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In silico study of lutein as anti-HER-2 receptors in breast cancer treatment

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Abstract: Human Epidermal Receptor-2 (HER-2) overexpression is implicated in breast cancer progression; thus, HER-2 is widely used as the target of anticancer therapy. Lapatinib is a drug widely used to inhibit the HER-2 receptor and tyrosine kinase; however, it develops drug resistance. Lutein is promising to be developed as breast cancer therapy. This study aims to determine the mechanism of inhibition of HER-2 receptor overexpression by lutein *in silico*. Molecular docking was carried out by optimizing the lutein and lapatinib, preparing of protein target HER-2 (PDB ID 3PP0), validating of molecular docking protocol, and docking of lutein and lapatinib on HER-2. The study resulted in the binding energy of -12.37 kcal/mol, while the binding energy of the native ligand and lapatinib to HER-2 was -10.43 kcal/mol and -12.25 kcal/mol, respectively. The binding energy showed that lutein has potential as breast anticancer suggested from the stronger affinity to HER2.

Keywords: breast cancer, HER-2, lutein, lapatinib, molecular docking, in silico

Introduction

Breast cancer is the fifth leading cause of cancer death worldwide, with 685,000 deaths [1]. Fifty percent of breast cancer cases are caused by estrogen receptor overexpression, and 30% of them are caused by overexpression of Human Epidermal Receptor-2 (HER-2). Cancer cell invasion may occur due to overexpression of HER-2 receptors. The overexpression of HER-2 leads to increased proliferation, metastasis, as well as induces angiogenesis and anti-apoptosis [2]. HER-2 overexpression can induce spontaneous dimerization and autophosphorylation, thereby triggering the activation of focal adhesion kinase (FAK) involved in the migration and metastasis of cancer cells [3].

Breast cancer is mainly treated with chemotherapy or surgery, which are also used in combination. On the other side, various chemical drugs are used in chemotherapy to destroy the cancer cell tissue, but these chemotherapeutic agents cannot distinguish normal cells from cancer cells. This condition will cause several side effects such as vomiting, diarrhea, and alopecia [4]. Breast cancer therapy methods need to be developed to have specific targets and high selectivity against breast cancer. The therapy used in the treatment of HER-2 positive breast cancer is lapatinib. The mechanism of action of lapatinib as an oral dual tyrosine kinase inhibitor is to target the epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor-2 (HER-2) [5].

One strategy to overcome that problem is by exploring the potential of natural ingredients to be developed into pharmaceutical products. Lutein is one of the important xanthophyll present in many leafy vegetables and certain fruits. Lutein also has been proven to help combat several lifestyle complications, including cancer [6,7]. This lutein needs to be given special and intensive attention because of its benefits for health, one of which has anticarcinogenic activity [8]. Lutein has a very active anticancer activity against MCF-7 breast cancer cells with an IC_{50} value of 3.10 g/mL [9].

The mechanism of lutein to inhibit some human breast cancer cells has been explained. The growth inhibitory effect of lutein in MCF-7 and MDA-MB-231 breast cancer cells are facilitated by a disrupted balance between intracellular ROS and antioxidant defense system [10,11]. The growth inhibitory effect of lutein in MCF-7 and MDA-MB-231 breast cancer cells are linked with repressed redox response and survival signaling. This indication speculated that

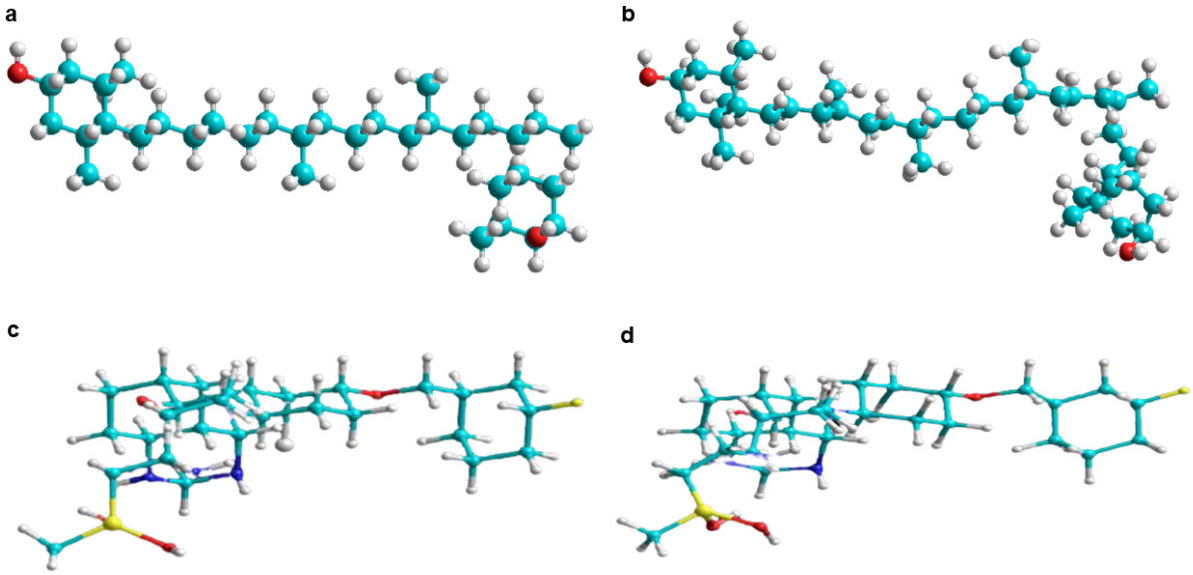

Figure 1. Optimized three-dimensional lutein and lapatinib structure. (a) Single point calculation of lutein, (b) geometry optimization of lutein, (c) single point calculation of lapatinib, (d) geometry optimization of lapatinib

lutein's attenuation of survival signals might force the breast cancer cells to undergo apoptosis [12].

However, the mechanism by which lutein inhibits the HER-2 receptor, a protein that plays a role in the growth and differentiation process of normal epithelial cells, is yet unknown [13]. Therefore, an *in silico* molecular docking can be utilized to examine the inhibition of HER-2 by lutein.

Methods

Optimization of the structure of lutein and lapatinib

Three-dimensional structure of lutein and lapatinib downloaded from https://pubchem.ncbi.nlm.nih.gov/. The three-dimensional structure of the lutein and lapatinib was optimized using the Hyperchem 8 program. Optimization was carried out using the AM1 (Austin Model 1) semi-empirical computational method, then single-point calculation and geometry optimization were performed.

Preparation of HER-2 protein

The HER-2 protein used in this study was downloaded from http://www.rcsb.org (PDB ID: 3PP0) containing native ligand (2-{2-[4-({5-chloro-6-[3-(trifluoromethyl) phenoxy]pyridine-3-yl}amino)-5H-pyrrolo[3,2d]pyrimidin-5-yl]ethoxy}ethanol) (03Q). The HER-2 protein was prepared using the Chimera 1.10.1 program to separate the protein from its native ligand.

Molecular docking validation

Molecular docking was validated by using Autodock 4.2. Grid box arrangement was also carried out by adjusting the coordinate size of the grid center and grid size, with the grid box size x = 80, y = 50, z = 50 and the grid center x = 1.259, y = 0.303, z = 0.565. 03Q was redocked on the HER-2 protein, and the protocol was valid by the value of Root Mean Square Distance (RMSD) ≤ 3.0 Å [14].

Docking lutein and lapatinib

The optimized lutein and lapatinib were then docked to the prepared HER-2 protein using Autodock 4.2 with a similar grid box size as the validation step. The docking study evaluated lutein or lapatinib's binding energy and interaction to HER-2 protein [15]. A lower binding energy value implies a stronger and more stable bond. The interaction was evaluated by analyzing the type of bonds such as hydrogen, Van der Waals, hydrophobic, and electrostatic [16].

Results

The lutein and lapatinib structures were optimized and resulted in single-point energy of -9541.96 and -8781.79 kcal/mol, and geometric energy of -11309.61 kcal/mol and -8860.12 kcal/mol, respectively. HER-2 protein was prepared by separating 03Q native ligand from protein. Chain A consisted of native ligand 03Q was selected for this study. The validation process produced

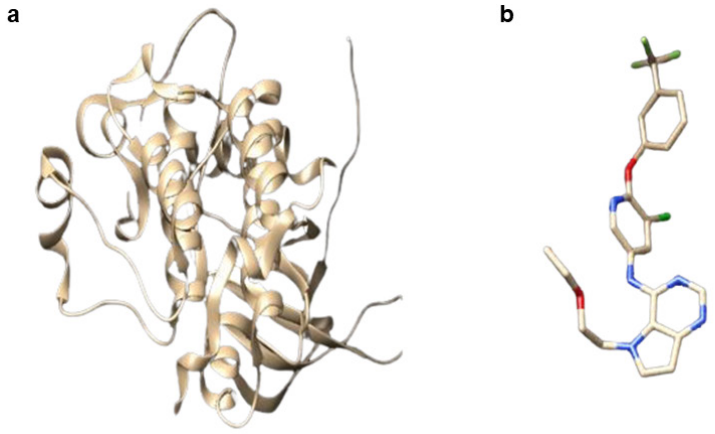

Figure 2. Preparation of three-dimensional structure of HER-2 protein. (a) structure of HER-2 protein without native ligand, (b) native ligand 03Q structure

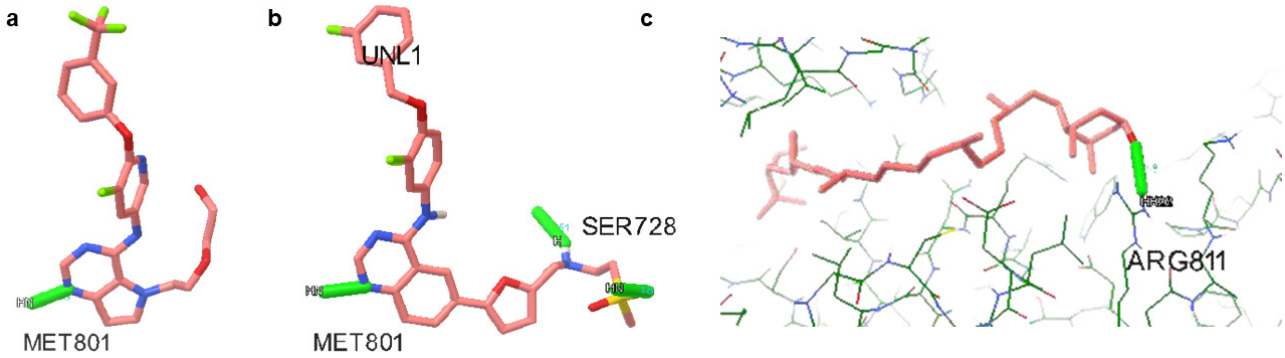

Figure 3. Interaction of native ligand, lapatinib, and lutein to HER-2. (a) native ligand 03Q, (b) lapatinib, (c) lutein

10 conformational 03Q native ligands towards HER-2 binding sites with different RMSD values and binding energies. The selected conformation had an RMSD of 0.73 Å value and met the validation criteria (RMSD ≤ 3 Å) [15]. The binding energy of 03Q native ligand to HER-2 was -10.43 kcal/mol (Table 1). The docking results showed that there were 10 conformations between lapatinib and HER-2 protein, as well as lutein and HER-2 protein. The binding energy of lapatinib was -12.25 kcal/mol (Table 2), while binding energy of lutein was -12.37 kcal/mol (Table 3).

The visualization analysis indicated that lapatinib and native ligand (03Q) interacted with methionine (MET 801) residue through hydrogen bonding interaction. This result suggests that lapatinib is able to occupy the active site of HER-2 protein as the original position of 03Q native ligand. The visualization analysis indicated that lutein interacted with arginine (ARG 811) residue through hydrogen bonding interaction. Other bonds that can form such as Van der Waals (vdw), hydrophobic (desolv), and electrostatic (elec) bonds.

Table 1. Validation of molecular docking

Protein	Ligand	RMSD (Å)	Bond energy (kcal/mol)
HER-2	03Q	1.60	-10.01
		0.73	-10.43
		2.67	-9.08
		2.28	-7.10
		1.70	-10.06
		2.67	-10.26
		2.54	-8.42
		1.84	-10.43
		2.72	-8.67
		2.85	-7.66

Discussion

Our results suggest that lutein shows the potential activity as an anticancer agent based on the lower binding affinity compared to lapatinib. The obtained binding energy of lutein was -12.37 kcal/mol lower

Table 2. Docking of lapatinib on HER-2 protein

Protein Target	Ligand	Van Der Waals, Hydrophobic Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Bonds Energy (kcal/mol)
HER-2	Lapatinib	-12.61	-0.33	-9.00
		-7.54	-0.22	-4.04
		-15.23	-0.30	-12.25
		-10.31	-0.33	-6.69
		-8.59	-0.92	-6.22
		-11.44	-0.56	-8.72
		-11.41	0.50	-7.62
		-15.02	-0.28	-11.73
		-12.75	-0.01	-9.48
		-13.05	-1.02	-10.79

Table 3. Docking of lutein on HER-2 protein

Protein Target	Ligand	Van Der Waals, Hydrophobic Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Bonds Energy (kcal/mol)
HER-2	Lutein	-14.66	-0.02	-11.97
		-15.02	-0.03	-12.37
		-14.40	-0.02	-11.71
		-13.82	-0.13	-11.14
		-8.63	-0.04	-5.99
		-10.74	-0.02	-8.05
		-12.97	-0.01	-10.29
		-10.57	-0.01	-7.89
		-10.90	0.01	-3.59
		-14.35	-0.04	-11.71

than 03Q native ligand (-10.43 kcal/mol) and lapatinib (-12.25 kcal/mol).

Lutein (xanthophyll, beta-carotene-3,3'-diol) is one of the carotenoids that have been known for their anticarcinogenic activity and is useful for combating several cancers. Carotenoid-enriched fractions of *Spondias mombin* inhibited ATP kinase domain of HER-2. By using the similar methods and protein target (HER-2, PDB ID 3PP0) with grid coordinates x = 17.1, y = 16.55, z = 26.6 of 03Q native ligand, the binding energy of astaxanthin, 7,7',8,8'-tetrahydro-β,β-carotene, β-carotene-15,15'-epoxide, and lapatinib were of -3.0, -8.5, -11.5, and -10.6 kcal/mol, respectively [17]. Another study using bioactive compounds of *Panax bipinnatifidus* and *P. pseudoginseng* indicated that luteolin 7-*O*-glucoside showed the highest binding affinity towards receptor HER2 by using Schrödinger suite 2020-3 of Glide module with docking score and Glide g score (G-Score) of -13.272 kcal/mol [18]. These two findings support that lutein as a carotenoid compound can inhibit HER-2.

In the present study, there are similarities in binding amino acid residues between 03Q native ligand and lapatinib. The hydrogen bonding interaction occurred

via methionine (MET 801) residue (Figure 3a, b). This interaction similarity indicates that lapatinib occupied the same active site of the 03Q position. Binding amino acid residues between lutein and HER2 occurred via arginine (ARG 811) residue (Figure 3c). This interaction was difference due to differences in the 3D conformational structure of the native ligand with lutein for determine the most stable state in binding to the pocket binding site HER-2. We found that lutein interacted with HER-2 protein through Van der Waals (vdw), hydrophobic (desolv), and electrostatic (elec) bonds. Future *in silico* study by using molecular dynamics is needed to further clarify the potential of lutein as anti-breast cancer.

Conclusion

In summary, this study reveals lutein interacted with HER-2 protein and promising to be developed as an HER-2 inhibitor in cancer therapy.

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Declaration of interest

The authors declare no competing interests.

Author contributions

NKNC, NPDM, NPLL conceptualized the study design; IKDD and WNEP investigated the data; NKNC and WNEP wrote the original draft; NKNC, NPDM, IKDD reviewed and edited the final version; NKNC acquitted the funding; NPLL supervised all experiments. All authors read and approved the final manuscript.

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