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***Research Article** (Open Access)*

IN SILICO MOLECULAR DOCKING ANALYSIS FOR REPURPOSING CHALCONE DERIVATIVES AGAINST BREAST CANCER

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ABSTRACT
Breast cancer is one of the most growing and lethal variety of cancer around the world causing over 7 lakhs death around the world. The majority of breast cancer cells and healthy breast cells both have receptors for circulating oestrogen and progesterone. Aromatase inhibitors, which directly interfere with the activation of the oestrogen signalling pathway and its synthesis, are among the most important targets for treating breast cancer. Very few synthetic drugs have been produced over the years to inhibit the aromatase receptor, however the all of them have shown some levels of toxicity. So, combating these problems, repurposing of earlier reported molecules can be prioritized. Based on a literature survey, the current study was undertaken to explore the anti-breast cancer potential of the repurposed derivatives from chalcones against breast cancer target aromatase protein (PDB ID:3EQM). In this study, eight chalcone based ligands were chosen, and the structure of the human aromatase receptor was acquired from the protein data bank. For all ligands, molecular docking, drug-likeness, toxicity, and molecular dynamics were used to evaluate and analyse their anti-breast cancer activity. Two ligands showed outstanding anti-breast cancer properties with low toxicity and drug likedness. So, these compounds can be explored clinically for breast cancer in future.

***Keywords**: Chalcone, Molecular Docking, ADME Prediction, Toxicity Prediction, Molecular Dynamics*

INTRODUCTION
Breast cancer is one of the most recurring deadliest types of cancer around the world only second to lung cancer with high mortality rates among woman. At global level, more than 2.3 million new cases of breast cancer have been recorded as per the reports of WHO. In India, as per the reports of 2020, around 1.78 lakhs new cases have been registered every year with 90 thousand mortalities (Mehrotra and Yadav, 2022).

As per various reports, it can be observed that elevated levels of hormones like estrogen and androgen in body can become the primary cause of breast cancer. So, to control endocrine-responsive and resistant breast cancers, two key receptors for inhibition are aromatase and estrogen receptors (Chan *et al*., 2016). The aromatase receptor plays a vital role in conversion of androgens to estrogens thereby controlling the levels of estrogen in body. Hence, the inhibition of aromatase receptor has become a major approach for preventing and treating breast cancer (Chumsri *et al*., 2011).

There are very few drugs in the market to target aromatic receptor like Letrozole, Anastrozole, Exemestane (Simpson and Dowsett, 2002), and with increasing market demand as well as reports of few interstitial lung toxicity and liver toxicities (Mukherjee *et al*., 2022). So, in order to fill the gaping gap in the market, repurposing of drugs can provide a major relief for the same (Suganya *et al*., 2014). In this study, eight earlier reported derivatives of chalcone moiety were utilized through *in silico* studies like molecular docking, ADME, oral toxicity and molecular dynamics to predict their utilities as aromatase inhibitor in cases of breast cancer.

MATERIALS AND METHODS

Pyrx software was used for molecular docking investigations of chalcone derivatives. ADME is predicted by SwissADME, Protox III was used in predicting oral toxicity and MDweb was useful for running the molecular dynamics simulation

Identification of protein target for breast cancer

Human aromatase cytochrome P450 receptor protein (PDB ID: 3EQM) has been obtained from protein data bank as seen in Fig. 1. Aromatase cytochrome P450 is the only enzyme in vertebrates that catalyses the production of all oestrogens from androgens. Aromatase inhibitors are thus a first-line treatment for estrogen-dependent breast cancer. In order to prepare the protein for molecular docking study, the Discovery studio Visualizer software from Dassault systemes was used to remove water molecules and hetero atoms while hydrogens were added (Omer *et al*., 2022; Sastry *et al*., 2013).

Figure 1: Human aromatase cytochrome P450 receptor protein (PDB ID: 3EQM)

Ligand optimization and molecular docking studies

As reported by Li, Zhuang and Qian, 2019, the novel eight derivatives from chalcone were selected as ligands for the anti-breast cancer studies and were drawn in Chem Draw Professional 15.0 (Fig. 2.) and names as Chl1 to Chl8 (Li *et al*., 2018). Three-dimensional structures of the ligands were created in Open Babel and saved in SDF format for further preparation and molecular docking analysis. The PyRx application was used to perform molecular docking using the Auto dock tool once the proteins were saved in pdb format and loaded. The PyPx stable was used to determine which conformer was the most stable. A grid dimension of 57.02 Å x 66.94 Å x 51.40 Å was chosen for the experiment. The intermolecular interactions between the chalcone derivatized ligands and 3EQM protein (Human aromatase cytochrome P450) were identified and visualized using the Discovery Studio 2022 visualization software (Samajdar and Mondal, 2023).

ADME studies

The physicochemical properties of all ligands were subjected to SwissADME, a freely accessible tool for assessment of pharmacokinetics, drug likedness and medicinal chemistry friendliness of any ligands. The bioavailability score, molecular weight, partition coefficient and Lipinski's rule of five violations were

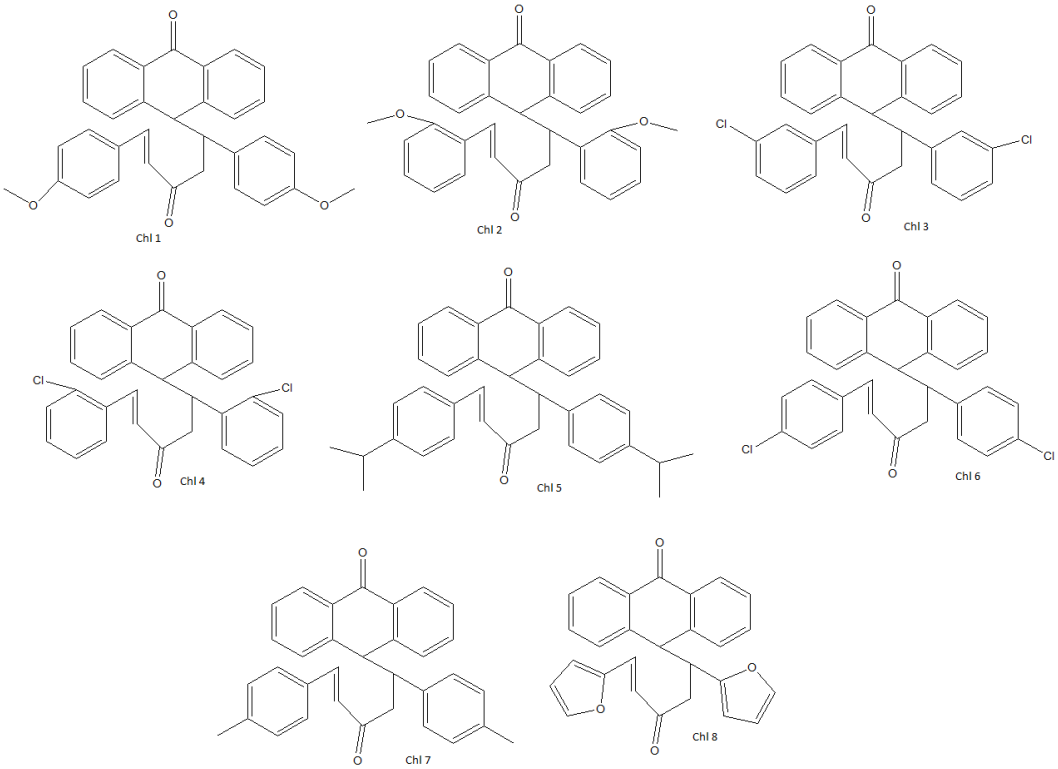

Figure 2: Different synthetic ligands derived from chalcone

used to determine the cutoff values for the physicochemical attributes of all the ligands. The MW (molecular weight), HBD (hydrogen bond donor), HBA (hydrogen bond acceptor), log P (lipophilicity log), and log S (aqueous solubility log) molecular characteristics were used to calculate drug likeness. The parameters were generated using the SWISSADME server (www.swissadme.ch/index.php) (Samajdar, 2023).

Toxicity prediction

All the chalcone based ligands were subjected to oral toxicity prediction using ProTox III software in specific end point human cells (https://tox.charite.de/protox3/). The platform accepts a two-dimensional chemical structure as input and returns the possible toxicity profile of the chalcone derivative molecule for eight models along with confidence scores (Ghosh *et al*., 2019).

Molecular dynamics

The top-scoring chalcone-based ligands were subjected to a 100 ns MD simulation with the GROMOS 99SB* force field and GROMACS software to investigate the stability and interactions of the ligand-receptor complexes. MD simulations were used to investigate structural characteristics like Root Mean Square Deviation (RMSD) for complex stability (Samajdar and Kumar, 2023).

RESULTS

Molecular Docking analysis

The aromatase inhibitor protein (3EQM), which is a target breast cancer receptor, and the chalcone derivatives as ligands were found to have substantial interactions and binding affinities using PyRx docking. Letrozole, a common aromatase inhibitor for breast cancer, and the acquired ligands' binding affinities were assessed. The binding affinities between the conventional medication Letrozole and the protein-bound ligands with grid dimensions of 69.8921Åx69.5881Åx50.4826Å are displayed in Table 1. The binding capacity of Chalcone derivatives were shown to have binding affinities ranging between -8.4 to -12.5 kcal/mol. Out of all the ligands Chl5 and Chl1 had the best binding affinity of – 12.5 kcal/mol

and -11.6 kcal/mol respectively. The lowest binding capacity was observed in Chl2 with binding capacity of -8.3 kcal/mol. As compared to the standard Letrozole (– 10.3 kcal/mol) a total of seven compounds (Chl1, Chl3, Chl4, Chl5, Chl6, Chl7, Chl8) were found to have higher binding affinities indicating major breast cancer inhibition properties of the derivatives of chalcone (Skariyachan *et al*., 2020).

Table 1.: Results of compounds docking in aromatase inhibitor target

Ligands	Binding Affinity (cΔG in kcal/mol)
	3EQM
Chl1	-11.6
Chl2	-8.3
Chl3	-11.3
Chl4	-10.9
Chl5	-12.5
Chl6	-10.8
Chl7	-11.1
Chl8	-10.4
Letrozole	-10.3

Furthermore, Chl1 and Chl5 were selected for their interactions studies based on their high binding capacity.

Compound Chl1

The interaction study between compound Chl1 and the aromatase receptor protein indicated some key interaction of the compound with the amino acids present. The two heterocyclic rings attached to the chalcone moiety showed multiple interactions including a pi-alkyl bond with Val A: 370 with one of the

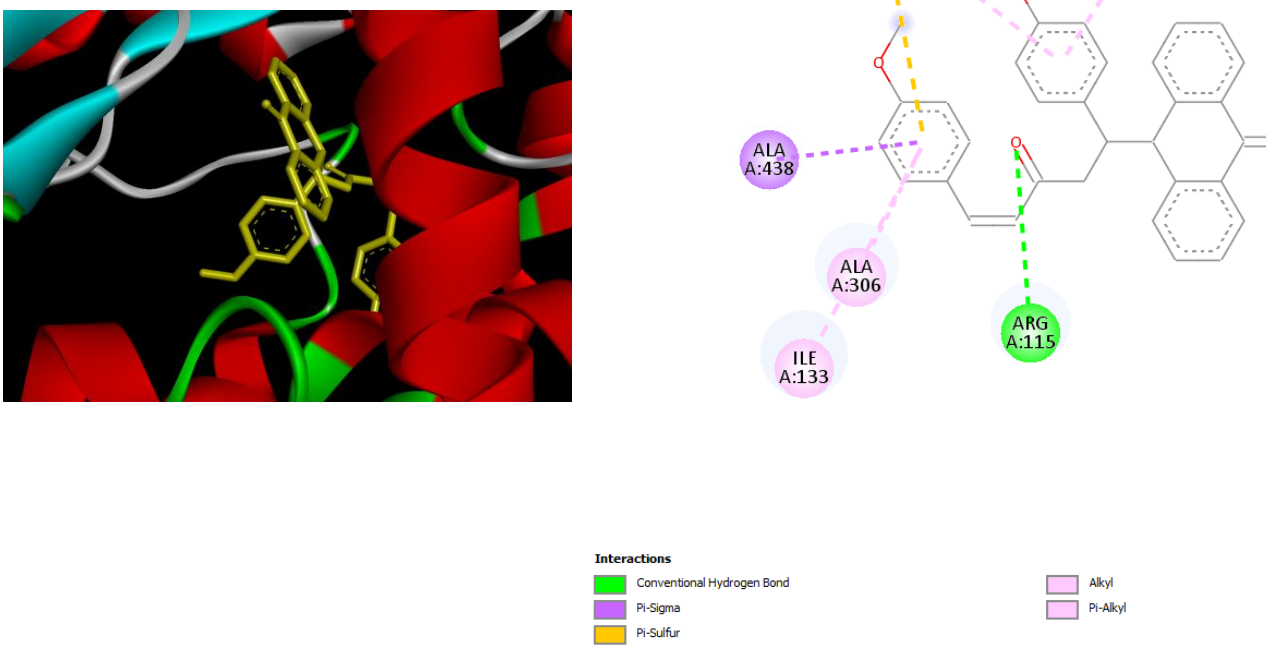

Figure 3: Molecular interaction of 3EQM and Chl 1

ring while another ring interacts by pi sigma and pi sulfur bond with Ala A:438 and Cys A:437 respecively (Fig. 3.). Also, the compound has a hydrogen bonding interactions with Arg A:115.

Compound Chl5

The interaction of the compound Chl5 and the protein indicate two pi donor hydrogen bonds (Thr A:310 and Cys A: 437) while one conventional hydrogen bond (Ala A:438) is also present. Two major pi alkyl interactions were observed in the main chalcone moiety with amino acids Ile A: 133 and Val A: 373 (Fig. 4.). Other interactions include pi-sulfur and pi-sigma interactions.

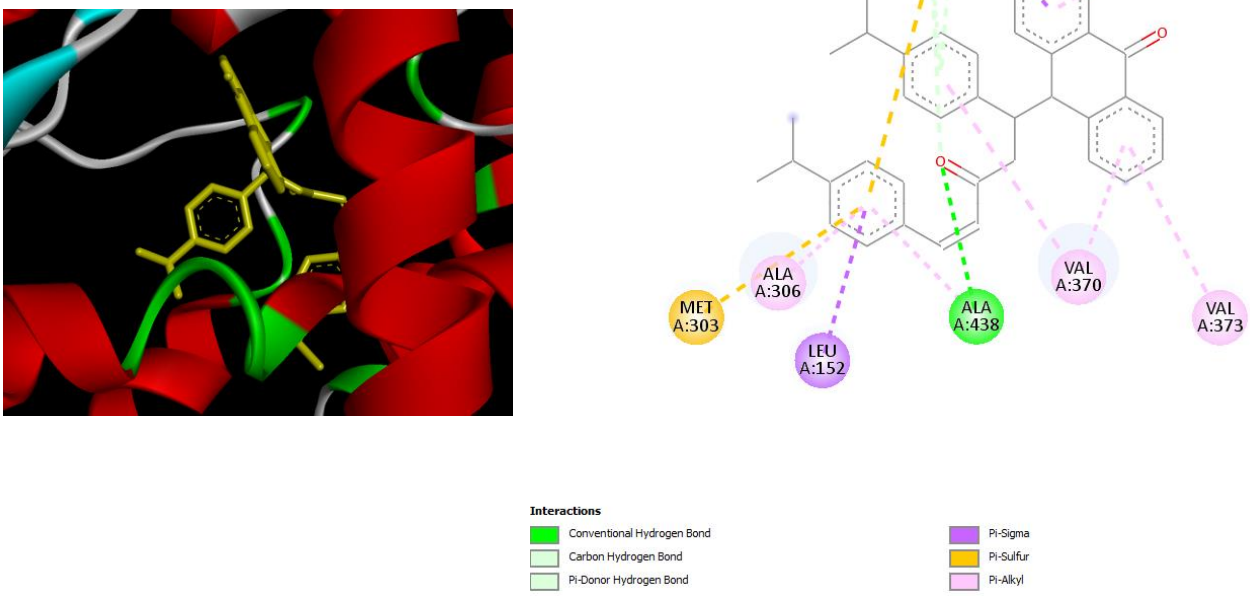

Figure 4: Molecular interaction of 3EQM and Chl 5

ADME Studies

The lipophilicity of the ligands from SwissADME was shown by their LogP values, which ranged from 3.9 to 5.1. Their molecular weights ranged from 440.53 to 530.74 g. Every ligand set's predicted value came inside Lipinski's rule's five cutoff marks. This shows that all ligands will quickly be absorbed into the gastrointestinal tract, more or less, and that the ligands with high log P values within the range are highly soluble in fats, oils, and lipids (Table 2). According to recent ADME research and molecular docking score values for ligand detection, compounds like Chl5 and Chl1 may have anti-breast cancer qualities (Yadav *et al*., 2013).

Table 2: ADME parameters for each ligand from SwissADME

Ligands	Mol Wt. (g)	Log P	HBD	HBA	Violation	BB barrier Yes/No	GI Ab-sorption	Log S
Chl1	520.66	4.64	0	4	2	No	High	-8
Chl2	520.66	4.69	0	4	2	No	High	-8
Chl3	529.5	4.47	0	2	2	No	Low	-8.97
Chl4	529.5	4.25	0	2	2	No	Low	-8.97
Chl5	530.74	4.56	0	2	2	No	Low	-8.97
Chl6	529.5	5.1	0	2	2	No	Low	-9.67
Chl7	488.66	4.72	0	2	1	No	Low	-8.42
Chl8	440.53	3.9	0	4	0	No	High	-6.36

Prediction of Toxicity

All the ligands were studied for their oral toxicity using ProTox III software showed all eight ligands had a predicted class 4 toxicity with high LD50 values indicating their safe usage in human (Table 3) (Samajdar, 2022).

Table 3: Toxicity study of the synthetic ligands

Ligands	Level of Toxicity (1=highly toxic; 6= safe)	Predicted LD50 (µg/ml)
Chl1	4	2000
Chl2	4	435
Chl3	4	1520
Chl4	4	1520
Chl5	4	1520
Chl6	4	2000
Chl7	4	2000
Chl8	4	1200

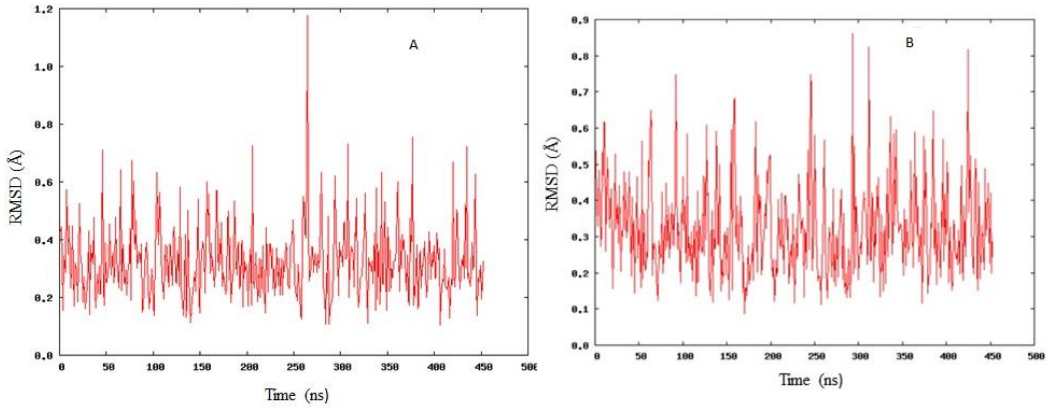

Figure 5: RMSD plot during molecular dynamics simulations of A. Chl 1 B. Chl 5

Molecular Dynamics

The root mean square deviation (RMSD) values of the top scoring ligands derived from chalcone in association with the aromatase receptor 3EQM are shown in Fig.5. Using computations of the backbone atom RMSD for the ligand-protein combination depicted in the image, the stability of the two-molecule

simulated model was evaluated. The RMSD is the most widely used quantitative technique for assessing the similarity of two superimposed protein structures (the reference and target structures). It monitors shifts in the atomic distances between two stacked arrangements. The RMSD of A (Chl 1) shows a sharp rise at 260 ns, then stabilizes at 0.85 Å and 350 ns, while B (Chl 5), shows steady rising peaks until 430 ns, with a short stabilization between 430-450 ns at 0.5-0.6 Å (Fig. 5.). This demonstrates that these ligands do not alter their orientation in the active site of proteins and stay stable inside the pocket agreeing with the visualization of molecular docking studies. With the smallest average and total RMSD and the greatest number of peaks in the 0.5 to 0.8 Å region, the 3EQM-ligand complexes demonstrated the best stability (Greatest left shift) (Samajdar and Kumar, 2023; Vieira, 2023).

DISCUSSION

Molecular docking, molecular dynamics and some of the other *in silico* prediction techniques are widely used to understand the ligand receptor interactions during drug discovery process (Altaher and Kandeel, 2016; Yadav and Chowdhury, 2022; Khater and Nassar, 2021). These processes of virtual screening and repurposing of earlier reported drugs are the need of the hour for prevention and cure of the ever-increasing disease of breast cancer (Rasul *et al*., 2022; Awasthi et al., 2015). Additionally, drug repurposing is a particularly efficient drug discovery method because it requires less capital investment and time than *de novo* drug discovery. In this study eight ligands derived from the base chalcone moiety (Chl 1- Chl 8) reported by Li, Zhuang and Qian in 2019 has been repurposed for their usage in breast cancer using multiple *in silico* approaches. Molecular docking studies using aromatase receptor protein using all eight ligands was compared against standard aromatase inhibitor Letrozole indicated outstanding results for the ligands with Chl 1 and Chl 5 showing stand out binding energy of of - 11.6 kcal/mol and - 12.5 kcal/mol respectively indicating high potential usage of these molecules in breast cancer (Sahu et al, 2023). The oral toxicity studies on the ligand performed by Protox III database software indicated that all eight ligands were in toxicity category of 4 with high IC_{50} values indicating safe for human use (Samajdar, 2024). ADME studies indicated that all compounds had no or less number of Lipinski violation with Log P values on higher side indicating within the range of highly soluble in fats, oils, and lipids (Shah *et al*., 2020). Molecular dynamics (MD) study of the top scoring molecules showed stable interaction around 350 to 400 ns (Vieira et al., 2023).

CONCLUSION

Molecular docking, ADME profiling, toxicity prediction and MD simulation have been performed to discover new agent against breast cancer sourced from earlier reported chalcone derivatives. Compound Chl 1 and Chl 5 high negative binding energy as compared to the standard Letrozole in 3EQM aromatase protein. MD simulation also approves that these two top scoring compounds (Chl 1 and Chl 5) maintains the same interaction with specific stability around 350-400 ns. In ADME studies all the ligands including Chl 1 and Chl 5 have shown drug like properties with minimum structural violations. The oral toxicity studies indicated the top scoring Chl 1 and Chl 5 are safe to use in human. In the future, reducing the toxicity potency and improving the ADME profile might necessitate structural modification or a different drug delivery strategy. Subsequently, the in vivo toxicity assessment remains necessary to guarantee the precision of the human mutagenicity, carcinogenicity, and hepatotoxicity assessments.

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DECLARATION OF INTEREST

None

REFERENCES

Altaher Y, Kandeel M (2016). Molecular analysis of some camel cytochrome P450 enzymes reveals lower evolution and drug-binding properties. *Journal of Biomolecular and Structural Dynamics.* **34**(1) 115-24. *https://doi.org/10.1080/07391102.2015.1014423.*

Awasthi M, Singh S, Pandey VP, Dwivedi UN (2015). Molecular docking and d 3D-QSAR-based virtual screening of flavonoids as potential aromatase inhibitors against estrogen-dependent breast cancer. *Journal of Biomolecular and Structural Dynamics.* **33**(4) 804-19. *https://doi.org/10.1080/07391102.2014.912152*.

Chan HJ, Petrossian K, Chen S (2016). Structural and functional characterization of aromatase, estrogen receptor, and their genes in endocrine-responsive and–resistant breast cancer cells. *The Journal of Steroid Biochemistry and Molecular Biology* **161** 73-83. *https://doi.org/10.1016/j.jsbmb.2015.07.018*

Chumsri S, Howes T, Bao T, Sabnis G, Brodie A (2011). Aromatase, aromatase inhibitors, and breast cancer. *The Journal of Steroid Biochemistry and Molecular Biology*, **125**(1-2) 13-22. *https://doi.org/10.1016/j.jsbmb.2011.02.001*

Ghosh S, Tripathi P, Talukdar P, Talapatra SN (2019). In silico study by using ProTox-II webserver for oral acute toxicity, organ toxicity, immunotoxicity, genetic toxicity endpoints, nuclear receptor signalling and stress response pathways of synthetic pyrethroids. *World Scientific News*. **132** 35-51.

Khater I, Nassar A (2021). In silico molecular docking analysis for repurposing approved antiviral drugs against SARS-CoV-2 main protease. *Biochemistry and Biophysics Reports*, **27** 101032. https://doi.org/10.1016/j.bbrep.2021.101032

Li J, Zhuang R, Qian Y (2018). Synthesis of novel Chalcone derivatives by organic catalysis. *Materials Chemistry and Physics*, **1**(1) 37-43.

Mehrotra R, Yadav K (2022). Breast cancer in India: Present scenario and the challenges ahead. *World Journal of Clinical Oncology*, **13**(3) 209. https://doi. org/10.5306/wjco.v13.i3.209

Omer SE, Ibrahim TM, Krar OA, Ali AM, Makki AA, Ibraheem W, Alzain AA (2022). Drug repurposing for SARS-CoV-2 main protease: Molecular docking and molecular dynamics investigations. *Biochemistry and Biophysics Reports*. **29** 101225.

Rasul HO, Aziz BK, Ghafour DD, Kivrak A (2022). In silico molecular docking and dynamic simulation of eugenol compounds against breast cancer. *Journal of Molecular Modelling*. **28**(1) 17. https://doi.org/10.1007/s00894-021-05010-w

Renu K, Dey A, Vellingiri B, Gopalakrishnan AV (2022). Letrozole: Pharmacology, toxicity and potential therapeutic effects. *Life Sciences*. **310**:121074. *https://doi.org/10.1016/j.lfs.2022.121074*

Sahu A, Ahmad S, Imtiyaz K, Kizhakkeppurath Kumaran A, Islam M, Raza K, Easwaran M, Kurukkan Kunnath A, Rizvi MA, Verma S (2023). In-silico and in-vitro study reveals Ziprasidone as a potential aromatase inhibitor against breast carcinoma. *Scientific Report.* **13**(1) 16545. https://doi.org/10.1038/s41598-023-43789-1.

Samajdar S, Mondal P (2023). In silico studies on the phytochemical components of *Lagenaria siceraria* targeting aromatase receptors against breast cancer. *In Silico Pharmacology*. **11**(1):19. *https://doi.org/10.1007/s40203-023-00155-3.*

Samajdar S. (2023). In silico ADME prediction of Phytochemicals present in *Piper longum*. *Asian Journal of Research in Pharmaceutical Science*. **13**(2)1 89-93.

Samajdar S, Kumar KJ (2020). Impact on stabilization of ionic gum emulsions by natural and thermally modified Chironji gum. *International Journal of Biological Macromolecule* **156** 233-8. *https://doi.org/10.1016/j.ijbiomac.2020.04.042*

Samajdar S (2024). Anti-fungal potential of phytochemicals present in *Mangifera indica*: An in-silico approach, *Journal of Botanical Society of Bengal* **78**(1) 50-55.

Samajdar S (2023). Structure based Molecular Docking Analysis for Selected Phytochemicals from *Murraya koenigii* Targeting Aromatase Receptor against Breast Cancer, *Indian Journal of Natural Science* **75** 49605-49611.

Sastry GM, Adzhigirey M, Day T, Annabhimoju R, Protein WS. (2013). ligand preparation: Parameters, protocols, and influence on virtual screening enrichments., *Journal of Computer-Aided Molecular Design.* **27**:221-34. *https://doi. org/10.1007/s10822-013-9644-8. 221-34.*

Skariyachan S, Gopal D, Chakrabarti S, Kempanna P, Uttarkar A, Muddebihalkar AG, Niranjan V. (2020). Structural and molecular basis of the interaction mechanism of selected drugs towards multiple targets of SARS-CoV-2 by molecular docking and dynamic simulation studies-deciphering the scope of repurposed drugs. *Computers in Biology and Medicine*. **126** 104054. *https://doi.org/10.1016/j.compbiomed.2020.104054*

Shah U, Patel S, Patel M, Gandhi K, Patel A (2020). Identification of chalcone derivatives as putative non-steroidal aromatase inhibitors potentially useful against breast cancer by molecular docking and ADME prediction. *Indian Journal of Chemistry -Section B.* **59**(2) 283-93. *https://doi.org/10.56042/ijcb.v59i2.27865.*

Simpson ER, Dowsett M. (2002), Aromatase and its inhibitors: significance for breast cancer therapy. *Recent Progress in Hormone Research*. **57** 317-38.

Suganya J, Radha M, Naorem DL, Nishandhini M (2014). *In silico* docking studies of selected flavonoids-natural healing agents against breast cancer. *Asian Pacific Journal of Cancer Prevention.* **15**(19) 8155-9. *https://doi.org/10.7314/APJCP.2014.15.19.8155.*

Vieira IH, Botelho EB, de Souza Gomes TJ, Kist R, Caceres RA, Zanchi FB (2023). Visual dynamics: a web application for molecular dynamics simulation using GROMACS. *BMC Bioinformatics*. **24**(1) 107. *https://doi.org/10.1186/s12859-023-05234-y.*

Yadav DK, Mudgal V, Agrawal J, Maurya A K, Bawankule D U, Chanotiya C S, Khan F, Thul ST (2013) Molecular docking and ADME studies of natural compounds of Agarwood oil for topical anti-inflammatory activity. *Current Computer-Aided Drug Design* **9**(3) 360-70.

Yadav P, Chowdhury P (2022). Effectivity of repurposed drugs against SARS-CoV-2 infections, A hope for COVID 19: inhibitor modelling studies by docking and molecular dynamics. *Heliyon*. **8**(12) e12327. *https://doi.org/10.1016/j.heliyon.2022.e12327.*

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