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INSILICO MOLECULAR DOCKING OF N-ACETYL CYANOACETY HYDRAZONE DERIVATIVES SCAFFOLDS AS PROSPECTIVE ANTI-BREAST CANCER AGENT

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ABSTRACT

The present study is concerned with the docking of synthesized molecules ((E)-2-(2-chlorobenzylidene) hydrazine-1-carbonyl cyanide **[1]**, (E)-2-(4-bromobenzylidene) hydrazine-1-carbonyl cyanide **[2]**, (E)-2-(2-bromobenzylidene) hydrazine-1-carbonyl cyanide **[3]**, (E)-2-(4-methylbenzylidene) hydrazine-1-carbonyl cyanide **[4]**, (E)-2-(2-methylbenzylidene) hydrazine-1-carbonyl cyanide **[5]**, (E)-2-(4-chlorobenzylidene) hydrazine-1-carbonyl cyanide **[6]**, (E)-2-benzylidene hydrazine-1-carbonyl cyanide **[7]** and in order to arrive at an effective drug such as a molecule targeting the Crystal Structure of the BRCT Domains of Human BRCA1 primarily responsible for Breast Cancer, this application as an anticancer agent. Protein Data Bank, to retrieve the protein structure; Pubchem compound database, to retrieve the chemical structure of estrogen receptor inhibitors; Discovery Studio 2017 for docking and ADMET research are the methods and applications used. The findings indicate that both compounds have a strong binding affinity with the Human Estrogen Receptor protein's active site and can be used in breast cancer as a possible estrogen receptor inhibitor.

Key words: Molecular docking, Breast cancer, ADMET, BRCA1, Schiff base

1. INTRODUCTION

Breast cancer is characterized by the uncontrolled growth of cells in the breast tissue forming a hard-painless lump, typically in the milk ducts or lobules supplying milk to them **[1]**. The status of three unique cell surface receptors is the most common practice for classifying breast tumours: the estrogen receptor (ER), the progesterone receptor, and the HER2/neu receptor for the human epidermal growth factor (EGF) receptor. Of all breast cancers, approximately 75 percent are hormone receptor-positive. HER2-positive breast cancer accounts for 20% to 30% of hormone receptor-positive breast cancer associated with HER2/neu protein overexpression **[2]**. Triple-negative breast cancer (TNBC), a rare type of breast cancer, contains tumour cells that lack estrogen and progesterone receptors and do not over-express the protein HER2 **[3]**. One of the factors shown to raise the risk of a woman getting breast cancer is age. In women, breast cancer has been found to develop at or above the age of 50. It is also understood that a good personal or family history presents an increased risk of developing breast cancer. Moreover, long menstrual life or the use of hormone replacement therapy after menopause raises the risk of breast cancer growth **[4]**.

The most common form of tumour in women is breast cancer (BC), but metastases are the primary cause of death. Metastasis is a complex mechanism in which cancer cells migrate into the vessels of the blood, invade other tissues, and identify secondary sites for a colony. Indeed, BC starts as a local disease but can spread to distant locations, such as lymph nodes and various organs, with metastases **[5]**. This process includes the expression of a series of genes that control cancer cells' survival and invasion. As possible drug targets in the drug development phase, drugs that modulate the genes/proteins that control cancer cell survival, metastasis, apoptosis, and invasion are therefore of great importance **[6]**. However while new therapies have been developed to dramatically reduce metastatic BC mortality, resistance to anticancer agents can lead to treatment failure **[7]**.

When there is a chronic disorder without an adequate cure, a drug discovery phase occurs. In academia, where a hypothesis is developed, the first phase of research always begins; for example, the inhibition or induction of a protein or pathway as a therapeutic effect in a condition of illness. Indeed, the selection of a target, which can be a variety of biological entities

such as proteins, RNA, and genes that can be selected through bioinformatics analysis, is a crucial point of the research process **[7]**. The putative drug molecule must have an ideal target available and the binding drug-target complex should induce a biological response **[5]** that can be quantified by in vitro models. Cell lines are the most used in vitro BC models, since they share many BC molecular and genomic features. With molecular docking, the binding affinity between the drug and the target can be computed in silico. In silico and in vitro screening can also help to determine the toxicity of the drugs/molecules examined rapidly, thus preventing further steps, such as in vivo and preclinical trials (in case of adverse effects of in silico and in vitro methods **[5]**. At least two components are needed in silico approaches with docking studies: a protein/drug database and a molecular docking algorithm. Protein and drug databases are a list of protein and drug structures.

Big data, which provides a broad variety of biological and chemical knowledge, has been generated by the rapidly growing number of structures and is a recent opportunity to gain a deeper understanding of the relationships between drugs and targets (usually proteins), drugs and diseases, and targets and diseases. However, although the available data is often heterogeneous and incomplete, this information can be exploited by computational methods to deepen these interactions **[8]**. High-performance computational algorithms for drug discovery processes are needed, given the cost and time consumption of experimental methods. The "docking" computational technique can predict the binding of drug-target complexes as well as the ligand's conformation upon binding to a protein target. The binding free energy of target-drug interactions determines an association's affinity and the conditions for a complex to form. Rated binding free energies are not always reliable, but they can be used in a virtual screening method to pick new drugs such as small molecules to be tested experimentally **[9-11]**. New drugs with a low molecular weight that allow them to easily penetrate cells are promising small molecules **[12]**. Molecular docking may also be used to predict the effects of a drug, such as detecting an unintended reaction between a compound and off-targets. 57,000 abstracts/papers on molecular docking have been published to date, showing the importance of this analytical approach in drug production **[13]**.

In silico methods have opened the way for a variety of biological problems to

be solved, leading to the discovery of novel inhibitors for multiple diseases. In this research, structure-based virtual screening (VS) screened active compounds against breast cancer targets to identify possible virtual hits. In addition, the ligands were examined for their absorption, delivery, metabolism, excretion, and toxicity (ADMET) profile, which determined the drug's ADMET efficacy. Potential hits have a greater chance of being potential drugs that suggest successful pharmacokinetic (PK) and pharmacodynamic (PD) properties. The results of the current study concluded that after testing through in vitro experiments, five multi-targeted compounds with high binding energies and a strong ADMET profile against all three targets were taken into account, suggesting them as possible hits for drug production against breast cancer.

2. METHODOLOGY

In our present study, *in silico* molecular docking and ADMET toxicity studies were carried out using BIOVIA Discovery Studio (DS) 2017 software.

2.1.Preparation of protein

In this analysis, the X-ray diffraction-based Crystal Structure of the BRCT Domains of Human BRCA1 in Complex with a Phosphorylated Peptide from Human Acetyl-CoA Carboxylase 1(PDB ID: 3COJ) with a resolution of 1.90 Å was selected. Hydrogens were applied to the 3COJ protein by applying the Forcefield algorithm and then using CHARM forcefield in DS, the protein energy was reduced. For molecular docking research, we followed previously mentioned parameter **[14]**.

2.2.Ligand preparation

The molecules ((E)-2-(2-chlorobenzylidene)hydrazine-1-carbonyl cyanide **[1]**, (E)-2-(4-bromobenzylidene)hydrazine-1-carbonyl cyanide **[2]**, (E)-2-(2-bromobenzylidene)hydrazine-1-carbonyl cyanide **[3]**, (E)-2-(4-methylbenzylidene) hydrazine-1-carbonyl cyanide **[4]**, (E)-2-(2-methylbenzylidene) hydrazine-1-carbonyl cyanide **[5]**, (E)-2-(4-chlorobenzylidene) hydrazine-1-carbonyl cyanide **[6]**, (E)-2-benzylidene hydrazine-1-carbonyl cyanide **[7]** and standard drug Tamoxifen were drawn in chemdraw software, subsequently, energy of the all the molecules were minimized and saved in SDF file format for further docking studies.

2.3. Docking study

In order to analyse the most common geometry of the protein-ligand complex, a molecular docking analysis was carried out. To understand the structural basis of these target proteins, a computational docking

study was used to analyse structural complexes of the 3COJ with 7 molecules along with Schiff base compounds. The CDOCKER (CHARMm-based DOCKER) protocol integrated within DS has examined potential binding modes between the ligands and these target proteins. The CDOCKER parameter to be run was tabulated in **Table 1**. The algorithm flexibly provides complete ligand and employs fields of CHARMm power. Using CDOCKER energy, CDOCKER Interaction energy, Hydrogen bonds, binding energies, protein energy and ligand-protein complex energy, ligand binding affinity was measured. The energy of CDOCKER is stated in negative values. More negative value energy is seen as the ligands' higher binding affinity to the target protein protein **[15, 16]**.

Table 1: Parameter of CDOCKER protocol

Input Receptor	**Input/3COJ.dsv**
Input Ligands	**/Input/Total_min_ligands.sd**
Input Site Sphere	**-23.9454, 29.2003, 7.29961, 9**
Top Hits	**1**
Random Conformations	**10**
Random Conformations Dynamics Steps	**1000**
Random Conformations Dynamics Target Temperature	**1000**
Include Electrostatic Interactions	**True**
Orientations to Refine	**10**
Maximum Bad Orientations	**800**
Orientation vdW Energy Threshold	**300**
Simulated Annealing	**True**
Heating Steps	**2000**
Heating Target Temperature	**700**
Cooling Steps	**5000**
Cooling Target Temperature	**300**
Forcefield	**CHARMm**
Use Full Potential	**Yes**
Grid Extension	**8.0**
Ligand Partial Charge Method	**CHARMm**
Random Number Seed	**314159**
Final Minimization	**Full Potential**
Final Minimization Gradient Tolerance	**0**
Parallel Processing	**False**
Parallel Processing Batch Size	**25**
Parallel Processing Server	**localhost**
Parallel Processing Server Processes	**2**
Parallel Processing Preserve Order	**True**
Random Dynamics Time Step	**0.002**

2.4.ADMET Toxicity Analysis

In drug development and environmental hazard assessment, prediction of the ADMET profile for drug candidates and environmental chemicals plays a major role. The ADMET properties of the filtered compounds were predicted using DS software in order to classify the potential

adverse effects of these compounds in humans. Solubility, Absorption, BBB, HIA, and ADMET parameters are included. Risks and toxicity. 95% and 99% confidence ellipses in the ADMET PSA 2D, ADMET AlogP98 aircraft are described by the absorption levels of the HIA model **[16]**.

3. RESULTS AND DISCUSSION

3.1.Molecular docking study

In the hope that effective and selective inhibitors will constitute a new class of therapeutics for cancers as well as other proliferative diseases, both pharmaceutical companies and university laboratories were involved in developing compounds that could inhibit the action of tyrosine kinase. As a new mode of cancer treatment, 3COJ inhibitors may also be implemented appropriately. To classify successful anti-breast cancer compounds, the current in silico research was conducted. The seven compounds such as ((E)-2-(2-chlorobenzylidene) hydrazine-1-carbonyl cyanide [Mol.1], (E)-2-(4-bromobenzylidene) hydrazine-1-carbonyl cyanide [Mol.2], (E)-2-(2-bromobenzylidene) hydrazine-1-carbonyl cyanide [Mol.3], (E)-2-(4-methylbenzylidene) hydrazine-1-carbonyl cyanide [Mol.4], (E)-2-(2-methylbenzylidene) hydrazine-1-carbonyl cyanide [Mol.5], (E)-2-(4-chlorobenzylidene) hydrazine-1-carbonyl cyanide [Mol.6], (E)-2-benzylidene hydrazine-1-carbonyl cyanide [Mol.7] and standard drug Tamoxifen were also evaluated for their binding interactions in 3COJ active site. After being tested via in vitro experiments, the virtual hits found in this study can be used as an alternative targeting agent for breast cancer. In addition, to philtre top hits to identify acceptable virtual hit candidate compounds, the ADMET profile was analysed. By extracting water molecules and repeating coordinates, the crystal structures have been refined. Atoms of hydrogen were added and charges were allocated to the atoms of proteins. The secondary structure of the active-site sphere target protein (radius 9) is shown in **Figure 1**. The -CDOCKER energy of each molecule with the target protein is described in **Table 2** as a result of the docking analysis.

The binding analysis indicates that the Human Estrogen Receptor was successfully docked with the seven compounds. From the docking results, the Mol.1 forms one strong Hydrogen bond with Arg 394 and Alkyl and Pi-Alkyl interactions with Leu 346, Met 421 and Leu 525 respectively. The -CDOCKER energy of this interaction is -19.0777 kcal/mole^{-1}. Out of seven molecules the Mol. 4 shows higher binding affinity in receptor 3COJ compare to standard drug Tamoxifen (-CDOCKER

energy -18.2547 Kcal/mol^{-1}) due to forms Pi-Alkyl and Pi-Pi T-shaped interactions. The remaining all compounds of 2D results are depicted in **Figure 4**. Here, the Mol. 1 revealed the poor binding affinity with binding energy being (is -19.0777 kcal/mole^{-1}) which predict its weak biological breast cancer activity (not measured yet), but it may be similar activity to Tamoxifen drug. Therefore it is easily seen that it is predicted that all compounds would have almost identical activity against Human Estrogen Receptor.

3.2.ADMET analysis

The good absorption or permeation of the compound through the blood brain barrier is measured by its LogP, which must be lower than 5 **[17, 18]**. Pharmacokinetic screening results showed the Mol. 1 to Mol.7 adopted the law of five for oral bioavailability of Lipinski. **Table 3** summarizes these ADMET screening findings. For drug likeness research, the ADMET descriptors of all the molecules were determined. Intestinal absorption and penetration of the blood brain barrier is predicted by the development of an ADMET model using 2D PSA and AlogP98 descriptors that include 95% and 99% confidence ellipses. These ellipses describe regions in which it is predicted that well-absorbed compounds will be contained. ADMET model screening findings have shown that all the compounds have 99 percent confidence levels for human intestinal absorption and penetration of the blood brain barrier (BBB). The polar surface area plot and ALogP for Mol.1 to Mol.7 are shown in **Figure 5**.

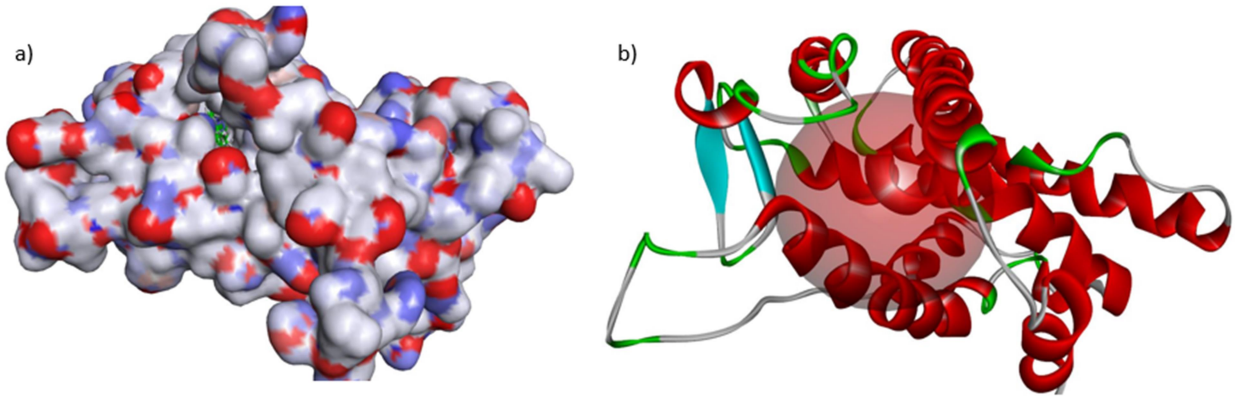
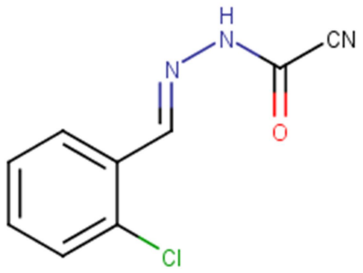
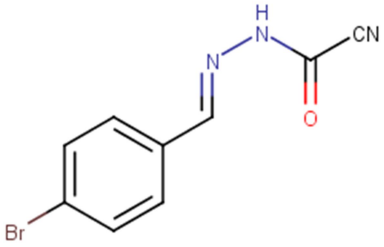
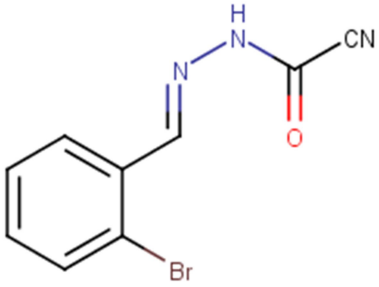
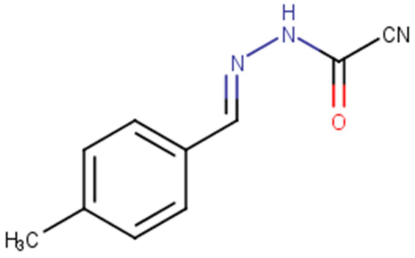
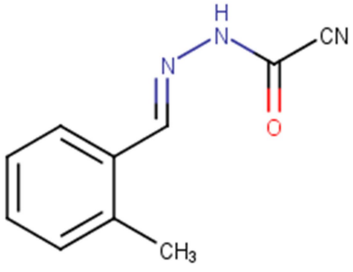
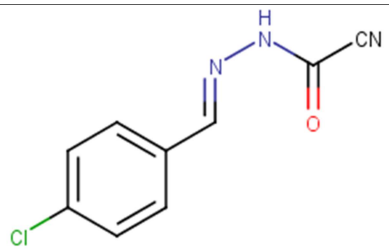
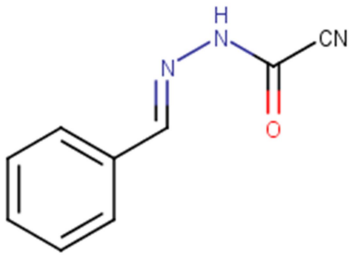
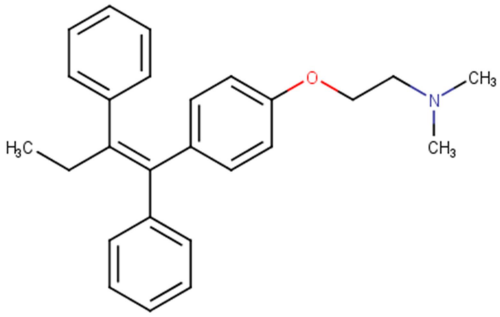


Figure 1: a) The secondary 3D structure of the target 3COJ protein with molecules and b) active site sphere of the protein

Table 2: CDOCKER energy of the molecules (Mol 1 to Mol.7)

S. No	Molecule Number	Structure	-CDOCKER Energy (Kcal/ mol^{-1})
1	Mol. 1		19.0777
2	Mol. 2		23.3452
3	Mol. 3		20.3915
4	Mol. 4		23.9721
5	Mol. 5		22.2153

6	Mol. 6		22.7068
7	Mol. 7		22.2919
8	Tamoxifen		18.2547

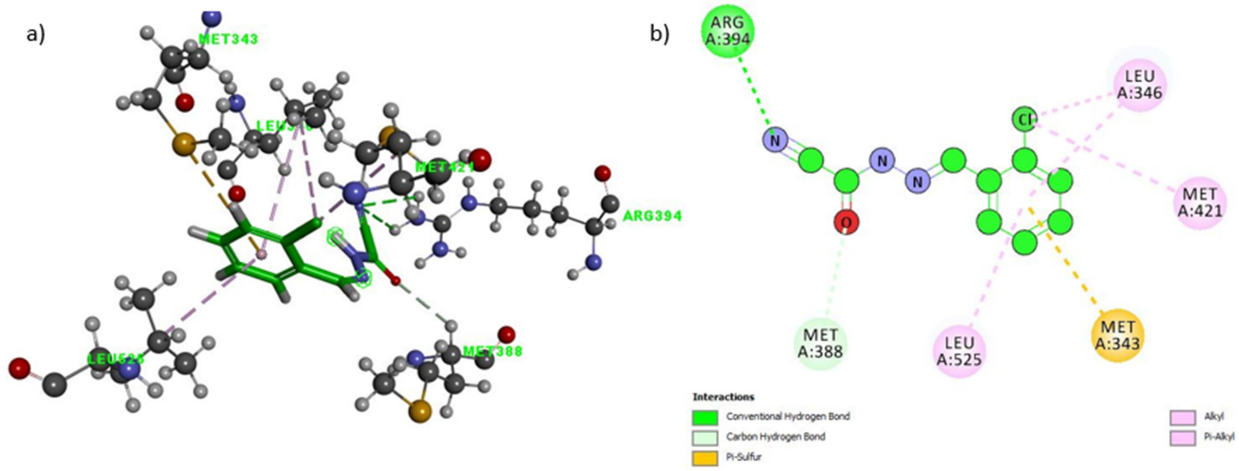


Figure 2: a) 3D and b) 2D binding site interaction of (E)-2-(2-chlorobenzylidene) hydrazine-1-carbonyl cyanide [Mol.1] in receptor 3COJ active site.

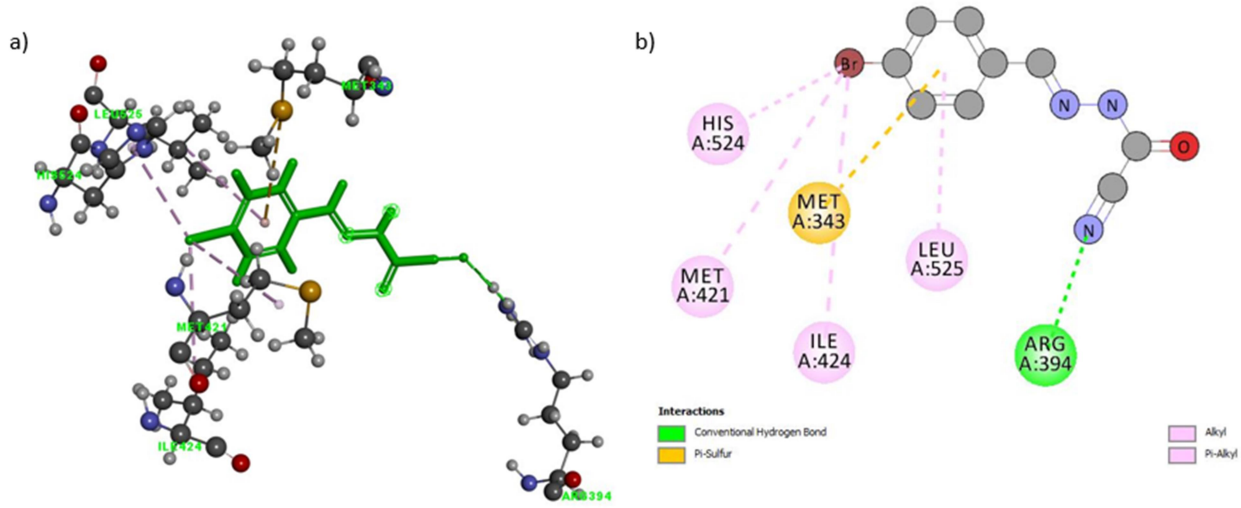


Figure 3: a) 3D and b) 2D binding site interaction of (E)-2-(4-bromobenzylidene) hydrazine-1-carbonyl cyanide [Mol.2] in receptor 3COJ active site

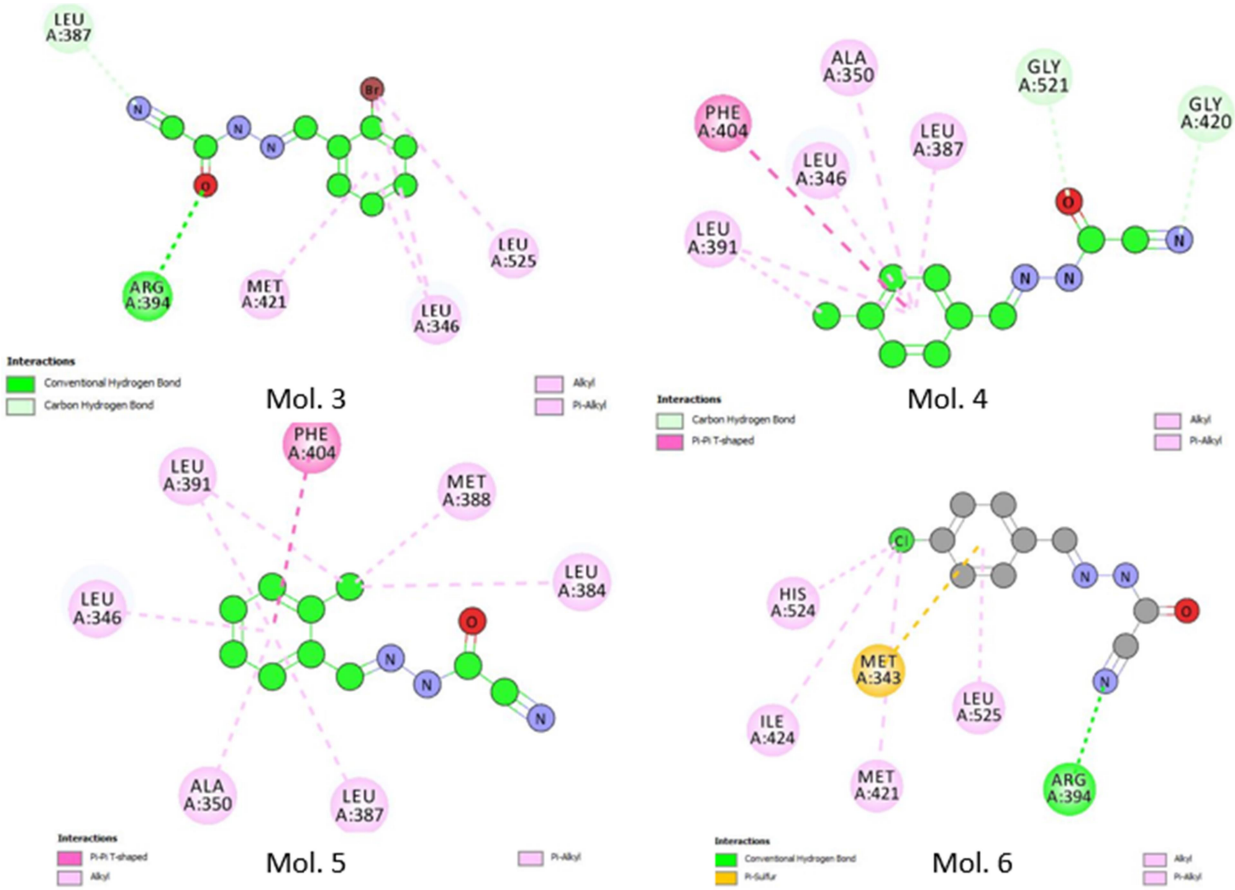


Figure 4: 2D interactions analysis of Mol.3, Mol.4, Mol.5 and Mol. 6in receptor 3COJ active site

Table 3: ADMET properties of the compound Mol. 1 to Mol. 7

Name	Absorption level	Solubility level	BBB level	PPB level	Hepatotoxic level	CYP 2D6	PSA 2D	AlogP98
Mol. 1	Good	good	Very Low	0	<90%	Non inhibitor	64.369	4.46
Mol. 2	Moderate	good	Very Low	0	<90%	Non inhibitor	64.369	4
Mol. 3	Moderate	good	Very Low	0	<90%	Non inhibitor	64.369	4
Mol. 4	Moderate	good	Very Low	0	<90%	Non inhibitor	64.369	4.66
Mol. 5	good	good	Very Low	1	<90%	Non inhibitor	64.369	5.48
Mol. 5	good	good	Very Low	0	<90%	Non inhibitor	64.369	4.539
Mol. 6	good	good	Very Low	1	<90%	Non inhibitor	64.369	4.492
Mol. 7	good	good	Very low	1	<90%	Non inhibitor	64.369	4.85
Tamoxifen	good	good	Very low	1	<90%	Non inhibitor	64.369	4.91

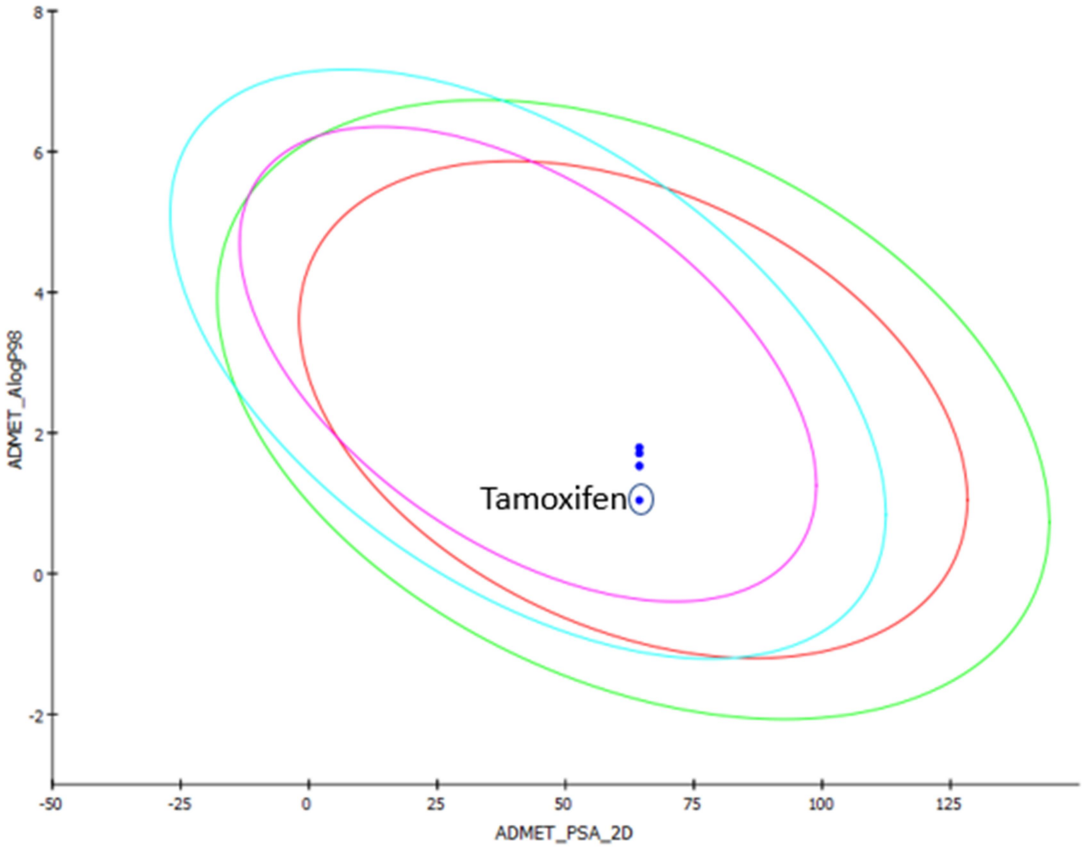


Figure 5: Plot of polar surface area (PSA) versus ALogP for capsazepine and its derivatives showing the 95% and 99% confidence limit ellipses corresponding to the blood brain barrier (BBB) and intestinal absorption

3.3.CONCLUSION

It can be concluded that ((E)-2-(2-chlorobenzylidene) hydrazine-1-carbonyl cyanide [Mol.1], (E)-2-(4-bromobenzylidene) hydrazine-1-carbonyl cyanide [Mol.2], (E)-2-(2-bromobenzylidene) hydrazine-1-carbonyl cyanide [Mol.3], (E)-2-(4-methylbenzylidene) hydrazine-1-carbonyl cyanide [Mol.4], (E)-2-(2-methylbenzylidene) hydrazine-1-carbonyl cyanide [Mol.5], (E)-2-(4-chlorobenzylidene) hydrazine-1-carbonyl cyanide [Mol.6], (E)-2-benzylidene hydrazine-1-carbonyl cyanide [Mol.7] have better binding interactions with crystal Structure of the BRCT Domains of Human BRCA1 in Complex with a Phosphorylated Peptide from Human Acetyl-CoA Carboxylase 1(PDB: 3COJ.) The protein-ligand interactions' binding energies also confirm that the ligands are fitted tightly into the receptor's active pockets. The Silico ADMET study concludes that when compared to myricetin, both analogues have better profiles. As drug candidates that inhibit the human estrogen receptor, these can hold better promise. In the future, further development and modification of these analogues may lead to the production of new, highly potent anti-breast cancer drugs.

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