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In Silico Drug Design and Virtual Screening of Novel Tamoxifen and Mitomycin Derivatives : ADMET Profiling, Molecular Docking, and Molecular Dynamics Simulations

Presented by:

Melle. ABDELLAOUI Ouijdane
Melle. BENKHALIFA Djoumana
Melle. DJOUGHI Yamna
Melle. OUAKOUAK Roummaissa

Jury Members:

President: Pr. CHOUIKH Atef	**MCA**	**El-Oued University**
Examiner: Pr. DJAHRA Ali Boutlelis	**MCA**	**El-Oued University**
Supervisor: Dr. LANEZ Elhafnaoui	**MAA**	**El-Oued University**

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شكر وتقدير

الحمد لله الذي بنعمته تتم الصالحات، وبفضله تتنزل الخيرات، والصلاة والسلام على خير خلق الله، محمد بن عبد الله، وعلى آله وصحبه أجمعين.

أتقدم بخالص عبارات الشكر والتقدير والامتنان

إلى أستاذنا الفاضل، المشرف الكريم، **الدكتور العانز الحفناوي،**

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.....

الملخص

يُعد سرطان الثدي أحد أبرز التحديات الصحية على مستوى العالم، خاصةً في أنواعه الفرعية التي تُظهر إيجابية لمستقبلات الهرمونات أو فرط تعبير بروتين HER2، ما يستدعي استراتيجيات علاجية موجهة وفعّالة. يُستخدم تاموكسيفين، كمعدِّل انتقائي لمستقبلات الإستروجين، بشكل واسع، إلا أن مقاومة الخلايا السرطانية تحدّ من فعاليته. في المقابل، أظهرت مادة الميتوميسين، كمضاد حيوي مضاد للأورام، نشاطًا واعدًا يجعلها محل اهتمام في تصميم أدوية جديدة.

تهدف هذه الدراسة إلى تصميم وتقييم 256 نظيرًا لكل من تاموكسيفين وميتوميسين باستخدام مقاربات حاسوبية متقدمة، تشمل تحليل الخصائص الصيدلانية-الدوائية (ADMET)، الإرساء الجزيئي، ومحاكاة الحركية الجزيئية (MD). أظهرت النتائج أن نظائر التاموكسيفين Tmx1 وTmx3 وTmx4 سجلت قيم LD_{50} تتراوح بين 1500 و1530 ملغ/كغ (التصنيف السمي: الفئة 4)، وكانت غير سامة للكبد (قيم hepatotoxicity بين -0.50 و-0.53)، وغير مناعية (immunotoxicity حتى -0.99)، مع احتمالية منخفضة للطفرة أو السرطان. كما أظهرت خلال المحاكاة الجزيئية RMSD أقل من 2.5 أنغستروم، مما يدل على استقرارها البنيوي. من جهة أخرى، تميز المركب Mitomycin6 بـ LD_{50} = 3000 ملغ/كغ (الفئة 5)، وقيم سمية منخفضة جداً (hepatotoxicity = -0.51، immunotoxicity = -0.99)، وفعالية عالية واستقرار قوي أثناء المحاكاة. بناءً على هذه المعطيات، يمكن اعتبار المركبات Tmx1 وTmx3 وTmx4 وMitomycin6 مرشحة واعدة كعلاجات موجهة من الجيل الجديد ضد سرطان الثدي، نظراً لتوازنها بين الفعالية والسلامة الدوائية.

الكلمات المفتاحية :سرطان الثدي، نظائر التاموكسيفين، Mitomycin6، HER2، ER-α، الارتساء الجزيئي، الحركية الجزيئية، تصميم الأدوية، ADMET، العلاج الموجه.

RÉSUMÉ

Le cancer du sein constitue l'un des défis majeurs en matière de santé publique à l'échelle mondiale, notamment dans ses sous-types caractérisés par la positivité des récepteurs hormonaux ou la surexpression de la protéine HER2, ce qui nécessite des stratégies thérapeutiques ciblées et efficaces. Le tamoxifène, un modulateur sélectif des récepteurs aux œstrogènes, est largement utilisé, mais l'apparition de résistances cellulaires en limite l'efficacité. En revanche, la mitomycine, un antibiotique antitumoral interférant avec l'ADN, a démontré une activité prometteuse, la rendant intéressante pour le développement de nouveaux médicaments.

Cette étude vise à concevoir et évaluer 256 analogues de chaque molécule (tamoxifène et mitomycine) à l'aide d'approches informatiques avancées, incluant l'analyse des propriétés pharmacocinétiques et toxicologiques (ADMET), le docking moléculaire, et la dynamique moléculaire (MD). Les résultats ont montré que les analogues du tamoxifène Tmx1, Tmx3 et Tmx4 présentent des valeurs de DL_{50} comprises entre 1500 et 1530 mg/kg (classe de toxicité 4), sont non hépatotoxiques (valeurs entre -0.50 et -0.53), non immunotoxiques (jusqu'à -0.99), et avec un faible potentiel mutagène ou cancérigène. Lors des simulations MD, ces composés ont montré des valeurs de RMSD inférieures à 2.5 Å, indiquant une grande stabilité structurale.

Par ailleurs, l'analogue Mitomycin6 s'est distingué avec une DL_{50} = 3000 mg/kg (classe 5), une hépatotoxicité faible (-0.51) et une immunotoxicité quasi nulle (-0.99), ainsi qu'une forte activité biologique et stabilité lors des simulations.

Ces résultats suggèrent que Tmx1, Tmx3, Tmx4 et Mitomycin6 représentent des candidats prometteurs en tant que thérapies ciblées de nouvelle génération contre le cancer du sein, alliant efficacité thérapeutique et profil de sécurité favorable.

Mots clés: *Cancer du sein, analogues du Tamoxifène, Mitomycin6, HER2, ER-α, docking moléculaire, dynamique moléculaire, conception de médicaments, ADMET, thérapie ciblée*.

Abstract

Breast cancer is one of the most significant global public health challenges, particularly in its subtypes characterized by hormone receptor positivity or HER2 protein overexpression, which require effective and targeted therapeutic strategies. Tamoxifen, a selective estrogen receptor modulator, is widely used; however, the development of cancer cell resistance often limits its efficacy. In contrast, Mitomycin, a DNA-interfering antitumor antibiotic, has shown promising activity, making it a molecule of interest for novel drug development.

This study aims to design and evaluate 256 analogues for both Tamoxifen and Mitomycin using advanced computational approaches, including pharmacokinetic and toxicological profiling (ADMET), molecular docking, and molecular dynamics (MD) simulations. Results revealed that Tamoxifen analogues Tmx1, Tmx3, and Tmx4 demonstrated LD_{50} values ranging from 1500 to 1530 mg/kg (toxicity class 4), were non-hepatotoxic (hepatotoxicity values between -0.50 and -0.53), and non-immunotoxic (up to -0.99), with low mutagenic and carcinogenic potential. During MD simulations, these compounds showed RMSD values below 2.5 Å, indicating high structural stability.

On the other hand, the Mitomycin6 compound stood out with an LD_{50} of 3000 mg/kg (toxicity class 5), very low hepatotoxicity (-0.51), negligible immunotoxicity (-0.99), strong biological activity, and excellent structural stability during simulations.

Based on these findings, Tmx1, Tmx3, Tmx4, and Mitomycin6 are considered promising next-generation targeted therapy candidates for breast cancer, offering a balanced profile of efficacy and drug safety.

Keywords: *Breast cancer, Tamoxifen analogues, Mitomycin6, HER2, ER-α, molecular docking, molecular dynamics, drug design, ADMET, targeted therapy*.

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Introduction

Introduction

Breast cancer remains one of the most prevalent malignancies among women worldwide, representing a major cause of cancer-related morbidity and mortality Its molecular subtypes are classified based on the expression of hormone receptors (ER, PR) and overexpression of the Human Epidermal Growth Factor Receptor 2 (HER2), which directly influences tumor behavior and response to therapy. Despite continuous advances in diagnostic and therapeutic strategies, certain subtypes—particularly hormone receptor-positive and HER2-overexpressing breast cancers—continue to pose significant clinical challenges due to recurrence, therapeutic resistance, and limited efficacy of conventional treatments[1] .

In this context, molecularly targeted therapies have emerged as promising strategies aimed at improving treatment precision and reducing toxicity compared to non- selective chemotherapeutics. Among the key molecular targets in breast cancer are Estrogen Receptor alpha (ER-α) and HER2. ER-α is involved in regulating gene expression related to cell proliferation and survival in hormone-responsive tumors, while HER2, a member of the ErbB receptor family, promotes cell growth and division, with its overexpression being linked to aggressive tumor phenotypes and poor prognosis[2].

Tamoxifen, a selective estrogen receptor modulator (SERM), has long been a first- line treatment for ER-positive breast cancer. However, its partial efficacy and the development of acquired resistance have underscored the need for novel analogues with enhanced binding affinity, improved pharmacokinetics, and reduced toxicity Accordingly, this study was designed to develop and evaluate a library of Tamoxifen analogues through advanced in silico approaches, including pharmacokinetic screening, molecular docking, and molecular dynamics simulations, with the goal of identifying promising candidates for targeting both ER-α and HER2 receptors.

In parallel, Mitomycin, a DNA cross-linking antitumor agent derived from Streptomyces caespitosus, has also shown potential in the treatment of various solid tumors, including breast cancer. Its mechanism of action involves the formation of covalent cross-links within DNA, thereby inhibiting replication and transcription. ultimately leading to apoptosis[3] .Despite its proven cytotoxic effects, the clinical use of mitomycin is limited by systemic toxicity .Recent efforts have focused on modifying its chemical structure or incorporating it into smart nanocarrier systems to enhance tumor-specific delivery and minimize side effects. Moreover, molecular modeling techniques are being employed to elucidate its interactions with cellular targets, which can aid in the design of more selective and potent analogues Based on these considerations, the present study explores the potential synergy between newly designed

Tamoxifen analogues and chemotherapeutic agents such as Mitomycin as part of a multi-targeted therapeutic strategy. This integrated approach holds promise for overcoming common limitations related to drug resistance and toxicity, and aligns with the growing shift toward personalized and precision medicine in the treatment of breast cancer

First part

Bibliographic synthesis

Chapter I :Definition of the disease

1.Breast Cancer:

is one of the most common types of cancer worldwide, characterized by the abnormal growth of cells in the breast.[4] It is a leading cause of cancer-related deaths, but early detection and effective treatment can significantly increase survival rates. The disease typically starts with abnormal changes in the cells of the breast, either in the milk ducts or the milk-producing glands. In the early stages, symptoms may not be noticeable, but as the disease progresses, a lump or changes in the shape of the breast may appear. If left untreated, breast cancer can spread to nearby tissues or other parts of the body via the lymph nodes. There are several types of breast cancer, with ductal carcinoma and lobular carcinoma being the most common.[5] Factors contributing to the development of breast cancer include genetic predisposition, such as mutations in the **BRCA1** and **BRCA2** genes, a family history of the disease, hormonal changes, and lifestyle factors such as obesity and lack of physical activity.[6] Regular self-examination and routine screenings like **mammograms** are essential for early detection, which significantly improves treatment outcomes. Treatment for breast cancer varies depending on the type and stage, and may include surgery, chemotherapy, radiation therapy, and hormone or immunotherapy. Lifestyle changes, including maintaining a healthy weight, regular physical activity, and avoiding smoking, can help reduce the risk of developing the disease.

2.Symptoms of Breast Cancer:

Breast cancer symptoms can vary, and individuals may experience a range of signs that should not be ignored. One of the most common symptoms is the presence of a lump in the breast or underarm, which can feel hard or have irregular edges. Changes in the size, shape, or contour of the breast are also indicators, with the skin sometimes appearing dimpled or puckered. Additionally, the skin on the breast may become red, swollen, or warm to the touch. Unexplained pain or discomfort in the breast, especially if it spreads to areas like the underarm or shoulder, can also be a warning sign. Some individuals may notice unusual discharge from the nipple, particularly if it’s clear, bloody, or happens without squeezing. Swelling in the lymph nodes under the arm or near the collarbone could indicate that the cancer has spread. Changes in the appearance of the nipple, such as inversion or changes in the skin around it, may also signal the presence of breast cancer. If any of these symptoms are observed, it is crucial to seek medical attention for proper evaluation and early detection, which significantly improves the chances of successful treatment.[7]

3.Causes of breast cancer:

The primary cause of breast cancer is the abnormal growth of breast cells, often triggered by genetic mutations that cause these cells to divide more rapidly than normal and form a mass or tumor. While the exact cause is multifactorial and not entirely understood, several risk factors contribute to the development and progression of the disease. These include inherited genetic mutations such as BRCA1 and BRCA2, advancing age, and a family history of breast cancer. Hormonal factors, like prolonged exposure to estrogen or the use of hormone replacement therapy after menopause, can also increase risk. Additionally, lifestyle factors such as obesity, physical inactivity, alcohol consumption, and an unhealthy diet may play a significant role. Environmental exposures and reproductive history may further influence an individual's susceptibility to breast cancer.[8]

Genetic predisposition: Some women may be genetically predisposed to breast cancer, making them more susceptible to it even with a healthy lifestyle.[9]

Hormonal changes: Hormonal fluctuations during puberty, pregnancy, menstruation, and menopause can affect breast health and increase the likelihood of developing breast cancer.[10]

Diabetes: Uncontrolled diabetes can impair the body's ability to fight infections and may be associated with an increased risk of breast cancer.[11]

Medications: Certain medications, such as anticonvulsants, immunosuppressants, and some calcium channel blockers, can affect breast tissue and increase the risk of breast cancer.[12]

Poor nutrition: A diet high in sugar and low in nutrients can weaken the immune system, contributing to abnormal changes in breast tissue and increasing the risk of breast cancer.[13]

Chronic stress: Chronic stress weakens the body's immune response and may exacerbate inflammation or trigger abnormal changes in breast tissue, thereby increasing the risk of breast cancer.[14]

4. Treatment of Breast Cancer:

Breast cancer is a serious condition, but it is treatable and, in many cases, manageable, entirely curable, advancements in treatment options have significantly improved survival rates and the quality of life for patients.[15] Treatment for breast cancer typically involves a combination of therapies aimed at controlling the disease, reducing tumor size, and preventing recurrence.Treatment for breast cancer often includes a multi-faceted approach that may involve surgery, chemotherapy, radiation therapy, hormone therapy, and targeted therapies. In addition to these, various medications may be prescribed to manage symptoms, reduce the risk of cancer recurrence, and improve overall outcomes. Here's a more detailed overview of drugs commonly used for treating breast cancer.

4.1. Chemotherapy Drugs

4.1.1. Doxorubicin: Doxorubicin is a commonly used chemotherapy drug for the treatment of breast cancer. It works by inhibiting DNA replication in cancer cells, preventing their growth and division. It is effective against a wide range of breast cancer subtypes and is often used in combination with other chemotherapeutic agents for better efficacy.[16]

4.1.2. Cyclophosphamide: Cyclophosphamide is frequently used as part of combination chemotherapy regimens for breast cancer. It is an alkylating agent that interferes with cancer cell DNA, and its broad-spectrum action is effective against both primary and metastatic breast cancer.[17]

4.1.3. Paclitaxel: Paclitaxel is a taxane chemotherapy drug that disrupts the microtubule structures in cancer cells, preventing them from dividing and growing. It is often used in combination with other drugs like doxorubicin or cyclophosphamide, especially for aggressive forms of breast cancer.[18]

4.1.4. Trastuzumab: Trastuzumab (Herceptin) is an effective monoclonal antibody used for HER2-positive breast cancers. It targets and inhibits the HER2 protein, which promotes cancer cell growth. This drug is used in both early and metastatic breast cancer treatments and may be used in conjunction with chemotherapy agents.[19]

4.2. Targeted Therapy

4.2.1. Tamoxifen: Tamoxifen is a selective estrogen receptor modulator (SERM) that is commonly prescribed for hormone receptor-positive breast cancers. By blocking estrogen from binding to cancer cells, it helps prevent tumor growth. It is frequently used as both an adjuvant therapy after surgery and in metastatic breast cancer.[20]

4.2.2. Aromatase inhibitors (e.g., Letrozole, Anastrozole): These drugs work by inhibiting aromatase, an enzyme responsible for producing estrogen. They are particularly effective in postmenopausal women with hormone receptor-positive breast cancer and are used as an alternative to tamoxifen for long-term treatment.[21]

4.2.3 Mitomycin: works by becoming activated inside the cell, where it acts as a DNA cross-linking agent. It forms covalent bonds between DNA strands, preventing them from separating during replication and transcription. This blocks cell division and triggers apoptosis (programmed cell death), especially in rapidly dividing cancer cells.[22]

4.3. Localized Treatment Options

4.3.1. Radiation Therapy: Radiation therapy is often used in the treatment of breast cancer after surgery to eliminate any remaining cancer cells in the breast or surrounding tissues. It uses high-energy rays to target localized cancer cells and reduce the risk of recurrence.[23]

4.3.2. Surgery: Surgical options for breast cancer include lumpectomy (removal of the tumor) or mastectomy (removal of the entire breast), depending on the stage and location of the cancer. Surgical intervention is often combined with other therapies like chemotherapy or radiation for more comprehensive treatmen. [23].

Chapter II :Definition of the medication

1.Definition of the two drugs:

1-1 Mitomycin C (MMC

Mitomycin C (MMC) is an antitumor antibiotic derived from Streptomyces caespitosus. It acts as an alkylating agent that binds to DNA, leading to the inhibition of cancer cell proliferation. [24]It is particularly effective under low-oxygen conditions and is considered one of the first agents with a bioreductive alkylation mechanism.[25]

1-2 Definition Tamoxifen:

Tamoxifen is a triphenylethylene-type anti-estrogen drug with partial estrogen agonist activity in certain species. It has been used since the late 1960s for the treatment and prevention of breast cancer, particularly in hormone receptor-positive types.[26] Tamoxifen works by binding to estrogen receptors in breast cells, thereby preventing estrogen from exerting its effects on these cells and reducing the growth of cancer cells.Tamoxifen is considered one of the pioneering drugs in the treatment of breast cancer and has been used as a first-line therapy for many years. Although it was the primary treatment for breast cancer for over 30 years, its use has gradually declined in favor of newer drugs such as aromatase inhibitors and selective estrogen receptor degraders (SERDs)[27]. Nevertheless, tamoxifen remains on the World Health Organization's list of essential medicines and continues to be an important option in the treatment of breast cancer, especially in cases where other drugs cannot be used or when the efficacy of newer treatments is limited.[28]

2-The uses of Mitomycin C **and Tamoxifen:**

2-1 The uses of Mitomycin C

1. Cancer Treatment (Chemotherapy): Mitomycin C is used as a chemotherapeutic agent in various cancers such as gastric, pancreatic, breast, lung (non-small cell), cervical, bladder, and prostate cancers.[29]

2. In Ophthalmic Surgery: Mitomycin C is applied in pterygium surgery, treatment of ocular surface neoplasia, and refractive surgeries (like LASIK and PRK) to reduce scarring. [30]

3. In ENT Surgery (Ear, Nose, and Throat): Used topically after sinus or vocal cord surgeries to minimize fibrosis and adhesion formation.[29]

4. In Urological Surgery: Administered intravesically for the treatment of superficial bladder cancer[31]

2-2 The uses of Tamoxifen:

1-Tamoxifen reduces the risk of contralateral breast cancer in women with BRCA1 or BRCA2 gene mutations, independent of the effect of oophorectomy.[32]

2-Tamoxifen is used at a dose of 5 mg daily for 3 years to reduce the recurrence of breast intraepithelial neoplasia by half with limited toxicity, providing a new treatment option for these disorders.[33]

3-Breast Cancer Treatment: It is used as an anti-estrogen treatment for estrogen-dependent cancers.[34]

4- Reduction of Breast Cancer Cell Growth: It helps reduce the growth of cancer cells in an estrogen-free environment.[34]

5-Resistance to Estrogenic Therapy: It counteracts the effects of estrogen in cases of cancer resistant to estrogen-based treatment[34]

3-Mechanism of Action of Mitomycin C **and Tamoxifen:**

3-1 Mechanism of Action of Mitomycin C:

Mitomycin C exhibits a multifaceted mechanism of action involving both direct effects on DNA and regulatory effects within the cell.[31] It acts as an alkylating agent, forming covalent cross-links between DNA strands, which inhibits helicase activity, thereby blocking DNA replication and transcription. This leads to cell cycle arrest and ultimately cell death. Additionally, Mitomycin C disrupts the arginase/nitric oxide synthase (Arginase/NO-synthase) system in lymphocytes by increasing arginase activity and inhibiting both the constitutive (cNOS) and inducible (iNOS) forms of nitric oxide synthase. This results in a reduction of nitric oxide (NO) production, limiting the formation of peroxynitrite ($ONOO^-$)—a toxic compound generated by the reaction of NO with superoxide radicals—thereby reducing oxidative stress and preventing cellular damage.[35]

3-2 Mechanism of Action of Tamoxifen:

Tamoxifen remains one of the most widely used selective estrogen receptor modulators (SERM) in clinical practice, both in pre- and postmenopausal women diagnosed with hormone receptor-positive (HR+) breast cancer[36]. Its therapeutic effect lies in its ability to competitively bind to estrogen receptors, leading to the disruption of the activation of estrogen-responsive genes, thereby inhibiting the growth of estrogen-dependent cancer cells.[37] This effect makes tamoxifen particularly effective in reducing the recurrence of hormone-dependent breast cancer. Despite the proven benefits of adjuvant tamoxifen treatment for at least five

years, which have contributed to improved survival rates and reduced relapse rates, the emergence of treatment resistance—whether acquired after a period of use or primary (de novo) resistance at the onset of treatment—remains a major obstacle limiting its clinical efficacy in many patients.[38] The mechanisms behind tamoxifen resistance are complex and multifaceted, primarily involving the abnormal activation of the estrogen receptor alpha (ERα) signaling pathway, which is the central target of hormonal therapy[39]. Additionally, the crosstalk between ERα signaling and growth factor receptor signaling pathways, such as the epidermal growth factor receptor (EGFR), HER2, and insulin-like growth factor 1 receptor (IGF1R), significantly contributes to the development of this resistance. Increased expression or mutations in these pathways can lead to the activation of alternative signaling pathways that bypass the inhibitory effects of tamoxifen[40][41]. Many studies have focused on growth factor receptor signaling mechanisms as some of the most closely associated pathways with tamoxifen resistance[42]. However, increasing scientific evidence in recent years highlights the role of transforming growth factor beta (TGF-β) signaling as a potential key player in the development of tamoxifen resistance.[43] Studies indicate that the TGF-β pathway not only regulates cell growth and differentiation but also enhances the characteristics of breast cancer stem cells (BCSCs), which are believed to contribute to the initiation and progression of cancer. [44] Furthermore, TGF-β interacts with estrogen receptors, which may contribute to enhancing cellular resistance to hormonal therapy.[45] Additionally, TGF-β's ability to alter the tumor microenvironment and its role in epithelial-mesenchymal transition (EMT) further complicates the development of resistance.[46] This review aims to analyze the central role of the TGF-β pathway in tamoxifen resistance mechanisms, focusing on the complex interactions between this pathway and estrogen receptors on one hand, [47]and breast cancer stem cells on the other We also discuss recent therapeutic strategies targeting TGF-β signaling as a promising approach to overcoming resistance challenges and improving treatment outcomes for HR+ breast cancer patients. Targeting TGF-β may help increase tumor sensitivity to tamoxifen and other hormonal therapies, offering new hope for overcoming resistance and improving patient outcomes.[48]

4-Side Effects of Mitomycin C and Tamoxifen:

4.1 Side Effects of Mitomycin C:

Mitomycin C is a potent antitumor agent; however, its clinical use is associated with several adverse effects that must be carefully considered. These side effects are categorized into common and rare but serious reactions :[49]

Hematologic Effects:

Thrombocytopenia: Increases the risk of bleeding due to a reduction in platelet count. Leucocytopenia: Leads to immunosuppression and a higher susceptibility to infections. [50]

Severe but Rare Effects:

Hemolytic Uremic Syndrome (HUS): A serious condition involving red blood cell destruction, renal failure, and thrombocytopenia.[51]

Pneumonitis: Inflammation of lung tissue, which may cause shortness of breath and pulmonary fibrosis.

Cardiac Failure: Rare instances of cardiac muscle damage have been documented.[52]

Other Systemic Effects:

Myelosuppression: Suppression of bone marrow function, resulting in decreased production of blood cells. [53]

Renal Toxicity: May manifest as impaired kidney function.

Local Toxicity: Extravasation of the drug can lead to severe tissue necrosis at the injection site.[54]

4.2 The side effects of Tamoxifen:

Tamoxifen is a selective estrogen receptor modulator (SERM).

, helping to combat breast cancer.[55]

Has estrogen-like effects on:

- The endometrium
- The skeletal system
- The coagulation system
- Lipid metabolism[56]

- **Common side effects**:
  - Hot flashes
  - Vaginal symptoms
  - Vaginal discharge
  - Irregular menstruation[57][58]
- **Serious but rare side effects**:
  - Venous thromboembolic events
  - Endometrial cancer (only in postmenopausal women)[59][60]
- An increased proportion of women experienced a noticeable increase in the severity of vasomotor and musculoskeletal symptoms.[61]

Administering tamoxifen with other chemotherapeutic agents, such as cytotoxic drugs, enhances the response rate but also increases the occurrence of adverse reactions.[24].

Second part:

Experimental part

Chapter I:

Materials & methods

1. Generation of Combinatorial Library

To expand the chemical space of potential analogues of tamoxifen and mitomycin, a virtual screening strategy was implemented using the SmiLib v2.0 software[62] . Using the parent drug structures as core scaffolds, a diverse range of substituents including hydroxyl (OH), carboxylic acid (COOH), nitro (NO_2), and amino (NH_2) groups—were systematically introduced, alongside vacant linker sites, to construct a comprehensive combinatorial library. This approach yielded a total of 256 structurally distinct analogues of tamoxifen and mitomycin.

To identify candidates with promising pharmacokinetic and toxicity profiles, the generated analogues were subsequently evaluated using in silico predictive tools such as SwissADME [63] and ProTox II [64]. These platforms enabled the assessment of critical drug-lie properties, including aqueous solubility, blood-brain barrier (BBB) permeability, cytochrome P450 (CYP) interactions, and gastrointestinal absorption

The generation of the tamoxifen and mitomycin analogue library for virtual screening and subsequent molecular docking studies was carried out according to the following workflow:

1.1. Scaffold Selection

The molecular scaffolds of tamoxifen and mitomycin were selected as the core frameworks to initiate the design of structural analogues .

1.2. Identification of Substituents

A series of chemically diverse substituents, including functional groups such as **carboxyl (COOH), amino (NH_2), hydroxyl (OH), and nitro (NO_2)**, were identified for potential attachment to the core scaffolds. These building blocks were chosen to enhance structural diversity and explore structure–activity relationships.

1.3. Combinatorial Enumeration

Using SmiLib v2.0 software[65], all feasible combinations between the chosen scaffolds and the defined substituents were computationally enumerated. This step enabled the generation of a large and diverse set of unique molecular entities by systematically combining each functional group with the tamoxifen and mitomycin backbones.

2. Filtering and Validation

The resulting molecular library was filtered through multiple criteria to ensure drug-likeness, synthetic accessibility, and chemical diversity. Compounds exhibiting undesirable physicochemical properties or violating fundamental drug design rules were excluded from further analysis.

3. Prediction of Pharmacokinetics and Toxicity

To assess the pharmacokinetic behavior and toxicity risk of the generated analogues, computational models such as SwissADME [66] and ProTox II [64] were employed. SwissADME facilitated the prediction of key parameters including aqueous solubility, blood-brain barrier permeability, cytochrome P450 (CYP) isoform inhibition, and intestinal absorption. Concurrently, ProTox II was used to estimate the median lethal dose (LD_{50}), a key toxicity index for preliminary safety profiling.

3.1 Final Selection

A refined subset of analogues was selected based on favorable pharmacokinetic predictions, low predicted toxicity, and representative structural diversity. These candidates were retained for subsequent virtual screening and molecular docking investigations to identify potential lead compounds with enhanced biological activity.

3.2 Database Compilation

The selected tamoxifen and mitomycin analogues were compiled into a structured database containing 2D chemical representations, identifiers, and predicted pharmacokinetic/toxicological properties. This database served as the foundation for the downstream stages of the virtual screening workflow.

3.3 Pharmacokinetics and Toxicity-Based Screening

Pharmacokinetic profiling of the generated tamoxifen and mitomycin analogues was conducted using SwissADME[66], developed by the Swiss Institute of Bioinformatics. This analysis aimed to identify CYP enzyme inhibitors and evaluate parameters such as gastrointestinal absorption, P-glycoprotein interaction, and BBB penetration. In parallel, ProTox II [64] was used to estimate the LD_{50} values for tamoxifen, mitomycin, and all synthesized analogues. This predictive toxicological assessment was critical for selecting analogues with minimal health risks, with a focus on LD_{50} as a decisive selection criterion.

4. Structural Optimization

At the outset of this study, structural refinement of the generated tamoxifen and mitomycin analogues was performed using molecular mechanics methods. This initial step employed computational algorithms to optimize the spatial arrangement of atoms, enabling the identification of low-energy conformations and structurally stable geometries.

Subsequently, a more rigorous geometry re-optimization was carried out using Density Functional Theory (DFT), a quantum chemical approach widely adopted for accurate

molecular modeling. In this context, the B3LYP hybrid functional was applied in combination with the 6-311++G(d,p) basis set, which offers an extended representation of electron density and polarization effects. All DFT calculations were performed using the Gaussian 16W software suite, a robust platform for high-level quantum chemical computations. This tool facilitated precise energy minimization and property evaluation of the optimized analogues.

To ensure the validity and comparative relevance of the computational results, the experimentally derived 3D structures of tamoxifen and mitomycin were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) [67]. These reference molecules provided a benchmark for assessing the geometric and electronic properties of the designed analogues.

By integrating molecular mechanics and quantum-level optimization with validated structural data, this computational approach allowed for a detailed analysis of the conformational stability, electronic distribution, and potential reactivity of tamoxifen and mitomycin analogues. These insights are essential for determining their suitability as candidate compounds for future biological evaluations and potential pharmacological or therapeutic applications.

5. Protein Selection

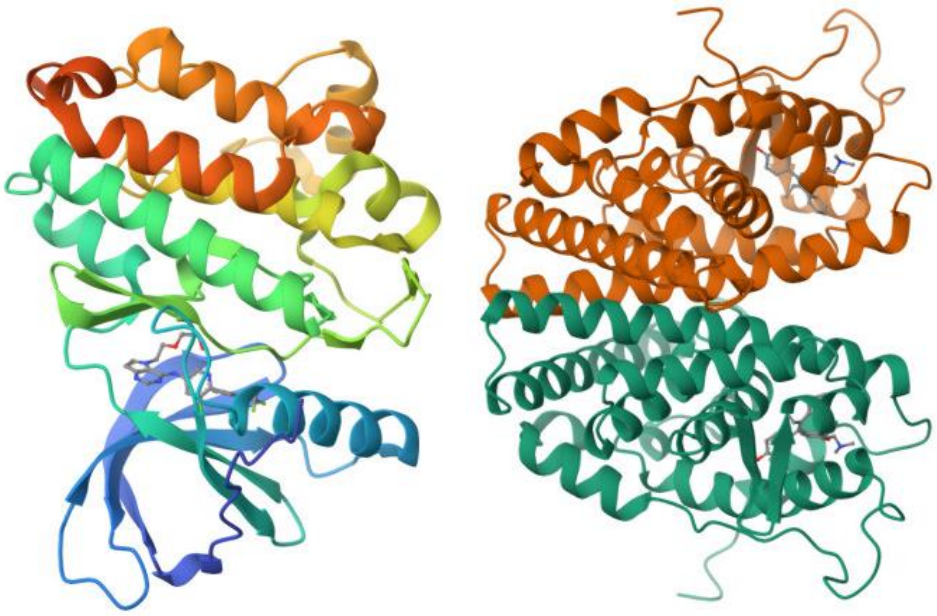
For this study, we selected two key protein targets: human epidermal growth factor receptor 2 (HER2) and estrogen receptor alpha (ER-α), given their critical involvement in breast cancer pathophysiology and their relevance to the mechanisms of action of tamoxifen and mitomycin analogues.

HER2 (PDB ID: 3PP0) is a transmembrane tyrosine kinase receptor that plays a pivotal role in the regulation of cell growth and differentiation. Its overexpression is often associated with aggressive tumor progression and poor prognosis in breast cancer. Estrogen receptor alpha (ER-α), retrieved using PDB ID: 3ERT, is a nuclear hormone receptor that mediates the biological effects of estrogens. It is a well-established therapeutic target in hormone-responsive breast cancers and is directly modulated by tamoxifen and related compounds.

The selection of these proteins was driven by their clinical relevance and their involvement in signaling pathways frequently dysregulated in cancer. The high-resolution crystal structures of HER2 and ER-α were obtained from the Protein Data Bank and are illustrated in **Figure 1**, ensuring accuracy and suitability for downstream molecular docking studies.

By focusing on HER2 and ER-α as target proteins, we aim to explore the potential binding interactions and inhibitory mechanisms of the designed tamoxifen and mitomycin analogues. The detailed structural data provided by these PDB entries will support reliable docking simulations,

enabling the prediction of binding affinities and interaction modes of the candidate molecules with their respective receptors.



HER2 **ER-alpha**

Figure 1. Three-dimensional structures of human epidermal growth factor receptor 2 (HER2, PDB ID: 3PP0) and estrogen receptor alpha (ER-α, PDB ID: 3ERT), selected as molecular targets for docking studies with tamoxifen and mitomycin analogues.

6. Steps of Molecular Docking

Molecular docking simulations were conducted using the Schrödinger Maestro software suite [68], employing a systematic workflow to predict the binding affinities and modes of tamoxifen and mitomycin analogues with the selected target proteins. The docking procedure consisted of the following key steps:

Step 1: Protein Structure Preparation

The crystallographic 3D structures of HER2 (PDB ID: 3PP0) and estrogen receptor alpha (ER-α, PDB ID: 3ERT) were imported into the Maestro workspace. Protein structures were prepared using the Protein Preparation Wizard at pH 7.0. During this process, water molecules and non-essential ligands were removed, hydrogen atoms were added, protonation states were adjusted, and bond orders were assigned.

Step 2: Protein Grid Generation

The receptor grid was defined using the Receptor Grid Generation tool. Active sites were

specified based on the co-crystallized ligand positions. The center of the grid box was set along the x, y, z coordinates at 13.21, 35.12, 35.90 for HER2 and 22.15, 26.84, 5.36 for ER-α, with a grid box dimension of 10×10×10 Å for both receptors.

Step 3: Ligand Preparation

Tamoxifen and mitomycin analogues were prepared using LigPrep, which included:

Generation of multiple 3D conformers to represent different spatial arrangements.

Geometry optimization to improve structural compatibility.

Adjustment of ionization states based on physiological pH.

Energy minimization to reduce steric clashes and obtain the most favorable conformers.

Step 4: Molecular Docking Simulation

The prepared ligands were flexibly docked into the receptor active sites using Glide SP (Standard Precision) mode. This was followed by XP (Extra Precision) docking to enhance the accuracy of ligand-receptor binding predictions. Glide scores were used to rank the docked poses.

Step 5: Pose Refinement and Scoring

Post-docking refinement was performed using Prime MM-GBSA to calculate the binding free energies and to further evaluate the interactions. Refined poses were scored based on binding affinities, hydrogen bonding, π–π stacking, and hydrophobic interactions.

Step 6: Analysis and Visualization

The best-ranked ligand-protein complexes were analyzed using the Ligand Interaction Diagram tool to identify critical residues involved in binding. The visualization facilitated understanding of the binding mode and interaction stability.

Step 7: Validation and Optimization

Docking reliability was assessed by re-docking the co-crystallized ligands into the respective binding sites to confirm the docking protocol's accuracy. Grid and scoring parameters were optimized accordingly.

Step 8: Reporting and Interpretation

A comprehensive report was compiled, including docking scores, interaction diagrams, and structural insights for both HER2 and ER-α complexes with tamoxifen and mitomycin analogues. These results provide a foundation for subsequent in vitro validations and structure-based optimization of lead candidates (Figure 2).

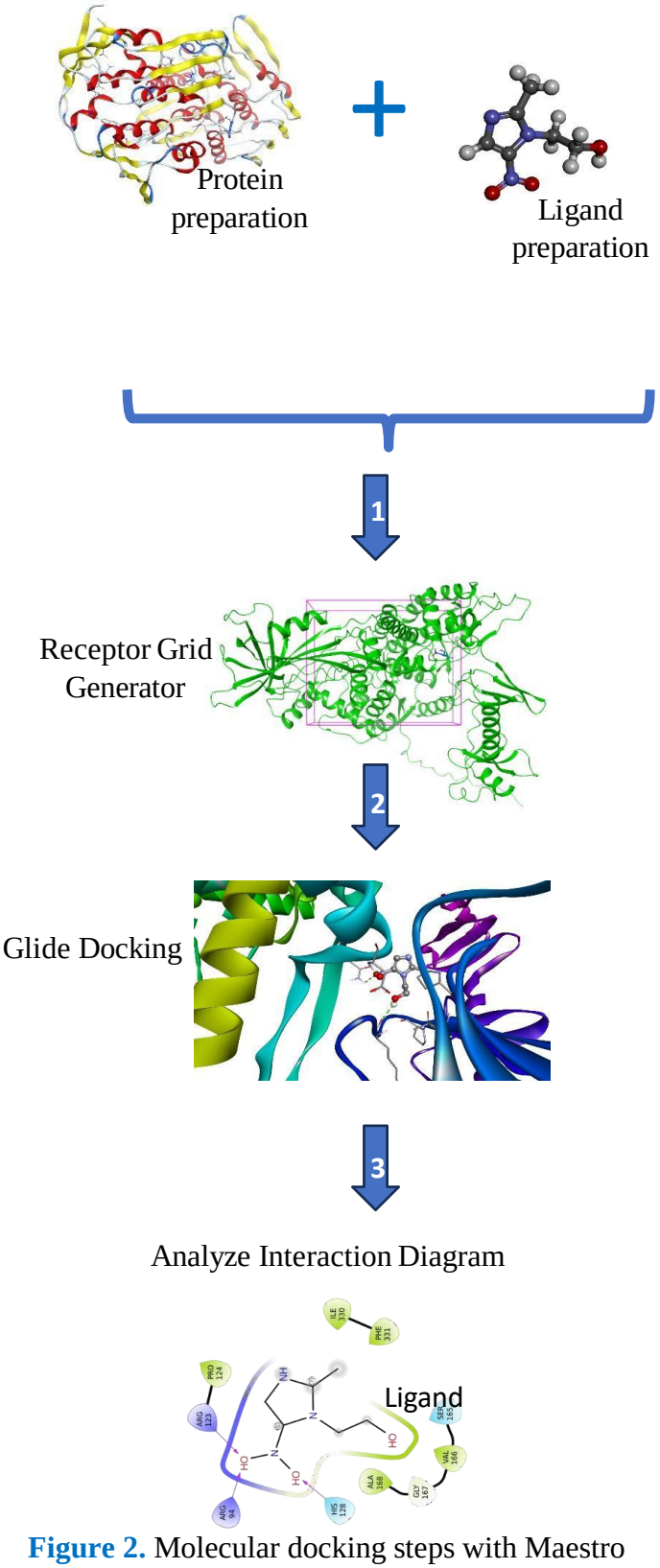


Figure 2. Molecular docking steps with Maestro

7. Steps of Molecular Dynamics Simulation:

To evaluate the dynamic behavior and stability of protein-ligand complexes under physiological conditions, Molecular Dynamics (MD) simulations were performed using the Desmond module integrated into Schrödinger Maestro. The simulations were run for 100 nanoseconds to analyze the conformational stability of tamoxifen and mitomycin bound to HER2 (3PP0) and ER-α (3ERT) targets. Key metrics such as Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and radius of gyration were monitored throughout the trajectories to assess stability and compactness of the complexes. The simulation process included the following steps, as illustrated in Figure 2.

Step 1: Complex Structure Preparation

Docked protein-ligand complexes from Glide (HER2-tamoxifen, HER2-mitomycin, ER-α-tamoxifen, and ER-α-mitomycin) were imported into the Maestro workspace and prepared using the Protein Preparation Wizard. The simulation pH was set to 7.0, and crystallographic water molecules were removed to avoid interference during solvation.

Step 2: Solvation and Neutralization

The prepared complex was solvated using the System Builder tool with the following conditions:

- Solvent Model: TIP3P water
- Box Type: Orthorhombic box with a 10 Å buffer region
- Force Field: OPLS4
- Ion Addition: Neutralization was achieved using Na^+ and Cl^- ions, with a final salt concentration of 0.15 M
- Ionization State: Adjusted to reflect physiological pH
- Energy Minimization: Performed to relieve steric clashes
- Equilibration: Conducted to stabilize solvent-protein-ligand interactions

The solvated and neutralized system is shown in Figure 2.

Step 3: Molecular Dynamics Simulation

Following equilibration, MD simulations were conducted under the following conditions:

- Simulation Duration: 100 nanoseconds
- Temperature: 300 K
- Pressure: 1 bar
- Recording Interval: 100 ps (total of ~1000 frames)

- Integrator: RESPA with default time steps
- Ensemble: NPT (constant Number of particles, Pressure, and Temperature)

The simulations were executed using the Desmond Molecular Dynamics Workflow. Default parameters were maintained unless otherwise specified.

Step 4: Simulation Interaction Analysis

Post-simulation analysis was conducted using the Simulation Interaction Diagram tool to extract detailed interaction profiles. Parameters including hydrogen bonding, hydrophobic contacts, salt bridges, and π–π stacking were visualized and quantified for both HER2 and ER-α complexes.

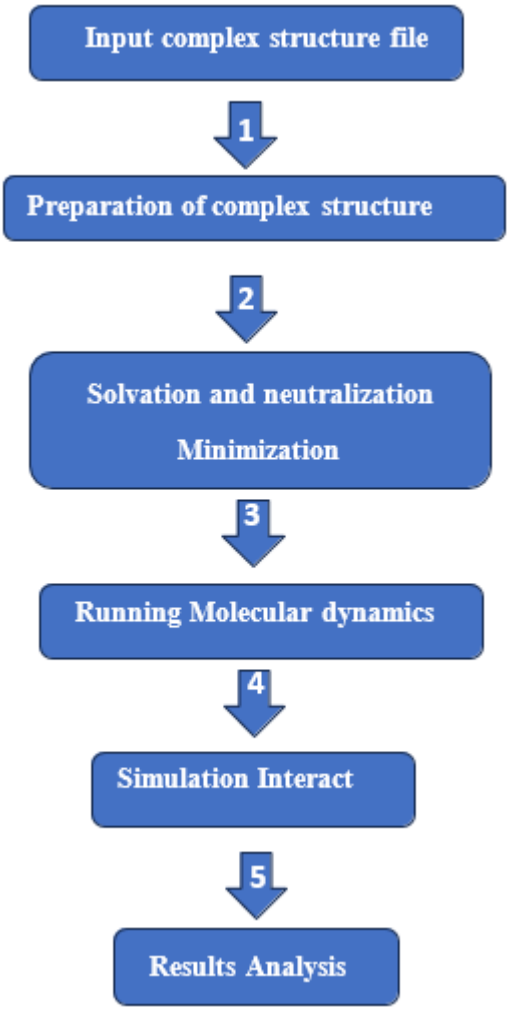


Figure 3. Molecular dynamics Simulation process

Step 5: Results Analysis

The output files generated from the Desmond MD simulation were analyzed to extract meaningful insights regarding the dynamic behavior of the HER2-tamoxifen, HER2-mitomycin, ER-α-tamoxifen, and ER-α-mitomycin complexes. Analysis focused on key

structural and interaction parameters, including:

- Root Mean Square Deviation (RMSD): To assess the overall structural stability of the protein-ligand complexes over time.
- Root Mean Square Fluctuation (RMSF): To evaluate the flexibility of individual amino acid residues and detect regions of significant motion.
- Radius of Gyration (Rg): To monitor the compactness and folding behavior of the protein during the simulation.
- Hydrogen Bonding Profile: To determine the number and consistency of hydrogen bonds formed between the ligand and key residues at the active site.

These analyses were visualized using Maestro's Simulation Event Analysis and Interaction Diagram tools, offering clear representations of time-resolved stability and binding behavior. The graphical results facilitated the selection of the most stable and biologically relevant conformations for further interpretation.

Figure 4 illustrates the solvation box encompassing the tamoxifen-HER2 complex during the MD simulation setup, highlighting the spatial arrangement of water molecules and ions around the system.

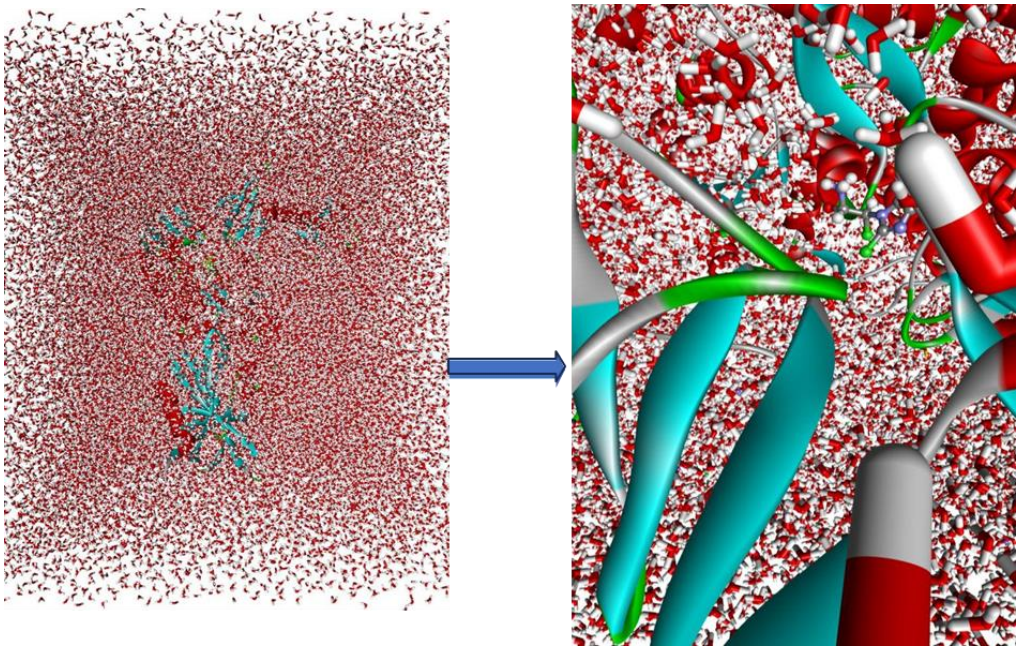


Figure 4. Solvation of tamoxifen-HER2 complex for the MD simulation.

Chapter II:

Results & discussion

1. Virtual Screening

The process of generating a series of **tamoxifen** and **mitomycin** analogues began with the use of the scaffold structures of **HER2** and **ER-α**, illustrated in **Figure 5**, as the foundational templates. Functional groups such as OH, COOH, NH_2, and NO_2 were selected as the primary building blocks, supplemented by empty linkers. To facilitate this process, Simplified Molecular Input Line Entry System (SMILES) structures representing both the scaffold and functional groups were obtained and integrated into the **SmiLib** software interface. Through this systematic approach, a comprehensive compound library consisting of **256 tamoxifen and mitomycin analogues** was created in SMILES format. This extensive library facilitated the exploration of various structural modifications, enabling the generation of diverse analogues with distinct chemical compositions.

Subsequently, these analogues were subjected to rigorous evaluation to assess their biological activities. This assessment specifically aimed to establish their potential in combating breast cancer and estrogen-related disorders, with a particular focus on their anti-cancer activity against **HER2** and **ER-α** proteins. These proteins play critical roles in the development and progression of breast cancer, making them ideal targets for anti-cancer drug development. This virtual screening step was crucial in identifying analogues with promising therapeutic properties, thereby paving the way for their potential application in breast cancer treatment and other estrogen-related conditions.

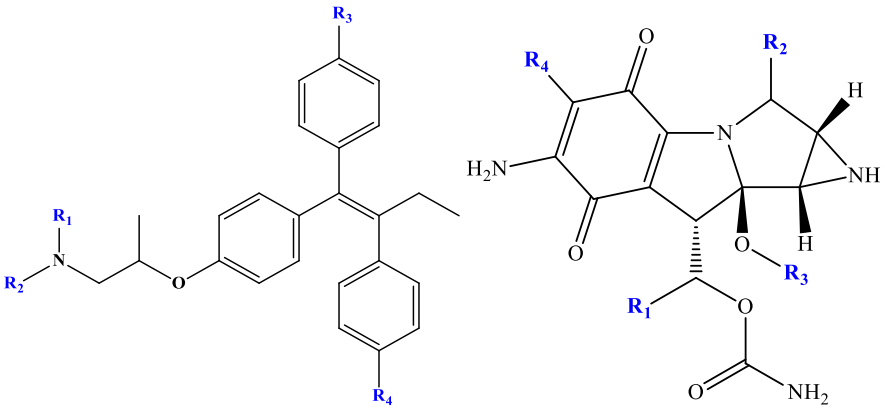


Figure.5. Scaffold structure used for the enumeration of **tamoxifen** and **mitomycin** analogues, (a) molecular structure presentation (b) SMILES presentation.

All generated molecules underwent a rigorous virtual screening process to evaluate their potential suitability as **anticancer agents targeting HER2 and ER-α proteins**, which are

critically involved in the progression of **breast cancer and estrogen-responsive tumors**. As part of this screening, a comprehensive toxicity evaluation was performed using the **ProTox II webserver** [64]. This platform enabled the prediction of **toxicity class (TC)** and **lethal dose 50 (LD_{50})**, the latter referring to the dose required to cause death in 50% of a test population, thereby serving as a key indicator of acute toxicity.

This analysis provided essential insights into the safety profiles of the compounds, allowing for the identification of analogues with acceptable toxicity thresholds. Only molecules exhibiting **higher toxicity class values and lower LD_{50} levels** compared to **tamoxifen and mitomycin** were retained for further pharmacokinetic analysis. Molecules that did not meet this toxicity criterion were excluded from subsequent stages.

Following toxicity filtering, the selected compounds were subjected to further evaluation using the **SwissADME webserver**, which predicted key pharmacokinetic properties such as gastrointestinal absorption, blood–brain barrier permeability, and drug-likeness. Based on the integrated in silico screening for both toxicity and pharmacokinetics, a subset of molecules with the **highest predicted anticancer potential and favorable ADME profiles** were identified (see **Table 1**).

Table 1. Selected tamoxifen and mitomycin analogues

Entry	Code	R1	R2	R3	R4
1	Tmx1	OH	OH	NH_2	COOH
2	Tmx 2	OH	NH_2	OH	OH
3	Tmx 3	OH	NH_2	OH	COOH
4	Tmx 4	OH	NH_2	COOH	OH
5	Tmx 5	NH_2	OH	OH	COOH
6	Tmx 6	NH_2	OH	COOH	OH
7	Tmx 7	NH_2	NH_2	OH	OH
8	Tmx 8	NH_2	NH_2	OH	COOH
9	Tmx 9	NH_2	NH_2	COOH	OH
10	Tmx 10	NH_2	NH_2	COOH	NH_2
11	Mty 1	OH	NO_2	NO_2	NO_2
12	Mty 2	OH	NH_2	NO_2	COOH
13	Mty 3	OH	NH_2	COOH	NH_2
14	Mty 4	NO_2	NH_2	COOH	NO_2
15	Mty 5	NO_2	COOH	OH	NO_2

16	Mty 6	NH_2	OH	NO2	NO_2
17	Mty 7	NH_2	NO_2	OH	NO_2
18	Mty 8	NH_2	NO_2	NO_2	OH
19	Mty 9	NH_2	NO_2	NO_2	COOH
20	Mty 10	NH_2	NO_2	COOH	NO_2

Another selection criterion was applied using the **ProTox-II online server**, which predicts toxicological endpoints including **cytotoxicity, mutagenicity, carcinogenicity, hepatotoxicity, and immunotoxicity**, in accordance with the **Globally Harmonized System (GHS)** classification. Compounds with **LD_{50} values between 2000–5000 mg/kg** were classified as **toxicity class 5**, while those ranging from **300–2000 mg/kg** were assigned to **class 4**. The predicted **probability of immunotoxicity** for all compounds was very low, ranging from **–0.88 to –0.99**. Moreover, all analogues were predicted to be **non-hepatotoxic** and **non-cytotoxic**, with **mutagenicity and carcinogenicity scores** remaining within **acceptable safety limits** (Table 2).

In comparison to **tmx** and **mty**, compounds with **lower LD_{50} values** and consequently **higher toxicity class (TC)**, in addition to **more negative values** for **hepatotoxicity** and **immunotoxicity**, as well as **less positive values** for **carcinogenicity**, **mutagenicity**, and **cytotoxicity**, were prioritized for further consideration. The most promising candidates, **highlighted in blue** in Table 2, include Tmx 10, Mty 1, Mty 2, Mty 5, Mty 7, Mty 8, and Mty9.

Table 2. Toxicity prediction probability, median lethal dose, and toxicity class of selected drugs analogues

Entry	Code	Hepato	Carcino	Immuno	Muta	Cyto	LD50	TC
1	Tmx1	-0.51	+0.69	-0.99	+0.85	-0.73	1530	4
2	Tmx 2	-0.62	+0.70	-0.98	+0.88	-0.72	3000	5
3	Tmx 3	-0.50	+0.66	-0.92	+0.79	-0.72	1530	4
4	Tmx 4	-0.53	+0.67	-0.99	+0.84	-0.71	1500	4
5	Tmx 5	-0.51	+0.72	-0.96	+0.84	-0.71	3000	5
6	Tmx 6	-0.53	+0.67	-0.98	+0.84	-0.71	3000	5
7	Tmx 7	-0.52	+0.75	-0.98	+0.85	-0.71	3000	5
8	Tmx 8	-0.63	+0.67	-0.99	+0.85	-0.71	3000	5
9	Tmx 9	-0.52	+0.68	-0.97	+0.80	-0.70	3000	5
10	Tmx 10	-0.61	+0.69	-0.98	+0.84	-0.70	3000	5
11	Mty 1	-0.50	+0.67	-0.93	+0.83	-0.70	2000	4

12	Mty 2	-0.50	+0.56	-0.98	+0.82	-0.69	2000	4
13	Mty 3	-0.51	+0.56	-0.92	+0.81	-0.69	1530	4
14	Mty 4	-0.52	+0.54	-0.94	+0.79	-0.69	3000	5
15	Mty 5	-0.53	+0.67	-0.98	+0.86	-0.69	3000	5
16	Mty 6	-0.51	+0.68	-0.99	+0.84	-0.69	3000	5
17	Mty 7	-0.50	+0.56	-0.98	+0.82	-0.69	2000	4
18	Mty 8	-0.77	+0.73	-0.88	+0.95	-0.69	3000	5
19	Mty 9	-0.71	+0.66	-0.96	+0.85	-0.65	3000	5
20	Mty 10	-0.76	+0.72	-0.97	+0.92	-0.66	3000	5

LD50 (mg/kg), - (Inactive toxic class (probability score)), + (Active toxic class (probability score)), TC: Toxicity Class

The evaluation of pharmacokinetic and toxicity parameters plays a pivotal role in identifying potential drug candidates during the early stages of drug development. In this study, special attention was given to the predicted toxicity profiles of the synthesized **tmx** and **mty** analogues, focusing on their **hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity**, and **LD_{50} values**, as estimated by the **ProTox-II server** and summarized in Table 2.

According to the **Globally Harmonized System (GHS)** classification, compounds with **LD_{50} values ranging from 300 to 2000 mg/kg** are categorized as **toxicity class 4**, whereas those within **2000–5000 mg/kg** fall under **class 5**. Most compounds in this study showed LD_{50} values of **≥2000 mg/kg**, placing them in **toxicity class 5**, indicative of low acute toxicity. Notably, a few entries such as **Tmx1, Tmx3, Tmx4, Mty3, and Mty7** fell into **class 4**, reflecting slightly higher acute toxicity yet still within an acceptable safety threshold.

In terms of organ-specific toxicity, all compounds were predicted to be **non-hepatotoxic** and **non-cytotoxic**, with hepatotoxicity values ranging from **–0.50 to –0.77** and cytotoxicity scores between **–0.65 and –0.73**. These negative values reflect a reduced probability of toxicity, suggesting that liver and cellular safety risks are minimal.

Additionally, all tested analogues showed **very low immunotoxicity probabilities**, ranging from **–0.88 to –0.99**, implying limited potential for triggering adverse immune responses. The **mutagenicity and carcinogenicity predictions** were also within acceptable safety margins, with most values below +0.85, suggesting a low likelihood of genotoxic or tumorigenic effects.

When compared to the reference compounds **tmx** and **mty**, several derivatives

demonstrated **improved safety characteristics**. Particularly, **Mty8, Mty9, and Mty10** exhibited **more negative values of hepatotoxicity and immunotoxicity**, as well as **lower carcinogenicity and cytotoxicity probabilities**, making them favorable candidates for further consideration. These compounds combined **low acute toxicity (LD_{50} = 3000 mg/kg, class 5)** with an overall **benign toxicological profile**, highlighting their potential for further pharmacological evaluation.

Taken together, the data indicate that the majority of the tested analogues possess **favorable toxicity profiles**, supporting their progression into subsequent **in vitro and in vivo** studies. Special emphasis should be placed on those analogues with **class 5 toxicity**, **non-hepatotoxic**, **non-cytotoxic**, and **low immunotoxic and mutagenic potential**, as they exhibit an optimal balance of **efficacy and safety**.

Table 3. Evaluation of Pharmacokinetics properties of **tamoxifen** and **mitomycin** and its generated analogues

Entry	Molecule	GI absorption	BBB permeant	P-gp substrate	CYP inhibitor				
					1A2	2C19	2C9	2D6	3A4
1	Tmx1	Low	No	No	No	No	No	No	No
2	Tmx 2	Low	No	No	No	No	No	No	No
3	Tmx 3	Low	No	No	No	No	No	No	No
4	Tmx 4	Low	No	No	No	No	No	No	No
5	Tmx 5	Low	No	No	No	No	No	No	No
6	Tmx 6	Low	No	No	No	No	No	No	No
7	Tmx 7	Low	No	No	No	No	No	No	No
8	Tmx 8	Low	No	No	No	No	No	No	No
9	Tmx 9	Low	No	No	No	No	No	No	No
10	Tmx 10	High	No	No	No	No	No	No	No
11	Mty 1	High	No	No	No	No	No	No	No
12	Mty 2	High	No	No	No	No	No	No	No
13	Mty 3	Low	No	No	No	No	No	No	No
14	Mty 4	Low	No	No	No	No	No	No	No
15	Mty 5	High	No	No	No	No	No	No	No
16	Mty 6	Low	No	No	No	No	No	No	No
17	Mty 7	High	No	No	No	No	No	No	No
18	Mty 8	High	No	No	No	No	No	No	No

19	Mty 9	High	No	No	No	No	No	No	No
20	Mty 10	Low	No	No	No	No	No	No	No

The in-silico pharmacokinetic analysis (Table 3) identifies compounds **Tmx1, Tmx3,** and **Tmx4**, **Mty1**, **Mty2**, **Mty5**, **Mty7**, **Mty8**, and **Mty9** as the most promising candidates for further investigation based on their favorable absorption profiles. These molecules exhibit **high gastrointestinal (GI) absorption**, an essential feature for effective oral bioavailability. Importantly, **none of the evaluated compounds** are predicted to cross the **blood-brain barrier (BBB)** or act as **P-glycoprotein (P-gp) substrates**, suggesting a reduced risk of central nervous system side effects and lower potential for efflux-related drug resistance.

Moreover, none of the tested molecules inhibit major **cytochrome P450 (CYP)** enzymes (1A2, 2C19, 2C9, 2D6, or 3A4), indicating a minimal likelihood of drug-drug interactions via CYP-mediated metabolism. This is particularly advantageous for clinical applications, where polypharmacy is common.

Taken together, these pharmacokinetic characteristics support further investigation of, **Tmx1, Tmx3,** and **Tmx4**, **Mty1**, **Mty2**, **Mty5**, **Mty7**, **Mty8**, and **Mty9** as potential therapeutic agents **for the treatment of breast cancer and tumors associated with estrogen receptors.**

1. Physicochemical Properties

Physicochemical characteristics, such as solubility, lipophilicity, and molecular flexibility, play a central role in determining a compound's suitability as a drug candidate [69]. Lipophilicity, in particular, significantly influences membrane permeability and bioavailability and is widely used in molecular design.[70] Key parameters evaluated in this study include XLogP (ideal range: 0.7–5), molecular weight (MW) (150–500 g/mol), solubility (log S) (optimal: 0 to −6), and molecular flexibility as indicated by the number of rotatable bonds.

As summarized in Table 4, all tested compounds—including tamoxifen, mitomycin, and their derivatives—fall within the recommended range for lipophilicity and molecular weight, suggesting adequate membrane permeability. Additionally, all molecules show good aqueous solubility, supporting their potential oral bioavailability. The number of rotatable bonds for each compound is below eight, implying low molecular flexibility and improved structural stability.

The acid-base nature of the molecules, which is influenced by hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA), is another critical factor in determining pharmacokinetic behavior[71] . According to Lipinski's rule of five[72], a favorable ADMET profile is associated with a lower number of HBDs and a moderate to high number of HBAs.

In the current analysis, all compounds exhibit a low number of HBDs and a higher number of HBAs, consistent with favorable drug-likeness and ADMET potential.

These findings collectively indicate that the designed analogues possess appropriate physicochemical profiles conducive to further pharmacological evaluation.

Table 4. Physicochemical Properties of **tamoxifen** and **mitomycin** and its generated analogues

Molecule	Water solubility (Log *S*)	Lipophilicity (Consensus Log *P*o/w)	Molar refractivity	H-bond acceptor	H-bond donor	Rotatable bonds	Molecular weight
1	-2.25	-1.36	49.84	7	4	3	218.17
2	-2.08	-1.75	48.25	5	4	3	203.16
3	-2.11	-2.41	47.74	8	5	3	219.16
4	-2.97	-1.14	48.29	7	4	3	219.15
5	-1.82	-2.33	50.96	6	5	3	218.17
6	-2.18	-1.46	50.68	6	4	3	218.17
7	-2.53	-1.40	53.06	5	4	3	218.17
8	-1.93	-2.00	49.80	5	4	3	202.17
9	-2.60	-1.51	52.22	6	4	3	217.18
10	-2.29	-0.84	49.51	5	3	3	202.17
11	-4.22	+0.61	54.90	5	2	3	276.46
12	-3.18	-0.44	52.82	6	3	3	257.03
13	-2.13	-1.58	50.73	7	4	3	237.60
14	-2.04	-2.38	48.58	7	5	3	219.16
15	-2.74	-0.60	54.32	5	3	3	216.19
16	-1.97	-2.28	49.41	6	5	3	219.16
17	-3.08	+0.83	57.80	4	1	3	254.07
18	-1.47	-0.96	42.68	4	2	3	172.14
19	-1.88	-1.17	44.71	5	3	3	188.14
20	-1.66	-1.40	47.09	4	3	3	187.16

2. Druglikeness

According to **Lipinski's Rule of Five**[72], all **tamoxifen** and **mitomycin** analogues comply with the established criteria for oral bioavailability, indicating a favorable drug-likeness profile. These rules assess molecular weight, lipophilicity, hydrogen bond donors, and acceptors, all of which were within the recommended thresholds for every compound analyzed.

However, when applying **Ghose's filter**[73], which includes parameters such as molar refractivity, total atom count, molecular weight, and logP, only **four compounds (Tmx1, Tmx3, and Tmx4 Mty1, Mty5, and Mty7)** met all the criteria, suggesting a narrower subset with optimal physicochemical balance.

In the context of **Veber's rule**[74], which emphasizes low molecular flexibility and optimal polar surface area (tPSA ≤ 140 Å² and ≤ 10 rotatable bonds), a larger subset—**Tmx1,Tmx3,Tmx4, Mty1, Mty2, Mty5, Mty7, Mty8, and Mty9**—exhibited favorable properties. This highlights their potential for efficient membrane permeability and oral absorption.

Similarly, applying **Egan's filter**[75], which focuses on logP and tPSA for absorption prediction, compounds **Tmx1,Tmx3,Tmx4, Mty1, Mty2, Mty5, Mty7, and Mty8** were identified as compliant, further validating their oral bioavailability potential.

Collectively, the application of the five drug-likeness rules—**Lipinski, Ghose, Veber, Egan, and Muegge**—yields an average compliance score of approximately **55%**, supporting the overall **good bioavailability and druglikeness** of the tested compounds, as detailed in **Table 5**.

Table 5 further refines the selection process, identifying **Mty1, Mty2, Mty5, Mty7, Mty8, and Mty9** as the most promising candidates based on their consistent performance across multiple filters. Notably, compounds like **Tmx1,Tmx3,Tmx4,** showed good initial potential but did not pass all selection thresholds. The retained compounds exhibit **favorable pharmacokinetic and non-toxicological profiles**, making them strong candidates for further preclinical evaluation. This strategic filtering approach ensures efficient prioritization for experimental validation and resource optimization in the drug development pipeline.

Table 5. Druglikeness properties of **tamoxifen** and **mitomycin** and its generated analogues

Molecule	Lipinski ≠violation	Ghose ≠violation	Veber ≠violation	Egan ≠violation	Muegge ≠violation	Bioavailability Score
Tmx1	0	1	1	1	1	0.55
Tmx 2	0	1	1	1	1	0.55
Tmx 3	0	1	1	1	1	0.55
Tmx 4	0	1	1	1	0	0.55
Tmx 5	0	1	1	1	1	0.55
Tmx 6	0	1	1	1	1	0.55
Tmx 7	0	1	1	1	1	0.55
Tmx 8	0	1	1	1	1	0.55
Tmx 9	0	1	1	1	1	0.55
Tmx 10	0	0	0	0	0	0.55
Mty 1	0	1	0	0	0	0.55
Mty 2	0	0	0	0	0	0.55
Mty 3	0	1	1	1	1	0.55
Mty 4	0	1	1	1	1	0.55
Mty 5	0	0	0	0	0	0.55
Mty 6	0	1	1	1	1	0.55
Mty 7	0	0	0	0	0	0.55
Mty 8	0	1	0	0	1	0.55
Mty 9	0	1	0	0	1	0.55
Mty 10	0	1	1	1	1	0.55

The **BOILED-Egg plot** shown in **Figure 6**, which maps **Total Polar Surface Area (TPSA)** against **lipophilicity (LogP)**, serves as an intuitive tool for predicting **human intestinal absorption (HIA)** and **blood–brain barrier (BBB)** penetration. The **white region** (egg white) indicates a high probability of passive absorption through the gastrointestinal tract (GIT), while the **yellow region** (yolk) represents compounds likely to permeate the brain. Additionally, **red-colored dots** correspond to molecules **not predicted to be P-glycoprotein (P-gp) substrates**, whereas **blue-colored dots** would signify **active P-gp substrates**.

In this study, **eight compounds— Tmx1,Tmx3,Tmx4, Mty1, Mty2, Mty5, Mty7, Mty8, Mty9, and Mty10**—as well as the reference drug metronidazole (Mtz) fall within the **white region**, suggesting

favorable passive GIT absorption. In contrast, the remaining compounds are positioned outside this region, indicating a **lower potential for passive intestinal absorption**.

Importantly, **none of the analogues fall within the yolk region**, which indicates a **low likelihood of brain penetration**, potentially minimizing central nervous system side effects. Moreover, all compounds are **colored red**, signifying that they are **not predicted to be substrates of P-glycoprotein (PGP−)**, which further supports their **favorable absorption and distribution profiles** with minimal risk of efflux-mediated drug resistance or interaction.

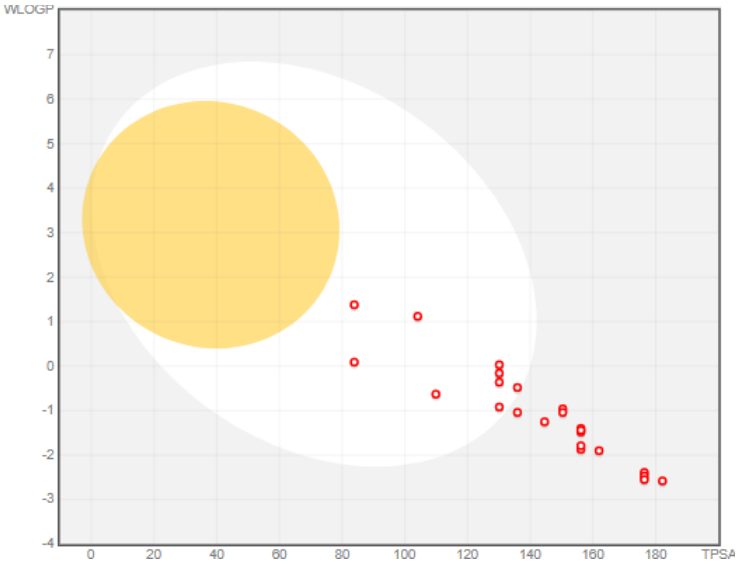


Figure.6. The Boiled-egg plot of **tamoxifen** and **mitomycin** and selected generated analogues.

Following the prediction of pharmacokinetic and toxicity properties, the **half maximal inhibitory concentration (IC_{50})** values of all selected compounds against **estrogen receptor alpha (ER-α)** and **human epidermal growth factor receptor 2 (HER2)** were calculated using **AutoDock 4.2** and **AutoDock Tools 1.5.6** software[76]. The obtained results are summarized in **Table 6**.

Table 6. Half maximal inhibitory concentration of **tamoxifen** and **mitomycin** and selected generated analogues

Molecule	HER2	ER-alpha
	IC50 (µM)	IC50 (µM)
Tmx1	47.24	66.21
Tmx 2	92.81	154
Tmx 3	39.90	47.24
Tmx 4	28.47	55.93
Tmx 5	66.21	130.1

Tmx 6	24.05	78.39
Tmx 7	55.93	109.9
Tmx 8	66.21	182.3
Tmx 9	66.21	55.93
Tmx 10	92.81	215.9
Mty 1	55.93	109.9
Mty 2	66.21	182.3
Mty 3	66.21	55.93
Mty 4	92.81	215.9
Mty 5	24.05	130.1
Mty 6	33.70	154.0
Mty 7	66.21	109.9
Mty 8	39.90	55.90
Mty 9	33.70	55.93
Mty 10	33.70	130.1

Table 6 presents the half maximal inhibitory concentration (IC_{50}) values of the synthesized compounds against HER2 and estrogen receptor alpha (ER-α), both of which are critical targets in hormone-related cancers such as breast cancer. The data reveal a range of inhibitory potencies, with several analogues showing promising activity toward one or both targets.

Among the tested compounds **Tmx1,Tmx3,Tmx4,** emerges as particularly potent, displaying the lowest IC_{50} value of 24.05 µM against HER2, suggesting strong binding affinity and potential efficacy in targeting HER2-positive cancer cells. Notably, Tmx1 ,Tmx3 and Tmx4 also demonstrate encouraging dual-target activity, with IC_{50} values of 28.47 µM and 39.90 µM against HER2, and 55.93 µM and 47.24 µM against ER-α, respectively. These results indicate a balanced and effective interaction with both receptors, positioning these molecules as promising multitarget therapeutic candidates.

Within the Mty series, Mty5 and Mty8 show compelling inhibition profiles. Mty5 exhibits strong HER2 inhibition (IC_{50} = 24.05 µM) and moderate ER-α inhibition (IC_{50} = 130.1 µM), while Mty8 displays a more balanced inhibitory effect across both targets (IC_{50} = 39.90 µM for HER2, and 55.90 µM for ER-α). This suggests their potential for further exploration as dual-target inhibitors.

The identification of compounds with greater inhibitory potential than reference standards across HER2 and ER-α underscores their therapeutic relevance, particularly in the context of estrogen-receptor-positive and HER2-overexpressing cancers. These findings nominate Tmx1, Tmx3, Tmx4, Mty5, and Mty8 as promising lead candidates for further molecular docking, dynamics simulations, and preclinical

evaluation.

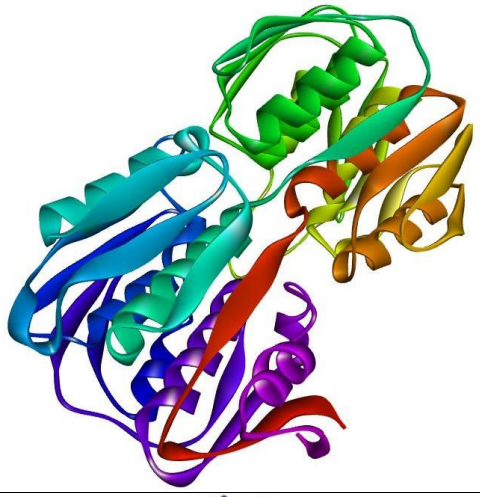
3.Molecular Docking Study

To elucidate the interaction mechanisms of the most potent tamoxifen and mitomycin analogues—TMX1, TMX3, and TMX4—with the HER2 and ER-alpha receptors, molecular docking simulations were conducted. These simulations aimed to predict the most stable conformations of these compounds when bound to the target receptors.

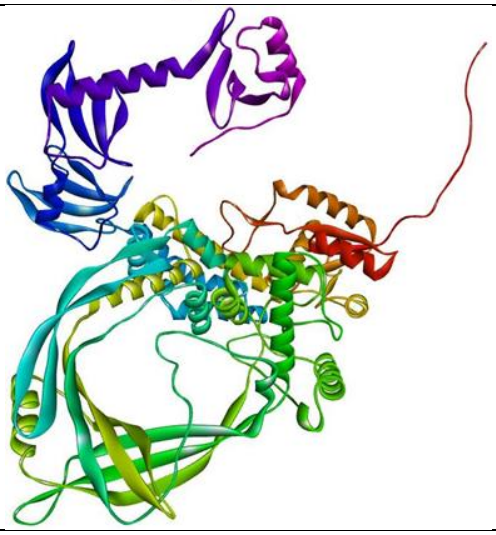
The protein sequences of HER2 and ER-alpha were retrieved from the RCSB Protein Data Bank. The docking simulations provided insights into the binding affinities and potential mechanisms through which these compounds may exert their therapeutic effects.

Figure 7 illustrates the docking results, showing the preferred binding modes of the compounds with HER2 and ER-alpha, along with the key molecular interactions contributing to binding stability.

Crystal Structure of the Kinase domain of Human HER2 (erbB2).



Crystal Structure of the Kinase domain of Human HER2 (erbB2).	
PDB ID: 3PP0 Gene HER2 Source organism Fusobacterium nucleatum Amino acids 423	Biological function Cell wall formation. Adds enolpyruvyl to UDP-N-acetylglucosamine



Human Estrogen Receptor Alpha Ligand-Binding Domain in Complex With 4-Hydroxytamoxifen	
PDB ID: Gene Source organism Amino acids	3ERT ER-Alpha Prevotella intermedia 800

Figure 7. Three-dimensional structure of HER2 and ER-alpha

The binding site was defined using **Ligand Binding Site Prediction** (https://prankweb.cz). The molecular docking procedure was conducted using the **Schrödinger Maestro** software, employing the **Glide SP (Standard Precision)** module. Initially, ligand molecules underwent preparation for docking calculations utilizing the **LigPrep** tool within the Schrödinger Software program, employing the **OPLS3** force field as described by Harder et al[77], This involved generating a maximum of 32 stereoisomers for each ligand after selecting the ionization states at pH 7.0 ± 2.0. Subsequently, receptor structures were prepared using the **Protein Preparation Wizard tool**[78], ensuring a solubility of 2.5 Å. Polar hydrogens were added to heavy atoms, and all water and ions were removed from the structure. Bond orders were assigned, charges were defined at pH 7.0, and the selected receptor was optimized using **PROPKA**. Heavy atoms in the receptor were constrained to a preferred 0.3Å RMSD using the **OPLS3** force field. Grid boxes were defined around the receptor using the grid generation tool in Maestro. Ligands were then docked into the receptor based on the defined grid using the **Standard Precision (SP)** docking algorithm, allowing for the ranking of ligands based on their interaction with specific conformations of the receptor molecule.[79]

The docking analysis depicted in **Figure 8** provides deeper insights into the interaction modes of the tested compounds within the binding site of **HER2** and **ER-alpha**. Notably,

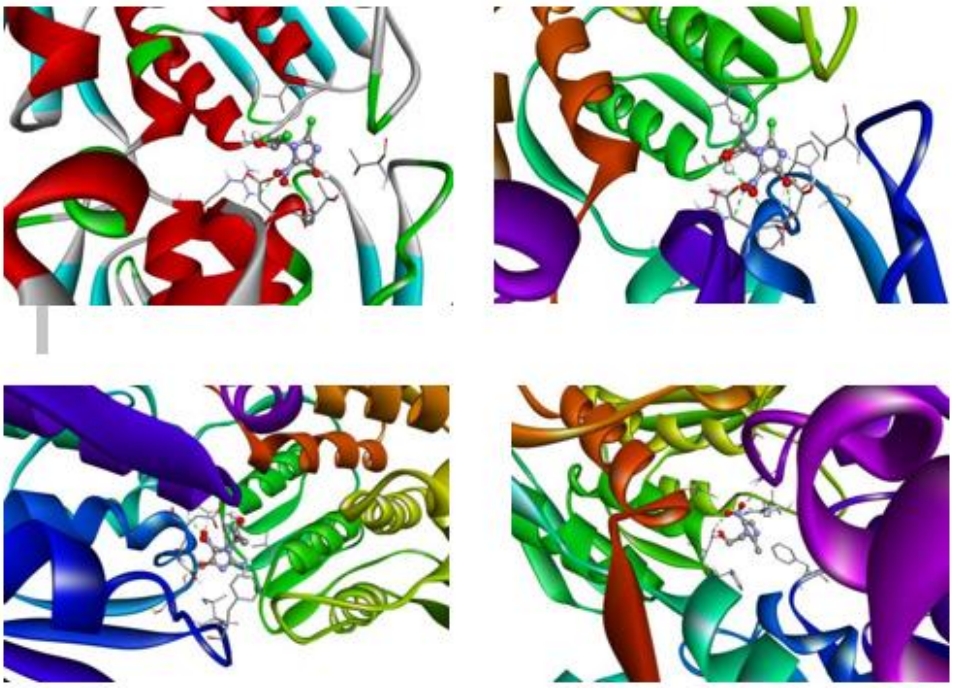


Figure 8. 3D interaction of tamoxifen the selected most potent analogues with the target the target HER2 and ER-alpha.

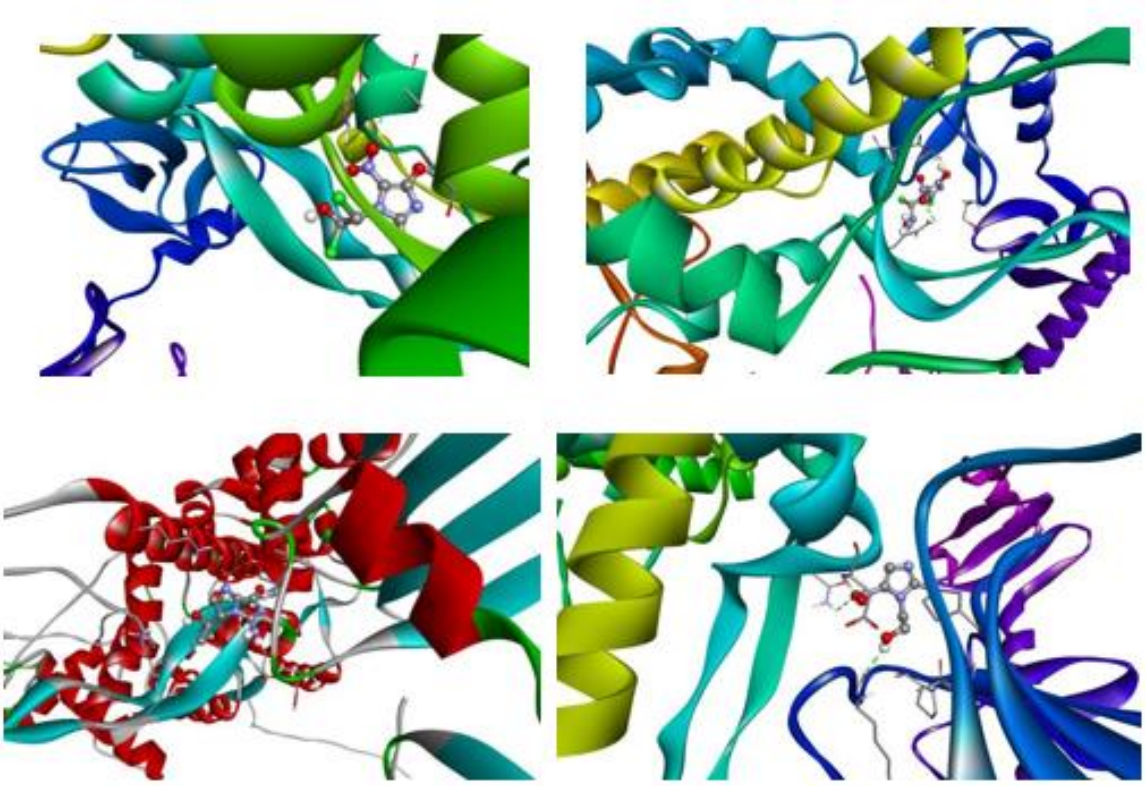


Figure 9. 3D interaction of mitomycin and the selected most potent analogues with the target HER2 and ER-alpha

4. Molecular Dynamic simulation:

Molecular dynamics simulation is a computational technique widely utilized to study the dynamic behavior of molecular systems over time. In molecular docking, the target protein is typically treated as a rigid structure, overlooking potential conformational changes that may occur during ligand binding. To better mimic physiological conditions and account for flexibility in both the protein and the ligand, molecular dynamics simulations were employed.

In this study, a molecular dynamics simulation was conducted using the **Desmond MD** program (version 2024-3) on the docked complex of the protein and ligand with the highest binding affinity, namely **HER2** and **ER-alpha**. This approach allowed for a more comprehensive analysis of the dynamic interactions between the protein and ligand, providing insights into their behavior under physiological conditions. By simulating the motion and interactions of atoms over time, molecular dynamics simulations offer valuable information about the stability and dynamics of protein-ligand complexes, aiding in the understanding of their functional mechanisms.

Preparing topology files and selecting appropriate force fields and parameters are essential steps to ensure the accuracy and reliability of molecular dynamics simulations. The

protein-ligand complex obtained from molecular docking was prepared for simulation, which involved generating topology files that describe the chemical structure and connectivity of the molecules in the system, along with assigning force field parameters that govern atomic interactions.

The protein structure was then solvated using the TIP3P water model to provide a realistic environment for the simulation. Similarly, the ligand molecules were parameterized using force field parameters compatible with the chosen force field for the protein. Special attention was given to handling non-standard residues or chemical modifications within the ligand structure to ensure accurate representation during the simulation.

Once the topology files for both protein and ligand were prepared, they were combined into a single system file along with water molecules and ions to construct the complete simulation system. The system was then subjected to energy minimization to eliminate any steric clashes or unfavorable interactions.

Subsequently, the molecular dynamics simulation parameters were defined, including the integration time step, temperature, pressure, and duration of the simulation. These parameters were selected based on the specific characteristics of the system and the desired balance between accuracy and computational efficiency.

Through careful preparation of topology files, appropriate selection of force fields, and precise definition of simulation parameters, the molecular dynamics simulations were able to accurately reflect the dynamic behavior of the protein-ligand complex and offer valuable insights into their interactions and stability under physiological conditions.

In this study, molecular dynamics simulations were performed for **100 nanoseconds** for each of **TMX1**, **TMX3**, and **TMX4** , with the objective of observing their interaction patterns and stability within the binding sites of **HER2** and **ER-alpha**. The dynamic behavior of the entire system was thoroughly analyzed using parameters such as **Root Mean Square Deviation (RMSD)**, **Root Mean Square Fluctuation (RMSF)**, and **Radius of Gyration (Rg)**.

4.1 Root mean square deviation (RMSD)

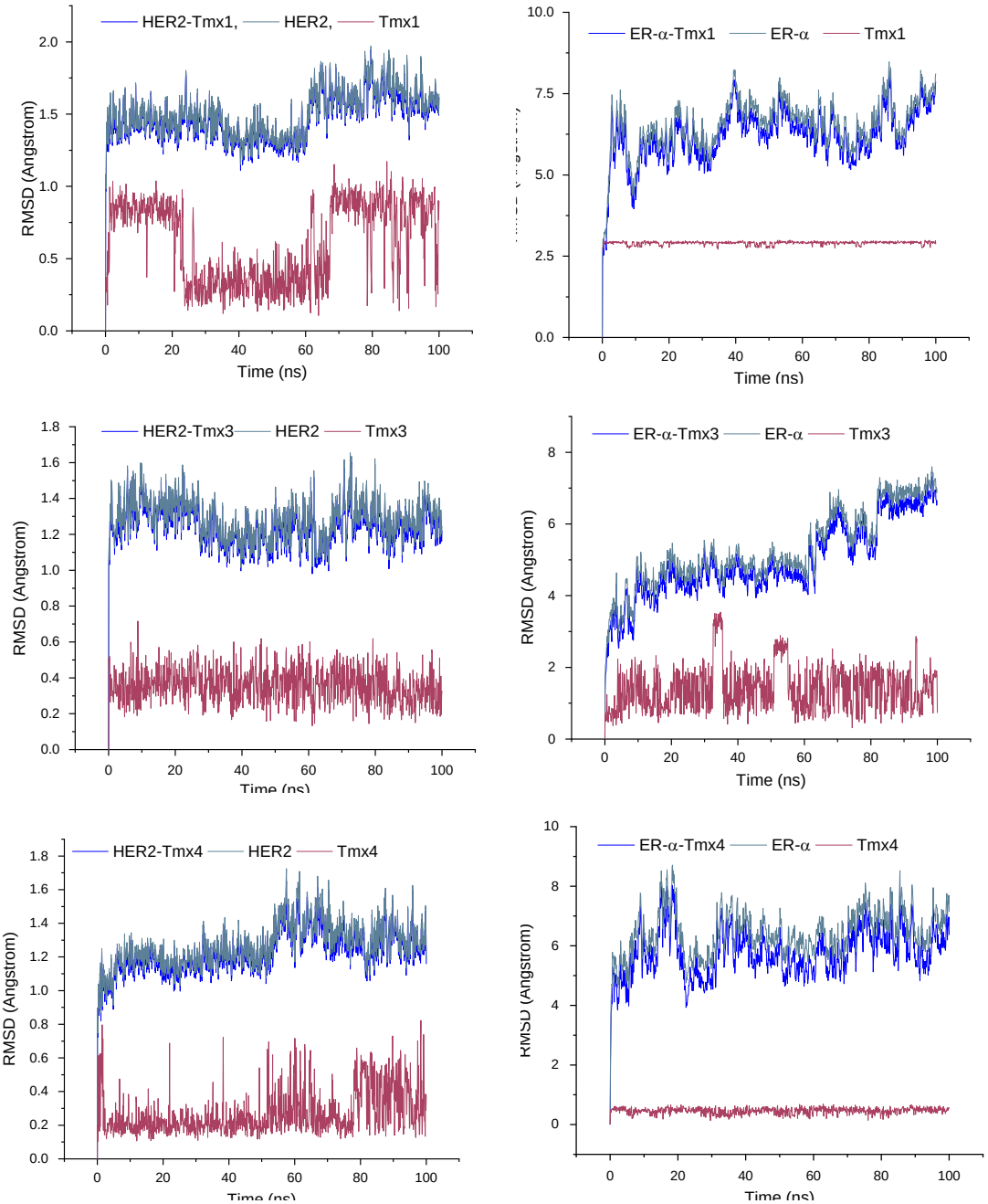
To assess the stability of the protein-ligand complexes formed with **Tmx1,Tmx3, Tmx4**, and the reference compound, the **RMSD of Cα atoms** was calculated from the molecular dynamics simulation trajectories (Figure 10). Following equilibration, the RMSD values for all complexes remained stable within the range of approximately **0.25–1.75 Å**, indicating a well-equilibrated system.

Notably, the **HER2–Tmx1,Tmx3, Tmx4** complex showed a gradual increase in RMSD

up to **5 Å**, maintaining this level consistently throughout the remainder of the 100 ns simulation. This trend reflects a possible conformational adjustment of the HER2 receptor in response to Tmx6 binding, followed by stabilization.

In contrast, the **ER-alpha–Tmx1,Tmx3, Tmx4** complexes exhibited relatively minor fluctuations, with RMSD values remaining below **5Å**, suggesting high structural stability.

These observations support the overall stability of the investigated protein–ligand complexes under physiological conditions, particularly for Tmx1,Tmx3,Tmx4 with HER2 .



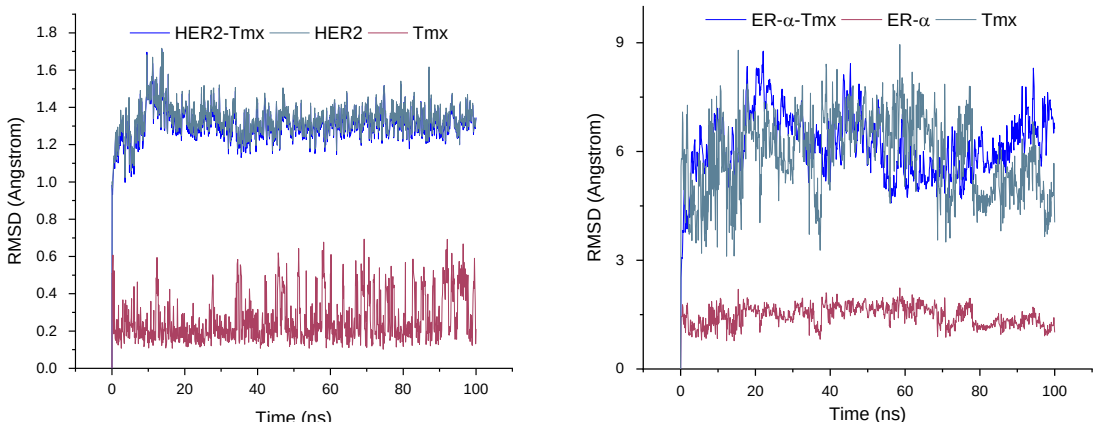


Figure 10. The RMSD plots of HER2 and ER-alpha complexes during 100 ns simulation of Tmx.

4.2 Root mean square fluctuation (RMSF):

To evaluate the dynamic behavior of the proteins when bound to the ligands, the **root mean square fluctuations (RMSF)** values were calculated. These values provide insights into the **flexibility and mobility of individual amino acid residues** throughout the simulation period.

Upon analysis of the RMSF profiles for both **HER2** and **ER-alpha**, it was observed that **most residues exhibited fluctuations below 2 Å**, indicating a relatively **rigid and stable conformation** during the 100 ns simulation with ligands **Tmx1,Tmx3,Tmx4** . This stability suggests that ligand binding does not induce major conformational changes in the protein backbones.

However, as expected, **loop and terminal regions** of both proteins showed **increased RMSF values**, peaking around **3.2 to 3.6 Å** (Figure 11).

These higher fluctuations in flexible regions are typical and do not affect the overall stability of the protein-ligand complexes. These results highlight the structural robustness of HER2 and ER-alpha when complexed with Tmx1,Tmx3, Tmx4 under simulated physiological conditions.

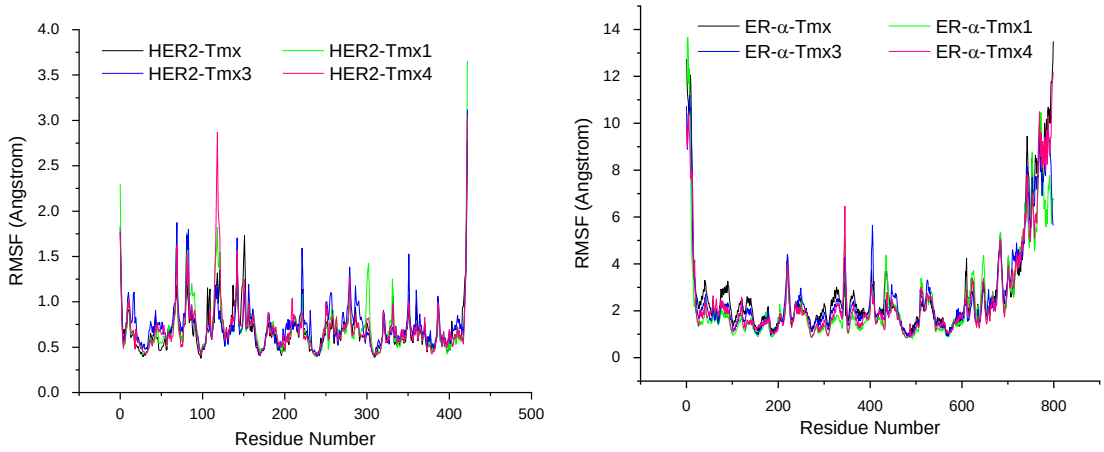


Figure 11. The fluctuation of protein residues of HER2 and ER-α during simulations as determined by RSMF values.

Conclusion

Conclusion

Studying the effectiveness of drugs in breast cancer treatment is a crucial step toward developing more efficient therapies for this complex disease, which is among the most prevalent cancers affecting women worldwide. Breast cancer remains a leading cause of cancer-related mortality among women and is characterized by its heterogeneity, often involving hormone receptor-positive (ER+) or HER2-positive subtypes. These characteristics offer therapeutic targets, making receptor-focused treatment strategies highly relevant. Among the most prominent drugs investigated for breast cancer treatment are Tamoxifen and Mitomycin, each exhibiting promising therapeutic potential through distinct mechanisms of action. Tamoxifen is a well-established therapeutic agent used primarily in treating estrogen receptor-positive breast cancer. It functions by binding to estrogen receptors (ER-α), blocking the proliferative effects of estrogen on tumor cells. In this study, a library of Tamoxifen-based analogs was designed to explore potential improvements in therapeutic efficacy. Computational screening revealed that analogs such as Tmx1, Tmx3, and Tmx4 demonstrated strong binding affinities to both HER2 and ER-α receptors, indicating their potential to effectively modulate cancer-related signaling pathways. Molecular dynamics simulations further confirmed the stability and persistence of these interactions within a biological environment, supporting their viability as future chemotherapeutic agents. On the other hand, Mitomycin is a potent anti-cancer compound known for its DNA cross-linking capabilities, which inhibit DNA replication and promote cancer cell apoptosis. It is especially valuable in cases resistant to conventional chemotherapy. In this study, novel Mitomycin analogs were developed and evaluated for their pharmacological and toxicological profiles using in silico screening. These analogs exhibited strong interactions with cancer-related target proteins, particularly those implicated in breast cancer progression. Furthermore, molecular dynamics simulations demonstrated the long-term stability of some Mitomycin analogs, suggesting that they could retain their effectiveness in vivo, potentially reducing the need for high dosages and minimizing adverse side effects. In summary, the newly developed Tamoxifen and Mitomycin analogs represent promising candidates for breast cancer treatment, particularly in resistant cases. The combination of receptor-specific targeting (HER2 and ER-α) with the DNA-targeting mechanism of Mitomycin offers a powerful therapeutic strategy. This integrative approach may pave the way for more effective, targeted, and less toxic treatments, ultimately improving patient outcomes and contributing to the advancement of personalized cancer therapies.

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