In silico molecular docking analysis of Imatinib to target the marker proteins of breast cancer

Abstract

Cancer is an uncontrolled over growth of abnormal cells elsewhere in the body. It is the second leading cause of death globally due to non communicable disease. Among the various types of cancers, the incidence of breast cancer is next to lung cancer. The most commonly used drugs to treat breast cancer are namely, Anastrozole, Arimidex, Letrozol, Imatinib, Tamoxifen, Raloxifene, Toremifene and so on. The hope is to establish the specificity of the drug Imatinib towards the selective potential breast cancers such as mammalian target of rapamycin, (mTOR), human epidermal growth factor receptor 2 (HER2), estrogen receptor (ER), poly (ADP-Ribose) polymerase (PARP) and phosphoprotein 53 (p53). To identify the promising target, the Schrodinger software was utilized for the study. The study helped to evaluate the pharmacokinetic properties and binding efficiency of Imatinib towards the breast cancer proteins. The results of study showed that the Imatinib exhibited better binding affinities to mTOR and HER2 as compared to ER, PARP and P53 proteins. The present study will be more useful to rationalize the anticancer therapy based on the expression levels of the target protein in the cancer microenvironment.

Keywords: Cancer, Breast cancer, Molecular docking, Schrodinger, *in silico*, Imatinib, Binding affinities, ADME.

Introduction

Cancer is an uncontrolled over proliferation of abnormal cells in the body. The six most widespread cancer types globally are lung cancer, breast cancer, colorectal cancer, prostate cancer, stomach cancer, and cervical cancer. Among the various types of cancers breast cancer is

a highly prevalent and second leading cause of death globally next to lung cancer [1, 2, 3, 4]. It is difficult to treat various cancers using a single drug, since the cancer has a large degree of variability in type of cancers as well as cancer patients [5, 6, 7]. The breast cancer targeted proteins were Serine-threonine kinase AKT, also known as protein kinase B (PKB), Cyclooxygenase 2 (COX2), Human epidermal growth factor receptor (HER/EGFR/ERBB)), Tumor necrosis factor receptor superfamily member 4 (OX40 or CD134), Programmed death-ligand 1 (PD-L1 or CD274 or B7 homolog 1), Vascular endothelial growth factor receptor 1 (VEGFR1), B7H3 (CD276or B7 homolog 3), Cytotoxic T-lymphocyte antigen 4 (CTLA-4 or CD152), Lymphocyte-activation gene 3 (LAG-3), Tumor protein P53 (p53), Cyclin-dependent kinase 4 (CDK4), Epidermal growth factor receptor (EGFR), The mammalian target of rapamycin (mTOR), Poly [ADP-ribose] polymerase 1 (PARP1), Tumor-necrosis factor related apoptosis-inducing ligand(TRAIL or CD253), Cyclin-dependent kinase 6 (CDK6), The transcription factor NF-κB, and Programmed cell death protein 1 (PD-1 or CD279) [37]. The most commonly used drugs to treat breast cancer are namely, Anastrozole, Arimidex, Letrozole, Imatinib, Tamoxifen, Raloxifene, Toremifene and so on [8, 9, 10]. The Scientists across the world are focusing their attention to develop an ideal drug to treat breast cancer using various tools. Among these the molecular docking analysis is playing a pivotal role in the process of new drug discovery and to establish drug/molecule and receptor interaction. Hence, in the present study i*n silico* analysis was adopted to establish the degree of specificity between Imatinib and the selective target protein such as mammalian target of rapamycin (mTOR) [11], human epidermal growth factor receptor 2 (HER2) [12], estrogen receptor (ER) [13], poly (ADP-Ribose) polymerase (PARP) [14] and phosphoprotein 53 (p53) [15].

Breast cancer most commonly occurs to women and to a small extent to men as well since the breast is composed of identical tissues in males and females. The occurrence of breast cancer in men is 100 times less common as compared to women, but the statistical survival rate of men was similar to women [**16**]. With 2.1 million new cases and 0.627 million mortality worldwide in 2018 is the foremost reason for the emergence of seriousness about breast cancer. Breast cancer is sorted out into 3 major subtypes based on the presence or absence of molecular markers for estrogen or progesterone receptors and human epidermal growth factor 2 (HER 2), which is

also known as erythroblastic oncogene B2 (ERBB2). The hormone receptor positive/ERBB2 negative patients are 70%, whereas ERBB2 positive alone accounts for 15-20% patients and the triple-negative is stands for a tumor lacking all three standard molecular markers associated with 15% of patients. During the diagnosis more than 90% of breast cancers are not metastatic and the therapeutic goals are tumor abolition as well as preventing the recurrence **[17]**.

The patients with non-metastatic and hormone receptor positive subtypes of breast cancer are treated with endocrine therapy and a minority of patients receive chemotherapy as well. Whereas, the patients with ERBB2-positive tumors receive ERBB2-targeted antibody or small-molecule inhibitor therapy combined with chemotherapy but the patients with triple-negative tumors receive chemotherapy only. Metastatic breast cancer is treated based on its subtype by extending life and reducing the pain of the symptoms. The mean survival time of patients with metastatic triple negative breast cancer is approximately 1 year, whereas 5 years for other two subtypes of cancer [**17**]. The important contributing factors associated with breast cancer must be taken into account while developing a new drug therapy. The prolonged exposure to endogenous estrogens leads to an early menarche; late menopause and first childbirth at late age are the most important risk factors for breast cancer. Exogenous hormones also exert a higher risk for breast cancer.

Current scenario on molecular therapies for cancer is aimed to alter the key functional molecules to arrest the progression of cancer cells. The eye catching targets are namely, platelet-derived growth factor receptors (PDGFRs) and c-Kit (CD117/stem cell factor receptor), which are causing an uneven signaling leads to either over expression or mutation that results tumorigenesis as well as cell proliferation [**18**].

Imatinib mesylate is a selective inhibitor of the tyrosine receptor kinase namely, PDGFR-β and c-Kit **[18**]. Imatinib (Gleevec® or Glivec®) is also known as a magical bullet developed by Nicholas Lyndon, a biochemist of Ciba-Geigy and the clinical trial was conducted with the patients of chronic myeloid leukemia (CML) by Brian Druker, an oncologist at the Dana-Farber

Cancer Institute, Harvard Medical School in USA. Since a successful result in treating the patients of CML, the scientists tried to explore the possibility of using imatinib to treat other types of cancer as well. Later it was proved that imatinib exhibited a miracle effect to treat other types of cancer which express the tyrosine kinases. The targeted therapy of Imatinib is in the form of selective tyrosine kinase inhibitors (TKIs). Hence by targeting the TKIs, it is the one of the first cancer therapy to treat various cancers. It is an oral targeted therapy of tyrosine kinases specifically BCR-ABL, c-KIT and PDGFRA. It also shows extraordinary success in chronic myeloid leukaemia (CML) and Gastrointestinal Tumor (GIST). It is also a beneficiary in other tumors caused by the Imatinib-specific abnormalities of platelet derived growth factor receptor A (PDGFR) and tyrosine-protein kinase Kit (c-KIT). It is also proved to be efficient in steroid-refractory chronic graft-versus-host disease because of its anti-PDGFR action [**19**].

Molecular docking is a pivotal tool used in molecular biology and computer-aided drug design. The objective of ligand-protein docking is to envisage the principal binding mode(s) of a ligand with known three dimensional (3D) structure of a protein. The successful docking protocols efficiently hunt high dimensional spaces with scoring properties, which suitably ranks the molecules/ligands. Docking is also used to execute virtual screening from a library of compounds, sort the results and suggest the structural hypotheses to inhibit the target protein, which is precious in lead optimization **[20]**. In addition to these, the docking is also employed for a broad set of *in vitro* assays to identify the key parameters such as lipophilicity, solubility as well as plasma stability during the absorption, distribution, metabolism and excretion of a ligand. These predictions assist the evaluation of the pharmacological parameters of a molecule and serve as a representative before it enters *in vivo* testing as well as clinical trials **[21]**. The computational protocol predicts probable ADME, toxicity issues and sinking the count of experiments that involve animal testing **[22]**.

The present study is focused on in silico molecular docking analysis of Imatinib on breast cancer specific proteins such as mTor, HER2, ER, PARP and P53 using Schrödinger software. These proteins were mined from protein data bank (PDB) and their identities (ID) are: 3jbz, 3pp0, 3ert, 2rcw and 2x0w respectively. In addition to these, the drug likeness as well as their absorption,

distribution, metabolism, and excretion (ADME) properties of Imatinib was also predicted using Lipinski rule.

Materials and Methods

Preparation and protein

Three dimensional (3D) crystal structure of the proteins namely, mammalian target of rapamycin (PDB ID: 3JBZ), epidermal growth factor receptor 2 (PDB ID: 3PP0), estrogen receptor (ER) (PDB ID: 3ERT), poly (ADP-ribose) polymerase (PDB ID: 2RCW) and phosphoprotein 53 (PDB ID: 2X0W). The resolution of these proteins are: 28.0 Å [23], 2.25 Å [24], 1.90 Å [25], 2.80 Å [26] and 2.10 Å [27] respectively. The proteins were mined from protein data bank (PDB) (https://www.rcsb.org). The 3D structures of the proteins were imported into "Protein preparation wizard" [20]. The protein was designed in such a way using appropriate modeling calculations of Schrodinger software.

The protein structure mined from the protein data bank contains heavy atoms, metal ions, missing hydrogen atoms, water molecules, co-crystallized ligand, incomplete and terminal amide groups. The wizard repair the bond orders, formal changes, adding the missing protons, metals treated, and the water beyond the 5Å from the hetero atom was removed. The probable ionization states were created for the ligand in the protein and the most stable state was preferred using Epik **[29].** Finally, a controlled minimization of the protein was carried using the force field OPLS-2005 by a limited root mean square deviation (RMSD) tolerance of 0.3 Å **[30].**

Receptor grid generation

The ligands found in all proteins were retained and the grid was generated over the ligand associated with an active site using the "receptor grid generation" module of Schrodinger software. The formation of centroid (cubical) shape over the ligand points out the active site of the protein **[7, 31]**

Preparation of ligand

The structure and molecular formula ($C_{29}H_{31}N_7O$) of Imatinib was obtained from the "PubChem" using the web link (https://pubchem.ncbi.nlm.nih.gov/compound/Imatinib) (**Fig.1)**

and saved in .SDF format. Then the ligand was imported into the "LigPrep" module of Schrodinger 2018.1. The 2D structure of the molecule was converted into a low energy 3D structure **[31]**. In addition to these, the multiple structures with possible ionization states, tautomers, stereo-isomers, and ring conformations were also created. Moreover, the ligand was energy minimized and optimized for their geometry. Using the "EPIK" module the ionization and tautomeric states were created at the pH between 6.8 to 7.2. Finally, the compounds were minimized using optimized potentials for liquid simulations-2005 (OPLS-2005) force field with a root mean square deviation (RMSD) of 1.8 Å was attained **[32]**.

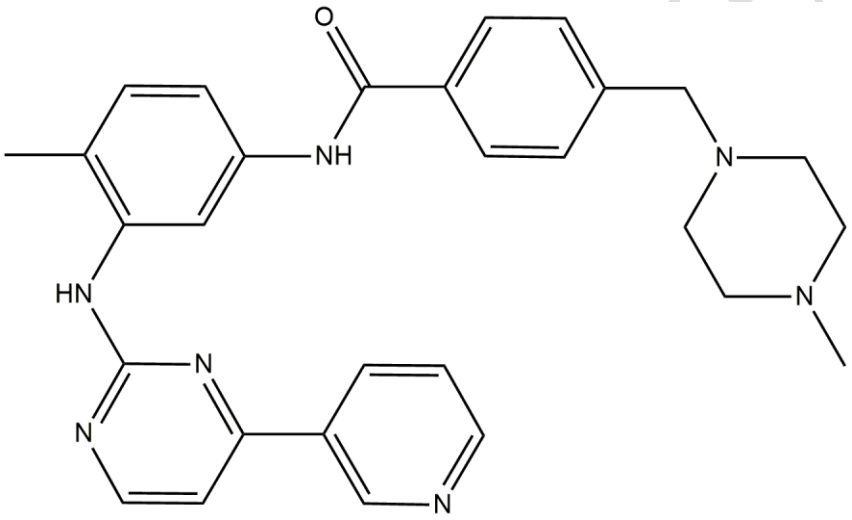

Fig.1 The structure of Imatinib

Molecular docking

The docking was performed using the "Glide" module of Schrodinger using the previously set receptor grid and ligand. The positive interactions between the ligand molecules were ranked using Glide "Ligand docking" program. The calculations concerning docking were achieved using the extra precision (XP) form and force field OPLS-2005. The flexible docking mode was performed to evaluate the ligand interaction with the receptor and the docking poses were undergoing a sequence of hierarchical filters. The algorithm identifies the good hydrophobic, hydrogen bonding and metal ligation interactions as well as penalizes the steric clashes. Followed by the final stage of the algorithm, minimization was performed using force field OPLS-2005 and the minimized poses were re-ranked with e Glide "Scoring function" **[33]**.

Lipinski rule and the analysis of ADME properties

The *in silico* analysis is a vital tool, which is employed for the early preclinical evaluation of a new chemical entity so as to avoid the failure of a molecule at the terminal stage of a drug discovery process. This technique considerably reduces the quantity of time, resources and rationalizes the entire expansion procedures of a new drug molecule. Almost 40% of drug candidates are unsuccessful due to their deprived absorption, distribution, metabolism and excretion (ADME) properties. Hence, the high-throughput screening (HTS) techniques are used to accurately predict the ADME parameters of a newly developed molecule and to screen the tricky drug candidates, which are not worthy to proceed further. Interestingly, this tool is used to redesign the failed compound to increase its preferred parameters to pass all ADME parameters **[34]**.

The bioavailability of a molecule is predicted more efficiently using Lipinski's rule of five, which is recognized as a filter of choice. The five rules denotes that the compound should have a molecular mass of less than 500 daltons, no additional 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors and an octonal/water partition coefficient "log P" should not be greater than 5 **[35]**. The Lipinksi's rule and pharmacokinetic parameters such as absorption, distribution, metabolism, and excretion (ADME) were predicted using the "QikProp" tool of Schrodinger 2018.1 **[36]**.

Results and Discussion

The molecular docking studies on the active sites of various target proteins in breast cancer and the ligand "Imatinib" was conducted using an advanced molecular docking tool "Schrodinger maestro version 11". The ligand Imatinib was prepared using the "LigPrep" tool of Schrodinger software and the optimized ligand was used for the docking analysis. The breast cancer proteins mTOR, HER2, ER, PARP and P53 were prepared using the protein preparation wizard tool of Schrodinger. The cube shape of the grid/active site region was generated using the receptor grid generation mode of Schrodinger. The generated Grid region/active site was selected and set accordingly to bind/interact with the "Imatinib", the drug of our interest. The study assessed the affinity of Imatinib towards all the target proteins and ranked the binding affinity.

The molecular docking was performed with all target proteins namely, mTOR, HER2, ER, PARP and P53 using the GLIDE tool of Schrodinger. The predicted docking score, Glide evdw (Van Der Waals energy), ecoul (Coulomb energy), Glide energy and the interacting residues (Hydrogen bond/ π- π bond) with target proteins were evaluated in detail to establish the degree of interaction between the target proteins and Imatinib. The results of the study showed that the ligand "Imatinib" perfectly docked and exhibited good binding interactions with the active sites of all the proteins. Results concerning mTOR, HER2, ER, PARP and P53 were referred to as A, B, C, D and E respectively (**Table 1**).

The results of the present study showed that the Imatinib exhibited good interaction with all the target proteins and the order of binding is mTOR>HER2>ER>PARP>P53. The docking scores of the proteins are -8.3, -8.1, -6.9, -5.3, and-5.2 respectively. The ligands which score the lowest binding energy are considered as a choice of high binding affinity. In the present study mTOR and HER2 showed higher binding affinities with imatinib as compared to ER, PARP and P53 proteins.

The Glide evdv values of the proteins mTOR, HER2, ER, PARP and P53 were -47.63,-46.32, -49.54, -46.44 and -28.58 respectively (Table 1). The Glide ecoul values f were -9.8, -10.71, 0.79, -11.64 and -6.55 respectively. Followed by the glide energy of the proteins such as mTOR, HER2, ER, PARP, P5 were also estimated and the scores are -57.45, -57.03, -48.75, -58.07 and -35.14 respectively. The amino acids of the target proteins namely, mTOR, HER2, ER, PARP and P53 demonstrated good interaction with Imatinib through hydrogen bonding, pi-pi bond and polar interactions. The hydrogen bonds and pi-pi interactions between the amino acids of target proteins and Imatinib is given in **Table 1.**

Name of the Drug	Target Protein	Docking Score	Glide evdw	Glide ecoul	Glide energy	Interacting residues/type (HB/Pi-Pi)
Imatinib	MTOR	-8.3	-47.63	-9.8	-57.45	CYS2243;ASP2252/ LYS2187 and TRP 2239

	HER2	-8.1	-46.32	-10.71	-57.03	THR862, HOH129 and ASP808/ LYS753
	ER	-6.9	-49.54	0.79	-48.75	LEU525
	PARP	-5.3	-46.44	-11.64	-58.07	GLY227, TYR228 and ARG217/ TYR235 and ARG217
	P53	-5.2	-28.58	-6.55	-35.14	HOH2254 and HOH20276

Table.1 The docking score, Glide evdw (Van Der Waals energy), ecoul (Coulomb energy), interacting residues and the type of interaction of Imatinib with breast cancer marker proteins. The docking scores calculated using Glide program of Schrodinger 2018.1. Where, HB denotes hydrogen bonding; Pi-Pi denotes π-π bond.

The 3D and 2D interactions of Imatinib with the proteins such as mTOR (A), HER2 (B), ER (C), PARP (D) and P53 (E) are shown in **Fig. 2 & 3.**

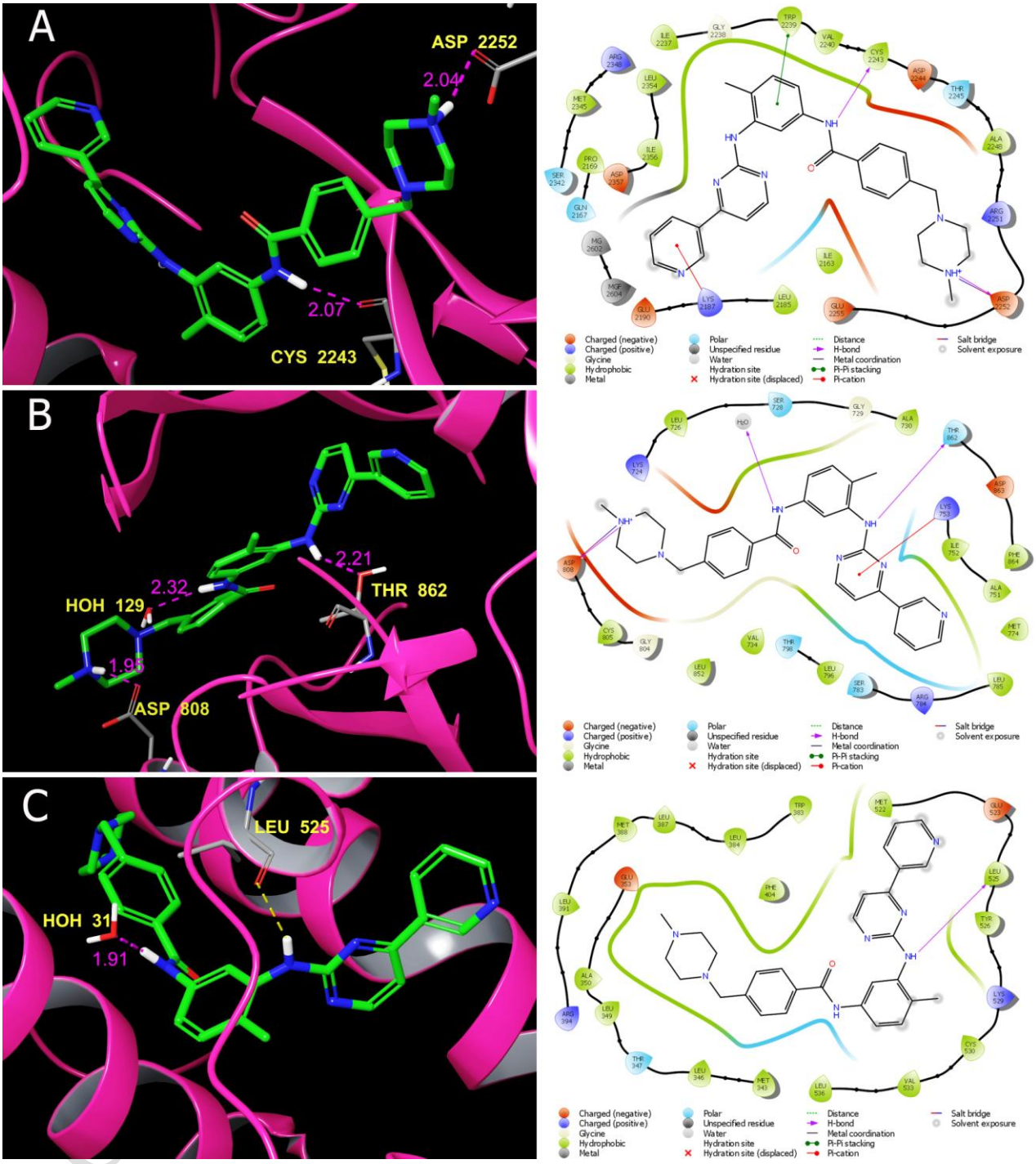

Fig.2 Binding orientations of Imatinib with the crystal structure of mTOR (A), HER2 (B), ER (C) and its hydrogen-bond interactions with amino acids.

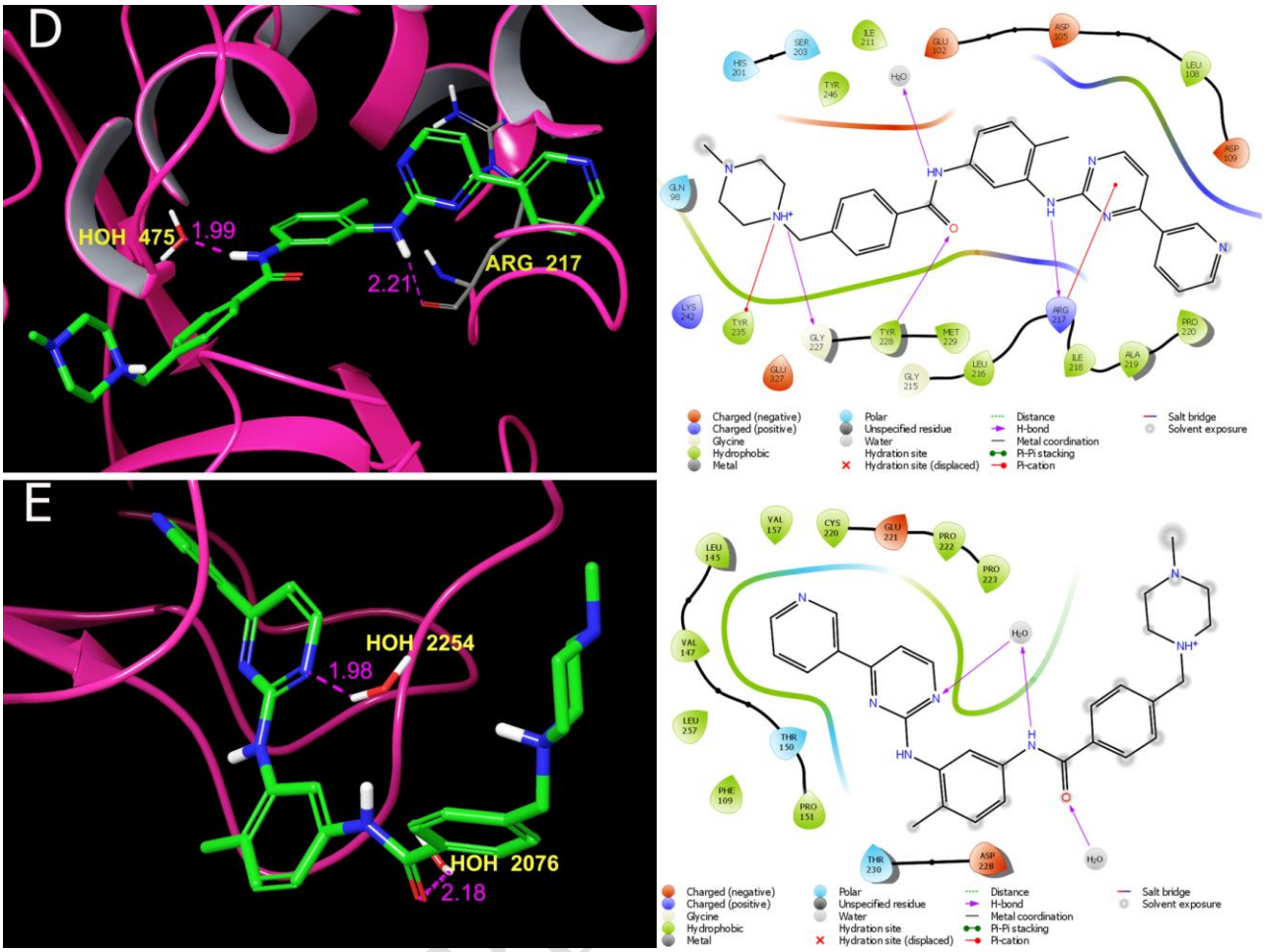

Fig.3 Binding orientations of Imatinib with the crystal structure of PARP (D), P53 (E), and its hydrogen bond interactions with amino acids.

Validation of the docking programme

The precision of the docking protocol was resolved by analyzing the binding conformation of the ligand and target proteins based on lowest energy poses using the scoring function. The Glide/docking score resembles the experimental binding as determined by X-ray crystallography. The results of the study were calculated using hydrogen bonding interactions, the root mean square deviation (RMSD) between the predicted and the experimental X-ray crystallographic conformations. Here the extra precision (XP) docking mode was demonstrated by taking away the co-crystallized ligand in its active site by docking "Imatinib" with its binding site **[31]**. The docking scores of each target protein with "Imatinib" were compared. Interestingly, "Imatinib"

showed a good binding interaction/affinity towards all the five breast cancer specific proteins and the order of binding interaction is as follows: mTOR>HER2>ER>PARP>P53.

Lipinski rule and ADME properties

The ADME properties of the test drug "Imatinib" was further assessed based on Lipinski's rule using the QikProp module of Schrodinger software. The Lipinski's properties such as molecular weight, hydrogen bond donor (HBD), hydrogen bond acceptor (HBA), partition coefficient (QPlogP (O/W) and the rule of five. The estimated values of the above parameters were 493.61 (<500), 2 (<5), 10.5 (<10), 3.70 (<5) and the rule of 5 is 0 (0) respectively (**Table.2)**. The predicted Lipinski values were below the limit given within the parentheses except a minor variation observed with HBA.

Name of the Drug	Factors of Lipinski rule of 5				
	MW (<500)	HB-Donor (<5)	HB-Acceptor (<10)	QPlogP (O/W) (<5)	Rule of 5 (0)
Imatinib	493.61	2	10.5	3.70	0

Table 2. The scores of Imatinib predicted by a QikProp module of Schrodinger based on the Lipinski rule. Where MW denotes molecular weight, HB-Hydrogen bond, QPLogP (O/W) - predicted octanol/water partition co-efficient logP respectively.

Name of the Drug	Pharmacokinetic properties				
	QPlogS (-6.5 to 0.5)	QpHERG (<-5)	QPlogBB (-3 to 1.2)	PHOA (>80 high, < 25 poor)	QPPCaco (>500 high, <25 poor)
Imatinib	-5.25	-9.61	0.33	81	66

Table 3: Predicted ADME values of Imatinib using a QikProp module of Schrodinger. Where, QPlogS denotes aqueous solubility, QpHERG-predicted IC_{50} value for blockage of HERG K^+ channels, QPlogBB-Brain/blood partition coefficient, PHOA- Predicted human oral absorption, QPPCaco- gut-blood barrier/cell permeability in nm/s respectively.

The Pharmacokinetic parameters such as aqueous solubility (QPlogS), Predicted IC_{50} value for blockage of HERG K^+ channels (QPlogHERG), Predicted brain/blood partition coefficient (QPlogBB), Predicted qualitative human oral absorption (PHOA) and gut-blood barrier/cell permeability in nm/s (QPPCaco) were calculated. The corresponding values are, -5.25 (-6.5 to

0.5), -9.61 (<-5), 0.33 (-3 to 1.2), 81 (>80 high, < 25 poor), and 66 (>500 high, <25 poor) respectively (**Table 3)**. The pharmacokinetic properties of Imatinib exhibited that the values were within the acceptable range and not deviated from the limit. From the overall study, the *in silico* analysis of Imatinib showed a good interaction with the target protein and qualified to all the parameters of LipinSki's rule and ADME.

Conclusion

The present study evaluated the molecular interaction and the pharmacokinetic analysis of Imatinib with the selected proteins namely mTOR, HER2, ER, PARP and P53 using Schrodinger Maestro. The programs namely, GLIDE and QikProp of Schrodinger were used to analyze the binding affinity as well as ADME properties. The results of the study showed good binding interaction/affinity through hydrogen bond, polar and the pi-pi bonds interactions with the respective proteins. Interestingly, the predicted scores for the drug Imatinib by Lipinski's rule and ADME properties were in acceptable range. To conclude this, the drug Imatinib effectively inhibits the target breast cancer proteins such as mTOR, HER2, ER, PARP and P53 with low energy. The interaction energies of Imatinib with the five proteins were in the increasing order of mTOR > HER2 > ER > PARP > P53. Additionally, the proteins mTOR and HER2 showed better binding affinities towards Imatinib as compared to ER, PARP and P53 proteins. The present study will be more useful to rationalize the anticancer therapy based on the expression levels of the target protein in the cancer microenvironment.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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