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ORIGINAL RESEARCH ARTICLE

Molecular docking, pharmacokinetic profiling and toxicity studies of 2-substituted benzimidazole derivatives against breast cancer

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ABSTRACT

The most prevalent malignancy among women is breast cancer, which had almost 1.3 million new cases in 2020. It is the second most common cancer in the world, followed by lung cancer. The survival rate would be 99% if the cancerous tumour was limited to the breast. If the cancer migrated to neighbouring lymph nodes, the survival percentage would be 85% and it would drop to 27% if it spread to distant regions. In fact, the most prevalent breast cancer subtype is that caused by excessive estrogen levels. The enhancement of pertinent treatment techniques depends on the estrogen receptors (ER) in both healthy and pathological conditions. There are two primary types of ER, ERα and ERβ, which are each encoded by a different gene. ER status is the most important indicator of breast cancer prediction. To develop novel therapeutics for breast cancer, 30 newly designed benzimidazole compounds targeting the ER were docked. Among them, a compound with a glide score of −9.293 was discovered to be the leading compound. ADME investigations provided additional validation of the docking results. The pyrazole fused benzimidazole nucleus is therefore suggested as a potential pharmacophore for the development of innovative anticancer treatment for breast cancer.

Keywords: docking; breast cancer; pyrazole; estrogen receptor alpha; pharmacokinetics; toxicity

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1. Introduction

Non-communicable diseases (NCDs) accounted for more than 71% of deaths globally. In India, NCDs accounted for more than 63% of all deaths. One of the primary causes of mortality from NCDs was cancer[1]. In 2020, more than 1.3 million cancer patients were reported in India[2]. The most prominent sites for carcinogenic growth are the breast, mouth, lungs, cervix, uterus, and tongue. Using incidence collected by central cancer registries and mortality data gathered by the National Centre for Health Statistics, the American Cancer Society estimates the number of expected cases of cancer to be 1,958,310 and 609,820 cancer-related deaths in 2023[3]. According to the report released by the Sri Shankara Cancer Foundation, out of the total of 29 different types of cancer, most cases of breast cancer were reported during 2019–2021. Breast ducts, ductules, and bud development are brought on by the female sex hormones estradiol and progesterone. Estrogen levels grow throughout puberty, encouraging the formation of estrogen and progesterone receptors in the mammary glands. These sex hormones might have a role in the development of breast cancer[4].

Pregnancy and breastfeeding, on the other hand, serve to lower the chance of cancer development. According to research, having a larger breast mass puts you at a higher chance of developing breast cancer[5]. A known risk factor for breast cancer in postmenopausal women is obesity and/or overweight, which is measured by the body mass index (BMI) or may be estimated by the distribution of fat in the body. Breast cancer is linked to the synthesis of estrogen from fat tissues. Breast cancer risk factors that are not mentioned include having children beyond the age of 30, having malignant tumours in the breast, not getting enough vitamin D, not getting enough sun, and more[6]. Treatment for breast cancer differs from other cancers in that it targets specific receptor functions, including those of the ER (estrogen receptor alpha), PR (progesterone receptor), EGFR (epidermal growth factor receptor), and others. Estrogen receptors (ER) are critical in developing and progressing breast cancer. According to studies, estrogen, particularly 17-estradiol, promotes cell cycle progression from G1 to the S phase in mammary epithelial cells by upregulating the expression and activity of c-Myc and cyclinD1. As a result, anti-estrogen therapy is a potential therapeutic option for ER-positive breast cancer and the first targeted therapy for human breast cancer[7]. PR overexpression is prevalent in breast cancer, and it is linked to ER overexpression Since PR is the result of estrogenic stimulation in the target tissues, this suggests that the ER pathway is active. Overexpression of PR and ER improves the prediction of PR-positive breast cancer and increases the likelihood of responding to hormone therapy[8]. Better prognosis and therapeutic choices may result from ER and PR antagonist therapy[9]. Aromatase inhibitors (AIs), such as exemestene, letrozole, and anastrozole, can prevent the production of estrogen by inhibiting its biosynthesis[10] (**Figure 1**).

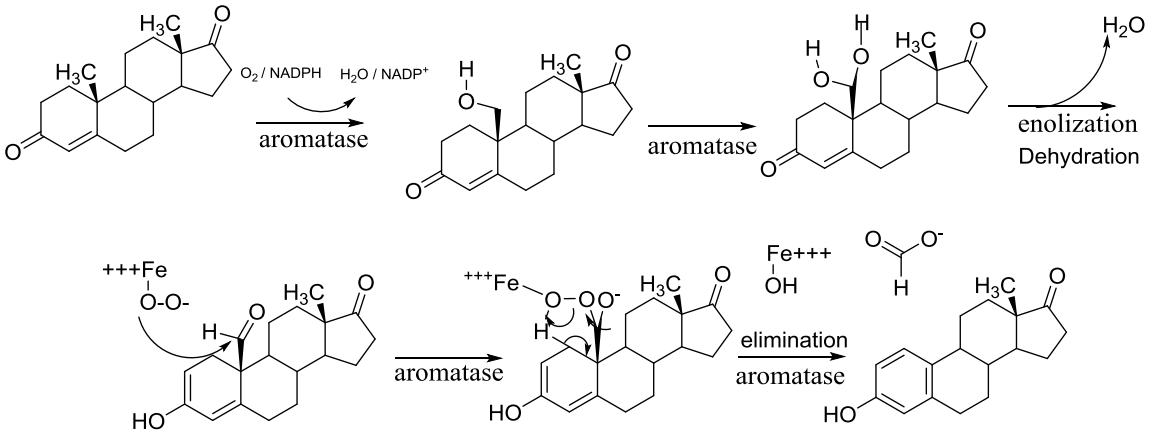

Figure 1. Biosynthesis of estrogen.

Tamoxifen, trastuzumab, paclitaxel, capecitabine, cyclophosphamide, gemcitabine, docetaxel, and other authorized marketed treatment medications for breast cancer have a variety of adverse effects[11]. Many natural and synthesized flavonoids have also been investigated and proven for their anti-cancer efficacy against breast carcinoma via blocking aromatase enzymes[12,13]. Benzimidazole is a heterocyclic compound consisting of benzene and imidazole rings[14]. It has a variety of biological actions like anti-inflammatory, analgesic, antiviral, antihelmentic, antiulcer etc., earning it the title of "strong moiety" in heterocyclic chemistry[15,16]. The discovery of N-ribosyl-dimethyl benzimidazole is said to have stimulated the interest in benzimidazole chemistry and as a scaffold or moiety in the discovery and development of pharmaceuticals[17]. Only one anticancer medication, bendamustine, has acquired FDA approval[18–20]. Selumetinib and Galeterone[21], two well-known benzimidazole agents that have proceeded to phase III clinical trials but have not so far been authorized as anticancer drugs. Surgery is currently the main therapeutic treatment for breast cancer; nevertheless, adjuvant chemotherapy is routinely used in these patients. These medical procedures are rather expensive. For instance, it was predicted that in 2030, the cost of treating breast cancer in the United States will be $245 billion[22]. Due to its high frequency, breast cancer therapy is the most expensive of all

malignancies[23]. The procedure has a significant possibility of failing as well. If this is not handled properly, the patient's life will be in danger. Additionally, surgical intervention may have a negative impact on the physical and mental health of female patients. Chemotherapy may cause breast cancer cells to become multi-drug resistance (MDR), which might lead to treatment failure[24]. Following surgery, anthracyclines, paclitaxel, and its semi-synthetic derivatives have demonstrated encouraging results in the treatment of breast cancer. Unfortunately, the effective use of these drugs was hampered by anthracycline and taxane side effects, notably haematological side effects (myelosuppression) and drug resistance. The failure of breast cancer treatments was frequently caused by unfavourable side effects[25]. One popular benzimidazole-based medication on the market is Pracinostat, which has adverse effects include weariness and myelosuppression. Thus, there is a great need to develop effective medicinal agent against breast cancer to overcome resistance and side effects. Through a variety of modes of action, including DNA alkylation, DNA binding, interfering with tubulin polymerization or depolymerization, enzyme inhibition, antiangiogenic effects, and signal transmission, benzimidazole scaffold demonstrated its anticancer activity[26,27]. In this article, we proposed thirty substituted benzimidazole derivatives and determine their anti-cancer potential using Maestro 13.1v against ER alpha.

2. Materials and methods

2.1. Data-set selection

Docking studies were performed with the following designed benzimidazole derivatives (**Table 1**). The basic structure of analogues is shown in **Figure 2** below:

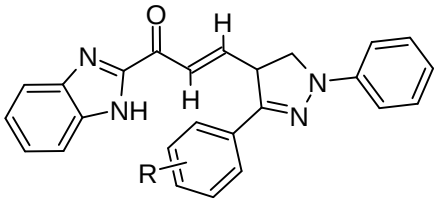

Figure 2. Basic structure of analogues given.

Table 1. 2-Substituted benzimidazole derivatives containing substituted pyrazole as substituent moiety.

Compound No.	R	Compound No.	R	Compound No.	R
1	H	**11**	4-Cl	**21**	2-Cl
2	4-NO_2	**12**	3-F	**22**	2-F
3	4-OAc	**13**	3-Br	**23**	2-Br
4	4-Br	**14**	3-OH	**24**	2-OH
5	4-OH	**15**	3-NH_2	**25**	2-NH_2
6	4-NH_2	**16**	3-NO_2	**26**	2-OAc
7	4-CH_3	**17**	3-OAc	**27**	2-NO_2
8	4-OCH_3	**18**	3-OCH_3	**28**	2-OCH_3
9	4-$CH(CH_3)_3$	**19**	3-tertbutyl	**29**	2-tertbutyl
10	4-$CH_2(CH_3)_2$	**20**	3-isopropyl	**30**	2-isopropyl

2.2. Ligand preparation

The LigPrep module of Schrodinger v13.1 was used to prepare the ligand structures. It is used for creating high-quality, all-atom 3D structures for a wide variety of drug-like compounds in SD or Maestro format. For each successfully presented input structure, Lig Prep creates a single, low-energy, 3D structures with proper chiralities. This stage involved determining chiralities from the 3D structure and retaining the original states

of ionization. Tautomers were created by eliminating existing conformers utilising the Macromodel techniques. The conformational space was explored using the Monte Carlo (MCMM) approach[28]. Using the truncated Newton Conjugate Gradient (TNCG), each search was repeated until the global energy minima were found.

2.3. Protein preparation

The study's major treatment focus for breast cancer was ER. The three-dimensional structure of ER alpha was downloaded from the protein data bank with PDB ID: 3ERT. The protein data bank's imported typical structure file of a protein is not appropriate for use right away in a molecular docking investigation. Protein downloaded from the protein data bank contains cofactors and water molecules, which could lead to inaccuracy and need to be removed from the MVD workspace before docking using the protein preparation wizard (preprocessed, optimised and minimised)[29].

2.4. Preparation of grid

Maestro version 13.1's receptor grid generation module was utilized to produce grids. A grid was formed around the current binding site of the co-crystallized ligand, allowing it (co-crystallized ligand) to be removed and other molecules to be linked to the same binding site of the protein[30].

2.5. Docking

To examine the binding mechanism of a compound with a chosen PDB ID: 3ERT[31,32] against breast cancer, molecular docking research was used. The binding location was chosen, the docking score from GLIDE (maestro v13.1) was acquired, and the grid was generated. The crucial amino acids interacting with receptors were covered by the active site grid. During the docking investigations, benzimidazoles were utilized as ligands, and their structures were designed using Maestro's workspace and translated to 3D form. Then the prepared ligands were docked into the generated grid in the prepared protein. The best-docked pose with the lowest glide score value was recorded for each ligand. Extra precision (XP) was performed using Schrodinger-maestro v13.1[33–35]. Coordinates of the complex of the protein with the ligand having the lowest binding free energy obtained from the docking results was selected to perform molecular dynamics simulations. The molecular dynamics simulation was carried out for 1 ps. The solvation was carried out before doing the dynamics by adding thirty-four Na++ counter ions to neutral the system. The final protein-ligand complex was placed into the periodic boundary conditions in all 3D space. Initially, the energy minimization step was carried out with 500 steps, followed by heating for 4ps for 300 K temperature and saved the result at every 2ps. To equalize the system equilibration step was carried out for 10ps and the final production was done for 10ps.

2.6. ADME prediction

ADME prediction is mainly done by using the Qik Prop of Maestro version 13.1. Qik Prop is a software programme that predicts ADME characteristics quickly, precisely, and easily. Qik Prop identifies organic compounds' physically significant descriptors and pharmaceutically relevant characteristics[36]. Qik Prop analyzed about thirteen physically significant descriptors and pharmacological characteristics of the compounds. Other parameters related to absorption, metabolism, excretion and toxicity were analyzed by the ADMET lab. 2.

3. Results and discussion

3.1. Docking study

Molecular docking research was performed to examine the drugs' binding mechanism against the human ER alpha. Docking is a computer tool for determining feasible binding mechanisms of drugs to the active region of the protein. It creates a picture of the active location with interaction points known as grid. The ligand

is then positioned in the binding site using either grid search or energy search. The binding energy is calculated by taking into account several forms of interactions between the receptor and the ligand, such as Van der Waal's interactions, electrostatic interactions, and aromatic interactions. To give practical answers to the problem of diversity sampling, computational techniques have been developed. Because it is difficult to synthesize and test all potential chemicals, molecular modelling facilitates this method while limiting it to a set number of compounds[28,29]. The docking protocol used in this study was first validated by redocking the co-crystallized ligand (4-hydroxy tamoxifen) with human ER protein (PDB ID: 3ERT). The re-docked ligand produced poses similar to those of the co-crystallized ligand with ER proteins, indicating that a rational docking protocol was used in this study. The interactions between 30 designed benzamide derivatives and standard 4-hydroxy tamoxifen for cancer were virtually scanned into the binding site of ER alpha, and the results were compared using the same cavity site of ER alpha protein. **Table 2** shows the Mol Dock score and H-bonding between breast cancer proteins and ligands (30 hypothetically designed benzimidazoles). Compounds 5, 6, and 15 were found to have the highest Mol Dock score (−8.573, −9.293, −9.063, respectively) in comparison to standard 4-hydroxy tamoxifen with Mol Dock score of −8.483. Compounds 1, 24, and 27 have comparable Mol Dock scores as that of the standard drug (−8.470, −8.160, and 8.248, respectively). The binding interactions of standard 4-hydroxy tamoxifen and compound 6 with the binding site residues of the ER alpha are shown in **Figures 3–6**. The molecular dynamics simulation of the best-docked pose of best-docked compound (**Figure 7**) was carried out to elucidate the stability of the docked complex. The plot between the total energy of the complex with respect to the time (ps) showed the stability of the complex (ligand-protein) sustained during the molecular dynamics simulations (**Figure 8**). The plot between temperature applied during the simulations and time (ps) confirmed the stability and sustainability of the complex with respect to the temperature (**Figure 9**). These molecular dynamics simulations confirmed the stability of the ligand-protein complex.

Table 2. Docking scores of 2-substituted benzimidazole derivatives using PDB ID: 3ERT for anticancer activity.

Sr. No.	Docking score	Glide energy (kcal/mol)	Ligand atom(s) involved in H-bonding	Residue(s) involved in H-bonding (bond distance, Å)	Other interacting residues
Std.	−8.483	−45.676	-	-	Leu354, Glu353, Asp351, Ala350, Leu349, Thr347, Leu346, Met343, Leu391, Met388, Leu387, Leu384, Trp383, Leu536, Leu428, Leu402, Phe404, Gly420, Gly521, Met421, Hie524, Leu525, Ile424
1	−8.470	−57.918	-	-	Arg394, Leu391, Met 388, Leu387, Leu384, Trp383, Leu539, Leu536, Val533, Leu354, Glu353, Asp351, Ala350, Leu349, Thr347, Leu346, Met343, Ile424, Gly521, Gly420, Hie524, Leu525
2.	−6.962	−65.917	C=O NH of imidazole	Cys530 (2.31) Cys530 (2.01)	Trp383, Leu354, Leu387, Asp351, Ala350, Thr347, Leu536, Pro535, Val533, Lys531, Cys530, Lys529, Tyr526, Leu525, Met522
3.	−4.890	−60.945	-	-	Leu525, Tyr526, Met528, Lys529, Cys530, Val533, Val534, Pro535, Leu536, Leu539, Leu354, Asp351, Ala350, Thr347, Leu346, Met343, Phe404, Leu391,

					Met388, Leu387, Leu384, Trp383
4	−6.893	−57.940	-	-	Cys530, Lys529, Met528, Tyr526, Leu525, Met522, Leu539, Leu536, Leu354, Asp351, Ala350, Thr347, Leu346, Met343, Phe404, Met388, Leu387, Leu384, Trp383
5	−8.573	−55.436	OH OH NH of imidazole	Glu353 (1.60) Arg394 (2.05) Asp351 (1.92)	Ile424, Met421, Gly420, Glu419, Val418, Arg394, Leu391, Met388, Leu387, Leu384, Trp383, Gly521, Hie524, Leu525, Met528, Lys529, Cys530, Val533, Met343, Leu346, Thr347, Leu349, Ala350, Asp351, Glu353
6	−9.293	−51.479	NH_2 NH_2	Gly420 (1.75) Gly521 (2.73)	Leu536, Leu539, Trp383, Leu354, Leu384, Glu353, Gly521, Lys520, Met421, Leu387, Asp351, Gly420, Glu419, Val418, Met388, Leu391, Arg394, Ala350, Leu349, Thr347, Leu346, Met343, Leu525, Hie524, Met522
7	−6.362	−56.451	-	-	Met388, Leu387, Leu384, Trp383. Asp351, Ala350, Thr347, Leu346, Met343, Phe404, Leu354, Leu539, Leu536, Cys530, Lys529, Met528, Tyr526, Leu525, Met522-
8	−6.323	−63.468	NH of imidazole	Asp351 (1.58)	Leu387, Leu384, Trp383, Ala350, Asp351, Thr347, Leu346, Met343, Phe404, Leu354, Leu539, Leu536, Val534, Val533, Cys530, Lys529, Met528, Leu525
9	−6.609	−55.209	NH of imidazole	Asp351 (1.85)	Met343, Phe404, Leu346, Thr347, Leu387, Leu384, Trp383, Ala350, Asp351, Leu354, Leu539, Leu536, Val534, Val533, Cys530, Lys529, Met528, Leu525
10	−6.629	−55.335	NH of imidazole	Asp351 (1.85)	Phe404, Met343, Leu346, Thr347, Ala350, Asp351, Leu387, Leu384, Trp383, Leu354, Leu539, Leu536, Val534, Val533, Cys530, Lys529, Met528, Leu525
11	−6.651	−65.332	-	-	Leu539, Leu536, Trp383, Leu384, Leu387, Met388, Leu391, Phe404, Met343, Leu346, Thr347, Ala350, Asp351, Leu354, Cys530, Leu529, Met528, Tyr526, Leu525, Met522
12	−6.985	−63.186	-	-	Leu539, Leu536, Trp383, Leu384, Leu387, Met388, Leu391, Phe404, Met343,

					Leu346, Thr347, Ala350, Asp351, Leu354, Cys530, Lys529, Met528, Tyr526, Leu525, Met522
13	−6.996	−63.794	-	-	Cys530, Lys529, Met528, Tyr526, Leu525, Met522, Leu539, Leu536, Leu354, Asp351, Ala350, Trp383, Leu384, Thr347, Leu346, Leu387, Met388, Met343, Leu391, Phe404
14	−6.295	−59.631	NH of imidazole OH	Asp351 (1.73) Leu525 (1.99)	Lys525, Tyr526, Met528, Lys529, Cys530, Val533, Val534, Leu536, Leu539, Leu354, Asp351, Ala350, Trp383, Leu384, Thr347, Leu346, Leu387, Met343
15	−9.063	−58.068	NH_2 NH of imidazole	Gly521 (2.09) Asp351 (2.17)	Arg394, Met343, Leu391, Leu346, Thr347, Leu349, Ala350, Met388, Leu387, Asp351, Glu353, Leu384, Trp383, Leu536, Leu539, Val418, Glu419, Gly420, Met421, Hie524, Leu525, Ile424, Gly521
16	−6.619	−68.822	-	-	Phe404, Met343, Leu346, Thr347, Ala350, Asp351, Leu387, Leu384, Leu354, Trp383, Leu539, Leu536, Cys530, Lys529, Met528, Tyr526, Leu525, Met522
17	−5.262	−66.129	NH of imidazole OAc	Asp351 (1.93) Cys530 (2.23)	Leu525, Met528, Lys529, Cys530, Val533, Val534, Pro535, Leu536, Leu539, Leu354, Asp351, Ala350, Trp383, Leu384, Thr347, Leu346, Met343, Leu387
18	−6.565	−63.55	-	-	Leu536, Leu539, Cys530, Lys529, Met528, Tyr526, Leu525, Met522, Leu354, Asp351, Ala350, Thr347, Leu346, Met343, Phe 404, Leu391, Met388, Leu387, Leu384, Trp383
19	−7.042	−64.345	-	-	Met522, Leu525, Tyr526, Met528, Lys529, Cys530, Val533, Leu536, Leu539, Leu354, Asp351, Ala350, Thr347, Leu346, Met343, Phe404, Leu391, Met388, Leu387, Leu384, Trp383
20	−6.322	−61.889	-	-	Cys530, Lys529, Met528, Tyr526, Leu525, Met522, Leu354, Leu536, Leu539, Asp351, Ala350, Thr347, Leu346, Met343, Phe404, Trp383, Leu384, Leu351
21	−6.489	−57.503	-	-	Leu525, Tyr526, Met528, Lys529, Cys530, Val533, Leu536, Leu539, Leu354, Asp351, Ala350, Thr347, Leu346, Met343, Ile424,

					Trp383, Leu384, Leu387, Phe404
22	−7.438	−60.524	-	-	Leu536, Leu539, Trp383, Leu384, Leu387, Met388, Leu391, Arg394, Phe404, Leu354, Glu353, Asp351, Ala350, Leu349, Thr347, Leu346, Met343, Val418, Glu419, Gly420, Met421, Ile424, Gly521, Hie524, Leu525
23	−6.7	−58.087	-	-	Val533, Leu536, Leu539, Leu354, Glu353, Asp351, Ala350, Leu349, Thr347, Leu346, Met343, Trp383, Leu384, Leu387, Met388, Leu391. Arg394, Phe404, Gly521, Hie524, Leu525, Met528, Ile424, Met421, Gly420, Glu419, Val418
24	−8.160	−59.714	OH NH of imidazole	Asp351 (1.66) Leu346 (2.06)	Met343, Leu346, Thr347, Leu349, Ala350, Asp351, Leu354, Trp383, Leu384, Leu387, Met388, Leu391, Phe404, Leu428, Leu536, Val533, Cys530, Lys529, Met528, Tyr526, Leu525
25	−6.339	−59.022	-	-	Leu525, Tyr526, Met528, Lys529, Cys530, Val533, Leu536, Leu539, Leu354, Asp351, Ala350, Thr347, Leu346, Met343, Ile424, Phe404, Trp383, Leu384
26	−7.114	−68.188	OAc NH of imidazole	Cys530 (2.17) Val534 (2.16)	Leu539, Leu536, Pro535, Val534, Val533, Cys530, Lys529, Met528, Tyr526, Leu525, Met522, Trp383, Leu384, Leu387, Met343, Leu346, Thr347, Ala350, Asp351
27	−8.248	−69.342	NH of imidazole	Asp351 (2.04)	Glu353, Asp351, Ala350, Leu349, Thr347, Leu346, Met343, Arg394, Leu391, Met388, Leu387, Leu384, Trp383, Leu539, Leu536, Ile424, Met421, Gly420, Glu419, Leu428, Phe404, Gly521, Hie524, Leu525
28	−6.358	−63.065	NH of imidazole	Asp351 (1.58)	Leu536, Leu539, Val534, Val533, Cys530, Lys529, Met528, Leu525, Asp351, Ala350, Thr347, Leu346, Leu384, Trp383, Leu387, Met343, Phe404
29	−7.713	−66.617	C=O NH of imidazole	Cys530 (1.90) Leu525 (2.00)	Leu354, Asp351, Ala350, Thr347, Leu346, Met343, Trp383, Leu384, Leu387, Leu539, Leu536, Val533, Cys530, Lys529, Met528, Tyr526, Leu525, Met522
30	−6.719	−69.573	C=O	Cys530 (2.14)	Val533, Leu536, Leu539, Cys530, Lys529, Met528,

	Tyr526, Leu525, Met522, Met343, Leu346, Thr347, Ala350, Asp351, Leu354, Trp383, Leu384, Leu387

Std.: 4-hydroxy tamoxifen.

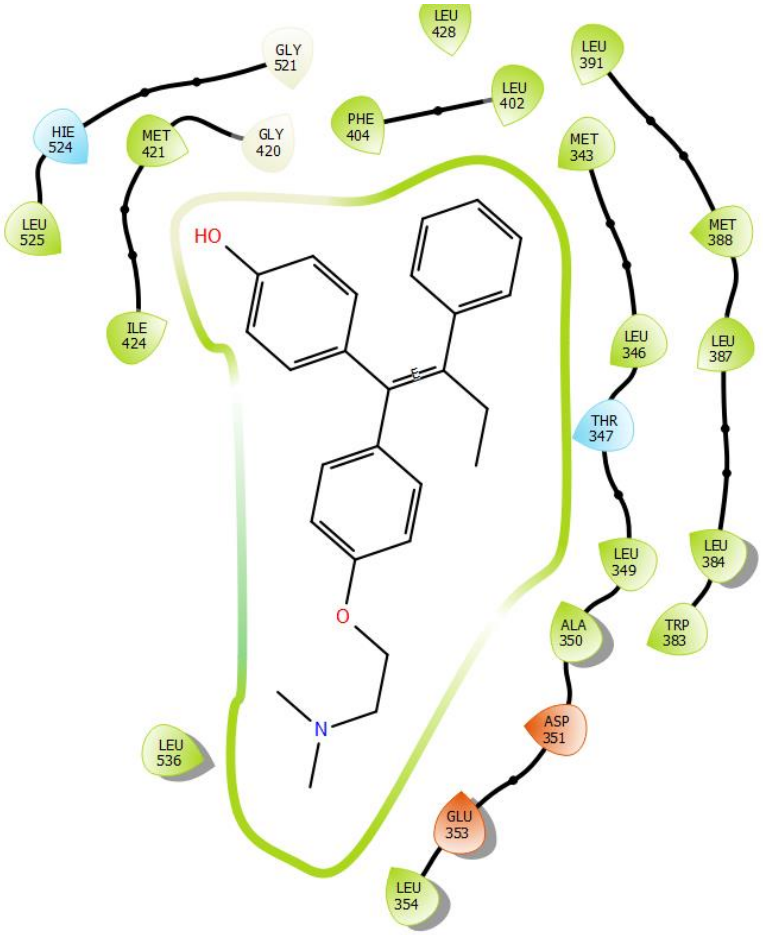

Figure 3. 2D-Ligand interaction diagram of compound 6 (highest Mol Dock score = −8.483) with ER alpha.

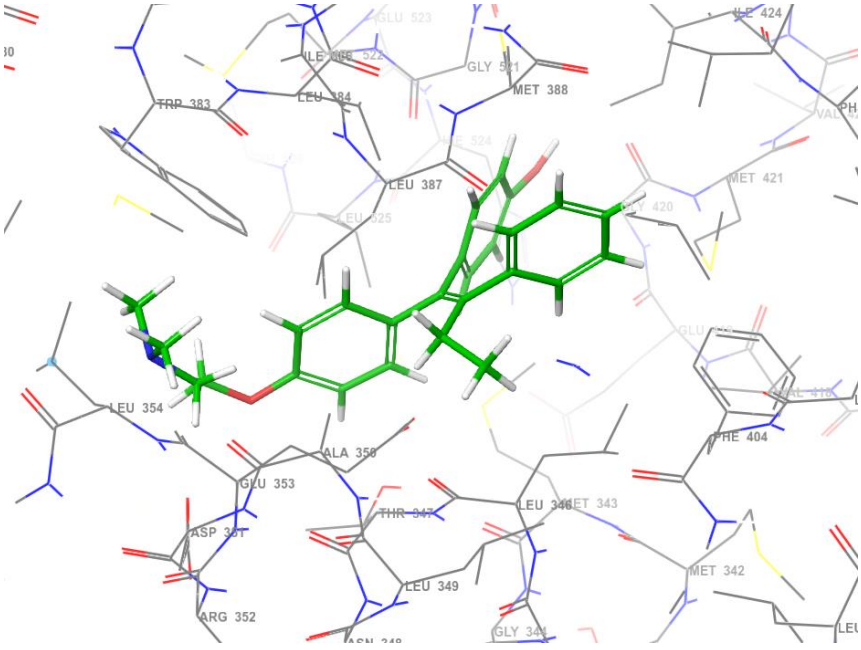

Figure 4. Binding mode of standard 4-hydroxy tamoxifen (highest Mol Dock score = −8.483) into receptor (ER alpha).

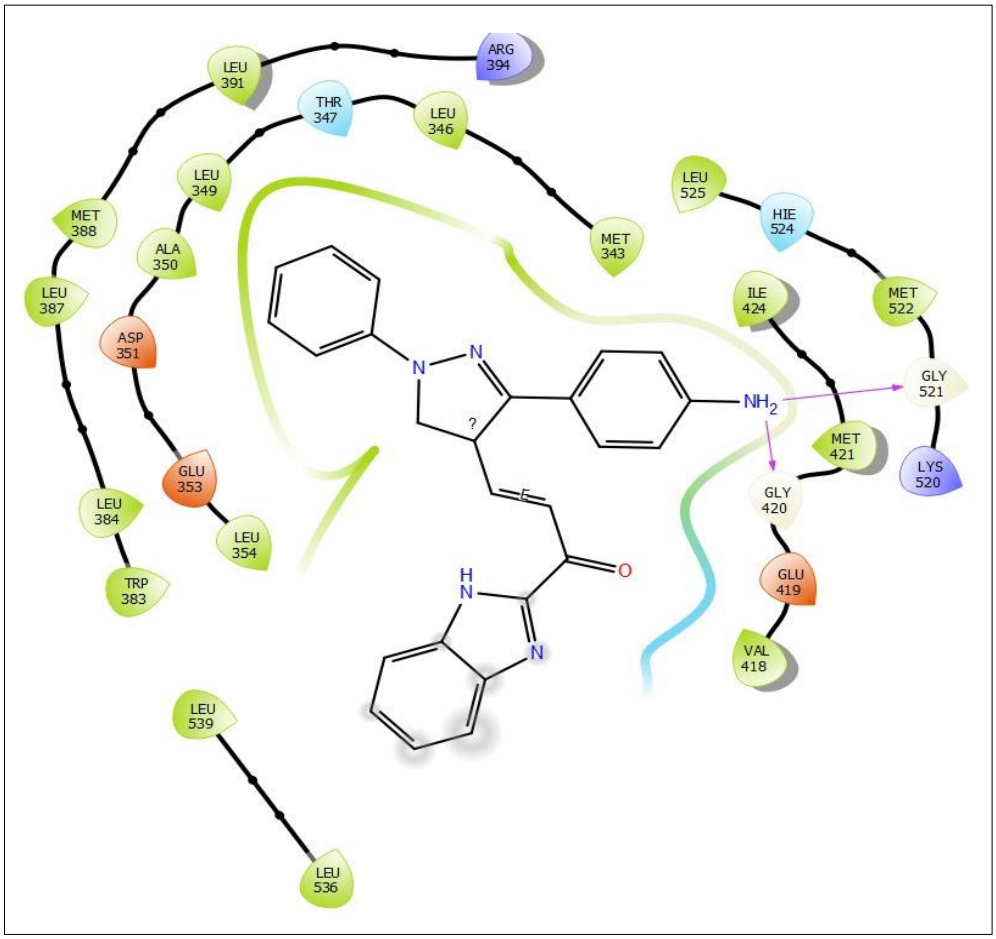

Figure 5. 2D-Ligand interaction diagram of compound 6 (highest Mol Dock score = −9.293) with ER alpha.

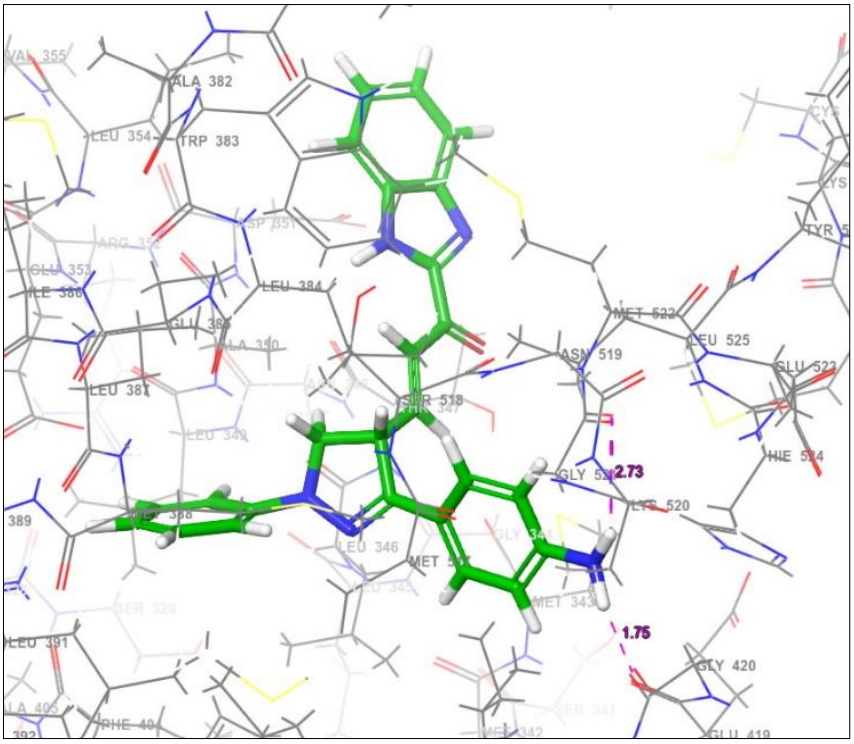

Figure 6. Binding mode of compound 6 (highest Mol Dock score = −9.293) into receptor (ER alpha).

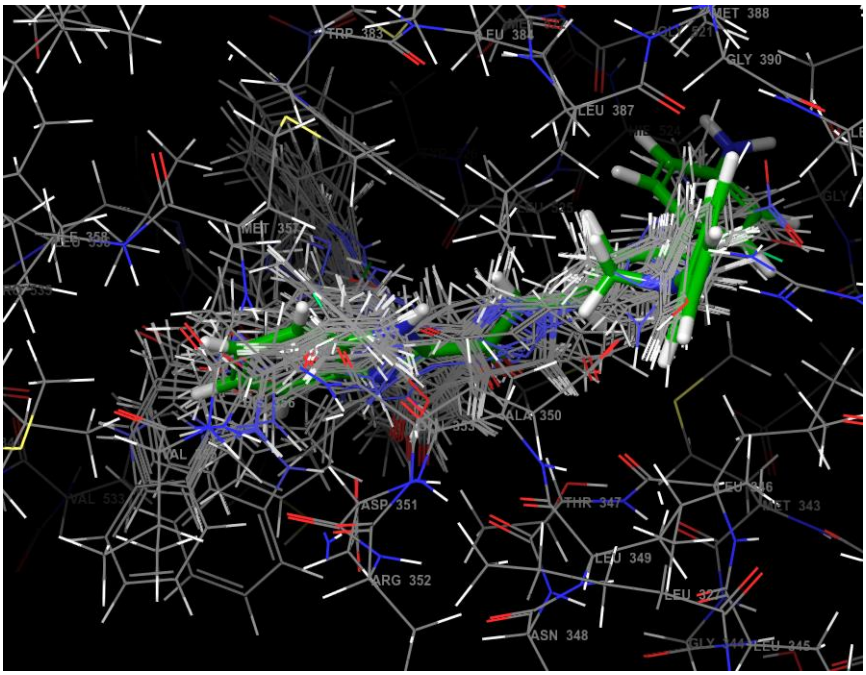

Figure 7. Binding mode of all novel designed ligand and reference into active region of 3ERT.

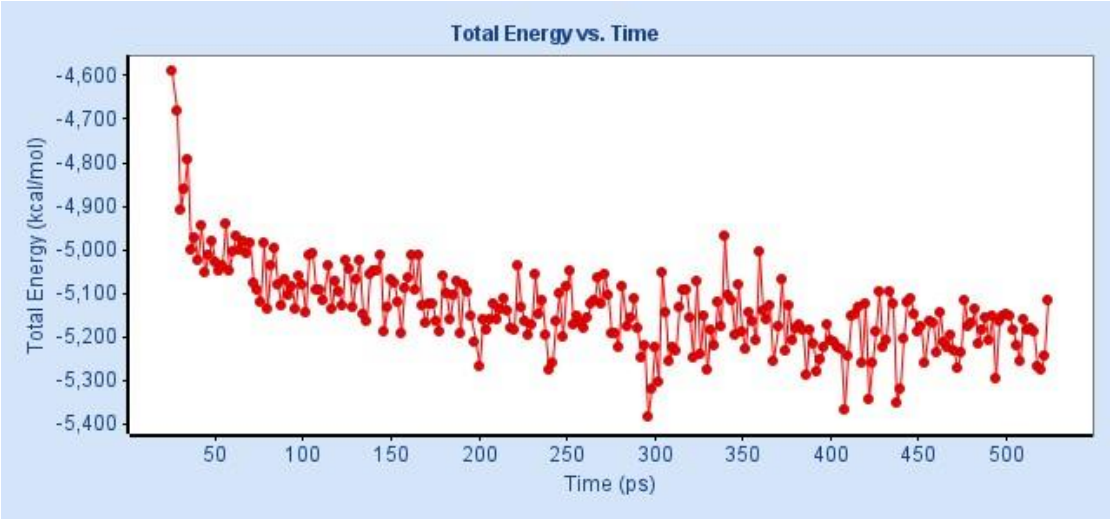

Figure 8. Plot between the total energy of the complex with respect to the time (ps).

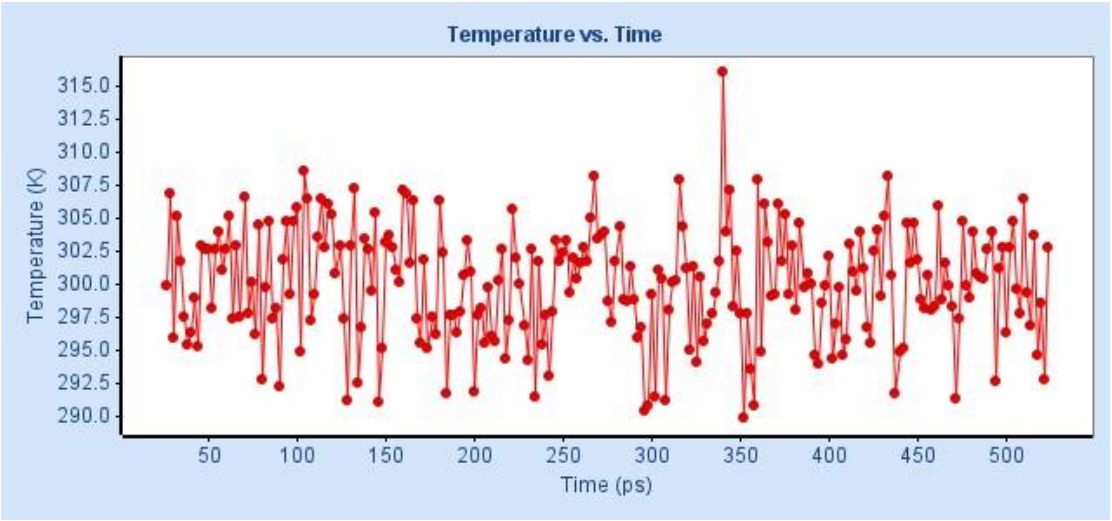

Figure 9. Plot between temperature applied during the simulations and time (ps).

3.2. Structure-activity relationships (SARs)

Based on the docking studies, the following SARs for novel-designed benzimidazole analogues were concluded in **Figure 10**.

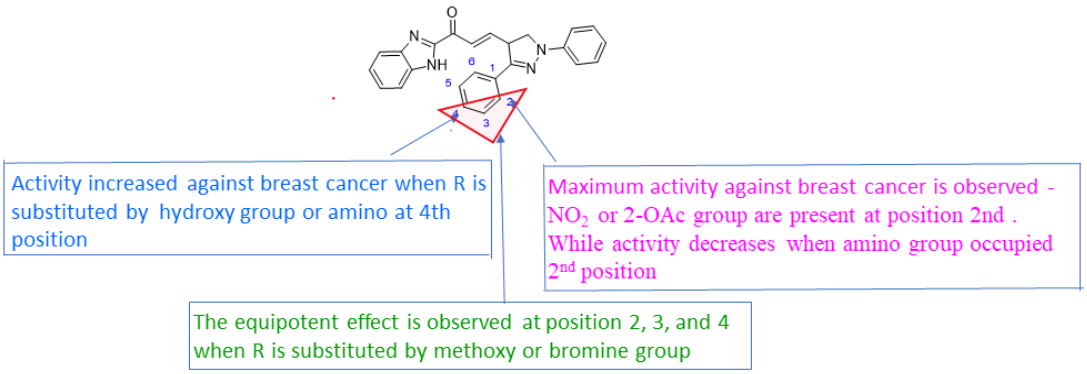

Figure 10. SAR analysis of target derivative.

1) If R = NH_2, maximum activity was observed at position 4, followed by 3 then 2nd. The order of activity was 4-NH_2 > 3-NH_2 > 2-NH_2.
2) If R = NO_2, maximum activity was observed at position 2.
3) Substitution with -Br and -OCH_3 at positions 2, 3 and 4 produced almost equivalent results.
4) If R = OH, order of activity was 4-OH > 2-OH > 3-OH.
5) If R = OAc, order of potency was 2-OAc > 3-OAc > 4-OAc.

3.3. ADME prediction

The research focused on performing in silico screening of several physicochemical parameters of substituted benzimidazole derivatives (**Table 3**). All of the compounds had molecular weights of fewer than 500 Daltons. The majority of molecules have LogPo/w values greater than 5, which contradicts the Lipinski rule of five. Compounds with LogPo/w in the range, i.e., LogPo/w 5, were shown to have excellent intestine absorption. The total number of hydrogen bond donors and acceptors in all tested compounds were within the permitted range i.e., not more than 10 and 5 respectively which is a reliable predictor of bioavailability, wPSA (a weakly polar component of SASA) for all compounds were within range 0–175. Most of the compounds were 0 wPSA, sum of O and N was between 5 and 8. Thus, few of the compounds (2, 3, 5, 6, 14, 15, 16, 24, 25, 26, 27) completely satisfied the Lipinski rule of five while except above compounds, most of them violate the Lipinski rule with respect to lipophilicity.

Table 3. Physicochemical characteristics predicted for the designed substituted benzamide derivatives (QikProp prediction).

Sr. No.	Mol. Wt.	wPSA	Volume	No. of hydrogen bond donors	No. of hydrogen bond acceptors	Log $P_{o/w}$	No. of N and O atoms	No. of violations of Rule of 5)
1	392.459	0	1285.217	1	4.5	5.483	5	1
2	437.457	0	1357.512	1	5.5	4.774	8	0
3	450.496	0	1440.023	1	7	4.986	7	0
4	471.355	77.264	1338.335	1	4.5	6.057	5	1
5	408.459	0	1303.222	2	5.25	4.673	6	0
6	407.474	0	1314.958	2.5	5.5	4.499	6	0
7	406.486	0	1345.958	1	4.5	5.802	5	1
8	422.485	0	1359.875	1	5.25	5.561	6	1
9	448.566	0	1446.289	1	4.5	6.425	5	1

10	434.540	0	1451.073	1	4.5	6.462	5	1
11	426.904	71.524	1329.512	1	4.5	5.98	5	1
12	410.450	46.805	1300.432	1	4.5	5.707	5	1
13	471.355	76.643	1337.664	1	4.5	6.045	5	1
14	408.459	0	1301.959	2	5.25	4.696	6	0
15	407.474	0	1314.579	2.5	5.5	4.497	6	0
16	437.457	0	1357.718	1	5.5	4.778	8	0
17	450.496	0	1434.713	1	7	5.018	7	1
18	422.485	0	1352.688	1	5.25	5.547	6	1
19	448.566	0	1497.861	1	4.5	6.731	5	1
20	434.540	0	1403.926	1	4.5	6.243	5	1
21	426.904	49.234	1305.254	1	4.5	5.807	5	1
22	410.450	28.071	1297.517	1	4.5	5.639	5	1
23	471.355	45.565	1304.890	1	4.5	5.813	5	1
24	408.459	0	1283.728	2	5.250	4.757	6	0
25	407.474	0	1292.311	2.5	5.5	4.573	6	0
26	450.496	0	1415.619	1	7	4.993	7	0
27	437.457	0	1347.790	1	5.5	4.919	8	0
28	422.485	0	1363.521	1	5.250	5.613	6	1
29	448.566	0	1451.075	1	4.5	6.509	5	1
30	434.540	0	1428.115	1	4.5	6.327	5	1

Absorption parameters (predicted % human oral absorption (%HOA) and brain/blood partition coefficient (QPlogBB)) for the 2-substituted benzimidazoles containing substituted pyrazole as a substituent are shown in **Table 4**.

Table 4. Predicted absorption parameters of the 2-substituted benzimidazole derivatives (Qik Prop).

Sr. No.	%HOA	QPlogBB	Sr. No.	%HOA	QPlogBB	Sr. No.	%HOA	QPlogBB
1	100	−0.645	**11**	100	−0.492	**21**	100	−0.376
2	94.130	−1.817	**12**	100	−0.592	**22**	100	−0.632
3	100	−1.373	**13**	100	−0.540	**23**	100	−0.345
4	100	−0.481	**14**	100	−1.248	**24**	100	−0.925
5	100	−1.310	**15**	100	−1.464	**25**	100	−0.985
6	100	−1.408	**16**	93.614	−1.883	**26**	100	−0.992
7	100	−0.675	**17**	92.405	−1.151	**27**	100	−1.463
8	100	−0.783	**18**	100	−0.658	**28**	100	−0.764
9	100	−0.558	**19**	100	−0.763	**29**	100	−0.607
10	100	−0.722	**20**	100	−0.512	**30**	100	−0.680

Volume distribution (VD) is useful in estimating the dosage required to achieve plasma concentration, and all derivatives were found to have excellent VD in the 0.4–20 LKg^{-1} range. The pharmacodynamic behaviour of a medication is strongly influenced by its binding to proteins in plasma. Because the free concentration of the medication is at stake when a drug binds to serum proteins in this process, PPB can have a direct impact on oral bioavailability. If a molecule has a projected value of 90%, it is deemed to have an

appropriate PPB, while medications with high protein-bound may have a poor therapeutic index. All derivatives were found to be a slightly higher value of plasma protein binding than the acceptable range, hence measures should be taken to reduce it. Fu is the proportion of medicines that are unbound in plasma, and all of the examined compounds were determined to be substantially bound to plasma since their values were less than 5%. The less medicine is linked to plasma, the more accessible it is for target activities (**Table 5**).

Table 5. Distribution properties of the 2-substituted benzimidazole derivatives (ADMET lab. 2 predictions).

Sr. No	PPB (%)	VD	BBB penetration	FU (%)	Sr. No	PPB (%)	VD	BBB penetration	FU (%)
1	99.118	0.601	0.70	1.728	16	99.249	0.279	0.09	1.477
2	99.286	0.340	0.08	1.400	17	98.985	0.349	0.47	1.716
3	99.108	0.366	0.40	1.643	18	98.925	0.411	0.44	1.864
4	99.247	0.660	0.53	2.308	19	99.411	0.525	0.42	1.090
5	98.667	0.428	0.24	1.603	20	99.218	0.625	0.40	1.636
6	98.257	0.438	0.62	2.172	21	99.288	0.543	0.46	1.619
7	99.260	0.554	0.65	1.538	22	99.296	0.582	0.68	1.597
8	98.965	0.465	0.39	1.765	23	99.270	0.597	0.66	2.476
9	99.512	0.556	0.40	1.154	24	99.016	0.378	0.49	1.629
10	99.317	0.637	0.42	1.620	25	98.110	0.405	0.65	2.804
11	99.235	0.608	0.44	1.495	26	98.586	0.333	0.49	1.780
12	99.924	0.632	0.59	1.618	27	99.134	0.256	0.23	1.455
13	99.213	0.606	0.68	2.294	28	98.846	0.395	0.48	1.801
14	98.487	0.402	0.28	1.713	29	99.379	0.520	0.38	1.271
15	98.280	0.383	0.48	2.235	30	99.285	0.596	0.42	1.662

Drug transformation, also called metabolism, is divided into Phase I and Phase II. Most of the drugs administered are fragmented down by the enzymes belonging to the family of cytochrome P450 which includes CYP1A2, CYP3A4, CYP2C9, CYP2C19 and 2D6 and are mainly present in the liver. The values presented are interpreted by the probability of the compound being an inhibitor (Inh.) or a substrate (Sub.) of these enzymes. Values closer to 0 indicate non-substrates or non-inhibitors while values closer to 1 indicate substrate or inhibitor properties (**Table 6**).

Table 6. Metabolism properties of 2-substituted benzimidazole derivatives (ADMET lab. 2 predictions).

Sr. No.	CYP1A2		CYP2C19		CYP2C9		CYP2D6		CYP3A4	
	Inh.	Sub.	Inh.	Sub.	Inh.	Sub.	Inh.	Sub.	Inh.	Sub.
1	0.98	0.70	0.97	0.08	0.98	0.45	0.28	0.29	0.79	0.38
2	0.83	0.27	0.96	0.07	0.98	0.96	0.08	0.67	0.75	0.57
3	0.85	0.27	0.98	0.065	0.97	0.86	0.28	0.42	0.63	0.61
4	0.96	0.22	0.98	0.078	0.96	0.61	0.40	0.38	0.79	0.64
5	0.95	0.24	0.94	0.08	0.93	0.82	0.42	0.67	0.78	0.66
6	0.99	0.29	0.98	0.09	0.96	0.60	0.26	0.68	0.97	0.41
7	0.84	0.85	0.95	0.067	0.96	0.78	0.22	0.69	74	0.79
8	0.92	0.84	0.98	0.078	0.95	0.93	0.30	0.77	0.79	0.90
9	0.85	0.57	0.95	0.077	0.93	0.82	0.39	0.46	0.84	0.86
10	0.85	0.90	0.95	0.079	0.92	0.84	0.29	0.40	0.87	0.78
11	0.94	0.46	0.96	0.081	0.97	0.82	0.45	0.43	0.84	0.57

12	0.98	0.62	0.94	0.093	0.98	0.45	0.49	0.47	0.89	0.46
13	0.96	0.44	0.98	0.067	0.96	0.44	0.43	0.46	0.90	0.45
14	0.98	0.49	0.98	0.086	0.98	0.84	0.59	0.59	0.99	0.42
15	0.97	0.23	0.95	0.067	0.98	0.46	0.27	0.62	0.97	0.40
16	0.90	0.29	0.93	0.094	0.94	0.84	0.25	0.44	0.84	0.39
17	0.95	0.27	0.92	0.069	0.92	0.85	0.42	0.41	0.86	0.44
18	0.93	0.88	0.95	0.076	0.94	0.81	0.28	0.84	0.92	0.58
19	0.94	0.84	0.96	0.084	0.93	0.82	0.45	0.49	0.92	0.83
20	0.95	0.81	0.95	0.087	0.97	0.62	0.43	0.24	0.84	0.62
21	0.96	0.49	0.94	0.091	0.95	0.44	0.45	0.48	0.87	0.66
22	0.98	0.55	0.95	0.093	0.98	0.60	0.090	0.47	0.86	0.46
23	0.99	0.48	0.98	0.086	0.96	0.41	0.25	0.43	0.84	0.40
24	0.95	0.49	0.98	0.078	0.98	0.83	0.55	0.45	0.88	0.46
25	0.96	0.48	0.98	0.095	0.98	0.62	0.49	0.76	0.98	0.49
26	0.98	0.28	0.98	0.090	0.98	0.85	0.47	0.29	0.84	0.45
27	0.85	0.49	0.98	0.096	0.98	0.86	0.28	0.62	0.98	0.46
28	0.94	0.98	0.98	0.27	0.98	0.87	0.26	0.64	0.98	0.78
29	0.93	0.98	0.98	0.27	0.98	0.63	0.66	0.47	0.98	0.79
30	0.86	0.84	0.98	0.29	0.98	0.64	0.49	0.44	0.84	0.65

Clearance (CL) is a pharmacokinetic measure that refers to the rate at which a drug is removed from plasma (mg/min) divided by the concentration of that drug in the plasma (mg/mL). The majority of the compounds under consideration had a promising half-life ($T^{1/2}$) between 0.109 and 0.690 (**Table 7**).

Table 7. Excretion characteristics of 2-substituted benzimidazole derivatives (ADMET lab. 2 predictions).

Sr. No.	CL	$T^{1/2}$	Sr. No.	CL	$T^{1/2}$	Sr. No.	CL	$T^{1/2}$
1	1.799	0.533	**11**	1.533	0.317	**21**	2.020	0.209
2	1.319	0.318	**12**	1.867	0.316	**22**	2.067	0.191
3	1.060	0.627	**13**	0.964	0.379	**23**	1.097	0.233
4	0.920	0.300	**14**	2.752	0.826	**24**	2.156	0.512
5	2.570	0.831	**15**	4.092	0.513	**25**	3.551	0.277
6	4.251	0.425	**16**	1.372	0.427	**26**	1.245	0.507
7	1.802	0.412	**17**	1.130	0.690	**27**	1.622	0.314
8	2.173	0.445	**18**	2.367	0.505	**28**	3.285	0.392
9	1.476	0.169	**19**	1.557	0.249	**29**	1.748	0.109
10	1.408	0.229	**20**	1.476	0.297	**30**	1.603	0.152

The compounds were also tested for the prediction of toxicity (**Table 8**). hERG refers to human ether a go-go gene, human hepatotoxicity, drug-induced liver injury and the AMES toxicity test is used as a mutagenicity test because it correlates with carcinogenicity. The rat oral acute toxicity test (RAT) is usually used to infer animal results from human counterparts for safety evaluation. All derivatives were found to show positive results for AMES mutagenicity, rat acute oral toxicity, hERG blockers and negative results for human hepatotoxicity (HT), drug-induced liver injury (DILI), respiratory toxicity (RT) and maximum recommended daily dose (Max DD) and eye toxicity.

Table 8. Predicted toxicity of the 2-substituted benzimidazole derivatives (ADMET lab. 2 predictions).

Sr. No.	hERG	Human HT	DILI	AMES	RAT	Max. DD	Eye corrosion	Eye irritation	RT
1	0.08	0.79	0.98	0.65	0.23	0.98	0.081	0.59	0.94
2	0.28	0.81	0.98	0.99	0.49	0.98	0.089	0.95	0.96
3	0.29	0.66	0.95	0.82	0.49	0.97	0.086	0.47	0.96
4	0.26	0.64	0.96	0.25	0.27	0.97	0.085	0.95	0.97
5	0.09	0.81	0.96	0.61	0.49	0.95	0.093	0.96	0.96
6	0.22	0.87	0.94	0.96	0.42	0.96	0.096	0.92	0.94
7	0.09	0.82	0.93	0.75	0.27	0.98	0.094	0.80	0.93
8	0.29	0.84	0.92	0.77	0.25	0.94	0.093	0.77	0.90
9	0.09	0.64	0.93	0.22	0.28	0.95	0.090	0.79	0.96
10	0.08	0.69	0.95	0.38	0.29	0.98	0.089	0.80	0.99
11	0.29	0.86	0.94	0.78	0.29	0.99	0.095	0.76	0.96
12	0.09	0.86	0.95	0.59	0.27	0.95	0.095	0.78	0.93
13	0.09	0.59	0.99	0.28	0.26	0.93	0.089	0.93	0.91
14	0.09	0.85	0.97	0.41	0.46	0.92	0.093	0.87	0.92
15	0.27	0.89	0.99	0.84	0.26	0.90	0.078	0.84	0.94
16	0.28	0.90	0.95	0.97	0.25	0.99	0.080	0.87	0.96
17	0.28	0.56	0.95	0.83	0.44	0.95	0.092	0.41	0.97
18	0.28	0.75	0.96	0.59	0.27	0.96	0.090	0.83	0.99
19	0.08	0.67	0.95	0.28	0.09	0.94	0.097	0.80	0.97
20	0.09	0.64	0.92	0.39	0.08	0.96	0.094	0.84	0.95
21	0.08	0.78	0.93	0.89	0.29	0.96	0.097	0.83	0.95
22	0.08	0.96	0.98	0.77	0.28	0.95	0.087	0.87	0.93
23	0.09	0.80	0.95	0.67	0.28	0.98	0.099	0.94	0.95
24	0.09	0.79	0.95	0.66	0.49	0.97	0.095	0.96	0.97
25	0.24	0.82	0.93	0.97	0.26	0.94	0.091	0.92	0.95
26	0.27	0.61	0.97	0.88	0.45	0.92	0.090	0.69	0.95
27	0.28	0.86	0.98	0.97	0.49	0.95	0.095	0.92	0.97
28	0.08	0.82	0.96	0.82	0.26	0.96	0.089	0.89	0.97
29	0.09	0.85	0.96	0.28	0.09	0.95	0.087	0.78	0.91
30	0.08	0.82	0.97	0.63	0.27	0.95	0.084	0.82	0.94

4. Conclusion

We might infer that breast cancer is an enticing topic for concern. It has been noted as being the most common cancer worldwide when compared to other malignancies. As a result, considerable testing and research were conducted to identify the best inhibitors for the treatment of breast cancer, especially against positive breast cancer. The literature revealed that 2-substituted benzimidazole derivatives may serve asestrogen receptor alpha antagonists to prevent breast cancer. In this study, thirty 2-substituted novel designed benzimidazoleswere molecularly docked on the estrogen receptor alpha (PDB ID: 3ERT) and compared to the reference drug, 4-hydroxytamoxifen, which served as a benchmark. From the results it was predicted that some compounds showed better binding affinity by interaction with estrogen receptor alpha, and the most potent compound was 6, which had a Glide score of −9.293. Even other parameters such as H-bonding are more commendable for inhibiting estrogen receptor alpha for compound 6 in comparison to standard 4-hydroxy

tamoxifen. Further these compounds were used for the development of highly active and potent benzimidazole derivatives against breast cancer targeting ER alpha. Next to this, supportable results were received in an ADME/pharmacokinetics study of hypothetically designed benzimidazole derivatives with pyrazole in contrast to the existing drug Tamoxifen. Depending on various characteristics such as binding affinity, volume distribution, metabolism, excretion and toxicity, compound 6 was concluded as being the most preferred anticancer agents based on the current study. To support the therapeutic potential of these compounds against cancer, in vivo and in vitro research are still needed.

Author contributions

Conceptualization, JM and NSG; methodology, JM; software, JM; validation, JM, NSG and GD; formal analysis, AKD; investigation, JM; resources, JM; data curation, ASG; writing—original draft preparation, JM; writing—review and editing, NSG; visualization, GD; supervision, ASG; project administration, NSG; funding acquisition, JM. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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