In-Silico Analysis of Active Compounds from Herbal Plants Against Penicillin Binding Protein 2a (PBP2a) of Methicillin-Resistant Staphylococcus Aureus

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Abstract

Herbal plants are frequently used for medication by people as they contain rich bioactive compounds. Turmeric (*Curcuma longa*) and bitter ginger (*Zingiber zerumbet*) are types of rhizome herbal plants with the highest amount of production in Indonesia. They contain bioactive compounds applicable for antibiotics against resistant bacteria, one of which is MRSA (Methicillin-resistant *Staphylococcus aureus*). This study aims to investigate the compound components in turmeric and bitter ginger, which might be anti-MRSA candidates against the PBP2a binding side by in-silico analysis. A total of 24 ligands of turmeric and bitter ginger are bound to the target protein, the PBP2a receptor. The binding results are followed with a test of biological activity, physicochemical properties, and toxicity of the herbal plant compounds. The study resulted in 12 ligands potentially being anti-MRSA with binding on the allosteric side of PBP2a. In sum, it suggested three compounds with the best potential for anti-MRSA candidates: curcumin, germacrone, and zerumbone and may be considered as drug candidates for therapeutic aims in several human infections associated with MRSA. Nevertheless, in vitro and in vivo confirmations are needed.

Introduction

Over the past few years, many antibiotic-resistance bacteria have risen due to excessive and irrational use of antibiotics in diverse sectors. One is Methicillin-Resistant Staphylococcus Aureus (MRSA) (Huan et al., 2020). MRSA is a pathogenic bacterium resistant to methicillin. It is responsible for various diseases, such as mild skin infections and invasive diseases threatening human life (Sousa, 2017). Therefore, MRSA has been a significant concern because it poses high morbidity, mortality, and resistance to all types of penicillin, mostly b-lactams (Lakhundi and Kunyan, 2018).

βlactam antibiotics target Penicillin Binding Proteins (PBPs) to inhibit the growth of MRSA. PBPs is one of the molecules that play a vital role in the synthesis of peptidoglycan so that the bacterial cell wall is formulated. β-lactam antibiotics work by inactivating transpeptidase action on PBPs thus preventing glycan and peptidoglycan biosynthesis chain cross-linking (Mohamed et al., 2019). However, there has been a resistance mechanism that resulted in MRSA immune to various antibiotics with the presence of penicillin binding protein 2a (PBP2a). The PBP2a molecule replaces the previous PBP function upon catalysing the transpeptidation reaction. This molecule has a low affinity for other β-lactam penicillin and antibiotics so that the synthesis of peptidoglycan in MRSA continues. Therefore, PBP2a becomes a potential target to inhibit MRSA growth.

Screening of active compounds has been successively made as an alternative to search for antibiotic agents, one of which is found in herbal plants. Some of the most widely produced herbal plants with antimicrobial activity in Indonesia are turmeric and bitter ginger (Arivoli et al., 2019; Filho et al., 2020; Tan et al., 2018; Rana et al., 2012). However, exploration on active compounds within those plants as anti-MRSA is rarely made. To know the potential of the herbal plants as anti-MRSA, we can start with in-silico method. This analysis method is reliable and cost and time saving to the process of developing and discovering new drug candidates (Dorchech et al., 2022).

Thus, this study employs in-silico analysis of turmeric and bitter ginger to find anti-MRSA candidates by targeting PBP2a molecules as the cause of MRSA resistance. To determine the binding interaction between herbal plant compound ligands and PBP2a receptors, this study analyses through molecular docking, physicochemical properties, biological properties, and toxicity of herbal plant compounds in-silico.

Materials And Methods

Modelling and validation of the receptor proteins

Some relevant articles, NCBI database (https://www.ncbi.nlm.nih.gov), and Protein Data Bank (PDB) (https://www.rcsb.org) were studied to obtain information about the structure of 3-dimensional proteins and the ligand binding site of staphylococcus aureus (PDB ID: 4CJN). Then, the receptor protein was validated with SAVES 6.0 program (https://saves.mbi.ucla.edu).

Exploration Of The Bioactive Compounds Of Herbal Plants

A total of 24 bioactive compounds from turmeric and bitter ginger plants were set based on the review of some articles by Gas Chromatography-Mass Spectrometry (GC-MS) technique to determine the compounds inside the herbal plants. The 3-dimensional and 2-dimensional structures of the 24 compounds were obtained from the PubChem Database (https://pubchem.ncbi.nlm.nih.gov).

Preparation And Analysis Of Molecular Docking

3-dimensional structure of PBP2a receptor was prepared for molecular docking analysis by removing all water molecules and other nuisance ions. The control molecule that bound to the receptor was also separated and stored in pdb format. A total of 24 ligands of bioactive compounds of herbal plants downloaded in SDF format on PubChem were included in open babel in PyRx 0.8 to do the energy minimization process and be converted to pdbqt. Then, the molecular docking analysis process followed with each ligand bound to PBP2a as anti MRSA using PyRx 0.8 software. The molecular docking results that showed the highest energy affinity were selected for further examination.

Physicochemical, Biological, And Toxicity Characteristics Test

The study was conducted using the SCFBio website (http://www.scfbio-iitd.res.in) by looking at the fulfilment of the Lipinski Rule of Five of each test compound. The biological properties test followed the Way2Drug website (http://www.way2drug.com/PASSOnline/) with the parameters of the resulting Pa and Pi values. The toxicity test adopted the ProTox-II website (https://tox-new.charite.de/protox_II/).

Results And Discussion

Validation of 3-dimensional model structure of macromolecules as receptors was performed using several analyses to determine the quality of macromolecular structure model globally and locally. The validation results of the 3-dimensional structure of macromolecules are described in Table 1.

Table 1
Validation Result of 3D PBP2a Macromolecule

Parameters	ERRAT	VERIFY3D	Ramachandran Plot		QMEAN
	(%)	(%)	Most Favoured Region (%)	Disallowed Region (%)	
Score	94.76	95.64	90.7	0.3	0.43

Based on Table 1, the ERRAT score is 94.76%. This score indicates that the resulted structural model is reliable and valid for use. According to Elengoe et al. (2014), the protein structure can be said to be good if the ERRAT score is over 91%, which indicates that the percentage of regional errors in the protein is relatively small and acceptable. ERRAT is used to detect error regions of protein structure based on errors that lead to random distribution of atoms (Al-Khayyat and Al-Dabbagh, 2016). The macromolecule VERIFY3D score is 95.64%. This result illustrated that the structure of the 3-dimensional model is compatible with the amino acid sequence so that it is classified as a good quality. A model can be said to have a good quality if the percentage value of amino acid residues from the Verify3D assessment is over 80% for the average score of 3D-1D residue ≥ 0.2 (Junaidin et al., 2019).

The validation measurement of protein model quality at atomic scale can be established through the conformation of amino acid residues drawn on the diagram at each dihedral angle of each residue. The combination of the dihedral angles can be interpreted by making Ramachandran plot (Arwansyah and Hasrianti 2014). The assessment is in the form of a percentage of amino acid residues in most favoured regions and disallowed regions/outliers from the Ramachandran plot (Junaidin et al., 2019). In Table 1, the percentage in the most favoured region is 90.7% and that in the disallowed region is only 0.3%. The data prove that the macromolecule has a good structural quality. According to Gaffar et al. (2016), a protein structure is stated to be fine if the number of residual plots in the most favoured regions is over 90%. Also, the percentage of less than 0.2% in the disallowed region is stated as a model with good structural quality (Yeni and Tjahjono, 2017). The greater the amino acid residues in the most favoured region and the lower in the disallowed region, the better and the more stable the quality of the structure.

In addition, this study employs the Quality Model Energy Analysis (QMEAN) test, which is a composite assessment function that describes the main aspects of the geometric structure of the protein as a whole. QMEAN assessment can estimate the global quality of the model and reflect the reliability of the predicted model. The QMEAN score is 0.43, so the 3-dimensional model within the range of a model is stated to be good. According to Benkert et al. (2007), a protein structure can be good once it has a QMEAN value of less than 1 and higher than − 4. The more positive the value of QMEAN, the better the quality of protein models produced, and it indicates that the produced model is reliable (Bordoli and Schwede, 2012).

Based on the validation results of the good 3-dimensional model of PBP2a receptor structure, molecular docking analysis is then performed to see the interaction of PBP2a binding with the test compound as an anti-MRSA. The best assessment of molecular docking results can be obtained from the interrelated assessment function, namely the ligand binding conformation on the active side of the receptor protein, and then a look at the ranking of scoring results (Meng et al., 2011). The result of molecular docking of herbal plants to PBP2a is available in Table 2.

Table 2
Receptor Interaction and Ligand Assay

Herbal Plants	Ligand	*Docking Score* (Kcal/mol)	Types of Interaction	
			Hydrogen Interaction	Hydrophobic Interactions
	Quinazolinone Control	-7.1	LYS316 TYR105	ASN104 ILE144 GLY296 TYR297 ASN146 ASP295 GLU294 LYS273
Turmeric	Curcumin	-5.9	LYS316 TYR105	ASP295 ASN104 ASN146 ILE144 GLU145 TYR297 LYS273
	Terpinolene	-4.4	-	ASN146 TYR105 LYS316 LYS273 ASP295 GLU294 GLY296 TYR297
	Curzerenone	-4.8	-	LYS316 ASP295 TYR297 ASN146 TYR105 LYS273
	β-Turmerone	-5.0	LYS273	GLY296 LYS316 TYR105 ILE144 GLU145 ASN146 ASP295 TYR297
	β-Phellandrene	-4.3	-	TYR105 TYR297 LYS316 LYS273 ASP295 GLY296 ASN146
	α-Curcumene	-5.1	-	GLU145 TYR105 ASN146 GLY296 LYS316 GLU294 LYS273 ASP295 TYR297 ILE144
	β-Curcumene	-5.1	-	TYR297 TYR105 ILE144 ASN146 GLU145 GLY296 LYS273 GLU294 LYS316 ASP295
	Germacrone	-5.3	-	TYR105 LYS273 GLU294 LYS316 ASP295 TYR297 ASN146
	α-Zingiberene	-4.7	-	LYS316 TYR297 GLU145 ILE144 TYR105 ASN146 ASP295 LYS273
	Ar-Turmerone	-5.1	LYS273	ASN146 TYR297 ASP295 LYS316 ASN104 ILE144 GLY296 TYR105
Bitter Ginger	Zerumbone	-5.4	LYS316	TYR297 TYR105 ASN146 ASP295 GLY296 LYS273
	Linalool	-4.0	ASP295	GLY296 LYS316 LYS273 TYR297 ILE144 TYR105 ASN146

Table 2 shows that the value of the control docking score is -7.1 Kcal/mol, while the ligand assay ranges from − 4.0 to -5.9 Kcal/mol. Assessment based on the docking score in molecular docking explains that the control has the lowest value of all test compounds from the herbal plants. However, the difference in value between control and test ligands is not too far, especially curcumin compounds that have a docking score value of -5.9 Kcal/mol, so it can be considered as a potential compound for an anti MRSA, such as its comparison compound, quinazolinone.

The docking score describes the strength or affinity of the bond from the interaction of the ligand and the receptor. The binding energy of the scoring results in the form of Gibbs free energy (ΔG) functions as a translation of the third law of thermodynamics and a parameter of conformational stability between ligand and binding site at PBP2a receptor (Arwansyah and Hasrianti 2014). Since the chemical compounds in herbal plants are all negative, which means that the reaction may occur and run spontaneously (the reaction goes to the product), the bond between the ligand assay of the herbal plant with PBP2a receptor can be formed and is stable.

Based on Table 2, the control compounds pose hydrogen interactions on the amino acids LYS316 and TYR105, and have hydrophobic interactions on the amino acids ASN104, ILE144, GLY296, TYR297, ASN146, ASP295, GLU294, and LYS273. Of these interactions, there are 5 interactions that are keys on the allosteric side that will affect PBP2a receptor activity, namely LYS273, ASN146, TYR105, TYR297, and LYS316 (Bouley et al., 2015). Of the 24 test compounds, only 12 ligands have a common bond with the control by making an interaction on the 5 key amino acid residues of the allosteric side of PBP2a. Turmeric becomes the plant with the highest number of compounds that show the most interactions in common with the controls, with 10 compounds out of a total of 14 ligand assays, which are curcumin, terpinolene, curzerenone, β-turmerone, β-phellandrene, α-curcumene, β-curcumene, germacrone, α-zingiberene, and Ar-turmerone. As for the bitter ginger, from a total of 10 ligand assays, 2 compounds show the same interaction as the control, namely zerumbone and linalool.

Visualization and interaction analysis of docking results aims to see the results of tethering between the comparison ligand (control) and the ligand assay used with PBP2a receptor. The visualization result is in the form of interaction of amino acid residues with ligands. The interaction of the amino acids involved allows a contact between the ligand and the receptor so that it has inhibitory activity. The binding site area shows amino acid residues that play a vital role upon creating interactions between macromolecules and ligands, such as hydrogen bonds and hydrophobic bonds (Sari et al., 2020).

The interactions among hydrogen bonds or hydrophobic bonds in the involved amino acids will affect the activity of proteins. It will be more impactful if the interaction occurs in the key amino acid residues on the active side and allosteric side of the protein. The mechanism of 18 herbal plant compounds in dealing with MRSA bacterial resistance can be known from the results of binding ligand molecules with PBP2a. PBP2a protein is a modified form of D-alanyl-D-alanine transpeptidase, which is involved in MRSA resistance (Alhadrami et al., 2020). The active side of this

protein has been modified, so it is highly protected and covered by a tyrosine residue (TYR466) which plays as a gatekeeper that prevents ligands, such as beta lactam antibiotics to enter the active side. However, the gatekeeper can be open through control on the allosteric side of the protein (~ 60A from the active side). Binding to the allosteric side can trigger a change in the overall conformation of the protein and will eventually let the active side of the protein reachable by inhibitors, such as antibiotics (Otero et al., 2013). After the active side is open, the inhibitor ligand will bind to the active side of the protein, the amino acid residue SER403, so that the catalysis process of PBP2a protein cannot occur. PBP2a protein catalyses the polymerization reaction of the peptidoglycan chain, so the bacterial cell wall can be formulated. If the reaction is not successful, the peptidoglycan as a constituent of bacterial cells is not formed, and it causes bacteria to die (Mahasenan et al., 2017).

The result of molecular docking analysis and visualization of residue interactions demonstrated that of the 12 potential compounds for anti-MRSA candidates, 3 best compounds show strong interaction and binding affinity with receptors, so they are highly potential for anti-MRSA. The The three best compounds are curcumin, with a docking score of -5.9 Kcal/mol and interaction of amino acid residues as in Fig. 1; germacrone compound with docking score of -5.3 Kcal/mol and residue interaction as in Fig. 2; zerumbone compound with a docking score of -5.4 Kcal/mol and bonding residue interactions as in Fig. 3.

Table 3. The test result of physicochemical, toxicity, and biological properties

Compound	MW (Da)	HBD	HBA	LogP	Hepato-Toxicity	Mutagenic	Carcinogenic	Sito-Toxicity	Biological Activity	Pa	Pi
Curcumin	368.4	2	6	3.369898	Non-Hepatoxic	Non-Mutagens	Non-Carcinogenic	Non-Cytotoxic	Antibacterial	0.272	0.071
									Antibiotics	0.097	0.081
									PGT Inhibitor	0.345	0.079
Terpinolene	136.23	0	0	3.452999	Non-Hepatoxic	Non-Mutagens	Non-Carcinogenic	Non-Cytotoxic	Antibacterial	0.348	0.044
									Antibiotics	0.163	0.042
									PGT Inhibitor	0.509	0.020
Curzerenone	230.30	0	2	3.478719	Non-Hepatoxic	Non-Mutagens	Carcinogenic	Non-Cytotoxic	Antibacterial	0.439	0.023
									Antibiotics	0.132	0.058
									PGT Inhibitor	0.238	0.169
β-Turmerone	218.33	0	1	4.070299	Non-Hepatoxic	Non-Mutagens	Non-Carcinogenic	Non-Cytotoxic	Antibacterial	0.396	0.031
									Antibiotics	0.197	0.029
									PGT Inhibitor	0.464	0.030
β-Phellandrene	136.23	0	0	3.164799	Non-Hepatoxic	Non-Mutagens	Non-Carcinogenic	Non-Cytotoxic	Antibacterial	0.369	0.038
									Antibiotics	0.203	0.028
									PGT Inhibitor	0.574	0.011
α-Curcumene	202.33	0	0	4.844920	Non-Hepatoxic	Non-Mutagens	Non-Carcinogenic	Non-Cytotoxic	Antibacterial	0.358	0.041
									Antibiotics	0.169	0.039
									PGT Inhibitor	0.550	0.014
β-Curcumene	204.35	0	0	5.035399	Non-Hepatoxic	Non-Mutagens	Non-Carcinogenic	Non-Cytotoxic	Antibacterial	0.415	0.027
									Antibiotics	0.217	0.025
									PGT Inhibitor	0.509	0.020
Germacrone	218.33	0	1	4.358499	Non-Hepatoxic	Non-Mutagens	Non-Carcinogenic	Non-Cytotoxic	Antibacterial	0.402	0.029
									Antibiotics	0.157	0.045
									PGT Inhibitor	0.414	0.046
α-Zingiberene	204.35	0	0	4.891299	Non-Hepatoxic	Non-Mutagens	Non-Carcinogenic	Non-Cytotoxic	Antibacterial	0.416	0.026
									Antibiotics	0.212	0.026
									PGT Inhibitor	0.542	0.015
Ar-Turmerone	216.32	0	1	4.023919	Non-Hepatoxic	Non-Mutagens	Non-Carcinogenic	Non-Cytotoxic	Antibacterial	0.296	0.062
									Antibiotics	0.146	0.051

Compound	MW (Da)	HBD	HBA	LogP	Hepato-Toxicity	Mutagenic	Carcinogenic	Sito-Toxicity	Biological Activity	Pa	Pi
									PGT Inhibitor	0.480	0.026
Zerumbone	218.33	0	1	4.214398	Non-Hepatoxic	Non-Mutagens	Non-Carcinogenic	Non-Cytotoxic	Antibacterial	0.415	0.027
									Antibiotics	0.202	0.028
									PGT Inhibitor	0.430	0.040
Linalool	154.25	1	1	2.669800	Non-Hepatoxic	Non-Mutagens	Non-Carcinogenic	Non-Cytotoxic	Antibacterial	0.385	0.034
									Antibiotics	0.140	0.054
									PGT Inhibitor	0.423	0.043

Abbreviations: MW (Molecular weight); HBD (hydrogen bond donor); HBA (hydrogen bond acceptor); PGT Inhibitor (Peptidoglycan glycosyltransferase inhibitor); Pa (Probable activity); Pi (Probable inactivity)

Table 3 proves that all the 12 compounds of ligand assay in the five herbal plants meet the Lipinski Rule of Five. The requirement of Lipinski Rule of Five includes having molecular weight of <500 Da, hydrogen bond donor of £5, hydrogen bond acceptor of £10, and LogP value of £5 (Lipinski, 2004). A compound with a low level of permeability, its process of absorption or permeation into the body is poor if it does not meet the Lipinski Rule of Five (Smelcerovic et al., 2017). Lipinski Rule of five analysis aims to determine whether a compound can be absorbed by the body by determining its physicochemical properties when it crosses the cell membrane in the body. In addition, it can also know the physicochemical properties, such as the hydrophobic or hydrophilic character of a compound to pass through the cell membrane by passive diffusion. The process of entering the drug into the body will go through several stages, absorption, distribution, metabolism, excretion, and safety within the body. This mechanism is often referred to as pharmacokinetics (Zhang and Wilkinson, 2007). Lipinski's rule becomes part of the pharmacokinetics stages of an active compound on the possibility of absorption activity in the body (Gupta et al., 2013).

Toxicity testing is a science-based approach that aims to describe the prospective side effects of a compound, especially for humans. It can serve as a foundation to determine if a compound can be used as a source of medicine (Schrenk, 2019). Undesirable bioavailability of the drug may occur due to inappropriate pharmacokinetic and pharmacodynamic properties. It is followed by a low level of drug safety, mainly due to the toxicity of a compound. Therefore, it is the main cause of the high failure rate in finding a new drug (Wu et al., 2020). Cook et al. (2014) stated that safety and toxicity are the most common causes of a lot of drug discovery project failures. Only about 8% of drug candidates that undergo clinical assessment are likely to be marketed, and 20% failed in the drug development stage due to toxicity (Drwal et al., 2014).

According to Table 3, no compounds have hepatoxic properties. Hepatoxicity can be the main reason for the cessation of drug circulation and the termination of the period of pre-clinical and clinical studies. This trait may result in organ failure and lead to fatal outcomes (Cheng, 2009). Hepatoxicity is related to its liver organ-harming properties, which can occur through various mechanisms, such as oxidative stress, mitochondrial dysfunction, transporter inhibition, and disruption of transcription processes (Luo et al., 2017). Based on mutagenic assays, all compounds show non-mutagenic properties. In the carcinogenic test, one compound has carcinogenic properties, which is curzerenone. It indicates that the compound may be harmful to the body. Mutagenicity or mutagenicity demonstrates the capability of a compound to induce the transmission of damage at the genetic level, such as gene mutations, DNA changes, chromosomal aberrations, and changes in the number of chromosomes (Benigni and Bossa, 2011). In addition, cytotoxicity tests are also performed on herbal plants to determine the side effects of compounds upon causing the desired or undesired damage to cells. Cytotoxic compounds can destroy both normal and abnormal cells (Banerjee et al., 2018). The test resulted that all compounds do not have cytotoxic properties. Therefore, they are not potentially harmful and causing cell damage once used.

The biological activity analysis shows that 12 bioactive compounds within herbal plants have a higher Pa value than that of Pi. This activity is indirectly related to the function of the compound as an anti-bacterial. The Pa value obtained indicated that the compounds within these herbs have the potential to be used as anti-bacterial. In addition, the high biological activity as an antibiotic also shows that the compounds within these herbal plants might be used as antibiotics with the perceived activity as an antibiotic. The other activity found in the test compound is peptidoglycan glycosyltransferase inhibitor. It is one of the enzymes used in the process of cell wall formation in S. aureus bacteria. This enzyme serves to catalyse the polymerization process of glycan chains to be peptidoglycan (Srisuknimit et al., 2017).

This enzyme belongs to one of the 2 membrane-related enzymes that are vital in the last stage of the peptidoglycan biosynthesis process for the integrity and stability of the bacterial cell wall (Zuegg et al., 2015). The enzyme peptidoglycan glycosyltransferase works by catalysing the polymerization process of Lipid II disaccharide units. The substrate binding region of peptidoglycan glycosyltransferase contains the side of

glycosyl acceptor (in which lipid II disaccharides are bound), and the side of glycosyl donor, where other lipids II are bound, and it accommodates glycan chain growth to be longer (Huang et al., 2012). The presence of peptidoglycan glycosyltransferase inhibitor activity in the chemical compound of herbal plants can increase its effectiveness and potential to be an anti-MRSA candidate.

Conclusion

Bioinformatics approach is currently a prominent and useful tool to discover new agents upon the fight against a wide range of diseases due to resistant bacteria, one of which is Methicillin-resistant Staphylococcus aureus. This study employs turmeric and bitter ginger herbal plants as alternatives to the prospective anti-MRSA compounds. Three compounds with the best activity are advisable for anti-MRSA candidates, which are curcumin, germacrone, and zerumbone. The current research is expected to be a starting point for the development of plant-based therapeutic agents and to be further studied with in-vitro and in-vivo analysis.

Declarations

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Competing Interests

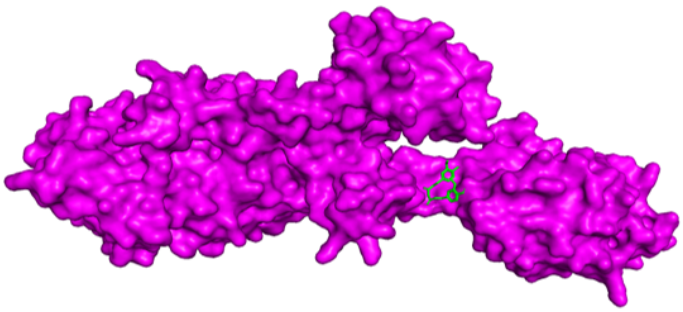
The authors have no conflict of interest to declare that relevant to the content of this article.

References

1. Alhadrami HA, Hamder AA, Hassan HM, Belbahri L, Rateb ME, dan Sayed AM. 2020. Flavonoids as Potential anti-MRSA Agents through Modulation of PBP2a: A Computational and Experimental Study. Antibiotics 9(562): 1-16. DOI:10.3390/antibiotics9090562
2. Al-Khayyat MZS, and Al-Dabbagh AGA. 2016. In silico prediction and docking of tertiary structure of LuxI, an inducer synthase of Vibrio fischeri. Rep. Biochem. Mol. Biol 4(2): 66-75
3. Arivoli S, Tennyson S, Divya S, Rani S, dan Marin G. 2019. GC-MS analysis of bioactive compounds of Curcuma longa Linnaeus (Zingiberaceae) rhizome extract. Journal of Pharmacognosy and Phytochemistry 8(6): 49-52
4. Arwansyah and Hasrianti. 2014. Simulasi molecular docking senyawa kurkumin dan analognya sebagai selective androgen receptor modulators (sarms) pada kanker prostat. Jurnal Dinamika 5(2): 60-75
5. Banerjee P, Eckert AO, Schrey AK, dan Preissner R. 2018. ProTox-II: a webserver for the prediction of toxicity of chemicals. Nucleic Acids Research 46: 257-263. DOI: 10.1093/nar/gky318
6. Benigni R, dan Bossa C. 2011. Mechanisms of chemical carcinogenicity and mutagenicity: a review with implications for predictive toxicology. Chemical Reviews 111(4): 2507- 2536. DOI:10.1021/cr100222q
7. Benkert P, Tosatto SCE, and Schomburg D. 2007. QMEAN: A comprehensive scoring function for model quality assessment. Proteins 71(1): 261-277. DOI:10.1002/prot.21715
8. Borodoli L, and Schwede T. 2012. Automated protein structure modeling with SWISS- MODEL workspace and the protein model portal. Methods Mol Biol 857: 107-136. DOI:10.1007/978-1-61779-588-6_5.
9. Cheng A. 2009. In Silico Prediction of Hepatotoxicity. Curr Comput Aided-Drug Des 5(2): 122– 127. DOI: 10.2174/157340909788451883
10. Cook D, Brown D, Alexander R, March R, Morgan P, Satterthwaite G, dan Pangalos MN. 2014. Lessons learned from the fate of AstraZeneca's drug pipeline: a five- dimensional framework. Nat. Rev. Drug Discov 13: 419–431. DOI: 10.1038/nrd4309
11. Dorcheh FA, Balmeh N, dan Sanjari S. 2022. In-silico investigation of antibacterial herbal compounds in order to find new antibiotic against Staphylococcus aureus and its resistant subtypes. Informatics in Medicine Unlocked 28. https://doi.org/10.1016/j.imu.2021.100843
12. Drwal MN, Banerjee P, Dunkel M, Wettig MR, Preissner R. 2014. ProTox: A web server for the in silico prediction of rodent oral toxicity. Nucleic Acids Res 42:53–58. DOI: 10.1093/nar/gku401
13. Elengoe A, Naser MA, dan Hamdan S. 2014. Modeling and docking studies on novel mutants (K71L and T204V) of the ATPase domain of human heat shock 70 kDa protein 1. Int. J. Mol. Sci. 15: 6797-6814. DOI:10.3390/ijms15046797
14. Filho JGDO, Almeida MJD, Sousa TL, Santos DCD, dan Egea MB. 2020. Bioactive Compounds of Turmeric (Curcuma longa L.). Dalam Murthy HN dan Paek KY (eds.), Bioactive Compounds in Underutilized Vegetables and Legumes. Springer, Switzerland
15. Gaffar S, Masyhuri AA, Hartati YW, dan Rustaman. 2016. Studi in silico single chain variable fragment (SCFV) selektif terhadap hormon basic natriuretic peptide (BNP). Chimica et Natura Acta 4(2): 52-59. https://doi.org/10.24198/cna.v4.n2.10671
16. Gupta S, Kesarla R, Omri A. 2013. Formulation strategies to improve the bioavailability of poorly absorbed drugs with special emphasis on self-emulsifying systems. ISRN Pharmaceutics. http://dx.doi. org/10.1155/2013/848043.

17. Huang CY, Shih HW, Lin LY, Tien YW, Cheng TJR, Cheng WC, Wong CH, Ma C. 2012. Crystal structure ofStaphylococcusaureustransglycosylase in complex with a lipid II analog andelucidation of peptidoglycan synthesis mechanism. PNAS 109(17): 6496- 6501. DOI: 10.1073/pnas.1203900109
18. Junaidin CS, dan Fadla NH. 2019. Studi homologi modeling enzim tirosinase (homo sapiens) dengan menggunakan swiss-model. Jurnal Farmagazine 6(1): 1-7. http://dx.doi.org/10.47653/farm.v6i1.125
19. Lakhundi S, dan Kunyan Z. 2018. Methicillin-resistant Staphylococcus aureus: Molecular characterization, evolution, and epidemiology. Clinical Microbiology Reviews 31(4): 1-103. DOI:10.1128/CMR.00020-18
20. Luo G, Shen Y, Yang L, Lu A, dan Xiang Z. 2017. A review of drug - induced liver injury databases. Arch Toxicol 91(9):3039-3049. DOI: 10.1007/s00204-017-2024-8
21. Lipinski CA. 2004. Lead- and drug-like compounds: the rule-of-five revolution. Drug Discovery Today: Technologies 1(4): 337-341. https://doi.org/10.1016/j.ddtec.2004.11.007
22. Mahasenan KV, Molina R, Bouley R, Bautuecas T, Fisher JF, Hermosho JA, Chang M, dan Mobashery S. 2017. Conformational dynamics in penicillin-binding protein 2a of methicillin-resistant Staphylococcus aureus, allosteric communication network and enablement of catalysis. J Am Chem Soc 139(5): 2102–2110. DOI:10.1021/jacs.6b12565.
23. Meng XY, Zhang HX, Mezei M, dan Cui M. 2011. Molecular Docking: A powerful approach for structure-based drug discovery. Curr Comput Aided Drug Des 7(2): 146–157.
24. Otero LH, Rojas-Altuve A, Llarrull LI, Carrasco-López C, Kumarasiri M, Lastochkin E, dan Johnson JW. 2013. How allosteric control of Staphylococcus aureus penicillin binding protein 2a enables methicillin resistance and physiological function. Proc. Natl. Acad. Sci. 110: 16808–16813.
25. Rana VS. 2010. GC and GC/MS analysis of the volatile constituents of the oils of Alpinia galanga (L.) Willd and A. officinarum hance rhizomes. Journal of Essential Oil Research 22: 521-524. DOI: 10.1080/10412905.2010.9700388
26. Sari IW, Junaidin, dan Pratiwi D. 2020. Studi molecular docking senyawa flavonoid herba kumis kucing *(orthosiphon stamineus)* pada reseptor glukosidase sebagai antidiabetes tipe 2. Jurnal Farmagazine 7(2)
27. Schrenk D. 2018. What is the meaning of 'A compound is carcinogenic'?. Toxicology Reports 5: 504-511. https://doi.org/10.1016/j.toxrep.2018.04.002
28. Sousa MAD. 2017. Methicillin-resistant Staphylococcus aureus among animals: current overview. Clinical Microbiology and Infection 23: 272-280. http://dx.doi.org/10.1016/j.cmi.2016.11.002
29. Srisuknimit V, Qiao Y, Schaefer K, Kahne D, dan Walker S. 2017. Peptidoglycan cross- linking preferences of Staphylococcus aureus penicillin-binding proteins have implications for treating MRSA infections. Journal of the American Chemical Society 139(29): 9791–9794. DOI:10.1021/jacs.7b04881
30. Tan JW, Israf DA, dan Tham CL. 2018. Major bioactive compounds in essential oils extracted from the rhizomes of Zingiber zerumbet (L) Smith: A mini-review on the anti- allergic and immunomodulatory properties. Front. Pharmacol 9(652): 1-8. https://doi.org/10.3389/fphar.2018.00652
31. Wu F, Zhou Y, Li L, Shen X, Chen G, Wang X, Liang X, Tan M, dan Huang Z. 2020. Computational Approaches in Preclinical Studies on Drug Discovery and Development. Front Chem 8(726): 1-32. DOI:10.3389/fchem.2020.00726
32. Yeni dan Tjahjono DH. 2017. Homology modeling epitop isocitrate dehydrogenase tipe 1 (R132H) 2 menggunakan modeller, I-TASSER dan (PS) untuk vaksin glioma. Farmasains 4(1): 21-32.
33. Zhang M, dan Wilkinson B. 2007. Drug Discovery beyond the rule of five. Current Opinion in Biotechnology 18(6): 478-488. https://doi.org/10.1016/j.copbio.2007.10.005
34. Zuegg J, Muldoon C, Adamson G, McKeveney D, Thanh GL, Premraj R, Becker B, Cheng M, Elliott AG, Huang XJ, Butler MS, Bajaj M, Seifert J, Singh L, Galley NF, Roper DI, Lloyd AJ, Dowson CG, Cheng TJ, Cheng WC, Demon D, Meyer E, Meutermans W, dan Cooper MA. 2015. Carbohydrate scaffolds as glycosyltransferase inhibitors with in vivo antibacterial activity. Nature Communications 6(7719): 1-11. DOI: 10.1038/ncomms8719

Figures



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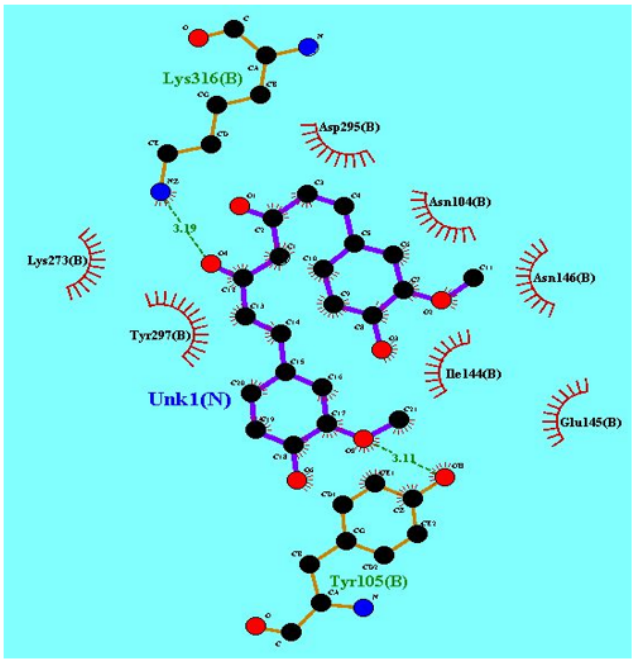
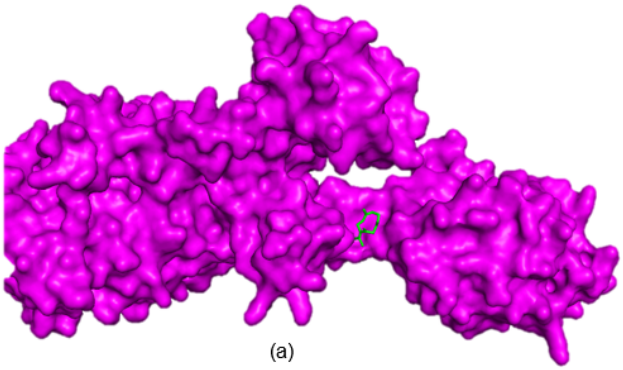


(b)

Figure 1

(a) 3D Visualization; and (b) 2D Visualization of PBP2a Binding Interaction with Curcumin



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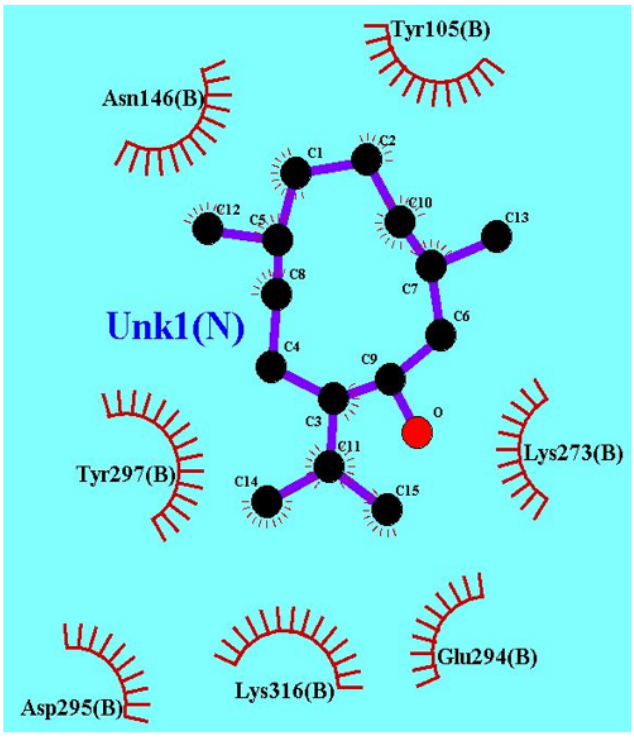
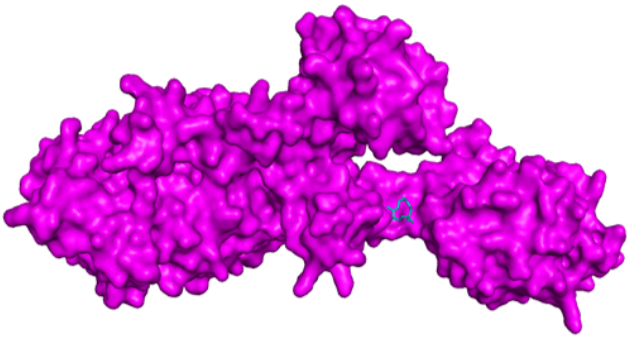


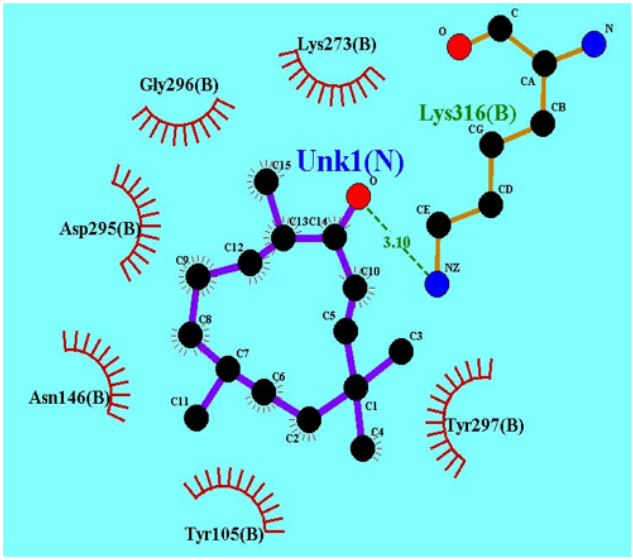
(b)

Figure 2

(a) 3D Visualization; and (b) 2D Visualization of PBP2a Binding Interaction with Germacrone



(a)



(b)

Figure 3

(a) 3D Visualization; and (b) 2D Visualization of PBP2a Binding Interaction with Zerumbone