Short Communication:

Molecular Docking Study of Eugenol and Its Derivatives as Potential Anti-Ischemia Agents for Angiotensin Converting Enzyme (ACE) Inhibition

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Abstract: *Mortality due to ischemic stroke has increased significantly, especially during the COVID-19 pandemic. Preventive measures are urgently needed to reduce the severity of ischemic stroke, which is mainly caused by blood vessel blockage due to increased secretion of angiotensin II (ANG II) by angiotensin-converting enzyme (ACE). This study investigated the potential of eugenol and its derivatives as ACE inhibitors using molecular docking, an* in silico *approach for drug discovery by using PLANTS software. The results showed that several eugenol derivatives, including (E)-1-(2-(4-allylphenoxy)acetyl)-4-cinnamoylthiosemicarbazide, exhibited potent ACE inhibition, with docking scores comparable to the native ligand (lisinopril) and superior to several commercial drugs. Physicochemical evaluation revealed that derivatives such as 5a, 5b, 7, and 9a had favorable molecular weight, total polar surface area, and lipophilicity (log P), thereby enhancing their permeability and bioavailability. Drug-likeness analysis confirmed that the compound meets several criteria, including Lipinski, Pfizer, and Golden Triangle rules, highlighting its potential safety and efficacy. Key binding interactions, including hydrogen bonds, hydrophobic interactions, and electrostatic interactions in the ACE active site, further support its candidacy as an ACE inhibitor. These findings suggest that eugenol derivatives are promising candidates for the development of therapies targeting ischemic stroke through ACE inhibition.*

Keywords: *ACE inhibitor; antioxidant; eugenol; ischemia; molecular docking*

■ INTRODUCTION

An increased risk of ischemia stroke has been reported in the Coronavirus disease 2019 (COVID-19) pandemic [1]. One of the cases reported by Qureshi [2] showed that the mortality rate in ischemic stroke patients who contracted COVID-19 could increase by 100%. Ischemia is a blockage of blood vessels that results in disruption of blood flow to parts of the body [3]. Reduced flow direction can interfere with oxygen demand in the myocardium, which causes weakening of the heart and can lead to death [4].

Blockages in blood vessels are caused by the secretion of the peptide angiotensin II (ANG II) from the Renin Angiotensin System (RAS) in the kidneys [5]. Peptide ANG II is the result of the conversion of peptide angiotensin I (ANG I) by Angiotensin Converting Enzyme (ACE) [6]. Peng et al. [7] showed that the formation of ANG II peptides can be stopped using ACE inhibitors. Evaluation of ACE inhibitors against antioxidant compounds has been reported [8-9]. The result is that compounds with high antioxidant activity have better potential as ACE inhibitors.

Eugenol is a natural compound known for its strong antioxidant activity [10-11], mainly due to its phenolic structure, allowing it to donate electrons and stabilize free radicals through resonance [12]. This

compound is widely found in extracts of medicinal plants such as cloves, cinnamon, basil, and nutmeg plants that are widely cultivated in Indonesia [13]. Previous studies, including those conducted by Dhiman et al. [14], have shown the antioxidant properties of eugenol with an IC_{50} value of 10.29 ± 0.011 μg/mL against DPPH radicals. In addition, chemical modification of eugenol, especially on the hydroxyl group, has produced derivatives with enhanced antioxidant activity. This highlights the potential of eugenol and its derivatives not only as antioxidants but also as candidates for other therapeutic applications, including inhibition of ACE.

Eugenol as an anti-ischemia has been proven in several studies. A study shows that eugenol shows cardioprotective potential in rat heterotopic heart transplantation by reducing myocardial edema, suppressing inflammation, and preventing apoptosis in heart tissue [15]. Moreover, methyl eugenol is able to protect the liver from liver ischemia by stimulating the PI3K/Akt pathway and reducing inflammatory and apoptotic responses [16]. Both studies strengthen eugenol and its derivatives for further study as anti-ischemia agents.

The search for new drug candidates with enhanced activity is a growing field of research, with *in silico* techniques such as molecular docking playing an important role [17]. The anti-ischemia docking research by salvianolic acid C compound [18], the active ingredient of *Gastrodia elata* Blume [19], and the active ingredient of *Dalbergia odorifera* [20], that has been conducted proves that the interaction between ischemia protein as a target and the compound as a ligand can be evaluated through binding interaction and energy with its active side. Molecular docking allows the prediction of ligand-receptor interactions at the atomic level, offering a cost-effective and efficient tool for drug discovery and development [21].

Protein-Ligand ANT System (PLANTS), one such molecular docking software, uses nature-inspired algorithms and provides flexibility in modeling amino acid residues, increasing its predictive accuracy [22]. PLANTS has advantages in the form of a nature-inspired docking algorithm, flexibility in parameter modification, use of the MOL2 format, explicit water molecule inclusion, NMR data integration, and scaffold hopping and molecular interaction fingerprint (IFP) features that improve the accuracy of ligand-protein interaction predictions [23]. A study showed that PLANTS can be used to identify the potential of chalcone derivatives in inhibiting angiogenesis in cancer cells, thus, this software helps in the process of discovering new drug candidates [24].

This study focuses on the evaluation of eugenol and its derivatives as potential ACE inhibitors using molecular docking. By analyzing several parameters, such as docking scores, binding interactions, and physicochemical properties of drug candidates, this study aims to predict their efficacy compared to commercial ACE inhibitors, thereby identifying promising candidates for further development.

■ EXPERIMENTAL SECTION

Materials

The protein-ligand complex used is an ACE complex with lisinopril. The protein-ligand complex was obtained from https://www.rscb.org with the code PDB of 1O86. The test ligands used are eugenol compounds and their derivatives that have been tested experimentally [14]. ACE inhibitors are used as commercial drugs from https://pubchem.ncbi.nlm.nih.gov. All ligands are shown in Table 1.

Instrumentation

The hardware is a computer set with AMD Ryzen 5 1.4 GHz processor, AMD Radeon Graphics, and 8 GB RAM. The software used is YASARA version 21.8.26 [25], PLANTS [26], Discovery Studio Visualizer version 24.1.0.23298 [27], and MarvinSketch version 5.2.5.1 (http://www.chemaxon.com).

Procedure

Preparation and validation of ACE protein complex and native ligand lisinopril

The ACE and lisinopril protein complexes were prepared using YASARA by separating the ACE and lisinopril proteins as native ligands into two files.

Individual files are saved in the mol2 sybyl Mol2 (.mol2) file format. The native ligand file is further prepared using Marvin Sketch (https://www.chemaxon.com). The ligands were optimized on the Clean 2D menu, and the ligand pH was adjusted to the body's pH of 7.4 in the Major Microspecies menu. The ligands are converted into 20 conformations in the Conformers menu and saved in the Tripos Mol2 (.mol2) file format.

ACE protein and lisinopril were validated first for binding site center and binding site radius using PLANTS with ant colony optimization (ACO) algorithm [23]. The result calculation for the binding site center is 41.0136, 34.3252, and 46.4412 Å; and the binding site radius is 12.8714 Å, stored in the command file (.txt). Furthermore, the ACE protein file and 20 conformational native ligands of lisinopril were docked with PLANTS. The conformation with the best docking value of the 20 conformations is compared with the native ligand lisinopril. Comparisons were made by calculating the root mean square deviation (RMSD) on the RSMD of Molecules menu in YASARA. Comparison and calculation files are saved in YASARA object (.yob) format.

Preparation of docking of test ligands and commercial drugs and visualization of docking results

Preparation docking of test ligands and commercial drugs was drawn using Marvin Sketch. The ligands were then optimized on the Clean 2D menu, and the ligand pH was adjusted to the body's pH of 7.4 in the Major Microspecies menu. The ligands are converted into 20 conformations in the Conformers menu and saved in the Tripos Mol2 (.mol2) file format. Test ligand files and commercial drugs were also docked to ACE protein using PLANTS via the ACO algorithm with the same command (.txt) file in the validation. The docking results for each compound are entered into Microsoft Excel. The best score from the docking results was then selected to compare the native ligand lisinopril, test ligands, and commercial drugs.

The best score for docking each compound was combined with a protein file using YASARA in (.pdb) format. Visualization is performed on each merged file with the Discovery Studio Visualizer. Visualization of ligand interaction with protein is stored in three dimensions through the Publication Quality menu and two dimensions through the Show 2D Diagram menu.

Molecular descriptor, drug-likeness, and toxicity of ligands

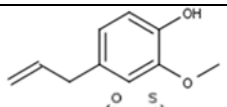
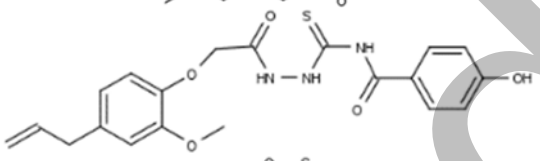
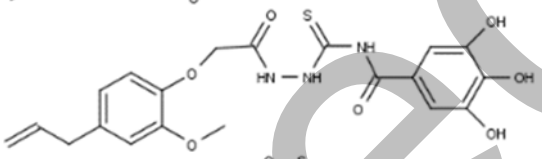
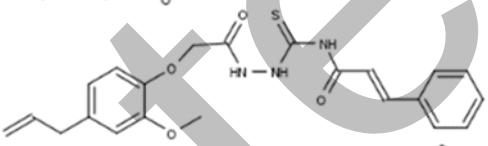
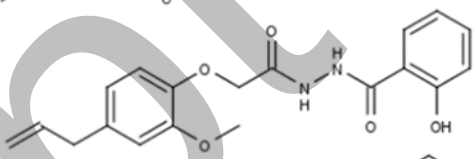
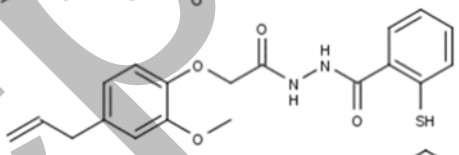
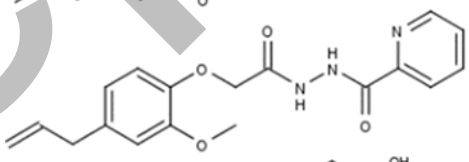
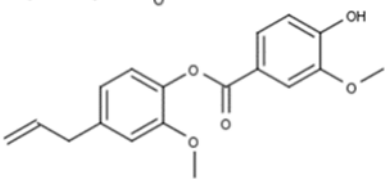
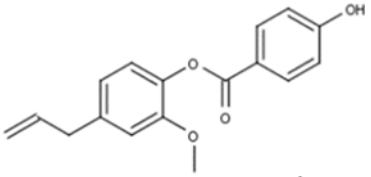
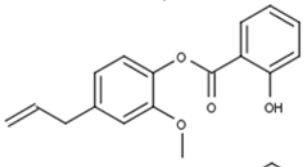
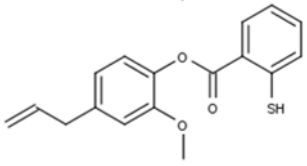
Molecular descriptors and drug-likeness were obtained by converting them into SMILE format and analyzed using ADMETLab3.0 with https://admetlab3.scbdd.com. Molecular descriptor parameters using molecular weight, hydrogen bond donor, hydrogen bond acceptor, total polar surface area, log P, and log D. Drug likeness parameters are used for Lipinski, Pfizer, GSK, and Golden Triangle. Predicted toxicity using SMILE format and entered into the Toxtree software. The toxicity methods use Kroes TTC decision tree and cytochrome P450-mediated drug metabolism (SMARTCyp).

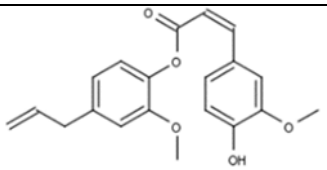
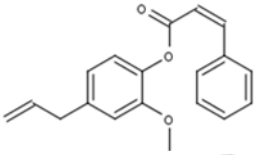
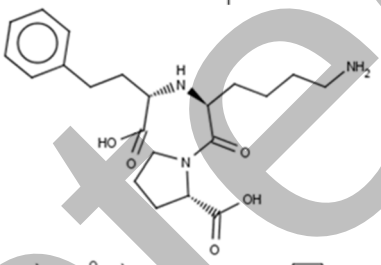
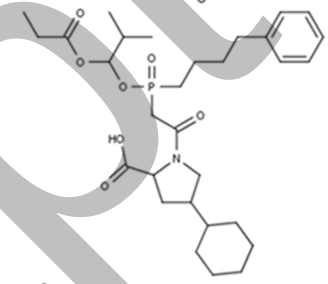
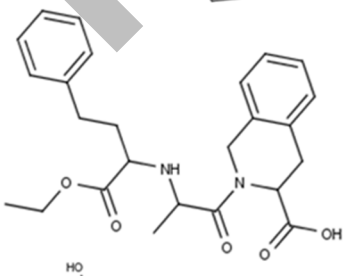
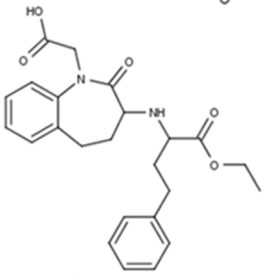
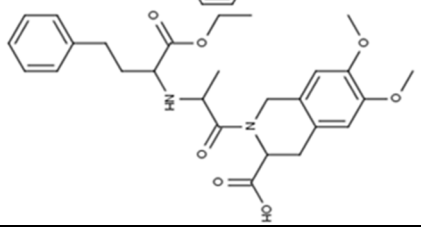
■ RESULTS AND DISCUSSION

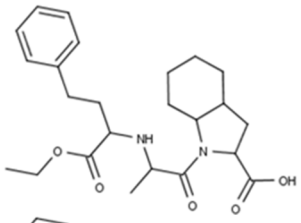
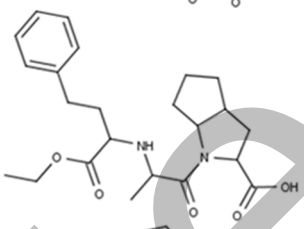
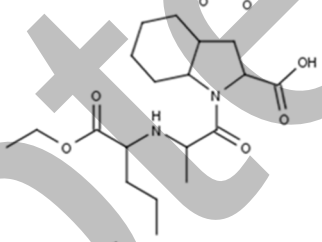
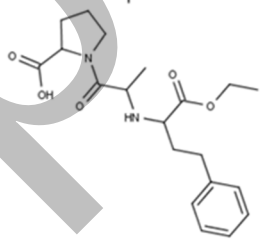
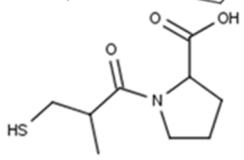
The material data shown in Table 1 with compounds 1–13 are eugenol and its derivatives based on experimental results used as test ligands [14]. Compound 14 is a native ligand of the ACE protein [28]. Compounds 15–23 are commercial drugs such as ACE inhibitors [29]. All ligands obtained in Table 1 were analyzed for docking of the ACE protein. Method validation is done first before docking analysis. Method validation serves to prove that the docking method can be trusted to perform analysis of other test ligands [30]. The method is validated by re-docking the native ligand to the protein is shown in Fig. 1 [31]. The parameter of the docking method validation is the RMSD value. The RMSD value obtained in the redocking native ligand is 1.3524 Å. The RMSD value < 2 Å indicates that the conformation of the native ligand carried out by re-docking is close to the conformation of the X-ray crystallography test [32] so that it can be trusted for molecular docking analysis [30].

Furthermore, the stable interaction between the ligand and ACE protein was analyzed using molecular docking and compared using a docking score [33]. The results of docking scores of all ligands with ACE protein are shown in Table 2. The results of docking with negative

Table 1. Visualization of native ligands, test ligands, and commercial drugs

No.	Compound	Structure	Symbol
1	2-methoxy-4-prop-2-enylphenol		Eugenol
2	1-(2-(4-allylphenoxy)acetyl)-4-(4-hydroxybenzoyl)thiosemicarbazide		5a
3	1-(2-(4-allylphenoxy)acetyl)-4-(3,4,5-trihydroxybenzoyl)thiosemicarbazide		5b
4	(*E*)-1-(2-(4-allylphenoxy)acetyl)-4-cinnamoylthiosemicarbazide		7
5	*N*′-(2-(4-allyl-2-methoxyphenoxy)acetyl)-2-hydroxybenzohydrazide		9a
6	*N*′-(2-(4-allyl-2-methoxyphenoxy)acetyl)-2-mercaptobenzohydrazide		9b
7	*N*′-(2-(4-allyl-2-methoxyphenoxy)acetyl)picolinohydrazide		11
8	4-allyl-2-methoxyphenyl 4-hydroxy-3-methoxybenzoate		13a
9	4-allyl-2-methoxyphenyl 4-hydroxybenzoate		13b
10	4-allyl-2-methoxyphenyl 2-hydroxybenzoate		13c
11	4-allyl-2-methoxyphenyl 2-mercaptobenzoate		13d

No.	Compound	Structure	Symbol
12	(*Z*)-4-allyl-2-methoxyphenyl 3-(4-hydroxy-3-methoxyphenyl)acrylate		16
13	(*Z*)-4-allyl-2-methoxyphenyl 3-phenylacrylate		17
14	(2*S*)-1-[(2*S*)-6-amino-2-[[(1*S*)-1-carboxy-3-phenylpropyl]amino]hexanoyl]pyrrolidine-2-carboxylic acid		Lisinopril
15	(2*S*,4*S*)-4-cyclohexyl-1-[2-[(2-methyl-1-propanoyloxypropoxy)-(4-phenylbutyl)phosphoryl]acetyl]pyrrolidine-2-carboxylic acid		Fosinopril
16	(3*S*)-2-[(2*S*)-2-[[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]-3,4-dihydro-1*H*-isoquinoline-3-carboxylic acid		Quinapril
17	2-[(3*S*)-3-[[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]-2-oxo-4,5-dihydro-3*H*-1-benzazepin-1-yl]acetic acid		Benazepril
18	(3*S*)-2-[(2*S*)-2-[[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]-6,7-dimethoxy-3,4-dihydro-1*H*-isoquinoline-3-carboxylic acid		Meoxipril

No.	Compound	Structure	Symbol
19	(2*S*,3*aR*,7*aS*)-1-[(2*S*)-2-[[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]-2,3,3*a*,4,5,6,7,7*a*-octahydroindole-2-carboxylic acid		Trandolapril
20	(2*S*,3*aS*,6*aS*)-1-[(2*S*)-2-[[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]-3,3*a*,4,5,6,6*a*-hexahydro-2*H*-cyclopenta[*b*]pyrrole-2-carboxylic acid		Ramipril
21	(2*S*,3*aS*,7*aS*)-1-[(2*S*)-2-[[(2*S*)-1-ethoxy-1-oxopentan-2-yl]amino]propanoyl]-2,3,3*a*,4,5,6,7,7*a*-octahydroindole-2-carboxylic acid		Perindopril
22	(2*S*)-1-[(2*S*)-2-[[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]pyrrolidine-2-carboxylic acid		Enalapril
23	(2*S*)-1-[(2*S*)-2-methyl-3-sulfanylpropanoyl]pyrrolidine-2-carboxylic acid		Captopril

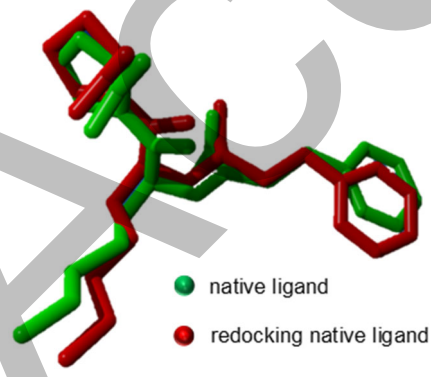


Fig 1. Comparison of the crystal structure of native ligands with the results of redocking native ligands

values indicate that all ligands can bind to ACE protein [34]. The more negative the docking value, the stronger the ligand bond on the active protein site [35].

Fosinopril had the most negative docking score, followed by native ligand. This shows a stronger interaction with the ACE protein than the native ligand. All the test ligands had more positive docking scores than the native ligands. However, some of the tested ligands had more negative docking scores than commercial drug docking scores. Test ligand 7 became the ligand that had the most negative docking score, and eugenol's test ligand had the most positive docking score.

The interaction of several amino acids of the ACE protein bound to the ligand was then analyzed [36]. According to previous work [37], the active site of ACE protein is divided into 3 active sites, i.e., the first active site consists of amino acids ALA 354, GLU 384, and TYR 523,

Table 2. Results of docking scores and amino acid residues bound to ligand

No.	Ligand	Docking score (kcal/mol)	Amino acid residue		
			Hydrogen bond	Electrostatic bond	Hydrophobic bond
1	Fosinopril	−114.474	HIS 353, GLN 281, LYS 511	LYS 511, HIS 383	HIS 353, HIS 383, PHE 457, PHE 527, TYR 523, VAL 379, TRP 279, VAL 380
2	Native Ligand (Lisinopril)	−109.653	ALA 354, TYR 523, GLN 281	GLU 384, LYS 511, GLU 162, ASP 377	TYR 523, HIS 383, VAL 518
3	Quinapril	−109.447	ALA 354, GLU 384, GLN 281		ALA 356, HIS 387
4	Benazapril	−107.179	ALA 354, GLU 384, GLN 281		HIS 383, HIS 410, HIS 387, VAL 380, ALA 356
5	7	−105.192	ALA 354, HIS 353, LYS 511, TYR 146	GLU 162	CYS 352, LEU 161, VAL 380
6	5b	−103.755	ALA 354, TYR 523, GLU 384, ASN 70	GLU 384, GLU 411	HIS 383, VAL 380, PHE 527, VAL 379, PHE 457, PHE 512
7	Meoxipril	−102.859	GLU 384, TYR 523, ARG 522	HIS 353, ARG 522	TYR 523, PHE 512, VAL 351, VAL 518
8	Trandolapril	−102.716	GLN 281, HIS 353, LYS 511		PHE 512, TYR 523, PHE 457, HIS 513
9	Ramipril	−101.241	ALA 354, GLN 281		PHE 457, TYR 523, PHE 527, HIS 383
10	5a	−99.8749	ALA 354, TYR 523, ALA 356, HIS 383	GLU 384, GLU 411	HIS 387, HIS 410, VAL 379, VAL 380, PHE 457, PHE 527
11	Perindopril	−97.5490	ALA 354, GLN 281	LYS 511	TYR 523, HIS 383, PHE 457, HIS 353
12	Enalapril	−93.7726	GLN 281		HIS 387, ALA 356, HIS 383, TYR 523
13	9a	−91.1606	ALA 354, GLU 411, GLU 384		HIS 383, HIS 387, ALA 356, VAL 380, VAL 379, TYR 523, PHE 457, PHE 527
14	11	−90.5970	GLN 281, TYR 523, LYS 511		PHE 457, ALA 354, VAL 380
15	9b	−90.0492	ALA 354, TYR 523, ARG 522	GLU 384, GLU 411	HIS 387, HIS 383, ALA 356, PHE 457, PHE 527, VAL 380, VAL 379
16	13a	−77.1591	GLN 281, ASP 415, LYS 511	LYS 511	HIS 353, HIS 383, VAL 380, LEU 161
17	16	−78.1483	GLN 281, LYS 511		PHE 457, TYR 523, PHE 527, VAL 518, PHE 512, HIS 387, ALA 356
18	13d	−74.8961	GLN 281, LYS 511	LYS 511	HIS 383, HIS 353, LEU 161, ALA 354, VAL 380
19	Captopril	−74.6613	GLN 281	HIS 353	TYR 523, PHE 457, HIS 383
20	13b	−74.6117	GLN 281, LYS 511	LYS 511	GLN 281, LYS 511, HIS 353, TYR 146, LEU 161, VAL 380
21	17	−74.4107	GLN 281, LYS 511	LYS 511	HIS 383, PHE 457, VAL 380, ALA 354
22	13c	−74.2184	ALA 356	GLU 384	TRP 357, HIS 513, TYR 523, HIS 353, PHE 391, HIS 387
23	Eugenol	−62.6668	CYS 352, SER 147		LEU 161, TRP 279, HIS 353, CYS 352

the second active site consists of amino acids GLN 281, HIS 353, LYS 511, HIS 513, and TYR 520, and the third active site consists of the amino acid GLU 162. Table 2 shows the amino acid residues that bind directly to the ligand. The fosinopril ligand was able to bind to both active sites, the test ligand 7 was able to bind to the three active sites, while the eugenol test ligand was able to bind to both active sites. Ligand binding that occurs at the active site of the ACE protein causes the protein to be inhibited in the mechanism of converting angiotensin I to angiotensin II [38]. Angiotensin II itself is the cause of hypertension, heart failure, chronic kidney disease, insulin resistance, and tumor development [39]. The visualization of the ligand interaction with the amino acids of the ACE protein is shown in Fig. 2.

The bonds that occur in ACE proteins with ligands are hydrogen bonds, hydrophobic bonds, and electrostatic bonds. Each of these bonds affects the stability of the ligand with the protein even though the bond is weak [40]. One of the bonds that has a strong bond between ligand and protein is the hydrogen bond [36]. Hydrogen bonds that are able to bind to all active sites can have a major effect on the docking score [41]. The fosinopril and 7 ligands have three hydrogen bonds while the eugenol ligands have no hydrogen bonds that bind to the active site of the protein. This correlates with eugenol's lowest docking score, while fosinopril and ligand 7 have the highest docking score. Ligand bonds with amino acid residues that often occur are hydrophobic bonds consisting of pi-sigma, pi-pi T-shaped, alkyl, and pi-alkyl

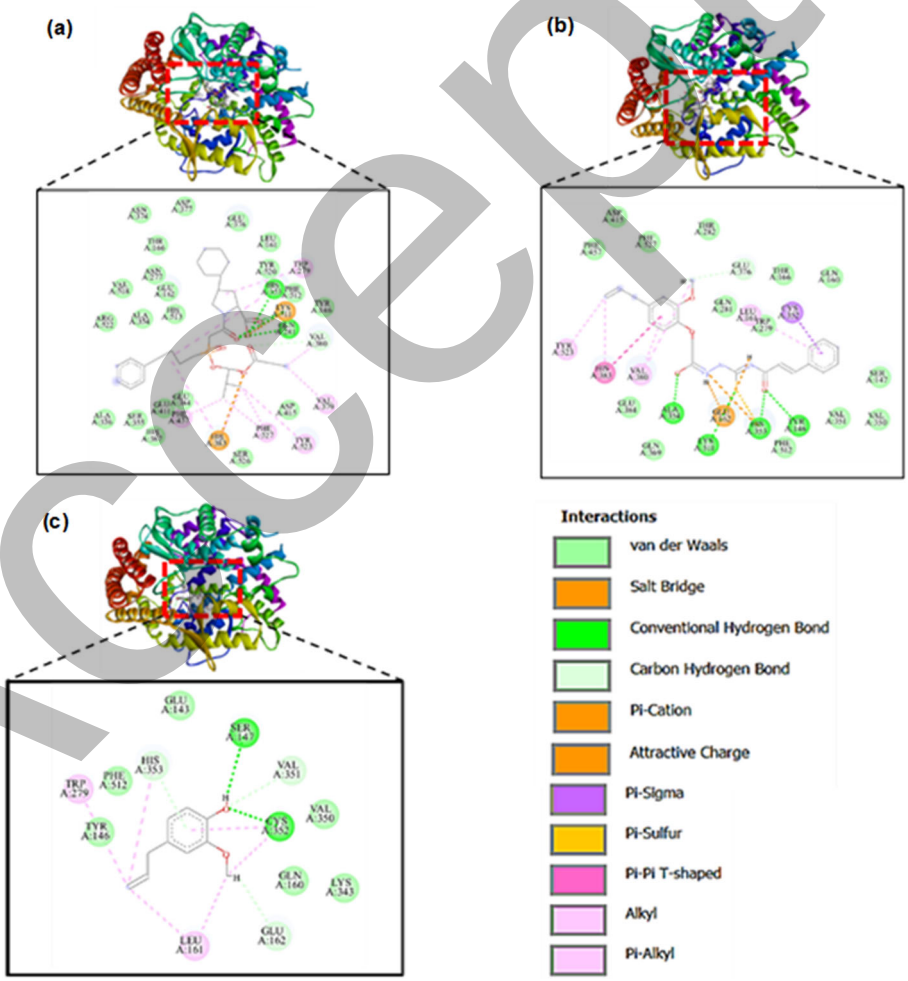


Fig 2. 2D interactions of (a) fosinopril, (b) test ligand 7, and (c) eugenol interaction with amino acid residues of ACE protein

interactions. Hydrophobic interactions can increase the binding affinity so as to stabilize the ligand on the active site of the target protein [42]. The fosinopril ligand has two hydrophobic bonds, ligand 7 has no hydrophobic bonds, while the eugenol ligand has 1 hydrophobic bond on the active site of the protein. This indicates that the hydrophobic bond has a small effect on the docking score of the three ligands.

In addition to hydrogen bonds and hydrophobic bonds, electrostatic bonds (attractive charge, pi-cation, and salt bridge) are also studied in this study. Electrostatic bonds play an important role in docking results compared to docking without electrostatic bonds [43]. The electrostatic bond in the fosinopril ligand has two bonds. The test ligand 7 has one bond, while the eugenol ligand has no bonds. This shows that the electrostatic bond also has a negligible effect on the docking score of the three ligands.

Table 3 provides a comparative analysis of the physicochemical properties of the tested compounds (derived from eugenol), the native ligand (lisinopril), and the commercial drug. The tested compounds, especially 5a, 5b, 7, and 9a, showed molecular weight (MW), hydrogen bond donor (HBD), hydrogen bond acceptor (HBA), total polar surface area (TPSA), and hydrophobicity (logP) values very similar to the native ligand, indicating their potential as effective ligands. Compounds 5a and 5b showed a good balance between lipophilicity (logP 2.415 and 1.91) and polarity (TPSA 108.92 and 149.38 Å^2), which improved the predicted permeability and bioavailability. Compound 7 also showed promising lipophilicity (logP 3.025) with a moderate TPSA of 88.69 Å^2. In particular, 9a stands out with lower MW (356.14 g/mol) and balanced hydrophobicity (logP 3.084), indicating enhanced membrane permeability. These findings highlight the

Table 3. The molecular descriptor of the proposed ligand obtained from ADMETlab 3.0

Compound	MW[a] (g/mol)	HBD[b]	HBA[c]	TPSA[d] (Å^2)	logP	logD
Native ligand (Lisinopril)	405.23	5	8	132.96	-1.104	1.249
5a	415.12	4	8	108.92	2.415	2.595
5b	447.11	6	10	149.38	1.910	2.108
7	425.14	3	7	88.69	3.025	3.133
9a	356.14	3	7	96.89	3.084	3.026
9b	372.11	2	6	76.66	2.797	2.793
11	341.14	2	7	89.55	2.235	2.168
13a	314.12	1	5	64.99	3.591	3.214
13b	284.10	1	4	55.76	3.545	3.223
13c	284.10	1	4	55.76	3..951	3.434
13d	300.08	0	3	35.53	3.912	3.505
16	340.13	1	5	64.99	3.445	3.176
17	294.13	0	3	35.53	3.836	3.536
Benazapril	424.20	2	7	95.94	1.178	2.038
Captopril	217.08	1	4	57.61	0.369	0.450
Enalapril	376.20	2	7	95.94	-0.105	1.299
Fosinopril	563.30	1	8	110.21	4.416	3.332
Perindopril	368.23	2	7	95.94	0.536	1.718
Ramipril	416.23	2	7	95.94	0.866	1.954
Trandolapril	430.25	2	7	95.94	1.122	2.089
Moexipril	498.24	2	9	114.4	1.669	2.165
Quinapril	438.22	2	7	95.94	1.170	2.092
Eugenol	164.08	1	2	29.46	2.321	2.146

[a]Molecular weight; [b]Hydrogen bond donor; [c]Hydrogen bond acceptor; [d]Total polar surface area

Table 4. Drug likeliness and toxicity analysis of the proposed ligands using various approaches

Compound	Approaches					
	Lipinski	Pfizer	GSK	Golden Triangle	Kroes TTC	SMARTCyp
Native ligand (Lisinopril)	Yes	Yes	No, MW>400	Yes	Negligible risk[a]	Yes[c]
5a	Yes	Yes	No, MW>400	Yes	Negligible risk[a]	Yes[c]
5b	Yes	Yes	No, MW>400	Yes	Negligible risk[a]	Yes[c]
7	Yes	Yes	No, MW>400	Yes	Negligible risk[a]	Yes[c]
9a	Yes	Yes	Yes	Yes	Negligible risk[a]	Yes[c]
9b	Yes	Yes	Yes	Yes	Negligible risk[a]	Yes[c]
11	Yes	Yes	Yes	Yes	Negligible risk[a]	Yes[c]
13a	Yes	No, LogP>3, TPSA<75	Yes	Yes	Negligible risk[a]	Yes[c]
13b	Yes	No, LogP>3, TPSA<75	Yes	Yes	Negligible risk[a]	Yes[c]
13c	Yes	No, LogP>3, TPSA<75	Yes	Yes	Negligible risk[a]	Yes[c]
13d	Yes	No, LogP>3, TPSA<75	Yes	Yes	Negligible risk[a]	Yes[c]
16	Yes	No, LogP>3, TPSA<75	Yes	Yes	Negligible risk[a]	Yes[c]
17	Yes	No, LogP>3, TPSA<75	Yes	Yes	Negligible risk[a]	Yes[c]
Benazapril	Yes	Yes	No, MW>400	Yes	Negligible risk[a]	Yes[c]
Captopril	Yes	Yes	Yes	Yes	Safe[b]	Yes[c]
Enalapril	Yes	Yes	Yes	Yes	Safe[b]	Yes[c]
Fosinopril	Yes	Yes	No, MW>400, logP>4	No, MW>500	Risk Assessment required	Yes[c]
Perindopril	Yes	Yes	Yes	Yes	Safe[b]	Yes[c]
Ramipril	Yes	Yes	No, MW>400	Yes	Safe[b]	Yes[c]
Trandolapril	Yes	Yes	No, MW>400	Yes	Safe[b]	Yes[d]
Moexipril	Yes	Yes	No, MW>400	Yes	Safe[b]	Yes[c]
Quinapril	Yes	Yes	No, MW>400	Yes	Safe[b]	Yes[c]
Eugenol	Yes	Yes	Yes	No, MW<200	Negligible risk[a]	Yes[c]

[a]low probability of a life-time cancer risk greater than 1 in 10^6; [b]Substance would not be expected to be a safety concern; [c]SMARTCyp predicted primary site of metabolism (rank 1), SMARTCyp predicted secondary site of metabolism (rank 2), SMARTCyp predicted tertiary site of metabolism (rank 3), SMARTCyp predicted site of metabolism with rank>3; [d]SMARTCyp predicted primary site of metabolism (rank 1), SMARTCyp predicted tertiary site of metabolism (rank 3), SMARTCyp predicted site of metabolism with rank>3

potential of this compound for further development, with physicochemical properties suitable for drug-like behavior.

Table 4 evaluates the drug-likeness and toxicity of the tested compounds, the native ligand (lisinopril), and the commercial drug based on four established frameworks: Lipinski, Pfizer, GSK, and Golden Triangle. Most of the tested compounds comply with the Lipinski, Pfizer, and Golden Triangle rules, indicating good drug-likeness profiles [44]. However, 5a, 5b, and 7 compounds fail to meet the GSK criteria because their molecular weights exceed 400 g/mol. In particular, compounds 13a–13d show deviations from the GSK framework due to high logP (> 3) and low TPSA (< 75 Å^2), which may affect their pharmacokinetics. The Kroes TTC [45] and SmartCyp [46] are toxicity parameters. Most of the tested compounds meet the safety and toxicity criteria based on these parameters. Overall, compounds 5a, 5b,

7, and 9a emerge as strong candidates, fulfilling most of the drug-likeness and toxicity criteria and showing promising profiles for further development.

A comprehensive analysis of molecular docking, pharmacokinetic properties, and drug-likeness assessment highlighted the potential of Eugenol derivatives as promising ACE inhibitors. This is appropriate with *in vitro* and *in vivo* research, which shows that Eugenol functions as a cardioprotective, suppresses inflammation, and prevents apoptosis in heart tissue [15]. Molecular docking results showed that derivatives such as (*E*)-1-(2-(4-allylphenoxy)acetyl)-4-cinnamoylthiosemicarbazide (compound 7) showed comparable docking scores to the native ligand and were superior to some commercial drugs, supported by strong hydrogen bonding and hydrophobic interactions in the ACE active site. Physicochemical analysis revealed that compounds 5a, 5b, 7, and 9a closely matched the molecular properties of the native ligand (lisinopril) and surpassed some commercial drugs, with balanced logP and TPSA, enhanced bioavailability, and membrane permeability. Drug-likeness evaluation (Table 2) confirmed that these compounds met the major criteria, including Lipinski, Pfizer, and Golden Triangle rules, indicating good pharmacokinetics and reduced toxicity risk. These findings position eugenol derivatives, particularly 5a, 5b, 7, and 9a, as viable candidates for further synthesis and evaluation as ACE inhibitors for ischemic stroke therapy.

■ CONCLUSION

Molecular docking analysis was performed to evaluate the potential of eugenol and its derivatives as ACE inhibitors. The findings revealed that several Eugenol derivatives, especially compounds 7 (−105.192 kcal/mol), 5b (−103.755 kcal/mol), 5a (−99.8749 kcal/mol), and 9a (−91.1606 kcal/mol), showed more negative docking scores than some commercial drugs, indicating stronger binding affinity. Among these, compound 7 showed one of the most promising results, forming a stable interaction with the active site of ACE through hydrogen bonding, hydrophobic interactions, and electrostatic forces. However, compounds 5a and 5b also showed potential based on physicochemical and drug-likeliness data. These interactions strengthened the potential of eugenol derivatives as effective ACE inhibitors, with compounds 7, 5a, and 5b emerging as strong candidates for further experimental validation.

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■ CONFLICT OF INTEREST

The authors declare that no conflict of interest could have influenced the research presented in this study.

■ AUTHOR CONTRIBUTIONS

Susy Yunita Prabawati conducted the conceptual study and revised the manuscript. Priyagung Dhemi Widiakongko conducted the data processing and wrote and revised the manuscript. Karisma Triatmaja performed the experiment, processed the data, and wrote the manuscript. All authors agreed to the final version of this manuscript.

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