Molecular Docking Analysis of 6-paradol, Zingerone and Zerumbone Against Human Estrogen Receptor Alpha (ERα)

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Abstract: Molecular docking was done to assess the binding affinity of 6-paradol (6PRD), Zingerone (ZGR) and Zerumbone (ZRB) ligand-ERα complex in comparison to Hydroxytamoxifen (HTMX). Docking results showed that Glu353 and Arg394 active residues forms hydrogen bonding with 6PRD and ZGR. Glu353, Leu387 and Arg394 were the three identical residues found to formed hydrophobic interaction in HTMX-ERα, 6PRD-ERα and ZGR-ERα. HTMX showed lowest binding energy (-10.71 ± 0.43 kcal/mol) followed by ZRB (-8.66 ± 0.04 kcal/mol), 6PRD (-6.92 ± 0.14 kcal/mol) and ZGR (-5.93 ± 0.31 kcal/mol). Inhibition constant (Ki) range of 6PRD-ERα was found to be drastically lower than HTMX-ERα, ZGR-ERα and ZRB-ERα. Based on the docking analysis, the three bioactive compounds were showed to poses low potential as substitute towards tamoxifen. Future study is recommended for analysing 6PRD potential in substituting estradiol as Hormone Replacement Therapy (HRT) for breast cancer.

Keywords: 6-paradol (6PRD), Zingerone (ZGR), Zerumbone (ZRB), Hydroxytamoxifen (HTMX), ligand-ERα complex, molecular docking.

1. Introduction

Breast cancer occurs when the cells in the lobules (milk producing glands) or the ducts become abnormal and divide uncontrollably. These abnormal cells begin to invade the surrounding breast tissue and may eventually spread via blood vessels and lymphatic channels to the lymph nodes, lungs, bones, brain and liver. As reported in the Breast Cancer Facts and Figure 2015-2016 by American Cancer Society, there are around 74% of breast cancer cases which estrogen receptors (ER) are over-expressed and it is categorized as "ER-positive". ER-positive cancer cell growth is dependent towards estrogen. The growth is treated by the use of drug such as tamoxifen and raloxifene to block the estrogen receptor [2].

Human estrogen receptor has two subtypes, alpha (ERα) and beta (ERβ). They have distinct tissue distributions, regulation different separate sets of gene and differ in their affinities and activity [4]. Ali & Combees [1] stated, "The presence of elevated levels of ERα in benign breast epithelium appears to indicate an increased risk of breast cancer, suggesting a role for ERα in breast cancer initiation, as well as progression." In addition, overexpression of ERα is recurrently detected in the early

stage of breast cancer as mentioned by Hayashi et al. [6]. They reported that the specific promoter of the ERα gene is important for enhanced transcription of the gene, and identified the cis-acting elements which play a crucial role in its transcription. Moreover, methylation of the ERα gene promoters also contributes to the regulation of gene transcription. Due to the critical role of estrogen in breast cancer development, it is necessary for the estrogen mechanism to be interrupted, for example by blocking of ERα.

Zingiberofficinale, from the family Zingiberaceae, is commonly called ginger and is one of the most widely used species. It is a common additive in a large number of food products and beverages due to its flavor, color and pungency. Ginger is also reported to have medicinal properties that contains numerous bioactive compounds that have been shown to have antioxidant, anti-inflammatory, antimicrobial, antiviral, antifungal, anti-arthritic, hypotensive, antiatherogenic, radioprotective and antiemetic properties [2]. It can treat digestive disorders, nausea and vomiting, rheumatism, migraine and headaches and diabetes. The cancer preventive activities of ginger are supposed to be mainly due to free radical scavenging, antioxidant pathway, alteration of

gene expressions and induction of apoptosis, all of which contributes towardsdecrease in tumor initiation, promotion and progression [3]. Ginger also contains components shown to have anti-cancer effects, including various gingerols, gingerdione, shogaols, and paradols, as well as caffeic acid, β-elemene and zingerone.

In this study, three common bioactive compounds found in ginger namely 6-paradol (6PRD), Zingerone (ZGR) and Zerumbone (ZRB) were examined for its application in the drug design against breast cancer using molecular docking tools to provide better analysis on their binding affinity against the human receptor alpha (ERα).

2. Methodology
2.1 Data collection

The three-dimensional (3D) molecular structures of 6PRD, ZGR and ZRB were downloaded from the PubChem database including the structure of hydroxytamoxifen (Figure 1). The compound identifier (CID) and other molecular information are listed in Table 1. 3D structure of the ERα (ID: 2IOK) was downloaded from the Protein Data Bank (PDB) database and visualized in Figure 2.
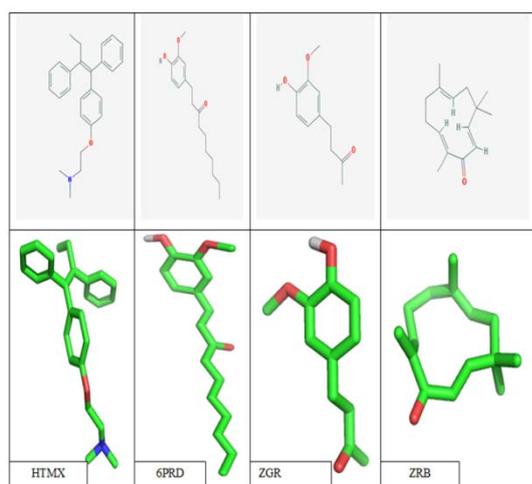
Fig. 1: Structural formula and the 3D structure of ligands molecule (carbon = green, oxygen = red, nitrogen = blue, polar hydrogen = grey).

Ligand Compound	PubChem CID	Molecular Formula	Molecular Weight (g/mol)	H-bond Donor Count	H-bond Acceptor Count	Rotatable Bond Count
HTMX	449459	$C_{26}H_{29}NO_2$	387.52	1	3	8
6PRD	94378	$C_{17}H_{26}O_3$	278.39	1	3	10
ZGR	31211	$C_{11}H_{14}O_3$	194.23	1	3	4
ZRB	5470187	$C_{15}H_{22}O$	218.34	0	1	0

Table 1: Molecular information of the ligands structure

Fig. 2: Three-dimensional structure of the dimeric human estrogen receptor alpha ligand-binding domain in complex with compound 1D (PDB ID: 2IOK)

2.2 Molecular docking

AutoDockTools (ADT) version 1.5.6, was used for molecular docking analysis [8]. ERα and ligands structures in PDB format were loaded into the docking system and extraneous water molecules were removed. To stabilize the protein system, hydrogen atoms and partial charges (Kollman and Gasteiger charges) were added to the protein. Then the molecules were exported and operated in PDBQT format. Binding region was specified within a grid map of 86 x 74 x 90 points with the default spacing of 0.375 Å. The grid box incorporates 26 of the following ERα residues; Met343, Leu346, Thr347, Leu349, Ala350, Asp351, Glu353, Leu354, Trp383, Leu384, Leu387, Met388, Leu391, Arg394, Leu402, Phe404, Val418, Glu419, Gly420, Met421, Ile424, Phe425, Leu428, Gly521, His524 and Leu525.

2.3 Molecular visualization of ERα complexes

The resulting structures from docking were viewed and analyzed using PyMOL[9]. PyMOL was also used to display the measurement of the hydrogen bond distance between the interacting atoms. Schematic 2D representation of the molecular interaction between the ligand and the ERα active site was displayed and examined using LigPlot+ software [10]. Hydrophobic interaction and hydrogen bond formation with the ERα residues are presented in the 2D schematic.

2.4 Ligand binding affinity analysis

Results of molecular docking were obtained, analyzed and visualized using ADT, LigPlot+ and PyMOL. The parameters for determining the ligand binding affinity involves the assessment of the hydrogen bonding, hydrophobic interactions, binding energy and inhibition constant (Ki) of ligand-ERα complexes.

3. Results and discussions
3.1 Hydrogen bond and binding energy

Hydrogen bond interaction was found to be crucial in the active site of ERα. The interaction may occur between hydrogen atom with electronegative atom such as nitrogen, oxygen or fluorine. Zhao and Huang [12] stated that hydrogen bonding is an exchange reaction whereby the hydrogen bonds and acceptors of the free protein and ligand break their hydrogen bonds with water and form new bonds in the protein-ligand complex.

Predominant binding modes for the HTMX-ERα, 6PRD-ERα, ZGR-ERα and ZRB-ERα ligand complexes were predicted using ADT version 1.5.6. The results were ranked according to the percentage of conformations formed in a cluster and by its correlating binding energy as presented in table 2.

Ligands	No. of H-bond	Distance (Å)	Binding Energy (kcal/mol)
HTMX	1	1.815	-10.71 ± 0.43
6PRD	2	1.809	-6.92 ± 0.14
		1.862	
ZGR	2	1.835	-5.93 ± 0.31
		1.853	
ZRB	0	-	-8.66 ± 0.04

Table 2: Docking simulation results based on the best rank of binding conformations and binding energy.

Figure 3 represent the structure of HTMX-ERα that shows one hydrogen bonds are formed between hydroxytamoxifen and one active site residue which is Arg394. HTMX-ERα has the lowest binding energy (-10.71 ± 0.43 kcal/mol). While 6PRD-ERα (Figure 4), two hydrogen bonds are formed from 6-paradol interaction with two active site residues, Glu353 and Arg394. However, the binding energy for 6PRD-ERα is higher (-6.92 ± 0.14 kcal/mol) compared to HTMX-ERα. There are also two hydrogen bonds formation between ZGR-ERα (Figure 5) and two active sites residues, Glu353 and Arg394 and the binding energy was highest of all four analyzed compound (-5.93 ± 0.31 kcal/mol). No hydrogen bond was formed from the docking of zerumbone and the binding energy was higher than HTMX-ERα but lower than 6PRD-ERα and ZGR-ERα.

The lowest energy binding required to form ligand-protein complex suggested better interaction in the active site [7]. In Table 2, it was observed that hydroxytamoxifen gave lowest binding energy followed by zerumbone, 6-paradol and zingerone respectively. However, this would be inconsistent with the second highest binding energy of zerumbone that has no hydrogen bond formation.
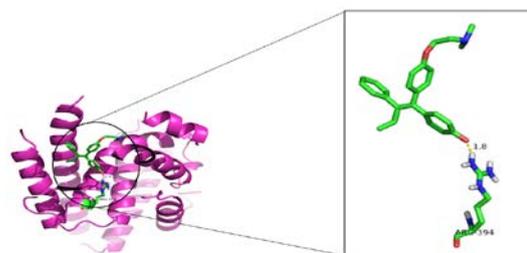
Fig. 3: Molecular representation exhibits the formation of h-bond (yellow dash line) with atomic distance of 1.8 Å between hydroxytamoxifen and ARG394 in HTMX-ERα. The ribbon structure represents the ERα.
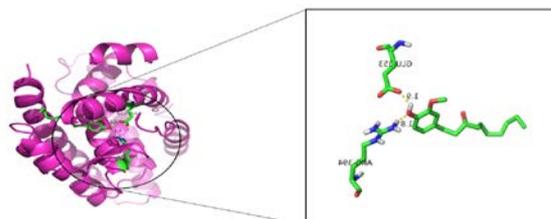
Fig. 4: Molecular representation exhibits the formation of h-bond (yellow dash line) with atomic distance of 1.8 Å between 6-paradol and two residues, GLU353 and ARG394 in PRD-ERα. The ribbon structure represents the ERα.
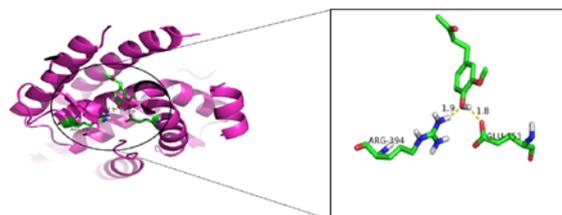
Fig. 5: Molecular representation exhibits the formation of h-bond (yellow dash line) with atomic distance of 1.8 Å between zingerone and two residues, GLU353 and ARG394 in ZGR-ERα. The ribbon structure represents the ERα.

3.2 Binding energy range and inhibition constant

As shown in Table 3, hydroxytamoxifen showed a binding energy of -10.71 ± 0.43 while 6-paradol, zingerone and zerumbone showed higher binding energy which are -6.92 ± 0.14, -5.93 ± 0.31 and -8.66 ± 0.04 respectively. The relationship between the binding energy and conformation is displayed in Figure 6. It is shown that zingerone have highest binding energy level followed by 6-paradol, zerumbone and hydroxytamoxifen respectively.

Compounds	Conformation	Binding Energy (kcal/mol)
HTMX	11	-10.71 ± 0.43
6PRD	3	-6.92 ± 0.14
ZGR	21	-5.93 ± 0.31
ZRB	56	-8.66 ± 0.04

Table 3: Binding energy of compounds based on docking ranks.

Compounds	Conformation	Inhibition Constant (µM, nM*)
HTMX	11	14.05
6PRD	3	8.51
ZGR	21	45.05
ZRB	56	449.08

Table 4: Inhibition constant of compounds based on docking ranks.
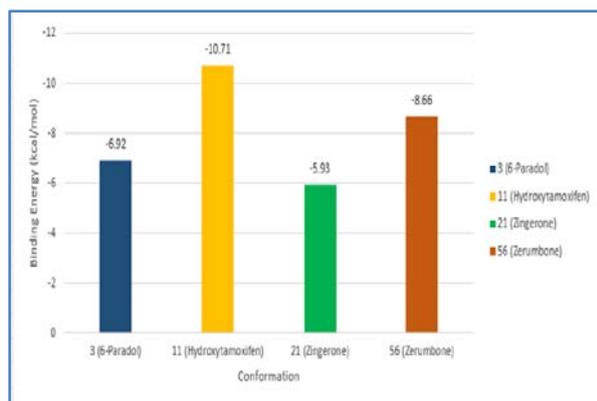
Fig. 6: Binding energy of the compounds according to their docking ranks.

Another parameter tested was the inhibition constant (K_i) of the compounds. As shown in Table 4, hydroxytamoxifen showed K_i value of 14.05 nM while 6-paradol, zingerone and zerumbone showed K_i value of 8.51 nM, 45.05 nM and 449.08 nM respectively. K_i value is reflective towards the compounds binding affinity. The smaller the K_i, the greater its binding affinity and the smaller the concentration needed to inhibit the activity of the ERα. In this study, paradol showed the lowest inhibition constant value compared to hydroxytamoxifen, zingerone and zerumbone. Based on the binding energy level and the related inhibition constant, it can be concluded that 6-paradol showed highest potential followed by hydroxytamoxifen, zingerone and zerumbone for the inhibition of ERα. Based on Table 2, there was no hydrogen bond formed between zerumbone and ERα because zerumbone has lowest K_i value which is 449.08 nM compared to hydroxytamoxifen, 6-paradol and zingerone.

3.3 Schematic 2D representation of H-bond and hydrophobic interaction

Apart from hydrogen bond interaction, hydrophobic interaction also plays essential part in the active site. Although the interaction is not as strong as hydrogen bond, hydrophobic interaction can show remarkable changes in docking free energy between ligand and enzyme [11].

The resulting hydrophobic interaction analyzed from the docking structure is as presented in Figure 7, Figure 8, Figure 9 and Figure 10. The active site residues involved with the hydrophobic interaction in HTMX-ERα, 6PRD-ERα, ZGR-ERα and ZRB-ERα were presented in Table 5.

Compounds	No. of Residues	Interacting residues
Hydroxytamoxifen	17	Leu346, Thr347, Leu349, Ala350, Asp351, Glu353, Leu354, Trp383, Leu384, Leu387, Met388, Leu391, Arg394, Phe404, Met421, Leu428, Gly521
6-Paradol	14	Leu346, Leu349, Ala350, Glu353, Leu384, Leu387, Met388, Leu391, Arg394, Phe404, Ile424, Gly521, His524, Leu525
Zingerone	12	Leu346, Leu349, Ala350, Glu353, Leu384, Leu387, Met388, Leu391, Arg394, Phe404, Met421, Leu428
Zerumbone	12	Pro324, Pro325, Ile326, Leu327, Glu353, His356, Mt357, Ile386, Leu387, Arg394, Gly398, Lys449

Table 5: Hydrophobic interacting residues of ERα

Hydroxytamoxifen formed hydrophobic interaction with more residues compared to 6-paradol, zingerone and zerumbone. The hydrophobic contacts in HTMX-ERα have 17 interacting residues whereas 6-PRD-ERα, ZGR-ERα and ZRB-ERα have 14, 12 and 12 interacting residues respectively. Three identical residues formed hydrophobic interaction in all complexes which are Glu353, Leu387 and Arg394. Although the number of molecules of 6-paradol, zingerone and zerumbone is comparatively lesser than hydroxytamoxifen, they showed relatively high number of hydrophobic contacts.

Hydrogen bonding and hydrophobic interactions are important factors that affect the thermodynamics of ligand-receptor binding [5]. The stability of ligand-receptor relationship is depends on hydrophobic contacts especially in the positioning of the ligands inside the binding site. The higher number of hydrophobic contacts in HTMX-ERα and the differing amino acid residues involved may have contributed to HTMX-ERα lowest binding energy and second lower in K_i value as compared to 6-PRD-ERα, ZGR-ERα and ZRB-ERα. Residues involved in hydrogen bonding in HTMX-ERα (Asp351 and Arg394), 6-PRD-ERα (Glu353 and Arg394) and ZGR-ERα (Glu353 and Arg394) also form hydrophobic contacts with its respective ligand which further stabilizes the hydrogen bond formation.
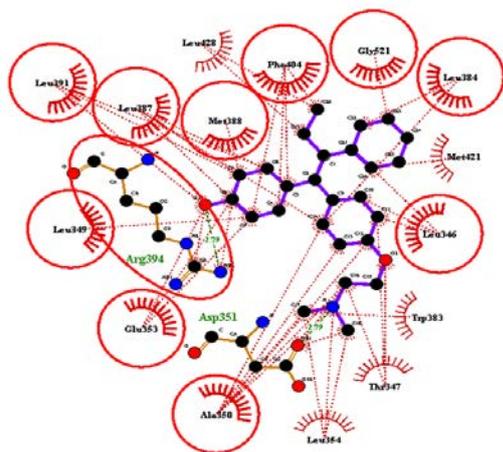
Fig. 7:Schematic 2D representation of hydrophobic interaction and hydrogen bonding from docking analysis of HTMX-ERα (Interactions labeled in dash line, red = hydrophobic contacts, green = hydrogen bond).

Fig. 8: Schematic 2D representation of hydrophobic interaction and hydrogen bonding from docking analysis of 6-PRD-ERα (Interactions labeled in dash line, red = hydrophobic contacts, green = hydrogen bond).
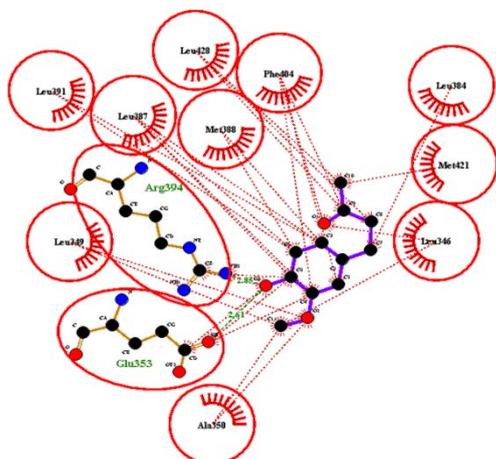
Fig. 9: Schematic 2D representation of hydrophobic interaction and hydrogen bonding from docking analysis of ZGR-ERα (Interactions labeled in dash line, red = hydrophobic contacts, green = hydrogen bond).

Fig. 10: Schematic 2D representation of hydrophobic interaction and hydrogen bonding from docking analysis of ZRB-ERα (Interactions labeled in dash line, red = hydrophobic contacts, green = hydrogen bond). No hydrogen bond was formed in ZRB-ERα.

4. Conclusion

The ligand binding affinity of 6-paradol, zingerone and zerumbone against estrogen receptor alpha (ERα) was evaluated using molecular docking analysis. The potential of the three bioactive compounds compared to the synthetic drug, hydroxytamoxifen was established. Results suggest that hydroxytamoxifen have high level of interactions with ERα active site in terms of hydrogen bonding and hydrophobic interactions. Three identical residues are involved in hydrophobic interaction in all four ligand-ERα complexes which are Glu353, Leu387 and Arg394. All hydrogen bonded residues in HTMX-ERα, 6-PRD-ERα and ZGR-ERα also formed hydrophobic contacts with its respective ligands which specify the importance of the two interacting residues (Glu353 and Arg394). However, the binding energy of three ginger bioactive compounds is exceptionally higher than hydroxytamoxifen and thus poses low potential as substitute. Binding energy for zingerone is marginally lower than 6-paradol but with high variation in Ki value. While 6-paradol gave the lowest inhibition constant value compared to hydroxytamoxifen, zingerone and zerumbone. Estradiol is a form of estrogen that is commonly used in hormone replacement therapy (HRT) along with the administration of hydroxytamoxifen as treatment against ER-positive breast cancer. The use of estradiol remains controversial due to the high risk of adverse effect. According to the findings of this study, zingerone show potential for further studies to determine a substitute for the estradiol in HRT against ER-positive breast cancer. Wide range of other bioactive compounds of ginger are also excluded in this research and thus can be used as an extension to this study.

Acknowledgement

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