Virtual Screening and Molecular Docking Studies with Organosulfur and Flavonoid Compounds of Garlic Targeting the Estrogen Receptor Protein for the Therapy of Breast Cancer

Diptendu Sarkar 1,*, Arpan Kumar Maiti 2

1 Department of Microbiology, Ramakrishna Mission Vidyamandira, Belur Marh, Howrah 711202, West Bengal, India; diptendu81@gmail.com (D.S.);
2 Department of Zoology, University of North Bengal, Raja Rammohunpur, Darjeeling 734013, West Bengal, India; arpankmaiti@nbu.ac.in (A.K.M.);
* Correspondence: diptendu81@gmail.com (D.S.);

Abstract: Breast cancer is becoming the leading risk factor for death, affecting millions of women. This cancer develops several desirable properties that impair the maintenance of a regular mammary gland in females. The overexpansion of ER-alpha protein can be driven by stimulating estrogen hormone gene expression in living organisms, which may lead to the improvement and advancement of various breast cancers. As a result, it covers a wide range of biochemical therapeutic targets in clinical research. The competence and binding capacity of several phytochemical constituents (organosulfur compounds and flavonoids) from Allium sativum L. (garlic) addressing the breast cancer target protein, ER-alpha (3ERT), were evaluated in the current research. The chemicals investigated in this study were found to have a significant association with the 3ERT molecule. Alliin had the best lipid-soluble compound contact with 3ERT (−4.8 Kcal/mol), whereas S-Allylmercaptocysteine had the best water-soluble compound interaction with 3ERT (−4.6 Kcal/mol). Among all flavonoids tested, kaempferol, a flavonoid phytocompound, had the maximum binding energy (−8.0 Kcal/mol). Flavonoid analogs have been found to have a higher affinity for protein 3ERT than the organosulfur compounds examined, leading to extensive in vitro research.

Keywords: EGFR; RTKs; garlic; lung cancer; organosulfur compounds; flavonoids.

© 2022 by the authors. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Carcinoma is a fatal illness characterized by longevity and unrestrained gene expression. Intrusive, malignant, and cancer metastases cells invade nearby tissues [1]. Breast cancer is the leading cause of mortality nowadays among women across the globe. This cancer becomes exceedingly variegated, disrupting the functionality of healthy mammary glands in women [2]. Regardless of the cancer type and the patient's maturity level, indicators include a mass in the mammary, an alteration in breast size, skin rash and pus leaking from the breast, a newly-inverted glans, or a reddish or rough spot of the epidermis [1]. Mammary cancer is the most prevalent cancer among women globally, contributing to twenty-five percent of all occurrences in 2020, involving two million individuals and around six lakh and twenty thousand fatalities [3]. This cancer is one of the second most popular causes of morbidity and...
mortality in western nations [2]. Over sixty percent of the total breast cancer cases identified in Asian nations comprise estrogen receptor alpha responsive tumors [3]. Among the principal sources of breast cancer would be the overexpression of estrogen. Estrogen is a sex hormone that has a role in the growth and maintenance of the female body and sexual function [4]. Estrone (also named as E1), estradiol (also termed as E2), and estriol (also called E3) are the 3 main biological estrogens with estogenic female hormones activity. When women are in the motherhood stage, their body synthesizes another estrogen hormone, called Estestrol or E4 [5]. Oestrogens, like every steroidal chemical messenger, quickly diffuse through biological membranes. They attach to and trigger estrogen receptors (ERs) while inside the membrane, therefore modulating the transcription of various genes [6]. Although estrogens have been found, including men and women, they are typically expressed in much larger concentrations in sexually active women. The estrogen receptors ER-alpha and ER-beta are found in the tissues of the human population innately. The mammary gland and the uterus are the primary sites where ER-alpha expression is maximum among women [7]. In the case of breast cancer in women, it has been found that the estrogen sensor appears involved in necrosis, inflammatory processes, equilibrium, polarization, catabolism, development, and multiplication [5]. As per the endocrinologist, ER-alpha protein was found to have various biological system activities like immune supervision, apoptosis resilience, malignancy, and gene expression [8]. The upregulation of estrogen hormone gene expression in human cells can ultimately trigger the overexpansion of ER-alpha protein, which might contribute to the development and progression of numerous forms of breast cancers [9]. Thus, it encompasses multiple molecular potential therapeutic targets in medical research.

Endocrinal medication, chemo, and radiation have developed a diverse combination in current history that really should be meticulously coordinated for each active treatment [4]. In the previous ten years, there has been a lot of momentum in drug design and the development of therapeutics against cancer research [7]. The application of structure-based high-throughput screening to locate effective molecules to remedy the development of a breast cancer medication is progressing [10]. For metastatic breast cancer or perhaps the early stages of breast cancer, hormonal medication gives better results. But such treatment was found very costly, and therefore everyone can not afford it [11]. Cancer therapy is attributed to the use of phytonutrients produced from fruits and vegetables and their propensity to impair a specific point of the involved proteins [12]. Garlic (*Allium sativum* L.) is among the most exemplary spices, including a variety of active substances which might aid in the management of cancer [13]. It has been frequently used more as a seasoning in meals, but it is being utilized like a medication across history, both past, and present, to prevent and handle a number of ailments and disorders [14]. Garlic has historically been used as anti pneumonia, antihypertensive, antituberculosis, giving protection to liver diseases as well as diarrhea, indigestion, spasm and not only that, it showed a beneficial effect on gastrointestinal disorders, osteoarthritis, hyperglycemia, and cough mostly in the Middle East, East Asia and even Nepal [15]. Garlic had been a valuable therapy in Hindu Mythology treatment. It has been used as a sedative and roborant for treating poor appetite, ordinary lethargy, bronchitis, skin infection, osteoarthritis, bowel problems, and other ailments [16]. Because of the wide range of impacts, a clove of garlic resembled an entire pharmaceutical sector at a period while antimicrobials and perhaps other drug treatments didn't even exist. As a result, there is a greater need for exploration into the histories of garlic to enhance chemists’ and doctors’ abilities to adapt to issues that arise in the development of appropriate solutions and improving human existence [17]. *Allium sativum*
L. has a number of functions. It possesses antimicrobial, bactericidal, plus fungicidal properties due to allicin as well as other sulfur compounds, as evidenced by in vitro research [18]. Garlic includes numerous aliphatic unsaturated sulfur substances, according to research. Alliin found in clove seems to have no antimicrobial effects, but when the enzyme alliinase from minced clove has been added, allicin with substantial antimicrobial effects would be formed. Allicin and other garlic components possess hypotension, cardioprotective, and antihyperlipidemic properties [19]. Ajoenes exhibit antithrombotic properties. *Allium sativum* L. inhibits the formation of free radicals but also promotes the body's natural defense systems against them [20]. Aqueous extracts prevent malignant cells from undergoing mitosis at all stages without creating adverse effects. Antioxidant properties of secondary metabolites such as flavonoids include stimulation of antioxidant enzymes, suppression of pro-oxidant enzymes, and amplification of antioxidant enzymes and many other detoxifying enzymes [21]. According to published research, allyl methyl-sulfide is the most effective organosulfur chemical in suppressing CYP2E1 molecule in animal cancer models [16]. Ajoene prevents cancer progression by triggering suicide in various cancer [13].

The use of docking studies allows researchers to comprehend how lead ligands interact with therapeutic targets fully. A simulation is an important tool in computer-assisted drug discovery and development [22]. Only molecular docking be used for virtual screening. Molecular docking would be a strategy for predicting non-covalent interaction processes, including affinities between two molecules [19]. The competence and binding capacity of several phytochemical constituents (organosulfur compounds and flavonoids) from *Allium sativum* L. (garlic) addressing the breast cancer target protein, ER-alpha (3ERT), were evaluated in the current research.

2. Materials and Methods

The in-silico study was carried out using the PyRx virtual screening tool [14]. Protein preparation was carried out by AutoDockTools [16]. Ligand preparation and grid generation were carried out by AutoDock Vina. Receptor-ligand docking was carried out by PyRx. The Swiss ADME server was used for drug-likeness prediction of all compounds selected for study [13,18].

2.1. Selection of target protein and preparation of protein target structure.

The human estrogen receptor alpha protein associated with its ligand 4-hydroxytamoxifen, whose PDB ID is 3ERT, was acquired from the Protein Data Bank (PDB) and used for docking. It seems to have a 1.90 Å resolution and one 261-amino-acid-residue protein chain (A) (Figure 1). The molecular weight of the protein is 30.24 kDa. The input files were prepared with AutoDockTools (ADT) 1.5.6. Molecules of water, bounded ligand, HET atoms, as well as ions have all been completely removed. The polar hydrogen atoms have been given Kollman charges.

2.2. Selection and Preparation of ligand molecules.

For the selection of ligand molecules, we used a target-based ligand selection approach. LEA3D-CNRS (https://chemoinfo.ipmc.cnrs.fr/LEA3D/index.html) was used for selecting ligand structure [23].
Figure 1. 3D picture of ER-alpha- a breast cancer targeting protein (PDB ID : 3ERT).

The three-dimensional structure of the most common organosulfur compounds plus flavonoids garlic contains were identified using PubChem. There were 19 molecules used in this study (lipid-soluble 8 molecules, water-soluble 5 molecules, and flavonoids 6 molecules) (Table1). All Ligands being uploaded (mol2 format) into the AutoDockTools, and to fix the torsional branch, non-polar hydrogen ion, charges, as well as atom type, the 'Ligand' option was employed. With all the structures, further ADME predictions were carried out by using SWISS ADME to filter all bioactive compounds based on the Lipinski rule of five [18].

Table 1. Ligand selected from garlic for the study with compound name, PubChemID, compound type, and molecular formula.

| Sl No | Compound Name            | PubChem ID | Compound Type      | Molecular Formula     |
|-------|--------------------------|------------|--------------------|-----------------------|
| 1     | 4-Hydroxytamoxifen       | 449459     | Anticancer drug    | C$_26$H$_{29}$NO$_2$  |
| 2     | Abemaciclib              | 46220502   | Anticancer drug    | C$_{37}$H$_{32}$F$_2$N$_8$ |
| 3     | Capecitabine             | 60953      | Anticancer drug    | C$_{15}$H$_{22}$FN$_3$O$_6$ |
| 4     | Diallyl Disulfide        | 16590      | Lipid soluble      | C$_{18}$H$_{18}$BrN$_3$O$_2$ |
| 5     | Diallyl Trisulfide       | 16315      | Lipid soluble      | C$_6$H$_{10}$S$_3$     |
| 6     | Alliin                   | 121922     | Lipid soluble      | C$_6$H$_{11}$NO$_3$S   |
| 7     | Allicin                  | 65036      | Lipid soluble      | C$_6$H$_{10}$OS$_2$    |
| 8     | Diallyl Sulfide          | 11617      | Lipid soluble      | C$_{37}$H$_{32}$F$_2$N$_8$ |
| 9     | Allyl Methyl Sulfide     | 66282      | Lipid soluble      | C$_6$H$_{10}$OS$_3$    |
| 10    | (Z)-Ajoene               | 988148     | Lipid soluble      | C$_6$H$_{10}$OS$_3$    |
| 11    | 2-Vinyl-4H-1,3-dithiane  | 133337     | Lipid soluble      | C$_6$H$_{10}$OS$_3$    |
| 12    | S-Allyl-L-cysteine       | 9793905    | Water soluble      | C$_{15}$H$_{10}$NO$_3$S |
| 13    | S-Methyl-L-cysteine      | 24417      | Water soluble      | C$_{15}$H$_{10}$NO$_3$S |
| 14    | S-Ethyl-L-cysteine       | 92185      | Water soluble      | C$_{15}$H$_{10}$NO$_3$S |
| 15    | S-Propyl-L-cysteine      | 125198     | Water soluble      | C$_{15}$H$_{10}$NO$_3$S |
| 16    | S-allylmercaptocysteine  | 9794159    | Water soluble      | C$_{15}$H$_{10}$NO$_3$S |
| 17    | Quercetin                | 5280343    | Flavonoids         | C$_{15}$H$_{10}$O$_7$  |
| 18    | Myricetin                | 5281672    | Flavonoids         | C$_{15}$H$_{10}$O$_7$  |
| 19    | Kaempferol               | 5280863    | Flavonoids         | C$_{15}$H$_{10}$O$_7$  |
| 20    | Apigenin                 | 5280443    | Flavonoids         | C$_{15}$H$_{10}$O$_7$  |
| 21    | Tangeretin               | 68077      | Flavonoids         | C$_{15}$H$_{10}$O$_7$  |
| 22    | Noblelein                | 72344      | Flavonoids         | C$_{15}$H$_{10}$O$_7$  |

2.3. Molecular docking investigation.

Molecular docking analysis was performed with a target like Epidermal Growth Factor Receptor tyrosine kinase domain using PyRx virtual screening tool [12,19]. This tool used Auto Dock for docking purposes. Here, we first uploaded the prepared protein structure as a macromolecule and then selected all bioactive compounds (ligand) one by one. The software first minimized ligand energy and then converted it into Auto Dock ligand format (pdbqt). Finally, started blind docking after covering the entire protein structure under the grid box to screen best fitted bioactive compounds based on the energy value. Each simulation was
conducted roughly ten times, resulting in ten docked conformations. The least energy configurations were deemed to be the highest binding conformations as a result of this. To achieve better ligand binding settings for 100 individual LGA variants with the greatest number of critical forecasts of 25000000, this docking methodology used an overall population of 300 and the largest frequency of assessments of 27,000. Intra-molecular interactions such as hydrogen bonds, van der Waals contacts, and hydrophobic interactions with that specific bioactive molecule have been clearly examined using Discovery Studio software. [24].

3. Results and Discussion

Garlic's organic properties have been ascribed to its organosulfur molecules. Previous research has shown to suppress carcinoma in the breast, colorectal, prostate, and lung of animal studies [25]. In the present research, we performed docking investigations to establish the binding affinity of organosulfur compounds and flavonoids prevalent in garlic with only a breast cancer biomolecule (3ERT). Table 1 lists all phytochemical compounds from garlic used during the in-silico study. Following formulation, all of the compounds were used to investigate the molecular origins of the substances using the SWISS ADME. As stated in Table 2, all of the substances fit the Lipinski 'Rule of Five.'

Table 2. Lipinski rule-related information of selected compounds studied from garlic along with three reference compounds such as 4-Hydroxytamoxifen, Abemaciclib, and Capecitabine. Given the emphasis on molecular weight, the number of hydrogen bond donors as well as acceptor, along with Log P<sub>o/w</sub> and Log S value of the compounds.

| SI No | Compound Name         | Molecular Weight (g/mol) | No. of H bond donor | No. of H bond acceptor | Log P<sub>o/w</sub> | Log S | Lipinski Rule violation |
|-------|-----------------------|--------------------------|---------------------|------------------------|-------------------|------|------------------------|
| 1     | 4-Hydroxytamoxifen    | 387.51                   | 1                   | 3                      | 5.36              | -6.45| 1                      |
| 2     | Abemaciclib           | 506.59                   | 1                   | 8                      | 4.04              | -5.36| 1                      |
| 3     | Capcetabine           | 359.35                   | 3                   | 8                      | 0.84              | -2.07| 0                      |
| 4     | Diallyl disulfide     | 388.26                   | 01                  | 04                     | 4.08              | -5.36| 0                      |
| 5     | Diallyl trisulfide    | 178.35                   | 00                  | 00                     | 2.68              | -2.21| 0                      |
| 6     | Aliin                 | 177.22                   | 02                  | 04                     | -1.33             | 1.62 | 0                      |
| 7     | Allicin               | 162.27                   | 00                  | 01                     | 1.61              | -1.34| 0                      |
| 8     | Diallyl sulfide       | 114.21                   | 00                  | 00                     | 2.14              | -1.64| 0                      |
| 9     | Allyl methyl sulfide  | 88.17                    | 00                  | 00                     | 1.53              | -1.21| 0                      |
| 10    | (Z)-Ajoene            | 234.40                   | 00                  | 01                     | 2.52              | -1.84| 0                      |
| 11    | 2-Vinyl-4H-1,3-dithiine| 144.26                   | 00                  | 00                     | 2.22              | -2.12| 0                      |
| 12    | S-Allyl-L-cysteine    | 161.22                   | 02                  | 03                     | -0.45             | 0.79 | 0                      |
| 13    | S-Methyl-L-cysteine   | 135.18                   | 02                  | 03                     | -1.06             | 1.23 | 0                      |
| 14    | S-Ethyl-L-cysteine    | 149.21                   | 02                  | 03                     | -0.68             | 0.98 | 0                      |
| 15    | S-Propyl-L-cysteine   | 163.24                   | 02                  | 03                     | -0.34             | 0.62 | 0                      |
| 16    | S-allylmercaptocysteine| 193.29                   | 02                  | 03                     | -0.22             | 0.64 | 0                      |
| 17    | Quercetin             | 302.24                   | 05                  | 07                     | 1.23              | -3.16| 0                      |
| 18    | Myricetin             | 318.24                   | 06                  | 08                     | 0.79              | -3.01| 0                      |
| 19    | Kaempferol            | 286.24                   | 04                  | 06                     | 1.58              | -3.31| 0                      |
| 20    | Apigenin              | 270.24                   | 03                  | 05                     | 2.11              | -3.94| 0                      |
| 21    | Tangeretin            | 372.37                   | 00                  | 07                     | 3.02              | -4.11| 0                      |
| 22    | Nobiletin             | 402.39                   | 00                  | 08                     | 3.02              | -4.18| 0                      |

Garlic (Allium sativum L) is known all over the world for its pungent odor and flavor [26]. This is a biennial vegetable that was first cultivated in Central Asia and is now grown worldwide [27]. We discovered that alliin, whose IUPAC name is (2R)-2-amino-3-prop-2-enyl sulfanyl propanoic acid, was the best appropriate phytocompounds (Table 3, Figure 2) to interact with target protein 3ERT with the lowest interaction energy, i.e., -4.8 Kcal/mol, among the lipid-soluble ligands from garlic examined, it also formed two hydrogen bonds. Alliin is nothing more than a cysteine amino acid variant [28]. It’s possible that it’s the first natural
compound with chiral molecules centered across carbon and sulfur. It may be capable of removing reactive oxygen radicals and thus is an antioxidant [29]. Alliin could help reduce inflammation and prevent reactive oxygen species from harming our body's tissues and organs, which can cause severe illness [30]. In terms of energy binding potentiality, Z-ajoene, which has a wide range of antimicrobial potential, was revealed to have the second-highest binding potentiality (~4.7 Kcal/mol) with protein 3ERT (Table 3, Figure 8). However, it did not form a hydrogen bond during the encounter, which could disadvantage when choosing a medication. However, anti-clotting capabilities have been discovered in Z-Ajoene, which may lower the risk of heart attack and peripheral artery disease by preventing platelets from forming thrombus in the arteries [31]. Conversely, the third rank molecule has a dithien ring like 2-vinyl-4H-1,3-dithiene. It had interaction energy of ~4.2 Kcal/mol for the 3ERT protein (Table 3, Figure 8) but did not create any hydrogen bonds during the interaction. Additional lipid-soluble compounds, e.g., diallyl disulfide, allicin, diallyl sulfide, and diallyl trisulfide, had virtually identical interaction energies, namely ~3.7 Kcal/mol (Table 3, Figure 3), ~3.5 Kcal/mol (Table 3, Figure 4), ~3.4 Kcal/mol (Table 3, Figure 5) and ~3.9 Kcal/mol (Table 3, Figure 6). None of these were observed to create hydrogen bonds during the interaction with the target protein. The allyl methyl sulfide compound had the lowest interaction energy with 3ERT, at ~2.9 Kcal/mol (Table 3, Figure 7).

Figure 2. 3D and 2D interaction between 3ERT protein and alliin.

Figure 3. 3D and 2D interaction between 3ERT protein and Diallyl Disulfide.
Figure 4. 3D and 2D interaction between 3ERT protein and allicin.

Figure 5. 3D and 2D interaction between 3ERT protein and Diallyl Sulfide.

Figure 6. 3D and 2D interaction between 3ERT protein and Diallyl Trisulfide.
Figure 7. 3D and 2D interaction between 3ERT protein and Allyl Methyl Sulfide.

Figure 8. 3D and 2D interaction between 3ERT protein and Z- Ajoene.

Figure 9. 3D and 2D interaction between 3ERT protein and 2-Vinyl-4H-1,3-dithiine.

Table 3. Lipid-soluble compounds from garlic used for molecular docking.

| Sl No | Compound          | Interaction energy (Kcal/mol) | Interacting residues                                      | No of H Bonds in interaction | Fig No |
|-------|-------------------|------------------------------|-----------------------------------------------------------|-----------------------------|--------|
| 1     | Alliin            | -4.8                         | Glu 353, Arg 394, Leu 525, Leu 334                       | 2                           | 2      |
| 2     | Diallyl Disulfide | -3.7                         | Leu 349, Leu 391, Arg 394, Phe 404, Gly 521, Leu 525, Leu 384, Ile 424, Met 388, Leu 428, Leu 387, Leu 346 | 0                           | 3      |
| 3     | Allicin           | -3.5                         | Ile 510, Leu 509, His 513, Arg 434, Ala 430, Thr 431     | 0                           | 4      |
Organosulfur compounds' antibacterial, antiviral, and other qualities make them effective in preventing and therapy various inflammatory conditions, including cardiovascular, cancer, neurological disorders, and diabetes [32]. The highest interaction energy among the water-soluble compounds from garlic was found for S-Allylmercaptocysteine (-4.6 Kcal/mol), and the lowest interaction energy was found for S-Methyl-L-cysteine (-3.8 Kcal/mol) in this research (Table 4 and Figure 13 and 14, respectively). S-allyl mercapto cysteine is a stable thioallyl compound. A subtype of acute myeloid leukemia cell cultures triggers cell death and works as an antitumoral medication [33]. S-Allylmercaptocysteine seems to be prospective cancer prevention but also a managerial food ingredient. Two other phytomolecule named S-Propyl-L-cysteine and S-Allyl-L-cysteine were found to have the same interaction energy (-4.4 Kcal/mol) with 3ERT receptor protein. But, S-Propyl-L-cysteine made 3 hydrogen bonds (Table 4, Figure 10) during the interaction, while S-Allyl-L-cysteine had 2 hydrogen bonds (Table 4, Figure 11). S-Propyl-L-cysteine has already been thoroughly investigated and found to have a wide spectrum of biological actions, including antitumor, antioxidant, lipid profile controlling, antihepatotoxic, as well as cognitive properties [34]. Both of these phytopharmaceuticals are nothing but modified L-cysteine amino acid derivatives and have the potential antitumor capability as per literature. Another amino acid derivative called S-Ethyl-L-cysteine was also found to have potentiality towards the 3ERT receptor (-4.1 Kcal/mol) along with making 2 hydrogen bonds during interaction (Table 4, Figure 13). It can be used to treat tuberculosis, as per the researcher. It is present in a lotion that's used to treat cervical injuries and inflammation [35].

| Sl No | Compound                  | Interaction energy (Kcal/mol) | Interacting residues                                                                 | No of H Bonds in interaction | Fig No |
|-------|---------------------------|------------------------------|------------------------------------------------------------------------------------|------------------------------|--------|
| 1     | S-Propyl-L-cysteine       | -4.4                         | Leu 387, Leu 391, Arg 394, Leu 346, Phe 404, Met 421, Glu 353, Leu 349, Ala 350 | 3                            | 10     |
| 2     | S-Allyl-L-cysteine        | -4.4                         | Glu 353, Arg 394, Leu 349, Phe 404, Leu 525, Trp 383, Leu 346, Leu 384, Ala 350, Met 388, Leu 387, Leu 391 | 2                            | 11     |
| 3     | S-Ethyl-L-cysteine        | -4.1                         | Lys 449, Glu 323, Pro 324, Ile 326, Pro 325, Ile 386, Met 357, Leu 387, Glu 353, Arg 394, Trp 393, Phe 445, Gly 390 | 2                            | 12     |
| 4     | S-Allylmercaptocysteine   | -4.6                         | Gly 521, Leu 384, Leu 525, Leu 346, Met 388, Ala 350, Leu 387, Leu 391, Glu 391, Glu 353, Arg 394, Leu 349, Phe 404 | 2                            | 13     |
| 5     | S-Methyl-L-cysteine       | -3.8                         | Arg 394, Phe 445, Lys 449, Met 357, Glu 353, Pro 324, Leu 387, Leu 391, Ile 386, Gly 390 | 2                            | 14     |

Table 4. Water-Soluble Compounds from garlic used for molecular docking
**Figure 10.** 3D and 2D interaction between 3ERT protein and S-Propyl-L-cysteine.

**Figure 11.** 3D and 2D interaction between 3ERT protein and S-Allyl-L-cysteine.

**Figure 12.** 3D and 2D interaction between 3ERT protein and S-Ethyl-L-cysteine.
Figure 13. 3D and 2D interaction between 3ERT protein and S-Allylmercaptocysteine.

Figure 14. 3D and 2D interaction between 3ERT protein and S-Methyl-L-cysteine

Flavonoids' favorable advantages as powerful antioxidants in normal situations and pro-oxidants in inflammatory conditions, ability to induce apoptosis, and reduction of proliferative and aggravation have been proven in a number of studies [9,17]. Flavonoids appear to be natural compounds that have been present in human diets and beverages since antiquity, suggesting that they may not have the same negative side effects as synthetic anticancer drugs [36]. The interaction range for all flavonoid molecules employed for docking was determined to be between -6.5 Kcal/mol to -8.0 Kcal/mol. The best interaction was discovered for kaempferol, which has a -8.0 Kcal/mol and two hydrogen bonds during interaction with 3ERT protein, among the six flavonoid phytomolecules from Allium sativum L. (Table 5, Figure 15). It appears to be an antioxidant polyphenol particularly abundant in fruits and leafy green vegetables. In scientific investigations, kaempferol consumption has been associated with lower cancer risk. It could help by bolstering the body's natural antioxidant responses against oxidative stress, which has been linked to cancer progression [37]. Both Quercetin (Table 5, Figure 17) and Apigenin (Table 5, Figure 20) created the same interaction energy (-7.6 Kcal/mol) and three interacting hydrogen bonds with the 3ERT protein. Flavonoid quercetin appears to be a pigment obtained from plants. Grapes, red onion, herbal tea, cherries, and blueberries are just a few examples of plants and foods that contain them [38]. It has mostly been used to address heart and lung diseases and perhaps to lower the risk of
developing cancer [27]. Quercetin offers anti-inflammatory and antioxidant properties, which may help reduce edema and cancer cell death [39]. Flavonoids, which contain apigenin, are the most abundant polyphenols found in nature. Apigenin shields plants from ultraviolet rays, sheltering them from illnesses and predatory insects, regulates cellular metabolism and attracts pollinators [40]. When used with the chemo medication cisplatin, kaempferol lowers cMyc mRNA expression while increasing CDKN1A mRNA expression in ovarian cancer cells [27]. Kaempferol facilitates cell death by inhibiting the actions of cMyc. Myricetin (Table 5, Figure 18) and tangeretin (Table 5, Figure 19) are two more molecules that have similar binding energies to the target protein 3ERT. During the interaction with 3ERT, myricetin provided -7.0 Kcal/mol with no hydrogen bond, and tangeretin generated -7.1 Kcal/mol without a hydrogen bond.

Myricetin has a number of roles connected to the central nervous system, and several studies have suggested that it may be useful in avoiding diseases like Parkinson’s and Alzheimer’s [29,41]. Tangerin, a real polymethoxyflavone, is detected in lemon peel oil. Tangeretin strengthens the cell wall of seedlings to protect them from disease-causing pathogens. It possesses a wide range of medicinal benefits, including cholesterol-lowering, antitumor activity, and neuroprotective capabilities [42]. Nobiletin, a flavonoid-containing phytomolecule from garlic, was discovered to have the lowest binding potentiality (-6.5Kcal/mol) with 3ERT. During contact, it formed two hydrogen bonds. On colon cancer cells, it was found to offer additive anticarcinogenic advantages [19]. Nobiletin has been demonstrated in animal and human studies to prevent osteoporosis, lower blood cholesterol levels, and improve diabetes and insulin levels [38]. The anti-inflammatory activities of nobiletin have been proven in fibroblast cells from human synovial fluids and the mouse J774A macrophage cell line [29].

| Sl No | Compound | Interaction energy (Kcal/mol) | Interacting residues | No of H Bonds in interaction | Fig No |
|-------|----------|------------------------------|----------------------|-----------------------------|--------|
| 1     | Kaempferol | -8.0                         | Gly 521, Ile 424, Met 388, Leu 428, Arg 394, Glu 353, Leu 349, Leu 391, Leu 387, Leu 384, Ala 350, Leu 346, Thr 347, Leu 525, Met 528, Met 343, Trp 383 | 2 | 15 |
| 2     | Nobiletin  | -6.5                         | Tyr 526, Lys 529, Cys 530, Val 533, Met 528, Ala 350, Thr 347, Leu 525, Leu 536, Trp 383, Met 522 | 2 | 16 |
| 3     | Quercetin  | -7.6                         | Asp 351, Thr 347, Lys 529, Leu 525, Tyr 526, Met 522, Leu 536, Ala 350, Leu 354, Trp 383, Leu 382 | 3 | 17 |
| 4     | Myrecetin  | -7.0                         | Ala 387, Asp 351, Thr 347, Val 533, Lys 529, Cys 530, Tyr 526, Met 522, Trp 383, Leu 525, Ala 350, Leu 536, Leu 354, Leu 387, Asp 351, Thr 347 | 0 | 18 |
| 5     | Tangeretin | -7.1                         | Val 534, Leu 539, Asp 351, Leu 354, thr 347, ala 350, Met 343, Trp 383, Leu 525, Leu 346, Leu 384, Leu 536, Met 528 | 0 | 19 |
| 6     | Apigenin   | -7.6                         | Leu 349, Glu 353, Arg 394, Leu 387, Phe 404, Leu 391, Leu 428, Met 388, Leu 384, Leu 525, Ile 424, Met 421, Gly 521, His 524, Gly 420, Met 343, Ala 350 | 3 | 20 |

Besides the phytocompounds from garlic, we have used three important drug molecules which may use for breast as well as other different cancer treatments to establish our findings in this research. These drugs are 4-Hydroxytamoxifen, Abemaciclib, and Capecitabine.
Figure 15. 3D and 2D interaction between 3ERT protein and Kaempferol.

Figure 16. 3D and 2D interaction between 3ERT protein and Nobiletin.

Figure 17. 3D and 2D interaction between 3ERT protein and Quercetin.
Figure 18. 3D and 2D interaction between 3ERT protein and Myrecetin.

Figure 19. 3D and 2D interaction between 3ERT protein and Tangeretin.

Figure 20. 3D and 2D interaction between 3ERT protein and Apigenin.

Among these, 4-Hydroxytamoxifen provided the best binding energy (-10.4Kcal/mol) towards 3ERT protein (Table 6, Figure 21). The second best binding energy was found for
Abemaciclib, i.e., -9.3 Kcal/mol (Table 6, Figure 22), and finally, Capecitabine was exhibited binding energy -8.2 Kcal/mol (Table 6, Figure 23). 4-Hydroxytamoxifen is a novel estrogen antagonist studied for a range of estrogen-dependent illnesses such as cyclic breast discomfort with gynecomastia [12]. Abemaciclib seems to be a pharmaceutical used to address the endocrine malignancy that has spread. Except malignancy has indeed been treated with Abemaciclib, a molecule termed human epidermal growth factor protein 2 that has been utilized to address this condition (HER2). The HER2 receptor does have the power to boost cancer cell progression [17]. Capecitabine would be a prescribed drug used to kill cancerous cells of the mammary, colon, and genital organs [30]. In this research, we observed that all the used three reference drug molecule has more potential to interact with 3ERT. But all these molecules were found to have many side effects as per literature. It helps to slow down or prevent cancer cell proliferation.

Breast cancer would be a squamous cell carcinoma in the mammary gland. This is the second-highest commonly diagnosed cancer amongst women worldwide, behind skin cancer [6]. This cancer would strike either females or males, although it happens to women more frequently [4]. According to data, physiological, cognitive, and environmental factors have all been related to an increased risk of breast cancer [3]. It is most apparently made by a complicated combination between biological profile and lifestyle [1].

**Table 6. Reference drugs used for molecular docking with 3ERT target protein.**

| Sl No | Compound            | Interaction energy (Kcal/mol) | Interacting residues                                                                 | No of H Bonds in interaction | Fig No |
|-------|---------------------|-------------------------------|--------------------------------------------------------------------------------------|------------------------------|--------|
| 1     | 4-Hydroxytamoxifen  | -10.4                         | Leu 354, Leu 536, Ala 359, Trp 383, Asp 351, Thr 347, Leu 525, Met 528              | 0                            | 21     |
| 2     | Abemaciclib         | -9.3                          | Lys 449, Glu 323, Pro 324, Ile 326, Pro 325, Ile 386, Met 357, Leu 387, Glu 353, Arg 394, Trp 393, Phe 445, Gly 390 | 4                            | 22     |
| 3     | Capecitabine        | -8.2                          | Lys 449, Glu 323, Pro 324, Ile 326, Pro 325, Ile 386, Met 357, Leu 387, Glu 353, Arg 394, Trp 393, Phe 445, Gly 390 | 4                            | 23     |

Figure 21. 3D and 2D interaction between 3ERT protein and 4-Hydroxytamoxifen.
A variety of congenital germline mutations that may increase the risk of breast cancer have indeed been discovered. Selective antagonists, which may be impairing the function of estrogen receptors and medicines that work by blocking the enzyme aromatase, for particular, help lower the risk of cancer in individuals with a high risk of developing the disease [9]. Mammograms help in early diagnosis [10]. The upregulation of ER-alpha gene transcription is thought to contribute to the emergence and spread of a number of breast cancers [6]. Therefore, we have used ER-alpha protein as a potential molecular therapeutic target in this research to screen organosulfur and flavonoid compounds from Allium sativum L through a molecular docking approach.

Molecular docking appears to be amongst the greatest widely adopted virtual screening procedures whenever the subject protein's ultrastructure seems to be reachable [19]. This approach was able to determine the ligand-protein binding modes as well as the topology of the protein-ligand interaction, providing useful information for finalizing the lead design [13]. The subject of computer-assisted development of new drugs (CADDD) is quickly expanding, with numerous breakthroughs in recent years. CADDD for drug lead discovery is used by several large pharmaceutical companies and academia [29]. We have used flexible docking activity in this entire study. It is founded on the idea that a protein is not a passively hard structure throughout the binding. However, both the ligand as well as the protein are flexible...
analogs. The rise of structural bioinformatics, genomics, and proteomics has aided in advancing modern-day drug designing [33]. Docking of proteins and ligands is a fairly recent phenomenon with various uses. It works as a lively exploration arena and its importance in structure-based drug design (SBDD), lead optimization, biochemical pathway analysis, and De Novo drug design. The goal of molecular docking approaches is to anticipate a ligand's optimum binding affinity to a macromolecular substrate (here, target protein). It entails creating a variety of potential ligand conformations/orientations, or poses, within the protein binding pocket [17,30]. While in the population of poses created by the sampling algorithm, grading mechanisms serve as a poses filter, separating putative valid complex formation and binders from non-binders [44]. Molecular biomechanics is a strategy for treating molecules that resemble their handling utilizing classical mechanics to reduce the processing cost of molecular mechanics simulations [13,25]. The aggregate of bound (intramolecular) and nonbonded terms of the energy approximates the energy stored with a simple method called force-field [26]. Van der Waals and Coulomb electrostatic repulsion between atom pairs are nonbonded concepts [32].

Herbal isolate has been used since the dawn of humanity [12]. It comprises the use of plant species to address diseases and improve health and wellbeing. Herbal drugs are becoming more widely used as part of alternative treatment around the globe [17]. Ayurveda is divided into many functional units, each having its ideology and traditions determined by the situations, habitat, and geographical place wherein it first originated. Herbs have been used to treat a wide range of mild and severe illnesses, as well as a variety of disorders and dysfunction, including atherosclerosis, prostate problems, nervousness, irritation, and immune system boosting, to mention only some [13,16,19]. Seedlings contain a wide range of phytochemicals. Most are biologically active and comprise aromatic compounds, which have been phenols or oxygen-substituted equivalents like tannins [22]. Garlic is an excellent therapeutic item. It has around two hundred compounds that give it these properties. It does not all have had the same number of bioactive chemicals. It has long been recommended to treat various illnesses, ranging from bronchitis to diphtheria. As a native antibacterial, it can be combined with prescription drugs to support the therapy and reduce the risk of adverse effects [25]. During the 1980s, a substantial investigation was performed in Japan, Germany, and the United States, although there are still debates over how garlic delivers particularly robust antimicrobial outcomes [7, 9]. *Allium sativum* L seems to be a calmer blood purifier, which means it can actually mitigate strokes and perhaps another cardiac arrhythmia while also lowering the risk of hypertension [42]. To support optimum health, nutritionists recommend continuously using minced garlic in cooked foodstuffs [20]. The current investigation assessed various phytochemical compounds' competence and binding affinity from garlic targeting breast cancer-causing protein. In vitro and in vivo research on gastrointestinal, hepatic, colorectal, rectal, epidermis, kidney, breast, and lung cancer, garlic was proven to have anticancer abilities [13–17]. Garlic prevents cancer by inhibiting the formation, biotransformation, and enzyme detoxifying of toxicants and preventing the formation of DNA-chemical complex. Garlic inhibits cancer promotion by antiangiogenic functions like celiac disease regulation, immunomodulation, and anti-inflammatory effect [12,41]. Further methods comprise histone acetylation-mediated reduction of cancer cell proliferation, angiogenesis prevention, immunomodulation, including anti-inflammatory action [19,40]. Garlic phytonutrients have previously been studied for their cancer chemopreventive qualities. However, there seems to be no published evidence of their pharmacological activity in cancer treatment. Some molecules of garlic have been suggested
to play crucial roles in the specific death of cancer cells, given their multitargeted carcinoma activities and lack significant toxicity [23]. However, the logical design of experimental investigations and clinical trials is essential to prove this hypothesis. In contrast to organosulfur compounds studied, flavonoid compounds were more potent to interact with protein 3ERT and therefore required further in vitro study more elaborately.

4. Conclusions

Breast carcinoma is the illness of the mammary gland, which is mainly predominant among females. Herbal drugs have become more widely used as alternative treatments since ancient ages. Investigators can understand fully how to lead ligands to connect with medicinal targets by using docking techniques. Docking appears to be significant in structure-based pharmaceutical research, where finding new physiologically active molecules becomes critical. The current study performed molecular docking between the protein molecule 3ERT and garlic’s organosulfur and flavonoid components for breast cancer therapy. This research found that the compounds tested significantly connect to 3ERT molecules. Under lipid-soluble compounds, alliin was found to have the best interaction with 3ERT (-4.8 Kcal/mol), as water-soluble compounds, S-Allylmercaptocysteine showed the best interaction potential (-4.6 Kcal/mol) towards 3ERT. Kaempferol, a flavonoid phytocompound, produced maximum binding energy (-8.0 Kcal/mol) among all flavonoids used in this research. Flavonoid derivatives were shown to be more efficient in their affinities for protein 3ERT than the organosulfur compounds studied, prompting more in vitro investigation.

Funding

This research did not receive any external funding.

Acknowledgments

The authors acknowledge the authority of Ramakrishna Mission Vidyamandira, Belur Marh, Howrah 711202, West Bengal, India, for providing accessibility to software used in the present research.

Conflicts of Interest

The authors affirm that there is no conflict of interest in this study.

References

1. Shylaja, R.; Loganathan, C.; Kabilan, S.; Vijayakumary, T.; Meganathan C. Synthesis and evaluation of the antagonistic activity of 3-acetyl-2H-benzo[g]chromen-2-one against mutant Y537S estrogen receptor alpha via E-Pharmacophore modeling, molecular docking, molecular dynamics, and in-vitro cytotoxicity studies. *Journal of Molecular Structure* 2021, 1224, 129289, https://doi.org/10.1016/j.molstruc.2020.129289.
2. Thomas, L.; Gonzalez.; James, M.; Rae; Justin, A.; Colacino; Rudy, J.; Richardson. Homology models of mouse and rat estrogen receptor-α ligand-binding domain created by in silico mutagenesis of a human template: Molecular docking with 17β-estradiol, diethylstilbestrol, and paraben analogs. *Computational Toxicology* 2019, 10, 1-16, https://doi.org/10.1016/j.comtox.2018.11.003.
3. Afaf, Z.; Dalal, H.; Samir, K.; Basil, A.; Saleh. QSAR modeling, docking, ADME and reactivity of indazole derivatives as antagonizes of estrogen receptor alpha (ER-α) positive in breast cancer. *Journal of Molecular Structure* 2020, 1217, 128442, https://doi.org/10.1016/j.molstruc.2020.128442.
4. Neelakantan, M.A.; Latha, V.; Thalamuthu, S. Polyaromatic ring containing β-diketone derivatives with antiproliferative activity toward human breast cancer cell lines: Synthesis, structure, DNA binding and molecular docking. *Journal of Molecular Structure* 2022, 1249, 131573, https://doi.org/10.1016/j.molstruc.2021.131573.

5. Adil, M.; Dhumad; Ahmed, M.; Jassem; Raed, A.; Alharis; Faeza, A.; Almashal. Design, cytotoxic effects on breast cancer cell line (MDA-MB 231), and molecular docking of some maleimide-benzenesulfonamide derivatives. *Journal of the Indian Chemical Society* 2021, 98, 100055, https://doi.org/10.1016/j.jics.2021.100055.

6. Belay, Z.S.; Sonia, K.; Pankaj, T.; Paratpar, S.; Neetu, K.T. Molecular docking, synthesis and anticancer activity of thiosemicarbazone derivatives against MCF-7 human breast cancer cell line. *Life Sciences* 2021, 273, 119305, https://doi.org/10.1016/j.lfs.2021.119305.

7. Waleed, M.; Serag; Faten, Z.; Yasmin, M.; Abdelghany; Reda, F.M.; Elshaarawy; Moustafa, S.; Abdelhamid. Synthesis and molecular docking of hybrids ionic azole Schiff bases as novel CDK1 inhibitors and anti-breast cancer agents: In vitro and in vivo study. *Journal of Molecular Structure* 2021, 1245, 131041, https://doi.org/10.1016/j.molstruc.2021.131041.

8. Mondal, A.; Banerjee, S.; Bose, S.; Mazumder, S.; Haber, R.A.; Farzaei, M.H.; Bishayee, A. Garlic constituents for cancer prevention and therapy: From phytochemistry to novel formulations. *Pharmacological Research* 2021, 105837, https://doi.org/10.1016/j.phrs.2021.105837.

9. Amani, M.; Shokati, E.; Entezami, K.; Khorrami, S.; Jazayeri, M.H.; SAFARI, E. The immunomodulatory effects of low molecular weight garlic protein in crosstalk between peripheral blood mononuclear cells and colon cancer cells. *Process Biochemistry* 2021, 108, 161-168, https://doi.org/10.1016/j.procbio.2021.06.008.

10. Zhang, Y.; Liu, X.; Ruan, J.; Zhuang, X.; Zhang, X.; Li, Z. Phytochemicals of garlic: Promising candidates for cancer therapy. *Biomedicine & Pharmacotherapy* 2020, 123, 109730, https://doi.org/10.1016/j.biopha.2019.109730.

11. Padmini, R.; Maheshwari, V.U.; Saravanan, P.; Lee, K.W.; Razia, M.; Mona, S.; Alwahibi B.; Ravindran; Elshikh. M.S.; Kim, Y.O.; Kim, H.; Kim, H.J. Identification of novel bioactive molecules from garlic bulbs: A special effort to determine the anticancer potential against lung cancer with targeted drugs. *Saudi Journal of Biological Sciences* 2020, 27, 3274-3289, https://doi.org/10.1016/j.sjbs.2020.09.041.

12. Ricciutelli, M.; Nzekoue, F.K.; Caprioli, G.; Sagratini, G.; Alesi, A.; Vici, G.; Polzonetti, V. Study of the effect of marination treatment on garlic bioactive compounds through an innovative HPLC-DAD-MS method for alliin and curcuminoïd analysis. *LWT* 2020, 131, 109788, https://doi.org/10.1016/j.lwt.2020.109788.

13. Mirzavandi, F.; Mollahosseini, M.; Abargouei, A.S.; Makiaabadi, E.; Khosravi, H.M. Effects of garlic supplementation on serum inflammatory markers: A systematic review and meta-analysis of randomized controlled trials. *Diabetes & Metabolic Syndrome: Clinical Research & Review* 2020, 14, 1153-1161, https://doi.org/10.1016/j.jsxs.2020.06.031.

14. Moosavian, S.P.; Arab, A.; Paknahad, Z.; Moradi, S. The effects of garlic supplementation on oxidative stress markers: A systematic review and meta-analysis of randomized controlled trials. *Complementary Therapies in Medicine* 2020, 50, 102385, https://doi.org/10.1016/j.ctim.2020.102385.

15. Sangouni, A.A.; Azar, M.R.M.H.; Alizadeh, M. Effects of garlic powder supplementation on insulin resistance, oxidative stress, and body composition in patients with non-alcoholic fatty liver disease: A randomized controlled clinical trial. *Complementary Therapies in Medicine* 2020, 51, 102428, https://doi.org/10.1016/j.ctim.2020.102428.

16. Chen, Y.T.; Chen, Y.A.; Lee, C.H.; Wu, J.T.; Cheng, K.C.; Hsieh, C.W. A strategy for promoting γ-glutamyltransferase activity and enzymatic synthesis of S-allyl-(L)-cysteine in aged garlic via high hydrostatic pressure pretreatments. *Food Chemistry* 2020, 316, 126347, https://doi.org/10.1016/j.foodchem.2020.126347.

17. Shereen, S.T.; Ahmed; Fahim, J.R.; Khayrya, A. Youssif; Amin, M.N.; Hossam, M.H.; Alexander, A.A.O.; Brachmann; Piel, J.; Abdelmohsen, U.R.; Hamed, A.N.E. Cytotoxic potential of *Allium sativum* L. roots and their green synthesized nanoparticles supported with metabolomics and molecular docking analyses. *South African Journal of Botany* 2021, 142, 131-139, https://doi.org/10.1016/j.sajb.2021.06.020.

18. Padmini, R.; Maheshwari, V.U.; Saravanan, P.; Lee, K.W.; Razia, M.; Mona, S.; Alwahibi, B.; Ravindran; Elshikh, M.S.; Kim, Y.O.; Kim, H.; Kim, H.J. Identification of novel bioactive molecules from garlic bulbs: A special effort to determine the anticancer potential against lung cancer with targeted drugs. *Saudi Journal of Biological Sciences* 2020, 27, 3274-3289, https://doi.org/10.1016/j.sjbs.2020.09.041.
19. Rouf, R.; Uddin, S.J.; Sarker, D.K.; Islam, M.T.; Ali, E.S.; Shilpi, J.A.; Nahar, L.; Tiralongo, E.; Sarker, S.D. Antiviral potential of garlic (Allium sativum) and its organosulfur compounds: A systematic update of preclinical and clinical data. Trends In Food Science & Technology 2020, 104, 219-234, https://doi.org/10.1016/j.tifs.2020.08.006.

20. Alghamdi, H.A.; Attique, S.A.; Yan, W.; Arooj, A.; Albulyom, O.; Zhu, D.; Bilal, M.; Nawaz, M.Z. Repurposing the inhibitors of COVID-19 key proteins through molecular docking approach, Process Biochemistry 2021, 110, 216-222, https://doi.org/10.1016/j.procbio.2021.08.015.

21. Nag, A.; Paul, S.; Banerjee, R.; Kundu, R. In silico study of some selective phytochemicals against a hypothetical SARS-CoV-2 spike RBD using molecular docking tools. Computers in Biology and Medicine 2021, 137, 104818, https://doi.org/10.1016/j.compbiomed.2021.104818.

22. Das, A.; Roy, A.; Mandal, A.; Mondal, H.A.; Hess, D.; Kundu, P.; Das, S. Inhibition of Bemisia tabaci vectored, GroEL mediated transmission of tomato leaf curl New Delhi virus by garlic leaf lectin (Allium sativum leaf agglutinin). Virus Research 2021, 300, 198443, https://doi.org/10.1016/j.virusres.2021.198443.

23. Verma, A.K.; Kuma, V.; Singh, S.; Goswami, B.C.; Camps, I.; Sekar, A.; Yoon, S.; Le, K.W. Repurposing potential of Ayurvedic medicinal plants derived active principles against SARS-CoV-2 associated target proteins revealed by molecular docking, molecular dynamics and MM-PBSA studies. Biomedicine & Pharmacotherapy 2021, 137, 111356, https://doi.org/10.1016/j.biopha.2021.111356.

24. Mahmoud, A.A.; Ibrahim, A.H.M.; Taha, A.A.; Hussien, E.A.A.; Tariq, B.A.; Hesham, M.R.; Seedi, E.I.; Pare, P.W.; Effert, T.; Hegazy, M.E.F. In silico drug discovery of major metabolites from spices as SARS-CoV-2 main protease inhibitors. Computers in Biology and Medicine 2020, 126, 104046, https://doi.org/10.1016/j.compbiomed.2020.104046.

25. Sarkar, D. Molecular Docking study to Identify Potent Fungal Metabolites as Inhibitors against SARS-CoV-2 Main Protease Enzyme. Int J Pharm Bio Sci 2021, 12, 78-85, http://dx.doi.org/10.22376/ijpbs.2021.12.2.78.

26. Akella, M.; Mall, R. Molecular modeling and in vitro study on pyrocatechol as potential pharmacophore of CD151 inhibitor. Journal of Molecular Graphics and Modelling 2020, 100, 107681, https://doi.org/10.1016/j.jmgm.2020.107681.

27. Araki, M.; Kanda, N.; Iwata, H.; Sagae, Y.; Masuda, K.; Okuno, Y. Identification of a new class of non-electrophilic TRPA1 agonists by a structure-based virtual screening approach. Bioorganic & Medicinal Chemistry Letters 2020, 30, 127142, https://doi.org/10.1016/j.bmcl.2020.127142.

28. Eman, Y.; Ahmed.; Weam, S.; Elserwy; Mohamed, F.; Mansy, E.I.; Aya, M.; Serr; Abdelrahman, M.; Salem; Andrew, M.; Abdou; Basel, A.; Abdelrahman; Kenzi, H.; Elsayed; Moaz; R.; Abd, Elaziz. Angiokinase inhibition of VEGFR-2, PDGFR and FGFR and cell growth inhibition in lung cancer: Design, synthesis, biological evaluation and molecular docking of novel azaheterocyclic coumarin derivatives. Bioorganic & Medicinal Chemistry Letters 2021, 48, 128258, https://doi.org/10.1016/j.bmcl.2021.128258.

29. Mohamed; Borai, E.I.; Hala, F.; Rizk; Seham, A.; Ibrahim; Amira, K.; Fares, Mohsen, M.T.; Tahawy, E.I.; Doha, M.; Beltagy. Assessment of anti-hemolytic, cytotoxicity, antioxidant activities and molecular docking study based on thiencyrazole scaffold as pharmacophore. Journal of Molecular Structure 2021, 1240, 130602, https://doi.org/10.1016/j.molstruc.2021.130602.

30. Taibi, N.; Balas, Q.A.A.; Bekari, N.; Taibi, O.; Jabal, G.A.A.; Benali, Y.; Ameraoui, E.; Hadjadji, M.; Taibi, A.; Bouteiba, Z.M.; Mustapha, M.A.; Khammar, F.; Dergal, F.; Hassaine, R.; Boukenna, L.; Bachar, K.; Silva, A.M.S. Molecular docking and dynamic studies of a potential therapeutic target inhibiting glyoxalase system: Metabolic action of the 3, 3'-[3-(5-chloro-2-hydroxyphenyl)-3-oxopropane-1, 1-diyl]- Bis-4-hydroxycoumarin leads overexpression of the intracellular level of methylglyoxal and induction of a pro-apoptotic phenomenon in a hepatocellular carcinoma model. Chemico-Biological Interactions 2021, 345,109511, https://doi.org/10.1016/j.ubiiosis.2021.109511.

31. Águila, I.D.; Mendiola, M.A.; Pradhan, S.; Sinha, C.; Torres, E.L. Synthesis, characterization, in vitro cytotoxic activity and molecular docking of dinuclear gold(I) complexes with terephthalaldehyde bis(thiosemicarbazones). Polixyderon 2021, 210, 115498, https://doi.org/10.1016/j.poly.2021.115498.

32. Zhao, L.; Xiao, S.; Jiang, S.; Jin, Y.; Fang, W.; Wang, Z. Detailed structural investigation of Crizotinib and the exploration of its antitumor potential by DFT calculations and molecular docking. Journal of Molecular Structure 2022, 1248, 131530, https://doi.org/10.1016/j.molstruc.2021.131530.

33. Oubella, A.; Mansouri, A.E.E; Fawzi, M.; Bimoussa, A.; Laamari, Y.; Auhmani, A.; Robert, H.M.A.; Riahi, A.; Itto, M.Y.A. Thiazolididinone-linked1,2,3-triazoles with monoterpenic skeleton as new potential anticancer
agents: Design, synthesis and molecular docking studies. *Bioorganic Chemistry* 2021, 115, 105184, https://doi.org/10.1016/j.bioorg.2021.105184.

34. Zilbeyaz, K.; Oztekin, A.; Kutluana, E.G. Design and synthesis of garlic-related unsymmetrical thiosulfonates as potential Alzheimer’s disease therapeutics: In vitro and in silico study. *Bioorganic & Medicinal Chemistry* 2021, 40, 116194, https://doi.org/10.1016/j.bmc.2021.116194.

35. Das, A.; Kuma, S.; Persoons, L.; Daelemans, D.; Schols, D.; Alici, H.; Tahtaci, H.; Karki, S.S. Synthesis, in silico ADME, molecular docking and in vitro cytotoxicity evaluation of stilbene linked 1,2,3-triazoles, *Heliyon* 2021, 7, e05893, https://doi.org/10.1016/j.heliyon.2020.e05893.

36. Govindarasu, M.; Ganeshan, S.; Ansari, M.A.; Mohammad, N.; Alomary, Yahya, S.A.; Alghamdi, S.; Almehmadi, M.; Rajakumar, G.; Thiruvengadam, M.; Vaityapuri, M. In silico modeling and molecular docking insights of kaempferitin for colon cancer-related molecular targets. *Journal of Saudi Chemical Society* 2021, 25,101319, https://doi.org/10.1016/j.jscs.2021.101319.

37. Suryanarayana, K.; Robert, A.R.; Kerru, N.; Pooventhiran, T.; Thomas, R.; Maddila, S.; Sreekantha, B.; Jomnalagadda. Design, synthesis, anticancer activity and molecular docking analysis of novel dinitrophenylpyrazole bearing 1,2,3-triazoles. *Journal of Molecular Structure* 2021, 1243, 130865, https://doi.org/10.1016/j.molstruc.2021.130865.

38. Águila, I.D.; Mendiola, M.A.; Pradhan, S.; Sinha, C.; Torres, E.L. Synthesis, characterization, in vitro cytotoxic activity and molecular docking of dinuclear gold(I) complexes with terephthalaldehyde bis(thiosemicarbazones). *Polyhedron* 2021, 210, 115498, https://doi.org/10.1016/j.poly.2021.115498.

39. Zhao, L.; Xiao, S.; Jiang, S.; Jin, Y.; Fang, W.; Wang, Z. Detailed structural investigation of Crizotinib and the exploration of its antitumor potential by DFT calculations and molecular docking. *Journal of Molecular Structure* 2022, 1248, 131530, https://doi.org/10.1016/j.molstruc.2021.131530.

40. Oubella, A.; Mansouri, A.E.E.; Fawzi, M.; Binnoussa, A.; Laamari, Y.; Auhmani, A.; Robert, H.M.A.; Riahi, A.; Itto, M.Y.A. Thiazolidinone-linked1,2,3-triazoles with monoterpenic skeleton as new potential anticancer agents: Design, synthesis and molecular docking studies. *Bioorganic Chemistry* 2021, 115, 105184, https://doi.org/10.1016/j.bioorg.2021.105184.

41. Das, A.; Kuma, S.; Persoons, L.; Daelemans, D.; Schols, D.; Alici, H.; Tahtaci, H.; Karki, S.S. Synthesis, in silico ADME, molecular docking and in vitro cytotoxicity evaluation of stilbene linked 1,2,3-triazoles, *Heliyon* 2021, 7, e05893, https://doi.org/10.1016/j.heliyon.2020.e05893.

42. Qun, L.; Zhengqing, C.; Jie, F.; Siyi, L.; Chunsheng, L.; Xiaolin, L.; Dongye, Z. Molecular docking and molecular dynamics studies on the interactions of hydroxylated polybrominated diphenyl ethers to estrogen receptor alpha. *Ecotoxicology and Environmental Safety* 2014, 101, 83-89, https://doi.org/10.1016/j.ecoenv.2013.12.018.