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In-silico STUDIES OF UNIQUE SCHIFF BASE OF BENZIMIDAZOLE DERIVATIVES AS ANTICANCER AGENTS

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ABSTRACT

One of the most hazardous illnesses affecting peopleis breast cancer, but there is currently no permanent treatment available. New drug therapies with better pharmacokinetic characteristics are needed to treat breast cancer. The current work made use of computer modeling ideas such as docking for evaluating the efficacy and potency of novel designed three series of benzimidazole derivatives against the crystal structure of Estrogen receptor alpha. The results of molecular docking showed that Schiff baseof 2-substituted benzimidazole with quinoline and substituted benzaldehyde produced subpar results when compared to internal standards, whereas Schiff base of 2-substituted benzimidazole with pyrazole as a substituent produced the greatest results. Therefore, series with pyrazole as a substituent might be thought of as the finest for creating new compounds. The results of the docking were further confirmed by molecular dynamicsimulationwhich examined the strength of the macromolecular complex with regard to time.Additionally, these compounds displayed an improved pharmacokinetic profile.

Keywords: Benzimidazole, Breast Cancer, Molecular Docking, Dynamics Simulation, Mol Dock Score, EstrogenReceptor

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INTRODUCTION

Non-communicable diseases (NCDs) accounted for more than 71% of deaths globally. In India, NCDs accounted for more than 63% of all deaths. Cancer was one of the leading causes of death from NCDs.[1,2] In 2020, more than 1.3 million cancer patients were reported in India.[3] The most prominent sites for carcinogenic growth are the breast, mouth, lungs, cervix, uterus, and tongue. According to the WHO, millions of new cases of breast cancer were detected in 2020, resulting in 68,500 deaths from the disease. The intensifyingfrequency of breast cancer is a public health issue that requires an abrupt response. Radiotherapy, surgery, and chemotherapy are available depending on the stage of the disease.[4] However, it is quite difficult for a single or combination of molecules to suppress all disease macromolecules at once since malignant development manifests itself in the human body through diverse routes. The hormone 17-estradiol (estrogen) activates the orphan nuclear hormone receptor known as the estrogen receptor (ER).[5] ER comes in two different varieties: ERα and Erβ. A ligand-activated transcription domain called estrogen receptor, which is involved in breast cancer and is overexpressed in around 75% of cases, is found to develop after menopause. On the Estrogen receptor, there are many different functional domains, including the hinge region, DNA-binding domain, N-terminal domain (NTD), activation function-1 (found in NTD), activation function-2 (placed in LBD), and the C-terminal ligand-binding domain (LBD). An accurate assessment of estrogen receptors is crucial for the diagnosis and dealingwith breast cancer patients. The three drugs used today in breast cancer patients that act as estrogen receptor inhibitors or modulators are tamoxifen, raloxifene, and toremifene.[6] Antiestrogens, also known as estrogen antagonists, block the estrogen receptor and decrease cancer growth. Bazadoxifene, clomifene, cyclofenil, epimestrol, lasofoxifene, nafoxifene, ormeloxofene, raloxifene, tamoxifen, and toremifene are antiestrogens that have been designed to suppress the estrogen signal.[7] They typically have

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low bioavailability and can cause a variety of adverse side effects, such as unexpected vaginal bleeding, chest pain, and shortness of breath.[8, 9]Thus,the research aims to find new benzimidazole compounds with better activity and efficacy against estrogen receptor for application in the treatment of breast cancer. In the present article, we describe variousnovel- designed benzimidazole derivatives and their potential to link with Erα-positive sites which were obtained from the Protein Data Bank. Additionally, we performed molecular dynamics and ADME analysis studies.

EXPERIMENTAL

Computational-based approaches, such as molecular docking and dynamics, were employed for drug discovery and drug design.[10]During development, drug molecules might fail due to poor pharmacokinetic properties.[11] As a result, determining ADME characteristics is necessary to ensure the drug's clinical success.[12] Nowadays, computer-based methods are used to determine the compound's ADME profile. In the present study, we will discuss the binding interaction of hypothetically designed 2-substituted benzimidazoles with a receptor, estrogen receptor alpha using Molegro virtual Docker 6.0[13] as well as ADME profile of best series compounds among three series by online Swiss ADME online web server.The challenge of diversity sampling has developed through computational approaches.[14] Molecular modeling simplifies this procedure and limits the number of compounds to some predetermined number of compounds because it is difficult to synthesize and test them all. It involves the following steps:

- Data-set selection
- Ligand preparation
- Protein preparation
- Docking
- Calculations of binding energy
- Results and discussions

Data-Set Selection

Docking studies have been performed with following designed benzimidazole derivatives as given in Tables-1 to 3.

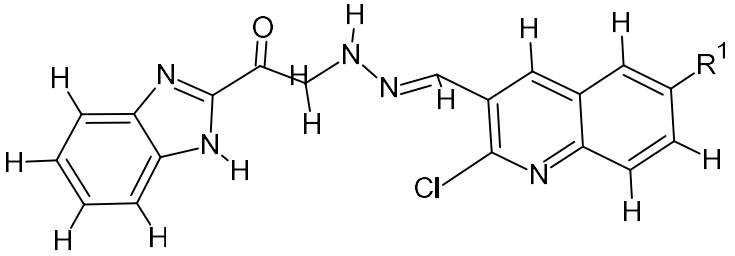

Table-1: Schiff Base of Benzimidazole Derivatives Containing Quinoline as Substituent Moiety

Compound No.	R^1-group
1	H
2	CH_3
3	Cl
4	Br
5	F
6	OH
7	OCH_3
8	NH_2
9	NO_2
10	$OCOCH_3$
11	SO_3H

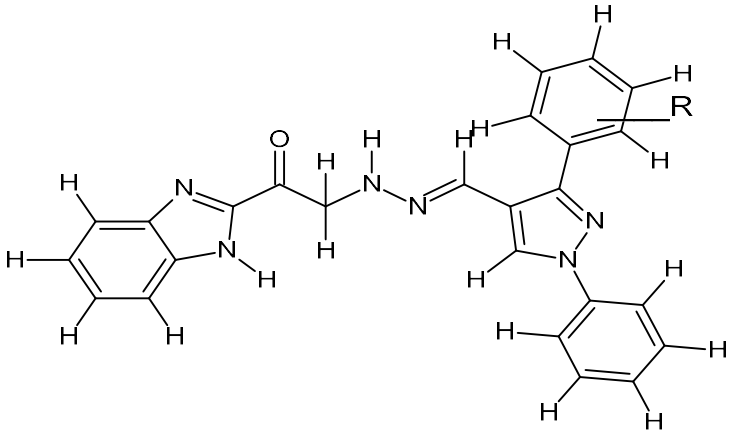

Table-2: Schiff Base of Benzimidazole Derivatives Containing Pyrazole as Substituent

Sr. No.	R-group
12	H
13	4-CH_3
14	4- Cl
15	4-Br
16	4-F
17	4-OH
18	4-OCH_3
19	4-NH_2
20	4-NO_2
21	4-$OCOCH_3$
22	4-SO_3H
23	4-OCF_3
24	3-CH_3
25	3-SO_3H
26	3-Cl
27	3-F
28	3-Br
29	3-OH
30	3-OCH_3
31	3-NH_2
32	3-$OCOCH_3$

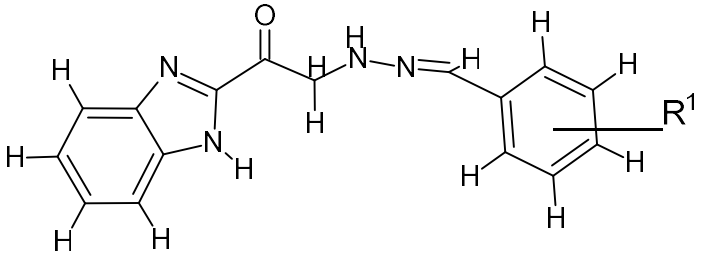

Table-3: Schiff Base of Benzimidazole Derivatives Containing Substituted Benzaldehyde as Substituent Moiety

Compound No.	R-group
33	H
34	4-Cl
35	4-OH
36	4-NO_2
37	4-F
38	4-NH_2
39	4-$OCOCH_3$
40	4-OCH_3

41	4-CH_3
42	4-isopropyl
43	4-tert butyl
44	3-Cl
45	3-OH
46	3-NO_2
47	3-NH_2
48	3-F
49	3-$OCOCH_3$
50	3-OCH_3
51	3-CH_3
52	2-CH_3
53	2-Cl
54	2-OH
55	2-NH_2
56	2-F
57	2-$OCOCH_3$

Ligand Preparation

The molecules were prepared in ACD Chem Sketch 12. v, converted from a 2D to a 3D structure using the build and optimization approach, and then the optimized structures were cleaned in 3D. The Molfile (mol) format was used to store the resultant structures. The constructed 3D structures were then imported into the Molegro virtual Docker 6.0 version. The introduced structures must be appropriatelyorganized, which means they must have the exact atom connections and bond sequencing. Here, the top ten conformations with minimum energy and maximum stability were preceded by the software for docking. Out of which only the best conformer was shown in the final result Tables-4, 5, and 6.

Table-4: Docking Result of Schiff Base of Benzimidazole Derivatives having Quinoline as Substituent (PDB ID:3ERT)

Comp. No.	R-group involved	Mol Dock Score	Docking Score	H-bonds	H-bond distance (Å)	Interacting Residue	Interacting Molecule
Standard	OHT_600[A]	-149.766	-90.31	3	3.51 2.55 2.89	O of Phe 404 N of Arg 394 O of Glu 353	O of OH O of OH O of OH
1	H	-130.007	-83.68	3	2.88 3.10 3.32	O of Leu 346 O of Thr 347 O of Thr 347	2.88 3.10 3.32
2	CH_3	-127.458	-82.94	0	-	-	-
3	Cl	-122.165	-86.99	0	-	-	-
4	Br	-121.888	-86.59	0	-	-	-
5	F	-120.479	-82.56	1	3.41	O of Thr 347	N of C=N
6	OH	-115.481	-83.53	2	2.96 3.14	N of Arg 394 O of Asp 351	O of OH N of NH of imidazole
7	OCH_3	-119.083	-80.96	0	-	-	-
8	NH_2	-123.901	-80.01	1	2.65	N of Arg 394	N of NH_2
9	NO_2	-131798	-83.07	2	3.27 2.58	N of Arg 394 N of Arg 394	N of NO_2 =O of NO_2
10	OAc	-132.806	-79.86	1	2.93	N of Arg 394	=O of $OCOCH_3$
11	SO_3H	-123.613	-84.42	4	3.50 2.85 3.57 2.13	N of Arg 394 N of Arg 394 O of Thr 347 N of Arg 394	-O of SO_3H =O of SO_3H =N of C=N -O of SO_3H

However, docking data showed that the docking score of all Schiff bases of benzimidazole derivatives with quinoline as a substituent moiety is lower than internal standard. All docked compounds demonstrated interactions with receptor. Fig.-5 demonstrated binding mode analysis of compound 10.
All the docked compounds showed strong interacting modes with receptor residue, out of which the docking studies revealed following information with respect to best benzimidazole as shown in Fig.-1.

Protein preparation

The Protein Data Bank was used to retrieve the human estrogen receptor alpha protein (PDB-3ERT). Using the protein production wizard in Molegro's virtual Docker, the protein structure was prepared. This step involved the assignment of bonds, bond order and hybridization, charges, and flexible torsion in the protein molecules. Then the electrostatic surface was created on the receptor site, and in order to determine the potential binding sites, the detect cavity algorithm was used.

Table-5: Docking Scores of Schiff Base of Benzimidazole Derivatives Having Pyrazole as Substituent using PDB ID: 3ERT

Comp. No.	R-Group involved	Mol Dock Score	Docking Score	H-bonds	H-bond distance (Å)	Interacting Residue	Interacting Molecule
Standard	OHT_600[A]	-149.766	-90.31	3	3.51 2.55 2.89	O of Phe 404 N of Arg 394 O of Glu 353	O of OH O of OH O of OH
12	H	-131.82	-78.69	0	-	-	-
13	4-CH_3	-159.495	-79.50	1	3.02	O of Thr 347	N of NH
14	4-Cl	-149.812	-84.57	0	-	-	-
15	4-Br	-156.423	-84.19	1	3.32	O of Thr 347	N of N=CH
16	4-F	-151.131	-95.39	0	-	-	-
17	4-OH	-158.717	-78.91	1	3.18	N of His 524	O of OH
18	4-OCH_3	-140.861	-72.61	1	3.17	N of His 524	O of OCH_3
19	4-NH_2	-134.035	-77.43	1	3.10	N of His 524	N of NH_2
20	4-NO_2	-148.217	-75.75	2	3.13 2.61	N of Arg 394 N of Arg 394	N of NO_2 O of NO_2
21	4-$OCOCH_3$	-104.058	-44.19	0	-	-	-
22	4-SO_3H	-112.786	-61.26	0	-	-	-
23	4-OCF_3	-143.483	-61.60	0	-	-	-
24	3-CH_3	-141.511	-92.33	0	-	-	-
25	3- SO_3H	-120.804	-61.68	1	3.24	N of His 524	=O of SO_3H
26	3-Cl	-152.829	-92.10	0	-	-	-
27	3-F	-150.901	-88.87	0	-	-	-
28	3-Br	-151.962	-88.64	0	-	-	-
29	3-OH	-152.53	-93.05	1	3.10	N of His 524	O of OH
30	3-OCH_3	-144.081	-77.81	1	2.60	O of Leu 346	N of NH of imidazole
31	3-NH_2	-134.496	-80.70	1	3.32	O of Thr 347	O of C=O
32	3-$OCOCH_3$	-143. 528	-44.08	0	-	-	-

Table-6: Docking Scores of Schiff Base of Benzimidazole Derivatives having Substituted Benzaldehyde as Substituent using PDB ID: 3ERT

S. No.	R-group involved	Mol Dock Score	Docking Score	H-bonds	H-bond distance (Å)	Interacting Residue	Interacting Molecule
Standard	OHT_600[A]	-149.766	-90.31	3	3.51 2.55 2.89	O of Phe 404 N of Arg 394 O of Glu 353	O of OH O of OH O of OH
33	H	-108.44	-78.0046	1	2.70	O of Leu 346	N of NH of imidazole
34	4-Cl	-109.535	-81.60	2	2.98 3.55	O of Leu 346 O of Thr 347	N of NH of imidazole N of NH
35	4-OH	-108.971	-77.45	1	2.91	O of Leu 346	N of NH of imidazole
36	4-NO_2	-110.139	-71.41	2	3.18 3.40	O of Thr 347 O of Thr 347	N of NH N of N=CH
37	4-F	-106.9	-74.71	2	2.80 3.25	O of Leu 346 O of Thr 347	N of NH of imidazole N of NH
38	4-NH_2	-105.29	-73.65	1	2.93	O of Leu 346	N of NH pf imidazole
39	4-$OCOCH_3$	-108.733	-66.47	No interaction	-	-	-
40	4-OCH_3	-116.768	-72.405	1	2.82	O of Leu346	N of NH of imidazole
41	4-CH_3	-111.101	-81.52	2	2.83 3.45	O of Leu 346 O of Thr 347	N of NH of imidazole N of NH
42	4-isopropyl	-114.942	-76.66	0	-	-	-
43	4- tert butyl	-97.57	-74.74	1	2.72	O of Leu 346	N of NH of imidazole
44	3-Cl	-105.25	-79.182	2	2.98 3.54	O of Leu 346 O of Thr 347	N of NH of imidazole N of NH
45	3-OH	-116.21	-77.63	1	2.91	O of Leu 346	N of NH of imidazole
46	3-NO_2	-109.082	-77.77	1	3.03	O of Leu 346	N of NH of imidazole
47	3-NH_2	-108.67	-76.88	1	2.79	O of Leu 346	N of NH of imidazole
48	3-F	-106.828	-78.03	1	2.89	O of Leu 346	N of NH of imidazole
49	3-$OCOCH_3$	-124.15	-77.53	1	2.84	O of Leu 346	N of NH of imidazole
50	3-OCH_3	-109.744	-79.05	1	3.51	O of Thr 347	N of NH
51	3-CH_3	-111.80	-78.96	1	2.77	O of Leu 346	N of NH of imidazole
52	2-CH_3	-113.979	-80.640	2	2.92 2.89	O of Leu 346 O of Leu 346	N of NH of imidazole N of NH of imidazole
53	2-Cl	-107.713	-78.51	1	2.86	O of Leu 346	N of NH of imidazole
54	2-OH	-113.893	-79.10	1	2.83	O of Leu 346	N of NH of imidazole
55	2-NH_2	-106.614	-77.87	2	2.73 3.24	O of Leu 346 O of Thr 347	N of NH of imidazole N of NH of imidazole
56	2-F	-113.365	-80.78	1	2.92	O of Leu 346	N of NH of imidazole
57	2-$OCOCH_3$	-121.428	-76.68	1	2.89	O of Leu 346	N of NH of imidazole

Docking

To determine the mechanism of action of a drug against cancer cells, a molecular docking study was conducted. Docking was performed by incorporating all the optimized conformers of ligands into the workspace of the docking wizard. In the docking wizard, all the ligands were selected, and scoring functions, *i.e.,* Mol Dock Score Grid, were adjusted by setting grid resolution at 0.30Å. The binding site was then established on three axes, and its radius was modified to a value of 15. The search algorithms were preceded by ten runs. Finally, the docking script was created and executed as an external process in the workspace. The generated script, log file, and poses so formed were saved in the output directory.

Molecular Dynamics Simulation

To evaluate the imperative strength and binding outlines of the lead found through virtual screening, a molecular dynamic (MD) simulation of the human estrogen receptor macromolecular complex with compound 13 was carried out for 100 ns.[15-19]For the 3D moving frame, a 10 Å gap was left between the wall and the ligand-protein complex, and counter ions were injected using 0.15 M sodium chloride to neutralize the present charge. Following that, 2000 iterations were performed using a merging threshold of 1 kcal/mol to decrease the system's energy. Then, over the next 100 ns, a pressure of 1.013 bar and a persistent temperature of 300K were maintained. To create simulation interaction graphs, the trajectory route was set to 9.6 with an input gap of 1.2 ps after the simulation process was completed.[20-34]

RESULTS AND DISCUSSION

The binding mechanism of the compounds to breast adenocarcinoma cancer cell lines was examined using a molecular docking analysis with PDB ID: 3ERT having a good resolution of around 1.9 Å containing standard reference 4-hydroxy Tamoxifen (OHT). Tables- 4to6 show the Mol Dock score and H-bonding between breast cancer proteins and ligands (57 hypothetically designed benzimidazoles).

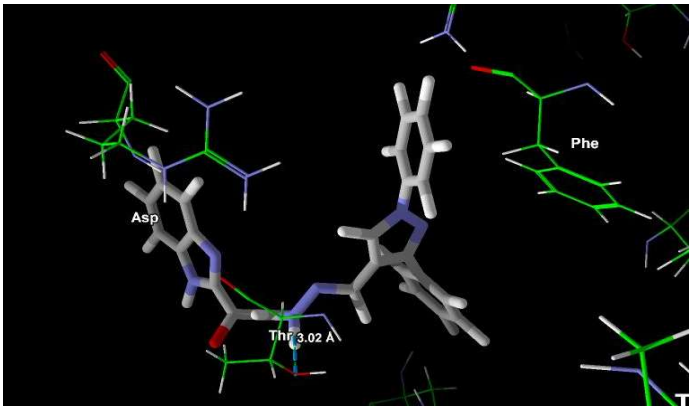

Fig.-1: Binding Mode of Compound 13 (R=4-CH_3) into the Binding Cavity of Estrogen Receptor Alpha (having Max. Mol Dock Score=-159.49)

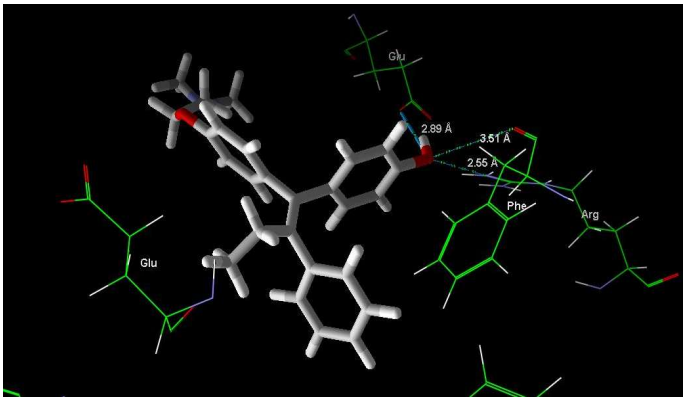

Fig.-2: Binding Mode of OHT_600[A] (Reference or Internal ligand) into the Binding Cavity of Estrogen Receptor Alpha (mol dock score =-149.76

The higher the mol dock score in terms of negative energy value, the better the binding affinity with the receptor. Results from docking with the protein estrogen receptor alpha (PDB ID-3ERT) revealed that the Schiff base of benzimidazole derivatives with pyrazole as a substituent had the greatest results that were equivalent to internal standards, whereas those with quinoline and substituted benzaldehyde had subpar outcomes. Among the series of Schiff bases of benzimidazole having pyrazole as a substituent, compound 13 had the best mol dock score with values of -159.495 (Fig.-1) comparable to the internal standard OHT_600 [A] (-149.76) (Fig.-2). Our most active drug molecule (compound 13) was further subjected to molecular dynamic simulation and has shown that it binds well with the receptor. From the results obtained from the wet lab, the designed molecules can be further subjected to synthesis and *in vitro* analysis for further validation of current experimental results.

Binding Mode Analysis of Internal Standard and Schiff Base of Pyrazole-Based Benzimidazoles

The estrogen receptor alpha's cavity-1 successfully received all of the filtered ligands. Following a visual examination of how 4-hydroxy tamoxifen and other ligands attached, it was found that all docked ligands as well as internal standard, 4-hydroxy tamoxifen displayed binding in the same cavity as seen in (Fig.-3 and Fig.-4).

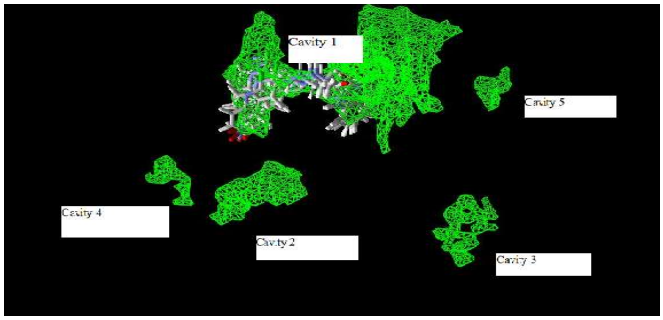

Fig.-3: shows Binding Mode Analysis of Internal Standard (4-hydroxytamoxifen) into Cavity I of Estrogen Receptor Alpha (PDB-3ERT)

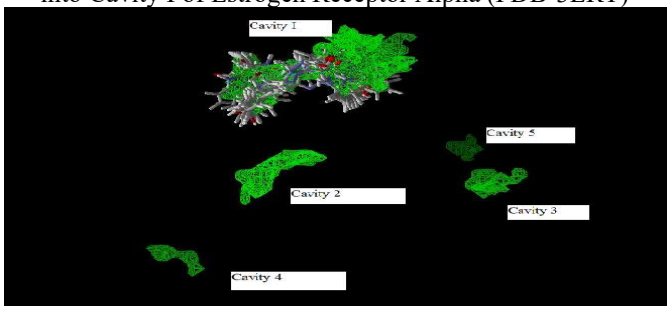

Fig.-4: Binding Mode of Schiff Bases of all (pyrazole based benzimidazole) Poses into the Binding Cavity 1 of Estrogen Receptor Alpha (PDB-3ERT)

Docking of Compound 10 (Quinoline-Based Benzimidazole) into PDB ID -3ERT

The outcomes of the docking investigation showed that there is no apparent distinction between compound 10 and reference tamoxifen in terms of their binding mechanisms. The oxygen in the acetoxy group of compound 10 shows a hydrogen bond interaction with the nitrogen atom of receptor residue Arg 394, which has a hydrogen bond distance of 2.93. (Fig.-5).

Binding Mode of all Poses of Schiff Base of (Substituted Benzaldehyde Based Benzimidazole) into the Binding Cavity 1 of Estrogen Receptor Alpha

All compounds in this series also bind in the same cavity as the internal standard but not with the same binding affinity as the internal standard, 4-hydroxy tamoxifen (Fig.-6).

Molecular Dynamic Simulation

The human estrogen receptor was found to have a macromolecular interaction with the inhibitor compound 13. This stability was determined by performing MD simulations with the Desmond module of the Schrodinger program. The strength and alterations of the macromolecule were computed using the RMSD. The composite was demonstrated to be stable during the simulations, with usual RMSD values

from 1.0 to 3.5 Å as represented in Fig.-7.There were some minor changes inside the receptor's cavity that generated swings of 3-10 Å, but the ligand's RMSD remained within the 8.0-10 Å range, when the ligand arrived at the active site. In the interest of estimating the vibrational frequency of the amino acids in reference to their initial conformations, the change of Cα atoms within the protein molecule was observed. The RMSF value of the macromolecular backbone was discovered to be between 1.0 and 2.5 Å. Following the investigation, it was discovered that the RMSF for the dynamic amino acids was lower for the complex system and that the compound 13 mean alteration was in the range of 3.0-4.0 Å, suggesting that the macromolecule had appropriate mobility in the active site.

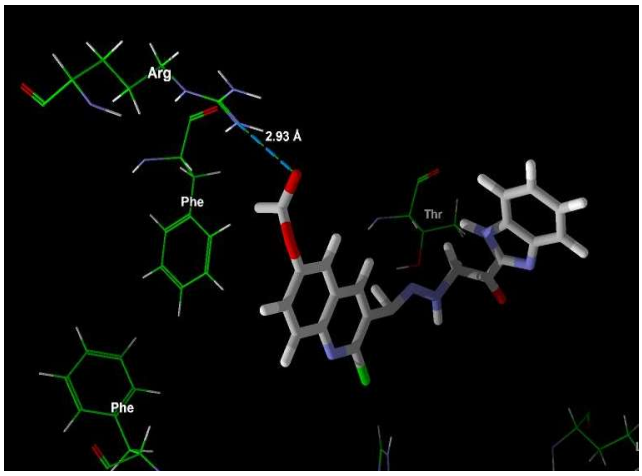

Fig.-5: Binding Mode of Compound 10 into the Binding Cavity of Estrogen Receptor Alpha

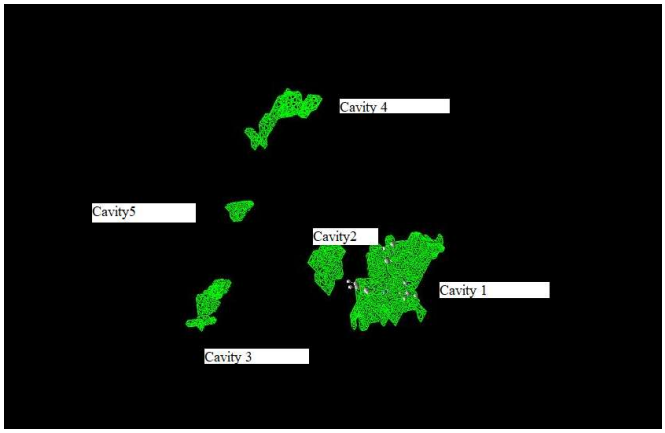

Fig.-6: Binding Mode of all Poses of Schiff Base of (substituted benzaldehyde substituent based benzimidazole) into the Binding Cavity 1 of Estrogen Receptor Alpha

Fig.-8 depicts the RMSF of the macromolecule's backbone as well as complex compound 13 detected over the 100 ns period of MD simulation.According to SSE analysis, the macromolecular structure included 55.5 percent SSE, with 53.2 percent alpha-helices and 3.3 percent beta-sheets remaining constant during the MD simulation. The stability of compound 13 was determined by measuring the strength of interactions created throughout the simulation procedure. From the results, it was concluded that the ligand molecule (compound 13) was hydrophobically attached to the amino acids Ala350, Leu354, Val355, Trp383, Leu525, Tyr526, Val533, Leu536, Leu539, and Met543, and Asp351 and Val534 through a hydrogen bond and a water bridge. The interaction between chemical 13 and the macromolecular target estrogen receptor is shown in Fig.-9.

In-silico ADME/Pharmacokinetic Predictions

It has been determined that a drug's appropriateness cannot be guaranteed by receptor inhibition .[35] To ascertain if a medication is physiologically active or not, pharmacokinetic studies are crucial in the drug

development process.[35,36] Additionally, the primary factor in many medication failures is inhibitors with significant biological system toxicity and low ADME properties. The results of pharmacokinetics attributes given in Table-7, it was detected that compound 22 and 23 violates Lipinski rule of 5 as they have molecular weight greater than 500 g/ mol.

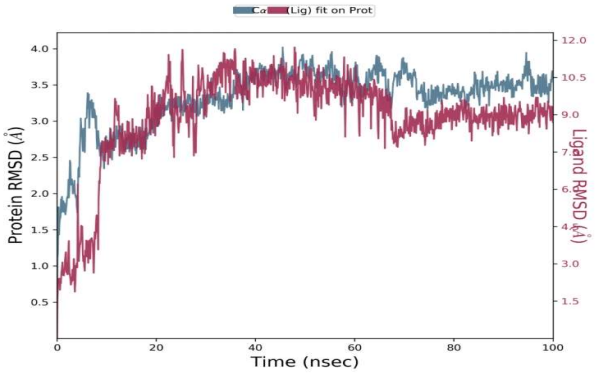

Fig.-7: RMSD of the Cα-backbone of the Human Estrogen Receptor and Complex Compound13 was measured over 100 ns of Molecular Dynamics Simulations.

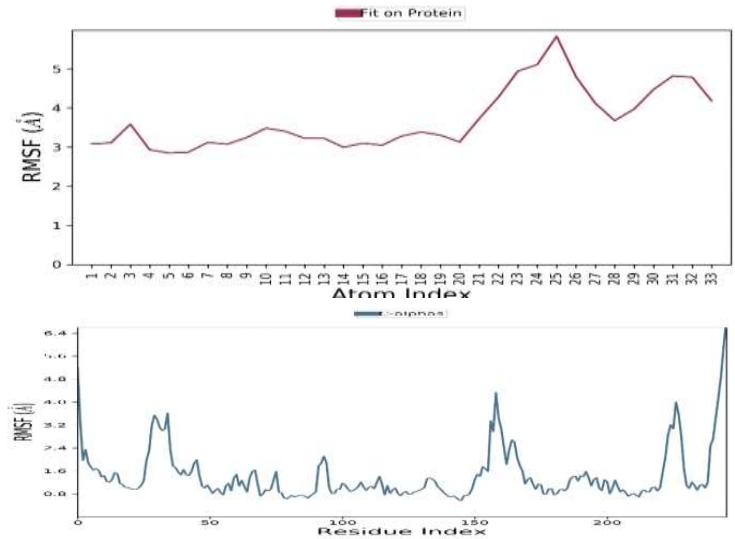

Fig.-8: RMSF of the Human Estrogen Receptor and its Complex with Compound 13 was Assessed after Conducting a Molecular Dynamics (MD) Simulation for 100 n

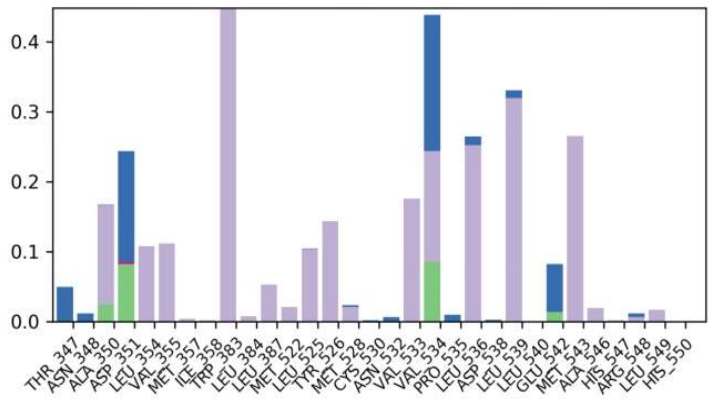

Fig.-9: Protein-Ligand Contacts Observed Between the Human Estrogen Receptor and Compound 13 during a 100 ns MD Simulation. Hydrogen Bonds are Shown as Green Bars, Water Bridges as Blue Bars, and Hydrophobic Interactions as Purple Bars

Additionally, the outcomes demonstrated a favourable pharmacokinetic profile, in which all compounds including reference 4-hydroxy tamoxifen exhibited substantial gastrointestinal absorption except (20, 22, 23), and none of them can pass through BBB. Moreover, through the SWISS ADME web server, the bioavailability radar of compound 13 was created as illustrated in Fig.-10 based on six physicochemical characteristics.[37] Coloured zone shown in bioactivity radar indicates suitable physico-chemical environment for oral bioavailability.

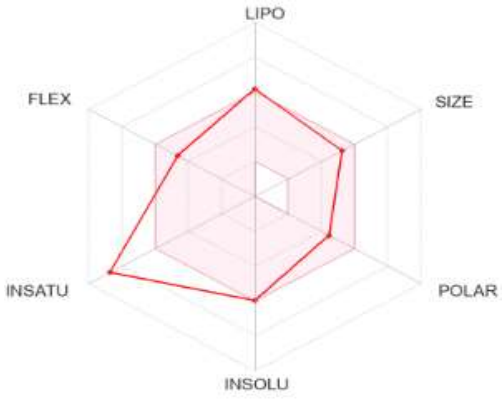

Fig.-10: Bioactivity RADAR of Compound 13 (Lipo: Lipophilicity, Flex: Flexibility, Insatu: Instauration, Insol-Insolubility, Polar-Polarity, Size)

Table-7: ADME And Drug Likeness Parameters of Schiff Base of Benzimidazole Derivatives having Pyrazole as Substituent

Comp. No.	R	SA	GI	BBB	Pgp	Bio-avalability	MW (g/mol)	nHBD	nHBA	TPSA (Å)	i log P	wlogp	nLV
12	H	3.44	High	No	No	0.55	420.47	2	4	87.96	2.92	4.22	0
13	4-CH_3	3.56	High	No	No	0.55	434.49	2	4	87.96	3.46	4.53	0
14	4-Cl	3.44	High	No	No	0.55	454.91	2	4	87.96	3.08	4.88	0
15	4-Br	3.48	High	No	No	0.55	499.36	2	4	87.96	3.67	4.98	0
16	4-F	3.45	High	No	No	0.55	438.46	2	5	87.96	3.39	4.78	0
17	4-OH	3.47	High	No	No	0.55	436.47	3	5	108.19	2.97	3.93	0
18	4-OCH_3	3.55	High	No	No	0.55	450.49	2	5	97.19	3.58	4.23	0
19	4-NH_2	3.51	High	No	No	0.55	435.48	3	4	113.98	2.75	3.81	0
20	4-NO_2	3.56	Low	No	No	0.55	465.46	2	6	133.78	2.75	4.13	0
21	4-$OCOCH_3$	3.65	High	No	No	0.55	478.50	2	6	114.26	3.45	4.15	0
22	4-SO_3H	3.66	Low	No	No	0.55	500.53	3	7	150.71	2.16	4.55	1Violation M.W. >500
23	4-OCF_3	3.57	Low	No	No	0.55	504.46	2	8	97.19	3.64	6.38	1
24	3-CH_3	3.59	High	No	No	0.55	434.49	2	4	87.96	2.95	4.53	0
25	3-SO_3H	3.75	High	No	No	0.55	500.53	3	7	150.7	2.10	4.55	1
26	3-Cl	3.47	High	No	No	0.55	454.91	2	4	87.96	3.53	4.88	0
27	3-F	3.44	High	No	No	0.55	438.46	2	5	87.96	3.35	4.78	0
28	3-Br	3.54	High	No	No	0.55	499.36	2	4	87.96	3.78	4.98	0
29	3-OH	3.51	High	No	No	0.55	436.47	3	5	108.19	2.73	4.57	0
30	3-OCH_3	3.61	High	No	No	0.55	450.49	2	5	97.19	3.60	4.23	0
31	3-NH_2	3.64	High	No	No	0.55	435.48	3	4	113.98	2.89	3.81	0
32	3-$OCOCH_3$	3.73	High	No	No	0.55	478.50	2	6	114.26	3.52	4.15	0

Boiled Egg Diagram

In the process of developing a medicine, GI absorption and blood-brain barrier penetration are essential milestones. The BOILED-EGG model is useful for determining the polarity and lipophilicity of derivatives. In the BOILED-Egg Diagram Fig.-11, the white region reflects GIT absorption, the yellow part denotes a high potential for BBB penetration, the blue dots denote molecules that are efficiently refluxed by P-glycoprotein (PGP+), and the red dots denote substances that are not P-glycoprotein substrates (PGP).

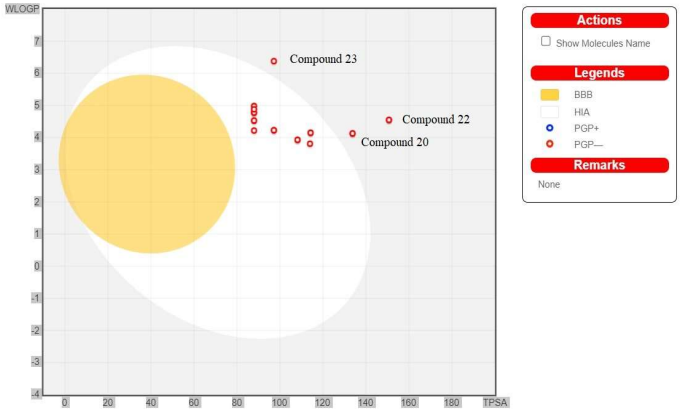

Fig.-11: illustrates Boiled Egg Diagram for Compound 13

CONCLUSION

The current study can be used further for the development of highly active and potent benzimidazole derivatives against breast cancer. Our *in-silico* approach has paved the path for shifting our current research to synthesis of these compounds and further *in-vitro* assays.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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List of Abbreviations

1. ADME : Absorption, Distribution, Metabolism, and Excretion
2. Ala : Alanine
3. Å : Angstrom
4. Arg : Arginine
5. ERT : Estrogen Receptor Alpha
6. Glu : Glutamate
7. Gln : Glutamine
8. HBD : Hydrogen bond donar
9. HBA : Hydrogen bond acceptor
10. His : Histidine
11. LBD : Ligand Binding Domain
12. log P : lipophillicity
13. Leu : Leucine
14. Mol.Form : Molecular formulae
15. Mol. Wt : Molecular weight
16. MR : Molar refractivity
17. MVD : Molegro virtual docker

18. nLV : No. of violations from Lipinski's rule of five
19. Pgp : P-glycoprotein
20. PDB : Protein data bank
21. Trp : Tryptophan
22. Tyr : Tyrosine
23. Val : Valine

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