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RESEARCH ARTICLE

Inhibition of Breast Cancer Proteins by the Flavonoid Naringenin and its Derivative: A Molecular Docking Study

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Abstract

Cancer is a multifaceted disease and is a major health burden in the world. Breast cancer is leading cause of mortality among women worldwide. Plant derived compounds have also been used in the treatment of cancer. Amongst them, flavonoids have been well documented for their therapeutic potential against cancer cells. Naringenin is a flavanone abundantly available in grapefruit and tomato among other sources. Several natural and synthetic derivatives of naringenin have been reported for anticancer activity. In this study, naringenin (Nar) and its derivative, naringenin 2-hydroxy benzoyl hydrazone (Nar-Bhz) were studied for their inhibitory potential against proteins involved in breast cancer. Molecular docking simulation by AutoDock was utilized to investigate the interaction of Nar and Nar-Bhz with Survivin, Estrogen receptor α (ERα), progesterone receptor (PR), Akt1, and Epidermal growth factor receptor (EGFR). Doxorubicin was used as positive control because of its clinical importance in breast cancer treatment. Discovery Studio Visualizer was used to visualize the interactions and the docking results showed that the protein ligand complexes were stabilized by hydrogen bonding and hydrophobic interactions. The binding energies ranged between -7.66 to -7.91 kcal/mol with Nar-Bhz and between -5.49 and -11.05 kcal/mol for Nar. Significant inhibition constant was observed for Nar-Bhz interaction with Akt1 and EGFR. Also, several residues of Akt1 interacted with both the ligands. It can be concluded that naringenin and its derivative have promising inhibitory potential against the breast cancer proteins. The findings of this study may pave the way for detailed exploration of naringenin as breast cancer drug and as a nutraceutical or dietary supplement in daily intake.

Keywords: Breast Cancer, Docking, Flavonoids, Molecular, Naringenin, Naringenin Derivative

1. Introduction

Cancer is a chronic disorder marked by immortal cells with uncontrolled proliferation. Cancer cells may turn invasive, aggressive and may become metastaticif untreated. Breast cancer occurs by uncontrolled proliferation of mammary epithelial cells with heterogeneous nature. It is the most common cancer and a leading cause of mortality among women worldwide[1]. Amongst the known risk factors of breast cancer such as age, genetics, familial history, lifestyle etc. estrogen and progesterone hormones also increase breast cancer risk[2,3]. Chemotherapeutic agents exert their activity against cancer by inducing apoptosis. But unfortunately, most agents do not distinguish between cancer and normal cells thereby affecting rapidly dividing normal cells such as hair follicle cells or bone marrow cells[4]. Toxicity remains to be a major concern in the use of these agents due to their synthetic origin. Plant derived compounds have shown remarkable therapeutic potential with minimal toxicity against

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normal cells[5]. One such group of plant secondary metabolite with widespread, pharmacologically important compounds are the flavonoids. Flavonoids containing foods can be consumed aspart of regular diet to control the proliferation of cells[6].

Flavonoids have exhibited antioxidant, anticancer, antimicrobial, and anti-inflammatory activities, to name a few[6,7]. Ideally, any substance used as anticancer agent should be able to specifically cause damage to cancer cells while exerting minimal toxicity to normal cells[8]. Flavonoids have been reported to possess specific cytotoxicity against cancer cells and hence are considered to be ideal for development of cancer chemotherapeutics[9,10].

In this research, one of the flavanones, naringenin which is present abundantly in fruits and vegetables was selected. Naringenin ($C_{15}H_{12}O_5$; molecular weight: 272.6 g/mol) is abundantly present in grapefruit, tomato skin, and oranges among other sources. Chemically it is known as 5,7-dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydrochromen-4-one. Naringenin is endowed with great therapeutic potential against various diseases and conditions[11]. Naringenin also has a notable breast cancer activity as reflected by studies in SKBR3 and MDA-MB-231 breast tumor cells, where it inhibited HER2 tyrosine kinase activity and also exerted antiproliferative and anti-apoptotic effects[12]. It also exhibited anticancer effects in Balb/c mice inoculated with breast carcinoma 4T1-Luc2 cells[13]. As seen from Figure 1(a), naringenin has three hydroxyl groups at 4', 5 and 7 positions. Substitutions at 4'- and 7- hydroxyl positions are relatively easier than substitutions at 5-hydroxyl which forms a H-bond with C4 ketone group.

Several natural derivatives of naringenin have been reported in literature[14]. In this study, a benzoyl hydrazine derivative of naringenin (Nar-Bhz) was studied to understand its antagonistic activity against breast cancer proteins (Figure 1b). Naringenin is substituted at the ketone group at C4 with a 2-hydroxy benzoyl hydrazone[15,16]. Reaction of hydrazine with carbonyl groups result in hydrazone formation. Hydrazone bonds are widely studied for their easy preparation, promising stability and sensitivity. These bonds hydrolyze at acidic pH and are used in preparation of acid sensitive carriers for stimuli responsive cancer therapies[17,18]. Nikolova-Mladenova *et al.* Synthesized 5-methoxysalicylaldehyde benzoyl hydrazones and demonstrated their efficacy against breast and leukemic cell lines[19].

This study investigated the inhibitory potential of Nar and Nar-bhz on proteins involved in cancer cell survival and growth using molecular docking. Doxorubicin was used as positive control. National Cancer Comprehensive Network, 2016 has recommended doxorubicin to be used as standard drug in breast cancer adjuvant chemotherapy[20]. The proteins Survivin, Estrogen Receptor α, Akt1, and Epidermal Growth Factor Receptor were used in the study. Expression of Inhibitors of Apoptosis Proteins (IAP) contributes to resistance in tumor cells. Survivin is one of the IAPs and plays a role in apoptosis inhibition, cell cycle regulation and chemo-resistance[21]. Hence,

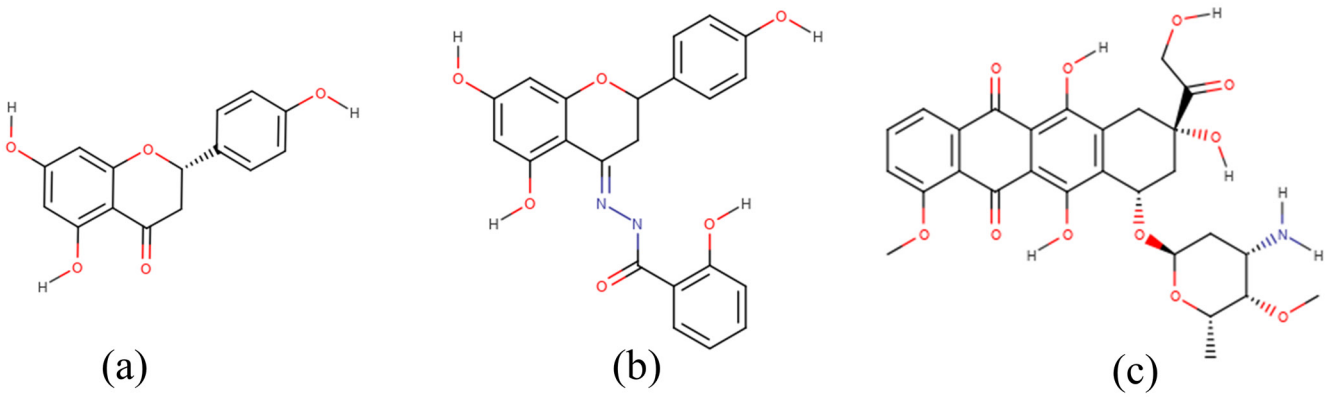

Figure 1. Chemical structures of the ligands used in the study, naringenin **(a)**, naringenin 2-hydroxy benzoyl hydrazone **(b)** and doxorubicin **(c)**.

survivin appears to be a promising strategy to ameliorate prognosis and overcome resistance in breast cancer.

Estrogen receptor α (ERα) is ubiquitously expressed and is involved in development and functioning of major organs[22,23]. The transcriptional activity mediated by ERα drives almost 75% of breast cancers[24]. Progesterone is a versatile hormone which exerts its functions in differentiation, development of female reproductive system. This hormone is capable of dual roles of cell proliferation and growth inhibition, depending on the cellular environment, cell type and stage of development[25]. Progesterone Receptor (PR), a member of the nuclear receptor superfamily, mediates the cellular biological effects of progesterone. Estrogen and progesterone may act as powerful mitogens in several tissues including the breast tissue wherein they are involved in breast cancer progress and development[26]. Based on histopathological evidences, it is now known that the breast cancer cells express either estrogen receptor or progesterone receptor or both. Hence, adjuvant cancer therapy involves starving these cells of hormones[27]. The phosphatidylinositol-3-kinase (PI3K) pathway plays an important role in survival of different cell types. The Akt protein (protein kinase B) is a serine/threonine kinase which regulates survival, proliferation, apoptosis and metabolism[28]. ErbB receptors (EGFR1, HER2, ErbB3, ErbB4) show abnormal signaling in many cancer types such as those of the breast, bladder, ovary, colorectal etc[29–31]. EGFR1 (commonly referred to as EGFR) is an attractive target owing to its tyrosine kinase activity.

Given the importance of these proteins in cell survival, proliferation and development, they were chosen as targets in the study. The aim of the study was to understand the inhibitory potential of Nar and Nar-Bhz against cancer proteins using molecular docking analysis in comparison to control. The binding affinities of the compounds were analyzed to elucidate the mechanism of inhibitory action. Both the ligands demonstrated significant inhibition of the proteins as evidenced by hydrogen bonding and hydrophobic interactions with the binding site residues of the proteins.

2. Experimental Method

2.1 Preparation of Proteins and Ligands

All the docked conformation of Nar/Nar-Bhz-protein interactions were obtained by AUTODOCK 4.0[32]. The structures of Nar and Nar-Bzh were drawn and optimized using MARVINSKETCH. The crystal structures of Survivin (PDB ID: IF3H), Estrogen receptor α (PDB ID: 1X7E), Progesterone Receptor (PDB ID: 4OAR), AKT1 (PDB ID: 3O96) and Epidermal Growth Factor Receptor (PDB ID: 6DUK) were obtained from RCSB PDB and optimized using Discovery Studio Visualizer.

2.2 Preparation of Input Files

AUTODOCK 4.0 tool (ADT) was used to add polar hydrogens and Kollman charges to the proteins. ADT adds Kollman charges for the protein and Gasteiger charges for ligand molecule. The torsions in ligand were detected and optimized. Both ligand and protein input files were generated and saved in the .pdbqt format for further analysis. Grid boxes of 40Å x 40Å x 40Å and 0.375 Å spacing were generated. The grid boxes were placed at xyz coordinates of 29.133, -3.836, 17.330 (Survivin); 27.424, 18.636, 20.307 (ERα); 10.993, 26.873, 16.019 (PR); 9.379, -8.847, 16.843 (AKT1) and 40.275, 93.643, -63.622 (EGFR).

2.3 Docking Study

Lamarckian Genetic Algorithm was used to perform the docking. The grid log files (.glg) and docking log files (.dlg) were generated by CYGWIN using grid parameter files (.gpf) and docking parameter files (.dpf) as input. The grid parameter file is required to specify the AUTOGRID program about the types of grid maps that need to be computed, the location and extent of these maps and potential energy parameters. This program creates one map for each element and an electrostatics map. Whereas, the AUTODOCK program needs the docking parameter file to identify the map files, ligand molecule, ligand center and torsions, algorithms and number of docking runs. The run with minimum binding energy was obtained using the RMSD Table. AutoDock uses the following

formulae (1) and (2) to calculate the binding energy (ΔG) and inhibition constant (K*i*) is obtained from ΔG[32].

ΔG = Final Intermolecular Energy + Final Total Internal Energy + Torsional Free Energy + Unbound System's Energy (1)

Final Intermolecular Energy = vdW + Hbond + desolv Energy + Electrostatic Energy

$Ki = \exp(\Delta G/RT)$ (2)

where, vdW is van der Waals, Hbond is Hydrogen bonding and desolv Energy is desolvation energy, R is the universal gas constant (1.985×10^{-3} kcal mol^{-1} K^{-1}) and T is the temperature (298.15 K).

2.4 Generation of Output and Visualization of Docked Complexes

CYGWIN was also used to create the final .pdb output file containing the ligand-protein complex. The interactions were visualized using Discovery Studio Visualizer.

3. Results and Discussion

Molecular docking study allows the study of interactions between ligand and target protein. The five target proteins Survivin, ERα, PR, Akt1 and EGFR were docked with ligands Nar and Nar-Bhz. The observed binding energies and inhibition constants are tabulated in Table 1. When docked with naringenin, the ΔG values were in the range of -7.66 to -7.94 kcal/mol whereas with Nar-Bhz, the values ranged between -5.49 and -11.05 kcal/mol. Maximum binding energy of -8.78 kcal/mol was obtained for naringenin-ERα interaction. Whereas, Nar-Bhz docking with Akt1 resulted in binding energy of -11.05 kcal/mol.

3.1 Naringenin has Better Inhibition against Survivin

Several H bond interactions were observed between target proteins and ligands. Naringenin was docked into the binding site of survivin (Figure 2). Naringenin-survivin interaction had ΔG value of -7.66 kcal/mol

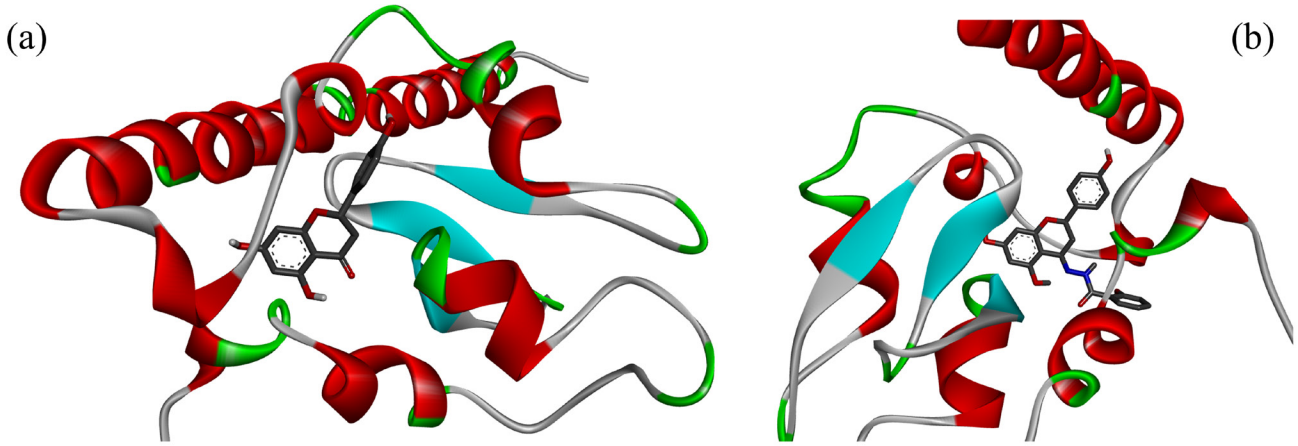

Figure 2. Docking of the ligands, naringenin **(a)** and naringenin 2-hydroxy benzoyl hydrazone **(b)** into the binding pocket of survivin.

Table 1. Binding energies (B.E) and Inhibition constants (K*i*) of the interactions between the proteins and ligands

Proteins	Naringenin (Nar)		Naringenin 2-hydroxy benzoyl hydrazone (Nar-Bhz)		Doxorubicin (Positive control)	
	B.E (kcal/mol)	K*i* (μM)	B.E (kcal/mol)	K*i* (μM)	B.E (kcal/mol)	K*i* (μM)
Survivin	-7.66	2.41	-5.56	84.16	+18.27	-
ERα	-8.78	0.36396	-5.49	94.25	-2.98	6570
PR	-8.48	0.61307	-9.66	0.0828	-11.09	0.007
AKT1	-7.94	1.52	-11.05	0.00798	-13.11	0.0002
EGFR	-7.91	1.59	-10.23	0.03154	-11.77	0.0023

and K*i* = 2.41μM. The residues involved in hydrogen bond interactions were Arg18, Leu87 and Phe93 (Table 2). Nar-Bhz interacted with five residues of Survivin including Gln92, Phe93, Phe86, Val89 and Phe101 with binding energy of -5.56 kcal/mol and K*i* = 84.16μM (Table 3). The residues Leu14, Arg18, Phe86, Leu87, Val89, Gln92, Phe93, Leu96 and Leu104 belong to the active site of survivin. Docking of doxorubicin with survivin resulted in unfavorable binding energy of +18.27 kcal/mol.

Interaction of the ligands with amino acid residues confirms their ability to interfere with the activity of survivin. Naringenin showed commendable inhibition against survivin as observed from K*i*. The inhibition constant (K*i*) helps to identify the ligand that might be able to bring about maximum inhibition at lower concentration. Nar-loaded nanolipid carriers were shown to lower the gene expression levels of survivin[33].

Survivin is highly expressed in cancer cells and its downregulation results in apoptotic pathway activation along with cell death signal sensitization[34]. Moreover, its high expression has been reported to result in poor prognosis and resistance in breast cancer cells[35]. Hence, by interacting with the residues of survivin, Nar and Nar-Bhz can interfere with protein activity.

3.2 Estrogen Receptor α is Effectively Inhibited by Naringenin in Comparison to Control

From the past few decades, estrogen receptor has been identified as a major player breast cancer disease progression[36]. Targeting this receptor is an important strategy to block breast cancer progress and recurrence[37]. Being a member of the nuclear receptor superfamily, ERα is regulated by estradiol (E2) binding.

Table 2. Protein residues interacting with Naringenin to form hydrogen bonding and hydrophobic interactions

Naringenin hydrogen bond interactions				
Protein	Protein residue: Atom	Ligand atom	Distance (Å)	Hydrophobic interactions
Survivin	Arg18:NH1	O	2.89533	Val89
	Arg18:NH2	O	3.38604	
	Leu87:O	H	2.14486	
	Phe93:CA	H	2.69263	
ERα	Arg394:NH2	O	3.01416	Phe404, Leu346, Ala350, Leu384, Leu525, Leu349, Ala350, Leu387, Leu391
	His524:ND1	O	3.00919	
	Gly521:O	O	2.15813	
PR	Cys891:N	H	2.79866	Phe778, Met759, Leu721, Leu797, Leu887
	Met759:O	H	2.38129	
AKT1	Thr81:N	O	2.60903	Ile84, Arg273
	Thr82:N	O	2.84373	
	Thr82:OG1	O	2.68937	
	Asp292:OD1	H	2.18525	
	Trp80:CD1	C	3.15112	
	Trp80:CD1	H	3.1078	
	Tyr272:CA	O	3.3505	
	Arg273:CD	C	3.15263	
	Arg273:CD	H	3.49999	
EGFR	Thr854:OG1	O	3.19348	Met766, Val726, Ala743, Lys745
	Asp855:N	O	2.9841	
	Phe856:O	H	1.8877	

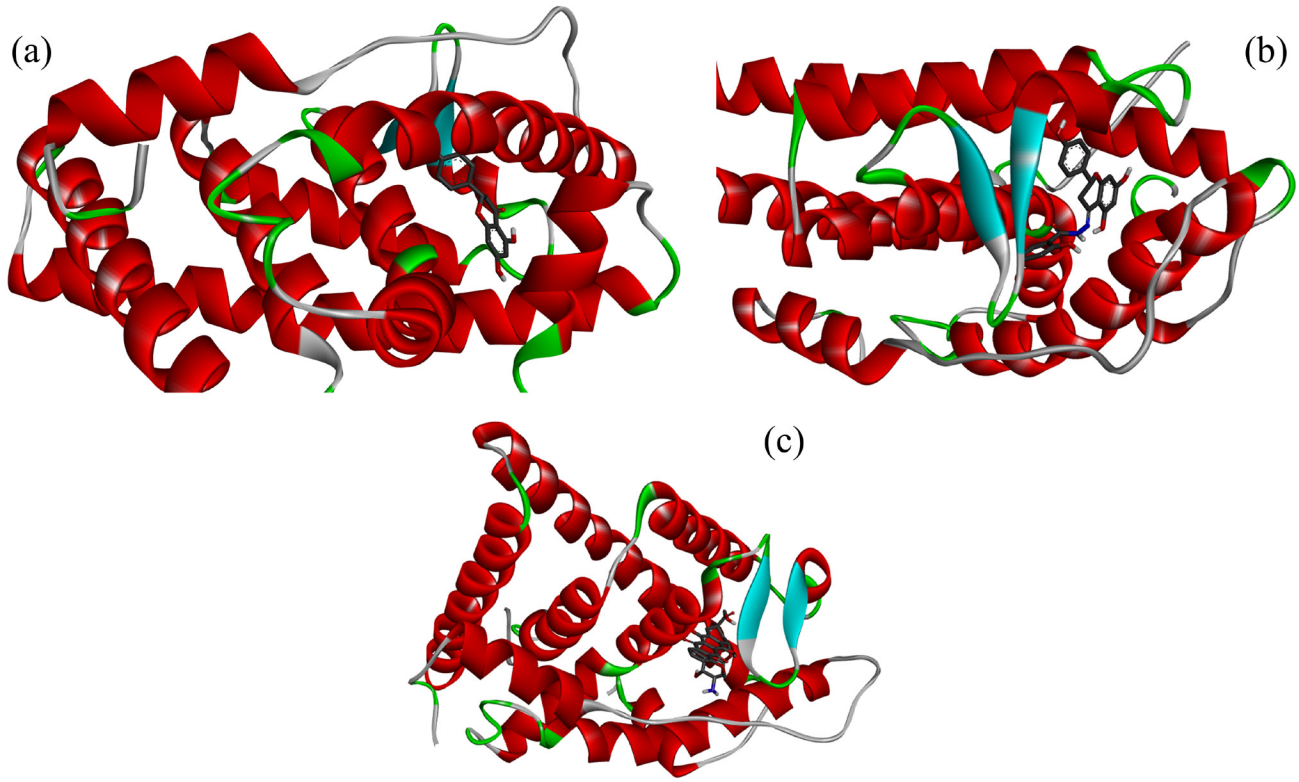

Figure 3. The best docked poses of naringenin (a), naringenin 2-hydroxy benzoyl hydrazone (b) and doxorubicin (c) within the binding site of estrogen receptor α.

Since E2 is involved in growth, development, and maintenance, sustained exposure to E2 is a stimulus for breast cancer growth[38,39]. Several Selective estrogen receptor modulators (SERMs) and selective estrogen receptor degraders (SERDs) have been developed to antagonize the ERα[40] with estrogen receptor positive (ER+. Nevertheless, as with any chemotherapeutic compounds, the compounds such as Tamoxifen, Trastuzumab, Paclitaxel etc. too exhibited mild to severe side effects. Hence, the need for alternative interventions as ERα antagonists is high.

ΔG values of -8.78 (K*i*= 0.36396 μM) and -7.91 kcal/mol (K*i* = 94.25 μM) were obtained for interaction of ERα with Nar and Nar-Bhz, respectively (Table 1). The residues Arg394, His521, Leu346 and Met421 are part of the ligand binding domain (LBD) of ERα[41]. Residues 303 to 593 are part of the LBD[42]. Nar formed hydrogen bonding with residues Arg394, His524 and Gly521 of ERα (Table 2). El-Kersh *et al.*[43] reported the interaction of naringenin with His524 of ERα.Naringenin has previously been reported to inhibit proliferation of estrogen receptor expressing MCF-7 human breast cancer cells and also demonstrated partial antiestrogenic activity in female rat uterus[44]. Nar-Bhz was docked into the binding site of ERα (Figure 3). The residues Arg394, Leu346, Met421 and Gly521were involved in hydrogen bonding interactions with Nar-Bhz (Table 3). As seen from Tables 2 and 3, both ligands were able to make several hydrophobic interactions with ERα residues. K*i* indicates that naringenin shows good inhibition against ERα compared to other proteins. The positive control doxorubicin showed high inhibitory constant compared to Nar and Nar-bhz which shows that the ligands had good inhibitory potential than the control. Both ligands were able to effectively interact with the residues involved in native ligand binding to the ERα receptor.

3.3 Naringenin 2-hydroxy Benzoyl Hydrazone Shows Higher Inhibition Against Progesterone Receptor

In breast cancer cells, significant crosstalk between ER and PR pathways have shown that their activation

has a significant impact on each other. PR interacts with ER after activation by its ligand in the presence of estrogen. This interaction implies modulation of ER activity by PR[45]. Like SERMs, selective progesterone receptor modulators (SPRMs) have also become an important element in breast cancer treatment. They act as antagonists or agonists to PR[46].

The ligands were docking into the binding site of PR (Figure 4). Hydrogen and hydrophobic interactions were observed between PR and the ligands. Nar-Bhz showed high binding affinity compared to naringenin. The obtained binding energies were -8.48 kcal/mol and -9.66 kcal/mol for nar and nar-bhz respectively (Table 1). The binding energy of nar-bhz was comparable to control doxorubicin (-11.09 kcal/mol). Naringenin interacted with only two residues of PR, Cys891 and Met759 (Table 2). Both residues interacted with H atoms of naringenin. On the other hand, Nar-Bhz and doxorubicin interacted with many residues out of which Gln725, Arg766, Thr894, Met801 were common interacting residues for all the three molecules. Doxorubicin exhibited very low K*i* value of 0.007 µm indicating its high inhibitory potential. However, in comparison to inhibition against other proteins, the inhibitory potential of Nar-Bhz was higher against PR. The residues Met759, and Phe778 involved in hydrophobic interactions with all three compounds. Leu797 interacted with nar and nar-bhz. Leu 763 and Cys891 interacted with only nar-bhz and doxorubicin. Cys891 however involved in hydrogen bonding with O atom of naringenin.

3.4 Naringenin 2-hydroxy Benzoyl Hydrazone Effectively Inhibits Akt1 Protein

18 H bond interactions occurred between Akt1 protein and the ligands. The binding energies were -7.90 and -11.05 kcal/mol for Nar and Nar-Bhz, respectively. The binding energy for doxorubicin was higher that the ligands with 13.11 kcal/mol. Nar-Bhz showed interaction comparable with the control. The K*i* for Nar and Nar-Bhz were 1.52 µM and 0.00798 µM, respectively, indicating reasonably high inhibition of Akt1 by Nar-Bhz compared

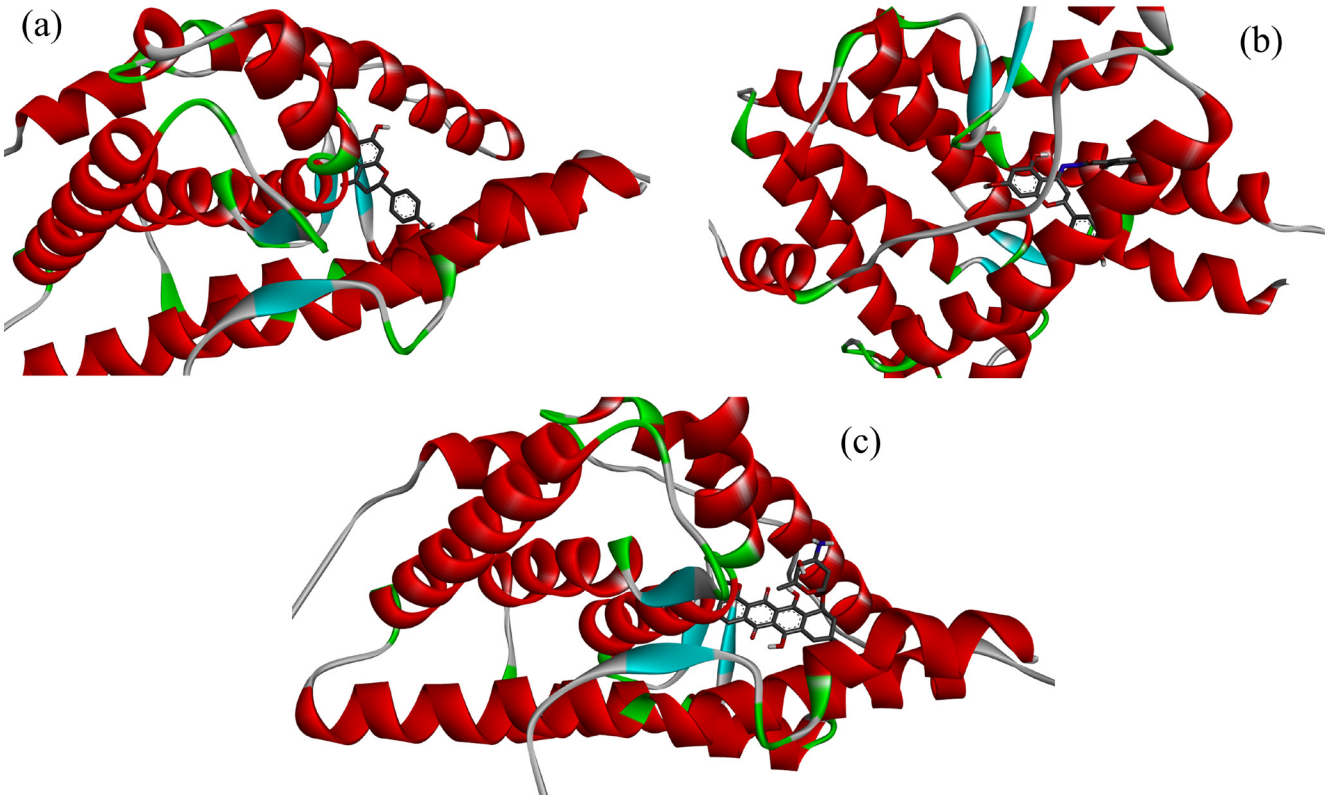

Figure 4. Representation of docking of naringenin (a), naringenin 2-hydroxy benzoyl hydrazone (b) and doxorubicin (c) within the progesterone receptor.

Table 3. Residues of the proteins interacting with Naringenin 2-hydroxy benzoyl hydrazone for hydrogen bonding and hydrophobic interactions

Naringenin 2-hydroxy benzoyl hydrazone interactions				
Protein	Protein residue: Atom	Ligand atom	Distance ( )	Hydrophobic interactions
Survivin	Gln92:NE2	O	2.67085	Leu96, Phe86, Phe93, Val89, Leu14, Leu104
	Phe93:N	O	2.67851	
	Phe101:O	O	2.72779	
	Phe86:O	H	2.6502	
	Val89:O	H	3.14993	
ERα	Arg394:NH2	C	2.67688	Leu384, Met522, Leu525, Leu349, Leu387, Leu391, Met421, Ile424, Leu428
	Leu346:O	H	2.73284	
	Leu346:O	H	2.36061	
	Met421:O	H	3.16132	
	Met421:SD	H	3.64802	
	Gly521:O	H	2.25766	
PR	Gln725:NE2	C	2.75019	Gly722, Leu797, Phe778, Met759, Leu763, Cys891
	Gln725:NE2	O	2.93888	
	Arg766:NH2	O	2.91433	
	Thr894:OG1	C	2.97597	
	Met801:SD	H	2.36725	
	Asn719:O	H	3.00309	
	Asn719:CA	C	3.24193	
AKT1	Asn54:ND2	H	3.17119	Phe120, Ile84
	Tyr272:OH	C	3.01406	
	Tyr272:OH	H	3.08798	
	Thr291:OG1	H	2.19013	
	Cys296:O	H	2.56629	
	Asp274:OD1	H	2.94872	
	AsP292:CA	C	3.55835	
	Cys296:CA	H	2.40603	
	Glu298:CA	H	2.65576	
EGFR	Asp837:OD2	H	1.76247	Leu747, Leu858, Leu788, Met766

to Nar (Table 1). However, doxorubicin control showed higher inhibition with 0.0002μM inhibition constant. The residues Tyr272 and Asp292 interacted with both the ligands and doxorubicin. Interestingly, only Nar interacted with the residues Trp80, Thr81, Thr82 via O atom which did not involve in the interactions with Nar-Bhz although nar-bhz interacted with other residues (Table 2). Thr82 also interacted with doxorubicin (Table 3). However, only three residues viz. Thr 82, Ile84, Phe120 and Arg273 were involved in hydrophobic interactions suggesting that hydrogen bonding were the major stabilizing interactions in all the complexes. While doxorubicin interacted with only one residue in the hydrophobic interactions, both Nar and Nar-Bhz interacted with two different residues, indicating their better ability to form hydrophobic interactions compared to control. However, the control had many hydrogen bonding interactions (15) compared to Nar and Nar-Bhz (Figure 5).

PI3K/AKT/mTOR pathway is most frequently activated in breast cancer[47]. Misregulation of AKT expression contributes to resistance in cancer cells[48]. Akt/protein kinase B is activated by PI3K which in turn triggers a downstream effect that ensures the survival of cancer cells by promoting cell growth, proliferation and motility[49]. Analysis of Nar and Nar-Bhz interaction with Akt1 show that they might be able to effectively antagonize Akt 1 in breast cancer cells and lower its activity. Moreover, mammary epithelial tumor cells deficient in Akt1 showed a reduction in size and proliferation[50]. Phenylhydrazone derivatives of naringenin have been reported to inhibit the PI3K/AKT signaling pathway in non-small cell lung cancer cells[51,52], which supports the results obtained in this study (Table 4).

3.5 Naringenin 2-hydroxy Benzoyl Hydrazone Shows Better Activity Against EGFR

Nar and Nar-Bhz formed H bond interactions with EGFR with binding energies and inhibition constants of -7.91 kcal/mol (K*i* = 1.59μM) and -10.23 kcal/mol (Ki = 0.03154 μM), respectively. Nar-Bhz showed higher affinity for EGFR compared to Nar. The affinity of Nar-Bhz was similar to that of doxorubicin control (-11.77 kcal/mol). While H bonding interaction was limited to one residue, Asp837 (Table 3), the EGFR-Nar-Bhz complex was stabilized by hydrophobic interactions with five residues (Leu747, Leu858, Leu788, Leu858, Met766). These five residues along with six other residues involve in hydrophobic interaction with doxorubicin control (Figure 6). On the other hand, Nar interacts with Thr854, Asp855 and Phe856 of the protein to form H bonding (Table 2). Nar shows better hydrogen bonding activity compared to doxorubicin. As seen already, the ErbB receptor family activates the MAPK and PI3K-AKT signaling pathways[30]. The downstream effects of this trigger include cell proliferation and survival, the key events required for malignancy[53]. Nar and Nar-Bhz might be able to antagonize EGFR in breast cancer cells and thereby prevent proliferation and survival. In conclusion, the results obtained in the study show that both the

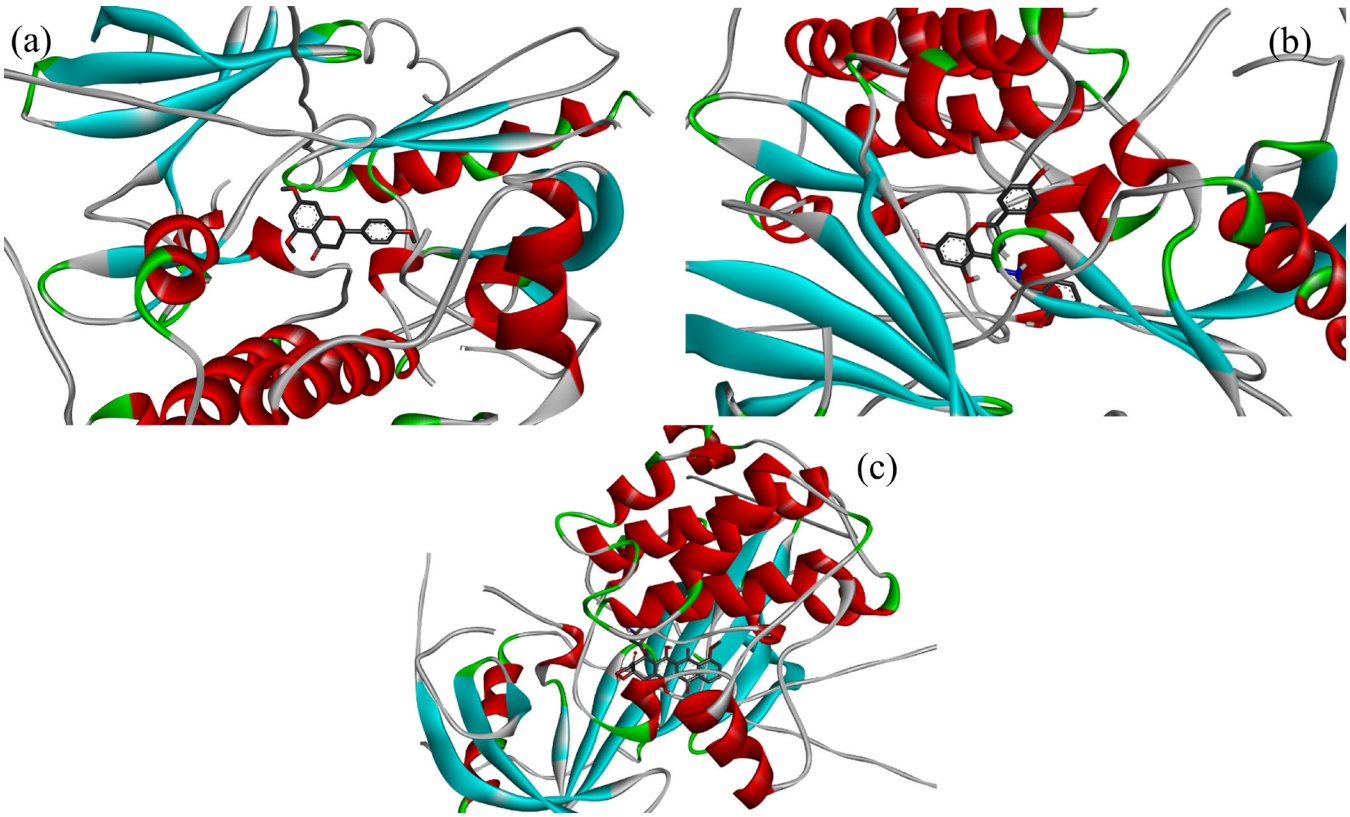

Figure 5. Docking pose of naringenin (a), naringenin 2-hydroxy benzoyl hydrazone **(b)** and doxorubicin **(c)** with Akt1.

Table 4. Residues of the proteins interacting with the positive control doxorubicin and the distances between the interacting atoms and hydrophobic interactions

Doxorubicin interactions				
Protein	Protein residue: Atom	Ligand atom	Distance ( )	Hydrophobic interactions
Survivin	-	-	-	
ERα	Arg394:NH2	C	3.28056	Leu384, Leu387, Leu391, Met 388
	His524:ND1	C	2.78149	
	Leu525:N	H	2.7715	
	Leu346:O	O	3.33879	
	Met421:CA	O	3.65577	
PR	Arg766:NH2	C	3.1276	Leu763, Phe778, Leu718, Met759, Leu763, Cys891
	Thr894:OG1	O	2.67894	
	Met759:SD	C	3.34522	
	Leu718:O	H	2.37298	
	Met801:SD	H	3.77723	
	Gln725:OE1	C	2.99506	
	Phe778:O	C	3.24399	
	Cys891:CA	CA	3.21664	
AKT1	Asn54:ND2	H	3.39395	Thr82
	Thr82:OG1	H	3.16122	
	Tyr272:OH	C	3.12322	
	Thr291:OG1	C	2.68957	
	Phe293:N	O	2.67977	
	Tyr326:OH	O	3.38718	
	Asp292:OD2	O	2.87352	
	Cys296:O	O	3.22136	
	Asp292:OD2	H	2.30366	
	Gln79:OE1	H	2.27403	
	Val271:O	O	3.21999	
	Cys296:O	H	3.36068	
	Thr291:O	C	2.88169	
	Arg273:CD	C	3.76852	
	Glu298:CA	C	2.82971	
EGFR	Lys745:NZ	O	3.07549	Leu788, Phe723, Val726, Lys745, Met790, Leu858, Leu862, Ile759, Leu861, Leu747, Met766
	Lys745:NZ	O	3.31814	

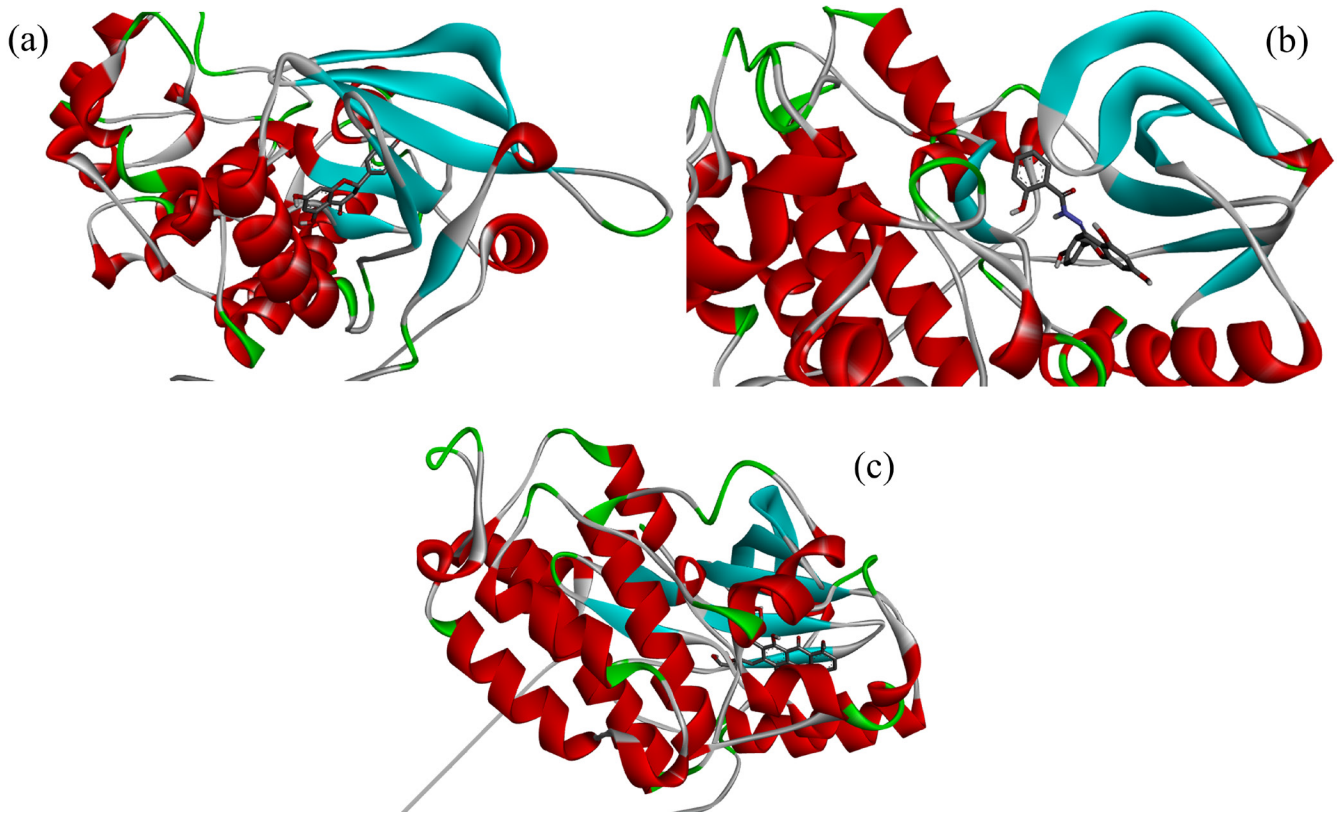

Figure 6. Molecular docking of the best docked pose of EGFR with naringenin (a), naringenin 2-hydroxy benzoyl hydrazone (b) and doxorubicin (c).

ligands, Nar and Nar-Bhz showed antagonistic activity against the proteins involved in development of breast cancer, evidence dintermolecular interactions. These compounds can be further developed as therapeutic agents for breast cancer and. However, future research is required to study their activity in cancer cells and animal models.

4. Conclusion

The present study utilized molecular docking simulations to identify the interactions between the ligands, Nar and Nar-Bhz, and proteins involved in breast cancer. Nar showed significant activity against Survivin and ERα, while Nar-Bhz showed remarkable inhibitor potential against Akt1 and EGFR. The binding interactions of the ligands and the proteins based on computational method is presented and interacting residues were identified. Nar showed high binding affinity with ERα with binding energy of -8.98 kcal/mol and with -8.48 kcal/mol upon interaction with PR. Nar-Bhz binds to Akt1 with binding energy of -11.05 kcal/mol and -10.23 kcal/mol with EGFR. Both ligands had better activity against survivin and ERα compared to doxorubicin. The results obtained in the study warrant the use of these compounds to study their activity in breast cancer cell lines in the future.

5. References

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