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Research Article

In silico analysis approach for screening new agents for breast cancer inhibitors based on 1,5-benzothiazepine

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ABSTRACT

Combination of similarity searching with docking and molecular dynamic simulations were performed. In this study, chalcone-based 1,5-benzothiazepine compound (i.e. MA9) was used as parent compound, since it exhibits potential enhancement and improvement of biological activity over doxorubicin (i.e. the common agent for cancer treatment). The main aim of this study was to explore a new potential inhibitor against breast cancer from a large database. To study this effect, several computational approaches were applied. Initially, seven compounds were observed according to the Euclidean distance and Tanimoto coefficient. Parent compound and all these seven compounds were docked into 1T46 protein active site. Docking results reported that ZINC4377306 and ZINC4377309 have exhibited binding free energy of -6.75 and -6.49 kcal/mol, respectively. In addition, they showed the binding interaction through hydrogen bond, van der Waals and other interactions with the notable residues around the active site. Both compounds were stable during the molecular dynamic simulation. Thus, ZINC4377306 and ZINC4377309 can be used as new potential agents against breast cancer as an early stage in drug discovery process.

Keywords:
Docking, Molecular dynamic, Similarity search, Pharmacophore, 1,5-benzothiazepine medicines

1. INTRODUCTION

Breast cancer is the most common type of tumour in women. To date, it is still a big issue around the world, as many illnesses and deaths in women caused by breast cancer[1]. Initially, it caused by a genetic abnormality and happens when the cells in the breast grow and divide in an uncontrolled way. Like the other cancers, breast cancer can grow and divide into the tissue around the breast. It also can move or travel into the other parts of the body and form new tumour[2].

Tyrosine kinase receptors belong to the epidermal growth factor receptor (EGFR) family[3]. The abnormal activation of these kinases can result in excessive cell growth and angiogenesis in epithelial cancer such as breast cancer[4]. In addition EGFR receptor family are also overexpressed in high percentages of human breast cancers. EGFR receptor family are connected with the plasma membrane and are involved in mitogenesis, it is thought that they work together to promote breast cancer development and progression[5].

1,5-Benzothiazepines are heterocyclic compounds of nitrogen and sulphur atoms[6]. This class of seven membered heterocycles has been reported to have ample range of biological activities, such as antimicrobial[7-9], anti-HIV[10] and also as breast cancer inhibitor. Based on our previous research, the *in vitro* assay revealed that one of 1,5-benzothiazepine derivatives i.e. MA9 exhibited an enhanced biological activity compared to doxorubicin (the common agent for treatment of breast cancer[11]).

In silico study contributes significantly to drug design process and reportedly, similarity search is considered as a tool to identify new lead compounds from a large database[12]. New agents against breast cancer have been discovered using docking study, but combination of similarity search with docking and molecular

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dynamic simulation have not been extensively investigated. In addition, there are not many reports concerning in silico study of chalcone-based 1,5-benzothiazepine compounds against breast cancer. Thus, the primary goal of this investigation is to discover new agents against breast cancer MCF7 from a large database and using 1,5-benzothiazepine as parent compound.

2. MATERIALS AND METHODS

2.1. Similarity Search

Based on our previous research, compound MA9 (i.e. depicted in Figure 1) has shown a better *in vitro* activity compare to doxorubicin as inhibitor for breast cancer MCF7. Therefore, it was used as parent compound to search new potential drugs from a large database. In this study, an available database, ZINC, which recorded more than 30,000 chemical structures, has been used to search new compound with the similar attributes to the parent compound.

Descriptors of all the compounds from database were generated using Chemdes[13] Before entering the model development stage, range scaling descriptors was carried out to prevent the manipulation between one descriptor to another. Moreover, this range scaling can also be used to avoid descriptor weighting in Euclidean distance calculations in a multidimensional space. The scaling was calculated as follows:

$$y = \frac{X - Xmin}{Xmax - Xmin} \qquad \text{equation 1}$$

Where y_i is scaled value, x_i is original value, min (x) is minimum of the collection of x object and max (x) is maximum of the collection of x objects.

Euclidean distance was utilized as the measure of similarity in the multi- dimensional descriptor space between parent compound and each structure in the database. The distance d_{ij} between any two compounds i dan j in N-dimensional descriptor space was calculated using the following equation:

$$d_{ij} = \sqrt{\sum_{n-1}^{N} \left(X_{in} + X_{jn}\right)^2} \qquad \text{equation 2}$$

Where x_{in} and x_{jn} are the values of nth descriptor for compounds i and j, respectively, and the summation is over all descriptors. Compounds with the shortest distance (highest similarity) from the parent compound were deliberated as hits.

Tanimoto coefficient was also applied to measure the similarity between parent compound and each compound in a database using the following equation

$$\text{Tanimoto} = \frac{\sum_{i=1}^{i=N} X_{iA} X_{iB}}{\sum_{i=1}^{i=N} (X_{iA})2 + \sum_{i=1}^{i=N} (X_{iA})2 - \sum_{i=1}^{i=N} X_{iA} X_{iB}}$$

equation 3

Where X_{iA} is the value of property i of molecule A, when i can take value of only 0 or 1 as in a bit string.

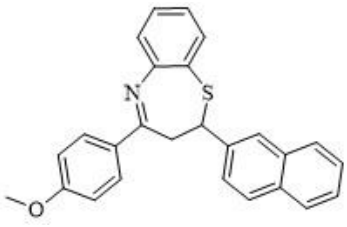
The applicability domain was applied for both distance (i.e. Eulidean distance and Tanimoto distance). The selected hits compounds are those with the shortest distance from the parent compound (i.e. they are considered to have the highest similarity with the possible leads for breast cancer inhibitors).

2.2. Molecular Docking

Seven compounds from a database were sketched using ChemDraw 2015, then converted into 3D structure using MOE 2020.0901 (Chemical computing group) with MMF94x force field and gradient 0.0001. These ligands are depicted in the Table 1.

The crystal structure of tyrosine kinase was downloaded from protein databank with PDB ID 1T46 for then the water molecules were removed. The molecular structure of co-crystal ligand was then removed and CHARMM27 was applied as force field with coordinates on the x, y, and z axes are 34.8122; -29.027 and -9.6847, respectively. Protein was prepared using the QuickPrep menu in MOE 2020.0901 software package (protonation was performed using protonate 3D and the RMS Gradient was set to 0.001 kcal/mol/A). The prepared structure is then saved in PDB format for then it can be used as a receptor.

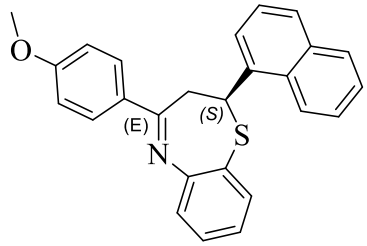
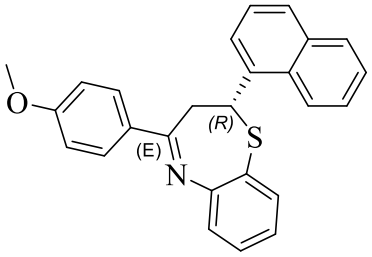
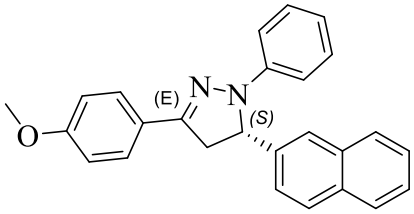
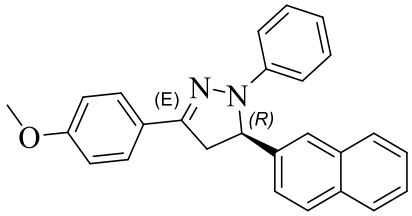
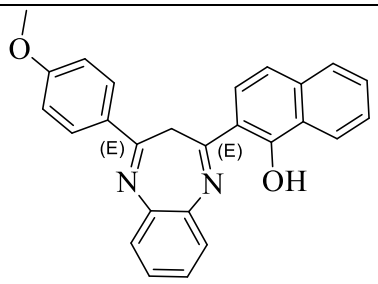
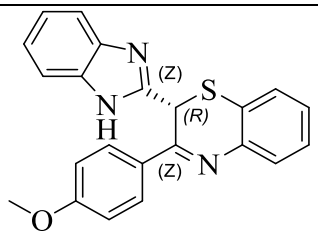
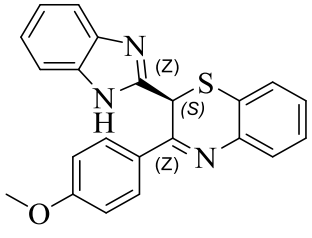
The protein active site was determined using a site finder before re-docking of the doxorubicin as a ligand to the prepared active site of the receptor. The selected



4-(4-methoxyphenyl)-2-(naphthalen-2-y1)-2,3-dihydrobenzo[b][1,4]thiazepine

Figure 1. Molecular structure of MA9.

Table 1. Selected compounds from database.

No.	Molekul	Struktur senyawa *hits*
1	ZINC4377306	
2	ZINC4377309	
3	ZINC95585154	
4	ZINC95585155	
5	ZINC4550853	
6	ZINC4149299	
7	ZINC4149300	

site consisted of some amino acids was set up as a dummy atom. Furthermore, this dummy atom was used as an docking protocols to perform the docking process. Docking was constructed using MOE 2020.0901 software package (Chemical computing group).

2.3. Molecular Dynamic Simulation (MD Simulation)

MD simulation was carried out to check the stability of protein and ligand complexes. Molecular dynamic was executed using NAMD (Nanoscale Molecular Dynamics Program v 2.9) with CHARMM27 (Chemistry at HARvard Macromolecular Mechanics) was selected as the best force field. The modeled protein was achieved using TIP3P water box with 2.5 Å water layer for each direction of coordinated molecular structure.

The modeled system was subjected to simulate gradual heating in the constant temperature and constant volume (NVT) was utilized as ensemble parameter from 0 to 300 K over 100 ps. The molecular dynamic simulation was scaled with 50-ns in time for each system, and it was in an iso-thermal iso-baric and with boundary conditions. The couplings of temperature and pressure were set at 1.0 ps. The sampling process was saved at every 0.1 ps. The simulations were generated the conformations for then they were used for further to calculate the binding free energy and also the decomposition process.

3. RESULTS AND DISCUSSION

3.1. Similarity Search

Scaling descriptor is a very delicate procedure as there might be underlying relationship between its descriptors and it may be impossible to foresee the results of manipulations. Range scaling may help to prevent the disproportional weightings of descriptors upon the Euclidean distance or Tanimoto coefficient calculations in multidimensional descriptors space.

The degree of similarity, based on Euclidean distance and Tanimoto similarity coefficient between the parent compound and those in database was computed using the same set of descriptors. In this study, log P, polarity, ndonr and naccr were selected as descriptors. Since the limited value for the distance is set to 0.5, compounds with the distance higher than 0.5 were rejected and classified as outliers[14]. Euclidean distance was applied with a distance of less than 0.5 units in a multi-dimensional descriptors space selected as the similarity cut-off value, a total 540 compounds were chosen from database. Through Tanimoto coefficient, 115 compounds were selected as initial hits.

Applicability domain is the last step in similarity search. It can be used to select the dissimilar compounds (i.e. substantially different structures as parent compound)[15-16]. By applying the applicability domain, the numbers of feasible candidates were narrowed down to 7 compounds. These candidates were further refined by finding compounds that appear within both similarity distances which resulted in as many as 7 compounds (i.e. depicted in Table 1).

3.2. Molecular Docking

The docking protocol is necessary to validate, in order to confirm the accuracy of the docking results. The validation of the docking protocol was carried out by re-docking the doxorubicin as positive control into the prepared receptor to determine whether the molecule can be properly bound to the active site of the protein. The validation results of the docking protocol showed that the re-docked ligand has similar interaction with the native ligand with root mean square deviation (RMSD) value of 1.19 (Figure 2). Thus, the proposed docking protocol can be used for further step.

In this study, seven compounds from the database were docked as ligands into proteins (PDB ID: 1T46) to elucidate the interactions of the ligands with the binding site of the protein. Interaction of these compounds which bind tightly to the active site as well as the allosteric site of the tyrosine kinase are listed as the docking results as presented in Table 2. The best pose of these complexes was selected based on the lowest binding free energy value and the Root Mean Squared Deviation (RMSD) value. The RMSD is an important statistic for predicting potentially bioactive chemicals, and it should preferably be less than two[17]. The RMSD measurement is commonly used to verify the docking technique. To validate a docking methodology, we must first consider a crystallographic complex protein including a ligand and then dock the same complex.

Based on the docking results, it was found that doxorubicin as positive control has binding free energy of -7.76 kcal/mol. The binding mode was shown that doxorubicin can bind well with Glu640 and Asp810 through van der Waals interaction. In addition, doxorubicin was also interact with Arg791, Leu644, Val643, Pro573, Ile789, Ile571, Leu783, Ile808, Ser639, Cys809, Cys788, His790, Asn787, Tyr570. Binding mode of doxorubicin is depicted in Figure 2.

Compounds ZINC4377306 and ZINC4377309 are estimated to be breast cancer inhibitor. Both of these compounds were bound well with the active site of tyrosine kinase with the binding free energy of -6.7535 and -6.4978 kcal/mol, respectively. Factor of binding is the main parameter in docking process. It is the probability of the receptor-ligand binds to the same amino acid residue that the positive control was bound18. This principal parameter for ZINC4377306 and ZINC4377309 were 11 and 12 respectively. ZINC4377306 has exhibited the hydrogen bond with

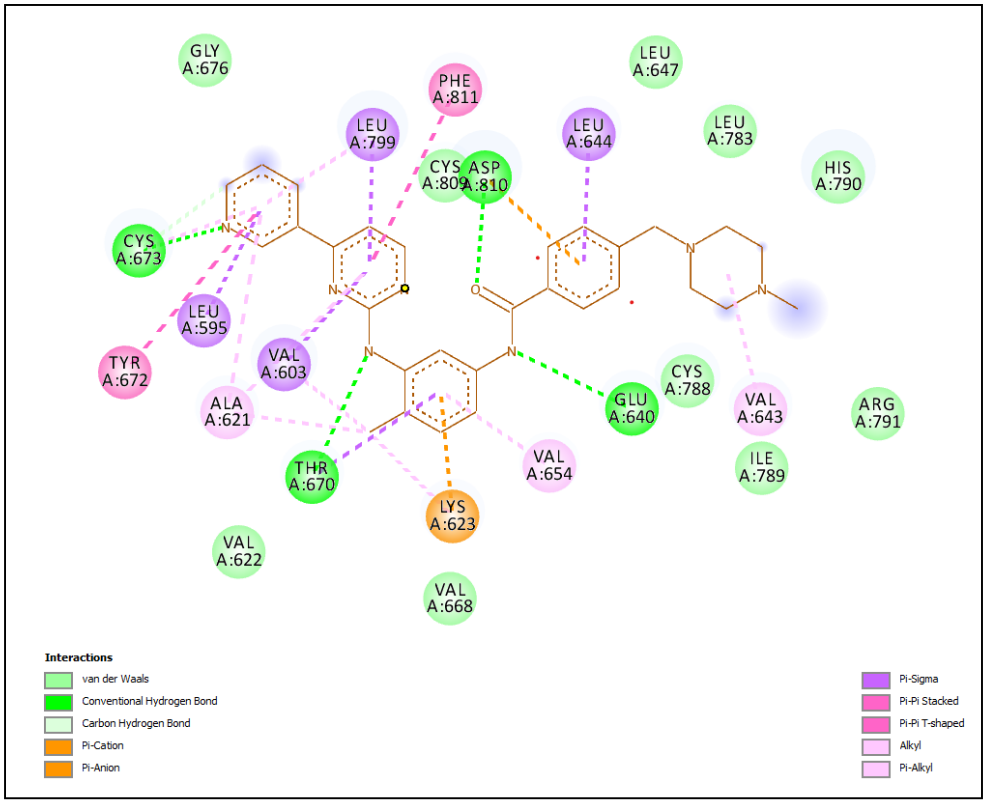


Figure 2. Binding mode of doxorubicin.

Glu640 and it is also observed to interact with Asp810 through van der Waals interaction. The spatial arrangement of ZINC4377306 is presented in Figure 3. ZINC4377309 observed to have two van der Waals interaction with amino acid residues Gl640 and Asp819. However, ZINC4377309 has not constructed any hydrogen bond with amino acid residues.

The estimated active compound ZINC4377306 and ZINC4377309 have interactions with important amino acids surrounding of active site such as Asp810, Glu640, Ser639, Leu647, Leu783, Ile571 dan Tyr570, Glu671, Ala621, Leu799, Val603, Leu595, Phe811, Asp677, Asn680, Cys674, Tyr657, Gly676, Cys673 and Cys788[8]. Hence, it presumably caused potential bioactivity of these compounds as breast cancer inhibitor. Figure 4 are depicted the spatial arrangement for compound ZINC4377309 which help to understand the binding site of this ligand with the protein active site.

Table 2. The Docking results.

Compound	S (kcal/ mol)	RMSD	H bond	van der Walls	The others interaction	Factor of Binding
Doxorubicin	-7,7632	1,1948	-	Glu640, Asp810	Arg791, Leu644, Val643, Pro573, Ile789, Ile571, Leu783, Ile808, Ser639, Cys809, Cys788, His790, Asn787, Tyr570	
ZINC4377306	-6,7535	1,7054	GLU640	Asp810	Arg791, Lys623, Tyr570, Cys788, His790, Ser639, Gly812, Ile789, Leu644, Ala636, Leu783, Leu813, Val643	11
ZINC4377309	-6,4978	0,8973	-	Glu640, Asp810	Gly812, Tyr570, Cys788, His790, Ala636, Pro573, Ile571, Ile789, Ile653, Leu783, Ile808, Leu644, Val643, Leu647, Leu813	12
ZINC95585154	-6,0259	1,0799	Ile789	Glu640, Asp810	Arg791, His790, Cys809, Tyr570, Val643, Leu644, Leu647, Ile808, Ile653, Ala636, Ile571, Pro573	12
ZINC95585155	-6,3218	0,8045	Ile789	Glu640, Asp810	Tyr570, His790, Cys788, Cys809, Gly812, Ile571, Leu644, Ala636, Leu813, Val654, Leu647, Ile653, Ile808, Val643, Leu783	12
ZINC4550853	-5,4360	1,0141	Glu640, Val643	Asp810	Tyr570, Ser639, Gly812, Cys809, Cys788, His790, Ile571, Ile789, Leu783, Ile808, Ile653, Leu647, Leu644, Leu813, Leu637, Ala636	13
ZINC4149299	-6,2694	1,0542	Glu640, Asp810	-	Arg791, His790, Ser639, Cys809, Ile789, Val643, Leu783, Leu647, Ile653, Ile808, Leu644	11
ZINC4149300	-6,1692	1,4898	Cys788, Ile789	Glu640, Asp810	Arg791, Ser639, His790, Cys809, Val643, Ile751, Leu783, Leu644, Ile653, Ile808, Ala636	12

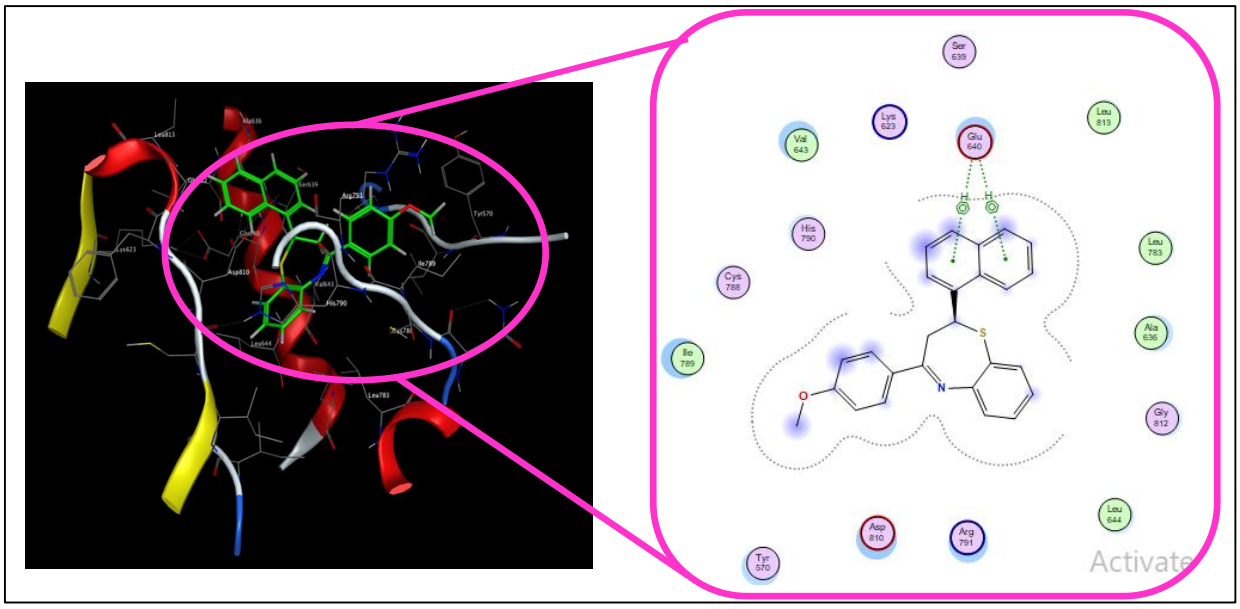


Figure 3. The spatial arrangement of ZINC4377306.

3.3. Molecular Dynamic

Currently, MD simulation is extensively used in drug design process in order to understand the drug-receptor interaction and also to explore the assessing predictive poses bonding from the docking results. Periodic boundary condition with isothermal-isobaric (NPT) scheme was used to stimulate for each system. The non-bonded interaction was set to 10Å and Ewald mesh method was used to manage the electrostatic interaction[19]. MD simulation was begun with the stable minimum energy; the system fluctuates around the initial conformation, and followed by binding mode of the initial ligand[20]. In current investigation, MD simulations were carried out at 300 K for 50 ns to determine the affinity of the ligand at the binding site.

Conformation of compounds ZINC4377306 and ZINC4377309 maintained to bind with the important residues as presented in Figure 5. Based on the estimated free energy of binding and MD simulation, the existence of the hydrogen bond of these molecules are 2.9 Å which is suggested that both of them can be used as potential inhibitor for breast cancer. MD results are listed in Table 3.

4. CONCLUSIONS

Combination similarity search with docking and molecular dynamic simulations were performed using 1,5-benzothiazepine compound (i.e. MA9) as parent compound. Based on Euclidean distance and Tanimoto coefficient by applying the applicability domain,

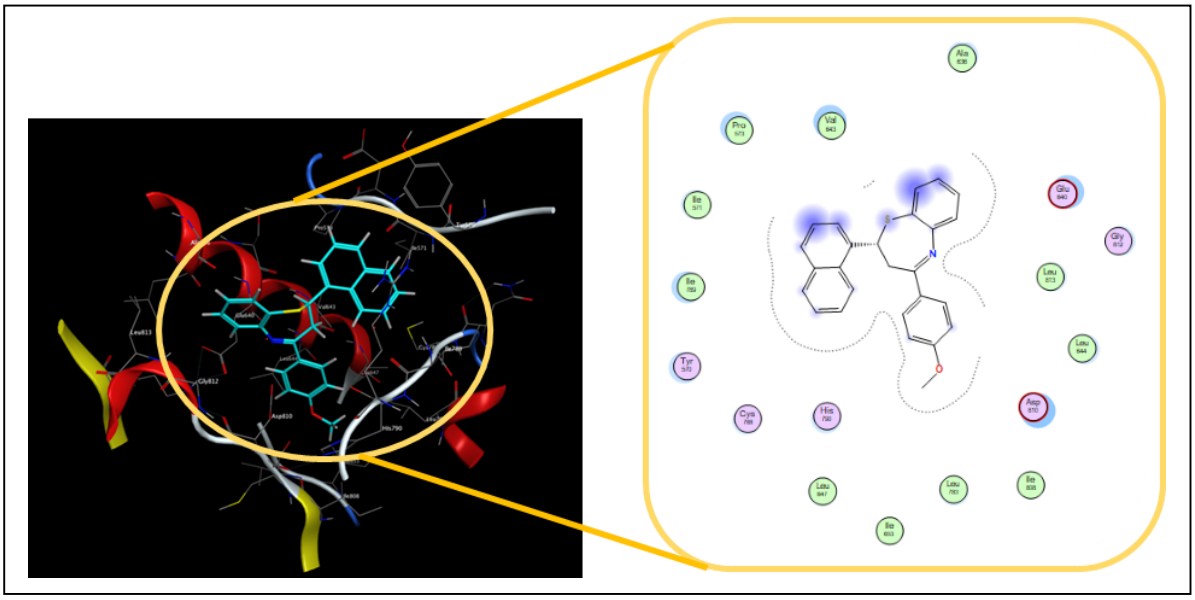
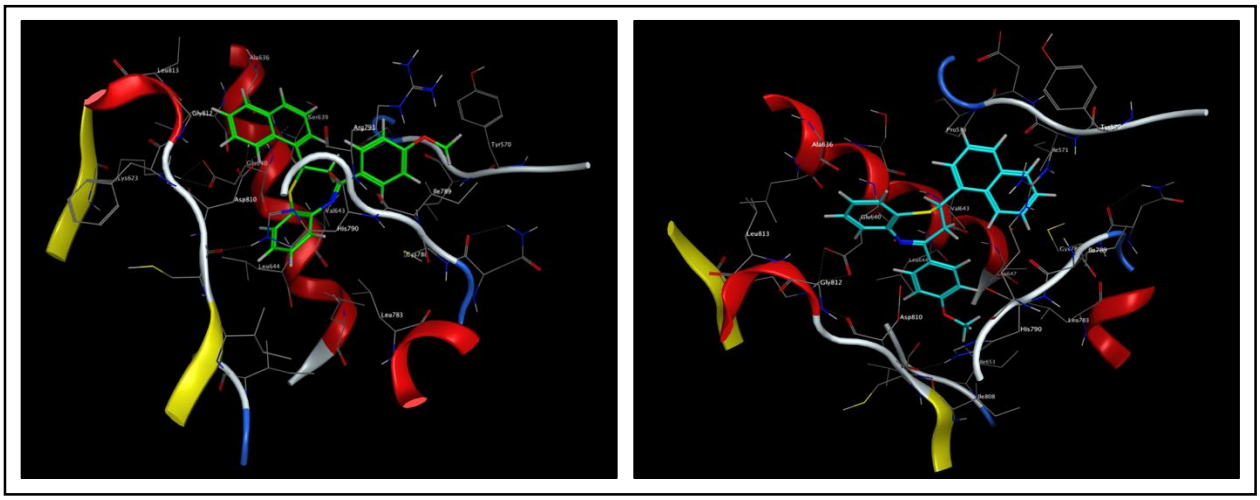


Figure 4. The spatial arrangement of ZINC4377309.

Table 3. MD results.

Compound	After docking	MD	H-bond distance
ZINC4377306	Glu640, Asp810	Glu640, Asp810	2.9 Å
ZINC4377309	Glu640, Asp810	Glu640, Asp810	-
ZINC95585154	Glu640, Ile789, Asp810	-	-
ZINC95585155	Glu640, Ile789, Asp810	-	-
ZINC4550853	Glu640, Val643, Asp810	-	-
ZINC4149299	Glu640, Asp810	-	-
ZINC4149300	Glu640, Cys788, Ile789, Asp810	-	-



(a) **(b)**

Figure 5. visualization of MD simulation for (a) ZINC4377306 (b) ZINC4377309.

numbers of possible candidates were further narrowed down to seven compounds. Based on docking results, only two compounds i.e. ZINC4377306 and ZINC4377309 were estimated as potentially active breast cancer showing the binding free energies of -6.75 and -6.49 kcal/mol, respectively. They also were also stable during the MD simulation. Hence ZINC4377306 and ZINC4377309 can be used as new potential agents against breast cancer as an inception stage in drug discovery process.

5. ACKNOWLEDGEMENT

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Author contribution

NF, MY, HN and AZ conceived and designed the study, conducted research, provided research materials, and collected and organized data. MC, MD and CO analysed and interpreted data. NF and MY wrote the initial and final drafts of the article. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript

Conflict of interest

None to declare.

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Ethics approval

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