Drug Repurposing: Search for Targeted Drug Candidates in the Treatment of HER2 Positive Breast Cancer

By

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A thesis submitted to the school of pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy

School of Pharmacy
Brac University
September 2023

Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis titled “Drug Repurposing: Search for Targeted Drug Candidates in the Treatment of HER2 Positive Breast Cancer.” submitted by Jahida Yeasmin (19346032) of Spring, 2023 has been accepted as satisfactory in partial fulfilment of the requirement for the degree of Bachelor of Pharmacy on 2023.

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Ethics Statement

The project does not involve any clinical trial or human participants, no animals were used or harmed.

Abstract

According to GLOBOCAN data, 2020, breast cancer (BC) is the leading cause of cancer-related mortalities worldwide, with HER2 positive breast cancer being very prevalent. The rising incidence of HER2 positive breast cancer has led to the search and development of targeted treatment strategies. The currently available targeted therapies have revolutionized treatment strategies. However, despite the remarkable progress in HER2 positive breast cancer management, challenges like drug resistance, and long-term side effects persist and need ongoing research attention. As such, drug repurposing could be a powerful strategy in the search for such compounds and thus enhancing the existing drug pool. Drug repurposing using computational biology was performed in the study, followed by analysis of the docking results, superimposition, assessing non-bonded protein-ligand interactions, and ADME properties were performed to propose potential candidates against HER2 positive breast cancer. Based on the results of this study, doxazosin was selected as a potential candidate to be further explored. Further studies such as MD simulation and biological assays will later need to be performed to validate the results of this study.

Keywords: HER2 Protein, Targeted-Therapy, Drug -Resistance, Drug Repurposing.

Dedication

Dedicated to my parents, who did all in their power for my education. They deserve a special mention in my thesis.

Acknowledgement

Bismillahir Rahmanir Rahim,

I want to start by thanking the Almighty Allah for guiding me through every challenge. I would not have progressed up to this point without His favor.

Next, I would like to convey my sincere thanks to my supervisor, Professor Dr Eva Rahman Kabir Ma'am (Dean, School of Pharmacy, Brac University), for her unwavering patience and support. Without her help, I would not have been able to complete my project. Her depth of expertise, continuous encouragement, devotion and appreciation have been quite helpful for me.

Also, I want to express my deepest gratitude to my co-supervisor, Nashrah Mustafa Ma'am (Lecturer, School of Pharmacy, Brac University), for being a constant guiding spirit throughout my journey. She has been incredibly helpful to me.

Finally, I am grateful to all of my faculty members of School of Pharmacy for their constant guidance, support and encouragement which helped me throughout this journey.

Last but not the least, I would like to take this opportunity to thank my family and friends who have helped me for all my educational achievements.

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List of Acronyms

HER2	Human Epidermal Growth Factor Receptor 2
AHTN	Antihypertensive
%HOA	Percentage of Human Oral Absorption
BC	Breast Cancer
PSA	Polar Surface Area
CNS	Central Nervous System
CT Scan	Computerized Tomography Scan
MDS	Molecular Dynamic Simulation
MCF-7	Michigan Cancer Foundation-7

Chapter 1

Introduction

Breast cancer is one of the major causes of deaths worldwide (Harbeck & Gnant, 2017). It was the most common cancer in 2020, contributing to 12.5% of the total number of newly diagnosed cases (*Worldwide Cancer Data | World Cancer Research Fund International*, May 27, 2023). According to WHO Reports 2023, each year, more than 2.3 million people suffer from breast cancer and in 95% countries, breast cancer is one of the main causes of cancer-related deaths among women (WHO, 2023). The different types of breast cancers include estrogen positive breast cancer (which accounts for 80%), progesterone positive breast cancer (which accounts for 65%), estrogen and progesterone negative breast cancer (which accounts for 25%), HER2 positive breast cancer (which accounts for 30%), HER2 negative and Triple-negative breast cancer (which accounts for 10 to 20%) (Gina Shaw, March 19, 2023). HER2 (human epidermal growth factor receptor 2) positive breast cancer is found to be quite common among women (Gina Shaw, March 19, 2023). Overexpression of HER2 receptor is a common feature of HER2 positive breast cancer, that accounts for the uncontrolled cell proliferation. The rate of HER2 positive breast cancer is different among different ethnicities with 10% in black women, 9% in white woman, 12% Hispanic women, 11% in Asian and Pacific Islander women and 10% in Asian women (Giaquinto et al., 2022). In Bangladesh according to a survey in 2022, among 90 participants of breast cancer patient, 18.9% participants were HER2 positive (Proteek et al., 2022).

1.1 HER2 Protein

HER family of receptors play a significant role in the pathogenesis of several human cancers, especially HER2 positive breast cancer. They regulate cell growth, survival, and differentiation via multiple signal transduction pathways and participate in cellular proliferation and differentiation. The family is made up of four main members: HER1, HER2, HER3, and HER4, which are also called ErbB1, ErbB2, ErbB3, and ErbB4, respectively. Like other types of HER receptors, HER2 receptor comprises of a cysteine-rich extracellular ligand binding site, and an intracellular domain with tyrosine kinase catalytic activity (Iqbal & Iqbal, 2014). The HER2 receptor consists of 1255 amino acids, 185 kD transmembrane glycoprotein located at the long arm of human chromosome (Schrohl et al., 2011). HER2 inhibitors bind with extracellular and intracellular cytoplasmic tyrosine kinase domains of HER2 receptor and therefore control the deregulated cell proliferation and cell division.

1.2 Signaling Pathway of HER2 Positive Breast Cancer

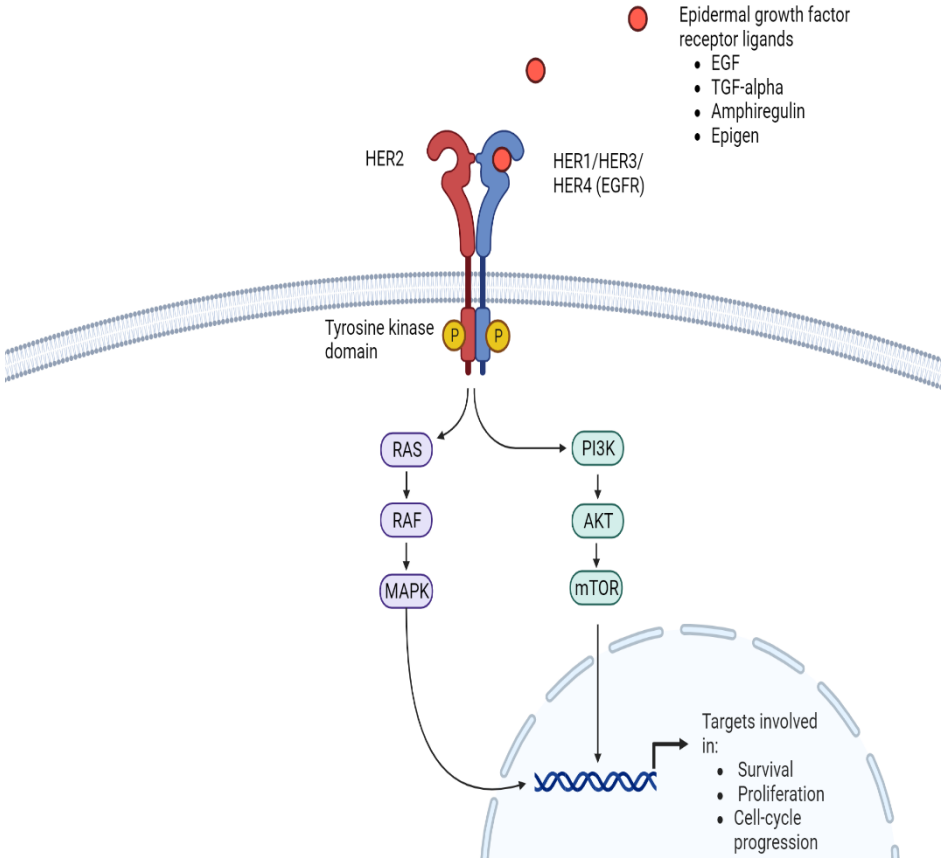


Figure 1:Signalling pathway of HER2 Positive Breast Cancer.

HER receptors exist as monomers on the cell surface. When ligand binds to extracellular domains of HER family receptors, the HER proteins undergo dimerization. Transphosphorylation then occurs in the intracellular tyrosine kinase domain that can directly activate the HER2 receptor and regulate cell division. HER2 receptor is only activated upon heterodimerization with other members of the family, such as HER1 and HER3. Dimerization causes autophosphorylation of tyrosine residues through the receptor's cytoplasmic domain, which in turns begins a number of signaling cascades, such as the mitogen-activated protein kinase (MAPK), phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), resulting in cell growth and proliferation. Overexpression of HER2 receptor modulates signaling pathways leading to uncontrolled cell growth and proliferation which may lead to HER2 positive breast

cancer (Shah & Osipo, 2016). HER2 overexpression can occurs via multiple mechanisms, including gene amplification, transcriptional activation, and protein stabilization. Gene amplification is a primary driver of HER2 protein overexpression, leading to augmented cellular signaling and oncogenic transformation.

1.3 Current Treatment Options for HER2 Positive Breast Cancer

The current treatment options of breast cancer are dependent on its stage and size, and whether the cancer cells are sensitive to hormones. The treatments include lumpectomy (removing the breast cancer), mastectomy (removing the entire breast), sentinel node biopsy (removing a limited number of lymph nodes), axillary lymph node dissection (removing several lymph nodes), removing both breasts, radiation therapy, chemotherapy, hormone therapy, immunotherapy, targeted drug therapy and supportive (palliative) care (Guide, 2023). Targeted drug therapy using monoclonal antibodies and tyrosine kinase inhibitors are also being used to block the overexpressed HER2 receptor and inhibit the downward signaling cascade of HER2 receptor (Figure 2).

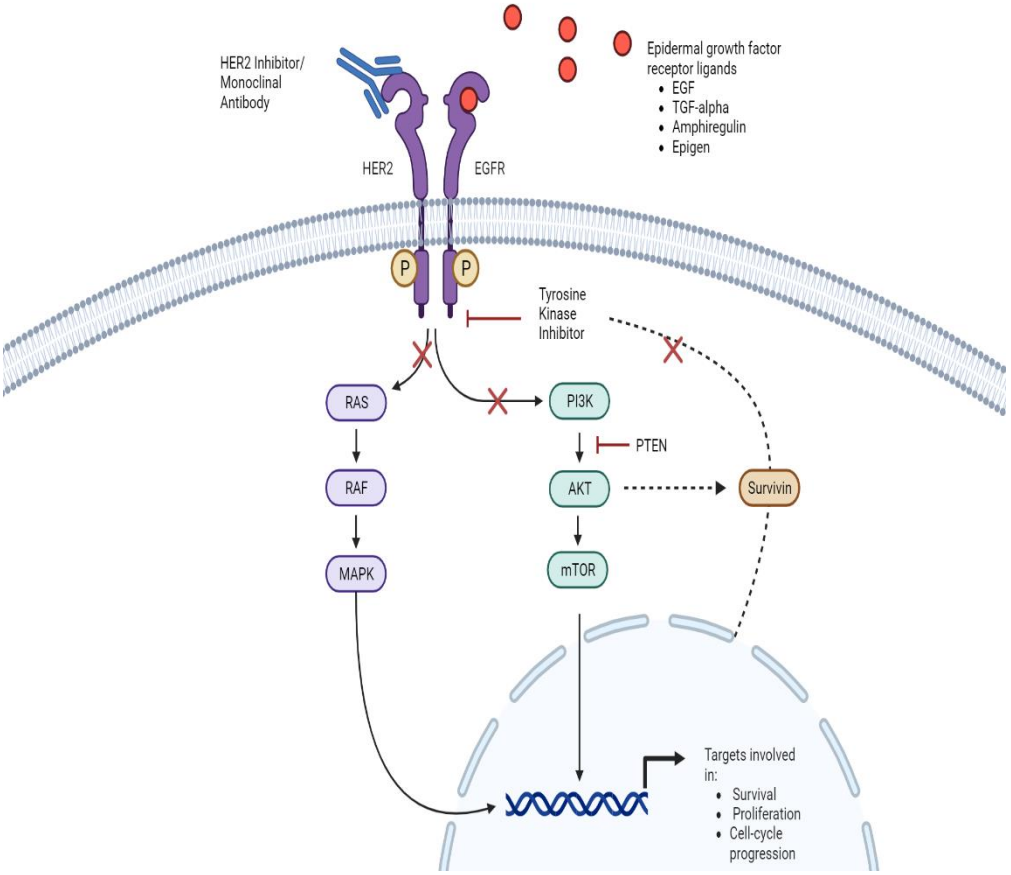


Figure 2:Targeted therapy blocks the overexpressed HER2 receptor.

Monoclonal antibodies such as pertuzumab**,** trastuzumab bind with the extracellular domain of HER2 receptor and inhibit the HER receptors' dimerization, which in turn, inhibits the transphosphorylation. This attenuates the downstream signaling cascades, leading to diminished activation of the PI3K/AKT pathway, which is crucial for cell survival and proliferation. On the other hand, small tyrosine kinase inhibitors like lapatinib, neratinib and tucatinib bind to the intracellular cytoplasmic tyrosine kinase domains of HER2 receptor and control the deregulated cell proliferation and cell division by inhibiting the signaling cascade (Name, 2012).

1.3.1 Drugs Approved for HER2 Positive Breast Cancer

The FDA has approved a number of drugs that can inhibit HER2, and these include

pertuzumab, trastuzumab (herceptin, herzuma, ontruzant), ado-trastuzumab emtansine (T-DM1), fam-trastuzumab deruxtecan-nxki (T-DXd), margetuximab-cmkab, neratinib, tucatinib and lapatinib. FDA has also approved fam-trastuzumab deruxtecan-nxki (marketed under the name Enhertu) for metastatic HER2-positive breast cancer patients (U.S. Food & Drug Administration, 2022).

1.4 Drug Repurposing

Drug repurposing, a strategy for new therapeutic applications of existing drugs, allows rapid development of effective treatments. In the context of HER2 positive breast cancer, where targeted therapies have revolutionized treatment paradigms, repurposing existing drugs as HER2 inhibitors presents another avenue. It is an important part of drug discovery, drug development and preclinical studies. In drug repurposing, a large number of drugs are screened and repositioned that are already approved based on the available pharmacological and toxicological data. *In silico* methods, which involve computer simulations, modeling, and data analysis, provide a robust foundation that not only expedites research but also optimizes resource utilization. They can serve as a precursor to lab-based investigations, streamlining processes, saving valuable time, and optimizing financial resources. It is safe to say that *in silico* experiments have a promising and bright future in pharmaceutical research. Molecular docking, part of computational biology, is used for drug repurposing that enables researchers to predict how molecules bind and interact with each other and with the target protein. It predicts the binding orientation and affinity between a small molecule (ligand) and a target biomolecule (usually a protein). By simulating their interactions, docking elucidates how these molecules interact, providing insights into potential therapeutic applications (Rudrapal et al., 2020).

1.5 Rationale of the Study

HER2 positive breast cancer is one of the most aggressive types of cancer in women. The objective of the study is to search for potential inhibitors of the HER2 receptor from the three classes of antidiabetic, antihypertensive and statins. The current FDA approved drugs include: lapatinib, neratinib, tucatinib are gradually becoming resistant. There is thus a need to increase the existing drug pool. This study used computational tools with the aim of screening statins, antidiabetic, antihypertensive classes of drugs to find out potential activity against HER2 receptor. Statins, antidiabetics, antihypertensive classes drugs were taken as candidates for computational study because these three pathological conditions (cholesterol, diabetes and hypertension) have a strong association with breast cancer. This is further explained in the following subsections.

1.5.1 Association of Cholesterol with Breast Cancer

A study showed that the incidence of HER2 positive breast cancer progression increases with increasing levels of cholesterol in serum due to their effect on signaling pathways (Zhao et al., 2019). Tumor cells have complex interactions with their adjacent tumor environment. Cholesterol-rich environments tend to alter immune cell morphologies and functions. This may assist breast cancer cell survival. Elevated blood cholesterol levels in HER2 positive cancer patients may be attributed to increased cholesterol production in tumor cells. As a result, cholesterol has the potential to be both a cause and an effect of cancer, though the type and stage of the tumor should not be disregarded. Since high cholesterol levels can affect cancer cell survival, growth and metastasis, prescribing drugs that lower cholesterol, such as statins, may be helpful in treating breast cancer. It has been suggested that targeting cancer cell cholesterol metabolism pathways could be a good way to understand the relationship (Halimi & Farjadian, 2022).

1.5.2 Association of Diabetes with Breast Cancer

Diabetes also worsens tumor prognosis and progression. Patients with diabetes specifically have higher levels of bioavailable IGF-1(Insulin-like Growth Factor-1), which raises their chances of developing cancers such as breast, colorectal, and prostate cancers. A recent study of 26,968 breast cancer patients, with 11.6% diabetic cases, showed that the chances of having both cancer and diabetes have increased over time. Patient with diabetes had a higher chance of being diagnosed with stage III-IV breast cancer than non-diabetes ones. The study showed diabetic women between the ages of 45 and 69 were more likely have breast cancer. The high amount of glucose in the tumor microenvironment (TME) is the main source of energy for tumor growth, and associated with rapid tumor proliferation. Therefore, any diabetes medication that also lowers blood glucose levels, may be beneficial in breast cancer treatment (Bashraheel et al., 2023).

1.5.3 Association of Hypertension with Breast Cancer

A meta-analysis with 11,643 breast cancer patients showed that hypertension increased the chances of breast cancer. Studies show that the incidence of breast cancer is 13%-15% higher in hypertensive women (Yue et al., 2022). Currently available antihypertensive (AHTN) drug products are considered a potential reservoir of agents that makes a significant contribution in the oncology field (Fan et al., 2022).

Chapter 2

Methodology

This section highlights the steps that were taken during the study.

2.1 Preparation of the Protein Structure

The protein, HER2 (PDB ID: 3P0Z) was retrieved from RCSB PDB (Resolution: 1.50 Angstroms). The protein was curated by using PyMOL by removing the co-crystallized ligand, TAK-285 and water molecules.

2.2 Preparation of the Ligands

The 3D structures of the reference drug (TAK-285), statins, antidiabetic and antihypertensive drugs, were retrieved from PubChem drug database. All compounds were optimized and converted to PDB format.

2.3 Molecular Docking

Docking and screening of around 100 drugs from therapeutic classes, anticholesterol, antidiabetic and antihypertensive against HER2 protein were performed using the Autodock Vina (Version 1.2.0) software. The drugs with better binding affinity values compared to the reference drug were chosen for further studies.

2.4 Superimposition

In this step, the selected drugs with better binding affinity values were superimposed with the reference drug, TAK-285 through PyMOL, a visualization software. The drugs were superimposed with the reference drug to assess if they bind to the same pocket as the reference drug. The drugs that overlapped with the reference drugs were chosen for the next steps.

2.5 Ligand-Protein Interaction

Drugs selected from the previous step were visualized in Discovery Studio Visualizers (Version 22.1). The molecular interactions of the protein-ligand complexes were identified. Ligand-protein interactions were then compared to the reference drug, TAK-285 to find out the common amino acids involved in binding with HER2 receptor and to assess the types of interactions involved in the binding. The drugs which had similar amino acids as the reference drugs were selected.

2.6 Analysis of Pharmacokinetic Parameters

The next stage was to determine the pharmacokinetic (ADME) properties of the selected drugs. Pharmacokinetics parameters of potential drug candidates were predicted using QikProp and compared with the reference drug. Based on the QikProp results, potential candidates were proposed.

Chapter 3

Results

Molecular docking is an important tool in drug discovery which predicts the orientation, interaction and binding affinities of ligands in their targeted binding sites. Statins, antidiabetic and antihypertensive drugs were successfully docked against the HER2 receptor, and the results are shown in Table 1.

TAK-285, a novel investigational small molecule which inhibits EGFR and specifically targets HER2 was the co-crystallized structure in the protein and was chosen as the reference drug. It has shown antiproliferative activity *in vitro* and *in vivo* cytotoxic activity against breast cancer cells (IC50: 17nM -23nM) (Ishikawa et al., 2011). Here, at first TAK-285 which was the co-crystalized reference ligand was docked against HER2 protein and the binding affinity was found to be -9.7 kcal/mol. This value was considered the cut off value and docking scores above this cut off value were chosen. The reference drug, TAK-285 binding involved the following amino acid residues: ARG803, ASN842, ASP800, VAL726, PHE723, LEU799, LEU844 and LEU718. These amino acid residues were considered to be present in the active binding pocket of the HER2 receptor. Finally, seventeen compounds were selected from statins, anti-diabetic and antihypertensive drugs based on their binding affinities.

3.1 Docking Results

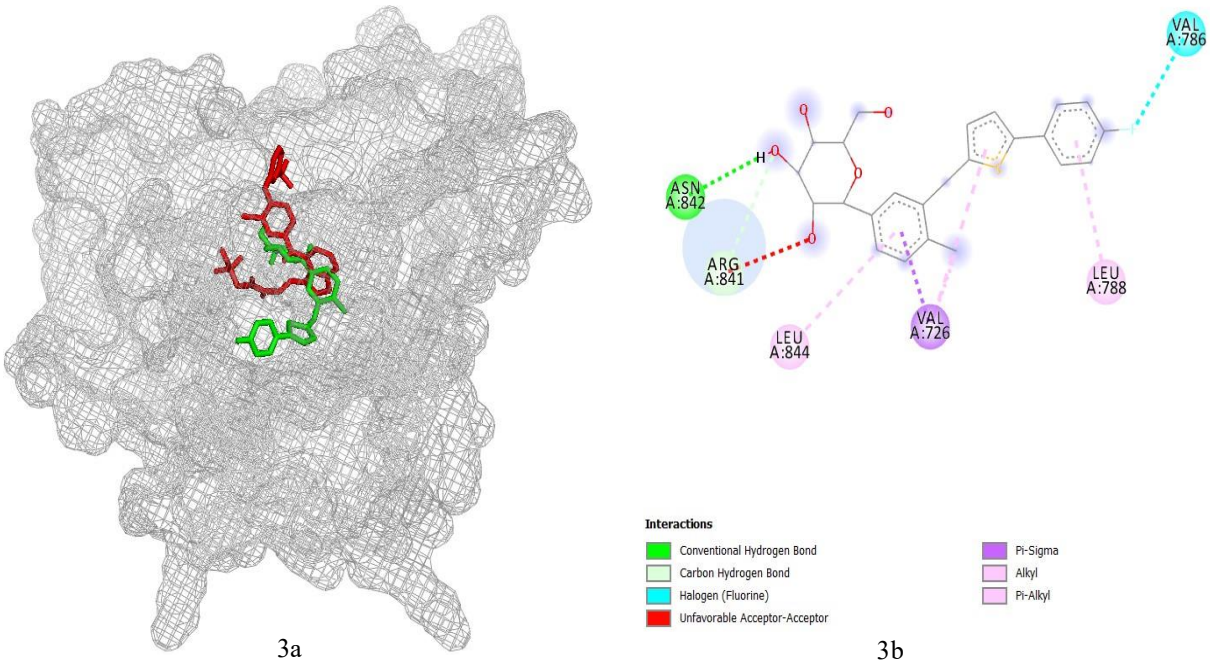
The compounds that showed better binding affinity than the reference drug, TAK-285 are listed in the table (Table 1).

Table 1:Binding Affinitity of the Drugs.

Drugs	**Binding affinity (kcal/mol)**
TAK-285 (Reference Drug)	**-9.7**
Canagliflozin (anti-diabetic)	-10.4
Dapagliflozin (anti-diabetic)	-10.0
Empagliflozin (anti-diabetic)	-10.0
Linagliptin (anti-diabetic)	-10.3
Doxazosin (antihypertensive)	-10.0
Lisinopril (antihypertensive)	-10.6
Moexipril (antihypertensive)	-10.5
Trandolapril (antihypertensive)	-9.8
Benazepril (antihypertensive)	-9.8
Reserpine (antihypertensive)	-10.3
Telmisartan (antihypertensive)	-10.0
Valsartan (antihypertensive)	-11.1
Candesartan (antihypertensive)	-10.1
Fluvastatin (anti-cholesterol)	-10.5
Pitavastain (anti-cholesterol)	-11.3
Lovastatin (anti-cholesterol)	-9.9
Pravastatin (anti-cholesterol)	-10.6

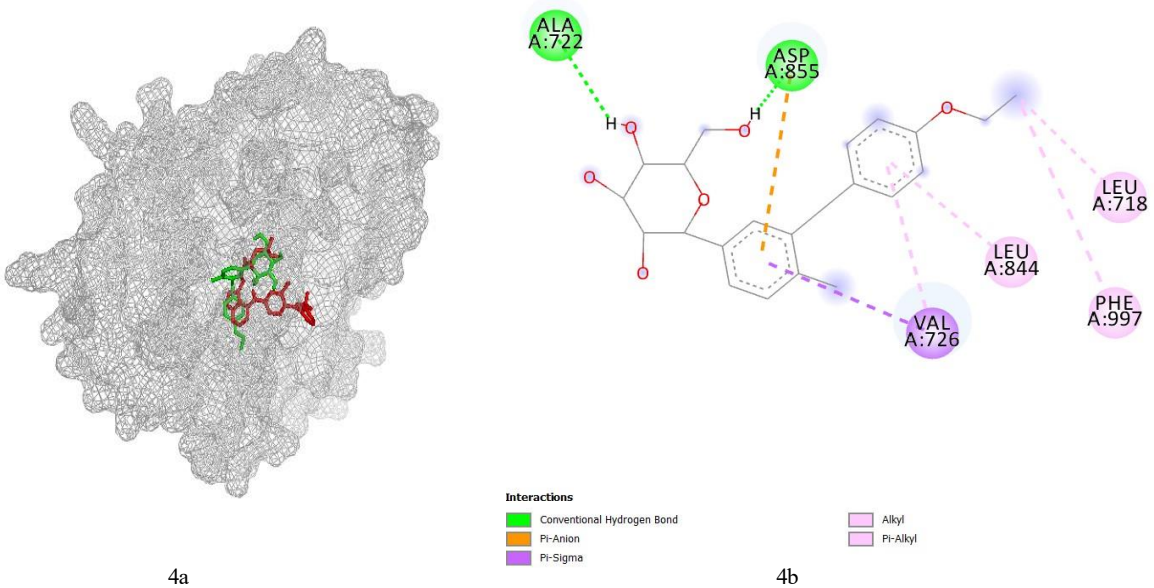
3.2 Superimposition

Drugs that had better binding affinities such as benazepril, canagliflozin, candesartan, dapagliflozin, empagliflozin, doxazosin, fluvastatin, linagliptin, lisinopril, lovastatin, moexipril, pitavastain pravastatin, reserpine, telmisartan, valsartan and trandolapril were chosen for superimposition. From there, eleven molecules were selected based on their binding with the active binding pocket and overlap with the reference drug. Some drugs that showed better superimposition with reference drug are given in Figures 3-8.



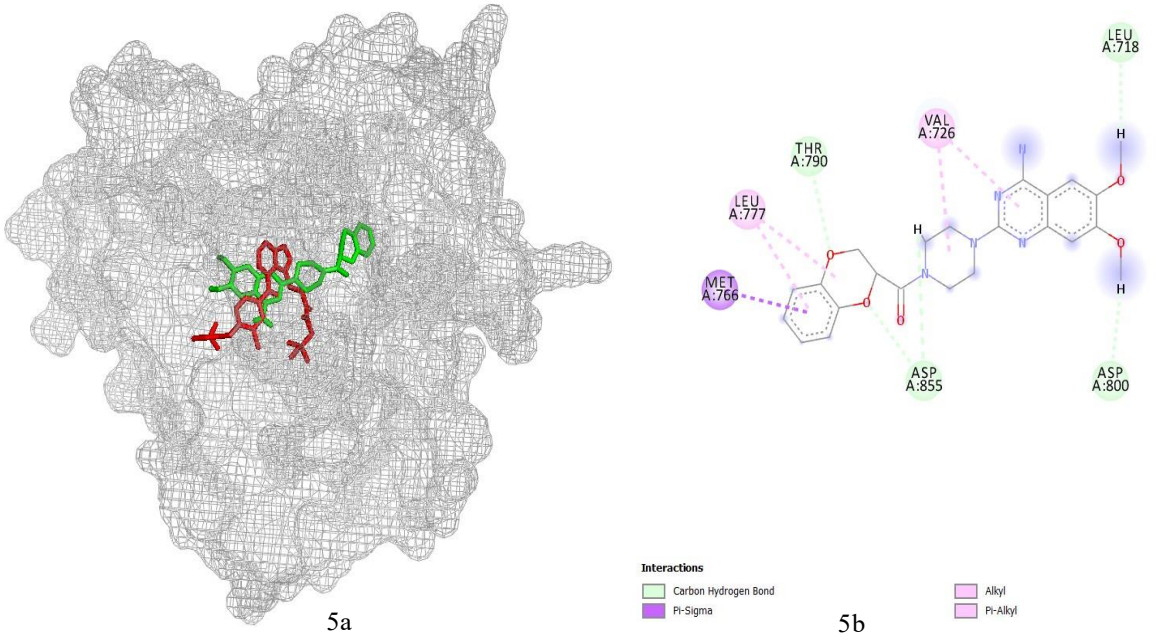
3a 3b

Figure 3a: Canagliflozin superimposed with TAK-285 (Red: TAK-285 and Green: canagliflozin. 3b: Interactions involved in the binding of canagliflozin with HER2 (PDB ID: 3POZ)



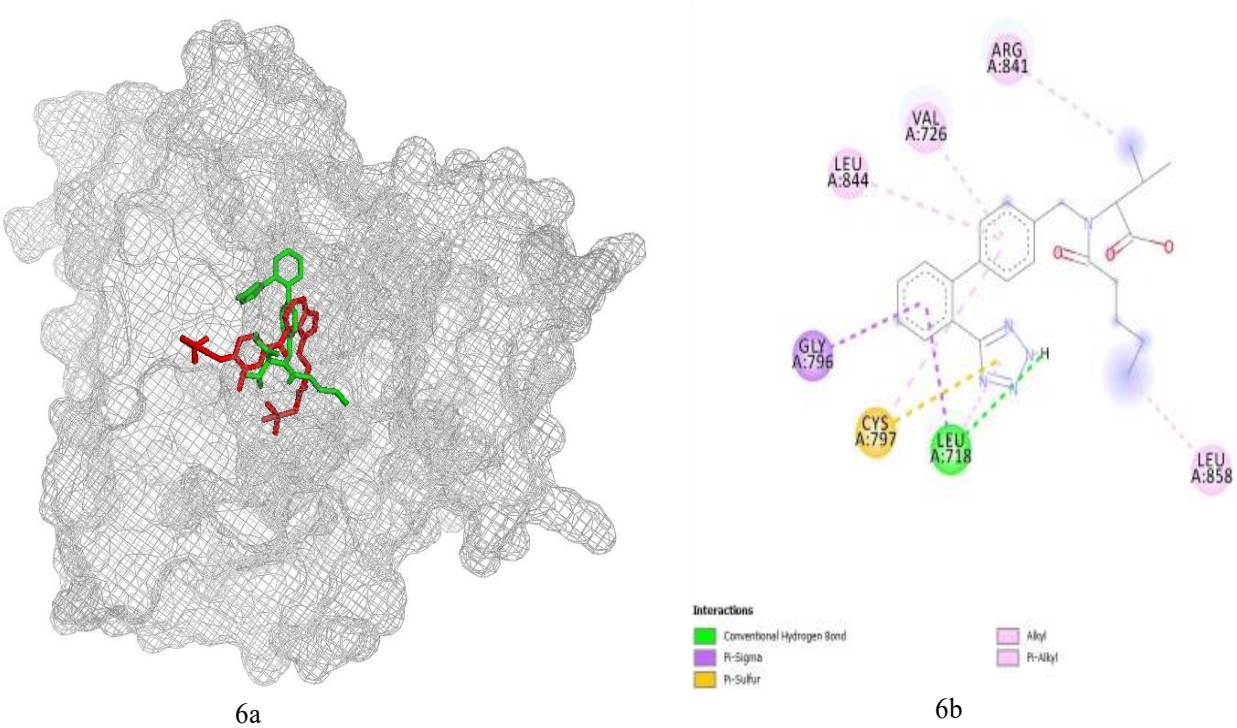
4a 4b

Figure 4a: Dapagliflozin superimposed with TAK-285 (Red: TAK-285 and Green: dapagliflozin). 4b: Interactions involved in the binding of dapagliflozin with HER2 (PDB ID: 3POZ)



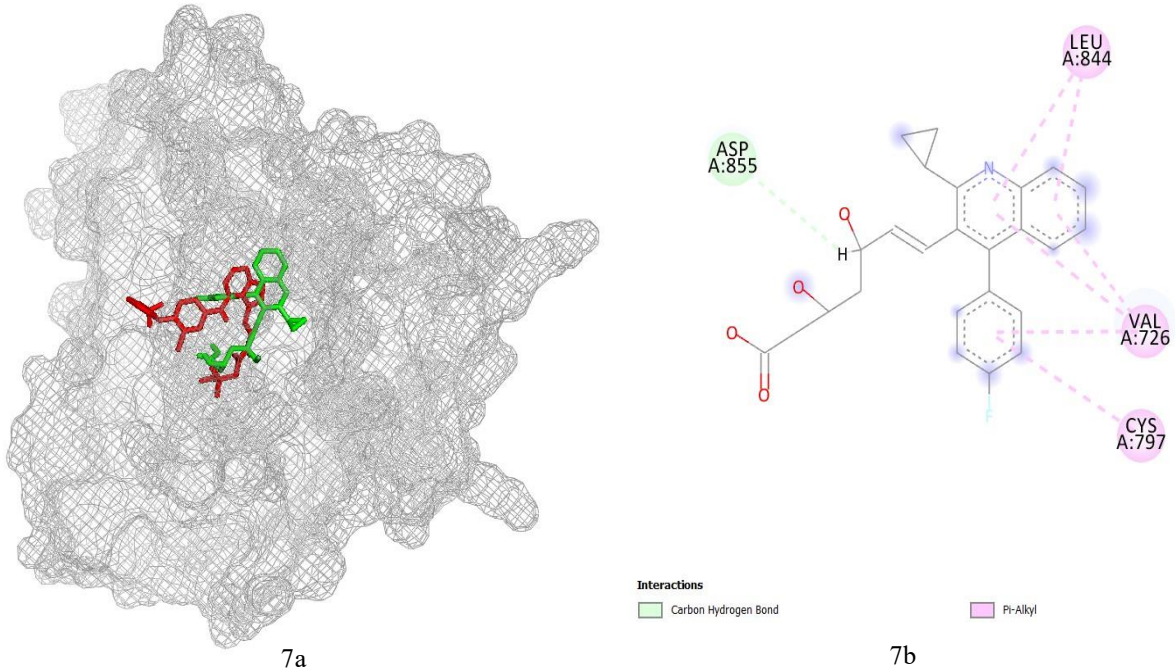
5a 5b

Figure 5a: Doxazosin superimposed with TAK-285(Red: TAK-285 and Green: doxazosin.) 5b: Interactions involved in the binding of canagliflozin with HER2 (PDB ID: 3POZ)



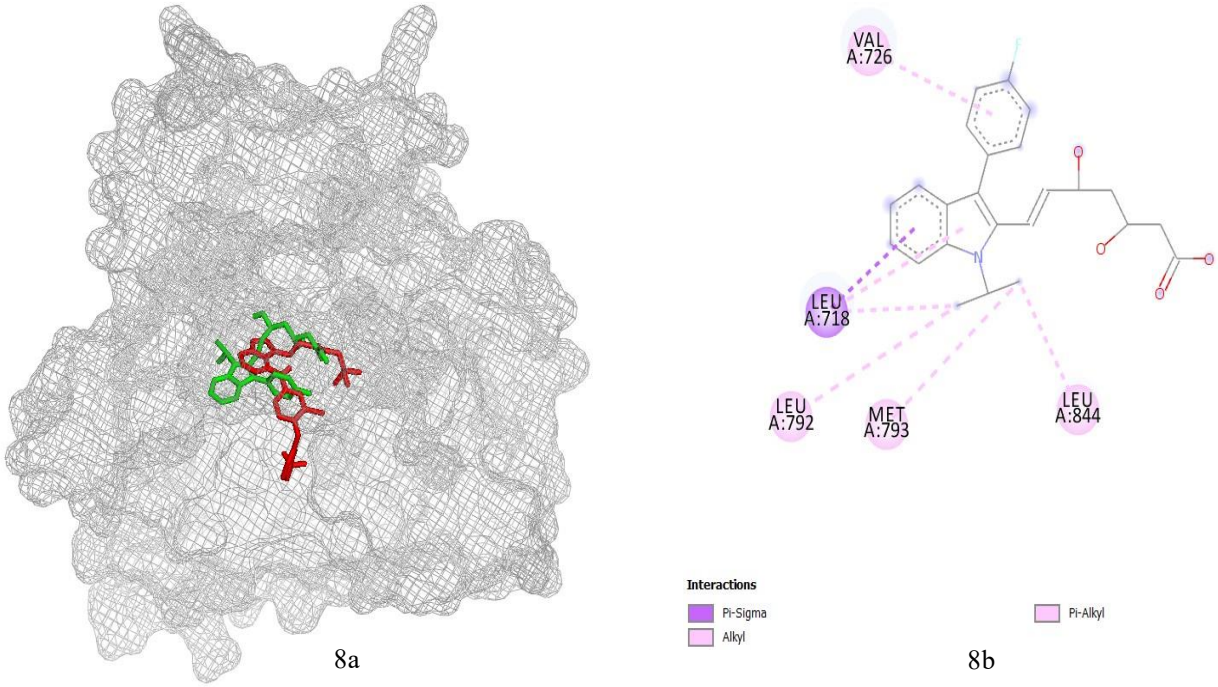
6a 6b

Figure 6a: Valsartan superimposed with *TAK-285(Red: TAK-285 and Green: valsartan). 6b: Interactions involved in the binding of canagliflozin with HER2 (PDB ID)*



7a 7b

Figure 7a: Pitavastatin superimposed with TAK-285(Red: TAK-285 and Green: pitavastatin). 7b: Interactions involved in the binding of canagliflozin with HER2 (PDB ID: 3POZ)



8a 8b

Figure 8a: Fluvastatin superimposed with TAK-285(Red: TAK-285 and Green: fluvastatin). 8b: Interactions involved in the binding of canagliflozin with HER2 (PDB ID: 3POZ)

3.3 Protein-Ligand Interaction

The amino acid residues involved in binding of TAK 285 with HER2 protein included ARG803, ASN842, ASP800, VAL726, PHE723, LEU799, LEU844 and LEU718. By considering these amino acid residues as parts of the active pocket, screening was performed. Selected molecules were screened from statins, anti-diabetic and antihypertensive drugs. These include canagliflozin, candesartan, dapagliflozin, doxazosin, empagliflozin, fluvastatin, linagliptin, pitavastain, pravastatin, reserpine and valsartan. They demonstrated common amino acid residues with the co-crystallized ligand TAK-285 in the binding with the mutated HER2 (Table 2).

Canagliflozin showed three common amino acid residues ASN842, LEU844, VAL726 common with TAK-285 and formed hydrogen bond, pi-alkyl and pi-sigma bond respectively. Dapagliflozin shows two common amino acid LEU718, VAL726 and formed pi-alkyl, alkyl and pi-sigma bond. Empagliflozin and linagliptin each had three common amino-acid residues LEU844, LEU718, VAL726 and ASP800, LEU718, VAL726 respectively. They formed hydrogen-bonds, alkyl, pi-alkyl and pi-sigma bonds respectively. On the other hand, anti-hypertensive drugs doxazosin and valsartan showed three common amino acids with TAK-285. The common amino acid of doxazosin included ASP800, LEU718, VAL726; valsartan had LEU844, LEU718, VAL726 amino acids in common. Valsartan formed conventional hydrogen bond, pi-alkyl and pi-sigma bond; where doxazosin showed hydrogen bonds, alkyl bond, pi-alkyl bond and pi-sigma bonds respectively with the HER2 receptor. Candesartan and reserpine from anti-hypertensive class had four common amino-acid residues. The common amino acid of candesartan included ASN842, LEU844, LEU718, VAL726 and common amino acid residues of reserpine included ASP800, LEU844, LEU718 and VAL726 with TAK-285. They formed hydrogen-bond, pi-alkyl and pi-sigma bonds respectively. The cholesterol lowering agent, fluvastatin also had three common amino acids, LEU844, LEU718, VAL726

and it formed pi-sigma, pi-alkyl and alkyl bonds respectively. Pitavastatin and pravastatin are also cholesterol lowering agent. Pitavastatin had two common amino acids LEU844, VAL726 with TAK-285. Pravastatin had four common amino residues with TAK-285 (ASN842, LEU844, LEU718, VAL726).

Table 2: Non-bonding Interaction Between HER2 protein and Ligand.

Drug Name	**Amino- Acid**
TAK- 285 (Reference-Drug)	ARG803, ASN842, ASP800, VAL726, PHE723, LEU799, LEU844, LEU718.
Canagliflozin (Anti-diabetic)	ASN842, LEU844, VAL726.
Dapagliflozin (Anti-diabetic)	LEU718, VAL726.
Empagliflozin (Anti-diabetic)	LEU844, LEU718, VAL726.
Linagliptin (Anti-diabetic)	ASP800, LEU718, VAL726.
Fluvastatin (Anti-cholesterol)	LEU844, LEU718, VAL726.
Pravastatin (Anti-cholesterol)	ASN842, LEU844, LEU718, VAL726.
Pitavastatin (Anti-cholesterol)	LEU844, VAL726.
Candesartan (Anti-hypertensive)	ASN842, LEU844, LEU718, VAL726.
Doxazosin (Anti-hypertensive)	ASP800, LEU718, VAL726.
Reserpine (Anti-hypertensive)	ASP800, LEU844, LEU718, VAL726
Valsartan (Anti-hypertensive)	LEU844, LEU718, VAL726.

3.4 Pharmacokinetics (ADME) Parameters

After analyzing the pharmacokinetic parameters of selected drugs, doxazosin was chosen. But canagliflozin, dapagliflozin, pitavastatin, fluvastatin, and valsartan could also be good options in the treatment of HER2 positive breast cancer as the pharmacokinetic properties were within the satisfactory range. The ADME properties of these drugs are tabulated below:

Table 3: Pharmacokinetics Properties of the Drugs.

Molecules	**Absorption**	**Distribution**			**CNS permeability**		
	%HOA	**QPPCaco2**	**QPPMDCK**	**QPlogKhsa**	**CNS**	**QPlogBB**	**PSA**
TAK-285 (Reference-drug)	89.533	935.401	4862.2	0.848	0	-0.569	94.991
Pitavastatin	90.403	95.108	83.54	0.439	-2	-1.224	99.365
Fluvastatin	82.792	130.112	105.621	0.643	-2	-1.081	91.121
Dapagliflozin	81.347	227.225	185.755	-0.226	-2	-1.657	101.808
Canagliflozin	89.635	251.467	260	0.195	-2	-1.411	93.913
Valsartan	81.627	87.744	58.935	-0.243	-2	-1.432	121.895
Doxazosin	93.152	610.112	532.117	-0.019	-1	-0.749	107.377

** Pharmacokinetic properties of the reference and candidate drugs. %HOA: Percentage of Human Oral Absorption; QPPCaco2: intestinal permeability in nm/s; QPPMDCK: renal permeability in nm/s; QPlogKHSA: Binding to human serum albumin; CNS: Central nervous system activity; QPlogBB: brain/blood partition coefficient; PSA: Van der Waals surface area of polar nitrogen and oxygen atoms.

3.4.1 Pitavastatin and Fluvastatin

Pitavastatin and fluvastatin are cholesterol lowering agents which exhibited good pharmacokinetic properties. According to the data (Table 3), they showed good human oral absorption (above 80%). The human oral absorption of pitavastatin was 90.403% that is higher than our reference drug, TAK-285. Fluvastatin also showed a satisfactory human oral absorption (82.792%). They showed good intestinal cell and renal cell permeabilities but were less compared to the reference drug. The intestinal and renal cell permeabilities of pitavastatin were 95.108 nm/s and 83.54 nm/s respectively. On the other hand, the intestinal cell and renal cell permeabilities of fluvastatin were 130.112 nm/s and 105.621 nm/s which are within

acceptable range. The binding to serum albumin were also within the range (-1.5 to 1.5). Also, they had a predicted brain/blood partition coefficient within the acceptable range –3.0 to 1.2. Van der Waals surface area of polar nitrogen and oxygen atoms (PSA) results were within the range (7.0 to 200) and the value indicates these drugs are CNS inactive. They will not show any effect on central nervous system.

3.4.2 Canagliflozin and Dapagliflozin

Canagliflozin and dapagliflozin are antidiabetic drugs which showed good results. The oral absorption of reference drug, TAK-285 was found to be 89.533%. Even though canagliflozin and dapagliflozin showed good human oral absorption, 88.235% and 81.347% respectively, the values were less compared to the reference drug. They showed good permeabilities through the intestinal cells and renal cells. None of them bind to serum albumin. They had a predicted brain/blood partition coefficient within the acceptable range –3.0 to 1.2 and showed no major activity in central nervous system. This indicates they are too polar to cross the blood-brain barrier.

3.4.3 Doxazosin and valsartan

Doxazosin and valsartan are antihypertensive drugs which showed good pharmacokinetic properties. Both of them have good oral absorption and distribution. But among valsartan and doxazosin, doxazosin showed better oral absorption than the reference drug (93.152% vs 89.533%). It showed good intestinal cells and renal cells permeability (above the 500 nm/s). It demonstrated good distribution properties. But both doxazosin and valsartan have a predicted brain/blood partition coefficient within the acceptable range which is -0.749. PSA result also within range (7.0 to 200) thus means both drugs are CNS inactive. They will not cross the blood brain barrier. Considering all of these, doxazosin was chosen as a potential drug candidate.

Chapter 4

Discussion

HER2 (human epidermal growth factor receptor 2) positive cancer is quite prevalent among women worldwide (Gina shaw, March 19,2023). Overexpression of HER2 receptor due to mutation is a common feature of HER2 positive breast cancer, leading to uncontrolled cell division and proliferation. Compared to normal cell, the HER2 receptors are amplified up to 40 – 100 folds in HER2-positive breast cancer cells (Furrer et al., 2018). HER2-HER3 heterodimer is the most potent stimulator of downstream signaling cascade, particularly the PI3K/Akt, which is the main regulator of breast cancer cell growth and survival.

This study explored different therapeutic classes of drugs and targeted the mutated HER2 protein by inhibiting extracellular domain of HER2 receptor and downward intracellular signaling cascade. To propose a potential drug against HER2 protein, different classes of drugs- anti-diabetic, statins and antihypertensive drugs were explored. The chosen reference drug was TAK-285 (EGFR/HER2 inhibitor). By analyzing the docking results, initially seventeen drug molecules, were chosen from 100 drug molecules based on their binding affinities (Table 1). After molecular docking, the chosen drugs with better binding affinity values were superimposed with the reference drug, TAK-285 (Figure 3, 4, 5, 6, 7 and 8). Then non-bonded protein-ligand interactions of selected drug candidates were visualized using Discovery Studio (Version 22.1) (Table 2). After that, the pharmacokinetic properties were evaluated using QikProp, Schrodinger (Table 3). Analyzing the docking results, superimposition, non-bonded protein-ligand interaction and ADME properties, initially six drugs were chosen. These include canagliflozin, dapagliflozin, doxazosin, fluvastatin, pitavastatin and valsartan. The binding affinity values of these selected candidates were higher compared to the other candidates. They demonstrated common amino acid interactions with TAK-285 (co-crystallized structure) when

bound to the HER2 receptor. Canagliflozin, doxazosin, fluvastatin and valsartan had three common amino acids and dapagliflozin, and pitavastatin had two common amino acids with TAK-285. They showed better oral absorption than the reference drug except dapagliflozin, fluvastatin and valsartan. The selected molecules also showed good permeability through the intestinal cells and renal cells but the permeabilities were less compared to the reference drug. However, all of them were in the recommended range of intestinal and renal cell permeability. And among the six drugs, none of them showed binding to serum albumin. This indicates the drugs have good distribution properties.

Based on the results obtained, doxazosin was chosen as a potential candidate for HER2 inhibition. Doxazosin demonstrated good binding affinity (-10.0 kcal/mol). It showed three common amino acids with TAK-285. The interaction results show that it formed hydrogen bonds and hydrophobic interactions (alkyl bonds, pi-alkyl bonds and pi-sigma bonds) with the HER2 receptor. Hydrogen bonds play an important role in determining the specificity of ligand binding with HER2 receptor (Wade & Goodford, 2016) and hydrophobic interactions helping to increase the affinity of the ligand bond with protein (Varma et al., 2010). Doxazosin also showed higher values for human oral absorption (93.1525%) than the reference drug. It showed good distribution properties (above than 500 nm/s). The CNS value of doxazosin is -1 and it has a predicted brain/blood partition coefficient within the acceptable range which is -0.749. The value indicates the drug is CNS inactive. Based on the QikProp results, doxazosin showed good pharmacokinetic properties and was selected as potential candidate to be further explored in the treatment of HER2 positive breast cancer.

Doxazosin, however has some minor side effects reported, such as dizziness, blurred vision, irregular heartbeat, shortness of breath, sleeping problem and numbness. No major side effects were observed for this selected drug candidate. Drug repurposing using *in silico* methods is

one of the powerful strategies to identify a potential candidate from our known existing drug database.

However, this study has some limitations. An *in silico* study using a wildtype HER2 could be performed and used as a control for better comparison. It would have been interesting to perform Molecular Dynamic Simulation (MDS), but this was beyond the scope of the project. This study further invokes future experiments with breast cancer cell lines, such as MCF-7. The study opens new avenues for research, expanding on the present findings to further elucidate doxazocin's role in BC using *in vitro* and *in vivo* models.

Chapter 5

Conclusion

HER2 inhibitors can inhibit the dimerization of the HER proteins that could lead to HER2 positive breast cancer. The study provides evidence that the drug, doxazosin could help with the inhibition of the HER2 protein. The chosen drug showed better binding affinity compared to the reference drug. It showed hydrogen bonds, and hydrophobic interactions include: alkyl bonds, pi-alkyl bonds and pi-sigma bonds with the HER2 receptor; that specify of ligand binding with receptor and increase the affinity of the ligand bond with protein. It demonstrated good human oral absorption, intestinal and renal cell permeability. This indicates the drug has good distribution properties. The CNS value suggested, doxazosin is a CNS inactive and it is too polar to cross the blood-brain barrier.

Given that, there is a continuous need to search for new drugs as the currently available ones pose the risk of becoming resistant over long term use. The next step would be to perform MD simulation to know how the ligand interacts with the protein. If successful, *in vitro* and *in vivo* evaluations could be performed to confirm the proposed action of the drug. Therefore, prospectively exploring doxazosin as a treatment may help overcome the crisis of drug resistance with specific drug therapies from our existing drug database.

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