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In Silico Approaches for the Identification of Novel Inhibitors against Breast Cancer Up-Regulated Protein

Bandar Hamad Aloufi and Ahmed Mohajja Alshammari

Department of Biology, Faculty of Science, University of Hail, Hail, Saudi Arabia

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ABSTRACT

Breast cancer is a type of cancer that develops in the breast tissues. When some breast cells begin to grow abnormally, breast cancer develops. These cells grow and divide at a faster rate than healthy cells and continue to grow, generating a lump or mass. Cancer cells in the breast may spread to lymph nodes or other places of the body. The hormone estrogen encourages cancer growth when it binds to the receptor of the targeted protein. The purpose of this study is the rational screening of a 15,000 phytochemicals library against the estrogen receptor alpha protein. The library was employed for molecular docking to find the binding affinities and simulation analysis of the top-selected compounds. The top four compounds, Mangostenone E, Exiguaflavanone M, Sanggenon A, and Flaccidine were identified as direct inhibitors of estrogen receptors as evident from their high binding affinity and occupancy of specific binding sites. Mangostenone E was the leading phytochemical that showed a high docking score—15.97 (kcal/mol)—and bonding interaction at the active site of Mangostenone E. Leading phytochemicals were subjected to analysis for drug-like properties that further reinforced their validation. Potential molecules identified in this study can be considered lead drugs for the treatment of breast cancer.

KEYWORDS

Bioinformatics, docking, drug candidates, molecular dynamic simulation, phytochemicals, protein data bank

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1. Introduction

Breast cancer is the most frequent type of cancer in women (Parkin, 2001), and early identification is a critical component of cancer treatment effectiveness (Michaelson *et al.*, 2022). Cancer is one of the most common causes of death in the modern world, accounting for around one-third of all deaths. The condition is extremely complicated, multifaceted, and linked to oxidative stress, as is the case with most human diseases (Tiwari, 2004).

The most prevalent type of cancer in women is mammary gland cancer, which originates in the gland itself. In developed countries, a woman's lifetime chance of developing breast cancer is estimated to be between 1 in 7 and 1 in 10. In Catalonia (Spain), the most recent estimates indicate that the cumulative lifetime risk of acquiring breast cancer is 1 in 11, with a 1 in 33 chance of dying from the disease (Gonzàlez *et al.*, 2005).

Breast cancer affects roughly 10% of females at some point in their lives (Feuer *et al.*, 1993). Around 30–40% of these people will die from this disease, mostly due to metastases, an irreversible condition in most cancers (Kleeff *et al.*, 2016). Breast cancer is one of the most significant health issues in our society because of its high incidence, complexity, and the financial implications of treatment. Over the previous two decades, there has been a noticeable decrease in the rate of breast cancer-specific death (Clèries *et al.*, 2006).

Patients' survival rates have been increasing in recent years, which might be due to both earlier diagnosis and increased treatment efficacy. Western countries have introduced widespread population-based screening programs during the last thirty years. As a result, the disease is being diagnosed at an earlier stage, with higher rates of curability (Brocklehurst *et al.*, 2013). Breast cancer treatment has improved due to better staging procedures, as well as developments in surgery and radiotherapy, which has resulted in higher local control rates while decreasing mutilation and the number of side effects associated with the disease. Improved systemic treatments have resulted in a significant breakthrough in patient outcomes, even when the disease is recognized as a systemic disease in its early stages (McDonald *et al.*, 2016). Chemotherapy and targeted techniques involving hormonal manipulations and anti-Her-2/neu medications are mainly used as systemic therapies in curative settings. Hormone therapy has the best-documented role in adjuvant settings for most patients, as well as the best toxicity/efficacy profile (Urruticoechea, 2008).

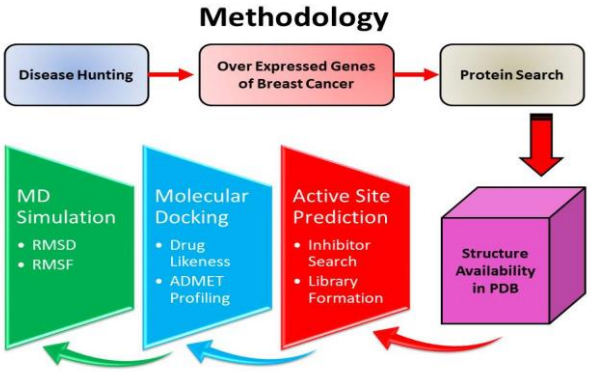
The number of molecular factors that are employed recently in diagnosis and treatment against breast cancer. Estrogen receptor alpha (ER-α) is the most used molecular marker for breast cancer. ER-α is a member of the nuclear receptor family that controls several physiological processes. Estrogen is the ligand of ER which activates the estrogen receptor. Overexpression of ER-α is seen in breast cancer (Joy *et al.*, 2001).

Computer-aided drug discovery is one of the most important methods for determining a drug's actions using computational structure-based drug discovery, and it is gaining popularity. Physics-based equations in various software programs were used to study how various chemicals interacted with their respective binding sites (Podlogar *et al.*, 2001). In several docking studies, breast cancer proteins, particularly ER-α, have been targeted in the hope of identifying the amino acids that are critical for the interactions in the active site in breast cancer. However, this has been unsuccessful. The use of medicinal plants and their extracts as a source of medication is widespread. Plants are used to make 25 percent of all medications in affluent countries, whereas the percentage in underdeveloped countries is substantially greater (Joy *et al.*, 2001). Phytochemicals are substances found in plants that control the disease. This study aimed to find effective bioactive chemicals that could be future ER-α inhibitors and potentially prevent breast cancer.

Corresponding Author: Ahmed Mohajja Alshammari 00966551355555, dr.mohajja@gmail.com

2. Materials and Methods

Figure 1: Flowchart of the methodology



2.1. Preparation of Protein:

From the Protein Data Bank, (RCSB PDB, http://www.rcsb.org) we obtained the crystal structure of breast cancer with the PDB ID: 1a52 (Berman *et al.*, 2000). After that, Discovery Studio 2019 was used to remove water molecules and heteroatoms and add polar hydrogens to the ER-α crystal structure in preparation for docking simulations.

2.2. Preparation of Compound Bank:

A library of 15,000 drug-like molecules was collected from ZINC PubChem, Mdpi, and PubChem databases, and their structures were downloaded. Before docking, these structures were optimized using the Gaussian 09 software using the B3LYP/6-31g basis set, which was then used to dock them (Frisch *et al.*, 2016). The Molecular Operating Environment (MOE) performed all computational operations, including ligand and protein synthesis and molecular docking (Vilar *et al.*, 2008).

2.3. Molecular Docking Studies:

The docking procedure was confirmed by redocking co-crystallized ligands into the protein structure using the MOE. Molecular docking studies are useful in determining the conformations and interactions that a ligand can have with a protein of interest (Venkatesan *et al.*, 2010 and Alamri *et al.*, 2021). The MOE found the active pocket on the receptor protein molecule. The MOE software was used to screen a library of 15,000 phytochemicals against the ER-α protein interaction residues. The MOE software used the "Triangular Matcher" technique to verify correct ligand confirmation before using it as the default ligand insertion approach (Vilar *et al.*, 2008). The London dG scoring algorithm in MOE was utilized to rescore simulated poses. The phytochemicals with the top and best conformations were identified once docking was completed based on their root mean square deviation (RMSD) values and S-score binding affinity. The MOE LigX tool was used to analyze and interpret two-dimensional plots of ligand-receptor interactions. The MOE was also used to make 3-D images of protein-inhibitor complexes.

2.4. Drug Toxicity Prediction:

The absence of toxicity of the chosen compound is regarded as a significant element in the selection of a component as a potential therapeutic (Segall and Barber, 2004). The current study examined the screened compounds' toxicity, including carcinogenicity, cytotoxicity, and mutagenicity. The Protox tool was used to assess the compounds' toxicity (Kumar *et al.*, 2018 and Sadeghi *et al.*, 2020).

2.5. Pharmacological Evaluation of the Chosen Compounds:

The evaluation of the pharmacological properties of the finalized compounds is the most critical and significant step in the in silico study process. The Lipinski parameter was used to investigate the compounds. The selected components met the Lipinski parameter's requirements and were tested for adsorption. The Lipinski characteristics of the selected components were assessed using the SwissADME database at http://www.swissadme.ch/index.php) (Yalcin, 2020).

2.6. Molecular Dynamics Simulation:

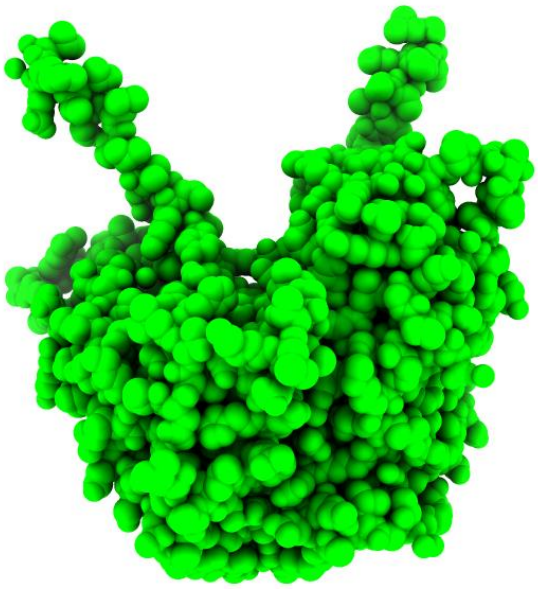
Molecular dynamics (MD) simulation is a successful in silico method for studying the dynamic behavior and stability of protein-ligand complexes under various conditions (Komanduri *et al.*, 2000). Molecular dynamics simulation of the best ligand poses was performed using the Desmond v3.6 program to verify the docking performance, as mentioned earlier (Srivastava *et al.*, 2021). The TIP3P solvent model was used in conjunction with an orthorhombic designed boundary box. By introducing Na + salt, the OPLS-2005 forcefield was used to counter the process. A hybrid algorithm of gradient descent and LBFGS algorithms was used to decrease the protein-ligand system (Blessy and Sharmile, 2015 and Sweke *et al.*, 2020). After docking, the MD simulation was run at 100 ns on Desmond for early confirmation of the protein-ligand complexes.

3. Results

3.1. Structural Retrieval:

The three-dimensional structure of estrogen receptor alpha was retrieved from the PDB database having PDB IDs: 1a52 as shown in Figure 2. The structure was chosen as a target because it has a higher resolution than other PDB structures. The resolution of 1a52 was 2.8 Å. The structure was optimized and then used as a receptor.

Figure 2: 3D visualization of the Estrogen Receptor Alpha

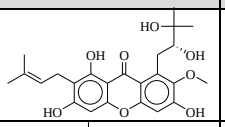
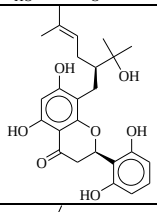
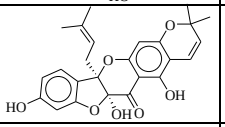
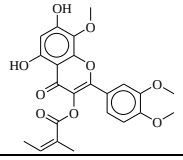


3.2. Molecular Docking:

This section involves the results obtained through docking the receptor protein structures with the phytochemicals library using MOE software. Ten different conformations were obtained for each compound. All these compounds' conformations were sorted based on binding affinity (S score), RMD values, and bonding interaction

with the active sites of the protein. The top four compounds Mangostenone E, Exiguaflavanone M, Sanggenon A, and Flaccidine were selected from each receptor protein for further analysis based on the lowest S value. These selected compounds have shown strong interactions with the binding pockets of the proteins and have minimum binding energies with the scoring function of each docked ligand as shown in Table 1.

Table 1: Detailed interactions and docking scores of the top four bioactive phytochemicals against Estrogen Receptor Alpha along with the reference drug

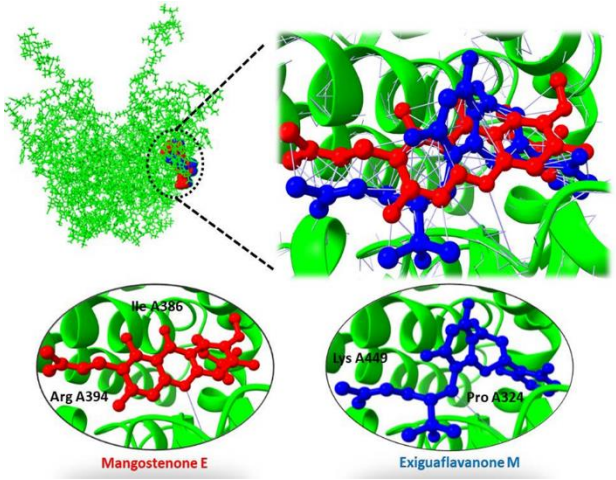
PubChem ID	Compound Name	Compound Structure	Docking Score	RMSD Value	Interacting Residues
11495983	Mangostenone E		-15.97	1.41	Arg A394 Ile A386
42607846	Exiguaflavanone M		-14.66	1.33	Lys A449 Pro A324
156707	Sanggenon A		-14.02	1.31	Leu A327 Arg A394 TrpA393
44260021	Flaccidine		-11.84	2.47	Arg A394 Lys A 449

3.3. Receptor-Ligand Interaction:

The ligand interactions of these top four compounds were checked with the receptor protein. The LigX tool was used to analyze the 2D plots of receptor-ligand interactions with the highest docked complexes to check their interactions.

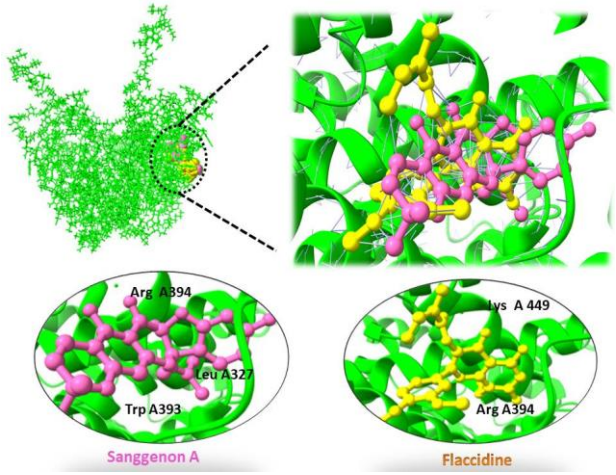
When the integral-alpha-6 protein was docked with the ready-to-dock library of 15,000 phytochemicals, the Mangostenone E docked complex showed good interaction. It was ranked at the top due to its lowest docking score of -15.97 kcal mol^{-1} and has shown potential interactions with Arg A394 and Ile A386. Exiguaflavanone M, which was ranked next to the Mangostenone E, and showed interactions with Lys A449 and Pro A324 with a binding score of -14.66 kcal mol^{-1} as shown in Figure 3.

Figure 3: Docked Mangostenone E and Exiguaflavanone M with Estrogen Receptor Alpha side chains atoms of Arg A394, Ile A386, Lys A449, and Pro A324 making hydrogen bonds. Docked poses of compound highlighting the most active residues of a protein's binding pocket



Moreover, Sanggenon A demonstrated the binding affinity of -14.02 kcal mol^{-1} and interaction with the sites Leu A327, Arg A394, and TrpA393. Flaccidine showed strong binding with Arg A394 and Lys A449 via hydrogen bonds, having a docking score of -11.84 kcal mol^{-1} as shown in Figure 4. All top four compounds showed great potential with the active site of the receptor protein.

Figure 4: Docked Sanggenon A & Flaccidine with Estrogen Receptor Alpha side chains atoms of Leu A327, Arg A394, Trp A393 Arg A394, and Lys A 449 making hydrogen bonds.



3.4. Drug-Likeness:

A drug scanning was performed via the Molinspration server to evaluate the drug-likeness properties of the top compounds. Lipinski's Rule of Five was used as a standard. This rule illustrates the significant properties of the drugs like pharmacokinetics properties, interactions with metabolism in the human body, and excretion. Selected compounds displayed zero-violations according to Lipinski's five rules and exhibited considerable drug-like properties, i.e., molecular weight (Table 2).

Table 2: Interacting compounds examined for the Lipinski rule of five by molinspiration

Compound I'D	Molecular Weight (g/mol)	Number of Hydrogen Bond Acceptor	Number of Hydrogen Bond Donor	miLogP
11495983	444.48	8	5	4.18
42607846	442.51	7	5	5.55
156707	436.46	7	3	4.60
44260021	442.42	9	2	3.32

3.5. Pharmacological Evaluation/Drug Toxicity:

The Swiss ADME and ADMETsar tools were utilized to forecast different types of pharmacokinetic features. The ADME and toxicity of the top therapeutic candidate molecules can be predicted using pharmacokinetic variables as shown in Table 3. The ADMET properties of derived phytochemicals are important for both targets. Because of poor pharmacokinetic qualities and toxicity, many medications do not involve this mechanism in their development. Early drug discovery relies on high-performance and quick ADMET profiling tests to identify active lead compounds. The ADMET profiling revealed that none of the candidate compounds had any absorption negative effects.

Table 3: ADMET profiling of Estrogen Receptor Alpha (includes drug-like properties such as absorption, metabolism, and toxicity)

Compounds IDs	11495983	42607846	156707	44260021
Gastro-Intestinal Absorption	Low	Low	High	High
Blood-Brain Barrier	No	No	No	No
P-glycoprotein-substrate	No	No	No	No
CYP1A2 Inhibitor	No	No	No	No
CYP2C19 Inhibitor	No	No	Yes	No
CYP2C9 Inhibitor	No	No	Yes	Yes
CYP2D6 Inhibitor	Yes	No	No	No
CYP3A4 Inhibitor	No	Yes	Yes	Yes
Toxicity				
Carcinogens	Non-Toxic	Non-Toxic	Non-Toxic	Non-Toxic
Cytotoxicity	Non-Cytotoxic	Non-Cytotoxic	Non-Cytotoxic	Non-Cytotoxic
Mutagenicity	No	No	No	No
Rat Acute Toxicity (LD50)	3.62 mol/kg	2.00 mol/kg	2.50 mol/kg	2.58 mol/kg

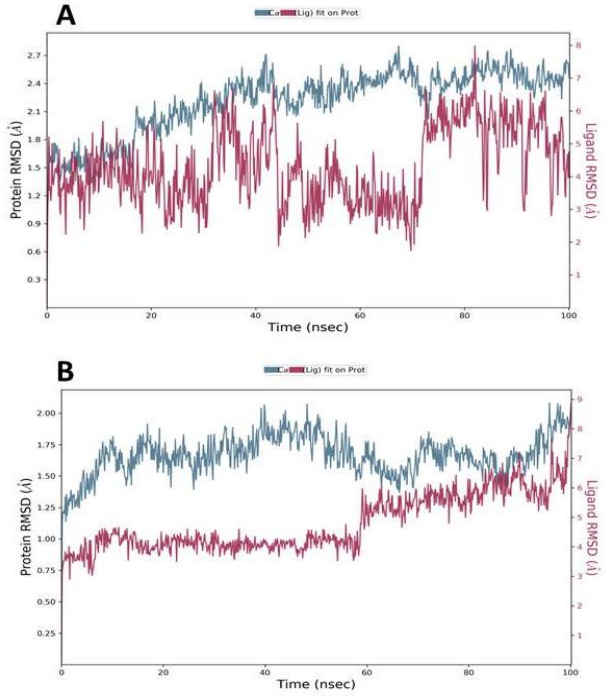
3.6. MD Simulation:

Docking analysis was performed to choose the best pose where the ligand can bind strongly with the receptor. Moreover, MD simulation was performed to determine the interaction patterns of ligands with the target protein Estrogen Receptor Alpha. Real-time MD simulation was performed by Desmond. In Desmond, the system builder used an overt aqueous medium to bring the complexes to the least energy level followed by the complex minimization step. The whole simulation process runs on three real-time simulations at 100 ns. Each of these simulations explicated the interaction patterns and stability of the complexes in terms of protein ligands RMSD and RMSF. RMSD was calculated during 100 ns simulations for each complex. The RMSD was used as a function of time for the best ligands (Mangostenone E and Exiguaflavanone M) with Estrogen Receptor Alpha protein.

3.7. Root Mean Square Deviation:

The RMSD results revealed that MD simulation was equilibrated, and conformational changes varied between 1.2 Å and 2.1Å for Mangostenone E/ ER-α; these conformational changes are tolerable for small globular protein as shown in Figure 5A. These RMSD results signify that estrogen receptor alpha did not undergo large conformational changes. The RMSD plot of the Exiguaflavanone M/ ER-α suggested that it showed a minor deviation of 0.75 Å up to 50 ns after that it becomes stable during simulation with respect to the protein binding pocket as shown in Figure 5B.

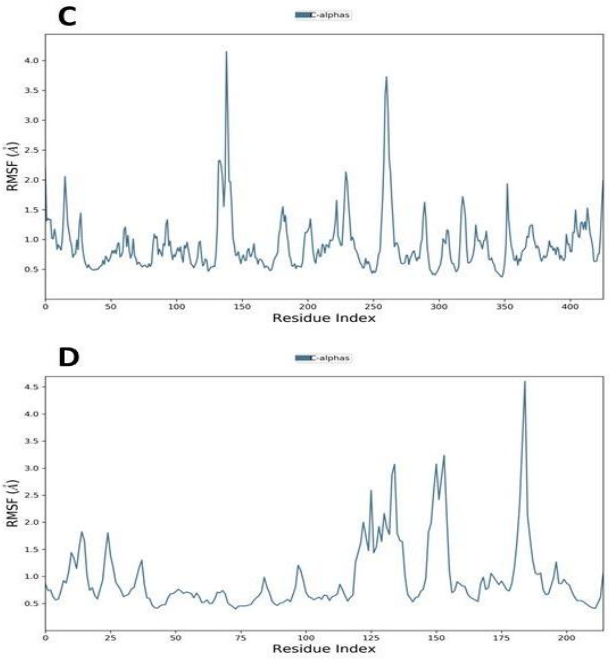
Figure 5(A,B): Statistical investigation of the intermolecular stability and dynamics of the two ER-α protein complexes using molecular dynamics simulations. The stability of the compounds is indicated by the Root Mean Square Deviation (A) Mangostenone E/ ER-α complex (B) Exiguaflavanone M/ ER-α complex.



3.8. Root Mean Square Fluctuation (RMSF):

The complex Mangostenone E/ ER-α study showed RMSF values ranging from 1.3 Å to 1.6 Å up to the residues number four hundred, as shown in the Figure 6C, while the complex Exiguaflavanone M/ ER-α revealed that its value ranges from 0.7 Å to 1.0 Å as shown in Figure 6D.

Figure 6(C,D): (C) RMSF plot trajectories of Mangostenone E/ ER-α complex, (D) Exiguaflavanone M/ ER-α complex reveals the RMSF plot trajectories



4. Discussion

Estrogen receptors have been known to play a role in the development of a high percentage of breast cancers (Ali and Coombes, 2000). Furthermore, the presence of elevated ERα receptors in breast epithelium appears to be associated with a greater risk of breast cancer, implying that ERα may play a key role in the beginning and progression of breast cancer (Tecalco-Cruz and Ramírez-Jarquín, 2017). Endocrine therapy does not work for all ER-positive cancers, and the majority of those that do respond become resistant over time (Zhao *et al.*, 2011). Most resistant tumors are ER-positive and react well to alternative endocrine therapy, indicating that ER continues to play a role in breast cancer cell proliferation (Skliris *et al.*, 2008). The problem of resistance has led to the search for and development of a variety of hormonal medications to block ER action, while research into the mechanisms that underpin resistance has revealed cellular mechanisms that control ER function with ligand binding.

In silico analysis has changed the drug formation method by the effective decrease in the expenditures as compared to the conventional drug formation procedure with the advancement in the bioinformatics field tools. Software developed by using these approaches targets for different drugs (Sliwoski *et al.*, 2014). Due to the development in the field of chemoinformatics, in silico compound libraries are present even though the modern computational methods screened the properties of these compounds for drug-likeness (Terstappen and Reggiani, 2001).

In this study, we used molecular docking for the overexpressed proteins against Estrogen Receptor Alpha for structure prediction of receptor-ligand complexes even though a protein receptor and ligand may be a small compound to attach to a receptor to show its activity (Kuntz *et al.*, 1982). Molecular docking studies were explored as a tool used for drug discovery and lead optimization of those compounds that are attached to the proteins (Levinthal *et al.*, 1975 and Salemme, 1976). An in silico approach for molecular docking is also used to prescreen the virtual compounds database.

All these protein molecules were docked in the same pocket. Four compounds were chosen from a library of 15,000 molecules and the

interaction analysis was used to further assess them. Lipinski's Rule of Five was applied to all the completed molecules (Chen *et al*., 2020). Four completed compounds demonstrated the qualities required for a therapeutic candidate. The four compounds had a lower docking value and more stable bonding with the overexpressed Estrogen Receptor Alpha protein, according to the current study.

In the current studies, the selected compounds showed the best docking scores along with closer interactions with the Estrogen Receptor Alpha protein as compared to the compounds first reported. Our studies showed that a comprehensive and integrated approach to the identification of novel inhibitors based on inhibitors of the Estrogen Receptor Alpha can serve as a starting point for the efficacy of these compounds for the in vitro test target. However, a more extensive description of compounds that interrelate is needed along with the permitted vibrant conformations over 100 ns MD simulations. These approaches are initially used to ensure safer and more practical inhibitors for the prevention of breast cancer.

5. Conclusion

In the current study, Mangostenone E, Exiguaflavanone M, Sanggenon A, and Flaccidine were identified as potential phytochemicals exhibiting good binding capability with Estrogen Receptor Alpha and found to possess all drug-like properties. Our present findings can be useful in designing and developing novel compounds with improved inhibitory action against breast cancer. However, in vitro and in vivo experimental analyses are strongly recommended to test for this study's effectiveness.

Biographies

Bandar Hamad Aloufi

Biology Department, College of Sciences, University of Hail, Hail, Saudi Arabia, 00966503306711, bandaraloufi@yahoo.com

Dr. Aloufi (Saudi) has been awarded his Ph.D. in the field of physiology. He is an expert in animal and molecular physiology and biotechnology, and his research interests in systematic biology and bioinformatics relate to biological and physiological studies. Dr. Aloufi has authored many articles in reputed journals. He has also published many textbooks in biology and physiology. He has participated in various national and international conferences and is a member of many national and international academic associations. ORCID: 0000-0003-3387-9034.

Ahmed Mohajja Alshmmari

Biology department, College of sciences, University of Hail, Hail, Saudi Arabia. 00966551355555, dr.mohajja@gmail.com

Dr. Alshammari (Saudi) is an associate professor of biology and has a Ph.D. in zoology in the field of ecological sciences. His research interests in systematic biology and bioinformatics relate to biological, toxicological, and physiological studies. He is the author of six books and forty manuscripts (including published and submitted). He has published many books in biology and physiology. He has participated in various national and international conferences and is a member of many national and international academic associations. ORCID: 0000-0003-2926-0861.

References

Alamri, M.A., Tahir ul Qamar, M., Afzal, O., Alabbas, A.B., Riadi, Y. and Alqahtani, S.M. (2021). Discovery of anti-MERS-CoV small covalent inhibitors through pharmacophore modeling, covalent docking and molecular dynamics simulation. *Journal of Molecular Liquids,* **330**(n/a), 115699. DOI: 10.1016/j.molliq.2021.115699

Ali, S. and Coombes, R.C. (2000). Estrogen receptor alpha in human breast cancer: Occurrence and significance. *Journal of Mammary Gland Biology and Neoplasia,* **5**(3), 271–81. DOI: 10.1023/A:1009594727358 gg –g

Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N. and Bourne, P.E. (2000). The Protein data bank. *Nucleic Acids Research,* **28**(1), 235–42. DOI: 10.1093/nar/28.1.235

Blessy, J.J. and Sharmila, D.J.S. (2015). Molecular simulation of N-acetylneuraminic acid analogs and molecular dynamics studies of cholera toxin-Neu5Gc complex. *Journal of Biomolecular Structure and Dynamics,* **33**(5), 1126–39. DOI: 10.1080/07391102.2014.931825

Brocklehurst, P., Kujan, O., O'Malley, L., Ogden, G.R., Shepherd, S. and Glenny, A.-M. (2013). Screening programmes for the early detection and prevention of oral cancer. *Cochrane Database of Systematic Reviews*, **n/a**(11), CD004150 DOI: 10.1002/14651858.CD004150.pub4

Chen, X., Li, H., Tian, L., Li, Q., Luo, J. and Zhang, Y. (2020). Analysis of the physicochemical properties of acaricides based on Lipinski's rule of five. *Journal of Computational Biology,* **27**(9), 1397–406. DOI: 10.1089/cmb.2019.0323

Clèries, R., Ribes, J., Esteban, L., Martinez, J.M. and Borràs, J.M. (2006). Time trends of breast cancer mortality in Spain during the period 1977–2001 and bayesian approach for projections during 2002–2016. *Annals of Oncology,* **17**(12), 1783–91. DOI: 10.1093/annonc/mdl303

Feuer, E.J., Wun, L.-M., Boring, C.C., Flanders, W.D., Timmel, M.J. and Tong, T. (1993). The lifetime risk of developing breast cancer. *JNCI: Journal of the National Cancer Institute,* **85**(11), 892–7. DOI: 10.1093/jnci/85.11.892

Frisch, M.J., Trucks, G.W., Schlegel, H.B., Scuseria, G.E., Robb, M.A., Cheeseman, J.R., Scalmani, G., Barone, V., Petersson, G.A., Nakatsuji, H., Li, X., Caricato, M., Marenich, A.V., Bloino, J., Janesko, B. G., Gomperts, R., Mennucci, B., Hratchian, H.P., Ortiz, J.V., Izmaylov, A.F., Sonnenberg, J.L., Williams-Young, D., Ding, F., Lipparini, F., Egidi, F., Goings, J., Peng, B., Petrone, A., Henderson, T., Ranasinghe, D., Zakrzewski, V.G., Gao, J., Rega, N., Zheng, G., Liang, W., Hada, M., Ehara, M., Toyota, K., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Honda, Y., Kitao, O., Nakai, H., Vreven, T., Throssell, K., Montgomery, J.A., Jr., Peralta, J.E., Ogliaro, F., Bearpark, M.J., Heyd, J.J., Brothers, E.N., Kudin, K.N. Staroverov, V.N., Keith, T.A., Kobayashi, R. Normand, J., Raghavachari, K., Rendell, A.P., Burant, J.C., Iyengar, S.S., Tomasi, J., Cossi, M., Millam, J.M., Klene, M., Adamo, C., Cammi, R., Ochterski, J.W., Martin, R.L., Morokuma, K., Farkas, O., Foresman, J.B., Fox, D.J. (2016). *Gaussian 16 Rev. C. 01*. Wallingford, CT: Gaussian Inc.

González, J.R., Moreno, V., Fernández, E., Izquierdo, A., Borrás, J., Gispert, R., and Grupo de Investigación sobre el Impacto del Cáncer en Cataluña (2005). *Probabilidad de desarrollar y morir por cáncer en Cataluña en el período 1998-2001* 'Probability of developing and dying of cancer in Catalonia during the period 1998-2001'. *Medicina clinica* [in Spanish], **124**(11), 411–14. DOI: 10.1157/13072840

Joy, P.P., Thomas, J., Mathew, S. and Skaria, B.P. (2001). Medicinal Plants. In: Bose, T.K., Kabir, J., Das, P. and Joy, P.P. (eds.) *Tropical Horticulture*. Calcutta, India: Naya Prokash, 449–632.

Kleeff, J., Korc, M., Apte, M., La Vecchia, C., Johnson, C.D., Biankin, A.V., Neale, R.E., Tempero, M., Tuveson, D.A., Hruban, R.H. and Neoptolemos, J.P. (2016). Pancreatic cancer. *Nature Reviews Disease Primers,* **2**(1), 1–22. DOI: 10.1038/nrdp.2016.22

Komanduri, R., Chandrasekaran, N. and Raff, L.M. (2000). MD simulation of indentation and scratching of single crystal aluminum. *Wear,* **240**(1), 113–43. DOI: 10.1016/S0043-1648(00)00358-6

Kumar, A., Behera, P.C., Rangra, N.K., Dey, S. and Kant, K. (2018). Computational tool for immunotoxic assessment of pyrethroids toward adaptive immune cell receptors. *Pharmacognosy magazine,* **14**(53), 124–8. DOI: 10.4103/pm.pm_62_17

Kuntz, I.D., Blaney, J.M., Oatley, S.J., Langridge, R. and Ferrin, T.E. (1982). A geometric approach to macromolecule-ligand interactions. *Journal of Molecular Biology,* **161**(2), 269–88. DOI: 10.1016/0022-2836(82)90153-X

Levinthal, C., Wodak, S.J., Kahn, P. and Dadivanian, A. K. (1975). Hemoglobin interaction in sickle cell fibers. I: Theoretical approaches to the molecular contacts. *Proceedings of the National Academy of Sciences,* **72**(4), 1330–4. DOI: 10.1073/pnas.72.4.1330

McDonald, E.S., Clark, A.S., Tchou, J., Zhang, P. and Freedman, G. M.

(2016). Clinical diagnosis and management of breast cancer. *Journal of Nuclear Medicine,* **57**(Supplement 1), 9S–16S. DOI: 10.2967/jnumed.115.157834

Michaelson, J.S., Silverstein, M., Wyatt, J., Weber, G., Moore, R., Halpern, E., Kopans, D.B. and Hughes, K. (2002). Predicting the survival of patients with breast carcinoma using tumor size. *Cancer,* **95**(4), 713–23. DOI: 10.1002/cncr.10742

Parkin, D.M. (2001). Global cancer statistics in the year 2000. *The Lancet Oncology,* **2**(9), 533–43. DOI: 10.1016/S1470-2045(01)00486-7

Podlogar, B.L., Muegge, I. and Brice, L.J. (2001). Computational methods to estimate drug development parameters. *Current Opinion in Drug Discovery & Amp; Development*, **4**(1), 102–9.

Sadeghi, M., Miroliaei, M. and Shorakai, Z. (2020). In silico investigation of flavanone compounds' inhibitory effects on alpha-amylase enzyme and predicting their inhibitory role in diabetes progression. *Journal of Fasa University of Medical Sciences,* **10**(4), 2786–95.

Salemme, F.R. (1976). An hypothetical structure for an intermolecular electron transfer complex of cytochromes c and b5. *Journal of Molecular Biology,* **102**(3), 563–8. DOI: 10.1016/0022-2836(76)90334-X

Segall, M.D. and Barber, C. (2014). Addressing toxicity risk when designing and selecting compounds in early drug discovery. *Drug Discovery Today,* **19**(5), 688–93. DOI: 10.1016/j.drudis.2014.01.006

Skliris, G.P., Leygue, E., Watson, P.H. and Murphy, L.C. (2008). Estrogen receptor alpha negative breast cancer patients: Estrogen receptor beta as a therapeutic target. *The Journal of Steroid Biochemistry and Molecular Biology,* **109**(1), 1–10. DOI: 10.1016/j.jsbmb.2007.12.010

Sliwoski, G., Kothiwale, S., Meiler, J. and Lowe, E. W. (2014). Computational methods in drug discovery. *Pharmacological Reviews,* **66**(1), 334–95. DOI: 10.1124/pr.112.007336

Srivastava, N., Garg, P., Srivastava, P. and Seth, P.K. (2021). A molecular dynamics simulation study of the ACE2 receptor with screened natural inhibitors to identify novel drug candidate against COVID-19. *PeerJ,* **9**(N/A), e11171. DOI: 10.7717/peerj.11171

Sweke, R., Wilde, F., Meyer, J., Schuld, M., Fährmann, P.K., Meynard-Piganeau, B. and Eisert, J. (2020). Stochastic gradient descent for hybrid quantum-classical optimization. *Quantum,* **4**(n/a), 314. DOI: 10.22331/q-2020-08-31-314

Tecalco-Cruz, A.C. and Ramírez-Jarquín, J.O. (2017). Mechanisms that increase stability of estrogen receptor alpha in breast cancer. *Clinical Breast Cancer,* **17**(1), 1–10. DOI: 10.1016/j.clbc.2016.07.015

Terstappen, G.C. and Reggiani, A. (2001). In silico research in drug discovery. *Trends in Pharmacological Sciences,* **22**(1), 23–6. DOI: 10.1016/S0165-6147(00)01584-4

Tiwari, A.K. (2004). Antioxidants: New-generation therapeutic base for treatment of polygenic disorders. *Current Science,* **86**(8), 1092–102.

Urruticoechea Ribate, A. (2008). *Description and Pre-clinical Validation of Dynamic Molecular Determinants of Sensitivity to Aromatase Inhibitors in Breast Cancer.* PhD Dissertation, Universitat de Barcelona, Barcelona, Spain.

Venkatesan, S.K., Shukla, A.K. and Dubey, V.K. (2010). Molecular docking studies of selected tricyclic and quinone derivatives on trypanothione reductase of *Leishmania infantum*. *Journal of Computational Chemistry,* **31**(13), 2463–75. DOI: 10.1002/jcc.21538

Vilar, S., Cozza, G. and Moro, S. (2008). Medicinal chemistry and the molecular operating environment (MOE): Application of QSAR and molecular docking to drug discovery. *Current Topics in Medicinal Chemistry,* **8**(18), 1555–72. DOI: 10.2174/156802608786786624

Yalcin, S. (2020). Molecular docking, drug likeness, and ADMET analyses of passiflora compounds as P-glycoprotein (P-gp) inhibitor for the treatment of cancer. *Current Pharmacology Reports,* **6**(6), 429–40. DOI: 10.1007/s40495-020-00241-6.

Zhao, Y., Deng, C., Wang, J., Xiao, J., Gatalica, Z., Recker, R.R. and Xiao, G.G. (2011). Let-7 family miRNAs regulate estrogen receptor alpha signaling in estrogen receptor positive breast cancer. *Breast Cancer Research and Treatment,* **127**(1), 69–80. DOI: 10.1007/s10549-010-0972-2.