Molecular Docking and Dynamics (MD) Simulation of 6-gingerol and 6-shogaol Against Human Estrogen Receptor Alpha (ERα)

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Abstract: Simulation and computational analysis of 6-gingerol and 6-shogaol is done to evaluate their binding affinity against ERα. Active site prediction was done using Computed Atlas of Surface Topography of Proteins (CASTp) to determine the binding pocket of ERα. Molecular docking and molecular dynamics (MD) simulation were done to assess the binding affinity and stability of the ligand-ERα complexes formed. Results showed that Tamoxifen have lowest binding energy (-9.61 ± 0.39 kcal/mol) followed by 6-gingerol (-6.59 ± 0.29 kcal/mol) and 6-shogaol (-5.70 ± 0.36 kcal/mol). Inhibition constant (Ki) range of TMX-ERα was found to be drastically lower than both 6GN-ERα and 6SG-ERα. Based on the difference in the binding energy range and inhibition constant, 6-gingerol and 6-shogaol showed less potential in substituting tamoxifen for the inhibition of ERα. Docking complexes formed was supported with stability in root mean square deviation (RMSD) and total binding energy of the complexes. The study is concluded that 6-gingerol have high level of interactions with the ERα active site in terms of hydrogen bonding whereas hydrophobic interactions are observed with both 6-gingerol and 6-shogaol. However, both ginger bioactive compounds poses low potential as substitute in comparison with tamoxifen against ERα.

Keywords: 6-gingerol, 6-shogaol, tamoxifen, molecular docking, molecular dynamics simulation, ginger

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1. Introduction

Most Asian countries are struggling with an increasing prevalence of breast cancer and it is becoming a major cause of morbidity and mortality amongst women [1]. Malaysian breast cancer patients are also struggling from getting the best of treatments. As reported by Abdullah et al.[1], Malaysian breast cancer patients have a lower overall 5-year survival rate as compared to those in developed countries in the cohort of 2000 to 2005. Yip et al.[16] stated an over 80% of 5-year survival rate for USA breast cancer patients which received optimal treatment. Most current treatments against breast cancer are mostly directed towards preventive strategy for early detection and intervention in order to increase the survival rates [16].

The use of "natural" or alternative medicines for treatment against breast cancer has increased over the last few years. Due to the adverse effects of the synthetic breast cancer drugs, the public have been more favorable to accepting drugs of natural sources especially from plants due to the fewer side effects and its abundant nature. Synthetic drugs like tamoxifen and raloxifine are usually associated with high level of toxicity and harmful adverse effects from its administration [3]. Furthermore, natural products have also shown to have high potential in designing a more effective drug compared to synthetic drugs [4].Ginger rhizome (*Zingiberofficinale*) is one of a natural plants that being promoted as a cancer treatment to help keep tumors from developing [8].The two well-known biologically active constituents of ginger are the gingerols and shogaols where many homologues of these compounds exist. 6-gingerol is a major pungent phenolic compound found in ginger which has been reported to exhibit antioxidant activity through inhibition of phospholipid peroxidation supported by in vitro and in vivo approach [8]. Shogaols are the product of dehydration reaction of the gingerols and is therefore present in larger amount in dried ginger. Moreover, ginger is widely used in many practices such as for making spices, cooking and as traditional medicine.

The constituents of ginger may show promising anti-cancer properties but pharmacological studies on its effects against breast cancer are scarce. Through the exploitation of bioinformatics, such potential can be established and further examined for its application in the drug design against breast cancer. In this study,in-silico analysis of 6-gingerol and 6-shogaol against the human breast cancer estrogen receptor alpha (ERα) were conducted to verify their potential interactions. Findings from this study will provide the scope for investigating these two compounds interaction against ERα which can be extrapolate for their suitability as alternative natural formulated breast cancer drugs.

2. Methodology

2.1 Data collection

The three-dimensional molecular structure of the bioactive compounds 6-gingerol and 6-shogaol were downloaded from the PubChem database including the structure of tamoxifen (Figure 1). The compound identifier (CID) and other molecular information are listed in Table 1. Three-dimensional structure of the human estrogen receptor alpha (ID: 2IOK) was acquired from the Protein Data Bank (PDB) database and was visualized as in Figure 2.
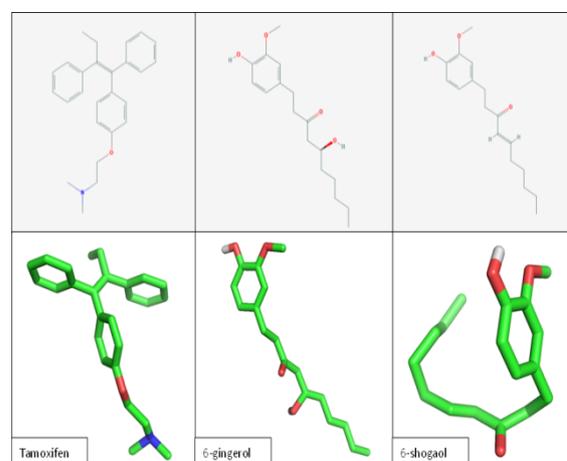
Fig. 1: Structural formula obtained from PubChem and the 3D structure of ligands molecule as viewed in PyMOL (carbon = green, oxygen = red, nitrogen = blue, polar hydrogen = grey).

Ligand Compound	PubChem CID	Molecular Formula	Molecular Weight (g/mol)	H-bond Donor Count	H-bond Acceptor Count	Rotatable Bond Count
Tamoxifen	2733526	$C_{26}H_{29}NO$	371.5145	0	2	8
6-Gingerol	442793	$C_{17}H_{26}O_4$	294.3859	2	4	10
6-Shogaol	5281794	$C_{17}H_{24}O_3$	276.3706	1	3	9

Table1:Molecular information of the ligands structures obtained from PubChem.

Fig. 2: Visualization of ERα structure and the key active site residues as obtained from CASTp server and reviewed using PyMOL (blue, green & red = ERα, green & red = CASTp predetermined active site residues, red = incorporated residues in AutoDock grid map).

2.2 Binding pocket prediction

The active site residues for the ERα were pre-determined prior to the docking analysis. The downloaded receptor structure was submitted to Computed Atlas of Surface Topography of Proteins (CASTp) online server. CASTp server enables the identification and measurements of the accessible pockets and voids on the 3D receptor structure that allows the visualization of the functional regions, surface structure and key residues of the active site [6, 10]. CASTp calculation (JobID: JIDSX87143Q) was done using the default probe radius of 1.4 Å.

2.3 Molecular docking using autodocktools

Molecular docking was done using AutoDockTools (ADT) version 1.5.6 [11]. ERα and ligands structures were loaded into the docking system in PDB format. Extraneous water molecules are removed from the protein system. The protein system was stabilized by adding hydrogen atoms and partial charges (Kollman and Gasteiger charges). The molecules are then exported and operated in PDBQT format.The binding region was specified within a grid map of 50 x 44 x 58 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. All of the incorporated residues coincide with the determined pocket from CASTp.Molecular docking was proceeded using Lamarckian Genetic

Algorithm (LGA) with an overall of 100 docking runs. The RMS tolerance for the docking analysis was set to 1.0 Å. The docking for all the studied compounds was done in triplicates. The structure of the ligand-ERα complexes resulted from the docking analysis was extracted for the molecular dynamics (MD) simulation.

2.4 Molecular dynamic (MD) simulations

The MD simulation was done using GROMACS 4.6.5 [2]. Topology files for both the receptor and the ligands were generated separately. GROMOS96 53A6 force field was used to generate the topology for the receptor while the topology for the ligands was generated using an online server, PRODRG [12].

A cubic simulation box was generated for the MD simulation where the protein complex was solvated with SPC/E water solvent. 8 Na^+ ions were added to neutralize the negatively charged ligand-ERα complex so as to produce a neutral net system charge. Energy minimization of the system was carried out to ensure no steric clashes or inappropriate geometry on the complex. Position restraint equilibration was performed on the complex so as to restrain particles/atoms at a fixed reference position. The system was equilibrated at constant temperature (303 K) and pressure (1 atm) and the MD simulation was finally operated to production stage of 10,000 pico-seconds (ps).

The resulting structures from docking and MD simulation were viewed and analyzed using PyMOL[13]. 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 [15].

Data results from the MD simulations were obtained using programs available within the GROMACS simulation package as listed in Table 2. An application called GRACE was used to plot and analyze the data.

Programs	Function
g_rms	Compares two structures by computing the root mean square deviation (RMSD).
g_energy	Extracts energy components or distance restraint data from an energy file.
g_hbond	Calculate hydrogen bonds formations as a function of time.
trjconv	Extract trajectory files into PDB format.

Table 2:List of GROMACS programs used in result collection and analysis.

3. Results and discussions

3.1 Molecular docking analysis

3.1.1 Hydrogen bond and binding energy

ADT version 1.5.6 was used to predict predominant binding modes for the ligand-receptor interactions. The results from the dockings are ranked according to the percentage of conformations formed in a cluster and by its correlating binding energy. The conformations cluster of the highest rank was selected for analysis (Table 3). The docking simulation between the ligands and ERα resulted in three ligand-receptor complexes, TMX-ERα, 6GN-ERα and 6SG-ERα.

Ligands	No. of H-bond	Interacting Residues	Distance (Å)	Binding Energy (kcal/mol)
Tamoxifen	1	Asp351	2.718	-9.61 ± 0.39
6-Gingerol	2	Glu353	1.855	-6.59 ± 0.29
		Arg394	1.933	
6-Shogaol	0	-	-	-5.70 ± 0.36

Table 3:Docking simulation results based on the best rank of binding conformations and binding energy.

Figure 3 represent the structure of TMX-ERα that shows one hydrogen bond formation between tamoxifen and an active site residue, Asp351. TMX-ERα has the lowest binding energy (-9.61 ± 0.39 kcal/mol). As compared to 6GN-ERα (Figure 4), two hydrogen bonds are formed from the 6-gingerol interaction with two active site residues, Glu353 and Arg394. However, the binding energy

for 6GN-ERα is higher (-6.59 ± 0.29 kcal/mol) compared to TMX-ERα. No hydrogen bond was formed from the docking of 6-shogaol and the binding energy was highest of all three analyzed compounds (-5.70 ± 0.36 kcal/mol).

Structure of proteins and their binding with ligands are primarily determined by hydrogen bonding. As affirmed by Zhao and Huang [17], ligand binding is influenced by the breaking of hydrogen bonds with water molecules and formation of new hydrogen bonds between ligand and receptor. Conversely, the higher binding energy of 6GN-ERα compared to TMX-ERα determines a lower binding affinity to ERα. Formation of hydrogen bond that leads to unfavorable geometry of the ligand-receptor interaction could be a factor decreasing the binding energy. As supported by Karaman et al. [5], ligand binding is improved by the detachment of group that forms hydrogen bond in incoherent geometry. However, this would be inconsistent with the highest binding energy of 6SG-ERα that has no hydrogen bond formation. Alternatively, the scoring function for the binding energy has its own limitations. Zhao and Huang [17] stated that a majority of scoring functions do not include the enthalpic loss of hydrogen bonding in ligand binding interaction adequately. According to the binding energy of the best ranked conformations, the binding affinity of tamoxifen is highest followed by 6-gingerol and 6-shogaol respectively.
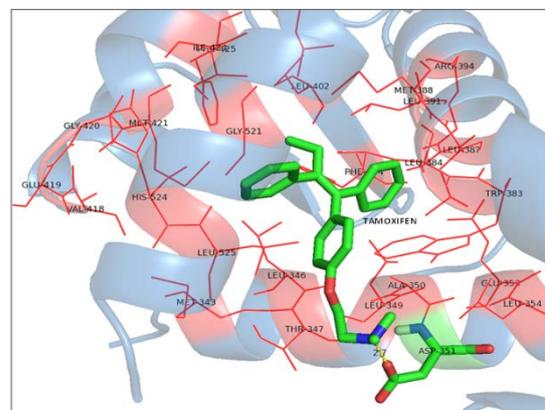
Fig. 3:Molecular representation exhibits the formation of h-bond (yellow dash line) with atomic distance of 2.7 Å between tamoxifen and Asp351 in TMX-ERα (carbon = green, oxygen = red, nitrogen = blue). The ribbon structure represents the ERα while the line structure (red) represents the pocket residues.
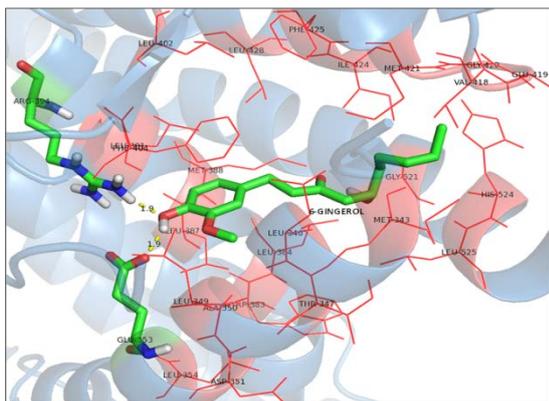
Fig. 4:Molecular representation exhibits the formation of h-bond (yellow dash line) with atomic distance of 1.9 Å between 6-gingerol and two residues, Glu353 and Arg394 in 6GN-ERα (carbon = green, oxygen = red, nitrogen = blue). The ribbon structure represents the ERα while the line structure (red) represents the pocket residues.

The interacting residues forming hydrogen bond in TMX-ERα is Asp351. As asserted by Liu et al. [9], "Amino acid Asp351 in the ligand binding domain of estrogen receptor alpha (ERα) plays an important role in regulating the estrogen-like activity of selective estrogen receptor modulator-ERα complexes". However, as documented by Dayan et al. [3], tamoxifen and most of its derivatives interact with Asp351 in an unfavorable mode.

The two active site residues determined in the hydrogen bonding with 6-gingerol are similar to that of estradiol. As stated by Kumar et al. [7], hydroxyl groups of the natural estrogen ligand, estradiol formed hydrogen bond with the active site residues Glu353 and Arg394 of ERα. Although the interacting residues for hydrogen bonding between 6-gingerol and tamoxifen differ, 6-gingerol formed stronger hydrogen bonding within the ERα pocket and thus could pose other ligand binding importance to the inhibition of the receptor.

3.1.2 Inhibition constant and binding energy range

As shown in Table 4, tamoxifen showed a binding energy range of -8.91 kcal/mol to -9.84 kcal/mol while 6-gingerol and 6-shogaol showed higher binding energy range of -5.12 kcal/mol to -7.22 kcal/mol and -5.66 kcal/mol to -7.58 kcal/mol respectively. The relationship between the binding energy is displayed in Figure 5. It is shown that tamoxifen have the highest binding energy level followed by 6-shogaol and 6-gingerol respectively. Another parameter tested was the inhibition constant (Ki) of the compounds. As shown in Table 5, tamoxifen showed Ki range of 65.79 nM

to 292.25 nM while 6-gingerol and 6-shogaol showed Ki range of 5.09 µM to 175.26 µM and 2.77 µM to 70.88 µM respectively. As according to Umamaheswari et al. [14], the inhibition constant is directly proportional to the binding energy. It is shown from the data that the decrease in inhibition constant of the compounds coincides with the decrease in the binding energy.
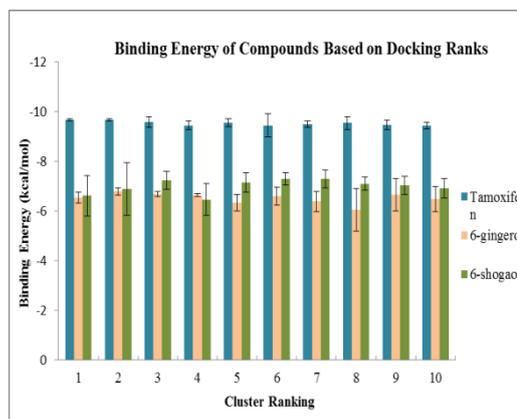
Fig.5:Bar chart exhibits the relationship of binding energy between the compounds according to the docking ranks.

3.2 Molecular dynamics (MD) simulation analysis

3.2.1 Ligand-ERα complex service

MD simulation was done to provide extensive data on the fluctuations and conformational changes of the ligand-receptor complexes. The atoms and molecules within the ligand-receptor system were allowed to interact within a timeframe of 10,000 ps. The parameters for determining the structural changes and stability of the ligand-receptor complexes involve the assessment of the root mean square deviation (RMSD) and the total energy.

3.2.2 Root mean square deviation

Root mean square deviation (RMSD) was calculated to evaluate the stability of the complexes obtained from the docking. It determines the deviation in the average distance of the backbone structure movement in the complexes. As confirmed by Morris and Lim-Wilby[18], success in docking is generally measured in terms of RMSD with arbitrary threshold of 2 Å or 0.2 nm.

As depicted in Figure 6, 6GN-ERα achieved stability at 2,000 ps with a standard deviation (SD) of ± 0.15 nm whereas the TMX-ERα achieved stability at 3,000 ps with SD of ± 0.15 nm. The 6SG-ERα achieved stability at 7,000 ps with SD of ± 0.20 nm. TMX-ERα, 6GN- ERα and 6SG-ERα showed minimal deviation in the RMSD which are

below 0.2 nm which confirms the docking structure stability. The results suggested that the simulation time established for the MD analysis was sufficient.
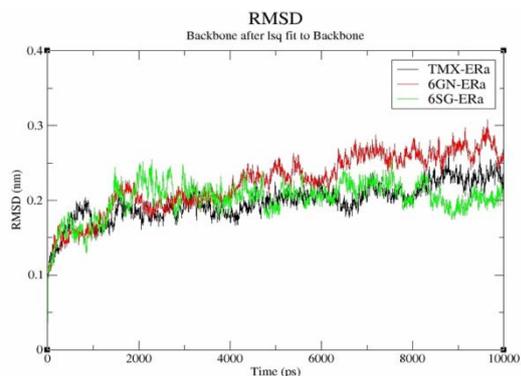
Fig. 6: Root mean square deviation (RMSD) graph against time for TMX-ERα, 6GN-ERα and 6SG-ERα complexes.

Compounds	Runs	Binding energy of compounds based on their rank (kcal/mol)									
		1	2	3	4	5	6	7	8	9	10
Tamoxifen	1	-9.61	-9.62	-9.35	-9.27	-9.44	-8.91	-9.59	-9.33	-9.31	-9.29
	2	-9.71	-9.67	-9.61	-9.45	-9.74	-9.69	-9.35	-9.47	-9.67	-9.57
	3	-9.68	-9.71	-9.80	-9.62	-9.51	-9.73	-9.56	-9.84	-9.44	-9.46
6-Gingerol	1	-6.30	-6.69	-6.61	-6.58	-6.55	-6.32	-6.22	-6.17	-5.94	-5.89
	2	-6.59	-6.95	-6.79	-6.68	-6.49	-6.44	-6.08	-5.12	-7.22	-6.82
	3	-6.74	-6.72	-6.65	-6.64	-5.95	-7.01	-6.84	-6.81	-6.78	-6.74
6-Shogaol	1	-5.70	-7.41	-7.39	-6.79	-7.33	-7.05	-6.88	-6.79	-6.62	-6.47
	2	-6.90	-7.58	-6.81	-5.73	-7.42	-7.31	-7.53	-7.26	-7.24	-7.18
	3	-7.25	-5.66	-7.49	-6.87	-6.71	-7.54	-7.47	-7.26	-7.23	-7.09

Table 4:Binding energy of compounds based according to docking rank (red = highest value, green = lowest value).

Compounds	Runs	Inhibition Constant of compounds based on their rank (μM, nM*)									
		1	2	3	4	5	6	7	8	9	10
Tamoxifen	1	89.84*	88.85*	141.18*	160.82*	119.4*	292.25*	93.07*	145.92*	150.16*	155.62*
	2	76.56*	81.05*	90.14*	118.24*	72.13*	79.30*	140.07*	114.01*	82.07*	96.26*
	3	80.84*	76.52*	65.79*	88.35*	107.64*	73.47*	97.52*	113.16*	121.14*	116.45*
6-Gingerol	1	24.07	12.46	14.29	15.13	15.80	23.23	27.65	29.94	44.37	47.92
	2	14.80	8.09	10.62	12.66	17.45	19.02	34.78	175.26	5.09	9.99
	3	11.42	11.83	13.43	13.68	43.15	7.28	9.70	10.12	10.69	11.44
6-Shogaol	1	66.17	3.71	3.80	10.62	4.21	6.76	9.0	10.47	13.98	17.95
	2	8.82	2.77	10.19	62.71	3.62	4.41	3.04	4.76	4.97	5.45
	3	4.86	70.88	3.25	9.15	12.04	2.98	3.33	4.80	5.01	6.33

Table 5: Inhibition constant, Ki, of compounds based according to docking rank (red = highest value, green = lowest value).

3.2.2 Total energy

Total energy was taken to analyze the equilibrium and stability of the ligand-ERα complexes along the duration of the simulation. According to Figure 7, the total energy for all three complexes is seen to average within miniscule range. The average total energy for 6GN-ERα is lowest (-661491 kJ/mol) followed by 6SG-ERα (-662214 kJ/mol) and TMX-ERα (-662178 kJ/mol) as shown in Appendix D. Constant equilibrium of the total energy was obtained for TMX-ERα, 6GN-ERα and 6SG-ERα along 10,000 ps of the simulationtimeframe. Stability of the complexes was shown in terms of its total energy throughout the simulation.
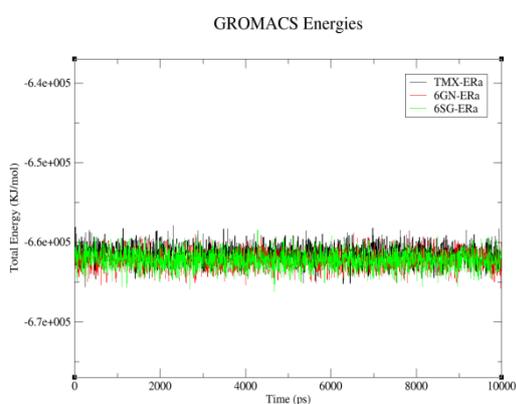
Fig. 7: Total energy graph against time.

4. Conclusion

The potential of the two bioactive compounds compared to the synthetic drug, tamoxifen was established. Results suggest that 6-gingerol have high level of interactions with the ERα active site in terms of hydrogen bonding. However, the binding energy of both ginger bioactive compounds is exceptionally higher than tamoxifen and thus poses low potential as substitute. Nevertheless,6-gingerol formed strong hydrogen bond with Glu353 and Arg397 residues of the ERα. Similar residues of ERα are found to form hydrogen bond with estradiol. Estradiol is a form of estrogen that is commonly used in hormone replacement therapy (HRT) along with the administration of tamoxifen as treatment against ER-positive breast cancer. The use of estradiol remains controversial due to the high risk of adverse effects. According to the findings of this study, 6-gingerol 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 extent to this study.

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Supplementary

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