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Structure-based multitargeted screening of bioactive compounds of *Catharanthus roseus* against breast cancer

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Abstract

Background: Breast cancer (BC) remains a major global challenge, with current treatments targeting hormone receptors with partial agonists/antagonists that frequently cause side effects and resistance.

Methods: This study investigates bioactive compounds of *Catharanthus roseus* as potential EGFR and ERα inhibitors. Protein-ligand interactions, which are important in drug design, were assessed using the PyRx 0.8 virtual screening tool. The LOTUS database was used to generate a bioactive compound library (N = 291) of *C. roseus* constituents. The physiochemical properties of selected hits were investigated to identify lead-like compounds.

Result: Among the screened compounds, five compounds namely, LTS0049153, LTS0192836, LTS0084120, LTS0052616, and LTS0199033 exhibited strong binding to ERα and EGFR, and interacted with key amino acid residues of ERα and EGFR proteins. These compounds have favorable physiochemical properties and meet Lipinski's criteria.

Conclusion: The compounds LTS0049153, LTS0192836, LTS0084120, LTS0052616, and LTS0199033 can be used as ERα and EGFR inhibitors for BC treatment. However, more experimental validation is needed to optimize these compounds as ERα and EGFR inhibitors.

Introduction

Breast cancer (BC) is the most common cancer in women, and it is the second biggest cause of cancer-related death after lung cancer [1]. The World Health Organization declares an alarming increase in BC prevalence in developing countries in early 2020, citing increased life expectancy, urbanization, and adoption of Western lifestyles. BC claimed the lives of an estimated 627,000 women in 2020, accounting for around 15% of all female cancer deaths [2].

Hence, establishing effective treatment solutions for this life-threatening condition is crucial. BC chemotherapy frequently targets important receptors, including ERα (estrogen receptor alpha), progesterone receptor, and epidermal growth factor receptor (EGFR).

Estrogen, notably 17 β-estradiol, plays a crucial role in BC initiation and progression by upregulating c-Myc and cyclin D1, allowing mammary epithelial cells to migrate from G1 to S phase more efficiently. Anti-estrogen treatments are a promising and core strategy to treat ER-positive BC [3].

EGFR is especially important in triple-negative BC, where its expression is often high [4,5]. Tamoxifen, Trastuzumab, Paclitaxel, Capecitabine, Cyclophosphamide, Gemcitabine, and Docetaxel are the approved treatment drugs for BC, albeit they come with a variety of side effects [6]. Furthermore, phytochemicals and their derivatives have shown promising anti-cancer potential as an alternative therapy [7].

Computer-Aided Drug Design (CADD) is gaining popularity because of its benefits in selectivity, efficiency, efficacy, less development timelines, lesser toxicity, and better alignment with pharmacokinetic parameters [8,9]. Achieving a balance between pharmaceutical chemistry and biological activity is critical for successful medication development. With the increasing demand for new medications, selecting physiologically active structures has become more crucial.

Utilizing structural biology and computational approaches enables the development of active chemical libraries and addresses the issue of molecular obesity [10]. *Catharanthus roseus* is a well-known medicinal plant that has been used since ancient times. It is well-known in herbal medicine due to its anticancer bioactive compounds. *C. roseus* is used in Ayurveda to treat cancer, diabetes, stomach disorders, kidney, liver, and cardiovascular diseases [11].

This study aims to find *C. roseus* compounds as ERα and EGFR inhibitors using in-silico tools to combat BC.

Methods

Protein preparation

The crystal structures of ERα (ID: 3ERT) and EGFR (ID: 2J6M) were retrieved from the Protein Data Bank.

The Discovery Studio Visualizer 2020 was used to remove co-crystal ligands and heteroatoms, and proteins were saved in .pdb format.

Bioactive compound library preparation

The LOTUS database acquired information about *C. roseus* constituents to create the bioactive compound library. 291 distinct compounds were discovered, with chemical structures, molecular weights, biological activities, and other relevant properties. The compounds were downloaded in .sdf format and then processed for energy minimization before being converted to pdbqt format with the PyRx tool.

Virtual screening

Molecular docking predicts the optimal binding mode between a ligand and a macromolecule. This technique requires a receptor and a ligand [12]. Virtual screening (VS) has emerged as a potentially successful technique for drug discovery, reducing time, cost, and resource requirements significantly [13]. The basic goal of screening is to explore chemical compound databases for new hits with the highest potential biological activity [14].

The PyRx 0.8 tool [15,16] was used to screen the prepared compound library against the ERα and EGFR active sites. Following that, a detailed interaction analysis and visual inspection were carried out to determine the most stable complex based on lower binding energy (BE) values.

Results

This study aimed to uncover natural inhibitors for two critical targets in BC treatment: ERα and EGFR. *C. roseus,* a well-known medicinal plant known for synthesizing vinca alkaloids with powerful anti-cancer capabilities, was chosen for study. This plant's bioactive constituents were accessed using the LOTUS database, yielding 291 compounds.

These compounds were computationally screened against the active sites of ERα and EGFR targets. Notably, 13 compounds exhibited significant efficacy and strong BE towards both EGFR and ERα (Table 1). Among them, the top 5 compounds were selected for detailed interaction analysis.

Interaction with EGFR

Figure 1 shows a superimposed view of the top 5 compounds and the control compound (AEE-788) in the EGFR binding pocket, indicating that they bind at the same pocket.

S. No.	Compounds	Binding energy (kcal/mol)	
		EGFR	ERα
1.	LTS0049153	-9.7	-8.9
2.	LTS0192836	-9.5	-8.6
3.	LTS0084120	-9.5	-8.8
4.	LTS0052616	-9.6	-8.8
5.	LTS0199033	-9.8	-8.7
6.	LTS0155203	-9.4	-8.2
7.	LTS0171444	-9.2	-8.1
8.	LTS0176726	-9.0	-8.4
9.	LTS0051708	-9.0	-8.1
10.	LTS0090234	-9.3	-8.3
11.	LTS0231121	-9.4	-8.5
12.	LTS0234774	-9.1	-8.5
13.	LTS0273091	-9.4	-8.0
14.	LTS0275464	-9.1	-8.0
15.	LTS0206376	-9.4	-8.4
16.	LTS0163993	-9.3	-8.1
17.	LTS0128010	-9.0	-8.3
18.	4-Hydroxytamoxifen (Control_ERα)	NA	-7.9
19.	AEE-788 (Control_EGFR)	-9.1	NA

Table 1: Top hit compounds with higher binding energies to ERα and EGFR compared to their controls

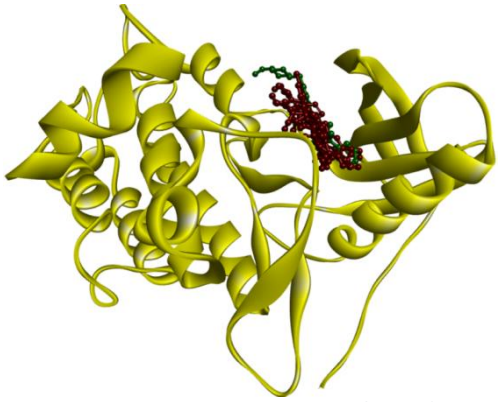

Figure 1: Superimpose representation of top 5 compounds LTS0049153, LTS0192836, LTS0084120, LTS0052616, and LTS0199033 along with control compound AEE-788 (green) in the EGFR binding pocket. The top 5 compounds are shown in red color.

LTS0199033 interacted with Pro794, Leu718, Ala743, Leu844, Val726, Thr854, Met766, Thr790, Glu762, Lys745, Phe723, Asp855, Met793, Gly796, and Leu792 residues of EGFR (Fig 2A). LTS0049153 was found to interact with Arg841, Asn842, Cys797, Asp800, Lys745, Thr854, Gly796, Glu762, Leu788, Thr790, Met766, Ala743, Met793, Leu792, Val726, Leu844, Asp855, and Phe723 residues of EGFR (Fig 2B). LTS0084120 interacted with Leu844, Leu792, Met793, Leu718, Pro794, His805, Glu804, Tyr801, Phe795, Gly796, Ala743, Leu788, Ile744, Thr790, Lys745, Met766, Glu762, Val726, and Gln791 residues of EGFR (Fig 2C). LTS0192836 binds with Met793, Leu718, Gly796, Asn842, Phe723, Arg841, Ala743, Asp855, Thr854, Leu844, Thr790, Gln791, Val726, and Leu792 residues of EGFR (Fig 2D). LTS0052616 was found to interact with Leu718, Pro794, Phe795, Leu792, Gly796, Met793, Val726, Ala743, Leu844, Glu762, Thr854, Met766, Thr790, Lys745, Asp855, and Phe723 residues of EGFR (Fig 2E). The control compound AEE-788 was found to interact with Gln791, Leu844, Leu792, Met793, Leu718, Pro794, His805, Glu804, Tyr801, Phe795, Gly796, Ala743, Met766, Leu788, Glu762, Thr790, Lys745, Lys745, Ile744, and Val726 (Fig 2F).

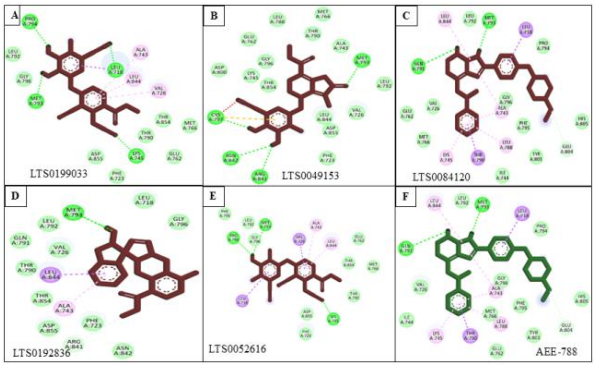

Figure 2: Interacting residues of EGFR with LTS0199033 (A), LTS0049153 (B), LTS0084120 (C), LTS0192836 (D), LTS0052616 (E), and the control compound AEE-788 (F).

Interaction with ERα

Figure 3 shows a superimposed view of the top 5 compounds and the control compound (4-Hydroxytamoxifenin) in the ERα binding pocket, indicating that they bind at the same pocket.

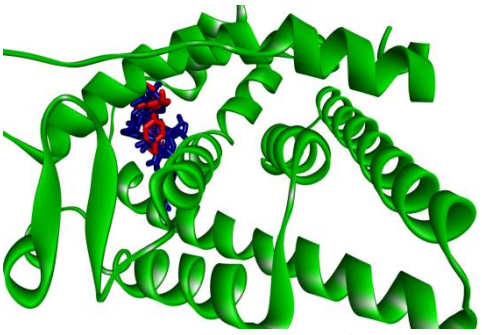

Figure 3: Superimpose representation of top 5 compounds LTS0049153, LTS0192836, LTS0084120, LTS0052616, and LTS0199033 along with control compound 4-Hydroxytamoxifenin (red) in the ERα binding pocket. The top 5 compounds are shown in blue color.

LTS0049153 interacted with Ala534, Leu539, Met528, Leu536, Trp383, Leu525, Met522, Tyr526, Lys529, Lys531, Cys530, Val533, and Pro535 residues of ERα (Fig 4A); while LTS0192836 interacted with Trp383, Met522, Leu536, Leu354, Leu539, Asp351, Cys530, Val533, Lys529, Tyr526, and Glu380 residues of ERα (Fig 4B). LTS0084120 was found to bind with Tyr537, Glu380, Val533, Pro535, Leu536, Val534, Leu539, Leu354, Asp351, Trp383, Met528, Leu525, Tyr526, and Met522 residues of ERα (Fig 4C). LTS0052616 interacted with Pro535, Cys530, Val533, Met522,

Lys529, Tyr526, Leu525, Leu536, Leu354, Trp383, Leu387, Ala350, Asp351, Leu539, and Val534 residues of ERα (Fig 4D). In addition, LTS0199033 was found to interact with Pro535, Cys530, Val533, Met522, Lys529, Tyr526, Leu525, Leu536, Leu354, Trp383, Leu387, Ala350, Asp351, Leu539, and Val534 residues of ERα (Fig 4E). Furthermore, Leu525, Met522, Leu387, Ala350, Asp351, Trp383, Leu354, Leu539, Leu536, Val534, Pro535, Val533, Cys530, Tyr526, and Lys529 residues of ERα was found interact with the control compound 4-Hydroxytamoxifen (Fig 4F).

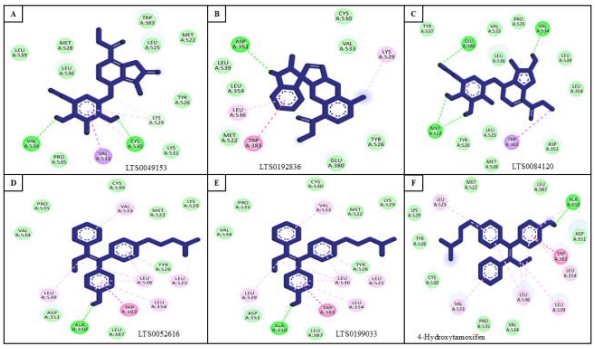

Figure 4: Interacting residues of ERα with LTS0049153 (A), LTS0192836 (B), LTS0084120 (C), LTS0052616 (D), LTS0199033 (E), and the control compound 4-Hydroxytamoxifenin (F).

Molecular Properties	**LTS0049153**	**LTS0192836**	**LTS0084120**	**LTS0052616**	**LTS0199033**
Total atom number	51	51	53	51	51
Heavy atom number	27	27	27	27	27
Bond count	29	31	29	28	28
Number of carbons	17	21	17	17	17
Minimal number of rings	3	5	3	2	2
Maximal number of rings	4	9	4	2	2
Molecular Descriptors					
NP-likeness score	1	0.98	0.97	0.97	0.97
Alogp	-2.26	1.29	-2.08	-1.98	-1.98
Alogp2	5.12	1.66	4.33	3.93	3.93
Apol	53.943	58.371	55.2766	53.943	53.943
Bpol	35.817	34.327	37.0454	35.817	35.817
Eccentric Connectivity Index Descriptor	526	519	526	512	512
Fmf_Descriptor	0.5926	0.7778	0.5926	0.4815	0.4815
Fsp3	0.7647	0.5238	0.8235	0.6471	0.6471
Fragment Complexity Descriptor	2107.1	2323.06	2323.1	2002.1	2002.1
PetitjeanNumber	0.5	0.4545	0.5	0.5	0.5
Lipinski violation	0	0	0	0	0
WienerPathNumber	1766	1609	1766	1810	1810
Xlogp	-1.505	2.019	-1.092	-0.647	-0.647
Zagreb Index	144	158	144	134	134
Topo_PSA	151.98	71.36	155.14	151.98	151.98

Table 2: Molecular properties and molecular descriptors of the selected compounds LTS0049153, LTS0192836, LTS0084120, LTS0052616, and LTS0199033.

Physiochemical properties

The physiochemical properties of selected hits were evaluated using the LOTUS database, which includes a variety of metrics used to identify lead-like compounds. These natural chemicals were assessed using molecular descriptors such as NP-like scores. The results reveal that all five compounds exhibit favorable properties within acceptable ranges of these parameters and adhere to Lipinski's criteria without exception (Table 2).

Discussion

BC is one of the most prevalent malignancies among women globally, with around 70% of cases being ERα^{+}. Deregulation of ERα signaling is crucial in malignancy progression. ERα is a transcription factor that promotes the production of estrogen-responsive genes, which are linked to tumor growth in BC cells [17]. EGFR is an important target for cancer treatments. Approximately 50% of triple-negative BC and inflammatory BC cases have EGFR overexpression, prompting extensive testing of EGFR inhibitors in several studies [18]. Bioactive compounds from *C. roseus* were screened against ERα and EGFR active sites, with 13 compounds showing strong BE towards both proteins. The top 5 compounds namely, LTS0049153, LTS0192836, LTS0084120, LTS0052616, and LTS0199033 are described in detail in this study.

The residues of ERα Leu525, Met522, Leu387, Ala350, Asp351, Trp383, Leu354, Leu539, Leu536, Val534, Pro535, Val533, Cys530, Tyr526, and Lys529 were found to interact with the control compound 4-Hydroxytamoxifen. Further, the EGFR residues Gln791, Leu844, Leu792, Met793, Leu718, Pro794, His805, Glu804, Tyr801, Phe795, Gly796, Ala743, Met766, Leu788, Glu762, Thr790, Lys745, Lys745, Ile744, and Val726 were found to interact with the control compound AEE-788. Notably, the hit compounds (LTS0049153, LTS0192836, LTS0084120, LTS0052616, and LTS0199033) interacted with the same ERα and EGFR residues, indicating that they bind to the same binding pocket as the control compounds.

In docking studies, BE is used to quantify the strength of the interaction between a ligand-protein complex, with a high negative BE indicating a stable ligand-protein complex [19-21]. Interestingly, the hit compounds (LTS0049153, LTS0192836, LTS0084120, LTS0052616, and LTS0199033) have high negative BE values for both ERα and EGFR, indicating strong interactions with both targets. In addition, H-bonding plays an important role in the stability of the ligand protein complex [22-24]. Notably, the hit compounds (LTS0049153, LTS0192836, LTS0084120, LTS0052616, and LTS0199033) form multiple H-bonds with ERα and EGFR residues.

Natural resources, including phytochemical-rich herbal plants, are thought to be safer and more effective than synthetic agents for treating chronic and infectious diseases due to their fewer side effects [25]. *C. roseus*, a medicinal plant from the Apocynaceae family, has a long history of use in Ayurvedic and traditional Chinese medicine. Research has demonstrated its potential in cancer treatment [26].

This study found that bioactive compounds from *C. roseus* bind strongly to ERα and EGFR proteins, suggesting their potential as anticancer agents.

EGFR and ERα play key roles in the progression of BC. EGFR increases cell proliferation and survival via a variety of signaling pathways and is frequently associated with aggressive malignancies. Endocrine therapies aim to target ERα, a nuclear hormone receptor that promotes hormone-dependent BC growth. Bioactive compounds from *C. roseus* were screened to target EGFR and ERα. Five compounds (LTS0049153, LTS0192836, LTS0084120, LTS0052616, and LTS0199033) demonstrated strong binding to ERα and EGFR, interacting with key amino acid residues of both targets ERα and EGFR. These compounds have favorable physiochemical properties and meet Lipinski's criteria, suggesting potential as ERα and EGFR inhibitors for BC treatment. Further experimental validation is required to optimize these compounds as ERα and EGFR inhibitors.

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Author Contributions

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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