Structural insights into conformational stability of ESR1 and structure base screening of new potent inhibitor for the treatment of Breast Cancer

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Abstract

Estrogen receptor alpha (ER α), a nuclear receptor protein encoded by the estrogen receptor1 (ESR1) gene, is an important biomarker in breast cancer diagnosis. Any dysregulation in its expression can actively implicate the development and progression of the disease. ER α is abnormally expressed in around 60% of the active cases, making it an important therapeutic target. In this study, we report the application of computational approaches to identify suitable drug-like molecules, which share similar ligand binding dynamics with ER α . Structure-based virtual screening (SBVS), docking, and inhibitor dynamics are used to study the ligand binding and interaction profiling of the anticipated ligand molecule, at the active site of the 4-hydroxytamoxifen (OHT) protein (PDB Id: 3ERT). SBVS analysis follows HTVS, SP, and XP protocol in comparison to the ZINC and NCI, to retrieve 20 best ligand hits as effective inhibitors; All the compounds have shown significant interaction with active site residues (Leu346, Thr347, Asp351, Glu353, Trp383, Leu387, and Arg394) of the 4-hydroxytamoxifen. Moreover, the docking study was used to screen the top 5 compounds: ZINC13377936, NCI35753, ZINC35465238, ZINC14726791, and NCI663569. We also, employed molecular dynamics simulations to explore the binding dynamics present at the atomic level. Our MDS results have revealed the compounds (ZINC13377936 and NCI35753) with outstanding binding stability and lesser fluctuations. Both above hits possess a high potential as future therapeutic agents, acting by the mechanism of competitive inhibition against the ER α protein in breast cancer.

1. Introduction

Among all malignant cancers, Breast Cancer is the most common cause of cancer-related deaths in women worldwide and it is a heterogeneous disease at the molecular level. Myriad studies have revealed that epigenetic alterations play a prominent role as an early and common mediator for multiple events in cancer. DNA methylation, an epigenetic modification, plays a chief role in deciding the carcinogenic potential, rate of progression, and overall prognosis of various human malignant tumours. The enrichment of DNA methylation at promoter regions of various tumour suppressor genes (p16, ESR1, GSTP1, and PITX) is shown to be associated with the development, and progression of breast cancer. Estrogen receptor- α (ESR1) belongs to the family of nuclear receptor proteins that are involved in the control and regulation of a majority of estrogen-responsive genes (ERG). Any aberrancy in its expression pattern is associated with the active development of breast cancer. The methylation of the ESR1 promoter leads to a poor prognosis in breast cancer [1].

Estrogen binding with the estrogen-receptor (ER) on the nuclear membrane generates a signaling cascade that increases the mitotic potential of the breast epithelium. The allelic polymorphism of the ESR1 gene decides the intensity of associated carcinogenicity developed as a response to the ligand binding. Different SNPs in the gene encoding ER α , in normal and neoplastic breast tissue, have been linked with the different clinical phenotypes [2].

Estrogen binds with the hormone-binding domain of ER α to form an intermediate complex; this, on dimerization associates with other co-regulatory proteins to form a final complex which can activate or repress the transcription of ERG. Co-activators (AIB1 or SRC3) assist in the binding of the DNA binding domain of ER α at the estrogen-response-elements located on the promoter of ERG [3]. The estrogen-independent activation of ER α is controlled by receptor tyrosine kinases (RTK). On stimulation by certain signalling molecules like Rg1, RTK induces downstream MEK/ERK signalling cascade which phosphorylates the AF-1 domain of ER α . The phosphorylated ER α regulates gene expression by the principle of protein-protein interaction with a specific group of transcriptional factors. The final regulated product of both of these pathways controls many important cross-talks taking place in the cell cycle, angiogenesis, differentiation, proliferation, apoptosis, and survival [4] [Fig. 1].

Augmented estrogen exposures have proved to escalate the risk associated with breast cancer. The underlying insightful mechanisms guiding this risk profile are not entirely characterised. However, immune histochemistry studies have shown that ER over expression increases the estrogen sensitivity and responsiveness of benign breast epithelium tissues [5]. Furthermore, a high percentage of the population exhibiting ESR1 amplification can be observed among all primary cases of breast cancer. Therapeutic agents targeting ESR1 in combination with optimised hormonal therapy can be used effectively for the treatment of this populational subtype [6]. The current investigation is aimed to identify high affinity and effective inhibitors towards ER α by structure-based virtual screening and molecular docking studies [7–15]. To explore the variations in the protein-ligand interaction, Molecular Dynamics Simulation (MDS) and Principal Component Analysis (PCA) methods were engaged [16–19]. A full-atom molecular dynamics simulation method was used to investigate the molecular mechanism of the interaction between the inhibitors with ESR1. The conformational changes and dynamical properties were calculated using principal component analysis and free energy landscape methods. The binding free energy was evaluated using MM/MMGBSA [20–28].

2. Results And Discussion

The performed study tends to report the appliance of structure-based virtual screening, docking and dynamics for ERα inhibitors, which results in the binding of ligands into the active site of the targeted protein. Based on structural and molecular information, effective compounds were identified leading towards the inhibition of Era which can be potential therapeutic agents in the future.

2.1. Re-docking and structure-based virtual screening (SBVS) analysis.

Docking studies provides a great understanding of the drug interaction mechanism with the protein. Thus, the re-docking of co-crystallized inhibitor (OHT) has shown the binding interaction within the same binding site in the targeted receptor with an RMSD value of 0.336 Å as illustrated in Fig. 2. Docking results were analyzed with perfect binding confirmation, higher docking score, glide energy and e-model; tabulated in Table 1.

Table 1
Re-docking of the co-crystallized inhibitor into the active site of the co-crystallized compound within the protein.

Name of Inhibitors	Docking Score (kcal/ mol)	Glide Energy (kcal/mol)	No. Hydrogen Bond present	Interactive residues for Hydrogen bond	Djstance (Å)
Co-crystallized Ligand (OHT)	-10.009	-62.124	02	Glu353,	1.81
				Arg394	1.93

Further, the SBVS was applied to identify the exact conformation and orientation of the drug-like molecule into the same binding site of targeted protein using HTVS, SP, and XP protocol with default parameters against the two different chemical libraries (ZINC and NCI), retrieved best 20 hits ligands as effective inhibitors in terms of the favorable condition of pharmacokinetic properties including highest docking score and glide energy are shown in Table 2. All the compounds have shown the significant interaction with active site residues (Leu346, Thr347, Asp351, Glu353, Trp383, Leu387, and Arg394) of the ESR1.

Table 2
Virtual screening top hits from Zinc and NCI Databases, respectively.

Zinc Virtual Screening.						
S. No.	Compound ID	Docking Score (kcal/mol)	Glide Energy (kcal/mol)	No. Hydrogen Bond present	Interactive residues for H-bond	Distance (Å)
1	ZINC35465238	-13.284	-46.171	04	Thr347 Glu353 Leu387 Arg394	2.09 1.77 2.11 2.09
2	ZINC13377936	-12.839	-53.516	04	Thr347 Glu353 Arg394	2.1 1.63 & 1.77 2.10
3	ZINC14726791	-12.522	-44.602	04	Thr347 Glu353 Arg394	1.68 1.62 & 1.86 2.05
4	ZINC14726789	-12.475	-50.847	04	Thr347 Glu353 Arg394	2.22 1.62 & 1.86 2.09
5	ZINC05359847	-12.357	-29.311	03	Glu353 Gly420	1.76 & 2.74 2.13
6	ZINC85489116	-12.267	-50.077	03	Asp351 Arg394 Gly420	1.85 1.67 1.78
7	ZINC03589221	-12.218	-51.246	04	Thr347 Glu353 Arg394	1.96 1.62 & 1.68 2.13
8	ZINC85569243	-12.058	-44.123	04	Glu353 Arg394 Gly420	1.62 & 1.66 1.88 2.19
9	ZINC00056472	-12.014	-47.512	04	Thr347 Glu353 Arg394	2.16 1.79 & 1.68 2.12
10	ZINC31169866	-12.011	-53.682	04	Thr347 Glu353 Arg394	1.87 1.63 & 1.58 2.16
NCI Virtual Screening						

Zinc Virtual Screening.						
S. No.	Compound ID	Docking Score (kcal/mol)	Glide Energy (kcal/mol)	No. Hydrogen Bond present	Interactive residues for H-bond	Distance (Å)
1	35753	-12.872	-47.536	04	Glu353 Arg394 Gly420	1.83 & 1.68 1.95 1.89
2	663569	-12.613	-44.045	04	Thr347 Glu353 Leu387 Arg394	2.05 1.92 1.76 2.16
3	747974	-12.55	-65.238	04	Asp351 Glu353 Arg394 Gly420	1.76 1.81 2.15 2.01
4	706725	-12.546	-64.12	04	Asp351 Glu353 Arg394 Gly420	1.71 1.81 2.14 2.00
5	665877	-12.53	-47.689	01	Glu353	1.49
6	211751	-12.475	-56.377	02	Leu346 Glu353	2.05 1.59
7	32653	-12.382	-47.57	04	Leu346 Glu353 Arg394 Gly420	2.03 1.66 2.20 1.85
8	286612	-12.076	-55.227	03	Asp351 Glu353 Arg394	1.73 1.58 2.10
9	153166	-12.022	-55.945	04	Asp351 Glu353 Arg394	1.69 1.66 & 1.65 1.90
10	32081	-11.761	-42.951	03	Glu353 Leu387 Arg394	1.77 2.16 2.00

2.2. ADME properties

Inadequate ADME properties of compounds are a major cause of clinical trial failures leading to wastage of resources during later stages of development. Prediction of ADME properties will help to improve compounds under consideration and to identify the lead compounds showing optimal performance during clinical trials. Therefore, these properties were predicted using the Schrodinger QikProp module. We

evaluated the properties within the standardized range defined for human use such as Molecular weight (MW 130–500), H-bond donor (< 5.0), H-Bond acceptors (< 10.0), the octanol/water partition coefficient log (-2 to 6.2), the aqueous solubility log (mol/L) (-6.5 to 0.5), Predicted Caco-2 cell permeability in nm/s (acceptable range: <25 is poor and > 500 is great), Predicted apparent MDCK cell permeability in nm/s, CNS activity – 2 (inactive) to + 2 (active), Lipinski's rule of five and Jorgensen rule of three, Percentage of human oral absorption (= > 80% is high, < 25% is low), demonstrated in Table 3, indicating screened compounds which can be a promising lead in drug discovery development.

Table 3
ADME profile of Screened hits and Reference Compound.

S. No.	Compound ID	MW	HBD	HBA	QP log Po/w	QP logS	QPP Caco	QPP MDCK	QP log BB	QP log KP	Rule of 5	Rule of 3	% HOA
1	ZINC35465238	316.353	4	4.7	1.772	-3.639	120.698	50.324	-1.474	-4.187	0	1	74.577
2	ZINC13377936	330.38	4	5	2.284	-4.018	59.399	23.386	-2.647	-3.861	0	1	72.067
3	ZINC14726791	300.354	4	3	2.054	-3.623	120.358	50.171	-1.453	-4.333	0	0	76.209
4	ZINC14726789	300.354	4	3	2.002	-3.556	120.36	50.172	-1.446	-4.344	0	0	75.904
5	ZINC03589221	330.337	4	6	1.14	-2.912	49.707	19.29	-2.114	-4.493	0	1	63.984
6	35753	302.369	4	3	2.403	-3.224	119.517	49.792	-1.785	-3.794	0	0	78.2
7	663569	330.337	4	6	1.066	-2.598	50.939	19.807	-1.98	-4.57	0	1	63.739
8	747974	473.586	2	6.25	4.7	-5.967	114.731	83.764	-0.962	-4.22	0	1	91.331
9	706725	473.586	2	6.25	4.43	-4.494	207.597	159.229	-0.49	-3.78	0	0	94.357
10	32653	286.37	3	2.25	3.219	-3.865	330.318	149.407	-1.343	-2.87	0	0	90.878
11	Reference OHT	387.521	1	3.5	5.59	-5.36	671.532	355.884	-0.259	-2.453	1	0	100

MW (Molecular Weight of the molecule) = (130.0 to 725.0)

HBD = Hydrogen Bond Donor = (0.0 to 6.0)

HBA = Hydrogen Bond Acceptor = (2.0 to 20.0)

QLogP o/w = (Predicted octanol/water partition coefficient) = (-2.0 to 6.5)

QLogS = (Predicted aqueous solubility, logS) = (-6.5 to 0.5)

QPPCaco = Predicted Caco-2 cell permeability in nm/s (acceptable range: <25 is poor and > 500 is great)

QPPMDCK = Predicted apparent MDCK cell permeability in nm/s.

QLogBB = (Predicted brain/blood partition coefficient) = (-3.0 to 1.2)

QLogKP = (Predicted skin permeability, logKp) = (-8.0 to -1.0)

Rule of 5 = (Number of violations of Lipinski's rule of five) = (maximum is 4)

Rule of 3 Violations = (Number of violations of Jorgensen's rule of three) = (maximum is 3)

% HOA = Percentage of human oral absorption (= > 80% is high, < 25% is low)

2.3. Enrichment Calculation

The virtual screening docking protocol was further evaluated by using an enrichment calculation process in terms of EF, ROC, and BEDROC metrics explained the area under ROC with 1.0, show the most active hits in ranking order based on quality. Since Trunchon and Bayly (2007) considered ROC with "≥ 0.7" as a satisfying metric value to determine the highest precision and predicting ability of virtual screening docking protocol. Enrichment curve and values of ROC are tabulated in Fig. 3 and Table 4, respectively.

Table 4
Evaluation of virtual screening protocol by Enrichment calculation.

EF 1%	RIE	ROC	Enrichment Matric	Value
68	16.99	1.0	BEDROC ($\alpha = 160.90$)	0.983
			BEDROC ($\alpha = 20.00$)	0.981
			BEDROC ($\alpha = 8.00$)	0.991

EF1%: Enrichment factor at 1% of the decoy data set.

RIE: Robust initial enhancement.

ROC: Receiver operating characteristic curve value.

BEDROC: Boltzmann-enhanced discrimination of receiver operating characteristic.

2.4. IFD

Top 5 screened compounds (ZINC13377936, NCI35753, ZINC35465238, ZINC14726791, and NCI663569) along with co-crystallized ligand (Reference OHT) were taken forward in the IFD platform to confirm the best binding interaction within the receptor's binding site based on the highest docking score within the range from - 11.314 to -14.527 kcal/mol, glide energy from - 51.648 to -62.526 kcal/mol, IFD score from - 532.191 to -535.418kcal/mol and interactive residues; shown in Table 5. To make protein-ligand interactions more understandable, the closed view of all the protein-ligand complexes, and their 2D interaction profile is presented in Fig. 4.

Table 5
Induced fit docking result of the compounds examined from Virtual screening.

S. No.	Compound ID	Docking Score (kcal/mol)	Glide Energy (kcal/mol)	Total No. of H-bond	Interactive residues for H-bond	Distance (Å)	IFD Score (kcal/mol)
1	ZINC13377936	-14.527	-62.526	05	Thr347 Asp351 Glu353 Trp383	1.85 1.61 & 1.83 1.81 1.90	-535.418
2	NCI35753	-14.017	-51.648	04	Leu346 Glu353 Glu419	1.75 1.66 1.85 & 2.03	-534.132
3	ZINC35465238	-13.960	-57.396	06	Thr347 Asp351 Glu353 Trp383	1.93 1.97 & 2.06 1.75 & 2.05 1.75	-534.440
4	ZINC14726791	-12.690	-53.666	03	Thr347 Glu353	2.10 1.71 & 1.78	-532.491
5	NCI663569	-12.566	-55.625	05	Thr347 Asp351 Glu353 Leu387	1.81 1.70 & 1.79 1.78 1.91	-532.191
6	Reference OHT	-11.314	-56.076	01	Glu353	1.80	-532.671

2.5. MM-GBSA

The binding free energy prediction was performed by a post-scoring method–MMGBSA for the evaluation of molecular docking process in both wild and mutant types which is applied to rank the best inhibition of targeted protein. In which, the value of MMGBSA (ΔG_{bind}) ranges from -13.802 to -65.359, are shown in Table 4 and represented graphically in Fig. 5. Results were correlated along with docking score to design relevant drug-like potent inhibitors because the value ΔG_{bind} defines the greater binding affinity of the ligand to bind with the receptor. The outcome of MMGBSA was more supportive to understand the binding mode analysis of the ligand with the receptor to help in the generation of potent inhibitors against ER α .

Table 6
Results of binding free energy analysis.

S. No.	Compound ID	ΔG_{Bind}^a	$\Delta G_{\text{Coulomb}}^b$	$\Delta G_{\text{Covalent}}^c$	ΔG_{VdW}^d
1	ZINC13377936	-65.359	-21.721	6.984	-39.766
2	NCI35753	-61.859	-23.281	6.439	-35.771
3	ZINC35465238	-53.601	-11.541	4.086	-39.429
4	ZINC14726791	-53.775	-20.872	6.016	-29.092
5	NCI663569	-47.967	-8.565	8.438	-35.177
6	Reference OHT	-13.802	-18.79	7.018	-10.915

- a- Free binding energy.
- b- Coulomb energy contribution to the binding free energy.
- c- Covalent energy contribution to the binding free energy
- d- Vander Waals energy contribution to the binding free energy.

2.6. MDS analysis

MDS is an effective method for studying conformational and interaction stability under specific conditions of the physiological environment. Thus, the best protein-ligand complexes of final hits along with the reference were incorporated for simulation of the time of 50 ns using MDS to compare the structural behavior and flexibility. Analysis of root mean square deviation (RMSD), root mean square fluctuation (RMSF), the radius of gyration (RGYR) and hydrogen contacts were carried out to determine the conformational, as well the interaction stability of the receptor-ligand complexes. Figure 6 (A) shows that the RMSD graph of the backbone of all complexes are in the range of 0.2 to 0.6 nm, in which the protein-ligand complexes with ZINC13377936 and NCI35753 have excellent stability in terms of RMSD as like known compound but the compound ZINC35465238 has shown significantly higher fluctuations. RMSF was calculated to get the residual stability of each complex. Figure 6 (B) of the RMSF graph represents that there are fewer changes in the residual level in all complexes. To find the compactness of protein's backbone atoms during the entire simulation period, the Radius of Gyration (RGYR) was calculated for all, represented in Fig. 6 (C), concentrate that all the complexes found to have less conformational changes and also, none of lost its compactness during the simulation, gives the significant results of stability in under an environmental condition. To determine the robust stability of hydrogen bond interactions between receptor-ligand, all complexes were monitored during trajectory analysis of 50 ns as shown in Fig. 6 (D) where compounds ZINC13377936, ZINC35465238 and NCI35753 have revealed one hydrogen bond and a maximum of two hydrogens throughout 50ns with high stability. But the Reference OHT showed one hydrogen bond throughout 50 ns with less stability. Resulted MDS reveals that the compounds ZINC13377936 and NCI35753 has outstanding binding stability and lesser fluctuations. Thus, both hits may be future therapeutic agents for the inhibition of ERα.

2.7. MM-PBSA

Herein, the examination of the stability of binding compounds next to simulation in the form of free energy was calculated using an effective methodology-MM-PBSA, shown in Table 7. Results reveal that the ZINC13377936 (-183.517±14.359 kJ mol⁻¹) and NCI35753 (-174.542±70.874 kJ mol⁻¹) compounds have less binding energy in comparison to ZINC35465238 (-128.596±29.284 kJ mol⁻¹) and Reference OHT (-117.642±127.121 kJ mol⁻¹). Likewise, the Van der Waal energies of ZINC13377936 (-217.606±14.362 kJ mol⁻¹) and NCI35753 (-184.590±59.838 kJ mol⁻¹) compounds were less in comparison to others. But the electrostatic energies of NCI35753 (-12.442±7.260 kJ mol⁻¹) and ZINC35465238 (-11.236±8.276 kJ mol⁻¹) were high in negative than ZINC13377936 (-6.782±9.359 kJ mol⁻¹) and the SASA energy of the ZINC13377936, NCI35753, and ZINC35465238 have higher in negative value than reference one. The polar solvation energy contributes in a positive way to the total binding free energy. Therefore, the predicted binding free energy strongly support the docking results.

Table 7
Binding free energy of selected complexes using the MM-PBSA approach. All energy in KJ mol⁻¹.

Compound ID	Binding energy	van der Waal energy	Electrostatic energy	Polar solvation energy	SASA energy
ZINC13377936	-183.517±14.359	-217.606±14.362	-6.782±9.359	60.988±9.369	-20.116±1.262
NCI35753	-174.542±70.874	-184.590±59.838	-12.442±7.260	39.016±14.783	-16.526±5.340
ZINC35465238	-128.596±29.284	-160.807±28.977	-11.236±8.276	59.500±14.775	-16.052±2.513
Reference OHT	-117.642±127.121	-132.132±131.402	-0.019±3.809	26.399±32.592	-11.891±12.138

2.8. PCA analysis

PCA analysis was done for the top-scoring ligands (ZINC13377936, ZINC35465238, NCI35753 and Reference OHT) for analyzing the atomic positional fluctuations. It clearly shows that the protein complex with ligands ZINC13377936 and NCI35753 were highly stable during the entire simulation and occupied very little space in the system as compared with other complexes. This study suggests that both the complexes have more stable complex for inhibitory activity. The PCA analysis result is shown in Fig. 7. An overall result suggests the variable conformation of the protein in connection with the compounds, leading to a change in potential interactions.

2.9 Boiled Egg-Plot

The boiled Egg plot is a statistically derived, expeditious and robust method to detect the passive gastrointestinal absorption. It is used to analyse the mechanism of small molecules which are being used for drug discovery in brain. The Boiled Egg plot is illustrated in Fig. 8, employing four chief inhibitors - ZINC90797260, ZINC14726791, ZINC13377936 and ZINC35753. It shows that all the four inhibitors lie in whitespace dictating a high potential for GI absorption. Furthermore, studies have revealed that P-glycoproteins play a paramount role in actively transporting the chemically tailored compounds out of the cell. Comparative analysis divulged that the compounds ZINC90797260, ZINC14726791 and ZINC35753 are P-glycoprotein substrates, whereas ZINC13377936 is a non-substrate inhibitor.

The pharmacological properties and physiochemical properties of all the hit ligands including the optimization for gastrointestinal absorption are listed in Table 8. The four inhibitors were selected based on some molecular properties such as Water Partition Coefficient (WlogP) – the values should be less than 5 indicating mitigated level of toxicity, specific binding, and possible oral administration. The topological Polar Surface Area (TPSA) of all the selected compounds should be less than 100 Å² indicating a high possibility for complete absorption. Therefore, based on these criteria, it is inferred that the selected ligands form good interaction with the protein ESR1. Moreover, CYP450 (CYP1A2, CYP2C9, and CYP2C19) plays a major role acting as a substrate model in the metabolism cycle. Excretion is predicted based on the total clearance model and renal ESR1 substrate. The toxicity of drugs was predicted using AMES toxicity test and LD50 in rat. Hence, all four tested parameters show a positive result for selected inhibitors.

Table 8
Calculated physiochemical and pharmacological properties of the hits ligands.

Ligands	WlogP	TPSA (Å ²)	GI absorption	BBB permeant	P-gp substrate	CYP450 inhibitors
ZINC90797260	2.95	90.15 Å ²	Yes	No	Yes	CYP1A2,CYP2C9,CYP2C19
ZINC14726791	3.47	80.92 Å ²	High	No	Yes	CYP1A2,CYP2C9
ZINC13377936	3.42	97.99 Å ²	High	No	No	CYP1A2,CYP2C9,CYP2C19
ZINC35753	4.20	80.92 Å ²	High	No	Yes	CYP1A2,CYP2C9,CYP2C19

2.10 Blood-Brain Barrier (BBB), HIA, Toxicity and LD50 comparison

The blood-brain-barrier (BBB) is used to describe the peculiar properties of the microvasculature of the central nervous system (CNS). This is also used for maintaining the homeostasis for CNS.

Inhibitor permeability for BBB is a paramount prerequisite for drug discovery. BBB values for the four compounds have been listed in Table 9 and illustrated in Fig. 9; compound ZINC14726791 has highest BBB value – 0.7154, whereas ZINC90797260 has lowest 0.5319. All the four inhibitors for ESR1 predicted BBB + compounds and have been found with direct CNS activities and functions. Development of oral drug requires human intestinal absorption (HIA) spectra which plays a chief role in designing, optimizing the compounds. Absorption in the small intestine is a predominant fundamental process, highly used for drug bio-availability for oral administration. The two inhibitors ZINC90797260 and ZINC1472679 display 98.89% of Human Intestinal Absorption (HIA), and ZINC13377936 displays 84.33% HIA which is lowest among all.

The comparative ADMET analysis is listed in Table 9 for the selected inhibitors (ZINC90797260, ZINC14726791, ZINC13377936 and ZINC35753) which is based upon four parameters; Blood-brain-barrier (BBB), Human Intestinal Absorption (HIA), AMES Toxicity, and LD50. The required parameters are procured from the admetSAR database and tabulated according to their predicted values and properties. These four compounds are also graphically estimated using R-programming as illustrated in Fig. 9.

LD50 values in rats for the selected compounds dictate mitigated values for oral acute toxicity.

Compounds ZINC14726791 has 2.3403 LD50 value, while the other three compounds show similar LD50 absorption in the rat. No toxic effects are observed in the predicted *In silico* model for chronic oral toxicity. Based upon mutagenicity and AMES toxicity analysis, all the four compounds are found to be non-carcinogenic indicating a positive result. Due to expensive and laboriousness of the experimental tests, therefore it is highly necessary to develop a robust *In silico* method to predict the chemical mutagenicity. Thus, if the docked compounds are synthesized or studied *in-vitro*, they may behave as HIT since there is a good correlation between desired ESR1 protein and the compounds.

Table 9
Comparative ADMET profile of the top four compounds.

Compound	BBB	HIA	CYPsubstrate /inhibition	AMES toxicity	Carcinogenicity	LD50 in rat
ZINC90797260	0.5319	0.9889	Non-substrate/inhibitor	0.8275	Non-carcinogenic	1.9470
ZINC14726791	0.7154	0.9948	Non-substrate/non-inhibitor	0.8114	Non-carcinogenic	2.3403
ZINC13377936	0.6046	0.8433	Non-substrate/non-inhibitor	0.6399	Non-carcinogenic	1.8502
ZINC35753	0.5902	0.9626	Non-substrate/non-inhibitor	0.9119	Non-carcinogenic	1.9670

3. Material And Methods

3.1. System Configuration

The presented *In silico* studies were performed using the specific software Schrodinger Drug Discovery Suite, NY and Gromacs installed on the CentOS Linux Enterprise version 7.0 with i5 processor, with 64 GB RAM. General workflow has represented in Fig. 10.

3.2. Target selection and Protein Preparation

X-ray crystal structure of the ER α in complex with 4-hydroxytamoxifen (OHT)(PDB Id: 3ERT [29] was selected for the present study by considering Resolution (1.9 Å), and R-Value Free (0.262) as specific selection parameters from the Protein Data Bank[30–38]. Before proceeding to dock, the PDB structure was prepared using the Protein Preparation Wizard module(Schrodinger, Inc., LLC, New York, USA) by applying criteria like removal of water molecules, assigning bond orders, filling missing hydrogens, side chains & loops, capping termini, selenomethionine to methionine reversion, optimization and energy minimization using the OPLS-2005 force field with default settings[39–50].

3.3. Preparation of Chemical libraries

Ligand preparation was carried out with two discrete libraries (ZINC and NCI) using LigPrep module (Schrodinger, Inc., LLC, New York, USA) for better optimization, conversion of 2D to 3D ring conformers and ionization states tautomer states with an OPLS-2005 force field at the pH range of 7 $\pm$ 2[39–45][46–58].

3.4. Receptor grid generation and redocking

Receptor grid was generated by picking the centroid OHT's in receptor using the Receptor Grid Generation Glide module(Schrodinger, Inc., LLC, New York, USA) in the XYZ coordinates (X = 31.91, Y=-1.8, Z = 25.17) with default settings of the OPLS-2005 force field and the Vander Waal's radius scaling factor of 1 Å with partial charge cut-off of 0.25Å[59–60]. Further, the ligand 4-hydroxytamoxifen (OHT) was docked into the generated grid for confirming the interaction between protein and ligand using Glide XP (Schrodinger, Inc., LLC, New York, USA)before the execution of virtual screening[39–45].

3.5. Structure-based virtual screening

The trio of VS protocol of HTVS, SP, and XP from Glide module (Schrodinger, Inc., LLC, New York, USA) was applied successfully on prepared chemical libraries to obtain new drug-like candidates. The VS protocol acts to pre-filter the choice of molecules by Lipinski's rule of 5 and removing ligand with a reactive functional group. To explain the arbitrary number of torsional degrees of freedom by transitional and rotational parameters, all the molecules were put authoritarian state [61–74]. Later, the best ligand docking poses were filtered using glide docking based on a receptor grid and minimized with Van der Waals as well as electrostatic energies. Finally, top hits were retrieved with a higher score of docking, glide energy and binding mode analysis [75–82].

3.6. ADME Prediction

QikProp module (Schrodinger, Inc., LLC, New York, USA) was applied to generate ADME (adsorption, distribution, metabolism, and excretion) properties of the compounds before failing at a later stage of the drug discovery [71]. It analyzes the relevant properties of drug-likeness and pharmaceutical for all the hits like octanol/water (QlogP), aqueous solubility (QPlogS), brain/blood partition coefficient (QPlogBB), Predicted Caco-2 cell permeability (QPPCaco), Predicted apparent MDCK cell permeability (QPPMDCK), % of Human Oral Absorption, molecular weight, number of hydrogen bond donors and acceptors. Besides, Jorgensen rule of three and Lipinski rule of five wasalso calculated. The QikProp module applies for the BOSS program with the OPLS-AA force field to perform Monte Carlo statistical mechanics simulation on various organic solutes in a periodic box of explicit water molecules [83–98].

3.7. Enrichment calculation

The combination of top hits compounds, co-crystallized inhibitor, and Schrödinger decoy sets (download from Schrödinger website) were taken into the platform of Glide XP docking and Enrichment calculator from Schrodinger, Inc., LLC, New York, USA (Toledo et al., 2014; Friesner et al., 2004; Truchon & Bayly, 2007) for validating the docking protocol to identify an active one [99–104].

3.8. IFD

IFD protocol (Schrodinger, Inc., LLC, New York, USA) was implied on the selected top hits compounds from screening to analyze the best confirmation of the protein-ligand complex. Before proceeding to IFD, the receptor centroid ligand was selected for generating the grid box for the workspace ligand and finally, IFD was carried forward to generate 20 poses of each compound within the range of 5 Å with default parameters of glide docking, constraints, prime energy refinement and re-docking [105–111].

3.9. MM-GBSA – free binding energy calculation

A prime module of Schrodinger Suite, MM-GBSA was selected to calculate the relative binding free energy for the improvement of the docking score of final IFD hits. It utilizes a surface generalized Born model for a more significant demonstration of a solvent surface area. The relative binding energy represents by ΔG_{bind} , with the following equation (Lyne et al., 2006) [112–120].

$$\Delta G_{\text{bind}} = \Delta E + \Delta G_{\text{solv}} + \Delta G_{\text{SA}}$$

In which,

ΔG_{bind} = Binding Free Energy,

ΔE = Difference of energy minimization between receptor-ligand complex & the energies of receptor and ligand

Where, $\Delta E = E_{\text{complex}} - E_{\text{receptor}} - E_{\text{ligand}}$,

ΔG_{solv} = Difference of electrostatic solvation energy of the receptor-ligand complex & the energies of receptor and ligand

Where, $\Delta G_{\text{solv}} = G_{\text{solv}(\text{complex})} - G_{\text{solv}(\text{receptor})} - G_{\text{solv}(\text{ligand})}$,

ΔG_{SA} = Difference of Surface area energies of the receptor-ligand complex & the energies of receptor and ligand

Where, $\Delta G_{\text{SA}} = G_{\text{SA}(\text{complex})} - G_{\text{SA}(\text{receptor})} - G_{\text{SA}(\text{ligand})}$

3.10. Molecular Dynamics Simulation

Groningen Machine for Chemicals Simulations (GROMACS) version 5.1.4 (<http://www.gromacs.org/>) was utilized in determining the structural dynamics of protein-ligand complexes (Jorgensen et al., 1996; Adcock et al., 2006). Top complexes of the screen along with reference, were taken for the MDS analysis. All the ligands' topologies were generated using a webserver – PRODRG (Schüttelkopf & Van Aalten, 2004). The GROMOS force field is applied for performing MDS. Explicit water molecules were defined employing SPC (Simple Point Charge) model and a cubic period box with 1.0 nm distance (minimum) were set between protein and edge of the box. Following these, the protonation states of amino acid residues were set as per pH 7.0 and the system was neutralized by adding counter ions [121–131].

Consequently, the energy minimization was performed for all protein-ligand complexes using the steepest descent energy approach (1,000 ps). Later on, the whole system was equilibrated by executing a position-restrained dynamics simulation (NVT and NPT) at 300 K for 300 ps. Finally, the equilibrated systems were subjected to MD to analyse the dynamics stability with 50 ns at a constant temperature of 300 K, the pressure of 1 atm, and an integration time step of 2 femtoseconds. Herein, other parameters like isothermal and isobaric coupling constants were set at 0.1 ps and 2 ps, respectively, within the minimum distance between box edges and any protein atom equal to 2.0 nm. Using Origin pro8 (Essmann et al., 1995) program, the statistical analysis like RMSD, RMSF, RGYR, and Hydrogen bond for each complex were analysed and respective graphs were plotted [132–140].

3.11 MM-PBSA – free binding energy calculation

The calculation of binding free energy of protein-ligand was carried out by using the MM-PBSA approach with the help of the gmmPBSA module in three steps. The potential energy in a vacuum is calculated in the previous step. Other energies (polar and non-polar solvation)

were predicted in respective steps. Herein, the non-polar solvation energy was generated using the solvent-accessible surface area (SASA) model [141–148].

The free energy of binding is coming from the following theory:

$$\Delta G_{\text{binding}} = G_{\text{complex}} - (G_{\text{protein}} + G_{\text{ligand}})$$

Where, G_{complex} = Total free energy of the protein-ligand complex

G_{protein} & G_{ligand} = Total free energies of the separated form of protein and ligand in the solvent, respectively.

3.12 Principal component analysis:

Principal component analysis (PCA) or essential dynamics (ED) is one of the most important approaches to reveal the dynamic nature of proteins. It is a specific method to explain the functionally relevant motions of protein by the combination of local fluctuations and collective motions. The protocol was applied to build the covariance matrix with the extraction of concerted motion from all trajectories using backbone with g-covar and g-anaeig modules of GROMACS on selected screened compounds as well as reference inhibitor. In this process, the diagonalization of the covariance matrix generates a set of eigenvectors. A specific eigenvalue explains the energetic impact of the component on the motion [149–158].

3.13 Boiled Egg-Plot:

In the present investigation, we have used Boiled EGG-Plot to predict gastrointestinal absorption and brain penetration [16][61]. Aside from distinction to efficacy and toxicity, many new drug development failures are responsible for indigent pharmacokinetics and bioavailability. Gastrointestinal (GI) absorption and blood-brain access are two pharmacokinetics behaviors that estimate various drug development mechanisms. The Brain or Intestinal Estimated permeation method (BOILED-Egg) is expected as a factual predictive model that works by computing two parameters, i.e., the lipophilicity and polarity of the molecules. Contemporary predictions for both brain and intestinal permeation are accessed from the two same physicochemical descriptors and impartially rendered into the molecular design, owing to the speed, accuracy, conceptual simplicity and clear graphical output of the BOILED-Egg plot model. It also contains several parameters such as MW, TPSA, MLOGP, GI, and BBB, to revamp the BOILED-Egg plot. It can be enforced in various frameworks, from the filtering of chemical libraries at the initial steps of drug discovery and development to drug candidates' evaluation for further development.

3.14 Blood-Brain Barrier (BBB), HIA, Toxicity and LD50 comparison

The physiochemical properties of the top 4 compounds such as ZINC13377936, ZINC14726791, ZINC35753, ZINC90797260 were analyzed using SwissADME server, and the properties such as Blood-Brain Barrier (BBB), HIA, CYP inhibition, AMES toxicity carcinogenicity, LD50 in rat etc. were used for a comparative study using R programming. Abar plot was created using the ggplot library in R programming to compare the above properties [16][61].

Conclusion

As stated by numerous researches, 70% of breast cancers express the estrogen receptor, many of which are sensitive to ER inhibition. To summarize the role of ESR1 in breast cancer, our study focused on identifying effective and potent inhibitors of ER α using rational *in silico* approaches. The key concept of structural features for ER α inhibition has been furnished by applying a combined molecular docking, virtual screening, and ADME, MMGBSA, dynamics simulation, MMPBSA and PCA studies. All the 20 compounds have shown significant interaction with active site residues of the 4-hydroxytamoxifen (OHT) protein. As a result of the docking study, we identified ZINC13377936, NCI35753, ZINC35465238, ZINC14726791, and NCI663569) as the top 5 screened compounds. Further, our Molecular Dynamics results suggest that the potent compounds ZINC13377936 and NCI35753 show the highly stable hydrogen bonding with the common residues of Glu353, Leu387, and Arg394. Moreover, PCA analysis suggests that both the complexes have a more stable complex for inhibitory activity. Thus, these results have important implications in future breast cancer diagnostics and treatments. We are hopeful that these notable findings could be a potent inhibitor of ER α , which may execute further for experimental investigations.

Abbreviations

ADME – Absorption, Distribution, Metabolism, Excretion

HTVS – High Throughput Virtual Screening

IFD - Induced Fit Docking

MDS – Molecular Dynamics Simulation

MM-GBSA – Molecular Mechanics energies combined with the Generalized Born and Surface Area continuum solvation

MM-BSA – Molecular mechanics–Poisson Boltzmann surface area

OPLS – Optimized Potentials for Liquid Simulations

RGYR – Radius of Gyration

RMSD – Root Mean Square Deviation

RMSF – Root-Mean-Square Fluctuation

SP – Standard Precision

VS – Virtual Screening

XP – Extra Precision

Declarations

AUTHOR CONTRIBUTIONS

IC, UP, AB, MM, LS was involved in Molecular docking, Molecular Dynamics Simulation, Writing – review & editing. KS, ASP, VPM, LP were contributed towards Inhibitors collection, Data curation, Formal analysis, Validation, Visualization. UP, IJ, AN was involved in Molecular dynamic simulation. RS, SA, SA, KJA and TH were involved in Molecular Docking, ADMET analysis, R Programming analysis, Writing – review & editing. AN, and SKS were contributed in investigation, supervision, writing – review & editing.

Conflicts of interest/Competing interests:

The authors declare that they have no conflict of interest.

AVAILABILITY OF DATA AND MATERIALS:

Not applicable.

Code Availability:

Code will be provided as per the request

ETHICS APPROVAL AND CONSENT TO PARTICIPATE:

Not applicable.

HUMAN AND ANIMAL RIGHTS:

No animals/humans were used in the studies that are the basis of this research.

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Conflict of Interest:

The Author(s) declare that there is no conflict of interest.

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Figures

Figure 1

ESR1 interacting Pathway and Regulation of Breast Cancer Metastasis

Figure 2

Superimposition of best-docked complex (blue) and known co-crystallized complex (pink) within the active site of ESR1.

Figure 3

Graphical view of Enrichment curve for evaluating docking protocol for virtual screening.

Figure 5

Graphical representation of binding free energy of all complexes.

Figure 6

MDS profile of RMSD (A), RMSF (B), RGYR (C) and H-Bond interaction analysis (D).

2D projection of trajectory

Figure 7

PCA plot constructed by eigenvector 1 vs. eigenvector 2 for the selected complexes.

Figure 8

Boiled egg plot, water partition coefficient (WlogP) vs. Topological polar surface area (TPSA) of the hits Legends.

Figure 9

Comparative ADMET studies of BBB, HIA, AMES toxicity and LD50 of the established and virtual screened compounds.

Figure 10

General workflow of the Current Study.