resolution (Å)	gridbox dimensions (Å)		
	X	Y	Z		X	Y	Z
4KL9	0.204	2.329	0.335	0.375	60	60	60
4KM2	14.866	-10.904	10.93	0.375	60	60	60

Docking on Ligand (Phytol, Chavibetol, Hydroxychavicol)

The results of molecular docking of Phytol, Chavibetol, Hydroxychavicol compounds against *Mycobacterium tuberculosis* with target proteins 4KL9 and 4KM2 obtained binding affinity as shown in **Table 2**. The binding affinity results of P33 and ATR were compared with ligands from the test plants where the original ligands each had a value of -0.90. and -7.84. While the binding affinity of the ligands (phytol, chavibetol, hydroxychavicol) to 4KL9 and 4KM2 is shown in **Table 2.** The binding affinity value of natural compound ligands has a lower value when compared to the P33 ligand on the target protein 4KL9, this illustrates the binding of the three test ligands to their binding with the target protein is stronger than the native ligand. The binding affinity value of the test ligand on the target protein 4KM2 was higher than the original ligand. This shows that the test ligand bonds are weaker than the original ligands.

The molecular docking results were visualized using the Discovery Studio Visualizer. The results of the visualization of the ligand with the target protein obtained in the 3D form shown in Figure 2., the 2D visualization shown in Figure 3., and the visualization of the interaction of residues involved in the interaction of the receptor ligand shown in Figure 4 and summarized in **Table 2.** target protein in **Table 3**. The visualization results showed the presence of the same residue between the three test ligands against the P33 ligand on the 4KL9 target protein (marked by the highlight color in **Table 2**). however, there was no residual similarity at all with the ATR ligand with the target protein. The similarity of these residues illustrates the similarity of the test ligand eels with the original ligand. From the binding affinity value and the similarity of the residues involved, the three ligands of these compounds can be said to be more potent as an alternative to antituberculosis in the Dihydrofolate reductase pathway with the target protein 4KL9 and less potent to the 4KM2 protein due to the absence of similar residues.

Table 2. Binding affinity obtained from ligands and target proteins

Ligand	target proteins	Binding affinity	Active compound
P33	4KL9	-0.90	-
ATR	4KM2	-7.84	-
Betel leaf	4KL9	-5.07	Phytol
Betel leaf	4KL9	-5.17	Chavibetol
Betel leaf	4KL9	-5.26	Hydroxychavicol
Betel leaf	4KM2	-3.99	Phytol
Betel leaf	4KM2	-3.99	Chavibetol
Betel leaf	4KM2	-4.18	Hydroxychavicol

Table 3. Types of interactions and residues involved in the category of ligands with target proteins

Ligand	Target protein	Interaction	Residue involved
P33	4KL9	Hydrogen bond	LEU131, GLN8
		Hydrophobic bond	TYR156, TRP6, PRO129, VAL130, GLY12, ALA128, LEU127
ATR	4KM2	Hydrogen bond	THR46, GLY96, ARG45, ARG44, GLN98
		Hydrophobic bond	SER81, LEU102, LEU65, SER66, GLN68, VAL99, ARG67, GLY43, GLY95
phytol	4KL9	Hydrogen bond	PRO129, LEU131
		Hydrophobic bond	GLU114, TYR156, GLN8, TRP6, ALA128, TYR154, VAL112, VAL130
	4KM2	Hydrogen bond	LEU57, ARG60, GLY59
		Hydrophobic bond	ALA52, ARG55, SER74, TRP47, LYS53, VAL54, PRO56, PRO58, ASN62, ARG61
chavibetol	4KL9	Hydrogen bond	ALA128
		Hydrophobic bond	LEU127, ARG121, GLY12, GLN8, VAL130, ASP132, TRP135, ARG136, TYR156, LEU131, TRP6, VAL112, PRO129
	4KM2	Hydrogen bond	TRP47, MET72
		Hydrophobic bond	PRO56, ALA52, ARG55, SER74, ALA73, PHE71
Hydroxychavicol	4KL9	Hydrogen bond	PRO129, LEU131
		Hydrophobic bond	GLU114, TYR156, GLN8, TRP6, ALA128, VAL130, TYR154, VAL112
	4KM2	Hydrogen bond	TRP47, MET72
		Hydrophobic bond	PRO56, ARG55, SER74 ALA73, PHE71, ALA52

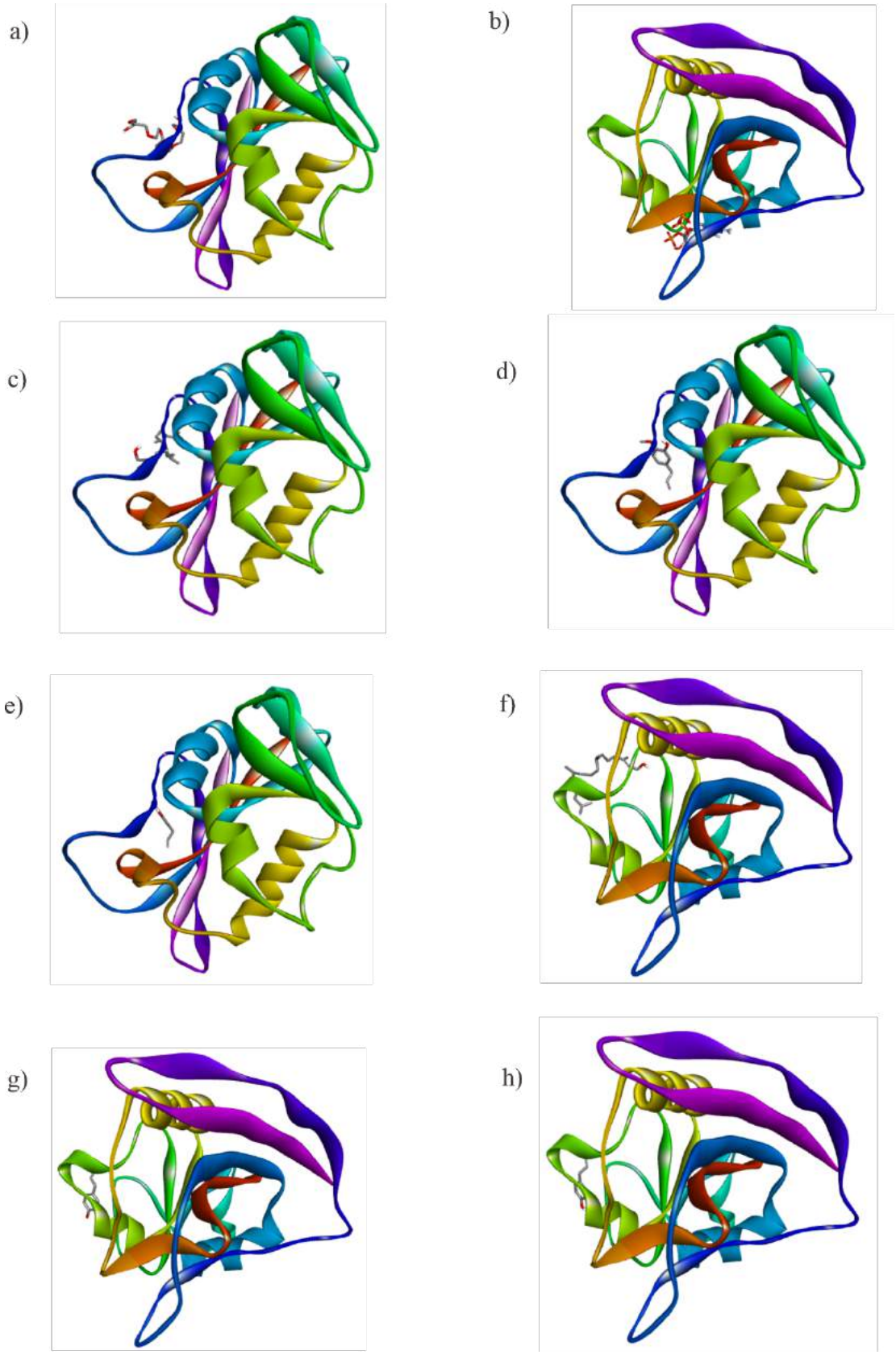

Figure 2. 3D visualization of ligands and receptors a) P33-4KL9, b) ATR-4KM2, c) Phytol -4KL9, d) Chavibetol-4KL9, e) Hydroxychavicol-4KL9 f) Phytol -4KM2 g) Chavibetol-4KM2 h) Hydroxychavicol-4KM2

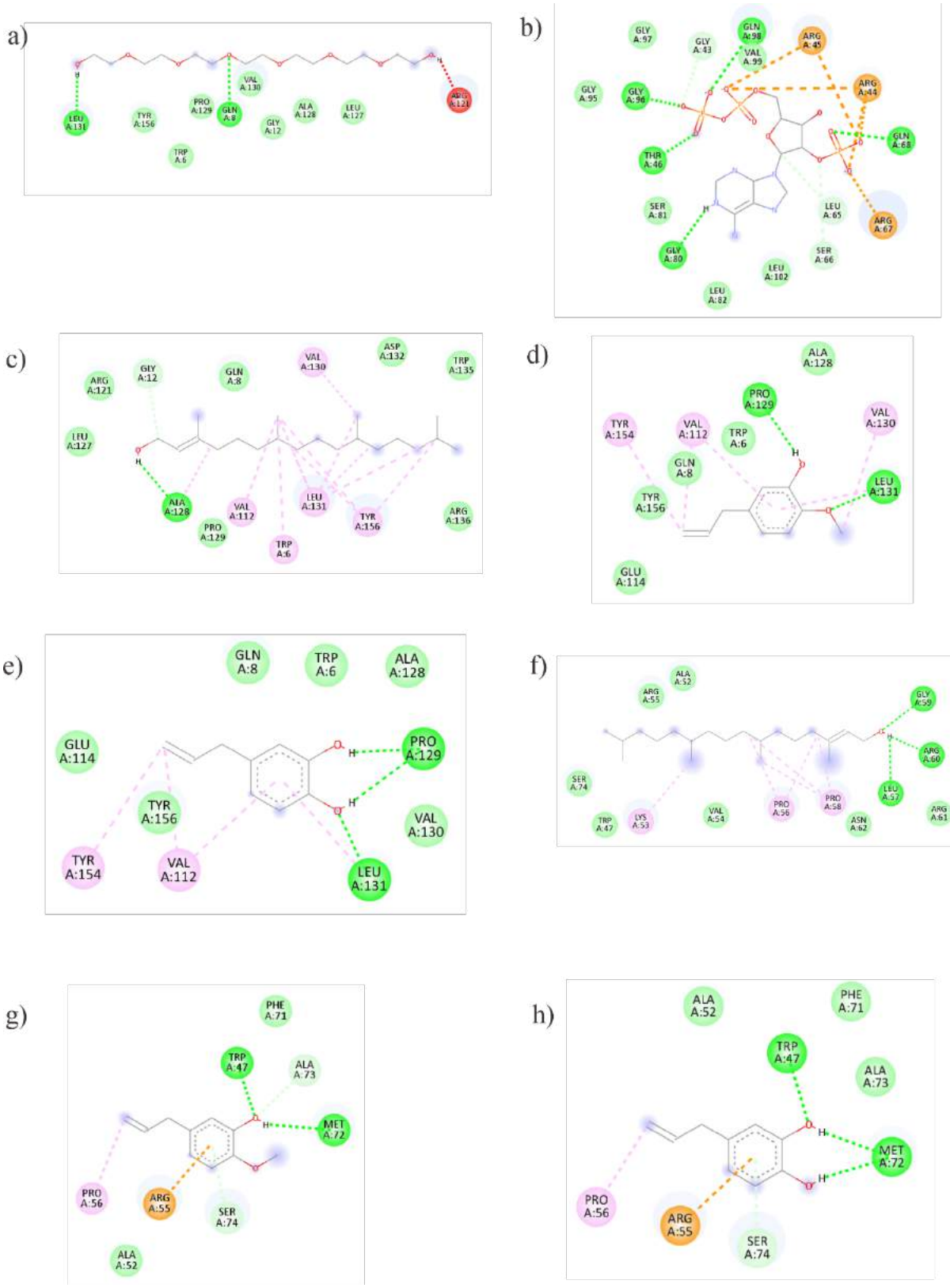

Figure 3. 2D visualization of ligands and receptors a) P33-4KL9, b) ATR-4KM2, c) Phytol -4KL9, d) Chavibetol-4KL9, e) Hydroxychavicol-4KL9 f) Phytol -4KM2 g) Chavibetol-4KM2 h) Hydroxychavicol-4KM2

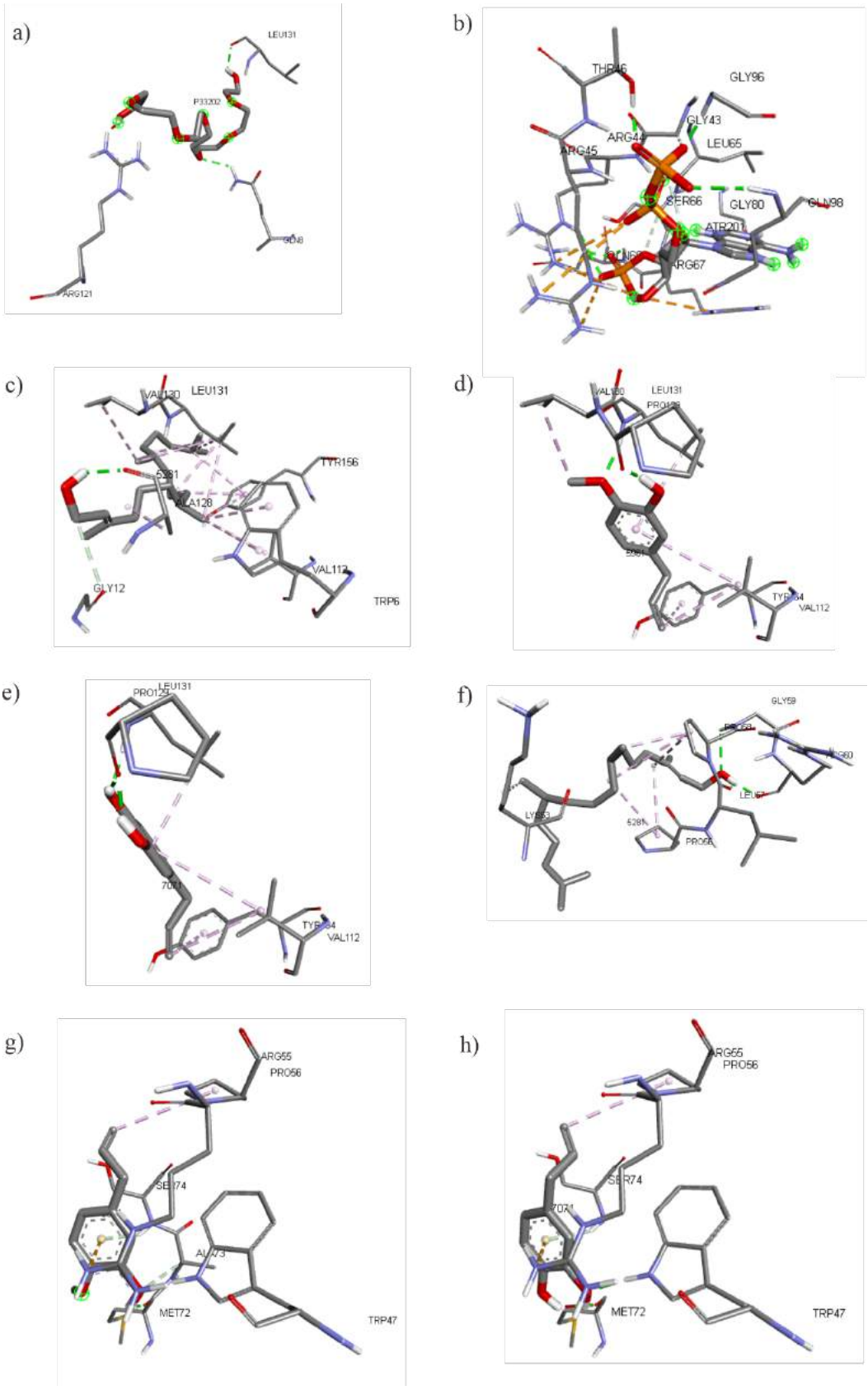

Figure 4. Visualization of 3D receptor and ligand interactions a) P33-4KL9, b) ATR-4KM2, c) Phytol -4KL9, d) Chavibetol-4KL9, e) Hydroxychavicol-4KL9 f) Phytol -4KM2 g) Chavibetol-4KM2 h) Hydroxychavicol-4KM2

CONCLUSION

The results of the molecular docking study of the dihydrofolate reductase pathway in *Mycobacterium tuberculosis* showed that the target protein 4KL9, phytol, chavibetol, and hydroxychavicol compounds had a lower binding affinity when compared to the original ligand and in the target protein 4KM2 the three compounds had a higher binding affinity value than the original ligand. This value indicates the strength of the ligand when it binds to the target protein, the smaller the value, the stronger the bond. In addition, there are similar residues involved in the target protein 4KL9 between the original ligand and the test compound, but in the target protein 4KM2 there is no similarity in the residues between the original ligand and the test compound. Residual similarity describes the similarity of the test ligand properties to the original ligand. it can be concluded that Phytol, Chavibetol, Hydroxychavicol compounds contained in betel leaf have potential as antituberculosis in the dihydrofolate reductase pathway on the target protein 4KL9 of *Mycobacterium tuberculosis.*

CONFLIC OF INTEREST

The authors state that the research was conducted without any commercial or financial relationship that could be construed as a potential conflict of interest.

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