



Exploring the Anti-Breast Cancer Potential of Chalcomoracin, Guangsangon E, and Morushalunin: A Computational Analysis of Compounds from *Morus sp.*

Rani Wardani Hakim^{1,2*}, Rizky Clarinta Putri^{1,6}, Wilzar Fachri^{1,2}, Fadilah Fadilah³,
Desak Gede Budi Krisnamurti^{1,2}, Rizki Fitriani⁴, Euis Holisotan Hakim⁴, Dewi Wulansari⁵

¹Department of Medical Pharmacy, Faculty of Medicine, Universitas Indonesia, DKI Jakarta, 10430, Indonesia

²Drug Discovery Research Cluster, IMERI, Faculty of Medicine, Universitas Indonesia, DKI Jakarta, 10430

³Department of Medical Chemistry, Faculty of Medicine Universitas Indonesia, DKI Jakarta, 10430

⁴Organic Chemistry Division, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung, Natural Product Research Group, Jalan Ganesha 10, Bandung, 40132, Indonesia

⁵Graduation School of Medicine, University of Tokyo, 1404-1 Katakuramatchi, Tokyo, Japan

⁶Master's Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, DKI Jakarta, 10430, Indonesia

Abstract. *Morus sp.* is a plant containing polyphenol compounds such as Chalcomoracin, Morushalunin, and Guangsangon E. These compounds play a crucial role in modifying proteins and signaling pathways that influence the progression of cancer cells, including breast cancer. Therefore, this study aimed to analyze the interaction between Chalcomoracin, Morushalunin, and Guangsangon E on PD-1 and PPAR- γ proteins as well as determine the physicochemical and pharmacological properties of these compounds. To achieve this, molecular docking was conducted on PD-1 (PDB ID: 57w9) and PPAR- γ (PDB ID: 5two) human proteins. The results showed that Chalcomoracin and Guangsangon E had binding capabilities to both PD-1 and PPAR- γ , while Morushalunin interacted exclusively with PD-1 protein. The $\Delta G_{\text{binding}}$ interaction between Guangsangon E and PPAR- γ was -12.29 (Kcal/mol), and for Chalcomoracin with PPAR- γ , it was -5.69 (Kcal/mol). Docking scores for Chalcomoracin, Morushalunin, and Guangsangon E on PD-1 were -6.21 kcal/mol, -8.91 kcal/mol, and -9.28/kcal/mol, respectively. Based on PASS analysis, Morushalunin had potential as an HIF1a-inhibitor, while Chalcomoracin demonstrated activity as an MMP-9 expression inhibitor. Guangsangon E showed activity on both proteins. Additionally, drug-likeness score (DLS) for Chalcomoracin, Morushalunin, and Guangsangon E were 1.14, 1.09, and 0.79, respectively. These concluded that the compounds could effectively interact with PD-1 and PPAR- γ , two important proteins in breast cancer.

Keywords: Breast cancer; *Morus sp.*; PD-1/PDL-1 ; PPAR- γ ; Triple negative breast cancer

1. Introduction

According to Globocan data from (2020), the incidence of new breast cancer cases in Indonesia reached 68.858 out of 396.914, accounting for 16.6% (Sung *et al.*, 2021; Giaquinto *et al.*, 2022). Peroxisome proliferator-activated receptor gamma (PPAR- γ), a component of the nuclear receptor superfamily, functions as a transcription factor and is implicated in cancers. Additionally, PD-ligand 1 or Programmed cell death protein 1 (PD-1)

*Corresponding author's email: rani.wardani1708@ui.ac.id, Tel.: +62 21 31901147; Fax: +62 21 31901147
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is a key target protein in immunotherapy and an important marker in breast cancer patients, especially the Triple Negative Breast Cancer (TNBC) subtype. (Łukasiewicz *et al.*, 2021; Schütz *et al.*, 2017). The use of herbal-based therapy is on the rise as a valuable and efficient method of treatment. This is because these herbs offer a vast array of phytochemical compounds, which serve as crucial elements in the development and exploration of novel drugs (Janakirama *et al.*, 2020). Among these compounds, polyphenols are known to have a role in modifying proteins and signaling pathways influencing the progression of cancer cells, including breast cancer. *Morus sp*, a plant species from the genus *Morus* and Family *Moraceae*, was confirmed to contain phenols, namely Morushalunin, Guangsangon E, and Chalcomoracin (See Figure 1). These compounds, classified as Type A: Dehydroprenyl-2-arylbenzofuran Dies-Alder type Adduct, are derived from the intermolecular [4+2]-cycloaddition of dienophiles such as chalcones and dehydroprenylphenol dienes to form a 6 membered ring (Fitriani, Happyana and Hakim, 2021; Yang *et al.*, 2014).

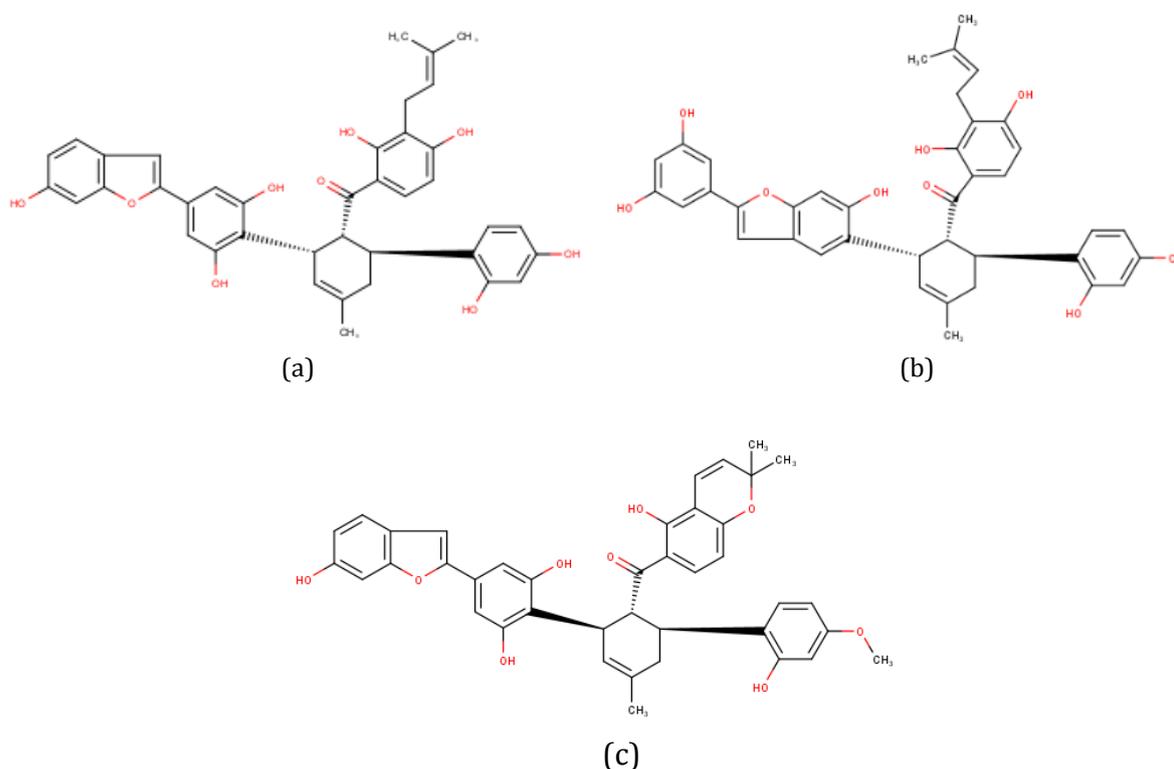


Figure 1 Chemical Structures of (a) Chalcomoracin, (b) Guangsangon E, (c) Morushalunin from *Morus sp*

A study showed that the inhibitory effects of the three compounds on leukemia P-388 cells, with Morushalunin, Guangsangon E, and Chalcomoracin having IC₅₀ values of 0.7 ppm, 2.5 ppm, and 1.7 ppm, respectively (Fitriani, Happyana and Hakim, 2021). Chalcomoracin demonstrated the ability to inhibit the MDA-MB-231 breast cancer cell line with an IC₅₀ of 6 μM. However, investigations on the anticancer potential of these compounds remain limited. Molecular docking, an essential tool in drug discovery, aids in predicting compound binding affinity with protein targets. Therefore, this study aimed to analyze the interaction of Chalcomoracin, Morushalunin, and Guangsangon E with PD-1 and PPAR-γ proteins, while also determining the physicochemical and pharmacological properties of these compounds. To achieve this, an in silico test was conducted to assess interactions, using Autodock software and for visualization, the Discovery Studio was adopted.

2. Methods

2.1. The Biological Activity Prediction Using PASS

The 3 isolated compounds from *Morus* sp, namely Chalcomoracin, Morushalunin, and Guangsangon E, obtained through tissue culture, were analyzed for their biological activity using the Prediction of Activity Spectra for Substances (PASS). The online platform, accessible at <http://www.pharmaexpert.ru/passonline>, was used for this purpose. Canonical SMILES of each compound were inputted on the website and a list of the biological activity was generated based on the existing database on PASS. The results included Pa (Probably active) and Pi (Probably inactive) values. Finally, when the Pa and Pi values are closer to 1 and 0, respectively, it signified better and good performance.

2.2. Prediction of Pharmacological Activity (ADME) of Compounds and Drug Likeness Score

ADME characteristics of the compounds were analyzed using SwissADME (<http://www.swissadme.ch/>). Canonical SMILES of each compound were incorporated into Swiss ADME. Swiss ADME, providing a predicted pharmacological profile. Additionally, <https://www.molinspiration.com/> and <https://molsoft.com/mprop/> were constituted to verify compliance with Lipinski's rules.

2.3. Molecular Docking

The proteins used were Human PD-1 (PDB ID: 57w9) and PPAR- γ (PDB ID: 5two), sourced from the RCSB Protein Data Bank (<https://www.rcsb.org/>). Removal of unnecessary water molecules, ligands, and chains was conducted. Autodock Vina 1.5.7 served as the docking software. Validation of grid box dimensions was performed, with the dimensions for PD-1 being $x=50$, $y=50$, and $z=50$, centered at $x=14.974$ Å, $y=30.713$ Å, $z=187.813$ Å, and for PPAR- γ $x=40$, $y=40$, and $z=40$, centered at $x=-23.939$ Å, $y=-20.434$ Å, $z=9.727$ Å. The grid box dimensions were selected based on the RMSD value (Sahlan *et al.*, 2020). After docking, visualization was performed using Discovery Studio.

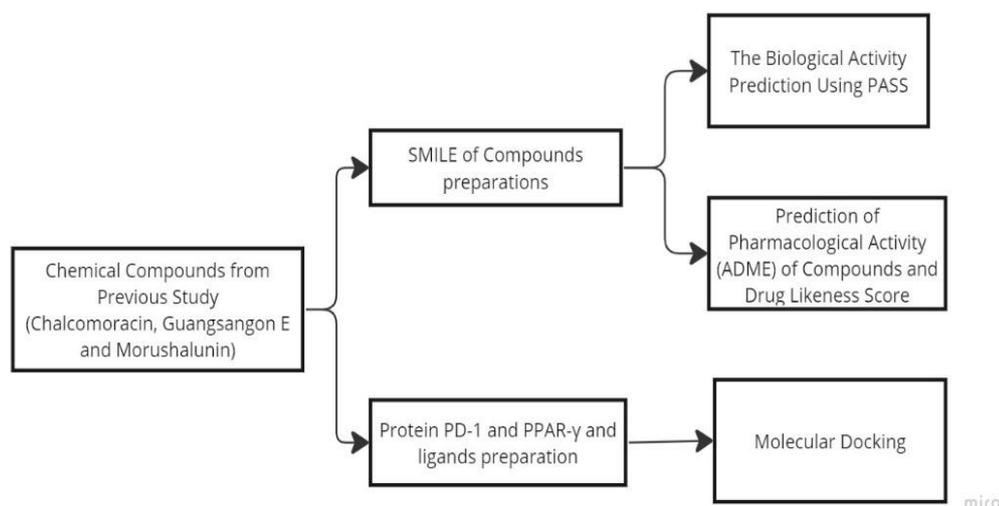


Figure 2 Research Frameworks of This Study

3. Results and Discussion

3.1. Prediction of the Biological Activity of Compounds

A computer program called PASS, accessible at (<http://www.pharmaexpert.ru/passonline/>) was used to predict bioactivity spectra based on chemical structures. This computational method facilitated potential *in vivo* bioactivity for chalcomoracin, guangsangon E, and morushalunin. Furthermore, this method produced

a comprehensive list of biological activities along with their Pa and Pi. From the PASS analysis, activities related to anticancer mechanisms were selected with a cut-off value of >0.6. The selected activities include free radical scavenger, HIF-1a inhibitor, apoptotic agonist, MMP9 Expression inhibitor, and chemopreventive. HIF-1a had a relationship with increased PD-L1 during hypoxia. Additionally, it can increase PD-1 protein expression (Guo *et al.*, 2022). Table 1 shows the result of the PASS analysis. Guangsangon E shows HIF-1a inhibitor effects, but its Pa value falls below Morushalunin. MMP-9, a crucial element in cancer metastasis, was influenced by chalcomoracin and guangsangon compounds, indicating their activity on this protein.

Table 1 Biological activity related to cancer prediction results analyzed using PASS.

Anticancer Activities	Chalcomoracin		Guangsangon E		Morushalunin	
	Pa	Pi	Pa	Pi	Pa	Pi
Free Radical Scavenger	0.620	0.005	0.631	0.005	-	-
HIF-1a inhibitor	-	-	0.623	0.029	0.876	0.007
Apoptosis Agonist	0.629	0.023	0.623	0.024	-	-
MMP-9 expression inhibitor	0.623	0.013	0.678	0.008	-	-
Chemopreventive	-	-	0.649	0.008	0.602	0.010

3.2. Prediction of ADME of Compounds and Drug Likeness Score

Bioavailability Radar of SwissADME showed 6 physicochemical properties, including lipophilicity, size, polarity, solubility, flexibility, and saturation. The pink area represented the optimal range for each property, which comprises lipophilicity: XLOGP3 between -0.7 and +5.0, size: MW between 150 and 500 g/mol, polarity: TPSA between 20 and 130 Å², solubility: log S not higher than 6, saturation: fraction of carbons in the sp³ hybridization not less than 0.25, and flexibility: no more than 9 rotatable bonds (Daina, Michielin and Zoete, 2017). Based on the SwissADME bioavailability radar, it was observed that the 3 compounds have poor bioavailability due to their physico-chemical properties. According to several studies, polyphenol indicated biological activity at low plasma concentrations. To enhance the bioavailability of phenolic compounds, various methods were adopted, such as modifying the formulation or engaging in chemical derivatization. Curcumin is an example of a beneficial polyphenol with poor bioavailability (Abourashed, 2013).

Drug-likeness scores (DLS) from the 3 compounds were assessed using the Molinspiration web server, as presented in Table 2. These scores compared the physicochemical properties of the compounds with those of existing drugs based on Lipinski's rules. DLS usually ranged from 0 to 1, where a score of 1 indicated a good candidate for drug development. Conversely, a score of 0 implies that the compound is less likely to be a drug (Sampat *et al.*, 2022). In the context of this study, a DLS score above 0 was observed. This information is valuable in predicting whether the compound can be synthesized or evaluated.

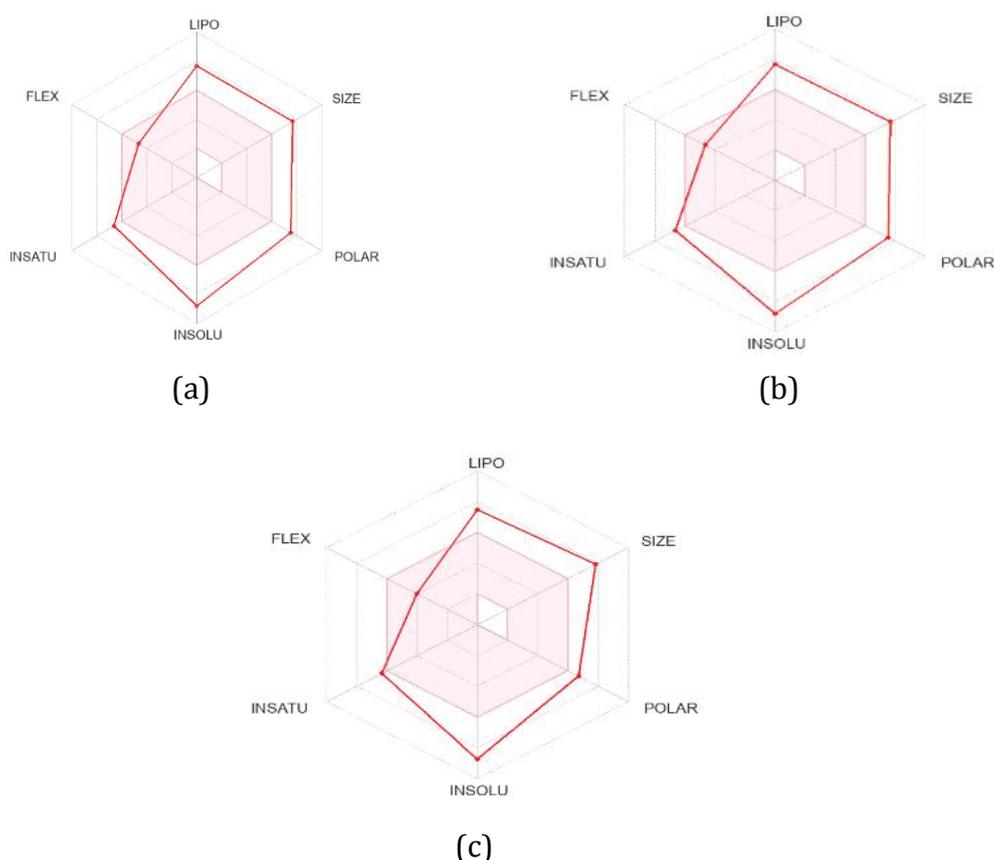


Figure 3 Bioavailability Radar of SwissADME analysis (a) Chalcomoracin, (b) Guangsangon E, (c) Morushalunin

Table 2 ADME of Compounds and Drug Likeness Score

No	Compounds Name	Software	MW	Log P	TPSA* (Å ²)	HBD	HBA	Rotatable Bond	DLS
1	Chalcomoracin	SwissADME	648.70	8.26	171.82	7	9	7	1.14
		Molsoft.com	648.24	8.86	138.28	-	-	-	
		Molinspiration cheminformatic	648.71	8.98	171.81	-	-	-	
2	Guangsangon E	SwissADME	648.70	8.26	171.82	7	9	7	1.09
		Molsoft.com	648.24	8.83	139.35	-	-	-	
		Molinspiration cheminformatic	648.71	8.79	171.81	-	-	-	
3	Morushalunin	SwissADME	660.71	8.43	149.82	5	9	6	0.79
		Molsoft.com	660.24	8.77	117.99	-	-	-	
		Molinspiration cheminformatic	660.72	8.97	149.82	-	-	-	

Notes: MW: Molecular Weight; TPSA: The Polar Surface Area; HBD: Hydrogen Bond Donor; HBA: Hydrogen Bond Acceptor; DLS: Drug Likeness Score

3.3. Molecular Docking

Molecular docking was conducted to explore the potential interactions between chemical compounds derived from *Morus* sp and the PD-1 and PPAR- γ proteins. Furthermore, it is a computational method aimed at identifying ligands that are geometrically and energetically suitable for a given receptor (Suhartanto *et al.*, 2017). Autodock Vina software was chosen for this analysis, as previous studies have showed its superior accuracy compared to other options such as PatchDoc (Sahlan *et al.* 2023). The

critical residues included in the PD-1/PD-L1 interaction were VAL64, ILE126, LEU128, ALA132, ILE134, ILE54, TYR56, MET115, ALA121, and TYR123. The result shows chalconoracin was bound to protein through 4 hydrogen bonds at amino acid residues such as ALA 121, ASP 122, SER 17, and TYR 123. Guangsangon showed hydrogen binding on ALA121, ILE54, and TYR123, while morushalunin interacted with ARG 125, ASP 122, and TYR 123. The 3 compounds had protein interaction through hydrogen bonds on several critical residues in the PD-1/PDL-1 interaction, as detailed in Table 3. They can bond with the TYR 123 amino acid residue. Morushalunin had the lowest $\Delta G_{\text{binding}}$ compared to chalconoracin and guangsangon E. Additionally, it had the highest inhibition constant (K_i) value.

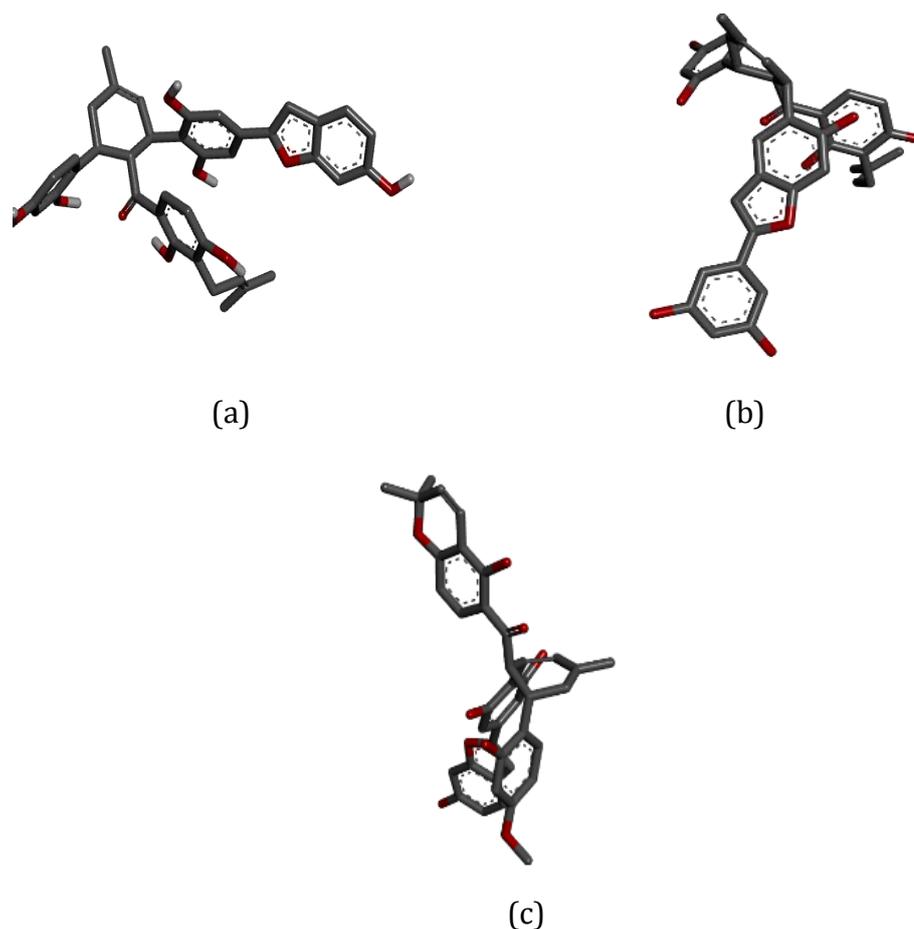


Figure 4 Compounds Three Dimension Structures (a) Chalcomoracin, (b) Guangsangon E, (c) Morushalunin

Tables 3 Molecular Docking Results of Chalcomoracin, Guangsangon E, and Morushalunin on PD-1/PDL1 Protein

Compounds Name	$\Delta G_{\text{binding}}$ (Kcal/Mol)	Inhibition Constanta (K_i)	Hydrogen Bond
Chalcomoracin	-6.22	27.41 μm	ALA 121, ASP 122, SER 17, TYR 123
Guangsangon E	-8.91	292.2 nm	ALA121, ILE54, TYR123
Morushalunin	-9.28	157.41 nm	ARG 125, ASP 122, TYR 123

Phenolic compounds such as chalconoracin, guangsangon E, and chalconoracin can form complexes with protein through covalent or non-covalent interaction, hydrogen, van der Waals, electrostatic, and hydrophobic bonding. The primary modes of interaction are

predominantly hydrophobic interaction and hydrogen binding (Shahidi and Dissanayaka, 2023).

Hydrophobic interaction between chalcomoracin and protein PD-1/PDL-1 occurs through pi-alkyl binding with amino acid residue MET115, ILE54, ALA121, and TYR56. Additionally, there was evidence of pi-cation interaction comprising LYS124 and ASP 122, which showed a high strength compared to hydrogen bonds. A P-alkyl binding pattern was identified between morushalunin and PD-1/PDL-1, interacting with residues MET115, ILE54, ALA121, and TYR56 similar to chalcomoracin. In the case of guangsangon, pi-alkyl interaction was specifically observed with residue ALA18.

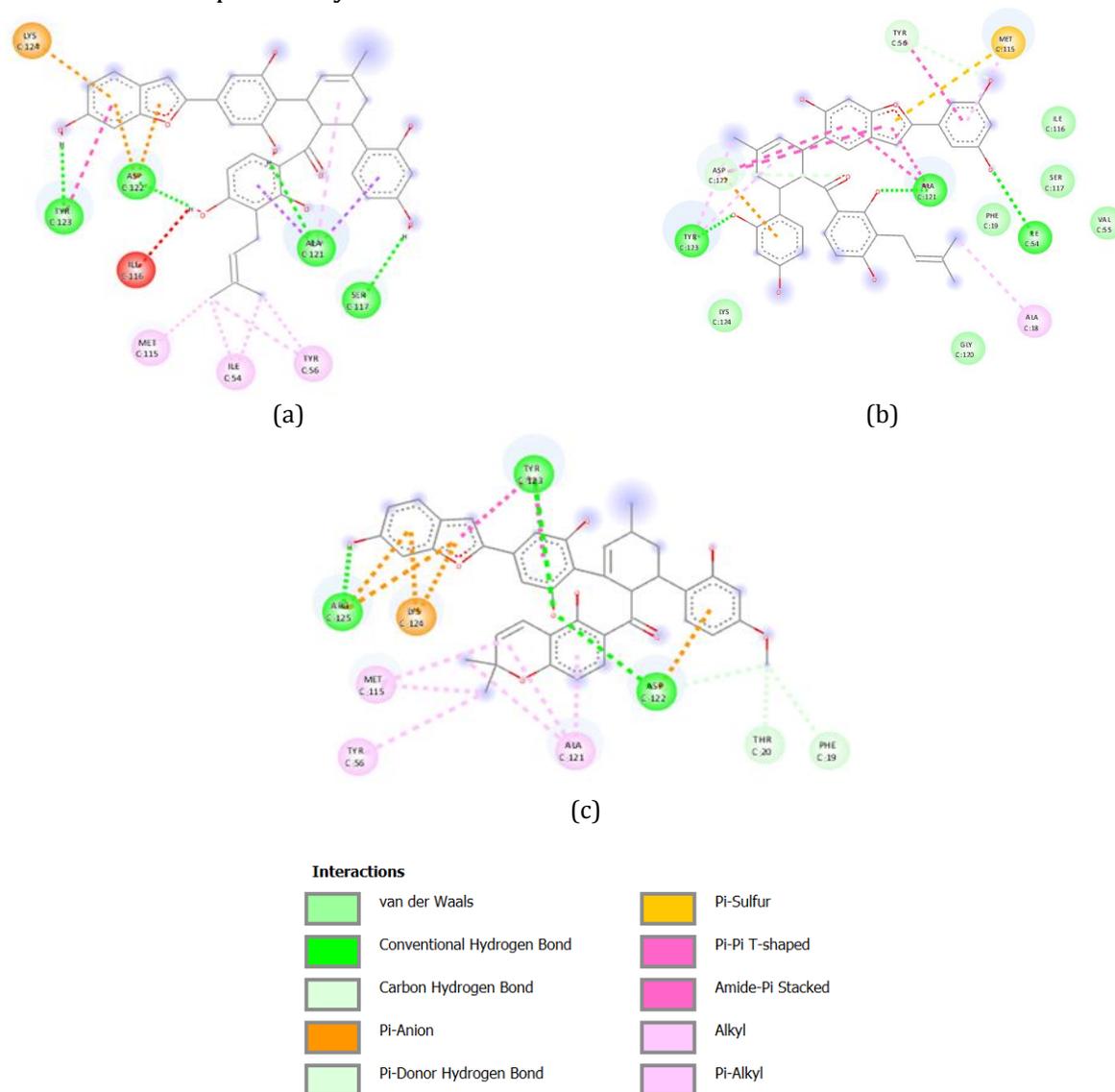


Figure 5 Two-Dimensional (2D) Visualizations Interaction with Protein PD-1 (a) Chalcomoracin, (b) Guangsangon E, (c) Morushalunin

Table 4 Molecular Docking Results of Chalcomoracin, and Guangsangon E on PPAR- γ

Compounds Name	$\Delta G_{\text{binding}}$ (Kcal/Mol)	Inhibition Constant	Hydrogen Bond
Chalcomoracin	-5.69	67.39 μm	ARG 280, CYS 285, SER299, TYR327,
Guangsangon E	-12.29	977.08 pm	GLU343, GLY284, HIS449

In the field of cancer study, PPAR- γ has been identified in various tumor types, including breast cancer. Its role after binding to a ligand has been associated with the initiation, progression, and spread of tumors. Furthermore, antagonists of PPAR- γ were considered effective drugs for breast cancer metastasis, particularly in the case of TNBC (Wang *et al.*, 2018). In the context of ligand binding affinity, Guangsangon E and Chalcomoracin had $\Delta G_{\text{binding}}$ values of -12.29 and -5.69 (Kcal/mol). However, Morushalunin data was excluded due to a positive $\Delta G_{\text{binding}}$ result. Guangsangon E demonstrated interaction with amino acid residue ARG 280, CYS 285, SER299, and TYR327, while Chalcomoracin bound to GLU343, GLY284, and HIS449. Therefore, it was concluded that Guangsangon E, with the lowest bonding $\Delta G_{\text{binding}}$ and K_i values, was the best ligand for PPAR- γ . This was the most spontaneous interaction and the most stable protein-ligand complexes compared to chalcomoracin (See Table 4).

This study showed that Guangsangon E, with its lower $\Delta G_{\text{binding}}$ and K_i values, were the superior ligand for PPAR- γ . This is supported by its more spontaneous interactions and the formation of more stable protein-ligand complexes, as evidenced in Table 4.

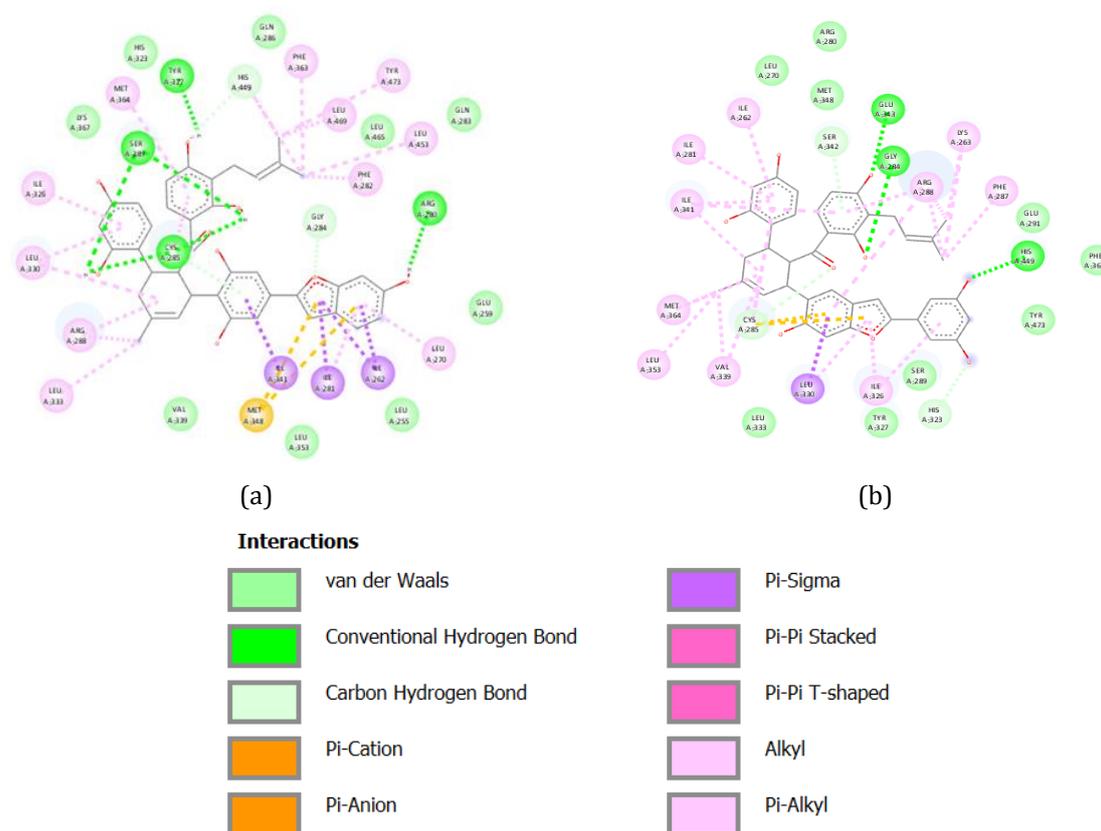


Figure 6 Two-Dimensional (2D) Visualizations Interaction with Protein PPAR- γ (a) Chalcomoracin, (b) Guangsangon E

4. Conclusions

In conclusion, the PASS analysis indicated that Morushalunin and Chalcomoracin had activity as HIF1a and MMP-9 expression inhibitors, respectively. Meanwhile, Guangsangon E showed activity on both proteins. DLS for Chalcomoracin, Morushalunin, and Guangsangon E were 1.14, 1.09, and 0.79 respectively. According to the SwissADME bioavailability radar, all three compounds demonstrated poor bioavailability due to their physicochemical properties. It is important to note that several polyphenols manifested

biological activity at low plasma concentrations. To address the issue of poor bioavailability, various strategies were adopted, such as modifying the formulation or chemical derivatization. In this study, the $\Delta G_{\text{binding}}$ interaction between Guangsangon E and PPAR- γ was -12.29 kcal/mol, while of Chalcomoracin with PPAR- γ was -5.69 kcal/mol. The docking scores for Chalcomoracin, Morushalunin, and Guangsangon E on PD-1 proteins were -6.21 kcal/mol, -8.91 kcal/mol, and -9.28 kcal/mol, respectively. Both Chalcomoracin and Guangsangon E are bound to PD-1 and PPAR- γ , suggesting potential significance in breast cancer pathogenesis. On the other hand, Morushalunin exclusively interacted with the PD-1 protein. These indicated the potential of the compound to relate with PD-1 and PPAR- γ , key proteins in breast cancer pathogenesis. Therefore, further study is needed to validate these predictions.

Acknowledgments

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