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Development of Benzimidazole a promising scaffold against Breast cancer via *in silico* approaches

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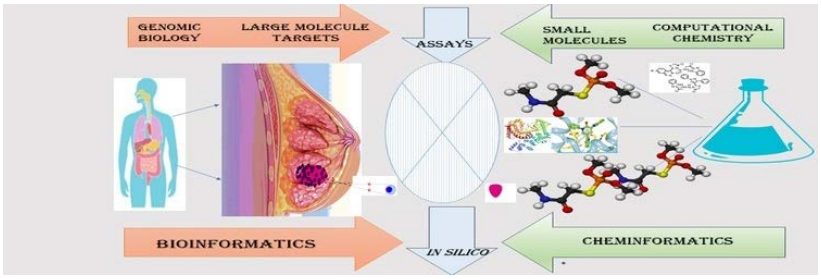
ABSTRACT



Computational techniques offer useful tools for lead identification, optimization, and target selection in the search for many therapeutic candidates for breast cancer. It is well known that benzimidazole and its derivatives are important players in the development of novel anticancer drugs. Computational methods help to streamline the drug discovery process, reduce costs, and increase the chances of identifying effective treatments for this complex disease. As is commonly accepted, discovering new drugs is a difficult, slow, and affluent process. According to estimates, the typical drug development pipeline takes 12 years and costs $2.7 billion to produce a new drug. The pharmaceutical sector is struggling to find a solution to the difficult and pressing issue of how to minimize research costs while expediting the development of new therapies. The development of computer-aided drug discovery (CADD), is a potent and optimistic technique for developing medications rapidly, inexpensively, and efficiently. Recent advances in computational drug discovery technologies have substantially influenced the development of drugs to treat Breast Cancer. To identify leads, computational methods offer useful tools. In the present study, a computational study on benzimidazoles and their derivatives against Breast Cancer targets have been provided.

Keywords: Breast Cancer, Docking, Targets, Computational Methods

INTRODUCTION

Cancer is becoming an increasing global burden due to a rapid rise in cancer incidence and mortality rates. There are reportedly more than 200 distinct forms of cancer, which are often called by the tissue where they were originally discovered. Globally, Non Communicable Diseases (NCDs) were responsible for more than 71% of fatalities. In India, more than 63% of fatalities were caused by NCDs. One of the primary causes of mortality from NCDs was cancer.[1,2] There were estimated to be more than 1.3 million cancer sufferers in 2020.[3] The breast, mouth, lungs, cervix, uterus, and tongue are the most common places for carcinogenic development. In 2020, there were 2.3 million new cases of Breast cancer (BC) discovered, resulting in 68,500 deaths as found by WHO. Out of a total of 29 distinct cancer kinds, the Shri Shankara Cancer Foundation's research indicates that the highest instances of BC were recorded between 2019 and 2021(**Table 1**).

Table 1. Cases of cancer during 2019-2021 as reported by Shri Shankara Cancer Foundation

Types of Cancer	Year 2019	Year 2020	Year 2021
Breast Cancer	1510	1167	1133
Lung Cancer	353	329	283
Ovarian Cancer	211	340	134
Lymphoma Cancer	257	212	172
Prostate Cancer	272	166	172

By minimizing global BC mortality by 2.5% yearly, the WHO - GBCI hopes to avert 2.5 million BC deaths between 2020 and 2040, out of which 25% of deaths due to BC among women under the age of 70 would be avoided by 2030 and 40% by 2040 if the worldwide rate of BC mortality was reduced by 2.5% annually. Promotion of

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health for early detection, prompt BC diagnosis and comprehensive BC management are the three factors that will help you achieve these goals.[4]

The progress of novel, efficient, and advanced small molecule continue to be a difficult, pricey, and time-consuming endeavor that requires the collaboration of numerous experts from multidisciplinary fields, such as medicinal chemistry, computational chemistry, biology, drug metabolism, clinical research, etc. This is true, despite the remarkable advancements in biotechnologies and the growing understanding of disease biology. It takes 12 years to effectively find and develop a new treatment, which requires a substantial financial investment.[5] Therefore, there is a great demand for novel medication development processes that are less expensive in terms of time and money as well as more effective.

Since successfully creating HIV protease inhibitor Viracept in the USA in 1997 the first medicine whose design was entirely determined by its target structure computational techniques have become an essential tool in drug discovery projects and an essential foundation for new drug development methodology. This expedites and lowers the price of drug development.[6] Due to their exceptional performance in offering fresh, hopeful viewpoints and treatments for fatal illnesses, this received a great deal of attention.

Benzimidazole is a heterocyclic compound consisting of benzene and imidazole rings.[7] It has a variety of biological actions, earning it the title of "strong moiety" in heterocyclic chemistry.[8,9] Due to its extensive biological profile and synthetic uses in medicinal chemistry, the benzimidazole heterocyclic nucleus is sometimes referred to as the ‘‘Master Key’’.

The first benzimidazole was created by Hoebrecker created in 1872 by reducing 2-nitro-4-methyl acetanilide.[10] Researchers have documented many techniques for synthesizing 1-substituted or 1,2-disubstituted benzimidazoles, 1,2,5 trisubstituted, and 1,2,5,6-tetra substituted derivatives by using different moiety under a different atmospheric reaction environment. Due to the presence of an electron-rich aromatic system and two hetero-nitrogen atoms, the distinctive fused benzene and imidazole rings can interact noncovalently with a variety of biological targets, which is thought to be the cause of the wide range of pharmacological activities of benzimidazole-containing agents.[11,12] Having a "privileged sub-structure moiety" refers to this particular azole drug's ability to interact with a number of unrelated chemical targets. The discovery of N-ribosyl-dimethyl benzimidazole is said to have stimulated interest in benzimidazole chemistry and as a scaffold or moiety in the discovery and development of pharmaceuticals.[13] Bendamustine, the only anticancer medication has acquired FDA approval.[14–16] The two well-known benzimidazole agents, Selumetinib and Galeterone[17] has been moved to phase III clinical trials but not been approved as anticancer drugs so far. As per literature survey, the different benzimidazole derivatives have been produced for their pharmacological properties.[18] Benzimidazoles have revolutionized the process of drug discovery, making this scaffold an essential component in the creation of novel therapies. Research has been focused on developing and synthesizing more potent derivatives with a wide range of pharmacological actions. Because of their huge synthetic value and extensive bioactivities, attempts to create libraries of benzimidazoles and their derivatives have rarely been made. Here, in this review, we discussed a general overview of computational studies involved in the drug discovery of benzimidazole and molecular targets against BC. Also, we summarize Clinical trials of benzimidazole derivatives against BC with its status in the last five years.

The bibliography was crucially analyzed from world-wide established scientific databases like SCOPUS, PubMed, ScienceDirect, Springerlink, Web of Science, Wiley, SciFinder, and Google Scholar. Both the reviews and the research articles on benzimidazole and its anticancer studies are considered. The search terms were benzimidazole, benzimidazole derivatives, benzimidazoles in breast cancer, clinical trials without narrowing or limiting search items.

COMPUTATIONAL TECHNIQUES INVOLVED IN DRUG DISCOVERY

Structure-Based Drug Discovery

A method called "structure-based" uses structural knowledge to specify how bioactive chemicals interact with their matching receptors.[19] Remarkable advancements have been achieved in this area with the introduction of spectroscopic techniques like nuclear magnetic resonance (NMR) and X-ray crystallography, which have greatly improved our structural understanding of the therapeutic target. By using the three-dimensional structure of protein, new ligands might be logically created to have satisfying effects. Therefore, by identifying and improving the initial lead compounds, structure-based design (SBD) might offer crucial insights into the design and development of novel drugs.[20,21] The high-affinity ligand specifically controls verified drug targets to affect certain cellular functions, resulting in achieving desired pharmacological and therapeutic effects.[22] One of the earliest successful instances of employing structural information to optimize medication designs was Capoten (captopril), the first ACEinhibitor, which was developed in the 1980s.[23] After this, structure-based drug development has emerged as a cutting-edge and potent algorithm and approach to support more rapid, less expensive, and more efficient drug development. Extensive efforts have been done in the last ten years to advance the SBD strategy, and an increasing number of successful applications have been crucial to the advancement of new medical research.[24–28]

Molecular Docking

A common structure-based strategy for rational drug design is molecular docking, which analyses and forecasts the interactions and binding affinities between ligand and receptor proteins.[29] According to the flexibility of the ligands used in the computational process, it may be divided into stiff docking and flexible docking.[30,31] Salmaso and Moro defines[32], the rigid docking technique as a binding model that solely takes into account the static geometrical, physical, and chemical complementarity between the ligand and the target proteins, ignoring flexibility and the induced-fit theory. Rigid docking, which is speedy and very effective, is frequently used in high throughput virtual screening with a large number of small molecule databases. While the flexible docking approach takes into account more precise and accurate data. Flexible docking techniques continued to advance and became

more widely available with the quick advancement of computer power and efficiency. Docking software comes in a variety of forms, including Molegro, Schrodinger, DOCK, AutoDock, etc.[32]

This process of docking is divided into three fundamental stages. Target proteins and small molecules should first have their structural details prepared. The open-access PDB library (http://www.rcsb.org) has a large number of experimentally solved structures that may be utilised at this level to understand a number of physiological processes based on crystal structures. If docking structures are of interest, these structures can also be used for homologous template models. Another application is that it serves as a prediction engine for the conformations, orientations, and positional spaces of the ligand binding site.[33] Conformational search algorithms achieve this objective of predicting the conformations of binary compounds by utilising the methods of systematic and stochastic search. Exhaustive search, fragmentation, and conformational ensemble are the three different categories of systematic search techniques. The former is more frequently utilized (i) Monte Carlo (MC) methods, (ii) Tabu search methods, (iii) Evolutionary Algorithms (EA), and (iv) Swarm optimisation (SO) methods are stochastic search methods.[29] As the last stage in identifying which substances are more likely to engage targets during the molecular docking process, these algorithms evaluate the predicted binding-free energy.[34]

According to Kortagere and Ekins,[35] these are four categories of scoring functions; Consensus scoring functions, empirical scoring functions, knowledge-based scoring functions, force-field based scoring functions. In addition, new mechanisms for scoring have been developed, including interactive fingerprints, machine learning, and quantum mechanical scores.[36]

Structure-Based 3D QSAR

The pharmacophore mapping approach has advanced over the past few decades and is now regarded as one of the most important technologies for the drug discovery process. To enhance pharmacophore modeling, a variety of structure-based techniques have been undertaken.[37] One of the important technique is the structure-based pharmacophore (SBP).[38] Target-ligand complex-based and target-binding site-based (without ligand) techniques for SBP modelling may be categorized depending on the availability of ligand structures.[39] LigandScout,[40] Pocket v.2[41] and GBPM[42] serve as examples of this. It is important to note that they cannot be applied to circumstances in which ligands are not known.

Ligand-Based Drug Discovery

Comparability Search

A notion known as molecular similarity is the driving force and inspiration behind ligand-based techniques in drug development. According to this idea, molecules tend to perform comparable biological actions because of their substantial structural similarity.[43] In other words, a compound with interesting biological properties can be used as a query template to find and predict new chemical entities with similar properties. This is because ligand-based drug discovery techniques rely on the structural details of the active ligand that interacts with the target protein. Since all that is needed to apply this method to find new drugs is an understanding of the structures of the known ligands, it is regarded as an indirective strategy. This method is used when the 3D target protein structure is not known. In order to improve the biological properties of ligands and improve medication pharmacokinetics, including ADMET attributes (absorption, distribution, metabolism, excretion, and toxicity), this method is widely employed to uncover new ligands with intriguing biological aspects. This is the most often-used approach which is simple and based on molecular descriptors. Physical and chemical characteristics such as molecular weight, log P, the energy of the highest occupied molecular orbital (EHOMO), the energy of the lowest unoccupied molecular orbital (ELUMO), and charges can be used as coordinates to represent the reference compounds in addition to 2D fingerprint and 3D shape-similarity searches. For molecular representation in virtual screening, the 2D fingerprint (Molprint2D and Unity 2D), 3D shape similarity approaches (MACCS), extended-connectivity fingerprints (ECFP), rapid overlay of chemical structures (ROCS), and phase shape are more often utilized.[44] To find new agonists of the GPR30 receptor, for instance, Bologa *et al*. used 2D fingerprint and 3D shape-similarity approaches.[45] Additionally, both approaches have been effectively used in virtual screens, and they both outperformed docking techniques in terms of scalability and computing speed against a variety of targets. However, the main problem with such methods is the difficulty in choosing the right input structures and their preference for input molecules.[46]

Ligand Based 3D QSAR

The pharmacophore-based technique develops a pharmacophore model built on a set of active compounds, is another method that is more accurate than the molecular descriptors. "A collection of spatial and electronic properties required to ensure optimal supramolecular interactions with specific biological targets and to activate (or inhibit) their biological reactions" is how pharmacophores are defined.[47] A pattern of structural overlap among crucial molecular features produced by an active compound or a binding site in space is employed to denote the chemical characteristics that are most likely to be present. The newly discovered compounds that complement the created pharmacophore and match it closely are likely to be active against the target. As a result, individuals can be chosen as potential subjects for more research. In the lack of macromolecular structures, this method has emerged as a crucial computational way to support and direct drug discovery.[48] The following is a succinct description of pharmacophore modeling: (i) Choosing an active and inactive set of ligands for training (ii) Low energy conformations in molecular preparation, (iii) Generating pharmacophore models and finding common pharmacophore (iv) Building QSAR model.[49] A strong training set of drugs exhibiting the same binding mode is essential for ligand-based pharmacophore modeling.

QSAR Modeling

Using a range of molecular descriptors (MDs) or fingerprints (FPs), QSAR is another ligand-based method that examines how medications affect biological processes. By computing the correlations between the features of the ligand-binding agent and the biological activity assessed during trials, QSAR was developed. Numerous ML and DL methods, including SVM, RF, PR, MLR, and ANN, have been utilised to develop QSAR model .[50] QSAR models, in contrast to pharmacophore models, can quantify

biological activities and even pinpoint favourable or unfavourable effects in line with certain criteria. QSAR has been used to optimise leads, predict new structural leads, and predict the activity of novel molecules analogues in addition to these different molecular design applications. In the conventional 2D-QSAR approaches, the biological activity is related to the steric, electronic, and hydrophobic properties of medicines.[51] The force field computations form the basis for more complex 3D-QSAR techniques including comparative molecular field analysis and a comparison analysis using molecular similarity indexes.[52]

Recent advances in major computational approaches for the prediction of functional sites, such as 3DLigandStie, COACH-D, and SiteMap, are available at the following URLs: http://www.sbg.bio.ic.ac.uk/3dligandsite/, http://yanglab.nankai.edu.cn/COACH-D/, (https://www.schrodinger.com/sitemap). However, it can be problematic for the operator to identify which site is really in charge of the chemical binding because these stated procedures classically result in a large number of possible ligand binding sites. In order to work around this limitation, methods based on MD have been developed recently.[53]

Molecular Docking Simulation

For many crucial biological processes, an understanding of drug-receptor interactions is essential. The cornerstone to understanding the function of internal(reference) ligands and syntheszed therapeutic compounds is to study the interactions between ligands and proteins. GPCRs play a crucial part in many physiological processes. According to Conn *et al.*, GPCRs[54] are a class of frequently exploited drug development targets. Recent research found that ligands might also attach to several regulatory sites that are far from the intended binding pockets in addition to orthosteric sites.[55–57] Unfortunately, without knowledge of experimental structures, the location of such an allosteric site is uncertain, and anticipating the presence of such sites might speed up the development of novel medications.[55]

TARGET PREDICTION

The idea of "one molecule - one target - one disease" dominates traditional drug research, which mostly ignores the interactions between medications and proteins. However, it has been neglected that a number of target proteins are linked to various complicated disorders.[58] Furthermore, due to the "poly-pharmacological" characteristics of some medications, which may induce unfavorable side effects, unanticipated drug purposes resulting from wrong selection of targets are unintended and irrepressible activities. These are especially noticeable with cancer medications.[59] On the contrary, there are some examples that benefit from the different pathways targeted by single given molecule. For instance, sildenafil (viagra), which was first created to treat angina, is now used to treat erectile dysfunction.[60] Additionally, pharmacological regulation still does not apply to some promising and maybe effective cancer targets.[61] The identification of all potential novel ligand binding sites has been emphasized as an important step in therapeutic repositioning and repurposing in order to make the most use of presently existing medicines to treat new indications. Different available databases, molecular simulations and docking softwares such as Drug Bank,[62] Therapeutic Taget database,[63] Supertarget,[64] MATADOR,[65] STITCH[66] TDR targets,[67] PDTD,[68] ChEMBL,[69] Integrity,[70] SIDER,[71] ChemBank,[72] IUPHAR guide,[73] CancerDR,[74] ZINC,[75] Binding DB,[76] CanSAR,[77] PDSP,[78] DCDB,[79] DINIES,[80] SuperPred,[81] Swiss Target Prediction,[82] are used to carry out the drug-target binding affinity evaluation *in-silico* studies.

SUCCESSFUL STORIES OF COMPUTATIONAL METHODS IN PROVING BENZIMIDAZOLE AS BC CELL TARGETS

Although surgery is the preferred course of treatment for BC, progress may be delayed by a number of variables, including the size of the tumour, the condition of the hormone receptors, and the frequency of metastases. Endocrine therapy has recently been added to the main procedure since it helps treat BC. ERs and HER2 are the primary targets that are taken into consideration for therapeutic development. Novel chemical classes known as AIs, SERM, and SERDs are commonly utilised against these targets.[83] TNBC, a particular subtype of BC, has just been discovered, and this subtype is resistant to these medication classes. Complex, diverse, and aggressive BC, lacks ER and PR expression or has overexpressed HER2 and does not react. Epidermal growth factor receptor,[84] heat shock protein,[85] poly-(ADP ribose) polymerase 1,[86] and vascular endothelial growth factor[87] and its receptor is among the molecular targets against TNBC. Since many compounds and significant efforts are wasted and abandoned during the traditional drug development process due to off-target effects, it is still highly desirable to develop target prediction at a much higher level in new drug exploration. This has shown to have appealing advantages.[88]

In this section, we provide some details on the molecular targets and pathways for BC (**Figure 1**). We have also discussed the ongoing research on medicinal chemistry compounds as promising leads. We assumed that this would be a useful resource for scientists working on BC drug discovery studies.

Targets against Breast Cancer

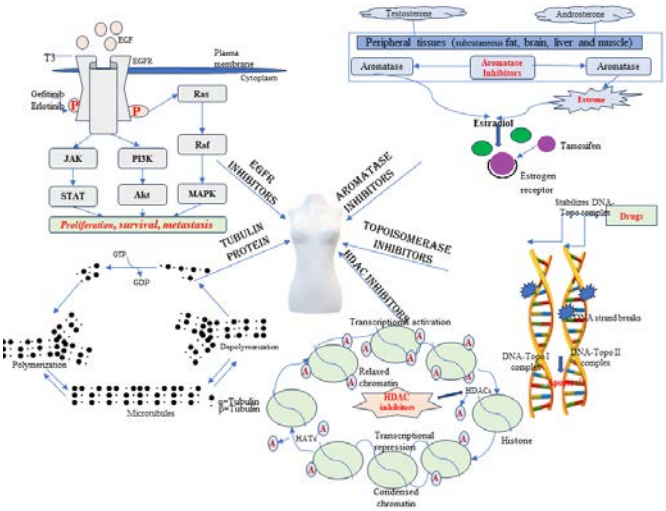


Figure 1: Various Mechanistic patways involving various targets against breast cancer

Tubulin Protein Inhibitors One of a few globular proteins in a tiny family is tubulin. There are many tubulin isoforms but α and β tubulins being the most prevalent ones. Tubulin, a cellular protein,

is crucial for replication. Microtubules are hallowing filaments made up of polar head and tail, configurations of α and β tubulins served as the basic building blocks. Tubulin is one of the most alluring and difficult ways for developing novel anticancer drugs at the molecular level.[89,90] A number of 1,2-diarylbenzimidazole compounds were created by Zhang *et al.* and described as possible anticancer drugs. It has been discovered that the target molecule (1) exhibits usual cytotoxicity towards healthy cells as well as substantial cytotoxicity towards human cancer cells such A549, HepG2, HeLa, and MCF-7 cells in the range of GI_{50} = 0.71-2.41μM. The target compound also significantly inhibited the polymerization of microtubules, with an IC_{50} value of 8.47 μM. To verify that the target chemical binds to the microtubule protein, molecular docking simulation studies were carried out.[91] Derivatives of dehydroabietic acid based on 2-aryl-benzimidazoles were described by Miao *et al*. as possible cytotoxic agents by targeting tubulin polymerization. Analytical and elemental methods were used to characterize these synthesized compounds. With an IC_{50} value of 0.08 ±0.01μM, the target compound (2) significantly inhibited the development of hepatocarcinoma cancer (SMMC-7721) cells. The target demonstrated effective cytotoxicity in the range of 0.04-0.07 μM against colon cancer (CT-26), BC (MDA-MB-231), and cervical cancer (HeLa). Additionally, with an IC_{50} of 5 μM, the target compound significantly inhibited microtubule polymerization. Based on robust electronic interactions between the target compounds and tubulin, the molecular docking investigations verified the target compound's selectivity to tubulin protein.[92] As possible inhibitors of tubulin polymerization, Wang *et al.*[93] described a novel family of benzimidazoles that comprise benzsulfamide-pyrazole ring derivatives. The target compound (3) significantly inhibited the development of A549 with an IC_{50} value of 0.15 ±0.05μM, and it also effectively inhibited the growth of Hela, HepG2, and MCF-7 cell lines at concentrations between 0.17 and 0.33μM. The target substance likewise showed a notable inhibition of microtubule polymerization with an IC_{50} value of 1.52 μM. The target chemical specifically stopped the proliferation of A549 cells at the G2/M phase when cell cycle analysis was performed. Based on tests of the annexin V/propidium iodide dual staining technique and cell cycle analysis, the target chemical demonstrated apoptosis in A549 cells. Based on the powerful interactions between the target molecule and amino acids such as Lys 352, Lys 254, Asn 258, and Cys 241, molecular docking studies were also supported the fact that target molecule (3) effectively inhibits tubulin polymerization. According to Baig *et al*., tubulin polymerization is inhibited by a class of imidazo [2,1-b] thiazole-benzimidazole derivatives, which have antiproliferative properties. The target molecule (4) showed considerable cytotoxicity against A549 with an IC_{50} value of 1.08 μM. It also displayed remarkable cytotoxicity against the cancer cells DU-145, MCF-7 (breast), A549and HeLa in the 1.65-7.55 μM range. When cell cycle analysis was done, the target compound precisely halted the proliferation of A549 cells in the G2/M phase. The target compound demonstrated apoptosis based on apoptosis studies such as Hoechst staining, mitochondrial membrane potential, and annexin V/propidium iodide dual staining assay, which was supported by morphological changes in A549-treated cells such as blebbing, cell wall deformation, and cell shrinkage. Additionally, with an IC_{50} of 1.68 μM, the target compound significantly inhibits microtubule assembly. The protein's colchicine binding site can readily be filled by the target compound, according to computer simulations.[94] Ren *et al*. have developed and compared the properties of many new imidazole and benzimidazole derivatives with colchicine and paclitaxel's cytotoxicity. Additionally, scientists looked into the compounds *in silico* to learn more about their binding behaviors.[95]. Compound 5 had the highest level of cytotoxicity on the cell lines and had an IC_{50} value (2.52 ± 0.63 μM) that was lower than colchicine's (IC_{50}=7.30±0.44μM) while still blocking tubulin polymerization. Additionally, the results of the interpretation of the X-ray crystallographic data from T2R-TTL in complex with compound 5 (PDB code: 7DBA, resolution: 2.45) were predicted. Compound 5 occupies the exact colchicine-binding site between αβ and -tubulin and offers numerous potent H-bonding and hydrophobic interactions. The efficacy of this compound may also be confirmed by its binding profile.[95] To prevent tubulin polymerization, Liu et al. have created several new benzimidazole compounds. When they first assessed the *in vitro* antiproliferative properties of these derivatives, they discovered that compound 6 had the lowest IC_{50} values, which ranged from 0.037 to 0.20μM. Additionally, this compound prevented cell migration by inhibiting tubulin polymerization, cell microtubule networks, cell cycle arrest, and apoptosis induction. On the other hand, *in vivo*, research indicated that compound 6, at a dosage of 2.5 mg/kg and a rate of 52%, also suppressed tumor development. This compound docked flawlessly at the colchicine binding site and had a higher docking score, which might account for the increased activity of 6, according to molecular modelling research using tubulin (PDB: 5gon).[96] (**Figure 2**)

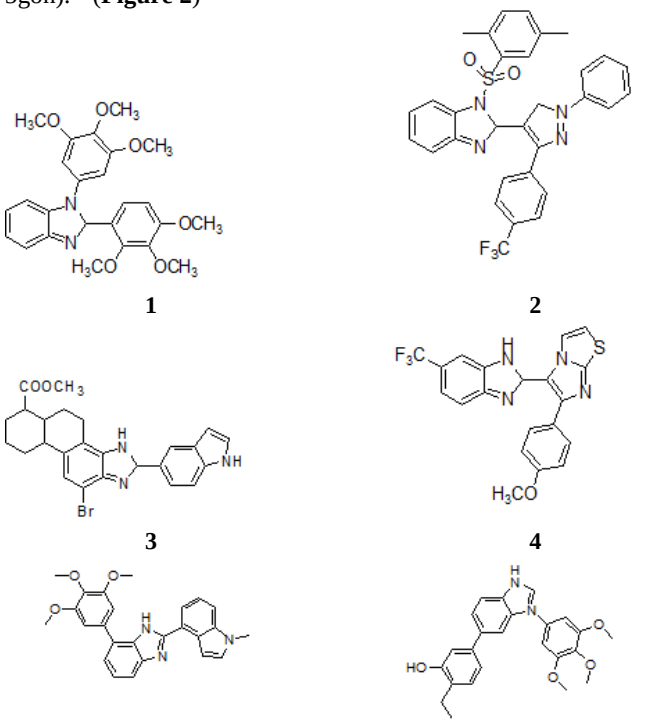


Figure 2: Chemical structures of benzimidazole derivative as Tubulin protein inhibitor

Estrogen Receptor

As a steroid hormone, estrogen can pass through the plasma membrane, interact with intracellular ER, and bind to DNA sequences and have direct effects. The GPER1 and/or ER can interact with estrogen to cause it to activate intracellular signaling cascades as an alternative. Estrogen-mediated signaling events can be classified as genomic and non-genomic due to differences in the cellular and molecular processes regulating gene expression, in which estrogen-receptor complexes can bind to DNA directly or indirectly. Malignant cells contain estrogen receptors (ER), which are cellular surface receptors with the unique capacity to bind important biomolecular compounds such as polypeptide growth factors, cytokines, and hormones. When estrogen binds to ER, the estrogen response element (ERE) on DNA would activate. Eventually, transcription-controlling genes are turned on, which increases BC cell proliferation.[97,98] ER is the main driving factor for tumor formation in more than 70% of BCs. ER activity can be prevented and ER function can be reduced using either aromatase inhibitors (AIs) or anti-estrogens.[99] Two classes of antiestrogen drugs, referred to as selective ER modulators (SERMs; Tamoxifen, raloxifene, ospemifene, and selective ER downregulation (SERDs; fulvestrant) (**Figure 3**)[100] SERDs should be used to treat breast tumors that are resistant to SERMs or Ais.

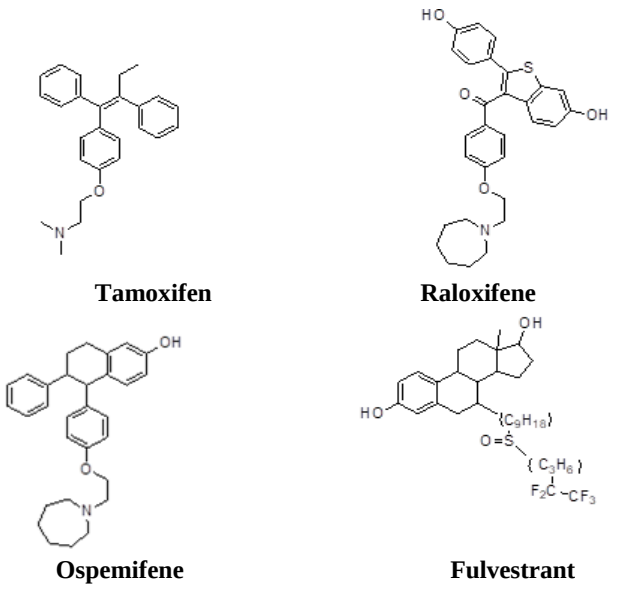


Figure 3: Examples of Marketed Antiestrogen Drugs

The benzimidazole and indole nuclei were combined to create several novel selective estrogen receptor modulators (SERMs), according to Singla *et al*. T47D BC cells that are ER-responsive were used to test the antiproliferative effect. Experiments on ER binding were also conducted. The most effective derivatives were 7 and 8 which could go through cell membranes and boost cytotoxicity.[101] By altering the amounts of mRNA and ER protein expression in T47D cells, they also stopped the transactivation and signaling pathways. Because they attach to ER with a conformation and interaction identical to bazedoxifene's, further docking study verified the antagonistic effect of 7 and 8.[102] In 2020 Karadayi et al. proposed new indole benzimidazole derivatives as SERMs that had ethyl and methylsulfonyl attachments at the fifth position of the benzimidazole ring and evaluated the effectiveness of the ethyl sulfonyl substituted compounds against the MDA-MB-231 ER cell line, the MCF-7 estrogen-sensitive cell line, and the HepG2 estrogen-sensitive cell line. The most potent compounds 9, 10, and 11 were selected as being substantially more active. *In silico* binding characteristics of these were also unswerving with bazedoxifene.[103](**Figure 4**)

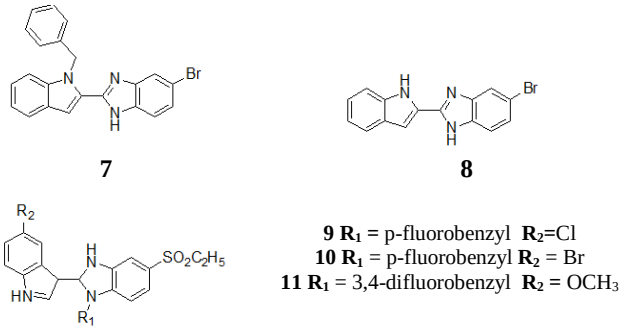


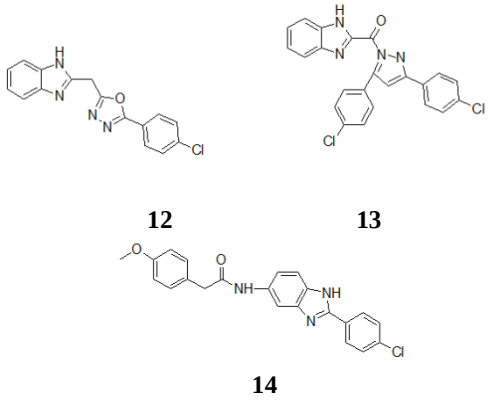
Figure 4: Chemical structures of benzimidazole derivatives as Estrogen Receptor Inhibitors

Epidermal Growth Factor Receptor

Other subfamilies of the transmembrane glycoprotein (ErbB-1) ErbB class of tyrosine kinase receptors include Her2 (ErbB-2), Her 3 (ErbB-3), and Her 4 (ErbB-4).[104] The epidermal growth factor receptor is one of these subfamilies. Internal ligands like EGF and TGF regulate epithelial tissue development and homeostasis by interacting with EGFR receptors and facilitating the growth-promoting signal to cells.[105,106] Due to an excess of EGFR ligands in the tumour microenvironment, which results in ongoing activation of (or alterations of) EGFR receptors, epithelial tumour growth, metastasis, and invasion are accelerated in cancer, particularly epithelial malignancies.[107,108]

The benzimidazole-oxadiazole hybrids were described by Akhtar *et al.* as selective EGFR and erbB2 receptor inhibitors. The target compound 12 showed a strong inhibition with an IC_{50} of 5.0 µM against BC (MCF-7) cells in *in vitro* cell inhibition tests. At concentration of 0.081 and 0.098µM, respectively, the target compound was shown to significantly block the EGFR and erbB2 receptors. According to cell cycle study, the target compound specifically stopped the proliferation of MCF-7 cells in the G2/M phase. The lead compound also displayed strong interactions with the EGFR enzyme at Asp831, Met769, and Thr830, according to computational and 3D-QSAR experiments.[109] Through a one-pot multicomponent synthesis, Akhtar *et al.* have created benzimidazole-based pyrazole derivatives and assessed them for possible anticancer properties. A549, MCF-7, MDA-MB231, HepG2, and HaCaT were among the human cancer cell lines against which the synthesised compounds were tested. For all the synthesised compounds, the EGFR inhibitory activities were assessed. Target compound 13 with an IC_{50} value of 0.97 mM

showed EGFR receptor inhibition. The target molecule had robust electronic properties in molecular docking tests.[110] The ability of a group of 6-amide-2-aryl benzoxazole/benzimidazole derivatives to specifically inhibit VEGFR-2 was investigated by Yuan *et al.* The library of compounds demonstrated a particular anticancer effect against the HepG2 and HUVECs of the liver, in contrast to the A549 and BC (MDA-MB-231) cancer cell lines. The target compound strongly reduced the growth of HepG2 and HUVEC, with IC_{50} values of 1.47 and 2.57 mM, respectively. The target compound 14 showed anti-angiogenesis action (79% inhibition at 10 nM/eggs) and excellent VEGFR-2 kinase inhibition with an IC_{50} of 0.051 mM utilizing the chick CAM test. The target substance interacted strongly with the VEGFR-2 kinase active site, according to the computational study.[111](**Figure 5**)



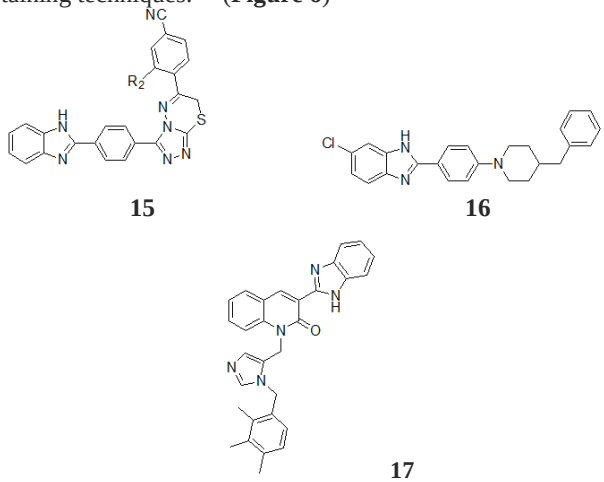
12 **13** **14**

Figure 5: Chemical structures of benzimidazole derivatives as Epidermal Growth Factor Receptor Inhibitors

Aromatase Inhibitors

BC depends on hormones, estrogens and in the absence of these hormones, cancer cannot proliferate, and disease would delay. In addition to the endocrine treatment, the option of inhibiting the aromatase enzyme has gained popularity. The latter method aims to inhibit the enzyme that turns androgens into estrogen, which then binds to the oestrogen receptor (ER) to carry out a certain function. Letrozole, anastrozole, and exemestane were observed to suppress androgen aromatization *in vivo by* >99%. .[112,113] These medications slow development and proliferation in hormone-dependent breast tumours.[114,115] Aromatase inhibitors (AIs) in particular have become more prevalent in current therapeutic regimens for the treatment of BC.[112,116] A cytochrome c subfamily member is the primary target for AIs. Aromatase enzyme belongs to the P450 family.[117,118] Its role is to catalyze the last stage of estrogen production sometimes referred to as androgen aromatization.

Steroid and non-steroidal AIs are the two subtypes that make up the class of AIs. Due to their structural resemblance to the aromatase enzyme, steroidal AIs (type I) attach to it. The nonsteroidal AIs saturate the binding site by attaching to the heme moiety and preventing androgen binding.[119] This kind of inhibition can be overridden by androgen competitive inhibition.[120]Aromatase inhibitory properties were found in a library of benzimidazole-triazolothiadiazine derivatives that had been synthesized. Compound 15 among the compounds showed a strong aromatase inhibitory action with an IC_{50} of 0.032± 0.01µM in BC cells. The 4-cyanophenyl substituent in compound 15 is located at the fourth position of the phenyl ring, which aids in the compound's inhibitory effect.[121] Gaikwad *et al.* developed 1,2,3-triazole compounds with quinoline-benzimidazole scaffolds and tested their cytotoxicity *in vitro* against NCI-60 humanoid cell lines. As a result, it was found that compound 16 had excellent GI_{50}, TGI, and LC_{50} values on several cell lines. The mechanism of action of this compound was then investigated using the BT-474 BC cell line. The MTT test yielded an IC_{50} value of 0.59± 0.01µM. Further research using the DAPI assay and acridine orange/ethidium bromide staining (AO/EB) revealed that 16 exhibits antiproliferative activity through apoptotic mechanisms, and the relationship between reactive oxygen species and apoptosis was clarified using the Dcfda and JC-1 staining techniques.[122] (**Figure 6**)



15 **16** **17**

Figure 6: Chemical structures of benzimidazole derivatives as Aromatase Inhibitors

Topoisomerase

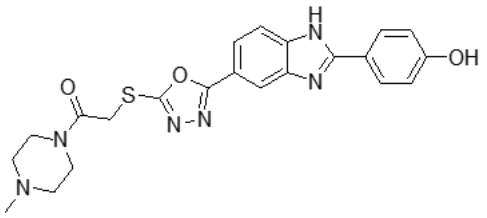
The DNA machinery includes topoisomerase II (topo II), which is extensively involved at numerous levels of DNA metabolism.[123–125] It transforms DNA structure from its storage (supercoiled) form to a more exposed (partially uncoiled) form by triggering single-strand DNA breaking and simultaneously passing another full double helix through the gap.[126,127]

Topoisomerase poisons, which primarily target topo II, belong to the anthracycline class of antitumor cytotoxic medicines.[124,128,129] These medications produce a cleavable complex made up of the medication, topo II, and DNA strands. The cleavable complex is thought to cause DNA damage, toxicity, and maybe death in tumor cells that are actively dividing.[128,130,131]

Few research has examined topo II as a potential prognostic marker in BC, aside from its potential relevance as a target for anticancer medications.[132]

Cevik *et al*. in 2020 synthesized benzimidazole-1,3,4-oxadiazole derivatives and evaluated them as human topoisomerase type I poisons. Their effects on a variety of cancer cell lines, including HeLa, MCF-7, A549, and HepG2, were examined. Compound 18 was singled out among the derivatives for being extremely

hazardous to the cell lines MCF-7 and HepG2, with IC_{50} values of 5.704 and 5.695 µM, respectively.[133](**Figure 7**)



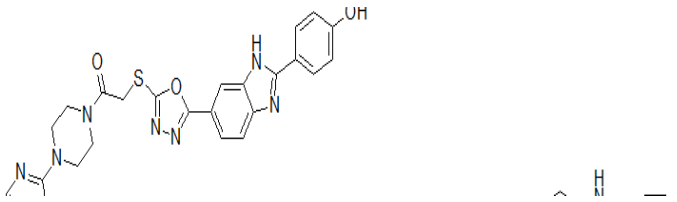
18

Figure 7: Chemical structures of benzimidazole derivatives as Topoisomerase Inhibitors

Additional research was conducted using DNA topo-I inhibition assay, DNA synthesis inhibition assay, and molecular docking. Various compounds and conventional doxorubicin were tested for their ability to block DNA synthesis in MCF-7, and they showed time- and dose-dependent inhibition of DNA synthesis. The same compounds were then examined using a flow cytometer. In comparison to doxorubicin (12.7%), the results showed that 18 had the highest levels of apoptotic characteristics (22.3%).[133]

HDAC inhibitors

HDAC enzymes have a substantial impact on the regulation of transcription at the estrogen and progesterone-mediated transduction pathways. At many points throughout this route, acetylation has been identified as a vital messenger that modulates ER transcription and turnover.[134] Acetylation and Deacetylation of histones are regulated by the enzyme histone acetyltransferase and histone deacetyltransferase. BC, gastric cancer, and AML are among the cancer types where the expression of HDAC is elevated.[135] On the other hand, increasing histone acetylation through the inhibition of HDAC by its inhibitors (HDACi) results in altered gene transcription. These inhibitors resulted in cell cycle arrest, inhibition of migration and invasion, and apoptosis whether given as monotherapy or in conjunction with another chemotherapeutic.[136] HDAC can be classified into four classes: class I HDACs (yeast Rpd3-like proteins: HDAC1-3, and HDAC8), class II HDACs with a single deacetylase domain at the C-terminus (yeast Hda1-like proteins: HDAC4-7, and HDAC9-10), class III HDACs (yeast silent information regulator 2 (Sir2)-like proteins: Sirtuin-1-7), and class IV (HDAC11). HDACi is a potent persuader of apoptosis and growth arrest, which can both be used to stop the differentiation of malignant cells.[137]One of the N-hydroxy-3-[3-(1-substituted-1*H*-benzoimidazol-2-yl)-phenyl]-acrylamide analogs, compound 19 is an HDAC2 inhibitor and demonstrated potent anticancer actions *in vitro* and *in vivo* in the nanomolar range. In HCT116 and PC3 models, compound 19 increased p21 activity, histone H3 and H4 hyperacetylation, and tumor suppression.[138] The competitive histone deacetylase inhibitor pracinostat (SB939) targets classes I, II, and IV HDACs.[139] In the phase II clinical trial, Pracinostat and Azacytidine were given together and demonstrated collaborative effects against AML, with reported reasonable safety and efficacy. However, patients did have mutual side effects such as infection, thrombocytopenia, and febrile neutropenia.[140] Unfortunately, the Pracinostat and Azacytidine phase III clinical trial in AML is obsolete as the treatment result was not anticipated to fulfill the primary endpoint of complete existence.[141] Class III histone deacetylases known as sirtuins are dependent on NAD to carry out their tasks. Sirtuin 1–7 mostly functions as a lysine deacetylase and/or mono-ADP-ribosyltransferase on both histone and non-histone proteins.[142,143] Anticancer effect of BZD9L1 as a sirtuin inhibitor is well described. Growing evidence has shown that sirtuins are critical for the genesis and progression of cancer,[144–148] making them a focus of interest in anticancer therapy.[149] BZD9L1 (20) is a versatile 1,2-disubstituted benzimidazole analogue that targets both the SIRT1 and SIRT2 proteins, with an IC_{50} of 42.9 µM for SIRT1 and 9 µM for SIRT2, respectively. The piperidinyl group, a crucial and potent electron-donating side chain, is substituted at the phenyl ring to create SIRT inhibitory action. The benzimidazole molecule is stabilised by this piperidinyl side chain, which also permits better contact with the SIRT protein's active site.[149] Comparable SIRT1 and/or SIRT2 inhibitory effects have been observed with BZD9L1 as that of known sirtuin inhibitors such AGK-2, EX527, and Tenovin-6. Tan *et al*.'s functional study has demonstrated its anticancer properties *in vitro* and *in vivo*, either alone or in conjunction with 5-FU, the first-line chemotherapy for colorectal cancer.[150,151] Additionally, BZD9L1 has been shown by Tan and colleagues to inhibit the feasibility, propagation, relocation, invasion, and induction of programmed cell death in colorectal cancer cells *in vitro* using the colorectal cancer cell lines HCT116 and HT-29, with IC_{50} values of 16.82 and 20.11 µM, respectively.[151] By preventing the growth of colorectal tumours by improving cell survival, cell cycle arrest, apoptosis, senescence, and micronucleation, the combination of BZD9L1 with 5-FU substantially improved the effectiveness of the treatment. Additionally, it was anticipated that BZD9L1 would alter the p53-dependent signaling pathways to cause cell death in CRC cells.[152] N, 2,6-Trisubstituted-1*H*-benzimidazoles was created by Em Canh Pham *et al*. in 2023 as an antibacterial and anticancer drug, and the researchers also used an *in silico* technique to evaluate the outcomes. Few derivatives were found to kill cancer cells like HepG2, MDA-MB-231, MCF7, RMS, and C26 with an IC_{50} of only 2.39-10.95µ M. In computational ADMET profiling, these compounds had similar drug-like characteristics to ciprofloxacin, fluconazole, and paclitaxel. To examine possible protein targets responsible for their biological activity, docking experiments were employed. From this, it was concluded that FGFR-1 and HDAC as a potential target both *in silico* and *in vitro*. [153] (**Figure 8**)



19 **20**

Figure 8: Chemical structures of benzimidazole derivatives as HDAC Inhibitors

CLINICAL TRIALS OF BENZIMIDAZOLES AGAINST BREAST CANCER

Cancer is becoming a major worldwide burden due to the fast rise in cancer incidence and death rates. Limiting factors that make treating cancer more difficult include the emergence of tumour resistance, therapeutic toxicities, cancer recurrence, and the poor success rate of drug development making it to clinical trials. The quest for new classes of anticancer medications is one of the areas of attention for increasing the treatment's effectiveness and the survival rate of cancer patients. Traditional non-targeting medications are damaging to healthy cells and may not be beneficial for all patients since they use a "one size fits all" approach. A personalised therapy strategy that aims to maximise results based on individual heterogeneity in genetic profile, lifestyle, and environmental variables is rapidly gaining recognition since cancer is a prominent focus of the precision medicine initiative[154]. Belinostat is a benzimidazole drug, which is a histone deacetylase inhibitor currently tested in clinical trials[155]. Various examples of clinical trials of benzimidazole against breast cancer is shown in **Table 2.**[156]

Table 2: Clinical trials of benzimidazoles against Breast Cancer

S. No	Drug	Condition	Trial type	Phase	Current status	Reference no.
1	Abemaciclib	HR +, HER2 Negative BC	Randomized	III	Active, Non-Recruiting	NCT02107703
2	Endocrine therapy with or without Abemciclib	HR Positive HER2 Negative BC	Randomized	III	Active, Non-Recruiting	NCT03155997
3	Abemaciclib + Nonsteroidal Aromatase Inhibitors	Recurrent or Metastatic BC	Randomized	III	Active, Non - Recruiting	NCT02246621
4	Abemaciclib	Previously treated, HER2 +, Negative Metastatic BC	N/A	II	Completed	NCT02102490
5	Abemaciclib	HR +, BC, Early-stage breast carcinoma	Randomized	II	Completed	NCT02441946
6	Abemaciclib	BC, Melanoma that has spread to the brain	Non-Randomized	II	Completed	NCT02308020
7	Abemaciclib+Fulvestrant	HER2 Negative BC (MONARCH 2)	Randomized	I	Active, not Recruiting	NCT02107703
8	Abemaciclib +Tamoxifen	HR+, HER2-, metastatic BC.	Randomized	II	Active, Non-Recruiting	NCT02747004
9	Abemaciclib + with or without food	Metastatic BC	Randomized	II	Active, Non - Recruiting	NCT03703466
10	Abemaciclib	Advanced and Metastatic cancer	Randomized	I	Completed	NCT02919696
11	Candesartan+ Metoprolol	BC+ heart failure	Randomized	II	Completed	NCT01434134
12	Debio 1347-101	Solid tumors	Non-randomized	I	Terminated	NCT01948297
13	Dovitinib + Fulvestrant	Metastatic BC	Randomized	II	Terminated	NCT01528345
14	Fulvestrant+ Selumetinib	MEK1/2 inhibitor, BC	Randomized	II	Completed	NCT01160718
15	Veliparib	Malignant solid Tumor	N/A	I	Completed	NCT00892736

16	Veliparib+Carboplatin	Solid tumors	Non-Randomized	II	Active, Non-Recruiting	NCT01149083
17	Veliparib+ Carboplatin+ paclitaxel	Metastatic BC	Randomized	III	Active, Non - Recruiting	NCT02163694
18	Veliparib and Cisplatin	Advanced Solid tumors	NA	I	Completed	NCT02723864
19	Veliparib+ Gemcetabine HCl	Adults solid neoplasm, BRCA -1 mutation carrier, BRCA-2 Mutation carrier	NA	I	Completed	NCT01154426
20	Veliparib+Lapatinib	Triple-negative BC	NA	NA	Active, Non - Recruiting	NCT02158507
21	Veliparib +temozolomide	Metastatic BC, BRCA-1Gene Mutation	NA	II	Active, Non Recruiting	NCT01009788
22	Veliparib+ Temozolomide	BC gene (BRCA)1 and (BRCA)2 mutation and Metastatic BC	Randomized	II	Completed	NCT01506609
24	Veliparib+ Pegylated Liposomal Doxorubicin Hydrochloride	Metastatic BC	NA	I	Completed	NCT01145430
25	Panobinostat	HER2-negative Locally Recurrent or Metastatic BC Conditions	Non-Randomized	II	Completed	NCT00777049

CONCLUSION

The application of *in-silico* methods demonstrated its benefits in the search for new drugs. *In silico* development is an essential tool in the search for innovative therapeutic options because of its quick screening, cost-effectiveness, and decreased reliance on conventional trial-and-error techniques. In this review, we investigated the *in-silico* synthesis of benzimidazoles as prospective therapeutic options for BC. Our investigation included this method's molecular design, computer simulations, and predictive modeling. According to the study, benzimidazoles have a lot of potential as BC preventatives. They may be effective, as shown by the complex interplay between the ligand-receptor interactions and the predicted data.

Benzimidazoles have been developed *in silico* as prospective treatments for BC, and this represents a promising new direction in the study of cancer. We believe that by showcasing the promise of benzimidazoles created *in silico*, researchers will be motivated to pursue this line of inquiry further and ultimately expand the range of therapeutic choices available to people with BC.

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List of Abbreviations

1. ACE Angiotensin Converting Enzyme
2. ADMET Absorption Distribution Metabolism Excretion and Toxicity
3. ANN Artificial Neural Network
4. ATC Anatomical Therapeutic Chemical
5. Ais Aromatase Inhibitors
6. AML Acute Myeloid Leukemia
7. A549 Lung Cancer cell line
8. Asn Asparagine
9. BC Breast Cancer
10. BRCA Breast Cancer Genes
11. CADD Computer-Aided Drug Design
12. CAM Chorioallantoic Membrane
13. CRC Colorectal cancer cells
14. Cys Cysteine
15. DCDB Medication Combination Database
16. Dcfda Dichlorofluorescein Diacetate
17. DINIES Drug-target interaction network inference engine
18. DL Deep Learning
19. DU-145 Prostate Cancer Cell line
20. EHUMO Energy of Highest Unoccupied Molecular Orbital

21. ELUMO Energy of Lowest Unoccupied Molecular Orbital
22. EO Evolutionary Algorithms
23. EGFR Epidermal Growth factor Receptor
24. ERs Estrogen Receptors
25. FAERS FDA Adverse Event Reporting System
26. FDA Food and Drug Administration
27. 5-FU Fluorouracil
28. GBCI Global BC Initiative
29. GPCR G-Protein coupled Receptor
30. GPER G-Protein coupled Estrogen Receptor
31. HaCaT Immortalized Human Keratinocytes
32. HCT116 Colorectal Cancer cell line
33. HDAC Histone Deacetylase
34. Hela Cervical Cancer Cell line
35. HepG2 Hepatocellular carcinoma
36. HER Human Epidermal Growth Factor Receptor
37. Hep G2 Hepatoblastoma cell line
38. HUVECs Human Umbilical vein endothelial cells
39. JAPIC Japan Pharmaceutical Information Centre
40. LBP Ligand Binding Protein
41. Lys Lysine
42. MATADOR Manual Annotation Online Resource
43. MC MonteCarlo
44. MCD Medication Combination Database
45. MCF Michigan Cancer Foundation
46. MD Molecular Dynamics
47. MDs Molecular Descriptors
48. ML Machine Learning
49. MLR Multiple Linear Regression
50. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
51. NAD Nicotinamide Adenine Dinucleotide
52. NCDs Non-communicable diseases
53. PC3 Prostate Xenograft Model
54. PDTD Prospective Drug Target Database
55. PDSP Psychoactive Drug Screening Programme
56. PR Polynomial Regression
57. QSAR Quantitative Structure Active Relationship
58. ROCS Rapid Overlay of Chemical Structures
59. RF Random Forest
60. SBD Structure-Based Design
61. SBP Structure-Based Pharmacophore
62. SERD Selective Estrogen Receptor Down regulator
63. SERM Selective Estrogen Receptor Modulators
64. SIRT Sirtuins
65. SVM Support Vector Machine
66. TGF Transforming Growth factor
67. TTD Therapeutic Target Database
68. TNBC Triple Negative BC
69. VEGFR Vascular Endothelial Growth factor receptor
70. WHO World Health Organisation

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