

# Molecular Docking and *in silico* Pharmacological Screening of Oleosin from *Cocos Nucifera* Complexed with Tamoxifen in Developing Potential Breast Chemotherapeutic Leads

Janee Myles D. Dela Cruz<sup>1</sup>, Sophia Allison A. Dones<sup>1</sup>, Rianne C. Villanueva<sup>1</sup>, Alexis M. Labrador<sup>1</sup>, Myla R. Santiago-Bautista<sup>1,2\*</sup>

## Abstract

**Objective:** Tamoxifen is a widely used drug for breast cancer therapy; however, concerns and controversies regarding its efficiency arise as it induces various side effects, including endometrial cancer. This study aimed to assess the application of Oleosin as a potential protein carrier of Tamoxifen by evaluating the pharmacokinetic and pharmacological properties of Tamoxifen and determining its intermolecular interactions with Oleosin through *in silico* techniques. **Methods:** The pharmacokinetic and pharmacological properties of Tamoxifen were assessed by using predictive applications such as SwissADME, PaccMann, and Way2Drug. On the other hand, Oleosin does not have a crystal structure in PDB. Thus, homology modeling was done through SWISS-MODEL to obtain a structure. The interactions between Oleosin (Accession no.: AZZ09171.1) and Tamoxifen (PubChem ID: 2733526) were studied by performing molecular docking using AutoDock4 to determine their feasibility as breast cancer drug combinations. **Result:** The chosen structure of Oleosin from the homology modeling resulted in an RMSD of 1.80Å. Tamoxifen was predicted to have the highest activity in MCF7 cell lines, direct interaction with cytochrome enzymes, mediated interaction with estrogen receptors and tyrosine-protein kinase FYN, and low toxicity hazards based on the acute rat toxicity assay. It has lowest binding affinity of -5.26 kcal/mol. The hydrophobic (Ala106, Leu77, Ile80, Val84, and Tyr81) and electrically charged (Lys107 and Asp108) amino acids were critical in binding in the Oleosin-Tamoxifen-complex. Heatmap revealed that phenyl, ether, amine, and alkenyl are the functional groups involved in the receptor-ligand interactions. **Conclusion:** The application of Oleosin as a potential drug carrier was demonstrated by assessing the intermolecular interactions between the Tamoxifen and Oleosin through molecular docking. The properties of Tamoxifen revealed that the molecular targets impact the efficiency and the mechanism of action of the drug. This can also be the basis for investigating and determining the serious adverse effects induced by the drug.

**Keywords:** Breast cancer- oleosin- tamoxifen- molecular docking- Estrogen receptor

*Asian Pac J Cancer Prev*, 23 (7), 2421-2430

## Introduction

Breast cancer is the most commonly diagnosed cancer among women worldwide, accounting for approximately 25% of all reported cancers (Wu & Lee, 2019). The World Health Organization (WHO) reported in 2021 that over 2.3 million women worldwide had breast cancer, and 685,000 were deceased in the year 2020. In 2012, a total of more than 600,000 new reported cases of breast cancer in Asia were accountable for 39% of the entire global incidence of breast cancer. According to these statistics, breast cancer is indeed one of the major threats among Asian women, making up for 21.2% of all cancer records in women (GLOBOCAN, 2012). According to the Philippine Cancer Society (PCS) breast cancer is the third leading cause of

cancer among Filipinos, with 20,267 new cases reported in 2015 and an estimated 7,384 deaths (Laudico et al., 2015). The development of breast cancer occurs when there is a growth of unregulated cells within any part of the breast. Around 70% of patients with breast cancer are estrogen receptor (ER) positive and benefit from chemoprevention drugs that are endocrine therapies such as Tamoxifen. However, Tamoxifen has posed potential risks and concerns regarding its efficiency as it elevates various side effects such as endometrial cancer (Fisher et al., 2001a). Tamoxifen is an ER antagonist and an aromatase inhibitor, which blocks estrogen production (Zhu et al., 2018; Ma et al., 2009). It is a selective estrogen receptor modulator (SERM) drug used to reduce the development of breast cancer (Winters et al., 2017).

<sup>1</sup>Department of Biochemistry, Faculty of Pharmacy, University of Santo Tomas, Manila Philippines. <sup>2</sup>Research Center for Natural and Applied Sciences, University of Santo Tomas, Manila, Philippines. \*For Correspondence: mrsantiago@ust.edu.ph

Tamoxifen is a triphenylethylenic antiestrogen with a high affinity for the ER and a binding affinity comparable to the microsomal antiestrogen binding site and inhibiting micromolar efficiency, protein kinase C calmodulin-dependent enzymes, and acyl-coenzyme A: Cholesterol acyltransferase (Medina et al., 2004).

*Cocos nucifera*, commonly known as coconut, is the fruit of a palm tree described as the tree of life. The Philippines is considered the second-largest producer of coconut worldwide since it is the most prominent harvest in the country. The oil produced from the coconut fruit has been a topic of interest for most researchers, and several studies have proven its benefits and applications in the pharmaceutical industry (Castillo & Ani, 2019; Ignacio & Miguel, 2021). Oil bodies (OBs), also known as oleosomes, are found in almost all plant tissues and are primarily triacylglycerols (Nikiforidis, 2019). An OB's core consists of hydrophobic triglycerides surrounded by a single layer of phospholipids containing three amphiphilic proteins: Oleosin, caleosin, and steroleosin.

Furthermore, OBs mainly consist of Oleosins, which are about 90% of the structural proteins that participate in the stability and function of OBs (Chen et al., 2019; Maurer et al., 2013). Oleosin are small proteins that consist of amphipathic N- and C- terminal sequences and a preserved central hydrophobic domain. The horizontally arranged amino acid sequence of Oleosin's C- terminal on the surface of OBs can induce alpha ( $\alpha$ ) helices. At the same time, the central domain comprises 72 nonpolar amino acids. These 33 amino acid residues of the said terminal end are likely to produce an attraction with charged species such as phosphate and choline on OBs.

Moreover, the length of the central hydrophobic domain of Oleosin is sufficient to form a hairpin structure that extends to the phospholipid layer until the matrix of the OBs, thereby stabilizing the entire OB. The hairpin structure has a single serine and three proline amino acid residues. It also contains two arms, both possessing 30 amino acids that interact with the highly conserved Pro knot, which holds 12 residues of PX5SPX3P, X being a very nonpolar residue, P as a proline amino acid, and S as a serine amino acid (Huang et al., 2013; Huang, C. & Huang A., 2017). Overall, it is presented that Oleosin performs a significant role in providing the stability that is important to the structure and function of OBs.

Due to the safety and stability of Oleosin from OBs, it may be a potential protein drug carrier of Tamoxifen. However, its capability as a drug carrier has not been extensively studied. Several studies, nonetheless, investigated the efficiency of Oleosin as a drug carrier, where it enhanced skin absorption among wounded rodents (Cai et al., 2018). A study also revealed that regeneration of tissues and wound healing had been improved through transdermal drug delivery due to the hEGF linked to an OB (Qiang et al., 2018). OBs have been investigated to determine their efficiency as a drug delivery system encapsulating various anticancer drugs (Acevedo et al., 2014; Aliman et al., 2021). However, utilizing and investigating Oleosin from OBs as a protein drug carrier for breast chemotherapeutics is not yet extensively studied. In addition, silico studies

investigating the effectiveness of Oleosin as a potential drug carrier have yet to be reported and explored. Thus, the aim of this *in silico* study was to assess the feasibility and application of Oleosin as a potential protein carrier of Tamoxifen by evaluating the pharmacokinetic and pharmacological properties of Tamoxifen and determine its intermolecular interactions with Oleosin through predictive software such as SwissADME, PaccMann, and Way2Drug, as well as AutoDock 4 for molecular docking. Moreover, this study did not focus on drug release and cell penetration mechanisms.

## Materials and Methods

### *In Silico Screening of Tamoxifen I*

The assessment of the drug's bioactivity was executed by introducing the Simplified Molecular-Input Line-Entry System (SMILES) sequence of Tamoxifen through online software, SwissADME® (<https://swissadme.ch>). The findings such as the Brain or IntestinaL estimated permeation method (BOILED-Egg) model, absorption, distribution, metabolism, and excretion (ADME) results, as well as the bioavailability radar were analyzed to determine the pharmacokinetics of Tamoxifen. Way2Drug (<http://www.way2drug.com/passonline/>), on the other hand, along with its embedded services such as Cell-Line Cytotoxicity Predictor (CLC-Pred), acute rat toxicity, and Prediction of Activity Spectra for Substances (PASS) Targets, were manipulated to predict the interaction with tumor and non-tumor cell lines, acute rat toxicity for four administration routes (intraperitoneal, intravenous, oral and subcutaneous), and projection of interaction with molecular targets, respectively. The SMILES sequence or Two-Dimensional (2D) structure of Tamoxifen taken from PubChem was introduced into the software for each service and immediately proceeded to the predictions. Afterward, a table of results for every service was generated, and each finding was taken into account by considering the Probability of Activeness (Pa), level of toxicity, and confidence scores. Also, Tamoxifen underwent a prediction through a web service called PaccMann (<https://ibm.biz/paccmann-aas>). Tamoxifen was selected in the drop-down drug list of the web service, and its corresponding SMILES sequence was shown on the interface. After proceeding to the prediction, model decipherability and evaluation were made possible through the generated attention heatmap, which displayed the intensities of the parts of the compound through a color map.

### *Molecular Docking 2.0*

#### *Preparation of Receptor and Ligand 2.1*

Up until this date, there is no existence of Three-Dimensional (3D)/crystal structures and Protein Data Bank (PDB) entry for Oleosin from coconut OBs; only primary and secondary structures were discovered, formulated, and studied. Herewith, the preparation of the receptor was executed through structure prediction in the form of homology modeling to visualize and match the protein sequence of Oleosin from *Cocos nucifera* (Accession no.: AZZ09171.1) that was obtained from

the National Center for Biotechnology Information (NCBI). The Fast-All (FASTA) sequence of Oleosin was submitted in SWISS-MODEL (<https://swissmodel.expasy.org/>) to construct a series of model results with various characteristics. Once the inspection of the individual results was performed, the 3D structure model with the highest sequence identity (>25%), ideal molecule size (< 2 (Å) Angstroms), and favored Ramachandran plots were chosen and downloaded as a PDB file format. On the other hand, the ligand preparation was done by taking the SDF format of Tamoxifen (PubChem ID: 2733526) from PubChem, incorporating it in BIOVIA Discovery Studio, and finally saving it as a PDB file. Prior to molecular docking, the energy of Oleosin and Tamoxifen was reduced via loading their PDB structures to the University of California, San Francisco (UCSF) Chimera (ver 1.15) and setting the steps of energy minimization to one thousand (1,000), whereas other parameters remained in default.

### Docking 2.2

Following the energy minimization step, the PDB structures of Oleosin and Tamoxifen were converted to a Protein Data Bank with partial charges' Q', AutoDock 4 atom types, T (PDBQT) file and were imported into the AutoDock 4.0 interface for molecular docking. Considering that there is no previous knowledge regarding the location of the binding site of Oleosin, blind docking was operated, and the grid box was set in a way that it surrounded the entire protein to calculate its target site. The parameters of the grid box were set into 0.719 Å for spacing, 50 points for x-dimension, 60 points for y-dimension, and 40 points for z-dimension. The criteria were then fixed in Grid Parameter File format, and the AutoGrid program was launched. The ligand docking parameters were settled in default condition to prepare for the docking procedure, while the search parameter was determined as a Genetic Algorithm (GA). The number of GA runs was 200, and other docking criteria were fixed via default setting. The AutoDock program was then initiated after the output was set as Lamarickian GA and saved as Dynamic Process Format. After the docking process, the binding affinities and conformations and several other vital results were analyzed and visualized by subjecting the docked compounds in BIOVIA Discovery Studio.

## Results

### Pharmacokinetics of Tamoxifen 1

The SwissADME predictions showed the BOILED-Egg model (Figure 1), ADME results (Figures 2 and 3), and bioavailability radar (Figure 3) of Tamoxifen. It can be observed that Tamoxifen is situated outside the model of the BOILED-Egg, denoting that it is not permeable and is poorly absorbed in the Blood-Brain Barrier (BBB) and the gastrointestinal tract (GIT). Based on the blue dot representing Tamoxifen, it is a substrate and is actively effluxed by P-glycoprotein (Pgp). Moreover, the topological polar surface area (TPSA) (Figure 2A), a physicochemical property, revealed that Tamoxifen only acquired 12.47 square angstrom (Å<sup>2</sup>). While the lipophilicity outputs (Figure 2B) had positive logP values, indicating that the drug is lipophilic. It was also shown that Tamoxifen is poorly soluble (Figure 2C) since it obtained numerical values ranging from -10 to -6. This then confirms that Tamoxifen is certainly a lipophilic drug as it has low water solubility and poor BBB and GIT penetration. On the other hand, the pharmacokinetic findings (Figure 2D) reported that Tamoxifen is a P-gp substrate and is not adequately absorbed in GIT and BBB, confirming the generated BOILED-Egg model. Additionally, the drug is an inhibitor of the two cytochrome P450 enzymes. The Druglikeness output (Figure 2E) of Tamoxifen revealed that it only violated one of Lipinski's five rules. The bioavailability of the drug is at 0.55, meaning it meets Lipinski's rule and is considered an excellent oral medicine according to the Abbot bioavailability score. The medicinal chemistry (Figure 2F) demonstrated that Tamoxifen did not satisfy lead likeness since it had three violations based on the software's criteria. Further, the bioavailability radar (Figure 3) disclosed that Tamoxifen acquired optimal results except for lipophilicity. Nevertheless, it is still considered a good orally bioavailable drug.

### Pharmacological Properties of Tamoxifen 2 Cancer Cell Line Prediction 2.1

The cancer cell line prediction by Way2Drug (Table 1) revealed different cancer cell lines that may form an interaction with Tamoxifen. The drug has the highest activity in MCF-7 breast cancer cell lines with

Table 1. CLC-Prediction Results of Tamoxifen from Way2Drug

Pa	Cell Line	Cell Line Full Name	Tissue	Tumor Type
0.811	MCF-7	Breast carcinoma	Breast	Carcinoma
0.573	SK-OV-3	Ovarian carcinoma	Ovarium	Carcinoma
0.469	T47D	Breast carcinoma	Breast	Carcinoma
0.374	HOP-92	Non-small cell lung carcinoma	Lung	Carcinoma
0.333	MCF7S	Breast carcinoma	Breast	Carcinoma
0.332	SNB-75	Glioblastoma	Nervous system	Glioblastoma
0.327	RPMI-8226	Multiple myeloma	Haematopoietic and lymphoid tissue	Myeloma

† Pa, probability of activeness; High Pa values indicate that a substance is highly active in a specific cell line; MCF-7, Michigan cancer foundation-7; SK-OV-3, ascites-derived ovarian cancer cell line from a 64-year old Caucasian female with an ovarian serous cystadenocarcinoma; T47D, Human breast cancer cells; HOP-92, Hopkins-92; MCF7S, parent MCF-7 subline; SNB-75, Surgical Neurology Branch-75; RPMI-8226, human B cells from a 61-year old male patient with myeloma/plasmacytoma

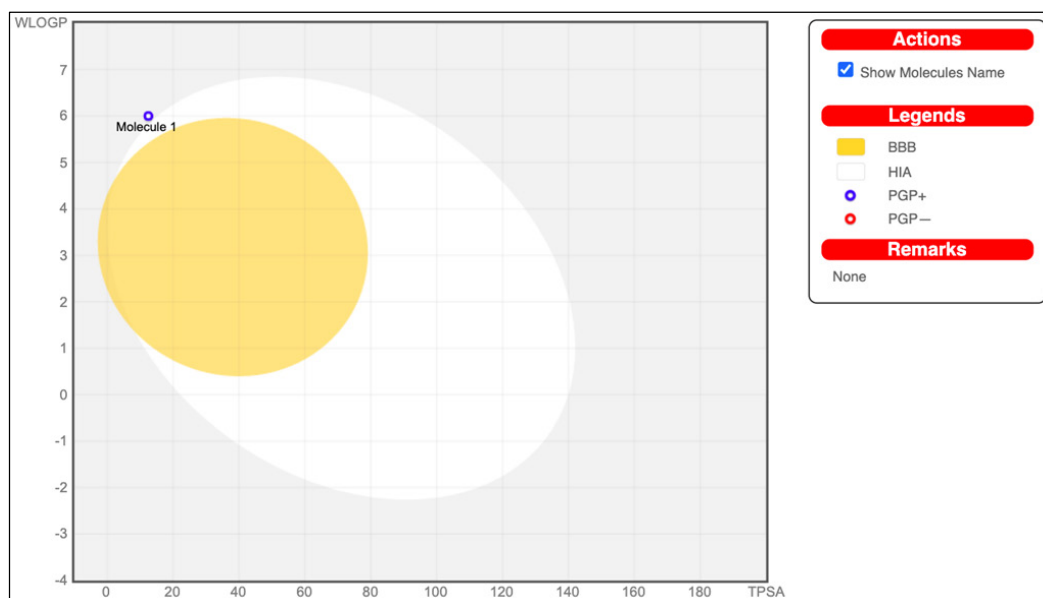


Figure 1. BOILED-Egg Model of Tamoxifen. The yellow or the yolk represents the Blood-Brain Barrier. The white area represents human gastrointestinal absorption (HIA).

Table 2. Acute Rat Toxicity results of Tamoxifen\*

Rat IP LD50 Classification	Rat IV LD50 Classification	Rat Oral LD50 Classification	Rat SC LD50 Classification
Class 4 in AD	Class 3 in AD	Class 5 in AD	Class 5 in AD

† IP, intraperitoneal route of administration; IV, intravenous route of administration; Oral, oral route of administration; SC, subcutaneous route of administration; in AD, compound falls in applicability domain of models; \*Tabulated results are based on Organisation for Economic Co-operation and Development (OECD) project from Way2Drug.

a probability of activeness (Pa) value of 0.811. While other predicted tumor cell lines, namely SK-OV-3 ovarian carcinoma, T47D breast carcinoma, HOP-92 non-small

cell lung carcinoma, MCF7S breast carcinoma, SNB-75 glioblastoma, RPMI-8226 multiple myeloma, possessed Pa values lower than 0.5. With this, if the selected group

<b>Physicochemical Properties</b> Formula C <sub>26</sub> H <sub>29</sub> NO Molecular weight 371.51 g/mol Num. heavy atoms 28 Num. arom. heavy atoms 18 Fraction Csp <sup>3</sup> 0.23 Num. rotatable bonds 8 Num. H-bond acceptors 2 Num. H-bond donors 0 Molar Refractivity 119.72 TPSA 12.47 Å <sup>2</sup>		<b>Lipophilicity</b> Log P <sub>OW</sub> (ILOGP) 4.64 Log P <sub>OW</sub> (XLOGP3) 7.14 Log P <sub>OW</sub> (WLOGP) 6.00 Log P <sub>OW</sub> (MLOGP) 5.10 Log P <sub>OW</sub> (SILICOS-IT) 5.99 Consensus Log P <sub>OW</sub> 5.77	
<b>Water Solubility</b> Log S (ESOL) -6.59 Solubility 9.56e-05 mg/ml ; 2.57e-07 mol/l Class Poorly soluble Log S (Ali) -7.22 Solubility 2.23e-05 mg/ml ; 5.99e-08 mol/l Class Poorly soluble Log S (SILICOS-IT) -8.92 Solubility 4.48e-07 mg/ml ; 1.21e-09 mol/l Class Poorly soluble		<b>Pharmacokinetics</b> GI absorption Low BBB permeant No P-gp substrate Yes CYP1A2 inhibitor No CYP2C19 inhibitor Yes CYP2C9 inhibitor No CYP2D6 inhibitor Yes CYP3A4 inhibitor No Log K <sub>p</sub> (skin permeation) -3.50 cm/s	
<b>Druglikeness</b> Lipinski Yes; 1 violation: MLOGP>4.15 Ghose No; 1 violation: WLOGP>5.6 Veber Yes Egan No; 1 violation: WLOGP>5.88 Muegge No; 1 violation: XLOGP3>5 Bioavailability Score 0.55		<b>Medicinal Chemistry</b> PAINS 0 alert Brenk 1 alert: stilbene Leadlikeness No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5 Synthetic accessibility 3.01	

Figure 2. The ADME Results of Tamoxifen. This includes the (A) Physicochemical properties, (B) Lipophilicity, (C) Water Solubility, (D) Pharmacokinetics, (E) Druglikeness, and (F) Medicinal Chemistry.



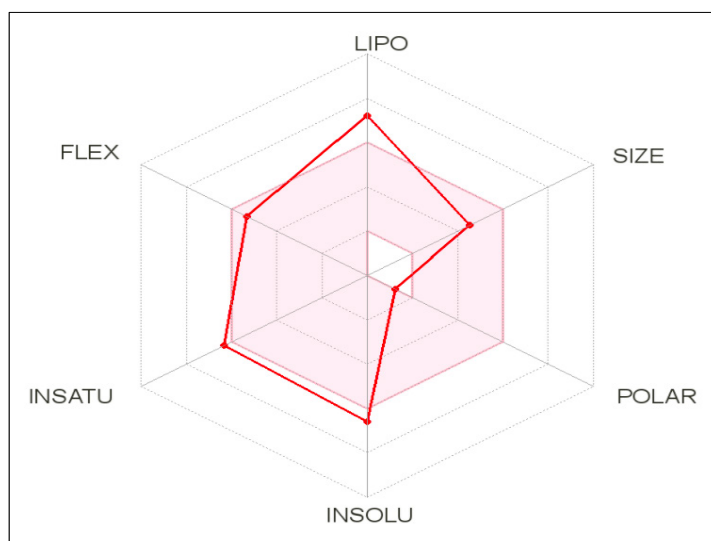


Figure 3. The Bioavailability Radar of Tamoxifen.

Table 3. PASS Target (Direct interaction) Results of Tamoxifen from Way2Drug.

Target Name	Confidence	ChEMBL id
Cytochrome P450 3A5	0.9031	CHEMBL3019
Cytochrome P450 2B6	0.833	CHEMBL4729
Proteasome component C5	0.5848	CHEMBL4208
Cytochrome P450 2J2	0.4877	CHEMBL3491
Proteasome Macropain subunit	0.4632	CHEMBL3492

†, Highest confidence values indicate that a substance is highly interactive in a certain molecule.

Table 4. PASS Targets (Mediated interaction) Results of Tamoxifen from Way2Drug

Target name	Confidence	ChEMBL id
Tyrosine-protein kinase FYN	0.9774	CHEMBL1841
Estrogen receptor	0.8846	CHEMBL2093866
Estrogen receptor alpha	0.7329	CHEMBL206
Sphingomyelin phosphodiesterase	0.5505	CHEMBL2760
Alpha-2b adrenergic receptor	0.4839	CHEMBL1942

†, Highest confidence values indicate that a substance is highly interactive in a certain molecule.

of compounds has a more excellent value of Pa, acquiring inaccurate results from biological testing is reduced (Basanagouda et al., 2011).

#### Acute Rat Toxicity Prediction 2.2

The acute rat toxicity results are based on the categories or classes set by Globally Harmonized

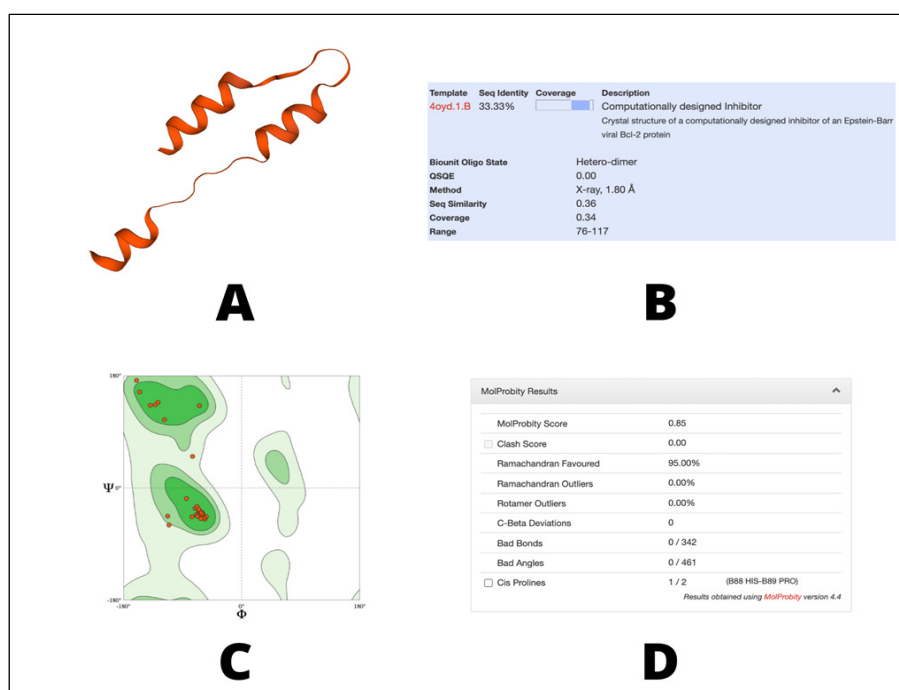


Figure 4. Results of Homology Modeling from SWISS-MODEL. (A) 3D/Crystal structure of Oleosin. (B) Information about the structure of the generated model. (C) and (D) Ramachandran plots of the model. The 3D model of Oleosin from chain B of Epstein-Barr viral Bcl-2 protein was selected as it produced an acceptable value of sequence identity, sequence similarity, molecule size, and is Ramachandran favored.

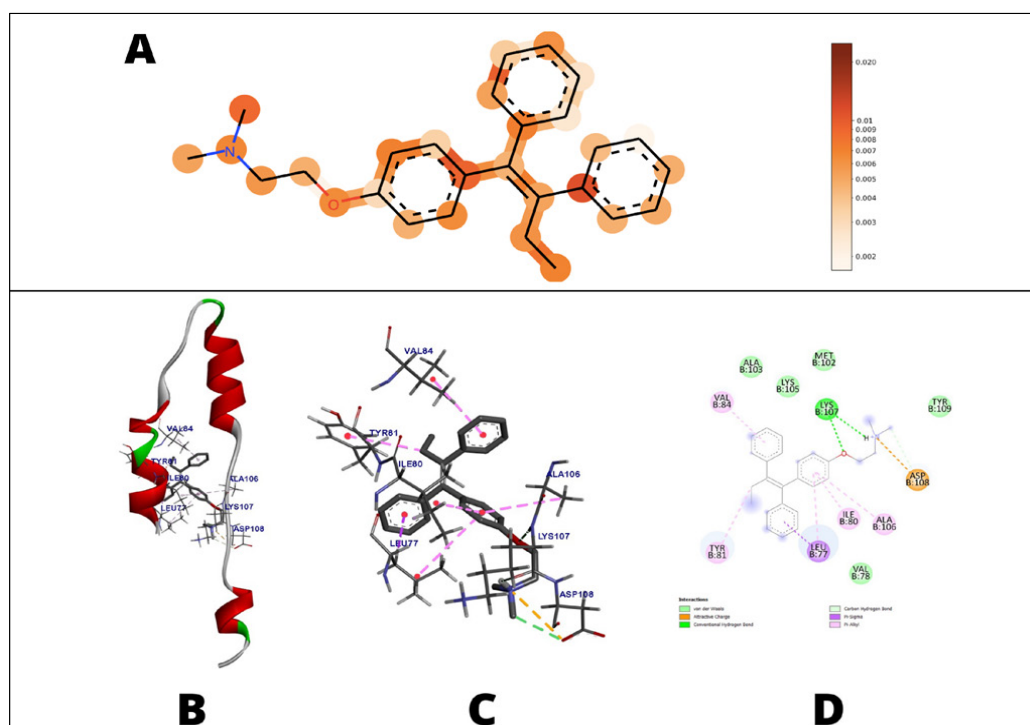


Figure 5. Predicted Molecular Interactions of Tamoxifen with Oleosin based on PaccMann Webserver and AutoDock 4.0 software. (A) Heatmap of Tamoxifen from PaccMann. Results showed the most and least interactive functional groups of Tamoxifen based on the intensity of the colors. Docking results from AutoDock 4 and BIOVIA Discovery Studio. (B) the best docking pose of Tamoxifen. (C) Close-up 3D visualization of Tamoxifen and Oleosin interaction. (D) 2D diagram of the receptor-ligand interaction with the formed bonds. Oleosin and Tamoxifen were successfully docked with -5.26 kcal/mol binding energy.

System for chemical substances and mixtures which cause acute toxicity established by the United Nations and Organisation for Economic Co-operation and Development (OECD). Based on the findings in Table 2, in terms of delivery route, Tamoxifen was determined to be practically nontoxic (class 4) for intraperitoneal administration, slightly toxic (class 3) for intravenous administration, and relatively harmless (class 5) for oral administration as well as for subcutaneous administration.

### Molecular Targets Prediction 2.3

Tamoxifen is a competitor of estrogen for binding sites and directly inhibits the ER. However, the results of PASS Targets (Table 3 & 4) revealed that cytochrome P450 enzymes, specifically cytochrome P450 3A5 and cytochrome P450 2B6, are the direct targets of Tamoxifen, while the ER and Estrogen Receptor Alpha (ER $\alpha$ ) were only in the list of possible targets. Based on the PASS Targets for mediated interactions of Tamoxifen (Table 4), the tyrosine-protein kinase FYN has the highest confidence level among the targets, with a value of 0.9774. In contrast, other mediated interactions such as estrogen receptor, estrogen receptor alpha, sphingomyelin phosphodiesterase, and alpha-2b adrenergic receptor acquired lower confidence values.

### Model Structure of Oleosin 3

In the results generated by the homology modeling of Oleosin in SWISS-MODEL (Figure 4), the model with the highest and most favorable sequence identity of 33.3%, the sequence similarity of 36%, and molecule size of 1.80

Å (Figure 4B) among the templates was from the B chain of computationally designed inhibitor of an Epstein-Barr viral Bcl-2-protein. The model (Figure 4A) only converted 34% of the Oleosin sequence with 41 residues (ALVWIYN YVMGKHPPGADRLDAARAAMARKAKDYGRYVQ TKS), ranging from amino acids in 76-117 positions. The model is also Ramachandran favored and has no outliers, bad angles, and bad bonds (Figures 4C & 4D).

### Predicted Moieties for the Interaction of Tamoxifen 4

The in silico screening of Tamoxifen using PaccMann revealed the heat map wherein the functional groups of Tamoxifen (Figure 5A) were shown in a certain intensity of heat or red color. The moieties involved in interacting with other compounds were the drug's phenyl, ether, amine, and alkenyl functional groups.

### Molecular Docking of Tamoxifen and Oleosin 5

The calculated binding affinity and interactions formed by the Tamoxifen-Oleosin complex were determined using a molecular docking simulation. The docked complex structure (Figures 5B and 5C) of Tamoxifen and Oleosin and the formation of different interactions (Figure 5D) were shown. Based on the docking results, the best docking pose had low binding energy of -5.26 kcal/mol. The 3D and 2D diagrams of the receptor-ligand interactions revealed that the involved amino acids were mostly hydrophobic and electrically charged in nature. Moreover, the docking pose identified the amino acids that were capable of interacting with Oleosin were alanine (106), leucine (77), isoleucine (80), and valine (84),

tyrosine (81), lysine (107), and aspartic acid (108).

## Discussion

Tamoxifen, an FDA-approved drug, is currently facing controversies regarding its treatment for patients with breast cancer due to its adverse side effects, induced drug resistance, and endometrial cancer (EBCTCG, 2011; Fisher et al., 2001b). To date, the intermolecular interactions of Oleosin with other molecules and its efficiency as a drug carrier system have not yet been fully explored. The present study used several *in silico* techniques to screen and assess the pharmacokinetic and pharmacological properties of Tamoxifen, which confirmed the challenges of Tamoxifen treatment against breast cancer. Through molecular docking, Oleosin was also evaluated as a potential drug carrier by determining its intermolecular interactions with Tamoxifen.

The pharmacokinetics of Tamoxifen were investigated through SwissADME. The absorption of Tamoxifen into the BBB and human intestine (HI) was demonstrated by the BOILED-Egg model. The BBB and HI are the common areas of absorption for a drug will be able to perform its function in the body. The model also evaluates a drug according to its property to be a substrate or inhibitor of Pgp. This membrane protein is responsible for managing the efflux of substances in cells. The bioavailability and retention time of a drug is affected by its decreased permeability as it is more extruded in the intestine and BBB, leading to drug resistance (Amin, 2013). In addition, chemotherapy is also disrupted due to the efflux of chemotherapeutic drugs due to overexpression of Pgp in cancer cells (Martins et al., 2010). Concerning this, the BOILED-Egg model results showed that Tamoxifen is a substrate of Pgp, which then depicts that it is effluxed by the cells. This then verifies the result of Tamoxifen that it is not within the range of the model; thus, it is not absorbed in BBB and HI. This also validates the Tamoxifen resistance as it is actively effluxed in cells. Furthermore, Lipinski's rule of five was used to evaluate the drug-likeness of Tamoxifen, as this parameter determines the physicochemical and drug-like properties of a compound (Lipinski et al., 1997). The ADME results showed that Tamoxifen only violated one rule from Lipinski's rule of five, making it an excellent oral drug. Moreover, the bioavailability radar (Figure 3) revealed that the lipophilicity of Tamoxifen did not meet the optimal range. The toxicity of a drug is due to its high lipophilicity, leading to low absorption and solubility (Gao et al., 2017). These findings depict that prolonged intake of Tamoxifen may impose adverse effects despite being an FDA-approved drug.

The prediction services of Way2Drug determined the pharmacological properties of Tamoxifen. The cancer cell line prediction (Table 1) revealed that Tamoxifen has the highest activity in MCF-7 breast cancer cell lines with a probability of activeness (Pa) value of 0.811. The rest of the predicted tumor cell lines have Pa values below 0.5, indicating that the drug has insignificant interactions with the cell lines. Generally, the mechanism of action of Tamoxifen is that it acts as an antagonist and blocks the

signaling pathway of positive alpha ER in breast cancer cells (Hu et al., 2015). However, Tamoxifen can also be an agonist and proestrogenic, activating the ER on endometrial cells, hence the rising concerns regarding the efficiency of the drug for cancer therapeutics (Sporn & Lippman, 2003). Due to its different effects on ER, Tamoxifen is appropriately labeled as a selective estrogen receptor modulator. The differences observed in Pa of the predicted breast carcinoma models such as MCF-7, T47D, and MCF-7S are likely due to the specific receptors contained in these cancer cells. MCF-7 cancer cell lines are susceptible and reliant on estrogen to multiply (Comşa et al., 2015). On the other hand, the T47D are progesterone-sensitive breast carcinoma models (Yu et al., 2017) and most MCF-7S cancer cell lines are Tamoxifen-resistant (Leung et al., 2014). With this, Tamoxifen is indeed highly active on MCF-7 cell lines as its primary role is to treat ER-positive breast cancer cells. Further, the categories/classes of the acute rat toxicity results are divided into five, where class 1 represents the most severe toxicity, and class 5 represents substances with low acute toxicity hazards but may present a danger to vulnerable populations (GHS, 2015; OECD, 2002). Tamoxifen presented class 4 toxicity through the intraperitoneal route of administration, class 3 toxicity through the intravenous route of administration, and class 5 toxicity to both oral and subcutaneous routes of administration. This indicates that Tamoxifen can be hazardous when injected into veins and may induce severe adverse effects. The administration through the abdominal cavity only possesses light toxicity, and the usual administration of the drug (oral) only has low acute toxicity and the subcutaneous route. This indicates that the drug may be relatively nontoxic when taken through the mouth and under or beneath the entire skin layers. According to the DrugBank, the acute rat toxicity of Tamoxifen is 1.9882 mol/kg LD50, which indicates and confirms that the drug has low toxicity based on the GHS classifications of hazardous materials (DrugBank Online Database, 2022).

The acquired results from PASS Target (Tables 3 and 4) indicate that the cytochrome enzymes metabolize Tamoxifen to its active form to promote its antiestrogenic effects (Singh et al., 2011). This then explains why the ER and ER $\alpha$  are predicted to have only a mediated interaction with Tamoxifen and are not necessarily regarded as ineffective. Tamoxifen undergoes oxidative metabolism to turn into its active metabolites, 4-hydroxytamoxifen and 4-hydroxy-N-desmethyl-tamoxifen, commonly known as afimoxifen and endoxifen, respectively (Serrano et al., 2011). Since they are the active metabolites, it has a higher affinity for ER rather than Tamoxifen itself. Moreover, endoxifen obtains a steadier plasma concentration state than afimoxifen. Thus it is commonly considered in investigating Tamoxifen's efficiency in clinical trials (Stearns et al., 2003; Wu et al., 2009). In addition to this, upon the metabolism of Tamoxifen in the liver, the active metabolite like 4-hydroxytamoxifen binds to the ER and makes a complex. The complex then activates corepressor proteins involved in regulating various genes in cancer (Ali et al., 2016; Wong et al., 2014; Shang et al., 2000). The anticancer effects of Tamoxifen are also

aided by Paired Box 2 (PAX2) protein to inactivate Erb-B2 Receptor Tyrosine Kinase 2 (ERBB2), a protein that promotes proliferation (Hurtado et al., 2008). Furthermore, tyrosine-protein kinase FYN was found to have the highest confidence level in the PASS Targets mediated interactions because tyrosine kinases play a role in many cellular pathways and signal transduction to induce cell growth and differentiation and apoptosis (Azevedo et al., 2019). It selectively phosphorylates tyrosine residues of various protein substrates, resulting in a change in activity and function in biological responses. The results specifically state that Tamoxifen possibly targets tyrosine-protein kinase FYN, wherein FYN is a part of the non-receptor tyrosine kinases (NRTKs) under the Src family (Nygaard et al., 2014). NRTKs are located in the cytoplasm of the cells and transduce intracellular signals for the FYN gene. The FYN gene's function depends on the interactions inside the cell, which includes the phosphorylation of tyrosine and is involved in cell adhesion and proliferation (Siveen et al., 2018). This then supports the predicted results for the direct and possible targets of Tamoxifen, as all the biomolecules involved have a vital role in cell proliferation in breast tissues. The PASS Target results also support the mechanism of action of Tamoxifen.

The structure of Oleosin contains amphipathic peptides in both amino-terminal and carboxyl-terminal sequences (Barre et al., 2018). The length of the terminal domains is dependent on the type of Oleosin isoforms, and the main domain is highly conserved (Huang et al., 2013). In addition, an alpha-helical structure is also formed by the carboxyl-terminal sequence, which interacts with the molecules present in the phospholipid layer of the OBs in plants. The central domain of Oleosin consists of a proline knot that contains 12 residues in total, which creates a loop and consists of  $PX_5SPX_3P$ , wherein P is a Proline residue, S is a Serine residue, and X is a nonpolar residue (Fang, 2014). The primary domain creates two alpha-helical structures with turn regions (Nikiforidis, 2019). Based on the results presented in Figure 4, Oleosin's established secondary structure, wherein the N- and C-terminal sequences contain amino acids with amphipathic side chains, and both formed alpha-helix structures. However, the central domain did not have a proline knot. The structure is only a model for Oleosin and did not come from other Oleosin isoforms yet created two alpha-helical structures separated by turn regions. Nevertheless, the selected model is Ramachandran favored and has no outliers, bad angles, and bad bonds.

The docking results showed hydrophobic and electrically charged amino acids present in the interaction of the Tamoxifen-Oleosin complex. The 2D diagram (Figure 5D) of the receptor-ligand interactions depicted that there were different interactions formed. There were two electrically charged amino acids, namely, aspartic acid, which has an attractive charge interaction with the amine functional group of Tamoxifen, and lysine, which formed a conventional hydrogen (H) bond with the ether functional group and the H atom from the amine functional group. Furthermore, the following amino acids formed hydrophobic interactions: leucine, which formed a pi-sigma bond with the phenyl functional

group, and alanine, isoleucine, and valine, which formed a pi-alkyl bond with the other phenyl functional groups. Additionally, similar amino acids also formed van der Waals (vdW) with Tamoxifen. Lastly, tyrosine formed a pi-alkyl bond with Tamoxifen's alkyl functional group. The interactions involved with the receptor-ligand were able to support the generated heat map of Tamoxifen from PaccMann. The notable interactions between Tamoxifen and Oleosin were vdW, pi-alkyl, and conventional H-bond. vdW was the most prominent interaction observed in the docked Tamoxifen-Oleosin complex, with five amino acids, namely, Ala103, Lys105, Met102, Tyr109, and Val78. Even though vdW is considered the weakest type of intermolecular force, its abundance may produce a strong force (Than, 2020). The formation of vdW is caused by fluctuations in the polarizations of two molecules adjacent to each other. With this, the formation of vdW in Met102 may be caused by its polarizable sulfur atom and hydrophobicity. On the other hand, the evident interactions formed by pi-alkyl with several amino acids, such as Val84, Tyr81, Ile80, and Ala106, add to the ligand's conformational stability as well as improve its hydrophobic interaction in the binding pocket of a receptor (Arthur & Uzairu, 2019; Arthur et al., 2021). Pi-alkyl bonds between the aromatic group and charged species of a molecule contribute to multiple protein functions such as folding and its structural and biological role (Ribas et al., 2002). According to Tsuzuki et al. (2008), the attraction in conventional H-bonds is due to electrostatic interactions. Lys107 is one of the amino acids that formed a conventional H-bond because of the positively charged  $\epsilon$ -amino group, which induces electrostatic force found in binding sites of protein (Betts et al., 2003). Moreover, the conventional H-bond provides structural rigidity and specificity due to its intermolecular interactions. Since conventional H-bond formation is considered the most vital type of interaction, the Tamoxifen-Oleosin complex may consider when constructing its optimal structure. Pi-sigma and attractive charge interactions were also observed between Tamoxifen and Oleosin. Determining the interactions of the protein and the drug is essential in investigating the ligand affinity associated with the protein's structural features (de Azevedo et al., 2009).

In conclusion, the study assessed the pharmacokinetics and pharmacological properties of Tamoxifen using various online prediction services. The application of Oleosin as a potential drug carrier was demonstrated by assessing the intermolecular interactions between the Tamoxifen and Oleosin through molecular docking. Furthermore, the properties of Tamoxifen revealed that the molecular targets have an impact on the efficiency and the mechanism of action of the drug. This can also be the basis for investigating and determining the serious adverse effects induced by the drug, along with molecular docking, additional *in silico* experiments such as molecular dynamics simulations to investigate the stable conformations of the Tamoxifen-Oleosin complex as well as its cell-penetrating and drug-release mechanisms could be performed for the future directions of the study. With the current findings and additional *in silico* experiments, *in vitro* and *in vivo* studies involving nanoencapsulation



using Oleosin could also be performed to improve further the drug carrier system design of the Tamoxifen-Oleosin complex as an alternative leads for breast cancer treatment.

## Author Contribution Statement

JMDD, SAAD, RCV, AML, and MRSB designed the model and the computational framework and analyzed the data. JMDD, SAAD, and RCV carried out the implementation. JMDD, SAAD, and RCV wrote the manuscript with input and guidance from AML and MRSB. MRSB conceived the study and was in charge of the overall direction, planning, and approval of the final version of the manuscript.

## Acknowledgments

The authors would like to thank the academic staff of the internship committee of the Mammalian Tissue Culture Laboratory of the University of Santo Tomas Research Center for Natural and Applied Sciences through the Department of Biochemistry, Faculty of Pharmacy, the University of Santo Tomas for providing the training for the use of in silico tools used in this study.

## Funding Statement

The authors received no financial support for this article's research, authorship, and publication.

## Approval

This study is part of an approved undergraduate thesis that has been approved and accepted as partial fulfillment of the requirements for the degree of Bachelor of Science in Biochemistry at the University of Santo Tomas, Manila, Philippines.

## Ethical Declaration

None.

## Data Availability

The model simulations based on this study are too extensive to archive. Instead, we provide all the information needed to replicate the simulations. Due to confidentiality agreements, supporting data can only be made available to bona fide researchers subject to a non-disclosure agreement. Details of the data and how to request access are available from the corresponding author at the University of Santo Tomas, Department of Biochemistry.

## Study Registration

The study is not registered in any registering dataset.

## Conflict of Interest

The authors declare that they have no competing interests.

## References

Acevedo F, Rubilar M, Jofré, I, et al (2014). Oil bodies as a potential microencapsulation carrier for astaxanthin

stabilization and safe delivery. *J Microencapsul*, **31**, 488–500.

Ali S, Rasool M, Chaoudhry H, et al (2016). Molecular mechanisms and mode of tamoxifen resistance in breast cancer. *Bioinformatics*, **12**, 135.

Aliman PT, Cada RFN, Devanadera MKP, Labrador AM, Santiago-Bautista, MR (2021). Characterization of Purified Coconut Oil Bodies as an Encapsulating Agent for Doxorubicin and Paclitaxel. *Acta Med Philipp*, **55**.

Amin ML (2013). P-glycoprotein Inhibition for Optimal Drug Delivery. *Drug Target Insights*, **7**, 27–34.

Arthur D, & Uzairu A (2019). Molecular docking studies on the interaction of NCI anticancer analogues with human Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit. *J King Saud Univ Sci*, **31**, 1151–66.

Arthur DE, Akoji JN, Sahnoun R, et al (2021). A theoretical insight in interactions of some chemical compounds as mTOR inhibitors. *Bull Natl Res Cent*, **45**, 67.

Azevedo, A., Silva, S., & Rueff, J. (2019). Non-receptor Tyrosine Kinases Role and Significance in Hematological Malignancies. In (Ed.), Tyrosine Kinases as Druggable Targets in Cancer. IntechOpen. <https://doi.org/10.5772/intechopen.84873>

Barre A, Simplicien M, Cassan G, Benoist H, Rougé P (2018). Oil bodies (oleosomes): Occurrence, structure, allergenicity. *Rev Fr Allergol*, **58**, 574-0.

Basanagouda M, Jadhav VB, Kulkarni MV, Rao RN (2011). Computer aided prediction of biological activity spectra: study of correlation between predicted and observed activities for coumarin-4-acetic acids. *Indian J Pharm Sci*, **73**, 88.

Betts MJ, Russell RB (2003). Amino Acid Properties and Consequences of Substitutions. *Bioinformatics for Geneticists*, 289–316.

Cai J, Wen R, Li W, et al (2018). Oil body bound oleosin-rhFGF9 fusion protein expressed in safflower (*Carthamus tinctorius* L.) stimulates hair growth and wound healing in mice. *BMC biotechnology*, **18**, 51.

Castillo MB, Ani PA (2019). The Philippine coconut industry: status, policies and strategic directions for development. FFTC Agri. Pol.

Comşa Ş, Cîmpean AM, Raica M (2015). The Story of MCF-7 Breast Cancer Cell Line: 40 years of Experience in Research. *Anticancer Res*, **35**, 3147–54.

Chen K, Yin Y, Liu S, et al (2019). Genome-wide identification and functional analysis of oleosin genes in *Brassica napus* L. *BMC Plant Biol*, **19**.

Daina A., Michielin O, Zoete V (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep*, **7**, 42717.

de Azevedo J, Walter F, Caceres RA, et al (2009). Protein-drug interaction studies for development of drugs against *Plasmodium falciparum*. *Curr Drug Targets*, **10**, 271-8.

DrugBank Online. (2022). Tamoxifen. Retrieved from <https://go.drugbank.com/drugs/DB00675>

Early Breast Cancer Trialists' Collaborative Group (EBCTCG) (2011). Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant Tamoxifen: patient-level meta-analysis of randomised trials. *The Lancet*, **378**, 771–784.

Fang Y, Zhu RL, Mishler BD (2014). Evolution of oleosin in land plants. *PLoS One*, **9**, e103806.

Fisher B, Dignam J, Bryant J, Wolmark N (2001). Five versus more than five years of Tamoxifen for lymph node-negative breast cancer: updated findings from the National Surgical Adjuvant Breast and Bowel Project B-14 randomized trial.

- J Natl Cancer Inst*, **93**, 684-0.
- Gao Y, Gesenberg C, Zheng W (2017). Oral Formulations for Preclinical Studies: Principle, Design, and Development Considerations. *Developing Solid Oral Dosage Forms, AP*, **2**, 455–495.
- Global Cancer Incidence, Mortality and Prevalence (GLOBOCAN) (2012). Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012 v1.0. Retrieved from <https://publications.iarc.fr/Databases/Iarc-Cancerbases/GLOBOCAN-2012-Estimated-Cancer-Incidence-Mortality-And-Prevalence-Worldwide-In-2012-V1.0-2012>
- Hu R, Hilakivi-Clarke L, Clarke R (2015). Molecular mechanisms of tamoxifen-associated endometrial cancer. *Oncol Lett*, **9**, 1495-1.
- Huang CY, Huang AH (2017). Unique motifs and length of hairpin in oleosin target the cytosolic side of endoplasmic reticulum and budding lipid droplet. *Plant Physiol*, **174**, 2248-60.
- Huang NL, Huang MD, Chen TLL, Huang AH (2013). Oleosin of subcellular lipid droplets evolved in green algae. *Plant Physiol*, **161**, 1862-74.
- Hurtado A, Holmes KA, Geistlinger TR, et al (2008). Regulation of ERBB2 by oestrogen receptor–PAX2 determines response to Tamoxifen. *Nature*, **456**, 663-6.
- Ignacio IF, Miguel TS (2021). Research opportunities on the coconut (*Cocos nucifera* L.) using new technologies. *S Afr J Bot*, **141**, 414-0.
- Laudico AV, Mirasol-Lumague MR, Medina V, et al. (2015). Philippine cancer facts and estimates. Philippine Cancer Society: Manila.
- Leung E, Kim JE, Askarian-Amiri M, et al (2014). Evidence for the Existence of Triple-Negative Variants in the MCF-7 Breast Cancer Cell Population. *Biomed Res Int*, 2014.
- Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev*, **23**, 3–25.
- Ma ZH, Yu DG, Branford-White CJ, et al (2009). Microencapsulation of Tamoxifen: Application to cotton fabric. *Colloids Surf B*, **69**, 85-0.
- Medina PD, Favre G, Poirot M (2004). Multiple targeting by the antitumor drug tamoxifen: a structure-activity study. *Curr Med Chem Anticancer Agents*, **4**, 491-508.
- Maurer S, Waschatko G, Schach D, et al (2013). The Role of Intact Oleosin for Stabilization and Function of Oleosomes. *J Phy Chem B*, **117**, 13872-83.
- Martins A, Vasas A, Schelz ZS, et al (2010). Constituents of *Carpobrotus edulis* Inhibit P-Glycoprotein of MDR1-transfected Mouse Lymphoma Cells. *Anticancer Res*, **30**, 829 LP – 835.
- Nikiforidis CV (2019). Structure and functions of oleosomes (oil bodies). *Adv Colloid Interface Sci*, **274**, 102039.
- Nygaard HB, van Dyck CH, Strittmatter SM (2014). Fyn kinase inhibition as a novel therapy for Alzheimer’s disease. *Alzheimers Res Ther*, **6**, 1-8.
- Organisation for Economic Co-operation and Development (OECD) (2002). Harmonised Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures, OECD J.
- Qiang W, Zhou T, Lan X, et al. (2018). A new nanoscale transdermal drug delivery system: oil body-linked oleosin-hEGF improves skin regeneration to accelerate wound healing. *J nanobiotechnology*, **16**, 1-18.
- Ribas J, Cubero E, Luque FJ, Orozco M (2002). Theoretical Study of Alkyl- $\pi$  and Aryl- $\pi$  Interactions. Reconciling Theory and Experiment. *J Org Chem*, **67**, 7057–65.
- Rye C, Wise R, Jurukovski V, et al (2018). Biology: Atoms, Isotopes, Ions, and Molecules: The Building Blocks. OpenStax. Retrieved from <https://openstax.org/books/biology/pages/2-1-atoms-isotopes-ions-and-molecules-the-building-blocks>
- Serrano D, Lazzeroni M, Zamboni CF, et al (2011). Efficacy of Tamoxifen based on cytochrome P450 2D6, CYP2C19 and SULT1A1 genotype in the Italian Tamoxifen Prevention Trial. *Pharmacogenomics J*, **11**, 100-7.
- Shang Y, Hu X, DiRenzo J, Lazar MA, Brown M (2000). Cofactor dynamics and sufficiency in estrogen receptor–regulated transcription. *Cell*, **103**, 843-52.
- Singh MS, Francis PA, Michael M (2011). Tamoxifen, cytochrome P450 genes and breast cancer clinical outcomes. *The Breast*, **20**, 111-8.
- Siveen KS, Prabhu KS, Achkar IW, et al (2018). Role of non receptor tyrosine kinases in hematological malignances and its targeting by natural products. *Mol Cancer*, **17**, 1-21.
- Sporn MB, Lippman SM (2003). Agents for chemoprevention and their mechanism of action. Holland-Frei cancer medicine. 6. Hamilton: BC Decker.
- Stearns V, Johnson MD, Rae JM, et al (2003). Active tamoxifen metabolite plasma concentrations after coadministration of Tamoxifen and the selective serotonin reuptake inhibitor paroxetine. *J Natl Cancer Inst*, **95**, 1758-64.
- Than, J. (2020). Van Der Waals Interactions. Chemistry LibreTexts. Retrieved from <https://chem.libretexts.org/@go/page/1664>
- Tsuzuki S, Fujii A (2008). Nature and physical origin of CH/  $\pi$  interaction: significant difference from conventional hydrogen bonds. *Phys Chem Chem Phys*, **10**, 2584–94.
- United Nations, Economic Commission for Europe, Secretariat (2015). Globally harmonized system of classification and labelling of chemicals (GHS). Copyright Law of the United Nations.
- Winters S, Martin C, Murphy D, Shokar NK (2017). Breast cancer epidemiology, prevention, and screening. *Prog Mol Biol Transl Sci*, **151**, 1-32.
- Wong MM, Guo C, Zhang J (2014). Nuclear receptor corepressor complexes in cancer: mechanism, function and regulation. *Am J Clin Exp Urol*, **2**, 169.
- World Health Organization (WHO) (2021). Breast Cancer. <https://www.who.int/news-room/fact-sheets/detail/breast-cancer>
- Wu TY, Lee J (2019). Promoting breast cancer awareness and screening practices for early detection in low-resource settings. *Eur J Breast Health*, **15**, 18.
- Wu X, Hawse JR, Subramaniam M, et al (2009). The tamoxifen metabolite, endoxifen, is a potent antiestrogen that targets estrogen receptor  $\alpha$  for degradation in breast cancer cells. *Cancer Res*, **69**, 1722-7.
- Yu S, Kim T, Yoo K, et al (2017). The T47D cell line is an ideal experimental model to elucidate the progesterone-specific effects of a luminal A subtype of breast cancer. *Biochem Biophys Res Commun*, **486**, 752–8.
- Zhu Y, Liu Y, Zhang C, et al (2018). Tamoxifen-resistant breast cancer cells are resistant to DNA-damaging chemotherapy because of upregulated BARD1 and BRCA1. *Nat Commun*, **9**, 1-11.



This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International License.