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Search for Novel Antagonist/S of HIF-1α from Selected Synthetic Analgesics/ Bioactive Flavonoids: An *In-Silico* Approach

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Abstract: Computer aided drug designing as well as drug repurposing implies the usage of molecular modelling techniques like analysis of the structures of receptor and ligand, molecular docking, pharmacokinetics and toxicity prediction, to explain the bioactivity of the synthetic molecules or plant secondary metabolites to design more efficient drug candidates or to repurpose an old drug in new diseases. Numerous studies have demonstrated that the low oxygen environment inside the cell is a key factor in developing breast cancer metastasis. To gain insight into the spread of breast cancer, hypoxia-inducible factor 1 (HIF-1), one of the master regulators of the hypoxic response, has been intensively explored. Our current research focuses on the *in-silico* analysis and comparative study to evaluate the effects of different cancer drugs, analgesics, and plant-derived flavonoid compounds on HIF-1α regulation of breast cancer metastasis. According to the study, Quercetin shows the maximum binding affinity, i.e., -8.2 kcal/ mol. followed by Letrozole (-7.3 kcal/mol.), Naringenin (-7.11 kcal/mol), Tamoxifen (-7.07 kcal/mol), Phenacetin (-6.16 kcal/mol), and Aspirin (-5.7 kcal/mol). The study highlighted that Quercetin has the strongest binding affinity whereas Aspirin has the least binding affinity with HIF-1α protein. Hence the least toxic compound Quercetin can be a good candidate to control breast cancer metastasis by modulating the HIF-1 pathway.

Keywords: Breast cancer metastasis, HIF-1α, Synthetic drugs, Flavonoids, Analgesics, Molecular docking, Pharmacokinetics.

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1. INTRODUCTION

Breast cancer is the number one cause of cancer and death in women worldwide. Still, sadly, many patients already have a secondary illness when they are first diagnosed, and some will develop metastasis during or after treatment.[1] In some specific breast cancer subtypes, it is usually linked to bone metastases even if prevention is not always achievable (such as triple-negative, one of the most aggressive types).[2] Tumours can easily arise when breast cancer tissue migrates and fuses with lung tissue. When tumor cells produce the HIF proteins (HIF-1 and HIF-2), they become more aggressive and capable of metastasizing to other organs.[3] Healthy cells' survival and subsequent adjustment to low oxygen conditions depend on these HIF proteins. Tumor formation in healthy mice has been linked to the absence of HIF-1α, according to studies on the functionality of HIF. Moreover, when HIF-2α was absent, they produced even more.[4] It was determined whether having more or less of each of these proteins would increase the likelihood of developing tumors by causing hypoxia (low oxygen situation) to activate HIF-1α or HIF-2α.[5, 6] Hypoxia-inducible factors (HIF-1α) is controlled by oxygen-dependent hydroxylation.[7] Certain genes, including those that code for vascular endothelial growth factor and erythropoietin, increase transcription during hypoxia.[8] It may be beneficial to alter the HIF-mediated hypoxia response in various diseases, such as cancer and cardiovascular conditions. [9] HIF-1α levels have been linked to aggressive tumor progression in several cancers, including cervical cancer, non-small cell lung carcinoma, and breast cancer. As a result, they have been suggested as a prognostic and predictive marker for resistance to chemotherapy and radiation therapy, as well as increased mortality. [10, 11] A schematic diagram shows the mechanism underlying the spread and progression of breast cancer cells (Figure 1). Based on the findings discussed above, it can be concluded that hypoxia-inducible factor 1α (HIF-1α) is a transcriptional factor with significant effects on the occurrence of cancers [9, 12], and metastasis disease, or the spread of tumor cells throughout the body, is accountable for the vast majority of cancer patient deaths and represents the key clinical challenge of solid tumor oncology. [13]

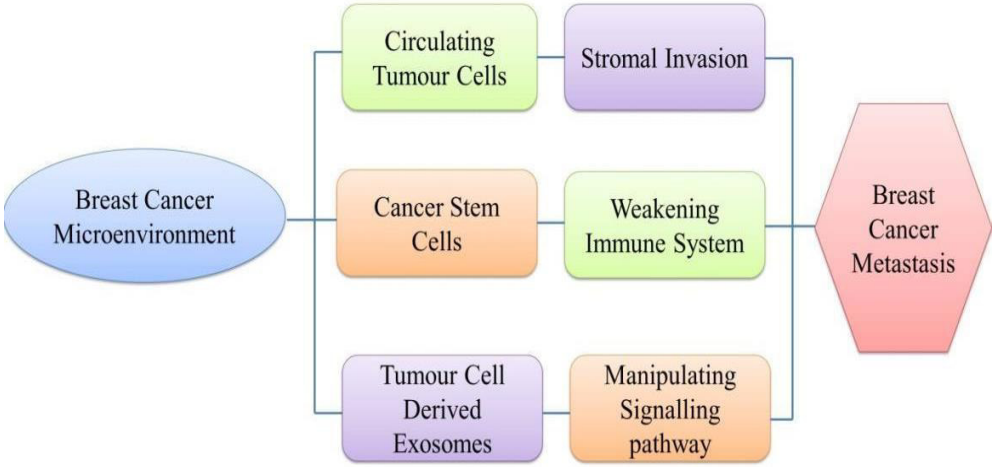

Fig 1: Process of Breast Cancer Metastasis

1.1. Standard Synthetic Drugs

AstraZeneca of the UK made Tamoxifen available for the first time. It is now commonly administered in Pakistani and Australian clinics as a hormonal therapy for estrogen-positive breast cancer.[14,15] Tamoxifen is a well-known example of a pro-drug that must undergo metabolic activation to begin acting pharmacologically. [16] The research shows that the effectiveness of Tamoxifen treatment depends on both hereditary and environmental (drug-induced) factors that alter CYP2D6 enzyme activity. [17] According to a study on women who developed breast cancer after using Tamoxifen, the risk of breast cancer decreases with the drug, resulting in less breast tissue density. [18] Another over-the-counter medication used in chemotherapy is letrozole. Femera is the brand name of the product. It is categorized as an aromatase inhibitor and used in hormone therapy. [19, 20] For the most part, postmenopausal women are given it as an oral pill to prevent breast cancer. This medication frequently causes weight gain, nausea, elevated cholesterol, and bone and joint pain. Numerous model cellular endocrine and tumor systems that include aromatase were used to investigate the relative potency of letrozole. [21, 22]

1.2. Group of Analgesics

According to several studies, taking aspirin regularly reduces the risk of developing cancer. Observational studies have also demonstrated aspirin's potential to prevent cancers other than colorectal cancer, such as melanoma, ovarian cancer, and pancreatic cancer.[23, 24] Inhibiting COX-1 and COX-2 enzymes, which are crucial components of the human inflammatory cascade, is the primary molecular mechanism by which aspirin demonstrates its anticancer potential.[25,] These investigations have identified [26] the major participants in this inflammatory cascade, and the changes they cause may be cancer risk markers. [27-29] The acetamide class of chemicals includes phenacetin. It functions as a cyclooxygenase 3 inhibitor, a peripheral nervous system medication, and a non-narcotic analgesic. N-acetyl-p-aminophenol, either conjugated or free, is the main metabolite of phenacetin detected in urine after oral treatment. [30]

1.3. Flavonoid Compounds

The category of polyphenolic substances known as flavonoids is primarily present in fruits, vegetables, herbs, cereals,

spices, and even dairy products. [31, 32] Scientists worldwide are interested in their function as an interconnected type of medicine. Various pharmacological actions, such as antioxidant, antibacterial, antiviral, anticancer, anti-atherosclerosis, antidiabetic, anti-inflammatory, anti-thrombogenic, hypolipidemic, and neuroprotective effects, are the key components of the health-promoting qualities. [33-35] Numerous studies have shown evidence to support the use of these chemicals as anticancer medications. One of the flavonoid groups widely distributed in various fruits, vegetables, and other foods is Quercetin. Naringenin is a member of the flavanones group, another group primarily found in *Citrus* fruits.[36,37] According to the literature, researchers looked into the anticarcinogenic effects of Quercetin on breast cancer stem cells and the potential mechanisms underlying those effects. Surprisingly, they discovered that Quercetin could reduce breast cancer stem cells' division, self-renewal, and invasiveness. Aldehyde dehydrogenase 1A1, C-X-C chemokine receptor type 4, and epithelial cell adhesion molecules were among the proteins whose expression levels were decreased. These proteins are linked to carcinogenesis and cancer progression.[38-40] Naringenin is present in either glycoside or aglycone form. The phenylpropanoid pathway synthesizes Naringenin and its derivatives among vegetables and fruits.[41] Naringenin inhibits breast cancer (MCF-7) cell generation by blocking the GLUT4 transporter. Naringenin prevented glucose absorption by reducing the phosphorylation of P44/P42 mitogen-activated protein kinase (MAPK), a crucial step in the insulin signaling cascade, and the activation of phosphoinositide-3-kinase[42], a major regulator of insulin-induced GLUT4 translocation. Similarly, Quercetin caused cell cycle arrest in the G0/G1 phase and reduced the expression of the survival gene in breast cancer. [43-46] Therefore, the present study aims to do a comparative *In-silico* analysis and detailed study on molecular docking with our protein of interest and comparison among the ligands to see the toxicity levels of these drugs and analyze the drug-likeliness of the synthetic compounds, i.e., Tamoxifen and Letrozole (which are of already widely used for the treatment of breast cancer patients), group of analgesics, i.e., Aspirin and Phenacetin and certain essential phytochemicals (flavonoids), i.e., Naringenin and Quercetin, represented through Figure 2.

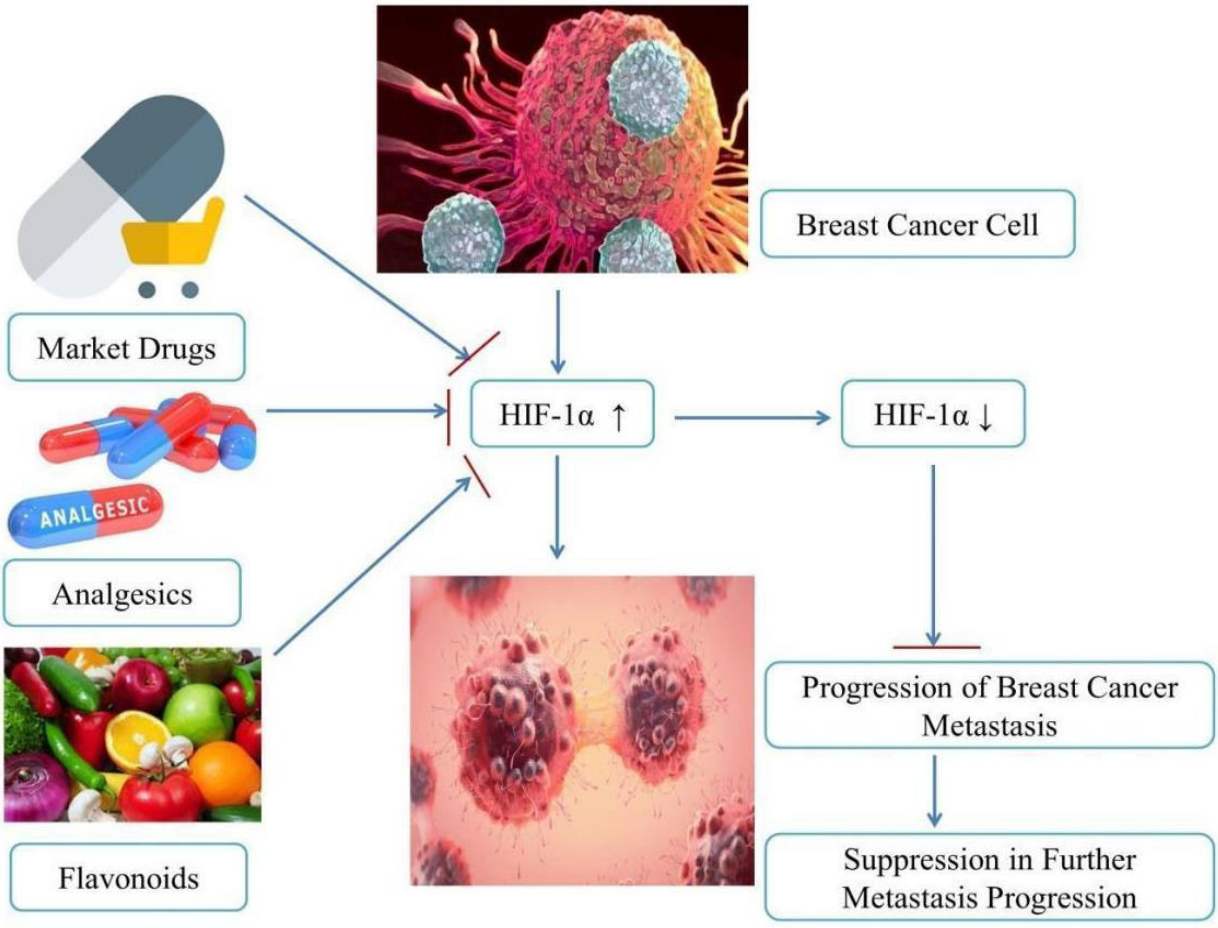

Fig 2: The Overall Workflow

2. MATERIALS AND METHODS

2.1. Selection and Preparation of the Ligands

Following a thorough literature review, six compounds from three different variants were selected. These include Quercetin and Naringenin under flavonoid compounds, Tamoxifen and Letrozole under standard synthetic market drugs, and Aspirin and Phenacetin under the category of analgesics. Next, all of the ligand structures' 2D Structure Data Format (SDF) [Figure 3] files were downloaded from PubChem (www.pubchem.ncbi.nlm.nih.gov/) and later translated to their 3D PDB format using Open Babel software. Finally, a PDBQT format file was created after adding [47] hydrogenating atoms and the desired torsion to a PDB format file.

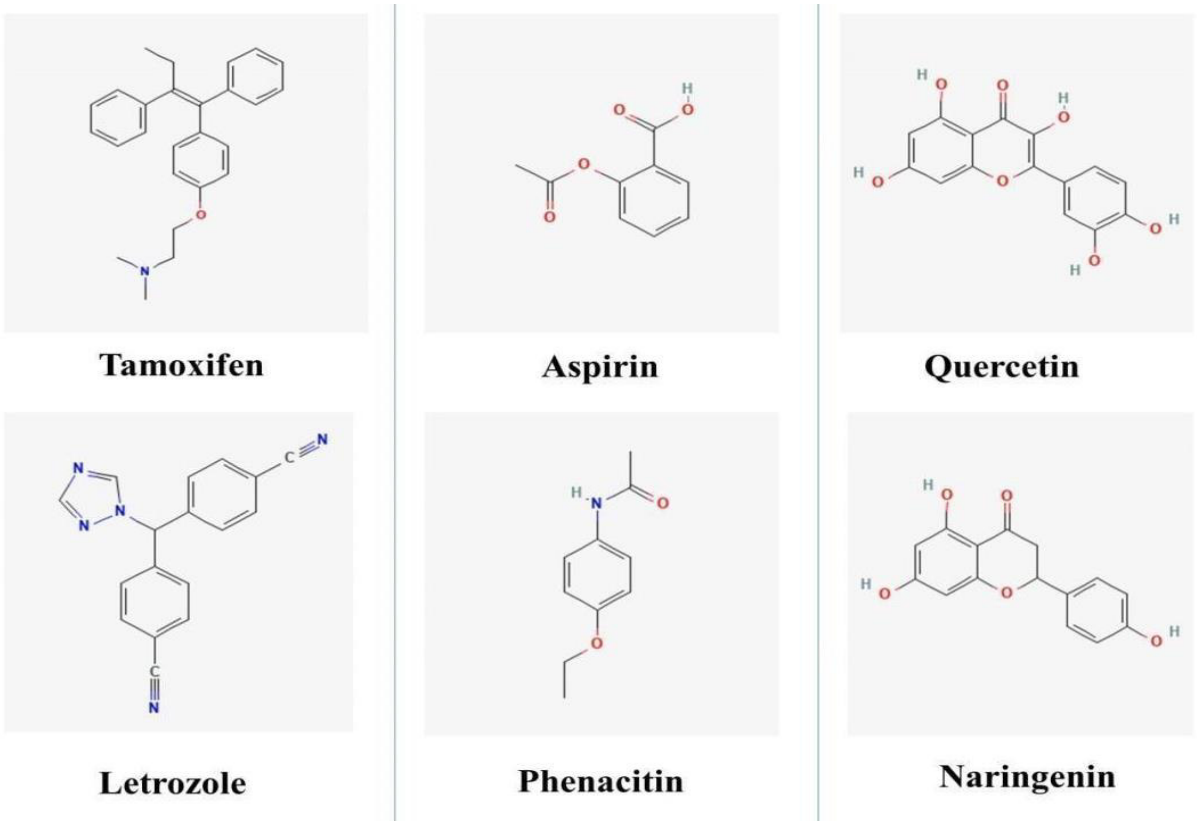

Fig 3: The Small Molecules or Compounds Selected based on Literature

2.2. *Selection of Receptor or Proteins*

For protein selection, 1H2M protein has been selected. This protein signifies the structure of HIF-1α. From Protein Data Bank (http://www.rcsb.org/), the 3D structure of 1H2M [Figure 4] has been obtained. Then to stabilize the receptor structures, the already attached ligands and water molecules were removed by BIOVIA Discovery Studio 2020 software (https://discover.3ds.com/discovery-studio-visualizer-download/).[48]

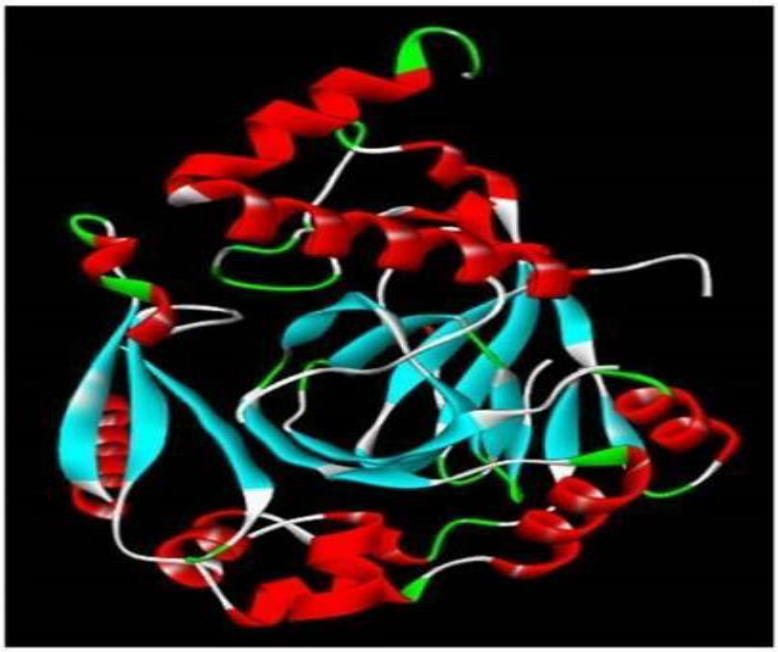

Fig 4: The 3D Structure of Protein 1H2M [Human Factor Inhibiting HIF-1α]

2.3. *Protein Preparation*

2.3.1. *Validation of Protein Structure*

The newly generated protein PDB structure was then undergone through a series of quality analyses, including ERRAT, Procheck using SAVES 6.0 (https://saves.mbi.ucla.edu/), QMEAN Z Score using SWISS-MODEL Server (https://swissmodel.expasy.org/qmean/) [49-52] and ProSA-web (https://prosa.services.came.sbg.ac.at/prosa.php/).[53]

2.3.2. SwissADME Prediction of the Compounds

Adsorption, Distribution, Metabolism, and Excretion all together, termed ADME, is a very profitable process to assess all those previously mentioned parameters of the ligands using the server of the SwissADME website (https://www.swissadme.ch/).[54, 55]

2.3.3. Toxicity Prediction of the Compounds

In the case of designing and establishing a suitable drug compound, it is a very necessary step to predict the toxicity level of the small compounds or rather ligands before investigating their endurance capacity when ingested into any animal model like a mouse or rat as well as in humans too. There are two online servers available for these purposes; they are: PreADMET server (https://preadmet.bmdrc.kr/).[56, 57]

2.3.4. Bioactivity Score Prediction Using Molinspiration Chemo Informatics Tool

Molinspiration chemoinformatics online tool (https://www.molinspiration.com/) helps to identify different bioactivity scores of a compound only upon submitting its SMILE structure to the server. Six various bioactivity scores can be predicted by supporting molecular manipulation, fragmentation, processing, and conversion.[58]

2.3.5. Molecular Docking Interaction Using AutoDock 4.2

With the help of AutoDock 4.2 online software [59], the molecular docking of the target protein (1H2M) with all the selected compounds has been carried out. This automation procedure was very effective for further investigation of the binding efficiency of respective ligands towards the macromolecule. Each PDB file has been changed into PDBQT format to carry out the docking, where all the non-protein elements are removed. The gasteiger partial charges and Hydrogen atoms were added using AutoDock tools (Version- 4.2). Following this, the LGA (Lamarckian Genetic Algorithm) has been implemented through AutoDock, ver-4.2 program by maintaining all default parameters, many algorithms, and techniques. This procedure involves many assembling tools to assemble the desired result by the conformations' similarities, conformational visualization, and protein-ligand interactions. To generate affinity potentials, the auto grid has been applied. For the interpretation of results of molecular docking, the AutoDock tool comprises. The final visualization of the docked structure was performed using BIOVIA Discovery Studio 2020 (https://discover.3ds.com/discovery-studio-visualizer-download/) [48] and PYMOL software (https://pymol.org/). [60]

2.3.6. Assessment of Structural Hotspots on the Receptor Protein

An internet service called CASTp 3.0 (http://sts.bioe.uic.edu/)[61] is used to predict active amino acid residues or structural hotspots on the receptor protein. In addition, the Computer Atlas Surface Topography of Protein (CASTp) often systematically quantitatively characterizes a protein's surface topography.

2.3.7. CABS-flex 2.0 Server prediction

One of the well-known tools for quick simulation is the CABS flex 2.0 webserver (http://biocomp.chem.uw.edu.pl/CABSflex2/), [62–65], where the MD-Simulation process is carried out. According to the results produced by the online server, the 'Fluctuation plot' tab offers a movable 2D RMSF plot following global superposition.

3. RESULTS

3.1. Validation of Protein Structure

The overall quality recognition of the 3D protein PDB structure, i.e., 1H2M, as predicted by several previously mentioned online tools, are represented in Figure 5, 6, and Table 1.

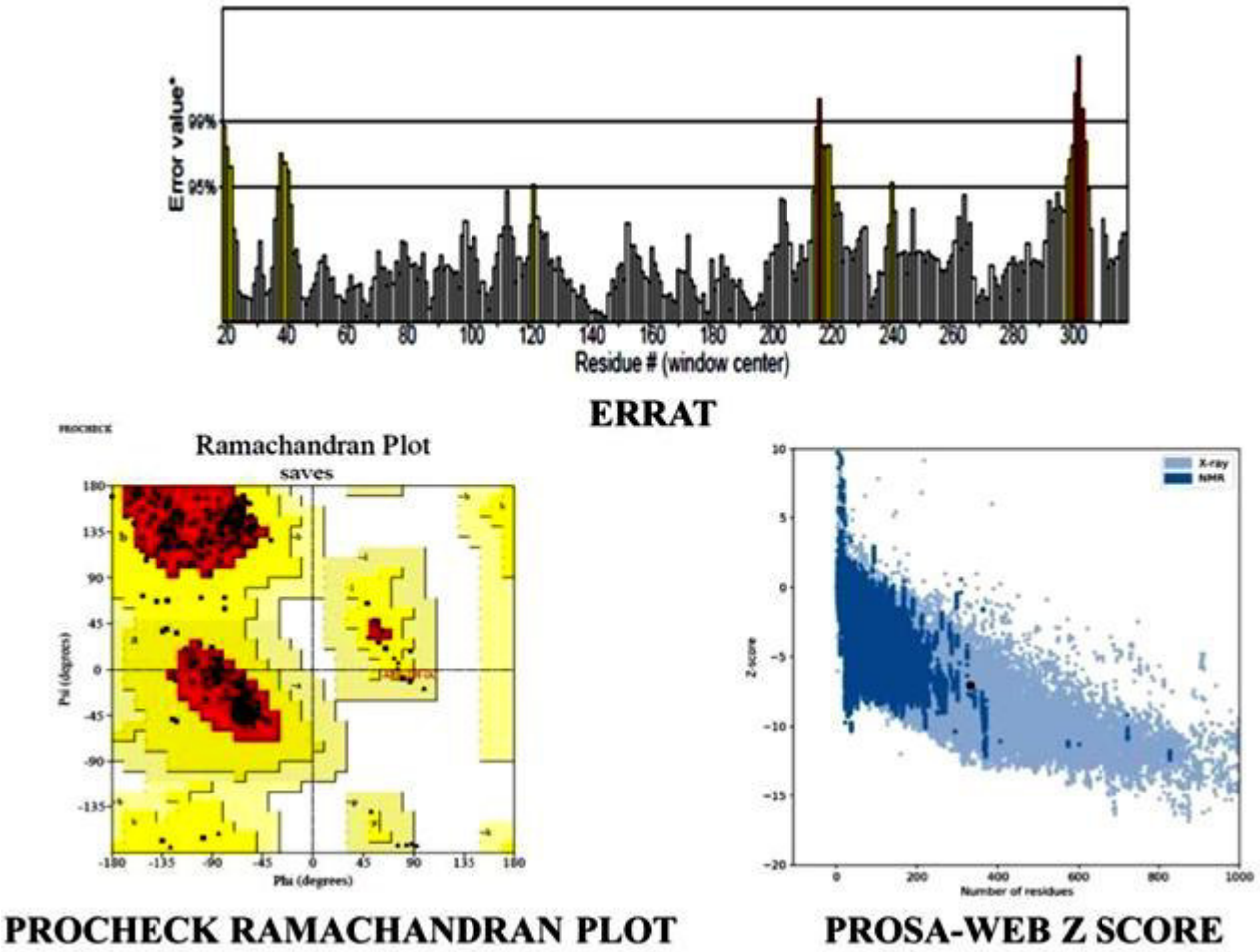

Fig 5: The Quality Checking Parameters of Protein 1H2M

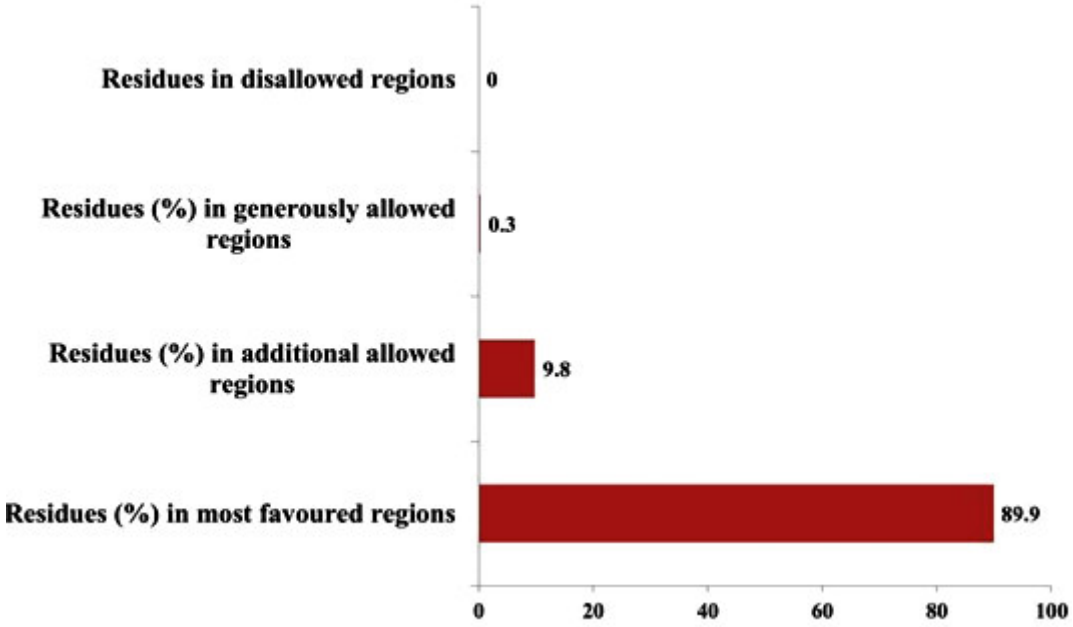

Fig 6: The RAMACHANDRAN Plot Statistics

Table 1: Table Showing ERRAT, and PROSA WEB and QMEAN Z Scores of Protein 1H2M			
Name of the protein	ERRATA Quality Score	PROSA WEB Score	QMEAN Z Score
1H2M	93.931	-7.03	-0.11

3.2. SwissADME Prediction Results

Upon submission of ligand structure in SMILEs format, SwissADME result is generated based on ADME/toxicity analysis and Lipinski filter analysis. Here in our result, we have given different tables for each of the result parameters: physicochemical properties [Table 2], lipophilicity [Table 3], water solubility [Table 4], pharmacokinetics [Table 5], and drug likeliness [Table 6]. The Boiled Egg Structure Showing Results of Gastrointestinal Tract Absorption and, afterward, Show BBB (Blood Brain Barrier) Permeation is represented via Figure 7.

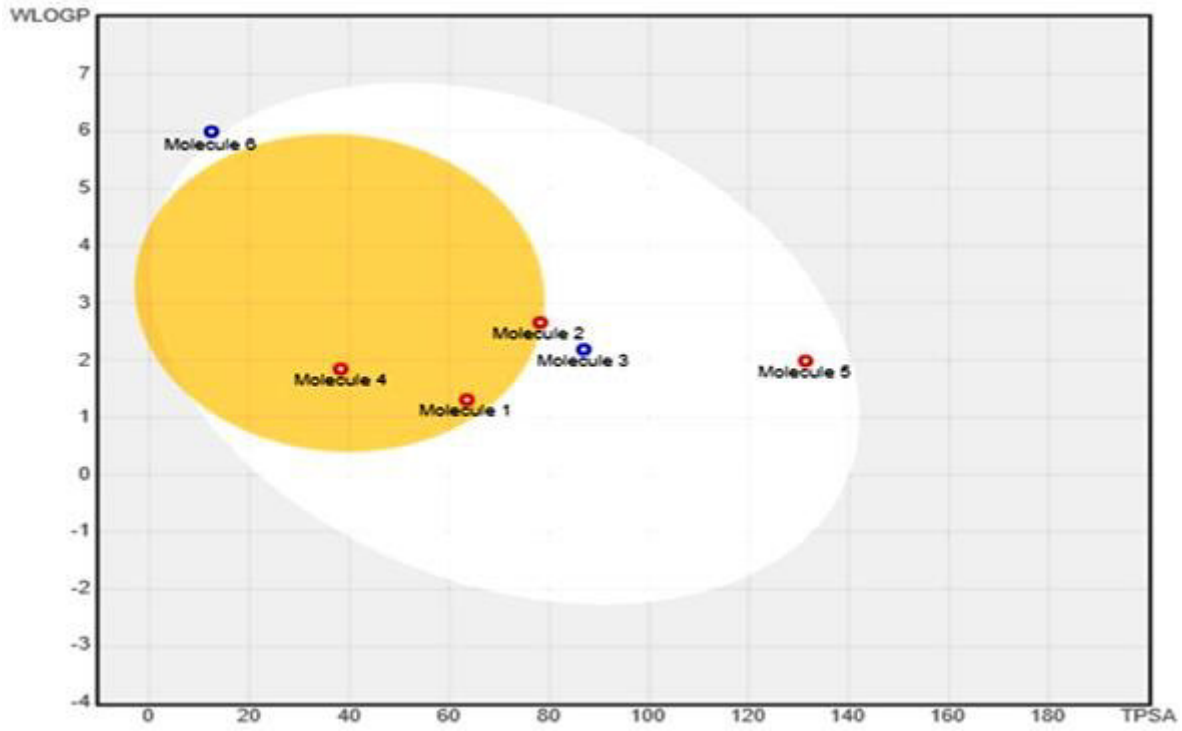

Fig 7: The Boiled Egg Structure Showing Results of Gastrointestinal Tract Absorption and after that Show BBB (Blood Brain Barrier) Permeation

Table 2: Table Showing Results of Physicochemical Properties of the Compounds

Parameters	Compounds					
	Tamoxifen	Letrozole	Aspirin	Phenacetin	Quercetin	Naringenin
Formula	C26H29NO	C17H11N5	C9H8O4	C10H13NO2	C15H10O7	C15H12O5
Molecular Weight	371.51 g/mol	285.30 g/mol	180.16 g/mol	179.22 g/mol	302.24 g/mol	272.25 g/mol
Num. heavy atoms	28	22	13	13	22	20
Num. arom. heavy atoms	18	17	6	6	16	12
Fraction Csp3	0.23	0.06	0.11	0.30	0.00	0.13
Num. rotatable bonds	8	3	3	4	1	1
Num. H-bond acceptors	2	4	4	2	7	5
Num. H-bond donors	0	0	1	1	5	3

Table 3: Table Showing Results of Lipophilicity of the compounds

Parameters	Compounds					
	Tamoxifen	Letrozole	Aspirin	Phenacetin	Quercetin	Naringenin
Log $P_{o/w}$ (iLOGP)	4.64	2.20	1.30	2.10	1.63	1.75
Log $P_{o/w}$ (XLOGP3)	7.14	2.73	1.19	1.58	1.54	2.52
Log $P_{o/w}$ (WLOGP)	6.00	2.66	1.31	1.85	1.99	2.19
Log $P_{o/w}$ (MLOGP)	5.10	1.49	1.51	1.54	-0.56	0.71
Log $P_{o/w}$ (SILICOS-IT)	5.99	2.53	1.10	1.74	1.54	2.05
Consensus Log $P_{o/w}$	5.77	2.32	1.28	1.76	1.23	1.84

Table 4: Table Showing Results of Water Solubility of the Compounds

Parameters	Compounds					
	Tamoxifen	Letrozole	Aspirin	Phenacetin	Quercetin	Naringenin
LOG S (ESOL)	-6.59	-3.70	-1.85	-2.02	-3.16	-3.49
Class	Poorly soluble	Soluble	Very soluble	Soluble	Soluble	Soluble
LOG S (Ali)	-7.22	-4.03	-2.12	-2.00	-3.91	-3.99

Class	Poorly soluble	Moderately soluble	Soluble	Very soluble	Soluble	Soluble
LOG S (SILICON-IT)	-8.92	-5.29	-1.85	-3.32	-3.24	-3.42
Class	Poorly soluble	Moderately soluble	Soluble	Soluble	Soluble	Soluble

Table 5: Table Showing Results of Pharmacokinetics of the Compounds

Parameters	Compounds					
	Tamoxifen	Letrozole	Aspirin	Phenacetin	Quercetin	Naringenin
GI absorption	Low	High	High	High	High	High
BBB permeant	No	Yes	Yes	Yes	No	No
P-gp substrate	Yes	No	No	No	No	Yes
CYP1A2 inhibitor	No	Yes	No	Yes	Yes	Yes
CYP2C19 inhibitor	Yes	Yes	No	No	No	No
CYP2C9 inhibitor	No	Yes	No	No	No	No
CYP2D6 inhibitor	Yes	Yes	No	No	Yes	No
CYP3A4 inhibitor	No	No	No	No	Yes	Yes
LOG K_P (skin permeation)	-3.50 cm/s	-6.10 cm/s	-6.55 cm/s	-6.27 cm/s	-7.05 cm/s	-6.17 cm/s

Table 6: Table Showing Results of Drug Likeliness of the Compounds

Parameters	Compounds					
	Tamoxifen	Letrozole	Aspirin	Phenacetin	Quercetin	Naringenin
Lipinski	Yes; 1 violation: MLOGP>4.15	Yes, 0 violation	Yes, 0 violation	Yes; 1 violation: NH or OH>5	Yes, 0 violation	Yes; 1 violation: NH or OH>5
Bioavailability Score	0.55	0.55	0.85	0.55	0.55	0.55

3.3. Toxicity Prediction of the Ligands

Evaluating a small compound's toxicity is a crucial step in the drug discovery process. Table 7 displays the results of the toxicological prediction using the PreADMET service, including the drugs' mutagenicity, carcinogenicity, and inhibition of hERG.

Table 7: Table Showing Results of Mutagenicity and Carcinogenicity along with hERG Inhibition of the compounds

Compounds	Ames Test	Carcino Mouse	Carcino Rat	Herg Inhibition
Tamoxifen	Mutagen	Positive	Negative	Medium Risk
Letrozole	Mutagen	Negative	Negative	Medium Risk
Aspirin	Mutagen	Negative	Positive	Low Risk
Phenacetin	Mutagen	Negative	Negative	Low Risk
Quercetin	Mutagen	Negative	Positive	Medium Risk
Naringenin	Mutagen	Negative	Positive	Medium Risk

3.4. Bioactivity Score Prediction Using Molinspiration Chemo Informatics Tool

The computed scores predicted by the Molinspiration chemo informatics online tool for different bioactivities of the compounds are displayed in Table 8.

Table 8: Table Showing Results of Bioactivity Score Prediction of the Compounds

Compounds	Gpcr Ligand	Ion Channel Modulator	Kinase Inhibitor	Nuclear Receptor Ligand	Protease Inhibitor	Enzyme Inhibitor
Tamoxifen	0.30	0.00	-0.01	0.57	0.04	0.32
Letrozole	-0.06	-0.07	-0.19	-0.27	-0.24	0.30
Aspirin	-0.76	-0.32	-1.06	-0.44	-0.82	-0.28
Phenacetin	-1.00	-0.61	-0.98	-1.15	-1.10	-0.72
Quercetin	-0.06	-0.19	0.28	0.36	-0.25	0.28
Naringenin	0.03	-0.20	-0.26	0.42	-0.12	0.21

3.5. Molecular Docking Interaction Using AutoDock 4.2

Based on the docking analysis done by AutoDock 4.2, The total result of different binding energy affinity is represented in Figure 10, and each docking interaction is presented in the 3D and 2D manner (Figures 8 and 9).

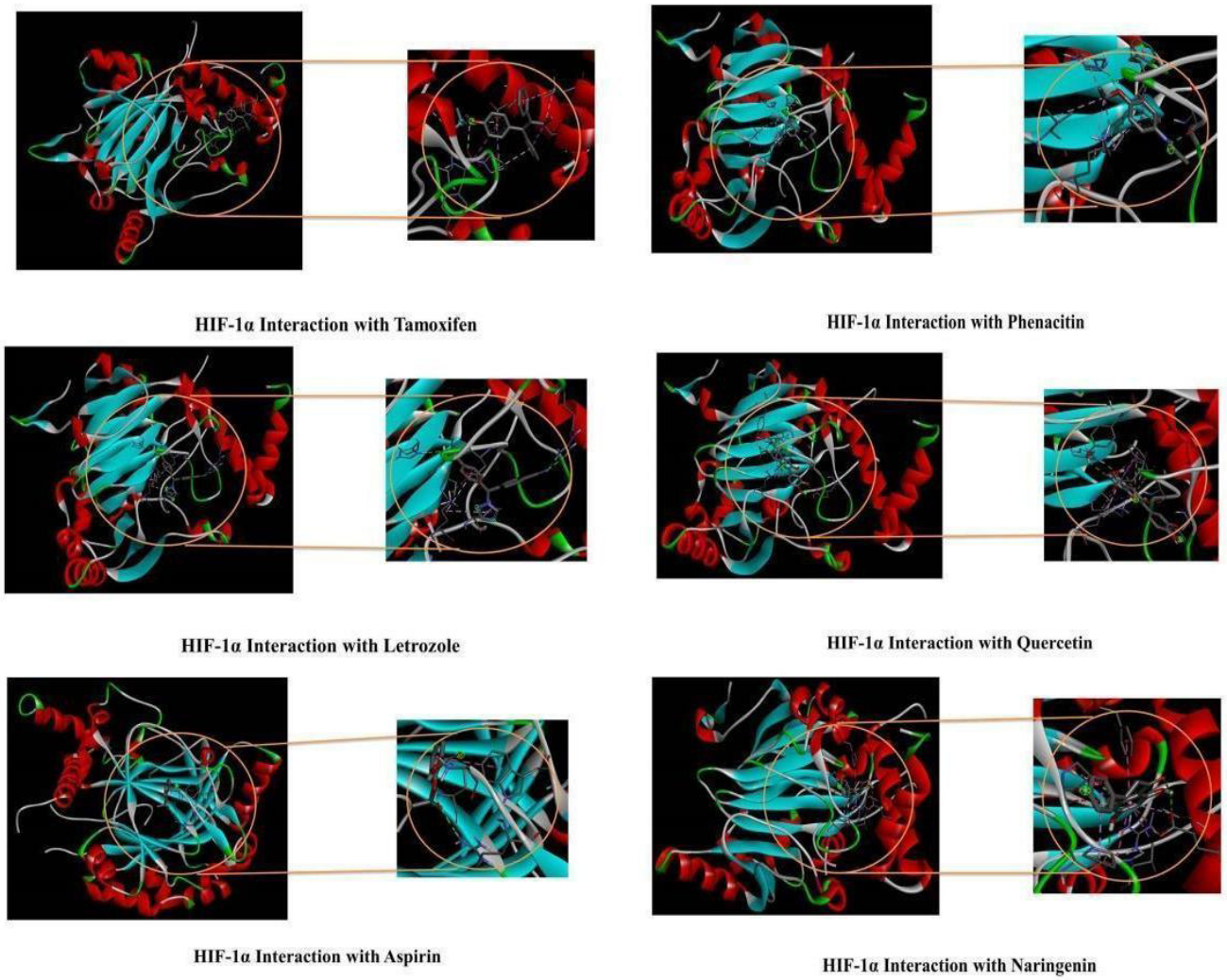

Fig 8: Complete Representation and Close Insight of 1H2M Interaction with the Respective Six Ligands

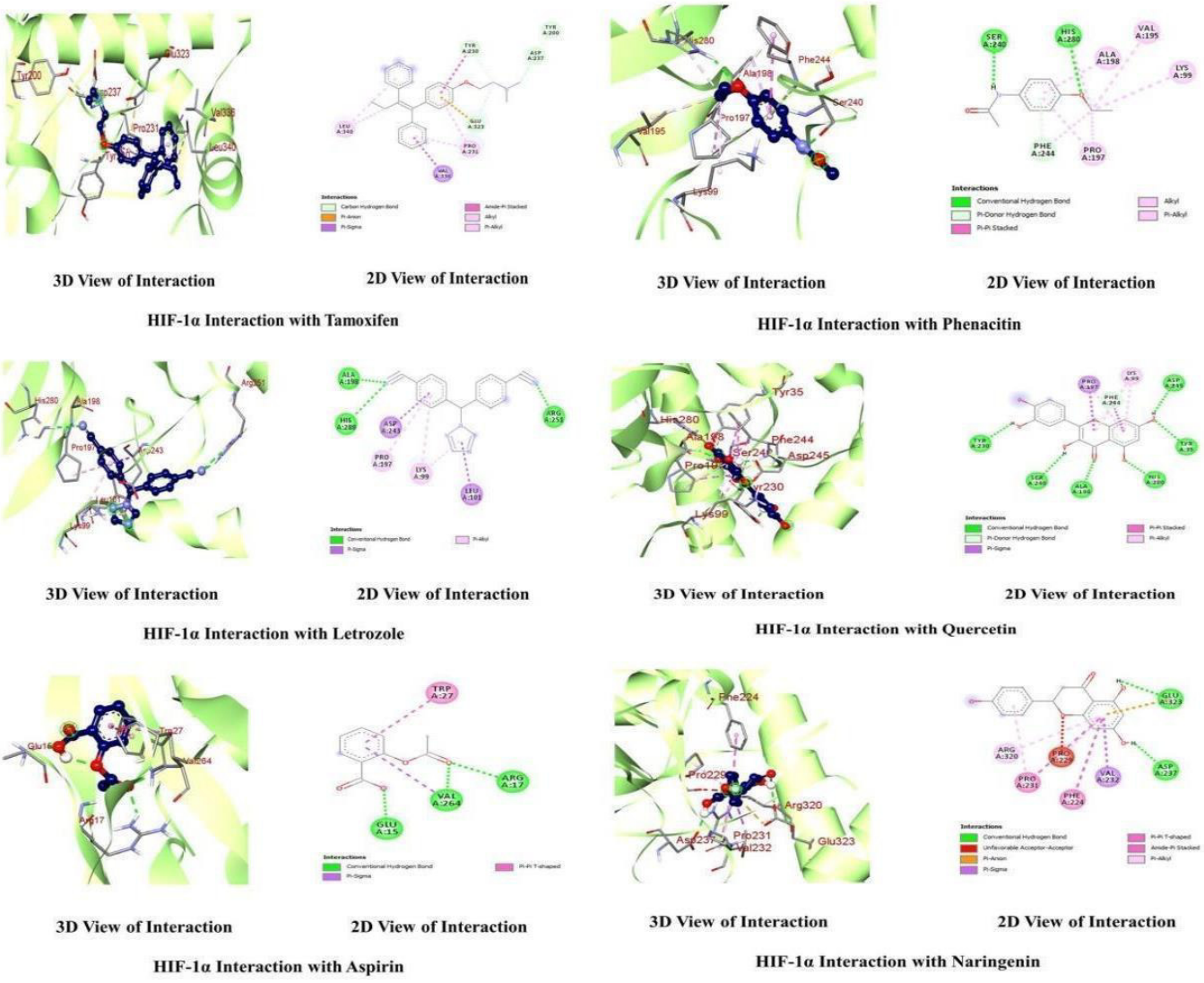

Fig 9: 3D and 2D View of interactions of 1H2M Protein with the Respective Six Ligands

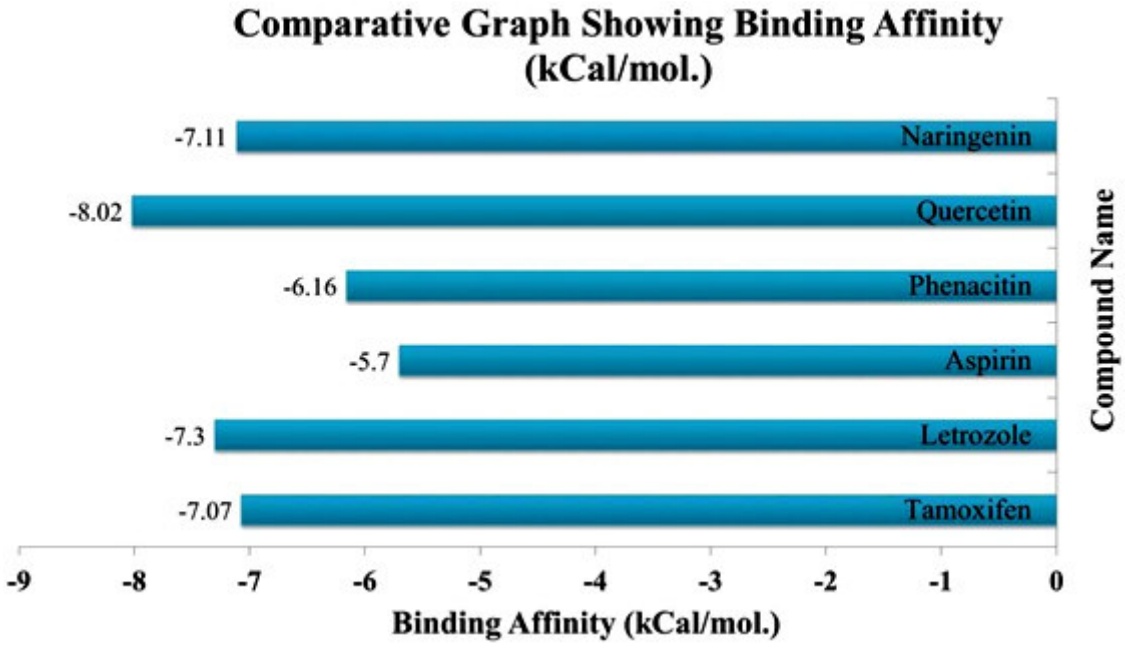

Fig 10: Graphical Representation of Binding Affinity along with the Score (kCal/ mol.) towards the Target Protein 1H2M by the Respective Compounds/ Ligands

3.6. Docking Calculation

Different energy values, including Binding energy, Torsional energy, Intermolecular energy, Vdw_hb_desolv_ energy, and Total Internal energy for each of the docked complexes, are represented in a tabulated form in Table 9.

Table 9: Results of Docking Calculations

Compound Name	Binding Energy (kCal/mol.)	Torsional Energy (kCal/mol.)	Intermolecular Energy (kCal/mol.)	Vdw_hb_desolv_ energy (kCal/mol.)	Total Internal Energy (kCal/mol.)
Tamoxifen	-7.07	2.39	-9.46	-8.37	-2.08
Letrozole	-7.3	0.89	-8.2	-7.95	-0.8
Aspirin	-5.7	1.19	-6.89	-5.18	-0.66
Phenacetin	-6.16	0.89	-7.06	-6.96	-0.32
Quercetin	-8.02	1.79	-9.81	-9.48	-1.99
Naringenin	-7.11	1.19	-8.3	-7.89	-0.91

3.7. Assessment of Structural Hotspots on the Receptor Protein

Table 10 displays the findings from the CASTp 3.0 online server for various protein PDB structures.

Table 10: Table Showing Active Amino Acid Residues Along with Different Categories of Bonds Obtained from Molecular Docking Interaction for Each of the Ligand Along with the Receptor Protein 1H2M

Name of the Protein	Name of the Compound/ Ligand	Active Amino Acid Residues
1H2M	Tamoxifen	TYR 200, TYR 230, PRO 231, ASP 237, GLU 323, VAL 336, LEU 340
	Letrozole	LYS 99, LEU 101, PRO 197, ALA 198, ASP 243, ARG 251, HIS 280
	Aspirin	GLU 15, ARG 17, TRP 27, VAL 264
	Phenacetin	LYS 99, VAL 195, PRO 197, ALA 198, SER 240, PHE 244, HIS 280
	Quercetin	TYR 35, LYS 99, PRO 197, ALA 198, TYR 230, SER 240, PHE 244, ASP 245, HIS 280
	Naringenin	PHE 224, PRO 229, PRO 231, VAL 232, ASP 237, ARG 320, GLU 323

3.8. CABS-flex 2.0 Server prediction

The MD-simulation study on CABS-flex 2.0 webserver was carried out, from where we derived the RMSF plots of each of the docked complexes, which is represented in a pictorial manner (Figure 11).

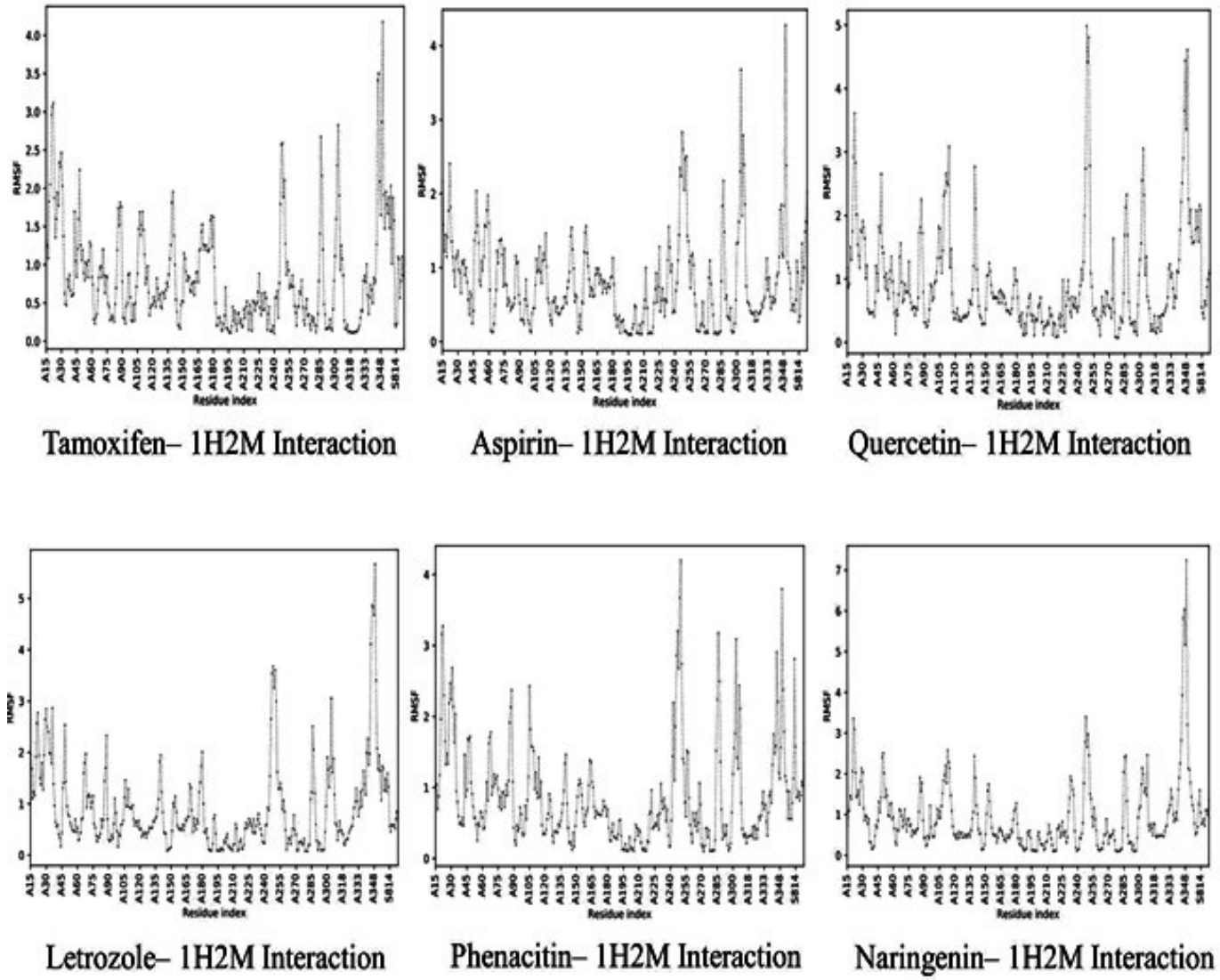

Fig 11: Results Showing 2D RMSF Plot of 1H2M with their respective ligand Complex Generated via CABS-flex 2.0 Server

4. DISCUSSION

4.1. Validation of Protein Structure

Perfect protein structure is approved by verifying the protein PDB model via a series of quality-checking parameters. ERRAT shows results of the "overall quality factor" that signifies proteins with higher scores have better quality; according to ERRAT results, each protein exhibits a quality score of more than 93.931%, which means each protein is well-modeled. Followed by VERIFY 3D result, which shows the result in the form of % of residues with an average 3d-1d score >= 2; according to the server data, it shows 92.72 % of residues bearing an average 3d-1d score >= 2. Next, talking about ProSA-web result showed the overall z score of the protein; here, the score is -7.03, which means the structure is under the X-ray region. The Ramachandran plot of the 1H2M protein model showed that 89.9% of the residues were present in the most preferred regions, followed by 9.8% in additional allowed, 0.3% in generously allowed, and 0.0% in disallowed regions, according to the PROCHECK result. The QMEAN Z Score is found to be -0.11. All the results accumulated from these parameters rectify that each of the proteins has a good quality and is suitable for studying further molecular interactions.

4.2. SwissADME Prediction Results of the Compounds

According to the SwissADME result from Table 2, it is observed that all six compounds have a molecular weight within an acceptable range (MW ≤ 500) [66] and also follow the Ro5, which states that the drug-like compounds ought to have nHBA ≤ 10 and nHBD ≤ 5. It indicates that all the compounds have the potential to be easily absorbed, diffused, and transported.[67] The number of rotatable bonds is a measure of molecular flexibility and is one of the widely used filters during the drug discovery process; [68] in this criterion, all four compounds have successfully passed as all of them fell within the acceptable range (nRB ≤ 15), indicative of their potential permeability and oral bioavailability.[69] From Table 3, it is concluded that each protein shows a value of CLogP ≤ 5, which influences their solubility, selectivity, potency, permeability, and promiscuity.[70] From Table 4, it is shown that all the compounds are within the range of moderately soluble to soluble, thus affecting drug absorption and distribution.[71, 72] According to Table 5, except for Tamoxifen, other compounds have a high potential to be absorbed by gastrointestinal tract but on the other hand tamoxifen and naringenin show low BBB (Blood Brain Barrier) permeation too. [73] One of the top priorities during the drug development process is analyzing metabolite prediction data of the compounds against five isoforms of cytochrome P450.[74] The

negative LogKp values indicate the skin impermeability of each compound.[75] The drug likeliness [Table 6] result reveals that all six compounds show satisfactory results with either 0 or 1 violation. According to Table 6, except for aspirin (0.85), the bioavailability scores describe the degree and rate at which administered compounds enter the systemic circulation and ultimately reach the targeted sites. The other five compounds have scores similar to aspirin's, i.e., 0.55. According to this figure, the compounds follow the Lipinski rule of five and have a 55% chance of being bioavailable. [76, 77]

4.3. Toxicity Prediction of the Ligands

The findings suggest that a positive prediction signifies that the molecule has no carcinogenic activity, while a negative prediction indicates carcinogenic activity. So from Table 7, it can be observed that in the case of rats, aspirin, Quercetin, and Naringenin show no carcinogenic activity, whereas Tamoxifen, letrozole, and phenacetin show carcinogenic properties. In the case of mice, except Tamoxifen, all other five compounds show carcinogenic activity. Talking about the mutagenic characteristics, all are mutagenic. In the case of hERG inhibition, all six compounds show medium to low probabilities of blogging the hERG gene often associated with sudden heart attacks in humans.

4.4. Bioactivity Score Prediction Using Molinspiration Chemo Informatics Tool

The bioactivity scores for substances are classified as being either effectively functioning (scores > 0), moderately functioning (scores: -5.0-0.0), or inactive (scores -5.0).[78]

4.5. Molecular Docking Interaction Using AutoDock 4.2

Based on the docking analysis done by AutoDock 4.2, the binding affinity of different compounds, including synthetic market drugs, analgesics, and plant-derived flavonoids, with the relevant protein Human Factor Inhibiting HIF-1α (1H2M), is determined. Ligands showing more negative binding energy exhibit the highest binding affinity towards the proteins. According to our study, we selected six ligands; each ligand shows a different result as their binding capacity with the target protein receptors differed. As reported by the docking result, Quercetin shows the maximum binding affinity, i.e., -8.2 kcal/ mol. followed by Letrozole (-7.3 kcal/mol.), Naringenin (-7.11 kcal/mol), Tamoxifen (-7.07 kcal/mol), Phenacetin (-6.16 kcal/mol), and lastly, Aspirin shows a minimum affinity energy of -5.7 kcal/mol.

4.6. Assessment of Structural Hotspots on the Receptor Protein

The key amino acids implicated in the particular protein-ligand interaction are displayed in this finding.

4.7. CABS-flex 2.0 Server prediction

This graph shows almost different patterns of fluctuation state of the amino acids involved in the interaction between Human Factor Inhibiting HIF-1α protein and small molecules/ ligands (market drugs, analgesics, and flavonoids).

5. CONCLUSION

After carrying out different methodologies such as PreADMET, toxicity prediction, energy minimization, and last of all, molecular docking interaction, our protein of interest with various compounds, we eventually found out that Quercetin being a basic phytochemical flavonoid compound which is mostly present in *Citrus* species fruits, gives the best possible outcome among all others. It provides almost similar efficacy and shows the least binding affinity among the other ligands when docked with the 1H2M receptor molecule. Next, talking about another phyto-compound, Naringenin also shows a more or less similar way of interacting with the preferred protein. Compared with the standard marketed drugs, their interaction status with the protein shows similar low binding affinity. However, as long as talking about a group of analgesics, they are not toxic and are approved as drugs to be prescribed. Still, they do not show such great activity or interaction with the HIF regulation factor of Breast cancer metastasis and hence cannot be considered a potent ligand. Furthermore, both *in vitro* and *in vivo* toxicological investigations should be used to validate these prediction results.

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7. AUTHOR'S CONTRIBUTION STATEMENT

Moumita Saha and Rumana Rahman have contributed substantially to the research work's concept and design. Moumita Saha, Anisha Mukherjee, and Chandreyi Ghosh helped with data analysis and interpretation of the data. Sirshendu Chatterjee and Moumita Saha have prepared and edited the manuscript's final draft.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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