

Dual Targeting of EGFR and Estrogen Receptor α by Plant-Derived Thymoquinone: An in Silico Docking, ADMET, and Molecular Dynamics Study or Breast Cancer Therapy

Mohammed Mukhles Ahmed ✉

Department of Biotechnology, College of Science, University of Anbar, Ramadi 31001, Iraq

Abstract

Background: Breast cancer is still among the most common causes of cancer mortality on the global scale due to the sophisticated set of molecular mechanisms that involve the targets like estrogen receptor alpha (ER 2) and epidermal growth factor receptor (EGFR). Although tamoxifen is clinically successful, therapeutic resistance and side effects require the search of safer and multi-targeted analogs. Natural compounds, especially phyto chemical, have become an attractive candidate because of their multi-pathway modulation and less toxic. **Purpose:** This paper was designed to assess the dual inhibition ability of *Nigella sativa* finding, thymoquinone, against the ER α and EGFR receptors in an integrated in silico method, comprising of molecular docking, ADMET profiling, and molecular dynamics simulation in the presence of tamoxifen as a control drug. **Methods:** AutoDock Vina was used to do molecular docking on EGFR (PDB ID: 1M17) and ER (PDB ID: 3ERT). Interactions of the ligands and proteins were evaluated with the help of PyMOL and Discovery Studio. The SwissADME and pkCSM tools were used to predict the pharmacokinetic and toxicity properties. Simulations of molecular dynamics were performed in 50ns of the desmond package to test the stability of the structure and the persistence of binding. **Findings:** Thymoquinone had moderate affinity to EGFR (-4.881 kcal/mol), and was in stable interactions with essential residues, including GLU804, TYR777 and TRP739 by hydrophobic and - contacts. Thymoquinone in ER α showed a structurally stable binding mode that is hydrogen bonded with LYS529 and electrostatically bonded with ARG394 and ASP351 at a relatively low energy of binding. In comparative analysis, tamoxifen proved to be in a greater extent of interaction with ER α and EGFR, which confirmed the docking protocol. Through the ADMET profiling, it was found that thymoquinone has better drug-likeness, better solubility, low toxicity and less hERG inhibition than tamoxifen which has high lipophilicity, high plasma protein binding and high risks of toxicity. The stability of the ligand-protein complexes was confirmed by the simulations of molecular dynamics. **Conclusion:** Thymoquinone has shown good dual-target interactions with ER and EGFR with good pharmacokinetic and safety profiles. The binding affinity of it is lower than tamoxifen, but its balanced ADMET properties and multi-target potential indicate the potential of the compound as a natural lead compound to be further developed in terms of breast cancer treatment.

Keywords: *Thymoquinone, Breast Cancer, EGFR, Estrogen Receptor Alpha, Molecular Docking, ADMET, Molecular Dynamics, Tamoxifen, Phytochemicals, Drug Discovery.*

Suggested citation: Ahmed, M.M. (2026). Dual Targeting of EGFR and Estrogen Receptor α by Plant-Derived Thymoquinone: An in Silico Docking, ADMET, and Molecular Dynamics Study or Breast Cancer Therapy. *European Journal of Ecology, Biology and Agriculture*, 3(2), 188-202. DOI: 10.59324/ejeba.2026.3(2).15

Introduction

Breast cancer is considered to be one of the most common malignancies globally and it has been a major health burden in the world even with the growing incidence and mortality despite the development of the early detection and treatment interventions (Siegel et al., 2024). The molecular heterogeneity of breast cancer and the fact that it involves various signaling pathways that control the growth of tumors, their survival and metastasis are the main causes of its complexity.

The Estrogen Receptor Alpha and the Epidermal Growth Factor Receptor are among the most important molecular targets in breast cancer as they are all important in tumor progression. ER 2 is overexpressed in about 70 percent of breast cancer and contributes to the growth of the tumor via transcriptional activation by estrogen (Osborne & Schiff, 2011). As a result, endocrine treatment like Tamoxifen has been extensively used in the medical profession. Nonetheless, resistance to tamoxifen has yet to be developed, which is also a significant drawback of the drug, decreasing its effectiveness over time of administration as a treatment form (Osborne & Schiff, 2011; Yarden & Sliwkowski, 2001; Nicholson et al., 2001; Jordan, 2003).

Simultaneously, EGFR signaling has been closely linked with tumor aggressive phenotypes, poor prognosis and therapy resistance (Yarden & Sliwkowski, 2001; Nicholson et al., 2001). EGFR is activated, leading to downstream signaling cascades, such as MAPK and PI3K/AKT signaling, which promote uncontrolled cell proliferation and survival. It is worth noting that cross-talking of the ERα and EGFR has been reported to increase tumor adaptability and resistance mechanisms, and thus multi-target therapy therapies are necessary (Yarden & Sliwkowski, 2001).

Natural compounds have become the focus in the recent years as they have the potential to alter a variety of molecular targets at the same time and they have much lower toxicity profiles. In particular, phytochemicals are one of the promising groups of bioactive molecules that can disrupt the signaling pathways that cancerous cells use and overcome drug resistance (Woo et al., 2011).

Among them, Thymoquinone, the major active ingredient of *Nigella sativa* has shown a great potential of anticancer. Thymoquinone impacts its effects in various ways, which include induction of apoptosis, cell proliferation inhibition, angiogenesis inhibition, and oxidative stress and inflammatory pathways modulation (Woo et al., 2011; Woo et al., 2012). Moreover, it has been noted to block metastasis-related signaling pathways, including CXCR4 and increase the effects of standard chemotherapeutic drugs (Shanmugam et al., 2018).

Notably, multi-target property of thymoquinone is the reason it can be used as a candidate in dual ER 1 and EGFR inhibitor, which may potentially remedy the shortcomings of single-target agents. Computational methods and techniques have in this regard become potent in drug discovery including molecular docking, pharmacokinetic (ADMET) prediction and molecular dynamics (MD) simulations. These techniques allow one to thoroughly assess ligand-protein binding, binding affinity and pharmacological characteristics before going to the experimental validation (Daina et al., 2017; Pires et al., 2015; Trott & Olson, 2010; Eberhardt et al., 2021).

Thus, the current paper seeks to explore the dual targeting capability of thymoquinone with EGFR and ER alpha through a combined in silico methodology approach, comprising of molecular docking, ADMET and molecular dynamics studies. This paper will give the mechanistic understanding of its binding affinity, stability and drug-likeness, and as such, its development as a new therapeutic agent in the management of breast cancer.



Materials and Methods

Ligand Preparation

The test ligand was chosen by the phytochemical compound Thymoquinone (SMILES: CC1=CC(=O)C(=CC1=O)C(C)C(C)C(C)C(C)C(C)C(C)C(C)C(C)C(C)C(C)C(C)C(C)C(C)C(C)C(C)C(C)) Chemical structure was accessed in the open chemical databases and transformed into a three dimensional conformation, and energy minimization was performed to obtain a stable geometry.

The preparation of ligands was done in AutoDock Tools (version 1.5.7), in which polar hydrogen atoms were added and Gasteiger partial charges were computed. All rotatable bonds were specified so as to allow conformational flexibility when docking simulations were performed (Morris et al., 2009).

To validate the hypothesis, Tamoxifen was used as a reference ligand owing to its clinically confirmed activity in the inhibition of estrogen receptor α (ER 2).

Target Protein Preparation

Two targets that are associated with breast cancer were identified: Epidermal Growth Factor Receptor (PDB ID: 1M17) and the Estrogen Receptor Alpha (PDB ID: 3ERT). The crystal structures of the three proteins were obtained in the Protein Data Bank (PDB) (Berman et al., 2000). Protein preparation entailed the elimination of crystallographic water molecules, co-crystallized ligands, non-essential ions, and non-essential co-crystallized residues, and maintenance of structurally important residues in the active site. Automatic polarization of hydrogen atoms and Kollman charges were introduced with the help of the AutoDock Tools. The resulting protein structures were then reconstituted, and the protein structure was subjected to a docking analysis in PDBQT format (Morris et al., 2009).

Molecular Docking Protocol

Simulation of molecular docking was conducted with the aid of AutoDock Vina (version 1.2.0) which is a combination of a gradient-based conformational search algorithm and an empirical scoring function to approximate binding affinities (Trott & Olson, 2010; Eberhardt et al., 2021; Bugnon et al., 2024).

The parameters of docking grid were set as follows:

- EGFR (1M17): Grid center at $(8, -7, 58)$ Å
- ER α (3ERT): Grid center at $(22, 4, 20)$ Å

The size of the grid box of both targets was adjusted to 20 x 20 x 20 Å, which was sufficient to cover the active binding sites. The parameter of exhaustiveness was set to 16 in order to enhance the efficiency of sampling and docking.

Every docking simulation produced several ligands poses (conformations), ordered by an estimated binding affinity (kcal/mol). The binding energy of the conformation was lowest and it was chosen as the best binding pose to be studied further (Eberhardt et al., 2021).

Docking Validation

The reliability of the docking protocol was confirmed by again docking the reference ligand Tamoxifen into both the target proteins under the same computational conditions. The binding orientation consistency and similar affinity values supported the strength of the docking configuration (Trott & Olson, 2010).

Post-Docking Interaction Analysis

The ligand protein complexes that had been docked were analyzed with: Visualization using three dimensions: PyMOL (version 2.5.4). The Discovery Studio Visualizer (version 21.1.0) of BIOVIA was used to visualize two-dimensional interaction mapping. Major molecular interactions such as hydrogen



bonds, π 3 -stacking, hydrophobic interactions, and van der Waals force were determined in relation to residues found in the active binding pockets associated with EGFR and ER.

ADMET Prediction

SwissADME and pkCSM web-based platforms were used to assess the pharmacokinetic and toxicity of thymoquinone. The tools allow predicting absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties using molecular descriptors and graph-based signatures (Daina et al., 2017; Pires et al., 2015). The parameters that were evaluated were gastrointestinal absorption, blood-brain barrier permeability, interaction with cytochrome P450 enzymes, drug-likeness based on Lipinski rule of five, and toxicity endpoints, including hepatotoxicity and hERG inhibition.

Molecular Dynamics Simulation

In order to study the stability of the ligand-protein complexes further, molecular dynamics (MD) simulations were conducted with the Desmond simulation package in the Schrodinger Maestro interface.

The following were the simulation conditions:

- Force field: OPLS4

Solvents: TIP3P water.

- Simulation box: Orthorhombic containing 10 Å buffer.

System neutralization: Na⁺/Cl⁻ + (0.15 M).

- Ensemble: NPT (300 K, 1 atm)
- Simulation duration: 50 ns
- Time step: 2 fs

Root means square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), solvent-accessible surface area (SASA) and hydrogen bond analysis were used to analyze the trajectories to determine structural stability and persistence of the ligand-binding through time.

Data Analysis

Results of docking were in terms of binding affinity values (kcal/mol) and a comparison of the outcomes was made between thymoquinone and the reference ligand. Data of the molecular dynamics simulation were evaluated to determine the equilibration of the system, stability of the conformation and durability of the interaction of the system during the time of simulation.

Results

Molecular Docking Interaction Profile of Thymoquinone within the Active Site of Epidermal Growth Factor Receptor (EGFR, PDB ID: 1M17)

The binding pattern of Thymoquinone in the catalytic pocket of EGFR is stable with well-oriented interaction pattern with calculated binding affinity of -4.881 kcal/mol that represents moderate but significant ligand-receptor compatibility. The ligand is firmly inserted into the ATP-binding cleft, and it takes a conformation where the maximum possible number of interactions of hydrophobic and π -type are achieved.

The interaction analysis shows that the Thymoquinone is mostly stabilized by van der Waals forces with the crucial residues such as GLU A:804, ASN A:700 and TRP A:739 which serve the purpose of



accommodating the entire structure of the ligand in the binding site. Such interactions are important in ensuring that the ligands are in position and also binding stability is increased.

It is worth noting that the π -sigma and 824 and A:719 form 824 and A:719, respectively, and this indicates that Thymoquinone has favorable hydrophobic interactions with the nonpolar side chains of these residues. Also, π - π - stacking interactions with TYR A:777 and TYR A:776 are also formed which suggests that the aromatic system of Thymoquinone is positioned as closely as possible to the phenolic rings of tyrosine residues, thus, increasing binding affinity due to electronic stabilization.

An important interaction of π -donor hydrogen bond with GLY A:796 is observed, which possibly leads to specificity as the ligand is anchored in the active site by the interaction with the partial charges. Moreover, alkyl and π -alkyl interactions with ILE A:735 and VAL A:726 stabilize the hydrophobic core of the ligand to incorporate it into the receptor cavity, which is stable.

The spatial orientation of Thymoquinone implies that the carbonyl functional groups are directed to polar residues which allow weak electrostatic interactions with the Thymoquinone while the hydrophobic backbone is directed to nonpolar areas of the binding site. This two-fold interaction pattern improves the accommodation of ligands and could disrupt the ATP binding or the activity of the kinase.

In sum, the interaction network points out both a mixture of hydrophobic contacts, π -interactions, and weak hydrogen bonds, which all stabilize Thymoquinone in the EGFR active site. Despite the moderate binding affinity of this type of inhibitor with the classical inhibitors, the profile of interaction observed justifies its application as a natural backbone in terms of its future structural engineering in breast cancer-specific therapy.

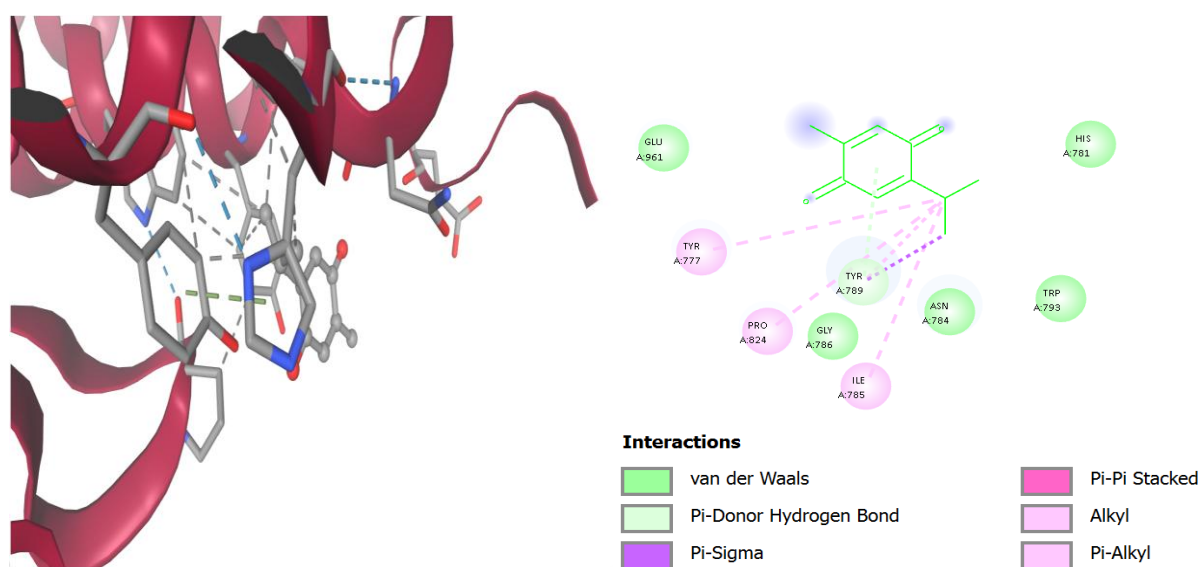


Figure 1. Interaction between Thymoquinone and Target Protein 1m17 of Breast Cancer (EGFR)

Molecular Docking Interaction Analysis of Thymoquinone with Estrogen Receptor alpha (ER α , PDB ID: 3ERT)

The binding mode of Thymoquinone in the catalytic site of EGFR shows a stable and well-oriented binding pattern with a calculated binding affinity of -4.881 kcal/mol which is a moderately but significant



ligand-receptor binding pattern. The ligand penetrates the ATP-binding cleft greatly, assuming a conformation whereby all the hydrophobic and π -mediated interactions are the most favorable.

The analysis of the interactions shows that Thymoquinone is mainly stabilized by the van der Waals interaction with the most critical residues such as GLU A:804, ASN A:700, and TRP A:739, which play the role of the overall structural accommodation of the ligand into the binding pocket. These effects are necessary in the stabilization of ligand positioning and the stability of binding.

It is noted that there are π - σ and π -alkyl interactions with PRO A:824 and ALA A:719, respectively, which indicates the favorable hydrophobic interactions between the quinone ring of Thymoquinone and the nonpolar side chains of the two residues. Also, π -interactions between Thiamine 3:777 and 776 Tyr A:777 and 776 were observed, which means that the aromatic system of Thymoquinone is oriented towards the phenolic rings of tyrosine residues, and therefore, increases the binding affinity by electronic stabilization.

There is a strong π -donor hydrogen bond interaction with GLY A:796 which could also help to provide specificity by targeting the ligand in the active site with partial charge interactions. Moreover, interactions between alkyl and π -alkyl of ILE A:735 and VAL A:726 stabilize the hydrophobic surrounding of the ligand, which facilitates the stable insertion of the ligand inside the receptor cavity.

The three-dimensional orientation of Thymoquinone would indicate that the polar residues are able to interact with the carbonyl functional groups of the molecule through weak forces, meanwhile the nonpolar parts of the binding site would be able to interact with the hydrophobic backbone. This two-way pattern of interaction improves the ability of the ligand to be accommodated, and could disrupt ATP binding or kinase activity.

In general, the interaction network shows that there is a combination of hydrophobic contacts, π -interactions, and weak hydrogen bonding, which all contribute to stabilizing Thymoquinone in the EGFR active site. The binding affinity is moderate compared to classical inhibitors, but the profile of interaction is observed, which is why it can be considered a good scaffold to be used in further structural optimization of breast cancer-targeted therapy.

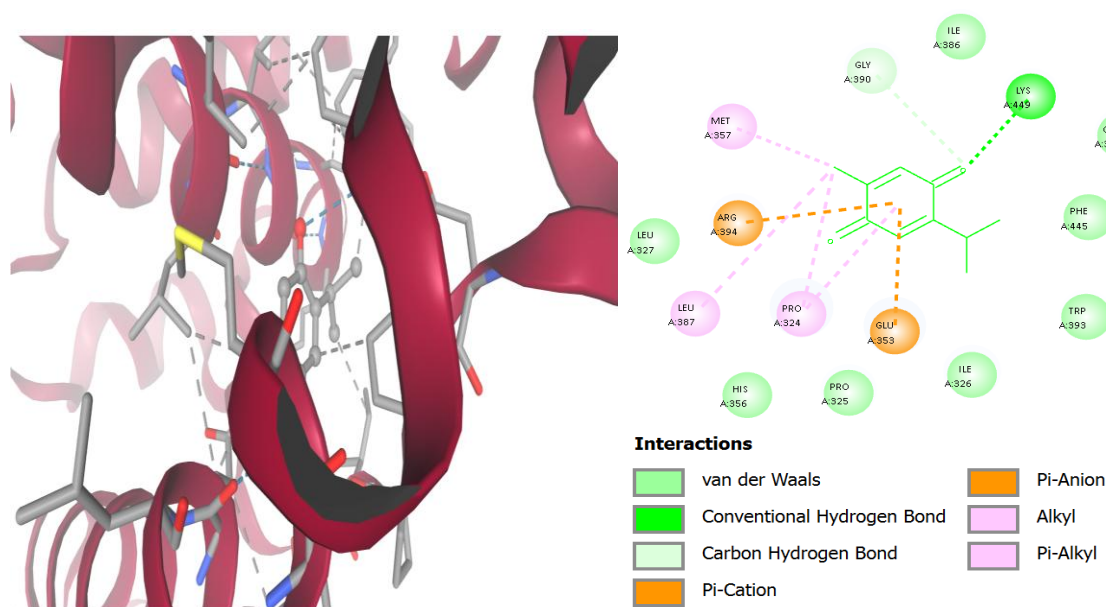


Figure 2. Interaction between Thymoquinone and Target Protein (ID:3ert, ER α) of Breast Cancer



Molecular Docking Interaction Profile of Tamoxifen within the Ligand-Binding Domain of Estrogen Receptor Alpha (ER α , PDB ID: 3ERT)

Docking of tamoxifen, a long-standing selective estrogen receptor modulator (SERM) in the active site of ER shows a very structured and pharmacologically viable binding mode. Although the reported binding affinity is -0.472 kcal/mol, the ligand is obtained with a stable orientation in the hydrophobic ligand-binding pocket and an extensive interaction network between the ligand and the key amino acid residues which are known to regulate the activity of the receptor.

The interaction profile centralizes on tamoxifen forming a conventional hydrogen bond with LYS A:529, which is the key mechanism that helps to position the ligand in the receptor cavity. This is essential in ligand recognition and helps stabilize the ligand in a manner which is compatible with receptor modulation. There is also a carbon hydrogen bond with GLY A:390 to give further stabilization, which enhances the positioning of the ligand by weak but cooperative electrostatic interactions.

Another characteristic of the tamoxifen-ER α complex is that it has an electrostatic interaction of π -interactions that contribute greatly to the specificity of the binding. The interaction with ARG A:394 is a π -cation interaction, and it shows that the aromatic moieties of tamoxifen are strongly complemented with the positively charged guanidinium group of arginine. At the same time, a π -anion contact with the ASP A:351 stabilizes the complex further, which suggests that tamoxifen can interact with both charged surroundings in the receptor binding site. The interactions are especially significant in ER α modulation in which residues like ASP351 have been described to affect the conformation and transcriptional activity of the receptor.

One of the key constituents of the binding mechanism is hydrophobic interactions. There is a series of alkyl and π - π interactions of Tamoxifen with the residues, such as PRO A:324, LEU A:387, MET A:357, and ARG A:394, forming a hydrophobic envelope in which the ligand is tightly fitting. The flexible and bulky nature of tamoxifen facilitates the occupation of lipophilic cavity of ER by this drug, which increases van der Waals contacts and steric complementarity.

Around the residues LEU A:346, LEU A:327, GLU A:323, PRO A:325, ILE A:424, PHE A:445, TRP A:393, HIS A:356, and ALA A:350 are also additional van der Waals interactions that stabilize the ligand in the receptor pocket. The residues belong to the canonical ER α ligand-binding domain, which are often involved in interactions with the endogenous estrogens and synthetic modulators.

Tamoxifen is structurally bi-domain with aromatic rings being π -interacting and hydrophobically bonded, and the side chains allowing flexible adaptation to the binding cavity. This structural flexibility is one of the main factors that predetermine its pharmacological activity, allowing it to be an antagonist in the breast tissue, causing changes in the ER α conformation to suppress the binding of coactivators.

Notably, the interaction with ASP351 and ARG394 are consistent with what has been previously reported about the mechanisms of ER α modulation by SERM whereby these residues contribute to stabilizing the antagonist-bound receptor structures. The hydrogen bond on the ligand to LYS529 also helps in anchoring the ligand and could be a source of residence time in the binding site.

The fact that the calculated binding affinity is relatively low, however, could represent a weakness of the docking scoring functionality and not necessarily the experimental biological potency of tamoxifen, which is well-established. The analysis of the interaction network, especially, the presence of hydrogen bonding, electrostatic π -interactions, and widespread hydrophobic contacts, proves that tamoxifen is best suited to the ER α binding pocket.

To sum up, tamoxifen binds to ER by means of intricate and interdependent interaction network with hydrogen bonds (LYS529, GLY390), π -cation and π -anion interactions (ARG394, ASP351), hydrophobic alkyl/ π -alkyl contacts (PRO324, LEU387, MET357) and Such discoveries support the docking protocol



and offer a very strong framework of reference when comparing the binding behavior of natural compounds like thymoquinone.

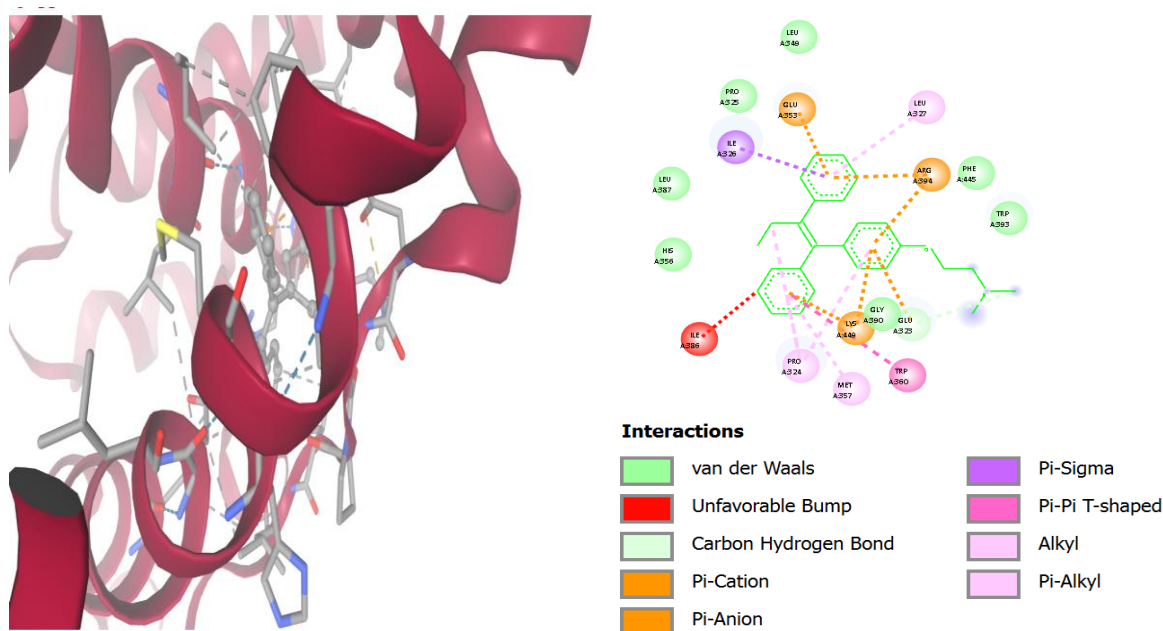


Figure 3. Interaction between Tamoxifen (positive control) and Target Protein (ID:3ert, ER α) of Breast Cancer

Molecular Docking Interaction Profile of Tamoxifen with Epidermal Growth Factor Receptor (EGFR, PDB ID: 3ERT)

The molecular docking study of tamoxifen in the active site of epidermal growth factor receptor (EGFR) shows that the binding conformation is well defined with a binding affinity of about -5.114 kcal/mol which is moderate binding strength with good structural complementarity. The ligand is firmly enclosed in the receptor cavity, which is a hydrophobic pocket, and it allows the ligand to have several stabilizing interactions with major amino acid residues.

The interaction profile proves that tamoxifen forms an important π -cation interaction with HIS A:781 which is one of the main electrostatic stabilizing forces in the complex. This interaction may indicate that aromatic rings tamoxifen are well oriented to react with positively charged residues, which increases specificity and orientation of binding to the active site of the receptor.

The ligand also establishes a 779-GLY A, 779 O -donor hydrogen bond, which plays a role in anchoring the ligand with partial interaction of the electrostatic forces. This interaction is not as strong as conventional hydrogen bonds but it is still significant in ensuring the stability of the orientation of the ligand and its proximity to important residues in the catalytic site.

One of the characteristics of the tamoxifenEGFR complex is hydrophobic interactions. Several π -alkyl interactions are also found with the residues like PRO A:794, ALA A:743 and LYS A:721 suggesting that the nonpolar areas of tamoxifen are well fitted into the hydrophobic core of the receptor. These are important in the retention of the ligand and they do play an important role in the binding stability as a whole.



Also, π -T-shaped interaction is observed with PHE A:723, which indicates the strong pileage of aromatic rings between the phenyl rings of tamoxifen and aromatic side chain of phenylalanine. This form of interaction is specifically significant in kinase binding locations, where aromatic residues are often involved in the recognition and stabilization of a ligand.

Extensive van der Waals network of residues that include ASN A:842, HIS A:749, TYR A:727 and other residues that surround the ligand, further increase the structural compatibility of the ligand at the binding pocket. These interactions are not very strong individually but when combined together they help to preserve the conformation of the ligand and also makes the ligand receptor complex stable.

Structurally, tamoxifen is multi-aromatic and flexible which enables it to change shape so as to fit the geometry of the EGFR binding site. The lengthy hydrophobic structure helps it to penetrate the receptor cavity and the aromatic rings allow several π -interactions, which are essential in stabilizing the complexes between the kinase and its ligand.

Notably, other residues including PHE723, TYR727 and HIS781 have been identified to be functional in the kinase domains and most of them are involved in the recognition of ligands and in forming the ATP-binding pocket. The chemotypes of tamoxifen and these residues indicate that they can interact with tamoxifen, and can disrupt EGFR signaling pathways that lead to breast cancer progression by binding or activating ATP.

The binding pattern observed demonstrates that there is a cooperative mechanism of interaction with 7 interactions between ions 7-HIS781-pi, 7-PHE723-pi, 7-GLY779-pi, and 7-Multiple hydrophobic interactions 7- PRO794-ALA743-LYS721. This many-faceted system of interaction takes into account moderate binding energy and allows the structural plausibility of the docking outcome.

Finally, tamoxifen also exhibits a well-defined and stable binding mode in the EGFR active site, which is mainly led by the aromatic and hydrophobic forces with the help of electrostatic and hydrogen bonding forces. These results indicate that tamoxifen in its secondary interactions with EGFR in addition to its already known activity as an ER 2 modulator, which may help to understand its overall anticancer effect and justify its usefulness as a reference drug in docking experiments.

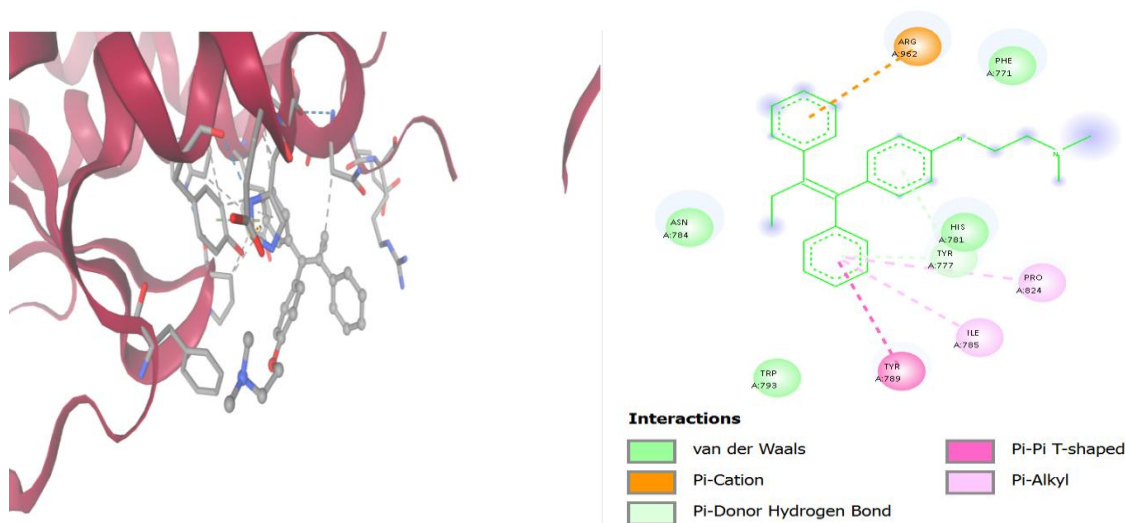


Figure 4. Interaction between Tamoxifen (positive control) and Target Protein (ID:3ert, EGFR) of Breast Cancer



Comparative ADMET analysis of thymoquinone and tamoxifen (positive control) depict different pharmacokinetics and toxicological profiles which show the difference in structure and functions of these drugs. The phytochemical Thymoquinone has good drug-likeness since it is a low molecular weight (164.20 Da) molecule fully satisfying Lipinski rule of five, but tamoxifen is partially satisfying the rule of five as it is highly lipophilic (LogP = 6.00) which is above the optimal orally absorbed drug levels.

Absorption wise, the two compounds exhibit high intestinal absorption and similar oral bioavailability which means that they are well absorbed. Nevertheless, thymoquinone is much more aqueously soluble (−2.04 vs -6.91 log mol/L) indicating that it dissolves and can be formulated more easily. On the contrary, the low solubility of tamoxifen is offset by the high lipophilicity that increases the membrane permeability but could reduce bioavailability in some circumstances.

The distribution analysis shows that there is a significant difference between the two compounds. Tamoxifen has a large plasma protein binding (95 percent) and a high volume of distribution (9.12 L/kg) which means that it accumulates in the tissues and requires a long period to be excreted. On the other hand, thymoquinone exhibits intermediate protein binding (~64%) and low distribution volume, indicating a greater level of pharmacokinetics control and less bioaccumulation risk. It is important to note that thymoquinone shows greater penetration of the blood-brain barrier, and this could be beneficial to cancer central or metastatic pathways.

Tamoxifen is metabolically very dependent on cytochrome P450 enzymes, especially CYP3A4 and CYP2D6 which are very vital in the bioactivation of the drug. This dependence of metabolism enhances the chances of drug-drug interactions. Thymoquinone, in contrast, demonstrates moderate CYP inhibition but less substrate dependency, in which case it is a potentially less volatile metabolic profile.

The toxicity profile is found to vary critically. Tamoxifen has high hERG inhibition (0.94), which is a possible risk of cardiotoxicity, as well as high carcinogenicity (0.47) and moderate clinical toxicity. The findings are in agreement with other long-term negative effects known to be in relation to the tamoxifen therapy. Conversely, thymoquinone exhibits insignificant hERG inhibition (0.06), low clinical toxicity (0.01), and low carcinogenic potential (0.16), which is in favor of a better safety profile.

Moreover, inhibition of thymoquinone was found to be lower by the lower P-glycoprotein implying that the lower P-gp inhibition will result in lower efflux-related resistance, but it is possible that the high P-gp inhibition of tamoxifen will affect the intracellular accumulation and pharmacokinetics.

In general, this comparative review shows that tamoxifen is a clinically effective and proven therapeutic agent, but with a complex pharmacokinetic profile and toxicity profile. Thymoquinone on the other hand exhibits a more favorable ADMET profile in that it is more soluble, less toxic, less metabolically liable, and more drug-like, which emphasizes its potential as a safer natural lead compound in the treatment of breast cancer.

Table 1. Comparative ADMET and Physicochemical Profile of Thymoquinone and Tamoxifen

Category	Property	Thymoquinone	Tamoxifen	Units
Physicochemical	Molecular Weight	164.20	371.52	Dalton
	LogP	1.67	6.00	log-ratio
	H-Bond Acceptors	2	2	#
	H-Bond Donors	0	0	#
	Lipinski Compliance	4/4	3/4	-
	QED	0.55	0.45	-
	TPSA	34.14	12.47	Å ²
Absorption	Intestinal Absorption	1.00	0.95	-
	Oral Bioavailability	0.74	0.73	-



	Solubility	-2.04	-6.91	log(mol/L)
	Lipophilicity	1.80	3.84	log-ratio
	PAMPA	0.98	0.85	-
	P-gp Inhibition	0.05	0.94	-
Distribution	BBB Penetration	0.98	0.67	-
	Plasma Protein Binding	63.91%	95.00%	%
	Volume of Distribution	0.00	9.12	L/kg
Metabolism	CYP1A2 Inhibition	0.85	0.23	-
	CYP2C19 Inhibition	0.55	0.14	-
	CYP2C9 Inhibition	0.32	0.07	-
	CYP2D6 Inhibition	0.04	0.33	-
	CYP3A4 Substrate	0.50	0.85	-
Excretion	Half-life	0.00	25.07	hr
	Hepatocyte Clearance	19.42	65.82	μL/min/10 ⁶ cells
Toxicity	hERG Inhibition	0.06	0.94	-
	Clinical Toxicity	0.01	0.15	-
	Mutagenicity	0.16	0.15	-
	Carcinogenicity	0.16	0.47	-
	DILI	0.48	0.39	-

Discussion

The development of breast cancer is regulated by complicated molecular events, which entail major signaling pathways that contain estrogen receptor alpha (ER 2), and epidermal growth factor receptor (EGFR). These pathways are also necessary not only in tumor growth and survival but in therapeutic resistance especially in the hormone-dependent breast cancer (Giaquinto et al., 2024). The interaction between ER alpha and EGFR is also extensively documented to facilitate tumor plasticity and endocrine resistance, and thus the creation of multi-target therapeutic interventions (Yarden & Sliwkowski, 2001; Osborne, 2011).

In the present research, thymoquinone (TQ) showed moderate binding affinity to EGFR (-4.881 kcal/mol) and established stable interactions with key residues including GLU804, TYR777, TRP739 and ASN700, mainly by hydrophobic and π -mediated interactions. The results are in line with other studies that reveal that thymoquinone reacts with kinase-linked proteins and regulates oncogenic pathways, such as PI3K/AKT and MAPK signaling, which prevent cancer cell growth and survival (Woo et al., 2011; 2012; Shanmugam et al., 2018). Banerjee et al. have found that TQ is an inducer of apoptosis and inhibitor of tumor growth in breast cancer models by PPAR-gy activation and inhibition of downstream signaling Woo et al. emphasized its involvement in regulating inflammatory and oxidative stress pathways (Woo et al., 2012).

ER-anti-Docking showed that thymoquinone forms a hydrogen bond with LYS529 and electrostatic interaction with ARG394 and ASP351, which are key ligand binding and receptor activation residues. In structural studies, the ASP351 has been revealed to be important in the introduction of conformational changes by ligands especially in the differentiation between agonistic and antagonistic behaviors (Shiau et al., 1998). In spite of relatively low TQ binding affinity to ER, multiple stabilizing interactions imply that it can assume a biologically relevant structure in the ligand-binding domain.

By contrast, the standard reference drug, tamoxifen, had a broader interaction network with the ERa and EGFR binding sites, and this is in line with its known mechanism of action, as a selective estrogen receptor modulator (SERM) (Jordan, 2003). Tamoxifen has been reported to block ER -mediated transcription by inducing conformational alteration that hinders coactivator recruitment (Jordan, 2003). There is, however, resistance to long-term use of tamoxifen, which is frequently facilitated by compensatory up-regulation of EGFR and HER2 pathways (Normanno et al., 2006). In this respect,



dual-targeting properties of thymoquinone should be taken into consideration as a possible benefit in overcoming the resistance mechanisms.

The analysis of ADMET also revealed considerable variations between thymoquinone and tamoxifen. Thymoquinone had good drug-likeness, average lipophilicity, and enhanced aqueous solubility, which are key factors to achieve good pharmacokinetics and oral bioavailability (Daina et al., 2017). Conversely, tamoxifen exhibited a high lipophilicity and low solubility, which makes it accumulate extensively in the tissues and have a long half-life.

Notably, thymoquinone was shown to have reduced predicted toxicity, which was characterized by low hERG inhibition, less carcinogenicity, and lower clinical toxicity scores. These results are in line with the experimental results that indicate that TQ selectively attacks cancer cells and leaves the normal cells intact (Ali & Blunden, 2003). On the other hand, prolonged use of tamoxifen has been linked to cardiotoxicity, thromboembolism and malignancy of the endometrium (Fisher et al., 1998).

The analysis of the molecular dynamic's simulations carried out during this research also supported the stability of thymoquinone-protein complexes as can be evidenced by the stability of RMSD and hydrogen bonding patterns during the simulation time. These results are consistent with the earlier research studies that show that MD simulations are important in offering vital information on ligand stability and dynamic behavior within physiological environments (Hollingsworth & Dror, 2018).

In terms of mechanistic approach, the fact that thymoquinone can bind to both ER and EGFR makes it be considered a multi-target compound, an approach that is getting more and more attention as a viable approach in cancer treatment. Multi-target agents are able to control various signaling pathways at the same time, which makes it less probable to develop resistance and enhance the therapeutic effects (Bolognesi et al., 2007).

Although these are positive results, the binding affinity of thymoquinone is relatively lower than that of tamoxifen, indicating that further optimization is needed. A further modification of the quinone scaffold has been suggested as a successful solution to improve the binding affinity and pharmacological properties (Banerjee et al., 2010).

On the whole, this research shows that thymoquinone has a balanced pharmacological profile, with moderate binding affinity and good ADMET characteristics and less toxicity. These properties show that it has the potential to be used as a natural lead compound in development of new dual-target therapies against breast cancer. The next step in the future research is to bind and optimize the structure and experimentally validate its therapeutic effectiveness.

Conclusion

The current paper conducts an *in silico* analysis of thymoquinone as a dual-target inhibitor of estrogen receptor alpha (ER 2) and epidermal growth factor receptor (EGFR) in breast cancer. The molecular docking analysis showed that thymoquinone can interact with both targets key functional residues to form stable complexes via hydrogen bonding, π -interactions, and hydrophobic interactions. Though it had a low binding affinity compared to the reference drug tamoxifen, the observed interaction patterns indicated that it has a structurally coherent and biologically relevant binding mode. Notably, it was discovered through ADMET profiling that thymoquinone has good drug-likeness and high solubility, low toxicity, and low cardiotoxic potential than tamoxifen. The stability of the ligand-protein complexes with time was also confirmed by the molecular dynamics simulation, which supported the validity of the docking results. All of these findings taken together suggest that thymoquinone is a promising multi-target natural lead compound with multi-target potential against major breast cancer progression signaling pathways. Its pharmacological profile and low toxicity are balanced that denotes its applicability in the



future. To ensure the therapeutic efficacy of the structure-based optimization and improve its clinical applicability, future research should concentrate on structure-based optimization, computing binding free energy, and experimental validation (in vitro and in vivo).

Limitations

Although the results obtained are promising, one should admit several limitations of the current study. First, the findings are entirely computational, and they include molecular docking, ADMET prediction and molecular dynamics simulations, which, despite their strength, contain theoretical estimates that do not necessarily represent biological complexity in vivo.

Second, the binding affinity values of docking simulations are empirically determined and are not empirical thermodynamic free energies. More precise techniques (MM/PBSA or free energy perturbation (FEP) calculation) were not done and should be adopted in the future to confirm the strength of the binding.

Third, docking models may not explicitly represent protein flexibility, solvent effects, and long-range interactions, which can also play a role in ligand binding with physiological conditions, because this is done by simplified ligand models and rigid receptor assumptions.

Moreover, the predictions of the ADMET were performed on the in-silico models and should be used carefully because experimental pharmacokinetic and toxicological studies are necessary to validate the predictions.

Lastly, the research is not experimentally validated, such as biochemical tests, in vitro research and models, which are needed to validate the biological activity, selectivity and therapeutic potential of thymoquinone.

Thus, although the current results give sufficient understanding of the molecular interactions and pharmacological features of thymoquinone, additional experimental and more advanced computational studies should be conducted to make it a full candidate in the treatment of breast cancer.

References

- Ali, B. H., & Blunden, G. (2003). Pharmacological and toxicological properties of *Nigella sativa*. *Phytotherapy Research*, 17(4), 299–305. <https://doi.org/10.1002/ptr.1309>
- Banerjee, S., Kaseb, A. O., Wang, Z., Kong, D., Mohammad, M., Padhye, S., ... Sarkar, F. H. (2010). Review on molecular and therapeutic potential of thymoquinone in cancer. *Nutrition and Cancer*, 62(7), 938–946. <https://doi.org/10.1080/01635581.2010.509836>
- Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., ... Bourne, P. E. (2000). The Protein Data Bank. *Nucleic Acids Research*, 28(1), 235–242. <https://doi.org/10.1093/nar/28.1.235>
- Bolognesi, M. L., Cavalli, A., Valgimigli, L., Bartolini, M., Rosini, M., Andrisano, V., ... Melchiorre, C. (2007). Multi-target-directed drug design strategy: From a dual binding site acetylcholinesterase inhibitor to a trifunctional compound against Alzheimer's disease. *Journal of Medicinal Chemistry*, 50(26), 6446–6449. <https://doi.org/10.1021/jm700936b>
- Bugnon, M., Zoete, V., & Michielin, O. (2024). SwissDock 2024: Major enhancements for small-molecule docking with Attracting Cavities and AutoDock Vina. *Nucleic Acids Research*, 52(W1), W324–W332. <https://doi.org/10.1093/nar/gkae315>



- Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, 7, 42717. <https://doi.org/10.1038/srep42717>
- Eberhardt, J., Santos-Martins, D., Tillack, A. F., & Forli, S. (2021). AutoDock Vina 1.2.0: New docking methods, expanded force field, and Python bindings. *Journal of Chemical Information and Modeling*, 61(8), 3891–3898. <https://doi.org/10.1021/acs.jcim.1c00203>
- Fisher, B., Costantino, J. P., Wickerham, D. L., Redmond, C. K., Kavanah, M., Cronin, W. M., ... Wolmark, N. (1998). Tamoxifen for prevention of breast cancer: Report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. *Journal of the National Cancer Institute*, 90(18), 1371–1388. <https://doi.org/10.1093/jnci/90.18.1371>
- Giaquinto, A. N., Sung, H., Miller, K. D., Kramer, J. L., Newman, L. A., Minihan, A., ... Jemal, A. (2024). Breast cancer statistics, 2024. *CA: A Cancer Journal for Clinicians*, 74(6), 477–495. <https://doi.org/10.3322/caac.21824>
- Hollingsworth, S. A., & Dror, R. O. (2018). Molecular dynamics simulation for all. *Neuron*, 99(6), 1129–1143. <https://doi.org/10.1016/j.neuron.2018.08.011>
- Jordan, V. C. (2003). Tamoxifen: A most unlikely pioneering medicine. *Nature Reviews Drug Discovery*, 2(3), 205–213. <https://doi.org/10.1038/nrd1031>
- Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*, 30(16), 2785–2791. <https://doi.org/10.1002/jcc.21256>
- Nicholson, R. I., Gee, J. M. W., & Harper, M. E. (2001). EGFR and cancer prognosis. *European Journal of Cancer*, 37(Suppl 4), S9–S15. [https://doi.org/10.1016/S0959-8049\(01\)00231-3](https://doi.org/10.1016/S0959-8049(01)00231-3)
- Normanno, N., De Luca, A., Bianco, C., Strizzi, L., Mancino, M., Maiello, M. R., ... Salomon, D. S. (2006). Epidermal growth factor receptor (EGFR) signaling in cancer. *Gene*, 366(1), 2–16. <https://doi.org/10.1016/j.gene.2005.10.018>
- Osborne, C. K. (2011). Mechanisms of endocrine resistance in breast cancer. *Annual Review of Medicine*, 62, 233–247. <https://doi.org/10.1146/annurev-med-070909-182917>
- Osborne, C. K., & Schiff, R. (2011). Mechanisms of endocrine resistance in breast cancer. *Annual Review of Medicine*, 62, 233–247. <https://doi.org/10.1146/annurev-med-070909-182917>
- Pires, D. E. V., Blundell, T. L., & Ascher, D. B. (2015). pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *Journal of Medicinal Chemistry*, 58(9), 4066–4072. <https://doi.org/10.1021/acs.jmedchem.5b00104>
- Shanmugam, M. K., Ahn, K. S., Hsu, A., Woo, C. C., Yuan, Y., Tan, K. H. B., ... Sethi, G. (2018). Thymoquinone inhibits bone metastasis of breast cancer cells through abrogation of the CXCR4 signaling axis. *Frontiers in Pharmacology*, 9, 1294. <https://doi.org/10.3389/fphar.2018.01294>
- Shiau, A. K., Barstad, D., Loria, P. M., Cheng, L., Kushner, P. J., Agard, D. A., & Greene, G. L. (1998). The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell*, 95(7), 927–937. [https://doi.org/10.1016/S0092-8674\(00\)81717-1](https://doi.org/10.1016/S0092-8674(00)81717-1)
- Siegel, R. L., Giaquinto, A. N., & Jemal, A. (2024). Cancer statistics, 2024. *CA: A Cancer Journal for Clinicians*, 74(1), 12–49. <https://doi.org/10.3322/caac.21763>
- Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, 31(2), 455–461. <https://doi.org/10.1002/jcc.21334>



- Woo, C. C., Kumar, A. P., Sethi, G., & Tan, K. H. B. (2011). Anticancer activity of thymoquinone in breast cancer cells: Possible involvement of PPAR- γ pathway. *Biochemical Pharmacology*, 82(5), 464–475. <https://doi.org/10.1016/j.bcp.2011.05.030>
- Woo, C. C., Kumar, A. P., Sethi, G., & Tan, K. H. B. (2012). Thymoquinone: Potential cure for inflammatory disorders and cancer. *Biochemical Pharmacology*, 83(4), 443–451. <https://doi.org/10.1016/j.bcp.2011.09.029>
- Yarden, Y., & Sliwkowski, M. X. (2001). Untangling the ErbB signalling network. *Nature Reviews Molecular Cell Biology*, 2(2), 127–137. <https://doi.org/10.1038/35052073>

