Indo. J. Chem. Sci. 15 (2) (2026)

Indonesian Journal of Chemical Science

http://journal.unnes.ac.id/sju/index.php/ijcs

Molecular Docking Study of Xanthone Targeting the Dihydropteroate Synthase (2VEG) Enzyme in *Streptococcus pneumoniae*

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Article Info

Accepted: 12-01-2026

Approved: 30-07-2026

Published: 03-08-2026

Keywords:
Molecular docking
Dihydropteroate synthase
Streptococcus pneumoniae
Xanthone
2VEG

Abstract

Streptococcus pneumoniae is a pathogenic bacterium causing serious infections, with rising antibiotic resistance highlighting the need for new agents. Dihydropteroate synthase (DHPS) is crucial in bacterial folate biosynthesis and a selective drug target. This study evaluated xanthone derivatives as DHPS inhibitors via molecular docking. Three derivatives namely 1,3,7-trihydroxy-9H-xanthen-9-one (Candidate 1), 1-hydroxy-9-oxo-7-sulfo-9H-xanthene-3-carboxylic acid (Candidate 2), and 9-oxo-1,7-disulfo-9H-xanthene-3-carboxylic acid (Candidate 3), were docked against DHPS (PDB ID: 2VEG) using UCSF Chimera, AutoDock Tools, and AutoDock Vina. Docking validation was conducted by re-docking the native ligand, and ligand–protein interactions were analyzed using BIOVIA Discovery Studio and LigPlot+.. All candidates exhibited favorable binding affinity toward the DHPS active site; −7.1 kcal/mol for the native ligand, −7.0 kcal/mol for Candidate 1, −7.7 kcal/mol for Candidate 2, −7.4 kcal/mol for Candidate 3, and −6.4 kcal/mol for the control compound sulfamethoxazole. Candidate 2 exhibited the strongest binding affinity and formed favorable interactions with key amino acid residues in the DHPS active site. These findings suggest that Candidate 2 is the most promising xanthone derivative for further development as a DHPS inhibitor against *Streptococcus pneumoniae*.

Abstrak

Streptococcus pneumoniae adalah bakteri patogen yang menyebabkan infeksi serius, dengan meningkatnya resistensi antibiotik yang menekankan perlunya agen baru. Dihydropteroate synthase (DHPS) sangat penting dalam biosintesis folat bakteri dan merupakan target obat yang selektif. Penelitian ini mengevaluasi turunan xanthone sebagai inhibitor DHPS melalui docking molekuler. Tiga turunan yaitu 1,3,7-trihidroksi-9H-xanthen-9-one (Kandidat 1), 1-hidroksi-9-oxo-7-sulfo-9H-xanthene-3-asam karboksilat (Kandidat 2), dan 9-oxo-1,7-disulfo-9H-xanthene-3-asam karboksilat (Kandidat 3), didocking terhadap DHPS (PDB ID: 2VEG) menggunakan UCSF Chimera, AutoDock Tools, dan AutoDock Vina. Validasi docking dilakukan dengan redocking ligan asli, dan interaksi ligan–protein dianalisis menggunakan BIOVIA Discovery Studio dan LigPlot+. Semua kandidat menunjukkan afinitas pengikatan yang baik terhadap situs aktif DHPS; −7,1 kcal/mol untuk ligan asli, −7,0 kcal/mol untuk Kandidat 1, −7,7 kcal/mol untuk Kandidat 2, −7,4 kcal/mol untuk Kandidat 3, dan −6,4 kcal/mol untuk senyawa kontrol sulfametoksazol. Kandidat 2 menunjukkan afinitas ikatan terkuat dan membentuk interaksi yang menguntungkan dengan residu asam amino kunci di situs aktif DHPS. Temuan ini menunjukkan bahwa Kandidat 2 adalah turunan xanton yang paling menjanjikan untuk pengembangan lebih lanjut sebagai inhibitor DHPS terhadap *Streptococcus pneumoniae.*

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p-ISSN 2252-6951
e-ISSN 2502-6844

Introduction

Streptococcus pneumoniae is one of the major pathogenic bacteria responsible for a range of serious infectious diseases, including pneumonia, meningitis, otitis media, and sepsis. The high morbidity and mortality associated with infections caused by this bacterium remain a global health concern, particularly due to the increasing prevalence of antibiotic-resistant strains. Resistance to conventional antibiotics, including sulfonamides and β-lactams, underscores the need for the discovery of new antimicrobial compounds that are more effective and possess distinct mechanisms of action (Olarte et al., 2021).

One of the critical targets in antibacterial drug development is the enzyme dihydropteroate synthase (DHPS), which plays a key role in the bacterial folate biosynthesis pathway. This enzyme is absent in human cells, making it a selective and safe target for antibacterial therapy. The crystal structure of DHPS from Streptococcus pneumoniae, available in the Protein Data Bank under the code 2VEG, allows a structure-based approach to study molecular interactions between the enzyme and potential ligands in silico Capasso and Supuran, 2019).

DHPS catalyzes the formation of dihydropteroate from dihydropteridine pyrophosphate and p-aminobenzoic acid, representing an early step in tetrahydrofolate synthesis. Tetrahydrofolate is essential for various bacterial metabolic processes, including nucleotide and amino acid synthesis, making DHPS crucial for bacterial growth and survival (Horra et al., 2021).

Xanthones are secondary metabolites widely found in plants, particularly in the genera Garcinia and Calophyllum. These compounds exhibit diverse biological activities, such as antibacterial, antioxidant, anti-inflammatory, and anticancer effects. Several studies indicate that xanthones and their derivatives have the potential to inhibit the growth of pathogenic bacteria. However, research examining the binding mechanisms of sulfonated xanthone derivatives to dihydropteroate synthase (DHPS) using a molecular docking approach remains very limited. As a result, comprehensive information regarding binding affinity, ligand orientation within the active site, and the amino acid residues involved in ligand–protein complex formation is not yet available (Ishak et al., 2023).

Molecular docking is a widely used computational approach for predicting the binding affinity and interaction modes between bioactive compounds and target proteins. This method allows the preliminary evaluation of a compound's effectiveness as a drug candidate by analyzing hydrogen bonding, hydrophobic interactions, and binding energies. Therefore, a molecular docking study of xanthone against DHPS (2VEG) from *Streptococcus pneumoniae* is expected to provide insights into the potential of xanthone as a DHPS inhibitor and its contribution to the development of novel antibacterial candidates (Chen et al., 2017).

Method

Equipment

Molecular docking was performed on an Lenovo ThinkPad X390 with Windows 11, equipped with an Intel(R) Core(TM) i5-8365U CPU @ 1.60GHz (1.90 GHz) and 8 GB of RAM. The software used was ChemDraw Ultra 22.0 for drawing ligands UCSF Chimera 1.17.3 for protein and ligand preparation, AutoDock Tools 1.5.7 and AutoDock Vina for docking simulations, BIOVIA Discovery Studio 2024 client for visualization of results, and LigPlot+ 2.3.1 for analyzing and identifying interacting amino acid residues.

Materials

The materials used in this study were Dihydropteroate Synthase (2VEG) protein, obtained from the Protein Data Bank website (PDB ID: 2VEG, https://www.rcsb.org/structure/2VEG)

Protein Preparation

The receptor preparation stage began with downloading the crystal structure of Dihydropteroate Synthase (2VEG) with PDB ID: 2VEG from the Protein Data Bank in .pdb format. Subsequently, the protein structure was processed using UCSF Chimera to remove the native ligand, water molecules, and unnecessary metal ions. Polar hydrogens were then added, and partial charges were assigned to the receptor. The prepared receptor was saved in receptor.pdb format and subsequently converted into receptor.pdbqt using AutoDock Tools version 1.5.7 for molecular docking purposes (Butt et al., 2020).

Ligand Preparation

Ligand preparation began with the creation of two-dimensional (2D) molecular structures of 1,3,7-trihydroxy-9H-xanthen-9-one (Candidate 1), 1-hydroxy-9-oxo-7-sulfo-9H-xanthene-3-carboxylic acid (Candidate 2), and 9-oxo-1,7-disulfo-9H-xanthene-3-carboxylic acid (Candidate 3) using ChemDraw Ultra version 22.0. Ligand optimization was subsequently performed using UCSF Chimera by adding polar

hydrogens and assigning partial charges to obtain the most stable 3D conformations with minimum energy. The optimized ligand structures were saved in *.pdb format and further converted into *.pdbqt format using AutoDock Tools version 1.5.7 to ensure compatibility as input files for molecular docking simulations using AutoDock Vina (Maharani et al., 2025).

Grid Box Setup

The determination of the grid box size begins by launching AutoDock Tools and loading the prepared receptor and ligand files. The "Grid" menu is then selected, followed by clicking the "Center" option once the Grid Options dialog appears. The next step involves selecting "Center on Ligand" to define the grid box position and dimensions based on the native ligand. The resulting grid parameters are saved in the conf.txt file, which will be used during the docking simulation to ensure that the ligand can optimally explore the protein's active site (Pebralia et al., 2024).

Docking Validation

Docking validation is done through a re-docking process using the original ligand. This validation aims to ensure that the docking parameters used, such as the size and position of the grid box, can accurately reproduce the orientation of the original ligand at the protein's active site. The comparison is expressed in terms of the root mean square deviation (RMSD), which is the average deviation of the ligand's atom positions between the crystal structure and the docking prediction. The smaller the RMSD value, the higher the match between the predicted pose and the original pose, showing that the docking protocol can accurately reproduce the ligand binding mode. A docking method is considered valid if it produces an RMSD value ≤ 2.0 Å (Yuanita et al., 2024).

Docking Simulation

Molecular docking is used to predict the interactions between ligands and target proteins with the assistance of AutoDock Vina. All required files, including the protein, ligands, and configuration file, are placed in a single folder. The docking process is then executed through the command prompt at the folder location. This procedure generates several ligand poses, typically five, which are stored in a single *.pdbqt file and can be separated for individual analysis or visualization (Pebralia et al., 2024).

Visualization and Analysis of Interaction

The results of molecular docking between the protein and ligands can be visualized using Discovery Studio software to examine both the 2D and 3D conformations and the types of interactions. Subsequent interaction analysis was supported by LigPlot+ version 2.2.4. LigPlot+ version 2.2.4 was employed to visualize and analyze two-dimensional interactions between the ligand and amino acid residues in the active site of protein 2VEG, including hydrogen bonds and hydrophobic interactions. This visualization also aimed to compare the similarity of interaction patterns between the test ligands and candidate ligands. The visualization process was carried out to clarify the formed interaction patterns and to support the interpretation of docking results (Abdullah & Bayuhansah, 2021).

Results and Discussion

Molecular docking is an important method in biochemistry and pharmacy that enables the rapid and efficient prediction of molecular interactions. Molecular docking is a computational approach aimed at predicting interactions between small molecules (ligands) and biological targets, typically proteins or enzymes (Majumdar et al., 2024). This technique plays a crucial role in biochemical and pharmaceutical research, particularly in the drug discovery process. In this molecular docking study, dihydropteroate synthase (DHPS) (PDB ID: 2VEG), isolated from *Streptococcus pneumoniae*, was used. *Streptococcus pneumoniae* is a pathogenic bacterium and a common cause of human respiratory tract infections. The crystal structure of 2VEG was determined using X-ray diffraction at a resolution of 2.4 Å, and it revealed the DHPS protein in complex with 6-hydroxymethyl-7,8-dihydropterin monophosphate (DHPP). The protein structure is presented in Figure 1. DHPS plays a crucial role as a key enzyme in the folate biosynthesis pathway and serves as the primary target of sulfonamide antibiotics. Sulfonamides act as structural analogs of p-aminobenzoic acid (pABA) and compete with pABA at the DHPS active site, thereby inhibiting bacterial folate production and microbial growth. These inhibitors exhibit high selectivity toward bacteria. However, resistance to sulfonamides has emerged as a significant clinical challenge.

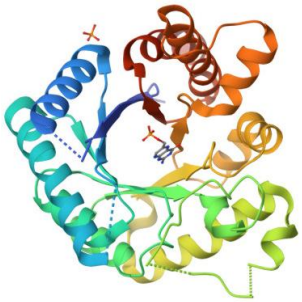


Figure 1. Stucture of dihydropteroate synthase (DHPS)

The initial step in molecular docking involves the careful preparation of both the receptor and the ligand, as this process is critical for ensuring the accuracy of predicted interactions between the ligand and the target protein. In this study, DHPS from *Streptococcus pneumoniae* was employed as the receptor, while DHPP served as the natural ligand. Receptor preparation was carried out by removing all non-essential molecules and the native ligand to provide unobstructed access to the protein's active site, allowing the candidate ligands to bind optimally. The protein structure was then examined and corrected to ensure that no atoms or residues were missing. Polar hydrogen atoms were added to facilitate accurate analysis of hydrogen bonding interactions between the ligand and the protein, and the partial atomic charges were assigned using Gasteiger charges (Butt et al., 2020). The optimized receptor was subsequently saved in PDB format. Structure of receptor is presented in Figure 2.

Ligand preparation is a critical step in molecular docking to ensure that the ligand structures are in an optimal state prior to simulation of their interactions with the receptor. In this study, the ligands used included pABA and a candidate sulfonamide inhibitor, specifically a xanthone derivative. Ligand preparation involved the addition of polar hydrogen atoms to facilitate the formation of hydrogen bonds with protein residues, as well as the assignment of partial charges using Gasteiger charges. The optimized ligands were subsequently saved in PDB format (Maharani et al., 2025). The structures of the native ligand and the candidate inhibitors are presented in Figure 3.

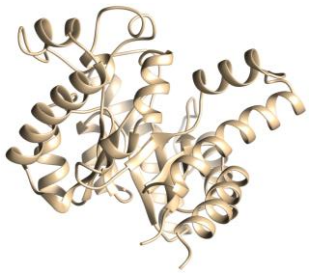


Figure 2. Structure of receptor

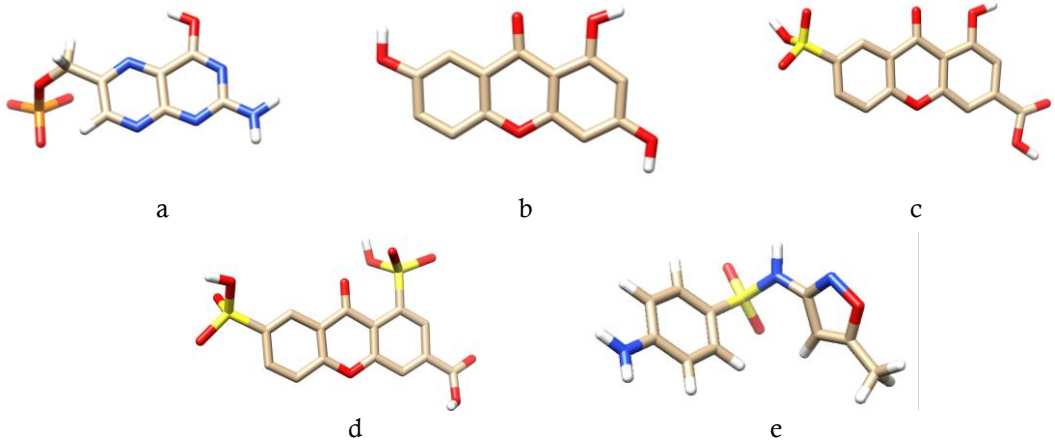


Figure 3. Structure of native ligand (a), candidate 1 (b), candidate 2 (c), candidate 3 (d), and control (e)

Molecular docking

The determination of grid box size is a crucial step in molecular docking, as it defines the active site of the protein where the ligand is expected to bind. The grid box serves as a three-dimensional spatial boundary that restricts the movement and orientation of the ligand during its interaction with the target protein. The placement of the grid box center is typically based on the location of the native ligand, catalytic residues, or active binding pockets previously identified through structural studies, thereby allowing the docking results to reflect binding mechanisms that closely resemble physiological conditions. Therefore, the appropriate selection of grid box size and position has a significant impact on the accuracy of binding affinity predictions, binding poses, and the overall validity of docking results. An optimally defined grid box also contributes to the consistency and comparability of docking outcomes across different ligands. When all ligands are evaluated using identical grid box parameters, differences in binding affinity values and interaction patterns can be attributed to variations in ligand chemical properties and structural features rather than differences in docking location or search space. This aspect is particularly important in comparative docking studies, especially when molecular docking is employed to screen potential inhibitor candidates. In this study, the grid box size was determined using AutoDock Tools, which generated the center coordinates (X, Y, and Z), as presented in Table 1.

Table 1. Grid center coordinate and grid box size

Ligand		X	Y	Z
Native ligand	Center	31.13	47.97	0.22
	Size	40	40	40
Candidate 1	Center	31.13	47.97	0.22
	Size	40	40	40
Candidate 2	Center	31.13	47.97	0.22
	Size	40	40	40
Candidate 3	Center	31.13	47.97	0.22
	Size	40	40	40
Sulfamethoxazole (Control)	Center	31.13	47.97	0.22
	Size	40	40	40

Based on the molecular docking results presented in Table 2, the binding affinity values obtained were within the negative range. From a thermodynamic perspective, a negative Gibbs free energy indicates that the ligand–protein binding process occurs spontaneously (Alifbi et al., 2022). Variations in the binding affinity values reflect differences in the ability of each ligand to interact with key residues within the active site of the protein, which are influenced by differences in ligand structure and physicochemical properties. The most favorable binding affinity was observed in mode 1, with binding affinity values of −7.1 for the native ligand, −7.1 for candidate 1, −7.7 for candidate 2, and −7.4 for candidate 3. Ligands exhibiting lower (more negative) binding energy values are predicted to have higher binding affinity toward the target protein. Accordingly, candidate 2 demonstrates the greatest potential to form a stable ligand–protein complex.

The difference in affinity is related to the difference in the chemical structure of each compound. Candidate 1 only has a hydroxyl group (-OH), so the chances of forming interactions with residues in the DHPS active site are relatively limited. On the other hand, Candidate 2 has a combination of hydroxyl (-OH), carboxyl (-COOH), and sulfonate ($-SO_3H$) groups. This combination of polar groups allows for the formation of hydrogen bonds and more effective electrostatic interactions with amino acid residues in the DHPS active site, resulting in a more optimal binding orientation and lower binding energy. The sulfonate group ($-SO_3H$) is a very polar functional group. Adding a sulfonate group makes the molecule negatively charged . It is negative charge and the presence of three oxygen atoms in the sulfonate group allow it to act as a hydrogen bond acceptor and form electrostatic interactions with positively charged amino acid residues in the protein's active site (Lehrer & Rheinstein, 2025). These characteristics can enhance the ligand binding affinity as well as contribute to the stability of the ligand–protein complex.

Candidate 3 contains two sulfonate groups and shows a more complex interaction pattern, but its binding affinity is still lower compared to Candidate 2. This result suggests that increasing the number of polar groups does not always enhance binding affinity. The effectiveness of ligand–protein interactions is

influenced by a combination of factors, such as the position of functional groups on the xanthone backbone, the orientation of the ligand in the binding pocket, and spatial compatibility with the protein's active site. Therefore, the combination of functional groups in Candidate 2 results in a more favorable binding configuration than in Candidate 3. These findings show that chemical structure modifications through the addition of certain functional groups can improve binding affinity to DHPS. However, the success of increasing affinity is not only determined by the number of polar functional groups, but also by the distribution and position of these groups so they can form the most optimal interactions with the residues on the enzyme's active site.

Sulfamethoxazole is used as a control compound because it is a sulfonamide antibiotic known to inhibit the enzyme dihydropteroate synthase (DHPS) in the bacterial folic acid biosynthesis pathway (Feng et al., 2022). Molecular docking results showed that Sulfamethoxazole has a binding affinity value of −6.4 kcal/mol. Compared to the control compound, Candidate 2 shows a higher binding affinity with a binding energy value of −7.7 kcal/mol. A lower binding energy value indicates that Candidate 2 has better binding potential to DHPS than Sulfamethoxazole in the molecular docking simulation.

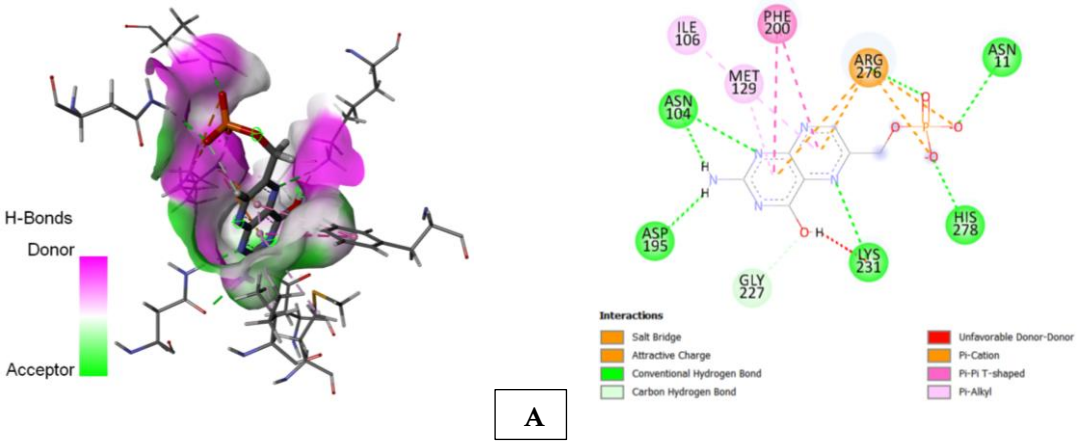
Furthermore, the Root Mean Square Deviation (RMSD) analysis provides important insight into the reliability of the docking results by describing the degree of deviation between the predicted ligand pose and the reference binding conformation. An RMSD value below 2 Å is generally considered acceptable in molecular docking studies, as it indicates that the predicted binding pose closely resembles the native ligand orientation within the active site (Maruyama et al., 2023). In addition, low RMSD values imply that the ligand maintains a consistent orientation within the binding pocket and that the predicted interactions are not the result of random or unstable conformations. Therefore, the combination of favorable binding affinity and low RMSD values strengthens the confidence in the docking predictions and supports the validity of the ligand as a potential inhibitor of the target protein.

Tabel 2. Docking result summary

Ligand	Mode	Affinity (kcal/mol)	Dist from best mode	
			rmsd l.b	rmsd u.b
Native ligand	1	-7.1	0.000	0.000
	2	-6.6	11.491	13.319
	3	-6.2	3.674	5.862
	4	-6.2	11.945	13.547
	5	-6.0	11.626	13.351
Candidate 1	1	-7.0	0.000	0.000
	2	-6.7	1.683	2.547
	3	-6.6	1.162	5.480
	4	-6.6	1.652	2.783
	5	-6.4	14.960	16.746
Candidate 2	1	-7.7	0.000	0.000
	2	-7.4	2.665	7.992
	3	-7.3	2.765	7.595
	4	-7.2	2.499	7.766
	5	-7.1	2.585	4.381
Candidate 3	1	-7.4	0.000	0.000
	2	-7.3	1.900	7.260
	3	-7.0	1.880	6.971
	4	-7.0	2.407	4.405
	5	-6.9	1.290	1.674
Sulfamethoxazole (Control)	1	-6.4	0.000	0.000
	2	-6.1	1.577	2.080
	3	-6.1	12.515	14.416
	4	-6.0	13.567	15.699
	5	-6.0	15.148	17.100

The molecular docking results were visualized using Discovery Studio software to provide a clearer representation of the interactions between the ligands and the target protein at the molecular level. This software enables the identification of various types of noncovalent interactions, including hydrogen bonds,

hydrophobic interactions, electrostatic interactions, as well as π-π and π-cation interactions formed between the ligands and amino acid residues within the protein active site (Baroroh, S.Si., M.Biotek. et al., 2023). In addition, Discovery Studio allows the visualization of interacting residues, bond distances, and ligand orientation within the protein binding pocket through 3D and 2D representations. The visualization of the docking results is presented in the corresponding figure 4. The binding interaction (amino acid residue) and hydrogen bond lengths are summarized in Table 3.



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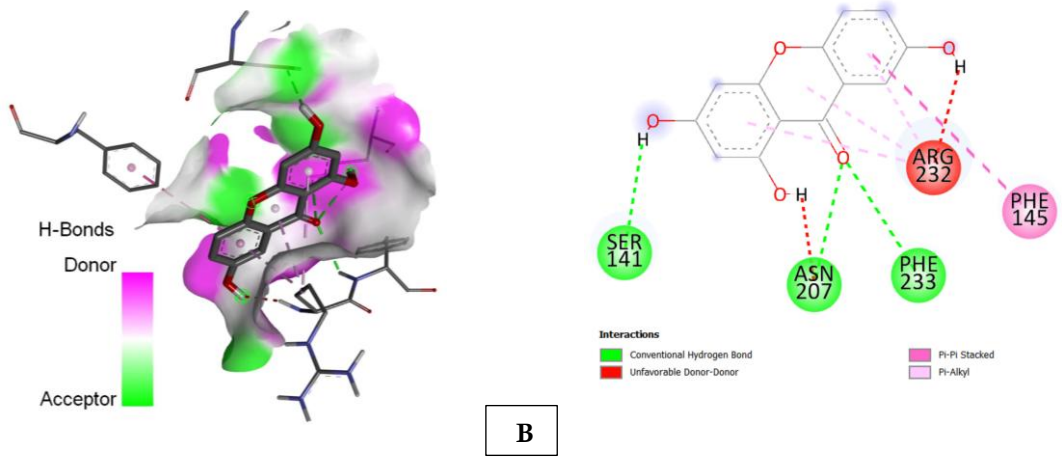

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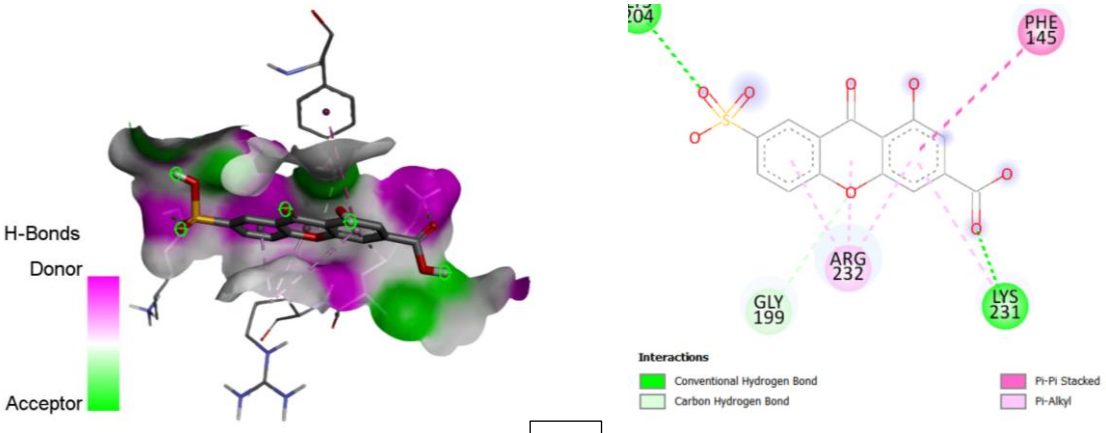

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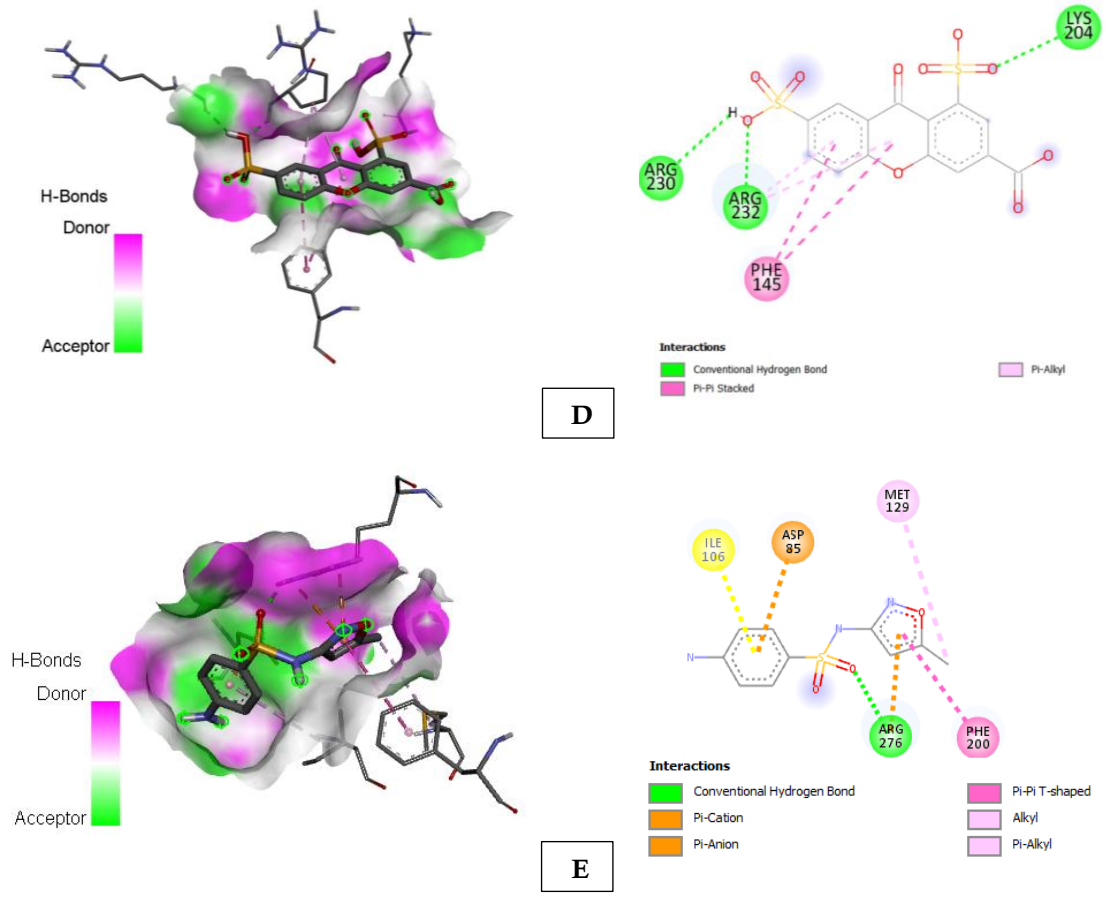


Figure 4. 2D and 3D predicted binding mode from docking simulation of (A) Native ligan, (B) Candidat 1, (C) Candidat 2, (D) Candidat 3, (E) Control the active site of Dihydropteroate Synthase (2VEG)

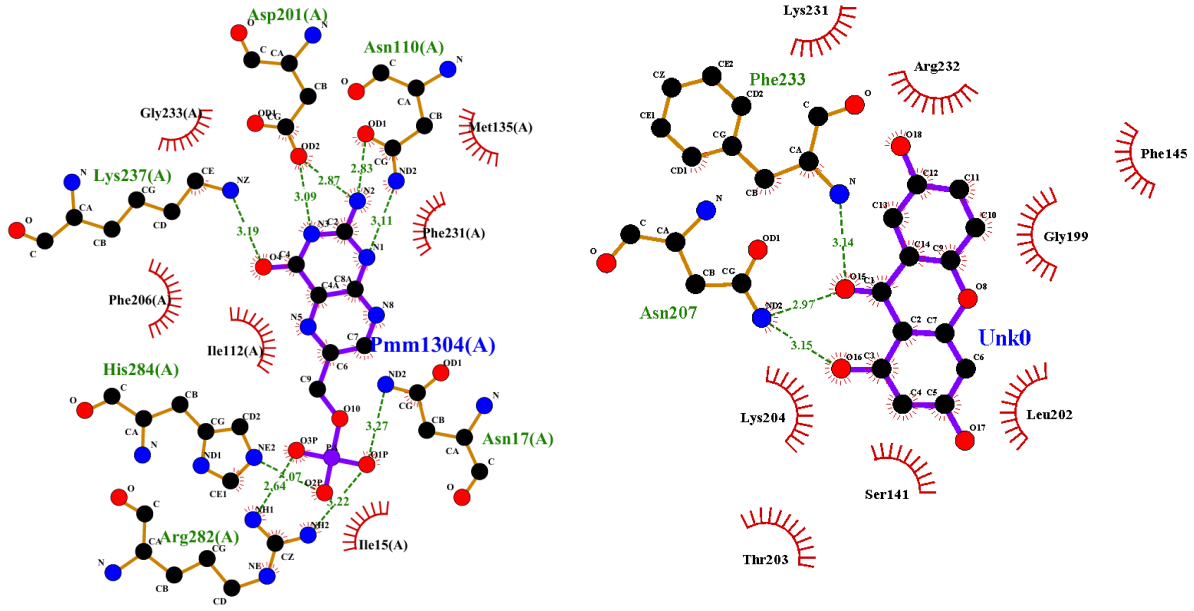
Tabel 3. Result of the interaction between protein and ligand

Ligand	Affinity (kcal/mol)	Binding intraction (amino acid residue)	Hydrogen bond length (Å)
Native ligand	-7.1	ASN11	2.43054
		ASN104	2.12313
		LYS231	2.67212
		ARG276	1.65329
		HIS278	2.10483
		ASP195	1.92042
		ASN104	1.84062
		GLY227	3.33449
Candidate 1	-7.0	ASN207	2.76852
		ASN207	2.57597
		PHE233	2.60637
		SER141	2.28529
Candidate 2	-7.7	LYS204	2.62609
		LYS231	2.13828
		GLY19	3.56182
Candidate 3	-7.4	LYS204	2.29741
		ARG232	2.14408
		ARG230	2.34469
Sulfamethoxazole (Control)	-6.4	ARG276	1.9739

The native ligand exhibited a complex and stable interaction pattern with the target protein through the involvement of several key amino acid residues, namely ASN11, ASN104, LYS231, ARG276, HIS278, ASP195, and GLY227. These residues are predominantly polar and charged, playing a crucial role in the formation of hydrogen bonds. The hydrogen bond lengths ranged from approximately 1.65 to 2.67 Å, indicating strong and biologically stable interactions. This interaction pattern reflects an optimal natural binding mechanism and serves as a reference for evaluating the performance of the candidate ligands. Candidate 1 interacted with the protein through residues ASN207, PHE233, and SER141. Notably, ASN207 participated in hydrogen bond formation more than once, highlighting its importance in stabilizing the ligand within the protein active site. The hydrogen bond lengths observed for candidate 1 ranged from approximately 2.28 to 2.77 Å, which remain within the acceptable range for stable hydrogen bonding interactions.

Candidate 2 demonstrated strong interactions with residues LYS204, LYS231, and GLY19. The involvement of positively charged residues such as LYS204 and LYS231 facilitates the formation of hydrogen bonds and significant electrostatic interactions. The hydrogen bond lengths ranged from approximately 2.13 to 2.63 Å, indicating relatively strong and stable interactions. Candidate 3 interacted with residues LYS204, ARG232, and ARG230, all of which are positively charged amino acids. These interactions were predominantly stabilized by hydrogen bonding, contributing to the stability of the ligand-protein complex. The hydrogen bond lengths ranged from approximately 2.14 to 2.34 Å, indicating strong and consistent interactions. Although the number of interacting residues for candidate 3 was lower compared to the native ligand and candidate 2, this ligand was still able to bind stably within the protein active site. This observation is consistent with the binding affinity value of candidate 3, which remains favorable but is less optimal than that of candidate 2.

Sulfamethoxazole (control) shows interaction with the protein through the ARG276 residue, which plays a role in forming hydrogen bonds at the DHPS active site. The hydrogen bond length formed is 1.97 Å, indicating that the interaction is relatively strong and stable. The binding affinity value of Sulfamethoxazole is −6.4 kcal/mol, suggesting that this compound can spontaneously bind to the target protein. However, the binding affinity of Sulfamethoxazole is still lower compared to Candidate 2, which has a binding affinity of −7.7 kcal/mol.



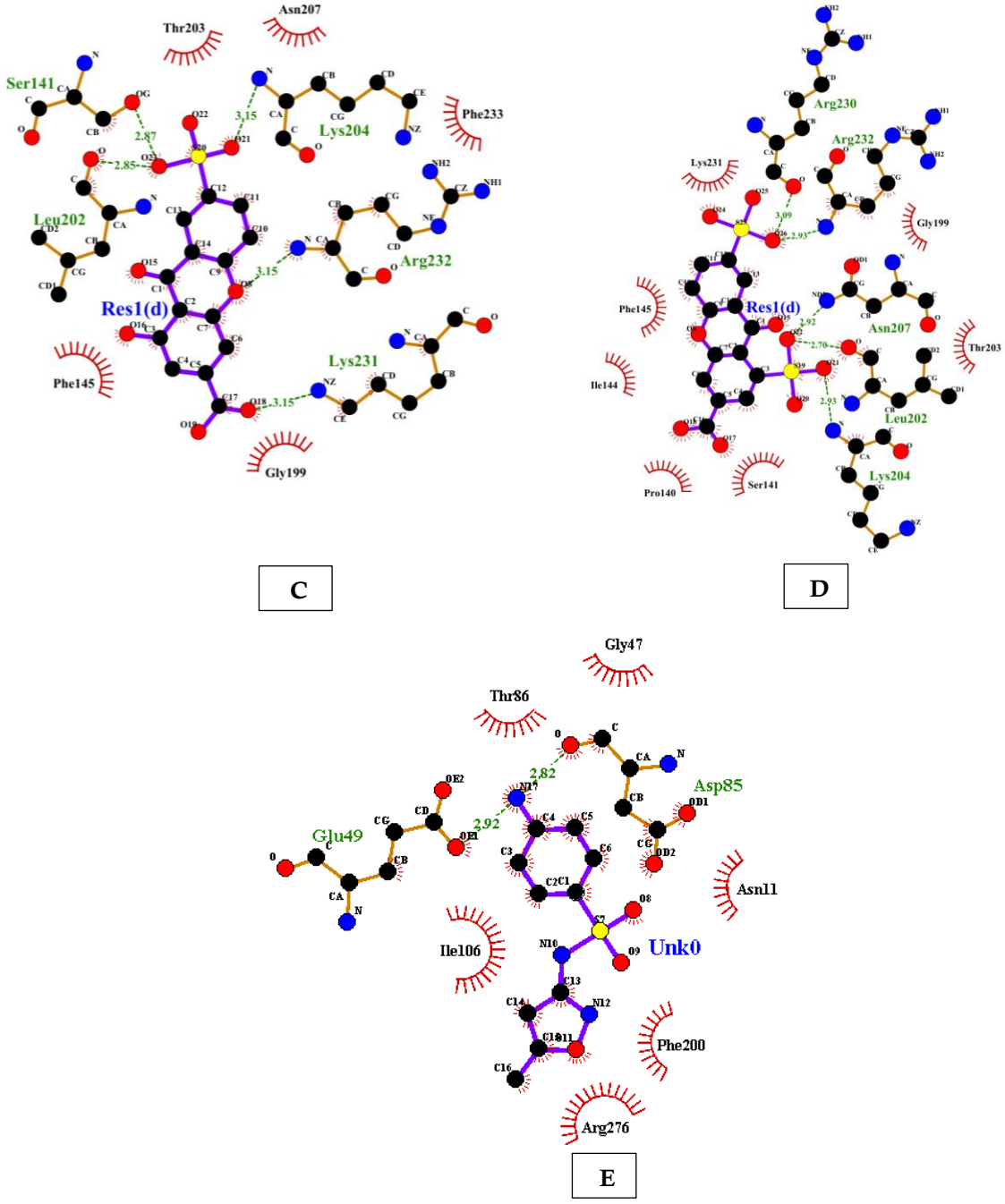


Figure 4. Visualization of1 Dihydropteroate Synthase (2VEG) interactions with (A) Native ligan, (B) Candidat 1, (C) Candidat 2, (D) Candidat 3 and (E) Control using LigPlot+

LigPlot+ was employed to visualize and analyze the two-dimensional interactions between the ligand and amino acid residues in the active site of protein 2VEG, encompassing both hydrogen bonds and hydrophobic interactions (Abdullah & Bayuhansah, 2021). Hydrogen bonds contribute to the binding specificity of the ligand, while hydrophobic interactions help to explain the inhibitor's potential toward the target protein and play a crucial role in the stability of the inhibitor–protein complex **(Alam & Al Balawi, 2022)**. Based on interaction visualization using LigPlot+, The native ligand shows interactions with residues LYS237, HIS284, ARG282, ASN17, ASN110, and ASP201 through hydrogen bond formation. The involvement of these residues helps increase the specificity and stability of ligand binding at the DHPS enzyme's active site. Positively charged residues like LYS237 and ARG282 can form favorable electrostatic interactions, while polar residues like HIS284, ASN17, ASN110, and ASP201 support hydrogen bond

formation, helping maintain the ligand's orientation in the binding pocket. In addition, the native ligand also forms hydrophobic interactions with residues GLY233, PHE206, PHE231, MET135, ILE112, and ILE15, contributing to the stabilization of the ligand–protein complex through nonpolar contacts around the enzyme's active site.

Candidate 1 demonstrates the ability to bind to the protein's active site through a combination of hydrogen bonds and hydrophobic interactions, although the intensity and number of interactions are relatively limited compared to the other candidates. The main hydrogen bonds are formed between the ligand and residues ASN207, PHE233, and LYS204, with bond distances ranging from 2.97 to 3.15 Å, indicating interactions of moderate strength. These hydrogen bonds play a role in facilitating the initial anchoring of the ligand and maintaining its orientation within the protein's active pocket. Additionally, Candidate 1 is surrounded by several nonpolar residues involved in hydrophobic interactions, including PHE145, GLY199, LEU202, SER141, THR203, LYS231, and ARG232. These hydrophobic interactions contribute to the stability of the protein–ligand complex by reducing the exposure of the ligand's nonpolar groups to the aqueous environment. Nevertheless, the relatively limited involvement of key residues and the smaller number of hydrogen bonds indicate that the complex stability of Candidate 1 is still lower compared to Candidates 2 and 3 (Dubey et al., 2023).

Analysis using LigPlot+ indicated that Candidate 2 exhibits a better and more stable interaction pattern compared to Candidate 1. This ligand forms several hydrogen bonds with key residues in the protein's active site, namely SER141, LEU202, LYS204, ARG232, and LYS231, with bond distances ranging from 2.85 to 3.15 Å. The presence of these hydrogen bonds suggests that Candidate 2 can interact specifically with residues that play a role in stabilizing the ligand within the protein's active pocket. In addition to hydrogen bonding, Candidate 2 also displays significant hydrophobic interactions with residues THR203, ASN207, PHE233, PHE145, and GLY199. These hydrophobic interactions contribute to reinforcing the stability of the protein–ligand complex by maintaining the ligand in an appropriate position during the docking simulation. The combination of relatively abundant hydrogen bonds and balanced hydrophobic interactions indicates that Candidate 2 has a more optimal binding orientation and higher affinity toward the target protein compared to Candidate 1.

Candidate 3 exhibits a more complex interaction pattern compared to the other candidates. This ligand forms several hydrogen bonds with residues ARG230, ARG232, ASN207, LEU202, and LYS204, with bond distances ranging from 2.70 to 3.09 Å, indicating strong hydrogen bonding. The involvement of positively charged residues, such as ARG230, ARG232, and LYS204, also indicates the contribution of electrostatic interactions that support ligand binding to the DHPS active site. In addition, Candidate 3 forms hydrophobic interactions with residues PHE145, ILE144, PRO140, SER141, THR203, GLY199, and LYS231, which contribute to the stability of the ligand–protein complex.

Although Candidate 3 exhibits a more diverse interaction pattern, the molecular docking results show that Candidate 2 has a higher binding affinity, with a binding energy of −7.7 kcal/mol, compared to Candidate 3, which has a binding energy of −7.4 kcal/mol. This indicates that the strength of binding affinity is determined not only by the number of interactions formed but also by the quality of the interactions, the ligand's orientation within the binding pocket, and the spatial complementarity between the ligand and the protein's active site. Thus, although Candidate 3 has a more complex interaction pattern, the binding orientation of Candidate 2 is estimated to be more optimal, resulting in an energy.

Analysis using LigPlot+ shows that Sulfamethoxazole, as the control compound, can interact with the active site of the DHPS enzyme through the residues GLU49, ASP85, ILE106, ARG276, PHE200, ASN11, GLY47, and THR86. The interactions formed suggest that Sulfamethoxazole can bind stably to the DHPS binding pocket through a combination of polar and nonpolar interactions that contribute to the stability of the ligand–protein complex. Nevertheless, the binding affinity value of Sulfamethoxazole (−6.4 kcal/mol) is still higher than that of Candidate 2 (−7.7 kcal/mol), which indicates that Candidate 2 has a better binding affinity to DHPS. These results suggest that the tested xanthone derivatives, especially Candidate 2, have the potential to be promising DHPS inhibitor candidates and are worthy of further investigation through biological testing.

Conclusion

Based on the analysis results, it can be concluded that xanthone derivatives have the potential as inhibitors of dihydropteroate synthase (DHPS) (2VEG) from *Streptococcus pneumoniae* based on molecular docking studies. Among the three candidates, Candidate 2 showed the most optimal performance with a binding affinity value of -7.7 kcal. Meanwhile, Candidate 1 and Candidate 3 showed relatively weaker interactions. Compared to the control compound Sulfamethoxazole, Candidate 2 has a better binding affinity

(−7.7 kcal/mol) than Sulfamethoxazole (−6.4 kcal/mol) under the docking simulation conditions used. Overall, these results indicate that Candidate 2 is the most promising candidate for further development as a new antibacterial agent targeting DHPS in Streptococcus pneumoniae.

Acknowledgments

The author would like to thank all parties who have provided support and contributions in the implementation and preparation of the article entitled "Molecular Docking Study of Xanthone Targeting the Dihydropteroate Synthase (2VEG) Enzyme in Streptococcus pneumoniae". Special thanks are extended to institutions that provided facilities and supporting resources, particularly in the implementation of computational studies and molecular docking analysis.

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