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Effectiveness In Silico Study of Eugenol Derivatives as Potential Breast Anticancer Agents Targeting Estrogen Receptor Alpha (ER-α)

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Abstract

Breast cancer is one of the leading causes of cancer-related mortality, with Estrogen Receptor alpha (ER-α) playing a critical role in ER-positive breast cancer. Resistance to endocrine therapies, such as tamoxifen, has encouraged the development of alternative agents targeting ER-α. Eugenol, a natural phenylpropanoid compound, has demonstrated anticancer activity, and structural modification of eugenol may enhance its binding affinity and therapeutic effectiveness. This study aimed to evaluate the interaction of quinoline-substituted eugenol derivatives with ER-α (PDB ID: 5W9D) using an in silico molecular docking approach. The docking results showed that all tested compounds exhibited better binding affinities than the native ligand. Among them, candidate compound (3) showed the lowest binding affinity value of −7.8 kcal/mol and hydrophobic interactions with key active-site residues such as Glu17, Ile20, Ile80, Trp87, and Arg88, indicating high complex stability. These results suggest that quinoline-based eugenol derivatives, particularly candidate compound (3), have potential as ER-α inhibitors and promising lead compounds for breast cancer therapy.

Abstrak

Kanker payudara merupakan salah satu penyebab utama kematian akibat kanker, dengan Estrogen Receptor alpha (ER-α) berperan penting pada kanker payudara ER-positif. Resistensi terhadap terapi endokrin seperti tamoksifen mendorong pengembangan agen alternatif yang menargetkan ER-α. Eugenol sebagai senyawa fenilpropanoid alami memiliki aktivitas antikanker, dan modifikasi strukturnya berpotensi meningkatkan afinitas ikatan serta efektivitas terapi. Penelitian ini bertujuan untuk mengevaluasi interaksi turunan eugenol tersubstitusi kuinolin terhadap ER-α (PDB ID: 5W9D) menggunakan pendekatan in silico melalui metode molecular docking. Hasil docking menunjukkan bahwa seluruh senyawa uji memiliki afinitas ikatan yang lebih baik dibandingkan ligan asli. Senyawa kandidat (3) menunjukkan nilai afinitas ikatan paling rendah sebesar −7,8 kcal/mol dan membentuk interaksi hidrofobik dengan residu-residu kunci pada sisi aktif seperti Glu17, Ile20, Ile80, Trp87, dan Arg88, yang mengindikasikan stabilitas kompleks yang tinggi. Hasil penelitian ini menunjukkan bahwa turunan eugenol berbasis kuinolin, khususnya senyawa kandidat (3), berpotensi dikembangkan sebagai inhibitor ER-α dan kandidat awal obat antikanker payudara.

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Introduction

Breast cancer remains a major global health concern due to its high incidence and mortality rates. According to the Global Cancer Observatory (GLOBOCAN 2022), breast cancer is the second most diagnosed cancer worldwide, with approximately 2.3 million new cases, and ranks fourth among the leading causes of cancer-related deaths, accounting for more than 666,000 deaths globally. The burden of breast cancer is particularly high in Asia, which contributes the largest proportion of new cases and mortality, followed by Europe and North America, indicating significant regional disparities in disease prevalence and outcomes. Breast cancer is a heterogeneous disease commonly classified based on the expression of key molecular receptors, including Estrogen Receptor alpha (ER-α), Estrogen Receptor beta (ERβ), Progesterone Receptor (PR), and Human Epidermal Growth Factor Receptor 2 (HER2) (Kunstič et al*.,* 2023). Approximately 75% of breast cancer patients exhibit positive estrogen receptor (ER) status, in which this receptor serves as a major driver of tumor growth and survival in (ER-α)-positive breast cancer (Lv et al., 2020).

ER antagonists such as tamoxifen are widely used as adjuvant endocrine therapy for patients with ER-positive breast cancer. However, a major limitation of this treatment is the development of acquired resistance, which occurs in approximately 40% of patients undergoing tamoxifen therapy (Mishra et al., 2021). As a selective estrogen receptor modulator (SERM), tamoxifen is initially effective in suppressing estrogen-mediated tumor growth, yet resistance remains a significant clinical challenge that can lead to reduced therapeutic efficacy and poor patient outcomes. The underlying mechanisms of tamoxifen resistance are complex and multifactorial, involving somatic genetic alterations, epigenetic modifications, and dynamic changes within the tumor microenvironment (Hanker et al., 2020).

The development of breast cancer therapies with specific targets and high selectivity is urgently required to enhance therapeutic efficacy while minimizing adverse effects. One promising strategy involves exploring bioactive natural products as alternative sources of anticancer agents. Eugenol ($C_{10}H_{12}O_2$) is a phenylpropanoid compound naturally found in clove oil (*Syzygium aromaticum*) (Bisergaeva et al., 2021) and has been widely reported to exhibit diverse biological activities, including antioxidant (Hernawan et al., 2012), anti-inflammatory (Filho et al., 2025), antifungal (Carrasco et al., 2012), antibacterial (Rahim et al., 2017), and anticancer effects (Suryasaputra et al., 2023). In breast cancer, eugenol exerts its anticancer activity through multiple mechanisms, such as inducing cell death and cell cycle arrest, as well as inhibiting cancer cell migration, metastasis, and angiogenesis in various breast cancer cell lines (Zari et al., 2021).

Molecular docking represents a key in silico approach for predicting ligand–receptor complexes and their binding interactions (Agu et al., 2023). This technique offers several advantages, including high computational speed, low cost, automation, and nearly unlimited scalability (Blanes-Mira et al., 2022). By estimating ligand binding poses and affinities within the active sites of target proteins, molecular docking enables the identification of compounds with favorable binding energies, making them promising candidates for further drug development (Nicholls et al., 2010; Precupas et al., 2017). In this study, the crystal structure of Estrogen Receptor alpha (ER-α), obtained from the Protein Data Bank (PDB ID: 5W9D), was employed as a biologically relevant target for molecular docking analysis to investigate ligand–receptor interactions (Mohtar et al., 2021).

Despite the growing interest in natural product–derived compounds for breast cancer therapy, *in silico* studies specifically investigating the interaction of eugenol derivatives with Estrogen Receptor alpha (ER-α) remain limited. Most existing studies focus on the general anticancer properties of eugenol, while its structure–activity relationship and binding behavior toward ER-α have not been thoroughly explored. This gap highlights the need for a systematic computational evaluation of eugenol derivatives as potential (ER-α)-targeted agents. Therefore, this study aims to evaluate the interaction of eugenol derivatives with Estrogen Receptor alpha using an *in silico* molecular docking approach to assess their potential as breast anticancer candidates.

Method

Equipment

Molecular docking was performed on an HP 14s-dq2622TU laptop (Intel® Core™ i3-1115G4 processor, 8 GB RAM, Windows 11). The software used was ChemOffice® 18.1 and HyperChem Professional for drawing candidate structures, GaussView 6.1 and Gaussian 09W for optimization candidates, UCSF Chimera 1.18 for protein and ligand preparation, AutoDock Tools 1.5.7 and AutoDock Vina for docking simulations, BIOVIA Discovery Studio 2024 for visualization of results, PyMOL and LigPlot+ 2.3 for analyzing and identifying interacting amino acid residues.

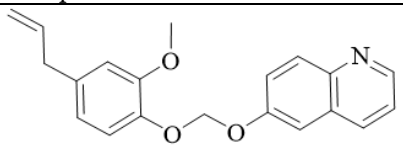
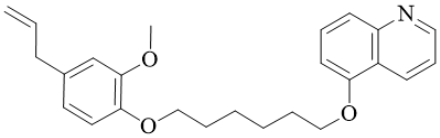
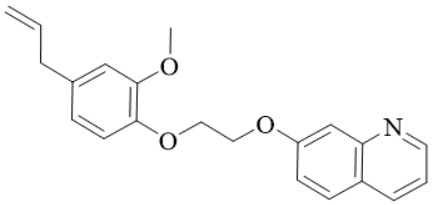
Materials

The techniques used for the identification and quantification of compounds (e.g., GC-MS, GC-FID, HPLC, NMR, UV-Vis, FTIR), as well as the main compounds identified and their percentages (Ghaffar *et al.*, 2015; Elaissi *et al.*, 2012; Čmiková *et al.*, 2023; Khedhri *et al.*, 2023). Differences in chemotypes were also noted (Hadj-Moussa *et al.*, 2024; Elaissi *et al.*, 2012).

Bioactivity Evaluation

The materials used were eugenol derivative compounds obtained from the study by Bendre et al. (2016). The data on eugenol derivatives are presented in Table 1. The primary target protein is Estrogen Receptor Alpha (ER-α) ligand-binding domain (LBD), obtained from the Protein Data Bank website (PDB ID: 5W9D, https://www.rcsb.org/structure/5W9D).

Table 1. The structures of eugenol derivative compounds (Bendre et al., 2016)

Candidate	IUPAC Name	Compounds Structure
1.	8-((4-allyl-2-methoxyphenoxy)methoxy)quinoline	
2.	8-((6-(4-allyl-2-methoxyphenoxy)hexyl)oxy)quinoline	
3.	8-(2-(4-allyl-2-methoxyphenoxy)ethoxy)quinoline	

Preparation of Protein

The initial stage of the molecular docking process involves the preparation of the receptor macromolecule and ligand structures. The ER-α macromolecule (PDB ID: 5W9D) was downloaded from the online Protein Data Bank in .pdb format (Mohtar et al., 2021). The receptor was required to meet several criteria, including a resolution of less than 2 Å, the absence of mutations, and determination by the X-ray diffraction method (Azzahra et al., 2024). The protein receptor was separated from unnecessary molecules, including the native ligand, water molecules, and metal ions. This separation was performed using UCSF Chimera. Subsequently, polar hydrogen atoms were added, and partial charges were assigned to the receptor. The prepared receptor was saved as a receptor.pdb file.

Preparation of ligand

The ligand downloaded from the PubChem database was initially converted from a two-dimensional (2D) structure to a three-dimensional (3D) conformation using MarvinSketch and subsequently saved in .pdb format. Further structural preparation was carried out by performing geometry optimization and adding hydrogen atoms using AutoDock Tools version 1.5.6 to ensure a stable and appropriate ligand conformation for docking analysis. The prepared ligand file was then converted and saved in .pdbqt format, making it suitable for use in the molecular docking process (Putri et al., 2024).

Grid Box Determination

The grid box was configured to encompass the active site region of the receptor. Grid box parameters included *center_x*, *center_y*, and *center_z* to define the box position on the protein macromolecule, as well as *size_x*, *size_y*, *size_z*, and *spacing* (angstrom) to determine the dimensions of the ligand docking space. The active site location was identified by determining the binding region on the receptor using Discovery Studio Visualizer, and the obtained x, y, and z coordinate values were subsequently input into AutoDock Tools for grid box setup. After completing the configuration, the configuration file was saved. Subsequently, the ligand file was converted to *.pdbqt* format, and the optimized receptor with added

hydrogen atoms was also converted to *.pdbqt* format using AutoDock Tools in preparation for the docking process (Prasetio et al., 2021).

Molecular Docking

Molecular docking between the receptor and ligand was carried out using AutoDock Vina. The initial step in this stage involved copying the receptor and ligand files in. pdbqt format into the Vina folder. The molecular configuration file and active site parameters were written using Notepad. The file names were freely assigned, provided that they clearly indicated the receptor and ligand used in the docking process. The file names specified in the configuration file had to match the corresponding files in the Vina folder. The predefined active site coordinates were included in the configuration file, which was saved as *conf.txt*. After completing the configuration setup, the docking process was executed via the Command Prompt using the command "vina --config conf.txt --log log.txt". Upon completion of the docking process, two new files were generated in the Vina folder, namely *log.txt* and *out.pdbqt* (Mohtar et al., 2021).

Visualization and Analysis of Interaction

Docking result analysis involved evaluation of the affinity values from multiple docking modes, as obtained from the *log.txt* file. A second analysis was conducted by examining the ligand positions of each docking mode using PyMOL software. The enzyme, ligand, and docking output files were loaded into PyMOL to visualize and compare the binding poses. The docking mode that most closely resembled the position of the native ligand was considered the best pose. Visualization of the interactions between the enzyme and the ligand was further performed using LigPlot, focusing on the amino acid residues involved in the interactions (Weni *et al.*, 2020).

Results and Discussion

Molecular docking is one of the in-silico methods widely applied in drug development based on Computer-Aided Drug Design (CADD). This technique aims to predict the most favorable orientation of a ligand when interacting with a target protein, thereby estimating binding affinity values and the interaction patterns formed (Raval & Ganatra, 2022). The primary target protein in this study was estrogen receptor alpha (ER-α) with PDB code 5W9D, as it plays a crucial role as a molecular binding site and the location of intermolecular interactions (Fitri et al., 2023). Estrogen receptor alpha (ER-α) is a member of the nuclear receptor family that functions as a transcription factor. Receptor activation occurs when the natural ligand, estrogen, binds to the ligand-binding domain (LBD), inducing ER-α dimerization and subsequent binding to DNA at estrogen response elements (EREs). This interaction activates the transcription of genes involved in cell proliferation and survival (Varriano et al., 2024). The crystal structure of estrogen receptor alpha (ER-α) was selected because it represents ER-α in complex with the antagonist 4-hydroxytamoxifen (4-OHT), making it relevant for evaluating the activity of tamoxifen derivatives against ER-α (Dermawan et al., 2019). Figure 1 shows the crystal structure of estrogen receptor alpha ligand binding domain C381S, C417S, C530S mutant in complex with endoxifen, determined at a resolution of 1.65 Å.



Figure 1. The crystal structure of ER-α (PDB ID: 5W9D)

Molecular docking was initiated with the preparation of ligands and receptors. The ligand represents the structure of the target compound, which is subsequently modified. Ligand preparation was carried out by adding or removing hydrogen atoms and selecting the relevant amino acid residues. The receptor represents the binding site that contains several active sites. Receptor preparation was performed by removing non-protein molecules, including water, metal ions, inhibitors, and native ligands, to allow the test ligands to bind to the active site of the protein (Nivatya et al., 2025). The addition of polar hydrogen

atoms was performed using Chimera software. The addition of polar hydrogen is necessary because protein structures obtained from databases generally lack hydrogen atoms, which are essential for forming hydrogen bonds with ligands. The ligands used were quinoline-substituted eugenol derivatives. The selection of these ligands was based on the role of eugenol as an active fragment and the function of quinoline as a versatile anticancer scaffold, as well as the suitability of this hybrid structure to occupy the aromatic ligand-binding pocket of ER-α (Bendre, et al., 2016). Structure ligands were optimized first by adding polar hydrogen atoms and assigning charges to obtain a minimum-energy conformation, ensuring that the ligands were in the most stable form and that their interactions with the receptor. The results of the ligand preparation are shown in Figure 2.

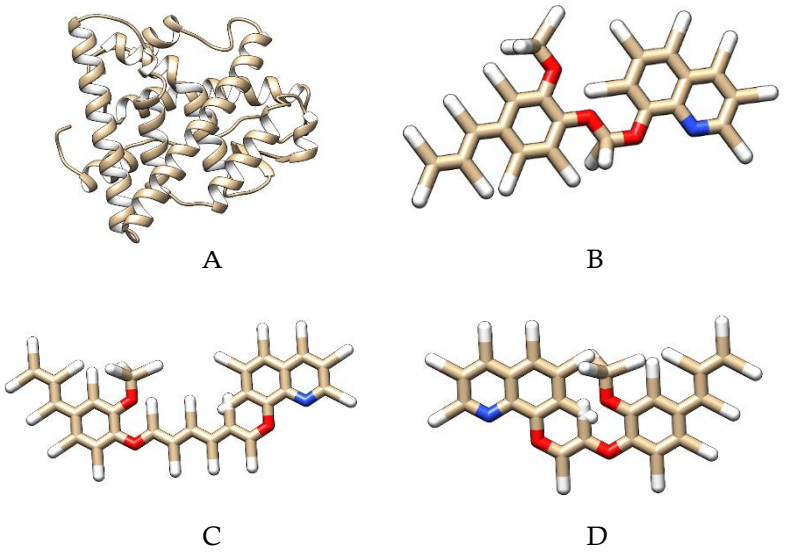


Figure 2. Visualization preparation of (A) receptor 5W9D, (B) ligand candidate (1), (C) ligand candidate (2), (D) ligand candidate (3)

The docking search space was defined by centering the grid box on the coordinates of the co-crystallized ligand within the experimentally resolved ER-α protein–ligand complex and expanding it to encompass the entire hormone-binding pocket, thereby ensuring inclusion of key interacting residues. This strategy follows the default grid box setup implemented in AutoDock Vina, in which the initial search space is constructed to enclose the bound ligand and subsequently adjusted to optimize docking simulations (Feinstein & Brylinski, 2015). In this study, the grid box center was defined at center_x = 17.226, center_y = 26.047, and center_z = −17.343, corresponding to the centroid of the native ligand binding position. Determination of these grid box center coordinates represents a crucial initial step in docking validation, as they define the spatial region in which the native ligand and candidate compounds adopt their binding conformations during interaction with the target protein (Hasan et al., 2022).

Subsequently, molecular docking results were evaluated based on binding affinity (ΔG) and Root Mean Square Deviation (RMSD) values. RMSD is a key parameter used to assess docking accuracy, where a docking protocol is considered valid when the RMSD value is ≤ 2 Å, indicating minimal deviation between predicted and reference binding poses (Muttaqin et al., 2019). Lower RMSD values reflect smaller conformational deviations and higher reliability of the docking results. Binding affinity represents the strength of ligand–receptor interactions, with lower (more negative) ΔG values indicating stronger and more stable binding, while higher ΔG values correspond to weaker interactions (Nuraini & Ruswanto, 2021). Negative ΔG values indicate the spontaneous formation of stable ligand–receptor complexes, and increasingly negative free energy values suggest stronger interactions that require greater energy to dissociate (Lestari, 2023).

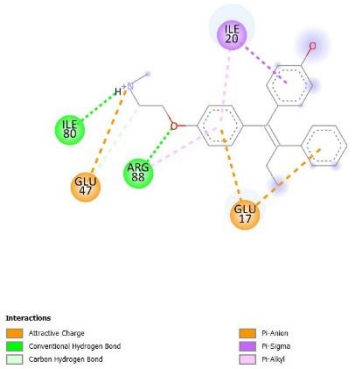
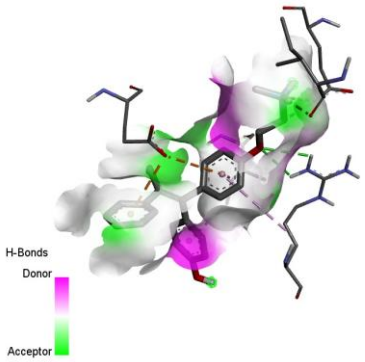
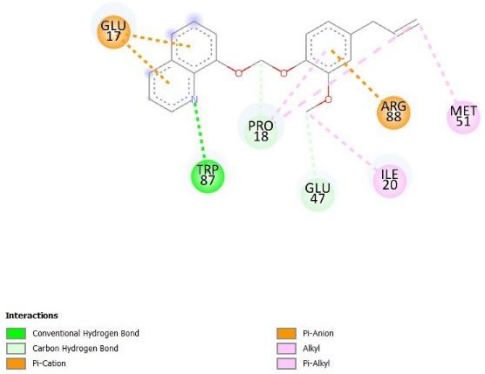
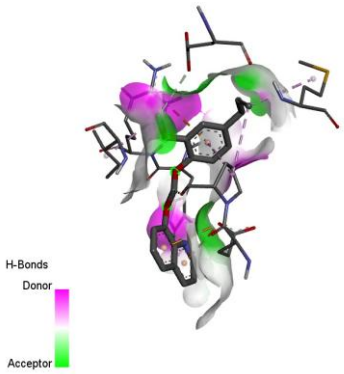
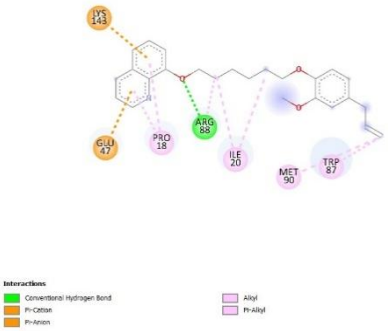
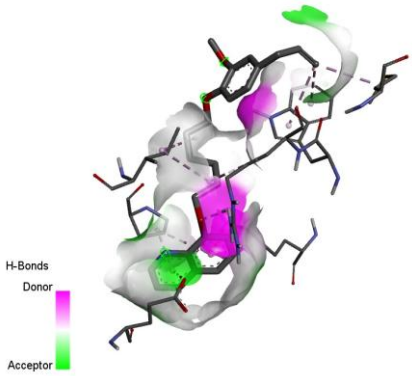
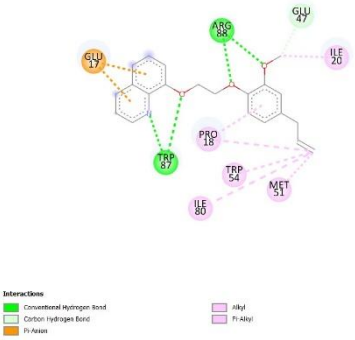
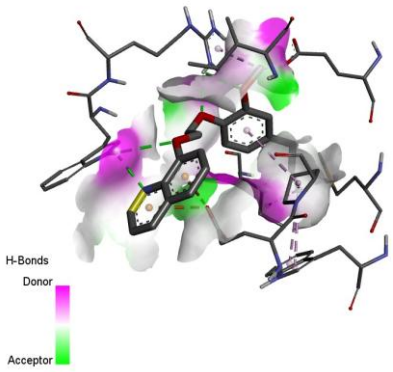
Table 2. Docking result summary

Ligand	Mode	Affinity (Kcal/mol)	Dist from best mode	
			rmsd I.b	rmsd u.b
Native ligand	1	-6.7	0.000	0.000
	2	-6.0	4.156	7.176
	3	-5.9	26.956	29.377
	4	-5.7	18.343	21.718
	5	-5.6	27.195	30.161
Candidate (1)	1	-7.4	0.000	0.000
	2	-6.5	8.808	11.933
	3	-6.4	20.691	23.426
	4	-6.4	12.464	16.915
	5	-6.3	1.826	2.027
Candidate (2)	1	-7.1	0.000	0.000
	2	-6.4	5.920	8.498
	3	-6.3	21.868	25.274
	4	-6.2	1.053	2.085
	5	-5.7	6.225	8.012
Candidate (3)	1	-7.8	0.000	0.000
	2	-6.5	1.902	2.606
	3	-6.4	8.863	12.047
	4	-6.4	1.934	2.309
	5	-6.3	2.273	3.235

The docking analysis results showed that candidate compound (3) exhibited the lowest binding affinity value of −7.8 kcal/mol toward the ER-α protein, indicating a strong and stable interaction. Binding energy serves as the primary parameter for comparing test ligands with the native ligand in order to evaluate the strength of their interactions. Candidate compound (3) can therefore be considered a promising compound for further investigation as a potential ER-α inhibitor, as it demonstrated a more favorable binding affinity than the native ligand, which showed an affinity value of −6.7 kcal/mol. The more negative the binding affinity, the stronger and more stable the protein–ligand complex formed, allowing the ligand to remain bound to the receptor for a longer duration and potentially resulting in higher biological activity.

Docking of the other test ligands yielded binding affinity values of −7.4 kcal/mol for candidate compound (1) and −7.1 kcal/mol for candidate compound (2), indicating stronger interactions than the native ligand and suggesting that both compounds are capable of effectively binding to the ER-α protein. The RMSD values obtained for all docked complexes were below 2 Å, confirming close agreement between the predicted binding poses and the reference conformation, thereby validating the reliability of the docking protocol. Nevertheless, further analysis of specific amino acid interactions is required to elucidate binding specificity and functional relevance.

Tabel 3. Visualitation 2D and 3D from docking simulation

Coumpund	**2D**	3D
Native ligand (9XY)		
Candidate 1		
Candidate 2		
Candidate 3		

Tabel 3. Docking Interaction Results of Ligands with ER-α (PDB ID: 5W9D)

Ligand	Affinity (Kcal/mol)	Binding Interaction (Amino acid residue)	Hydrogen Bond Length (Å)
Native Ligand	-6.7	Glu17	4.39215
		Glu47	4.09272
		Ile20	4.43956
		Ile80	2.21874
		Arg88	2.64942
Candidate 1	-7.4	Glu17	3.71272
		Pro18	3.76080
		Ile20	4.00021
		Glu47	3.35794
		Met51	4.10898
		Trp87	2.46357
		Arg88	4.00186
Candidate 2	-7.1	Pro18	4.49917
		Ile20	4.85189
		Glu47	3.65618
		Trp87	4.21764
		Arg88	2.57944
		Met90	3.91782
		Lys143	4.15938
Candidate 3	-7.8	Glu17	3.58973
		Pro18	3.64339
		Glu47	3.16866
		Met51	4.08641
		Trp54	4.31872
		Ile20	4.16463
		Ile80	5.31618
		Trp87	3.05573
		Arg88	2.73122

The redocking analysis revealed that the native ligand forms hydrogen bonds with five key residues within the ER-α ligand-binding domain, namely Glu17, Glu47, Ile20, Ile80, and Arg88, with hydrogen bond distances ranging from 2.22 to 4.44 Å. Hydrogen bonds with donor–acceptor distances of approximately ≤3.0 Å are considered strong interactions and play an important role in stabilizing ligand–receptor complexes through directional electrostatic forces (De Freitas & Schapira, 2017). The shortest hydrogen bond was observed with Glu17 (2.22 Å), indicating a strong stabilizing interaction that anchors the ligand within the hormone-binding pocket. The involvement of residues such as Glu17 and Arg88 supports that the native ligand occupies the biologically relevant binding site, thereby validating the reliability of the docking protocol.

Candidate (1) formed seven hydrogen bonds with ER-α, involving residues Glu17, Pro18, Ile20, Glu47, Met51, Trp87, and Arg88. The shortest hydrogen bond was detected with Arg88 (2.45 Å), suggesting a strong stabilizing interaction that contributes to ligand anchoring within the binding pocket. The presence of hydrogen bonds with residues shared with the native ligand, particularly Glu17 and Arg88, indicates that Candidate (1) interacts within the same hormone-binding pocket. The higher number of hydrogen bonds is consistent with its favorable binding affinity and supports a stable docking conformation.

Candidate (2) established hydrogen bonds with residues Pro18, Ile20, Glu47, Trp87, Arg88, Met90, and Lys143. The shortest hydrogen bond was observed with Glu47 (2.58 Å), reflecting a moderately strong stabilizing interaction. Compared to Candidate (1), Candidate (2) exhibited a different interaction pattern, involving additional residues such as Met90 and Lys143 that are located closer to the peripheral region of the binding cavity. This interaction distribution suggests that although Candidate (2) is capable of binding to ER-α, its ligand–receptor interactions may be less centralized within the hormone-binding pocket.

Notably, Candidate (3) exhibited the most extensive interaction profile, forming hydrogen bonds with residues Glu17, Pro18, Glu47, Met51, Trp54, Ile20, Ile80, Trp87, and Arg88. The shortest hydrogen bond was formed with Arg88 (2.73 Å), indicating strong ligand anchoring within the binding pocket. The

high number of hydrogen bonds, together with interactions involving conserved residues such as Glu17, Ile20, Ile80, and Arg88, explains the strongest binding affinity observed for this compound. These interactions suggest enhanced complex stability and a well-defined binding mode within the ER-α ligand-binding domain.

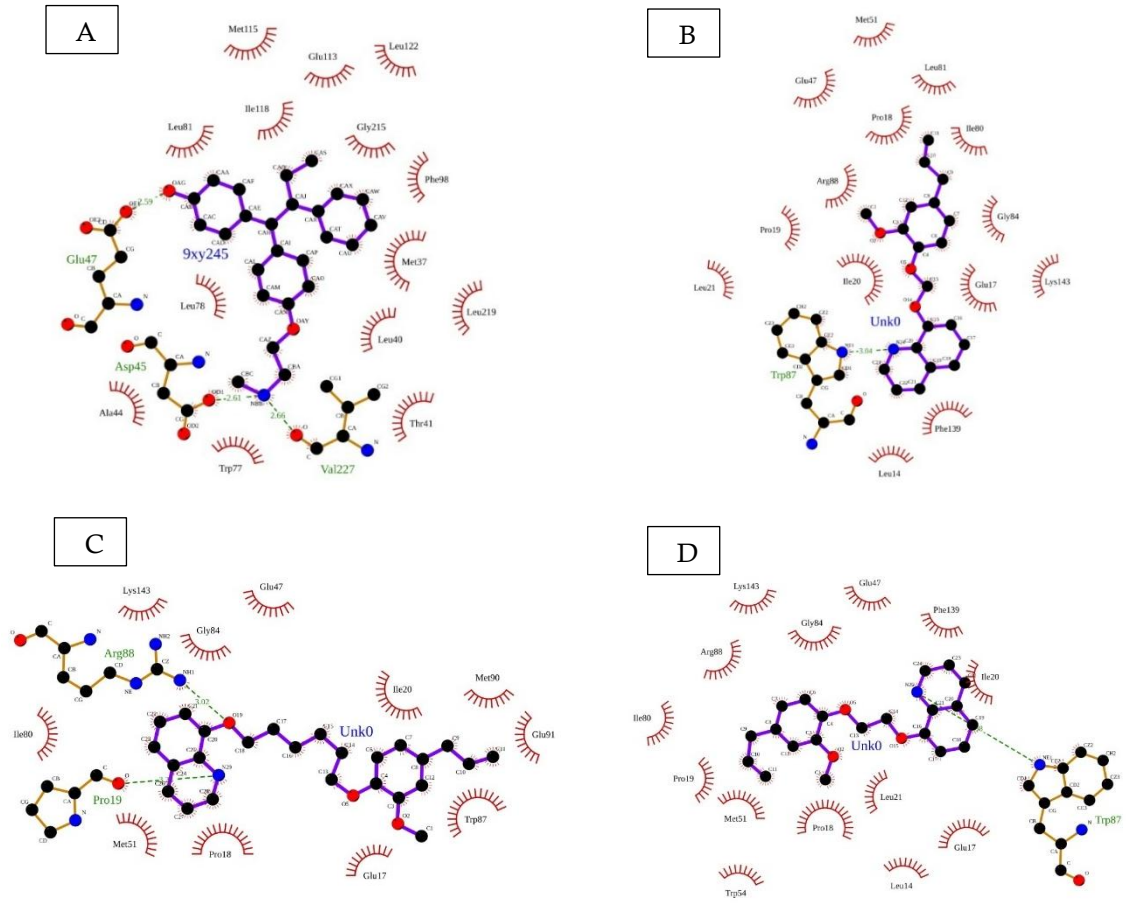


Figure 3. Visualization Using LigPlot of (A) Native ligand, (B) candidate (1), (C) candidate (2), (D) candidate (3)

Protein–ligand interactions were analyzed using LigPlot, a software tool designed to generate two-dimensional (2D) representations of the types of interactions formed between receptor protein residues and ligand atoms. The program displays hydrogen bonds as green dashed lines accompanied by bond length information in angstroms, while interacting protein residues are illustrated as arcs radiating toward the contacting ligand atoms. The analysis is not limited to hydrogen bonds but also encompasses all hydrophobic interactions occurring between the two molecules (Ongaba et al., 2022). These interactions contribute to the stability of the ligand–receptor complex and may influence the Gibbs free energy (ΔG) or binding affinity. In addition, hydrophobic interactions are also important in maintaining ligand stability at the target site and affect binding affinity and drug efficacy. An increased number of hydrophobic atoms on the surface of the active site is known to enhance the biological activity of a drug compound (Patil et al., 2010).

As shown in Figure 3, each ligand exhibits a distinct interaction pattern with the receptor, characterized by varying numbers of hydrogen bonds and hydrophobic contacts, indicating differences in binding stability. The native ligand formed several hydrogen bonds with residues Glu47, Asp45, and Val227, with bond distances ranging from 2.59 to 2.66 Å. These distances fall within the category of strong hydrogen bonds and significantly contribute to the stability of the ligand–receptor complex. In addition, the native ligand also established hydrophobic interactions with residues surrounding the active site, further supporting

its binding affinity to the receptor. Candidate compound (1) formed three hydrogen bonds with the Trp87 residue, accompanied by four hydrophobic interactions with residues within the active pocket. The involvement of aromatic residues such as Trp87 highlights the important role of π interactions and hydrogen bonding in stabilizing the ligand–receptor complex.

Candidate compound (2) formed hydrogen bonds with Arg88 and Pro19 residues. Although the number of hydrogen bonds was relatively lower, candidate (2) exhibited the highest number of hydrophobic interactions, totaling eight interactions. This indicates that the stability of the candidate (2) complex is predominantly governed by hydrophobic interactions rather than hydrogen bonding. The visualization results further revealed that candidate compound (3) formed five hydrogen bonds with the Trp87 residue and seven hydrophobic interactions. The combination of a high number of hydrogen bonds and extensive hydrophobic interactions suggests that candidate (3) possesses the most stable and optimal interaction pattern within the receptor's active site.

Overall, the LigPlot+ visualization results indicate that the stability of ligand–receptor complexes is determined not only by the number of hydrogen bonds but also by the contribution of hydrophobic interactions and the orientation of the ligand within the receptor's active pocket

Conclusion

This in silico study demonstrates that quinoline-substituted eugenol derivatives exhibit promising potential as estrogen receptor alpha (ER-α) inhibitors for breast cancer therapy. Molecular docking analysis revealed that all tested compounds showed stronger binding affinities than the native ligand, with binding energies ranging from −7.1 to −7.8 kcal/mol, compared to −6.7 kcal/mol for the native ligand. Among them, candidate compound (3) displayed the most favorable interaction with ER-α, evidenced by the lowest binding affinity (−7.8 kcal/mol) and extensive hydrogen bonding and hydrophobic interactions with key active-site residues such as Glu17, Ile20, Ile80, Trp87, and Arg88. These interactions indicate a stable and well-defined binding mode within the ligand-binding domain of ER-α. Overall, the results suggest that quinoline-substituted eugenol derivatives, particularly candidate (3), have strong potential to be developed further as ER-α-targeted breast anticancer agents, although experimental validation through in vitro and in vivo studies is still required.

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References

Abbaci, H., Nabti, E. H., Al-Bekairi, A. M., Hagras, S. A. A., Salem-Bekhit, M. M., Adjaoud, A., ... & Houali, K. (2023). Comparative Bioactivity Evaluation of Chemically Characterized Essential Oils Obtained from Different Aerial Parts of *Eucalyptus gunnii* Hook. f. (Myrtaceae). *Molecules*, *28*(6), 2638.

Agu, P. C., Afiukwa, C. A., Orji, O. U., Ezeh, E. M., Ofoke, I. H., Ogbu, C. O., Ugwaja & Aja, P. M. 2023. Molecular Docking as a Tool for the Discovery of Molecular Targets of Nutraceuticals in Diseases Management. *Scientific Reports, 13*(1):13398.

Azzahra, A. Y., Hidayat, T. S., & Saepudin, S. 2024. Penambatan Molekul Senyawa Aktif Sirih (Piper Betle L) terhadap Reseptor HSV-1 sebagai Kandidat AntiHerpes. *Pharmaceutical Science and Clinical Pharmacy, 2*(1):20-25.

Bendre, R. S., Rajput, J. D., Bagul, S. D., & Karandikar, P. S. 2016. Outlooks on medicinal properties of eugenol and its synthetic derivatives. *Nat Prod Chem Res*, *4*(3):1-6.

Bisergaeva, R. A., Takaeva, M. A., & Sirieva, Y. N. 2021. Extraction of Eugenol, A Natural Product, and The Preparation of Eugenol Benzoate. In Journal of Physics: *Conference Series*, 1889(2):1-6.

Blanes-Mira, C., Fernández-Aguado, P., de Andrés-López, J., Fernández-Carvajal, A., Ferrer-Montiel, A., & Fernández-Ballester, G. 2022. Comprehensive Survey of Consensus Docking for High-Throughput Virtual Screening. *Molecules*, 28(1):175.

Carrasco, H., Raimondi, M., Svetaz, L., Liberto, M. D., Rodriguez, M. V., Espinoza, L., & Zacchino, S. 2012. Antifungal Activity of Eugenol Analogues, Influence of Different Substituents and Studies on Mechanism of Action. *Molecules*, 17(1):1002-1024.

Chen, D., Oezguen, N., Urvil, P., Ferguson, C., Dann, S. M., & Savidge, T. C. 2016. Regulation of Protein-Ligand Binding Affinity by Hydrogen Bond Pairing. *Science advances*, 2(3):1-16.

De Freitas, R. F., & Schapira, M. 2017. A Systematic Analysis of Atomic Protein–Ligand Interactions in the PDB. *Medchemcomm*, *8*(10):1970-1981.

Dermawan, D., Sumirtanurdin, R., & Dewantisari, D. 2019. Molecular Dynamics Simulation Estrogen Receptor Alpha againts Andrographolide as Anti Breast Cancer. *Indonesian Journal of Pharmaceutical Science and Technology*, *6*(2):65-76.

Feinstein, W. P., & Brylinski, M. 2015. Calculating an Optimal Box Size for Ligand Docking and Virtual Screening Against Experimental and Predicted Binding Pockets. *Journal of cheminformatics*, *7*(1):18.

Filho, J. L. P., Pereira, R. B., Vieira, T. F., Sousa, S. F., Coelho, J. R., Pinto, N. F., & Pereira, D. M. 2025. Development of Eugenol Derivatives with 5-LOX Inhibitory Activity. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *40*(1):1-20.

Fitri, D. A., Hermanto, S., & Azizah, Y. N. 2023. Studi Penambatan Molekul Peptida Bioaktif Kacang Kedelai (Glycine max) Hasil Hidrolisis In Silico Terhadap Reseptor hER-α (3ERT), *Kimia Padjadjaran*, 1(2):122-130.

Hanker, A. B., Sudhan, D. R., & Arteaga, C. L. 2020. Overcoming Endocrine Resistance in Breast Cancer. Cancer Cell, 37(4):496-513.

Hasan, R., I'anah, F. C., & Bahi, R. R. R. 2022. Docking molekuler senyawa potensial daun kelor (Moringa oleifera) terhadap reseptor folat. *Journal of Innovation Research and Knowledge*, *2*(2):519-526.

Hernawan, H., Purwono, B., & Wahyuningsih, T. D. 2012. Synthesis of 2-methoxy-4,6-di(prop-1-enyl)phenol from Eugenol and its Activity as an Antioxidant. *Indonesian Journal of Chemistry*, 12(2):178-183.

Kai, Q. X. A., Rumengan, I. F., Lintang, R. A., Wullur, S., Sumilat, D. A., Pangkey, H., & Luntungan, H. A. 2021. Penambatan Molekul Glutation Fauna Laut Terhadap Reseptor dari Beberapa Penyakit Virus. *Jurnal Pesisir dan Laut Tropis*, 9(2), 53–58.

Kunstič, T. T., Debeljak, N., & Tacer, K. F. 2023. Heterogeneity in hormone-dependent breast cancer and therapy: Steroid hormones, HER2, melanoma antigens, and cannabinoid receptors. *Advances in Cancer Biology-Metastasis*, 7(1):100086.

Lestari, A. P. 2023. *Studi In Silico Senyawa Yang Terkandung Dalam Buah Mahkota Dewa (Phaleria macrocarpa) Sebagai Antikanker Payudara* (Doctoral dissertation, UNIVERSITAS BAKTI TUNAS HUSADA).

Lv, M., Mao, Y., Song, Y., Wang, Y., Liu, X., Wang, X., Nie, G., & Wang, H. 2020. Clinical features and survival of single hormone receptor–positive breast cancer: a population-based study of 531,605 patients. *Clinical breast cancer*, 20(5):589-599.

Mishra, A., Srivastava, A., Pateriya, A., Tomar, M. S., Mishra, A. K., & Shrivastava, A. 2021. Metabolic reprograming confers tamoxifen resistance in breast cancer. *Chemico-biological interactions*, 347(1):109602.

Mohtar, K., Fatimawali, F., Rumondor, E. M., Datu, O. S., & Tallei, T. E. 2021. Studi In Silico Senyawa Eugenol Cengkeh (Syzygium Aromaticum L.) terhadap Reseptor Er-Î±, Er-Î² Dan Her-2 Pada Kanker Payudara. *Pharmacon*, 10(3):1001-1008.

Muttaqin, F. Z., Ismail, H., & Muhammad, H. N. 2019. Studi molecular docking, molecular dynamic, dan prediksi toksisitas senyawa turunan alkaloid naftiridin sebagai inhibitor protein kasein kinase 2-A pada kanker leukemia. *Pharmacoscript*, *2*(1):49-64.

Nicholls, A., McGaughey, G.B., Sheridan, R.P., Good, A.C., Warren, G., Mathieu, M., Muchmore, S.W., Brown, S.P., Grant, J.A., Haigh, J.A., Nevins, N. 2010. Molecular shape and medicinal chemistry: a perspective. *J Med Chem*, 53(10):3862–3886.

Nivatya, H.K., Singh, A., Kumar, N., Sonam, Sharma, L., Singh, V., Mishra, R., Gaur, N. and Mishra, A.K. 2025. Assessing molecular docking tools: understanding drug discovery and design. *Future Journal of Pharmaceutical Sciences*, *11*(1):111.

Nuraini, M. 2021. Studi In Silico Senyawa Galangin Lengkuas (Alpinia galanga) Sebagai Antikanker Terhadap Kanker Payudara. In *Prosiding Seminar Nasional dan Penelitian Kesehatan 2018*.

Ongaba, T., Ndekezi, C., & Nakiddu, N. 2022. A molecular docking study of human STEAP2 for the discovery of new potential anti-prostate cancer chemotherapeutic candidates. *Frontiers in Bioinformatics*, 2(1):1-15.

Patil, R., Das, S., Stanley, A., Yadav, L., Sudhakar, A., & Varma, A. K. 2010. Optimized hydrophobic interactions and hydrogen bonding at the target-ligand interface leads the pathways of drug-designing. *PloS one*, *5*(8):1-10.

Prasetio, N. F., Kepel, B. J., Bodhi, W., Manampiring, A., & Budiarso, F. 2021. Molecular Docking terhadap Senyawa Isoeleutherin dan Isoeleutherol sebagai Penghambat Pertumbuhan SARS-CoV-2. *eBiomedik*, 9(1):101-106.

Precupas, A., Sandu, R., Leonties, A.R., Anghel, D.F., Popa, V.T. 2017. Complex interaction of caffeic acid with bovine serum albumin: calorimetric, spectroscopic and molecular docking evidence. *New J Chem*, 41(24):15003–15015.

Puspita, P. J., Liliyani, N. P. P., & Ambarsari, L. 2022. In silico analysis of active compounds of avocado fruit (Persea americana Mill.) as tyrosinase enzyme inhibitors. *Current Biochemistry*, 9(2):73-87.

Putri, T. Z. A. D., Findrayani, R. P., Isrul, M., & Lolok, N. 2024. Studi Molecular Docking Senyawa Kimia Dari Herba Putri Malu (Mimosa pudica) Terhadap Inhibisi Enzim A-Glukosidase Sebagai Antidiabetes Melitus. *Jurnal Pharmacia Mandala Waluya*, 3(4):225-233.

Rahim, N. H. C. A., Asari, A., Ismail, N., & Osman, H. 2017. Synthesis and Antibacterial Study of Eugenol Derivatives. *Asian Journal of Chemistry*, 29(1):1-20.

Raval, K., & Ganatra, T. (2022). Basics, Types and Applications of Molecular Docking: A Review. *IP International Journal of Comprehensive and Advanced Pharmacology*, *7*(1):12-16.

Suryasaputra, D., Mardatillah, A., & Nurfadilah, P. 2023. Iodination of Isoeugenol Compounds and Its Potential for Therapy of HER-2 Breast Cancer by Molecular Docking. *ICB-Pharma*, 3(1):227–239.

Varriano, S., Yu, A., Xu, Y. Q., Natelson, D. M., Ramadei, A., & Kleiman, F. E. 2024. Estrogen Receptor Alpha (ER-α) Regulates PARN-Mediated Nuclear Deadenylation and Gene Expression in Breast Cancer Cells, *RNA Biology*, *21*(1):1090-1099, DOI:

Weni, M., Safithri, M., & Seno, D. S. H. 2020. Molecular Docking of Active Compounds Piper crocatum on the a-glucosidase Enzyme as Antidiabetic. *Indonesian Journal of Pharmaceutical Science and Technology*, 7(2):64-72.

Zari, A. T., Zari, T. A., & Hakeem, K. R. 2021. Anticancer Properties of Eugenol: *A Review, Molecules*, 26(23):1-17.