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RESEARCH ARTICLE

Molecular docking of ferulic acid analogue compounds against epidermal growth factor receptor as a potential therapy for breast cancer

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Abstract

Background: Triple-negative breast cancer (TNBC) accounted for 18.1% of the breast cancer cases that occurred in Indonesia until 2020. Epidermal growth factor receptor (EGFR) overexpression is found in at least 50% of TNBC cases. So far, it is necessary to find an anti-cancer compound that has the potential against TNBC-type breast cancer to achieve good health and well-being. **Objective**: Ferulic acid derivatives were designed to be active on EGFR in silico study. **Methods**: Molecular docking was performed using Auto Dock 1.5.7 and PyRx 0.8 software, and visualisation was observed using Discovery Studio. FA ligand and its four derivatives were docked into the receptor EGFR (PDB ID: 3W33). **Results**: It was found that ferulic acid derivatives have high potential as an anticancer through EGFR inhibition in TNBC-type breast cancer. 4-(4-methyl) benzoyloxy-3-methoxycinnamic acid had the best potential among other derivatives, which showed the lowest binding free energy of -8.81 kcal/mol and the smallest Ki of 352.65nM. Methyl substitution at the benzoyloxy increased ligand interaction with amino acids in EGFR by increasing hydrophobic π-alkyl, π-π and alkyl binding with amino acids. **Conclusion**: The 4-(4-methyl) benzoyloxy-3-methoxycinnamic acid was the most prospective compound as an EGFR inhibitor and predicted as the most potential compound against breast cancer.

Introduction

Cancer is a highly lethal disease with significant fatality rates. Breast cancer is a frequently diagnosed kind of cancer, particularly prevalent among women. According to the International Agency for Research on Cancer (IARC) (Arnold *et al.,* 2022), in 2020, there were about 2.3 million new cases of breast cancer globally. Of these cases, as many as 685,000 were deaths from a variety of causes. Triple-negative breast cancer accounts for about 10-15% of all breast cancers. Triple-negative breast cancer (TNBC) is characterised by cells not having oestrogen or progesterone receptors (ER or PR), as well as the lack of overexpression of the Human epidermal receptor 2 (HER2) protein (Ali *et al.,* 2017).

Breast cancer exhibits the highest incidence rate in Indonesia. According to the Ministry of Health of the Republic of Indonesia (2022), data obtained from the Global Cancer Observatory in 2020 revealed that breast cancer accounted for 16.6% of the total number of cases, amounting to 68,858 individuals. Additionally, the number of fatalities resulting from breast cancer exceeded 22,000 cases. Based on research (Widiana & Irawan, 2020), 18.1% of TNBC-type breast cancer was obtained from 1260 cases of breast cancer that occurred in Indonesia during the 2014-2020 period.

EGFR (epidermal growth factor receptor) is more commonly overexpressed in highly aggressive TNBC and inflammatory breast cancer (IBC). EGFR

overexpression is found in at least 50% of TNBC cases, which is a higher level of expression than the levels seen in other breast cancer subtypes (Butti *et al*., 2018).

EGFR overexpression in breast cancer is associated with large tumour size, poor differentiation, and poor clinical outcomes. TNBC patients are initially treated with conventional chemotherapy, but the disease often recurs and leads to worse outcomes than patients with other breast cancer subtypes (Nakai *et al.*, 2016). Therefore, effective therapeutic strategies for TNBC are urgently needed (Hashmi *et al*., 2019).

A study by Sudhagar and colleagues investigated the effects of ferulic acid on EGF-induced MCF-7 cells in an in vitro setting. The results indicated that at a concentration of 10 µM, ferulic acid exhibited a cell proliferation inhibition ranging from 14% to 30% for 24 to 96 hours (Sudhagar *et al*., 2018). Similarly, at a concentration of 100µM, the inhibition ranged from 20% to 35% over the same time frame. This suggests that ferulic acid inhibits cell proliferation in breast cancer through EGFR receptor inhibition. This inhibitory activity is also supported by an in-silico study conducted by (Sudhagar *et al*., 2018), where ferulic acid showed a free energy value of -5.3 kcal/mol at the EGFR receptor (PDB ID: 1XKK) using Auto Dock 4.2.

Ferulic acid consists of one aromatic ring, one double bond (-C=C-), one carboxylic group (-COOH), one phenol group, and one methoxy group (-OCH3) (Figure 1A). In this study, modification of the phenolic -OH group on ferulic acid was carried out to form a carbonyl ester so that it became 4-benzoyloxy-3-methoxycinnamic acid as a derivative of ferulic acid (Figure 1B). Modification of ferulic acid to the carboxylic hydroxy group resulting in hexyl ferulic esters and feruloyl hexyl amide can increase cytotoxic activity against human breast cancer cells (Serafim *et al*., 2011; Serafim *et al.*, 2015). This is associated with increased lipophilic properties (Siswandono, 2016). Modifications to the position of the benzene ring were carried out to increase activity and produce compounds with variations in lipophilicity expressed in Log P values. The Log *p*-value of 4-(4-methyl) benzyloxy-3-methoxy cinnamic acid is 3.32, and 4-(4-methoxy) benzyloxy-3-methoxy cinnamic acid has Log P = 3.02; while the Log p-values of 4-benzoyloxy-3-methoxycinnamic acid and 4-(4-hydroxy) benzyloxy-3-methoxy cinnamic acid compounds are 3.01 and 2.90, respectively (Ekowati *et al.,* 2018). Based on these data, the most lipophilic compound is 4-(4-methyl) benzoyloxy-3-methoxycinnamic acid, expected to produce the highest activity.

Therefore, it is necessary to determine the ability of the derivative compounds to inhibit EGFR and then select the derivative compounds to be synthesised as targets in the treatment of TNBC. In the drug discovery and development process, developing new drugs takes a long time of 10-15 years (Kinch *et al.,* 2022) and a huge cost of about 12 trillion (Van Tonder *et al*., 2013; Siswandono, 2014). Computer-aided drug design (CADD) reduced the cost by up to 50% (Surabhi & Singh, 2018). One of the commonly used CADD methods is Molecular docking. Several docking programs, such as PyRx and Auto Dock, can be accessed for free. In the selection of derivative compounds to be synthesised, molecular docking studies were conducted by comparing the two docking programs.

Methods

Materials

The receptor used is EGFR. The protein structure of the receptor was downloaded from www.rcsb.org (Protein Data Bank). EGFR receptor, PDB ID: 3W33 with native ligand 4-{[4-(1-benzothiophen-4-yloxy)-3-chlorophenyl]a mino}-N-(2-hydroxyethyl)-8,9-dihydro-7H-pyrimi do[4,5-b]azepine-6-carboxamide with code W19. Whereas, the ligands used are ferulic acid and 4-benzoyloxy-3-methoxycinnamic acid derivatives with (-R = -H, -OH, -CH3, -OCH3). The ligand structure was visualised using the ChemDraw application version 19.1 and transferred to the Chem3D software application version 19.1 to generate a three-dimensional representation. The minimum energy of the ligand was determined using the MMF94 force field, and the resulting structure was saved as a mol2 file {SYBYL2 (*.mol2)}.

Tools

Some programmes used in this research are ChemDraw software version 19.1, Chem3D software version 19.1, AutoDock 1.5.7, PyRx 0.8 Automatics Molecular Docking, and Discovery Studio Visualizer (DSV) 2021 software.

Validation

The docking study is validated by re-docking the receptor with the native ligand with three replications. If the root mean square deviation (RMSD) value is < 2.0 Å, the validation is accepted (Susanti *et al.,* 2019).

Molecular docking

A molecular docking study was performed on the EGFR receptor (PDB ID: 3W33). The docking processes were done in triplicates. Molecular docking results showed ligand-receptor affinity as free binding energy observed with dg format. file, type of ligand-receptor

interactions, and type of amino acids interacting with the ligands. The lowest free binding energy indicates the most stable bond between the compound and the receptor, so the greatest activity can be predicted. The bonding of molecular docking results was analysed from the visualisation results between the parent compound ferulic acid and its derivatives.

Results

Receptor selection

The selected epidermal growth factor receptor is PDB ID 3W33, chosen based on the validation parameter.

Validation

Once the selected receptor protein was obtained, docking validation was performed using Auto dock 1.5.7. Validation aims to show the degree of accuracy of the instrument as a measuring tool against the content or what is measured. The validation results show that the average RMSD value is 0.999±0.08 (Figure 1). This means the docking procedure is correct, and the ligand and receptor bond are stable.

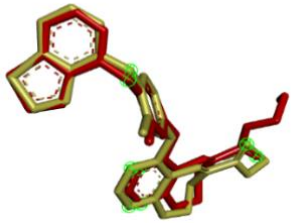


Figure 1: Overlay of native ligand from re-docking (yellow) to native ligand from crystallography (red)

Molecular docking

Docking results using Auto dock showed the ligand's affinity for the receptor (Table I). In addition to free energy, Auto dock showed the value of the inhibition constant indicated by the Ki value (nM). The results showed that almost all ferulic acid derivates have higher affinity than ferulic acid. The ligand showing the highest affinity against EGFR was FA3, with the lowest Ki value.

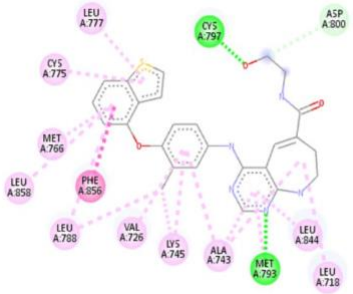
Docking was also conducted using PyRx. Based on the free energy results obtained can be seen in Table I. From these results, ferulic acid derivatives have lower free energy than ferulic acid**.**

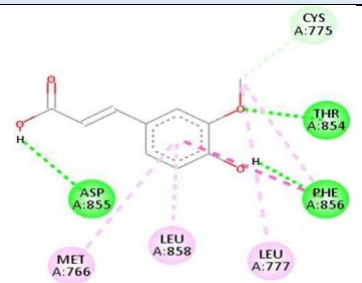
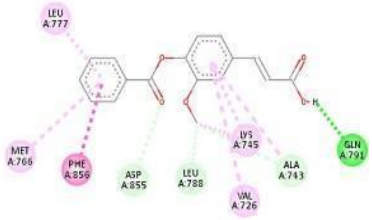
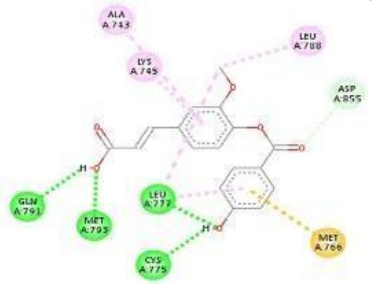
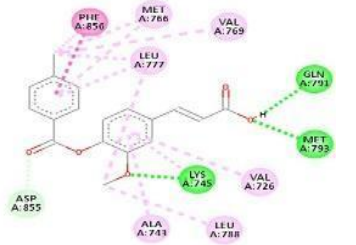
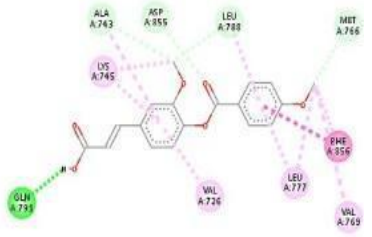
Table I: Docking results of 4-benzoyloxy-3-methoxycinnamic acid derivate with AutoDock 1.5.7

Ligand	AutoDock 1.5.7		PyRx
	Free binding energy (kcal/mol)	KI (nM)	Free binding energy (kcal/mol)
FA	-5,89	484,70	-7,10
FA1	-8,45	634,60	-8,40
FA2	-8,78	368,98	-8,40
FA3	-8,81	352,65	-8,40
FA4	-8,68	430,73	-8,20

Docking results showed the 2D interaction of ligands in the binding site of EGFR. Table II also displays the interactions and amino acids that interact with the ligands.

Table II: Interactive amino acids from Ligand-receptor Interaction (PDB ID: 3W33)

Compounds	Visualisation results	Number of bonds	Interactive amino acids	
			H-bonds	Van der Waals Interaction
Native Ligand (W19)		14	Met793, Cys797, Asp800	Leu718, Val726, Ala743, Lys745, Met766, Cys775, Leu777, Leu788, Leu844, Phe 856, Leu 858

Compounds	Visualisation results	Number of bonds	Interactive amino acids	
			H-bonds	Van der Waals Interaction
FA		7	Cys775, Thr854, Asp855, Phe856	Met766, Leu777, Leu858
FA1		9	Ala743, Leu788, Gln791, Asp855	Val726, Lys745, Met766, Leu777, Phe856
FA2		9	Cys775, Leu777, Gln791, Met793, Asp855	Ala743, Lys745, Met766, Leu788
FA3		11	Lys745, Gln791, Met793, Asp855	Val726, Ala743, Met766, Val769, Leu777, Leu788, Phe856
FA4		10	Lys745, Gln791, Met793, Asp855	Val726, Lys745, Val769, Leu777, Phe856

Discussion

Receptor selection

The process of selecting receptor protein PDB IDs is influenced by various factors. Firstly, the mechanism relies on receptors that form complexes with other molecules. Furthermore, the evaluation is based on the resemblance between the chemical group intended to be bound to the receptor's native ligand. Furthermore, the PDB validation results should be taken into account. The EGFR protein, identified by its Protein Data Bank (PDB) ID: 3W33, was selected for further analysis.

Validation

The RMSD value of the validation results obtained was 0.999 ± 0.089 Å. This indicated that the validation results were accepted because the RMSD < 2.0 Å showed that the molecular tethering was correct with

a small deviation, the smaller the RSMD value (Ekowati *et al*., 2018). Based on the overlay results between native ligand redocking and native ligand crystallography results in Figure 1, they are on the same active side. The smaller RMSD value shows that the native ligand conformation from re-docking is closer to the native ligand from crystallography with smaller deviations (Bao *et al.,* 2014; Susanti *et al*., 2019).

Molecular docking

Ferulic acid derivatives have lower free energy values than the parent compound, ferulic acid. This indicates that the bonds of ferulic acid derivatives are more stable and can be predicted to have greater biological activity. The lowest free energy among ferulic acid derivatives is shown by 4-(4-methyl)benzoyloxy-3-methoxycinnamic acid, which is -8.81 kcal/mol, while the free energy of ferulic acid is -5.89 kcal/mol.

The lowest MDS or energy value indicated the best binding between the receptor protein's ligand functional groups and amino acid residues. The lower the energy required to form a bond, the more stable the bond formed and the greater the resulting biological activity (Ekowati *et al*., 2018). In addition, 4-(4-methyl) benzoyloxy-3-methoxycinnamic acid has the smallest Ki value of 352.65 nM. The smaller the Ki value, the higher the inhibitory activity of the compound on the target receptor. The docking results by the PyRx program showed that FA1, FA2, and FA3 derivatives have the lowest free energy value of -8.40 kcal/mol compared to ferulic acid, with a free energy of -7.10 kcal/mol.

Auto dock and PyRx methods demonstrated that ferulic acid derivatives with substituted benzoyl were predicted to have greater biological activity than the parent compound by comparing free energy values. Both methods have their advantages and disadvantages. Auto dock, besides showing the value of free energy, can also provide the value of the inhibition constant (Ki). Meanwhile, PyRx can't predict Ki. However, the advantage of PyRx is that one or more compounds can be docked simultaneously, making it efficient in terms of time. Meanwhile, if AutoDock is used, molecular docking can only be done individually.

The free binding energy, which shows the stability of the ligand bond with the receptor, is also closely related to the type and number of bonds formed with the receptor. The more bonds between the ligand and the receptor, the lower the free energy. Based on the visualisation of ligand-receptor interaction (Figure 2), ferulic acid derivatives increase the interaction with the receptor. In the visualisation results, FA (ferulic acid) had seven interactions with three hydrogen bonds. The FA1 derivative (with -R = -H) adds an aromatic ring group, so the hydrophobic bonds are π-alkyl, π-π, and alkyl. In this case, carbon-hydrogen bonds are also added. However, only one hydrogen bond is formed compared to the parent compound.

In ligand FA2 (-R = -OH), there are nine interactions, where hydrogen bonds increase by four bonds due to the substitution of -OH. Bonds are formed with receptor amino acids in the presence of electronegative groups. Then, on the FA3 ligand (-R = -CH3), there are 11 interactions. The methyl group adds hydrophobic π-alkyl, π-π and alkyl bonds with the receptor amino acid. In addition, three hydrogen bonds were formed. While in the ligand FA4 (-R = -OCH3, the interactions formed amounted to 10. The methoxy group adds carbon-hydrogen (C-H) bonds and hydrophobic bonds. However, the hydrogen bond formed is reduced to one compared to the parent compound.

Prediction of the ferulic acid derivative compounds' pharmacokinetic properties parameters has been done using the pkCSM Online Tool program (Ekowati *et al.,* 2018). Absorption of ferulic acid derivatives is indicated by the intestinal absorption value obtained > 30%, so it can be concluded that the four derivative compounds have good absorption. However, FA2 have the smallest intestinal absorption value among others, namely 67.518%, while the other three derivatives obtained intestinal absorption successively, namely (FA1) 96.298%; (FA3) 94.509; (FA4) 94.089%.

The results of the distribution of compounds to the blood-brain barrier (BBB) showed that the four derivatives were classified as moderate with 0.3 > Log BBB > -1. The four ferulic acid derivatives did not act as inhibitors of CYP3A4 and CYP2D6, which are isoforms of CYP450 in metabolism. In the excretion phase, the four compounds were not renal OCT2 substrates. In LD50 values, ferulic acid derivatives with -R = -H, -CH3, -OCH3 are classified as mildly toxic (toxicity level 5), while derivatives substituting the R = -OH group are classified as non-toxic (toxicity level 6). The four ferulic acid derivatives do not potentially cause toxicity in AMES toxicity and hepatotoxicity parameters. Based on the ADMET prediction results, ferulic acid derivative compounds are feasible to synthesise.

Conclusion

The computational research findings suggest that ferulic acid derivatives have considerable promise as possible anticancer agents by inhibiting the EGFR pathway in triple-negative breast cancer (TNBC). Among the many compounds, 4-(4-methyl) benzoyloxy-3-methoxycinnamic acid exhibits the most potential. However, additional research is required to

assess the pharmacokinetic profile, efficacy, and safety through in vitro and in vivo testing.

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