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MOLECULAR DESIGN AND ANTI-BREAST CANCER ACTIVITY OF *TUPICHINOL E* IN HER2+ BREAST CANCER AND EGFR SENSITIZED TO OSMERTINIB THERAPY

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ABSTRACT: The epidermal growth factor receptor is a tyrosine kinase receptor and its overexpression triggers cancers. EGFR inhibition is one of the most unique and popular technique for cancer treatment. There are various approved EGFR inhibitors like osmertinib, lapatinib, afatinib, *etc*. Huge natural products are discovered every year and Rhamnocitrin derivative *Tupichinol E* obtained from the Northern Sikkim plant *Tupistra nutans* is structurally similar to EGFR inhibitors. Structurally similar EGFR inhibitors, their modes of binding and mechanism of actions are a new way to select lead compounds for better alternatives. Therefore molecular docking is done to find a potential anti-EGFR flavonoid. Osmertinib shows binding energy -107.23 kcal/mol with EGFR, while *Tupichinol E* shows binding energy -98.89 kcal/mol. *Tupichinol E* shows 1.5 times more binding affinity than osmertinib. The compound binds to the EGFR at the same exact position as osmertinib and has the same docking poses. Tupichinol E binding to EGFR stabilises the protein structure and is a pharmacologically active substance that can be used against cancer due to its potent activity.

INTRODUCTION: Cancer is the 2nd leading disease in the worldwide which causes death, among these about 29% of women dying every year suffering in breast cancer. The treatment mainly used for breast cancer therapy like chemotherapy, radiation therapy has a various side effect to our body and after frequent used they get resist to our body and not able to show potent action. Multiple disease processes involve protein kinase mutations, dysregulation, and overexpression. A protein kinase is encoded by approximately one in every 40 human genes, and nearly half of these genes map to cancer amplicons or disease sites [1].

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Tyrosine kinase inhibitors are a family of pharmacological agents that, through different modes of inhibition, disrupt the signal transduction pathways of protein kinases. Tyrosine kinase enzymes (TKs) are classified into three types: receptor tyrosine kinases (RTKs), non-receptor tyrosine kinases (NRTKs), and a small subgroup of dual-specificity kinases (DSKs) that can phosphorylate serine, threonine, and tyrosine residues.

RTKs are transmembrane receptors that include vascular endothelial growth factor receptors, platelet-derived growth factor receptors, the insulin receptor family, and the ErbB receptor family, which includes EGFR and HER2 [2, 3]. EGFR, a key component in cell signaling networks, is a member of the HER family. Binding of ligands (EGF and TGF-α) causes structural changes in EGFR as well as homodimerization or heterodimerization with other members of the HER family. The cytoplasmic

tyrosine kinase (TK) region is then autophosphorylated by adaptor proteins (such as SHC and GRB-2), resulting in downstream signaling [3]. Cancer development is also accelerated by EGFR. Cancers, particularly lung cancer, have been linked to EGFR gene mutations and protein over-expression, both of which trigger downstream pathways [19].

The significance of EGFR in breast cancers lends credence to the idea of "oncogene addiction." TKIs are used to treat those with cancer who have EGFR mutations or abnormal EGFR amplification. TKIs can reversibly block the EGFR TK domain via competitive interaction with ATP [4]. As a result, the goal of this research is to find potent molecules to TKI drugs that have the strength to bind both native and mutated EGFR, using the methodology of molecular docking to identify protein-ligand interaction and affinities.

MATERIALS AND METHODS:

Receptor Preparation: On the basis of review of literature, the EGFR and HER2 proteins were selected as molecular targets. The structures of L858R (identified by accession codes 4I20, X-ray crystal structure, resolution 3.34 A) and erbB2 (identified by accession codes 3PP0, X-ray crystal structure, resolution 2.25 A) receptors were acquired from the PDB database. Following that, the receptors were downloaded in PDB file. Previously, native receptors were created by altering the relevant residues with the USFC Chimera bioinformatics tool. PDB format proteins were also generated using Contreras-Puentes et al.'s method [5, 13].

Search and Selection of Ligands: Two drug osmertinib (first-line treatment drug) and *Tupichinol E* against cancer were selected for virtual screening. Ligand library was prepared by downloading in MDL Molfile (mol). from Drug Bank database manually. The drug-likeness of all the ligands was determined by using Lipinski's rule of five *via*. molecular weight < 500 Da, having up to 5 H-bond donors and less than 10 H-bond acceptors and logP not exceeding 5 [6, 17].

Molecular Docking: Molecular docking was carried out using iGEMDock, and 3D protein structures of all the proteins based on the study were put onto the iGEMDock interface for docking. Virtual Docker's built-in cavity detection method was used to discover potential binding sites. Before performing the analysis, each Protein Data Bank file was arranged using the default parameters in Virtual Docker.

The docking process was then carried out between different ligands and active sites of various protein structures using the MolDock score as the MVD scoring function with a grid resolution of 0.30. Each procedure was run ten times. Furthermore, the highest number of iterations was 2000, with a 100 Kcal/mol energy threshold. The conformations with the lowest docked binding energies were selected for each docking process, as best conformation [7, 8, 12].

Molecular Dynamics: The GROMACS force field was used in the Molecular Dynamics models of EGFR-TK and HER2 as well as their complexes with Tupichinol E. After charge neutralization, a cubic box containing 0.15 M NaCl was used for modeling. The Steepest Descent integrator was used to reduce the energy consumption of the devices. To achieve equilibrium at 300 K and 1.0 bar temperature and pressure, the NVT and NPT techniques were used. The simulation continued 100 ns and consisted of 1000 frames per evaluation. The cutoff number for calculating short-range interactions in these calculations was 10 A. The PME for electrostatic interactions estimates was 1.0 A [15, 16].

RESULT:

Molecular Docking: The docked model with the lowest binding energy and greatest binding affinity denotes the most stable binding between the ligand and the target protein. Ligands with significant binding affinities were chosen. The interactions of Osimertinib and *Tupichinol E* with EGFR are noticed in **Fig. 1** & **Fig. 2**, which displayed binding energies of -107.23 and -98.89, respectively. Similarly, the molecular interactions between *Tupichinol E* and Osimertinib compound HER2 are noticed in **Fig. 3** & **Fig. 4**.

So the molecules EGFR-*Tupichinol E* and HER2-*Tupichinol E* displays higher affinity -26.97 kcal/mol and -11.02 kcal/mol [9]. H-bond (conventional and carbon), π-sigma, alkyl and π-

alkyl and predominately Van der Waals forces were the interactions evident in compounds and the EGFR active site.

Osmertinib-EGFR Complex: Docking of Osmertinib with EGFR Complex displayed an affinity for binding of -107.23 kcal/mol. The ligand exhibited Van der Waals force with PHE-723, GLU749, ARG836, ARG858, GLY873, GLY874, LYS875 **Fig. 1.**

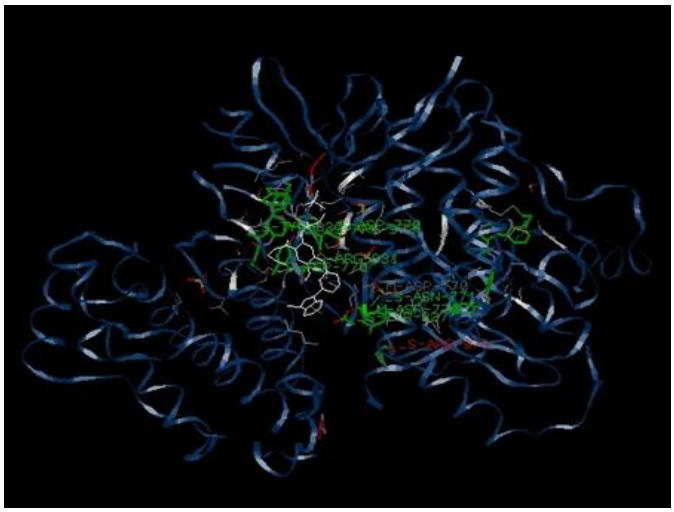

FIG. 1: MOLECULAR INTERACTIONS BETWEEN OSMERTINIB-EGFR COMPLEX

***Tupichinol E*- EGFR Complex:** Docking of *Tupichinol E* with EGFR Complex displayed an affinity for binding of -98.89 kcal/mol. The ligand exhibited H-bonding with ARG858, LYS860, TYR869, GLU872, GLY874. The ligand establishes Van der Waals force with PHE723, ARG836, GLY857, ARG858, GLY873, GLY874 **Fig. 2.**

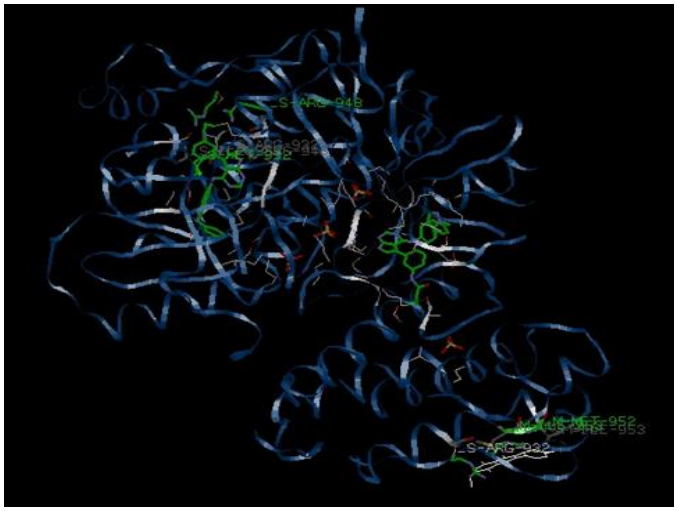

FIG. 2: MOLECULAR INTERACTIONS BETWEEN *TUPICHINOL E*- EGFR COMPLEX

Osmertinib-HER2 Complex: Docking of Docking of Osmertinib with HER2 Complex displayed an affinity for binding of -114.37 kcal/mol.

The ligand exhibits H-bonding with LYS 736 and PRO 802. The ligand establishes Van der Waals force with LEU726, VAL734, LEU800, PRO802, TYR803, GLY804, LEU852, THR862 **Fig. 3.**

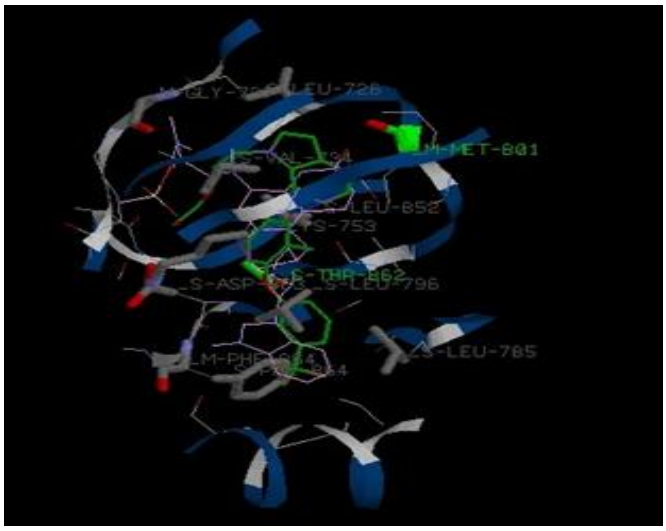

FIG. 3: MOLECULAR INTERACTIONS BETWEEN OSMERTINIB-HER2 COMPLEX

***Tupichinol E*-HER2 Complex:** Docking of *Tupichinol E* with HER2 Complex displayed an affinity for binding of −99.25kcal/mol. The ligand exhibited H-bonding with VAL797, ASP863. The ligand establishes Van der Waals force with LEU726, VAL734, LYS753, THR798, LEU852, THR862 **Fig. 4.**

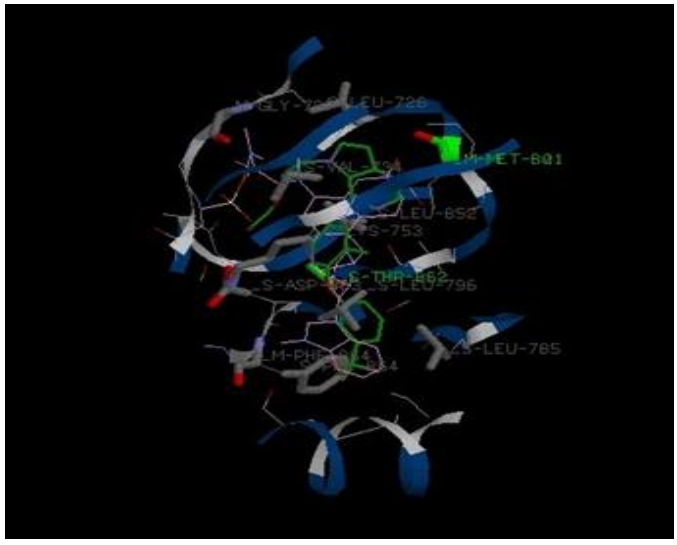

FIG. 4: MOLECULAR INTERACTIONS BETWEEN TUPICHINOL E-HER2 COMPLEX

TABLE 1: BINDING AFFINITY OF COMPOUNDS

Sl. no.	Compound	Energy	Protein-ligand interaction	H Bond
1	EGFR- Osmertinib	-107.23	-106.03	-1.21
2	EGFR- *Tupichinol E*	-98.89	-72.39	-21.85
3	HER2-Osmertinib	-114.37	-108.34	-6.73
4	HER2-*Tupichinol E*	-99.25	-88.23	-11.02

Tupichinol E showed high binding affinity for HER2 and EGFR Complex thereby suggesting that this compound is required for inhibiting cancer, and these could be validated as drugs for cancer treatment. According to reports, these anti-EGFR medications showed significant effects in a variety of therapeutic clinical situations [14].

Molecular Dynamics: For a better understanding of the stability of *Tupichinol E* in the EGFR catalytic site, a 100-ns molecular dynamics simulation was run. *Tupichinol E* was found to be stable in the active site in this research, and its conformation obtained from 100 ns MD simulations varies slightly from that docking obtained. The stability of EGFR TK- *Tupichinol E* can be measured using root-mean-square deviation and the molecular dynamics simulation findings of the EGFR and HER2 and its complex with *Tupichinol E* are shown in **Fig. 5** and **Fig. 6** [16].

The root-mean-square deviation values of free EGFR TK protein and its combination with *Tupichinol E* were 0.3-0.5 nm and 0.3-0.4 nm, respectively. The root-mean-square deviation of the EGFR TK - *Tupichinol E* combination is about 0.1 nm less than the free EGFR TK protein. In comparison to the complex, the RMSD variation with time is greater for free EGFR TK **Fig. 5A**.

This suggests that when *Tupichinol E* binds at the active site, the protein's conformational stability improves. *Tupichinol E*'s root-mean-square deviation changed during the simulation, with values changing at 10ns and 50ns due to binding and achieving a more stable conformer at the protein binding site **Fig. 5B** [16]. The simulations yielded the RMSF values of the amino acid residues in the protein and protein-*Tupichinol E* systems **Fig. 5C**.

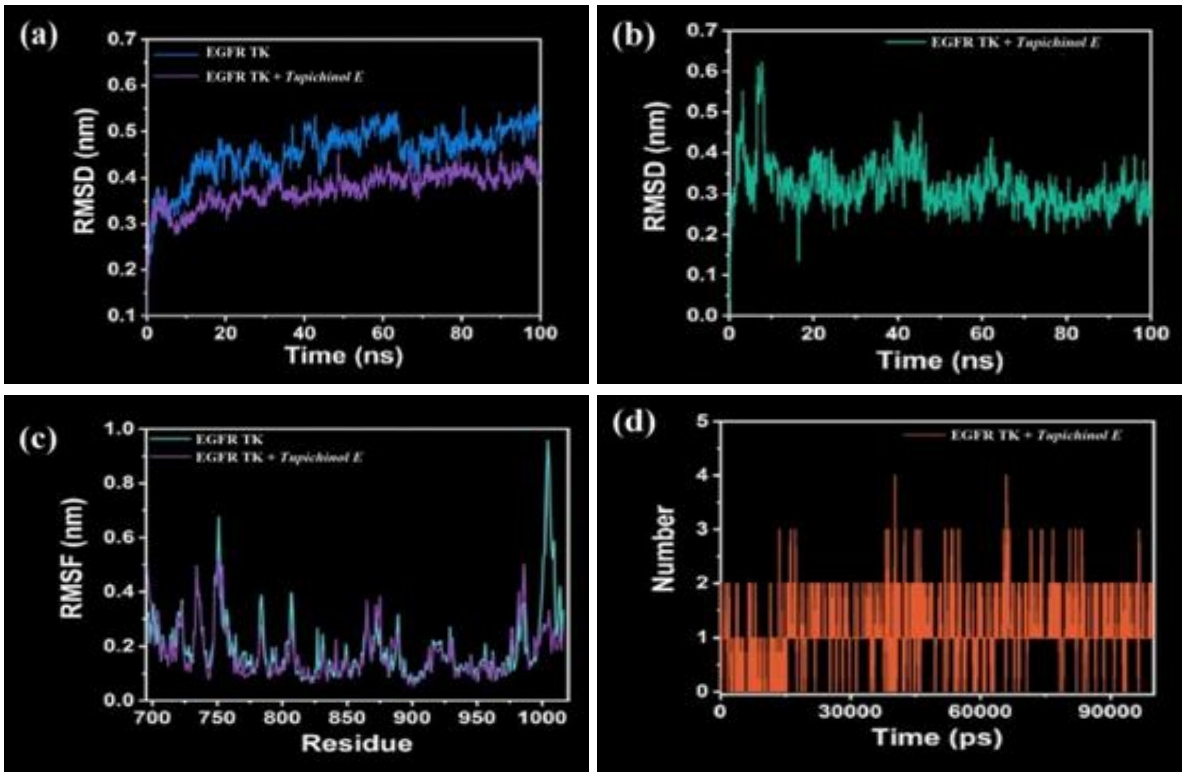

FIG. 5: (A) ROOT-MEAN-SQUARE DEVIATION PLOT OF FREE EGFR TK AND *TUPICHINOL E* BOUND PROTEIN. (B) ROOT-MEAN-SQUARE DEVIATION PLOT OF 10 IN THE EGFR TK- *TUPICHINOL E* SYSTEM. (C) ROOT-MEAN-SQUARE FLUCTUATION PLOT OF FREE EGFR TK AND EGFR TK- *TUPICHINOL E*. (D) HYDROGEN BONDING PLOTS OF *TUPICHINOL E* FORMED WITH EGFR TK DURING THE SIMULATION.

After *Tupichinol E* binding, the values of the majority of the EGFR TK protein residues dropped. It was also discovered that the RMSF of the residues 770, 771, 827 and 831 is among the lowest, having decreased considerably in the protein-*Tupichinol E* complex. This implies that these residues are involved in the interaction with *Tupichinol E*. Here aspartic acid is the amino acid sequence 770 in this case. The compound was able to establish at least two hydrogen bonding interactions during the simulation with the protein. *Tupichinol E* formed hydrogen bonds with S973, M770, S770, S827, and S831 in the docking research. It is important to note that M793 has the highest propensity to form H-bonds throughout the trajectory of these amino acid residues. It only has a 21.9% utilisation of these hydrogen bonds, which is adequate. As a result, the interaction between the EGFR TK protein and *Tupichinol E* is not triggered by conventional hydrogen bonding **Fig. 5D.** The Root-mean-square deviation values for free HER2 protein and its complex with *Tupichinol E* were 0.35-0.58 nm and 0.3-0.49 nm, respectively. The Root-mean-square deviation of the HER2 - *Tupichinol E* system is about 0.1 nm lower than that of free HER2. The Root-mean-square fluctuation with time is greater for free HER2 than for complex HER2 **Fig. 6A**. This suggests that when *Tupichinol E* binds at the active site, the protein's conformational stability improves. *Tupichinol E*'s Root-mean-square deviation changed during the simulation, with values changing at 10 and 50 ns due to binding and achieving a more stable conformer at the protein binding site **Fig. 6B**. The simulations yielded the root-mean-square fluctuation values of the amino acid residues in the protein and protein-*Tupichinol E* systems **Fig. 6C**. After *Tupichinol E* binding, the values of most HER2 protein residues dropped. The RMSF of residues 727, 729, 734, 753, 808, 849, 850, 852, 862, and 863 was also found to be among the lowest, decreasing considerably in the protein-*Tupichinol E* complex. This implies that these residues are involved in the interaction with *Tupichinol E. Tupichinol E* formed hydrogen bonds with M728, S808, M849, and S850 in the docking study. It is interesting to note that M849 has the highest propensity to form H-bonds throughout the trajectory of these amino acid residues. It only has a 22.02% occupancy of these hydrogen bonds, which is adequate. As a result, the interaction between the HER2 protein and *Tupichinol E* is not triggered by conventional hydrogen bonding **Fig. 6D** [20].

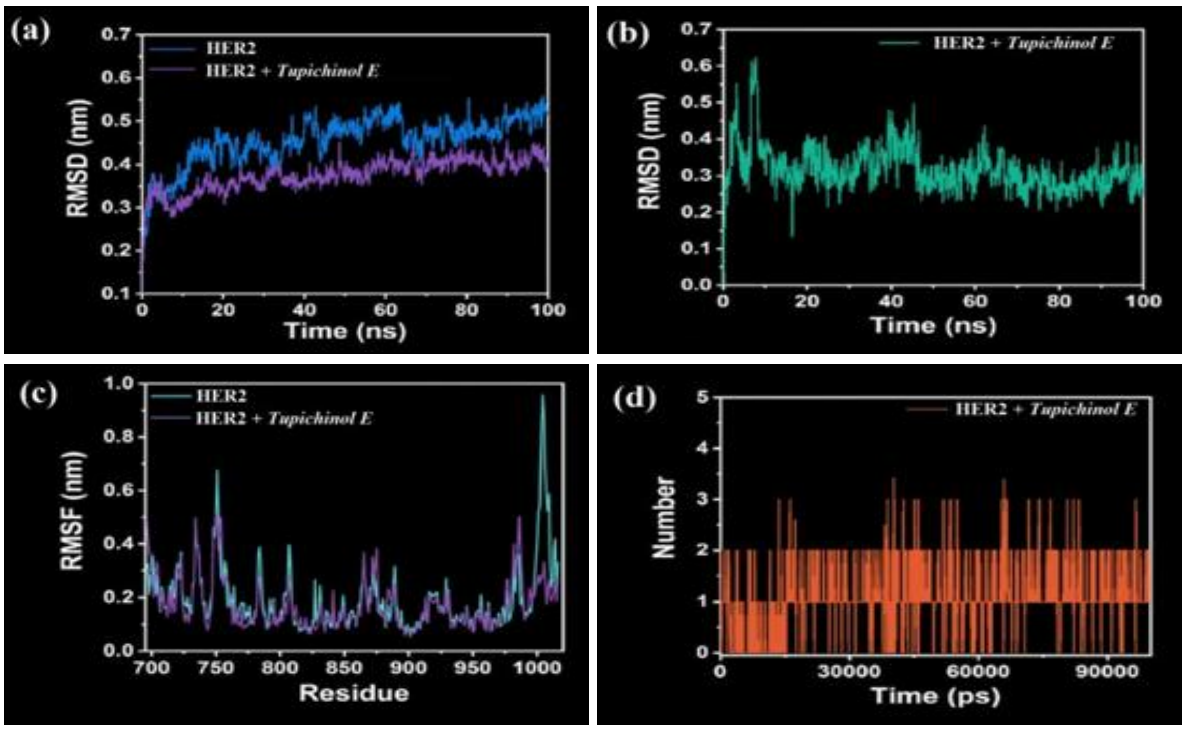

FIG. 6: (A) ROOT-MEAN-SQUARE DEVIATION PLOT OF FREE HER2 AND *TUPICHINOL E* BOUND PROTEIN. (B) ROOT-MEAN-SQUARE DEVIATION PLOT OF 10 IN THE HER2- *TUPICHINOL E* SYSTEM. (C) ROOT-MEAN-SQUARE FLUCTUATION PLOT OF FREE HER2 AND HER2- *TUPICHINOL E*. (D) HYDROGEN BONDING PLOTS OF *TUPICHINOL E* FORMED WITH HER2 DURING THE SIMULATION.

DISCUSSION: *Tupichinol E* has been proven to be a viable therapeutic target for the treatment of breast cancer and increases expression with point mutations like L858R and erbB2 that are correlated with disease progression. As a result, the TKI directed therapy against receptors can inhibit tumor growth at different stages, ensuring the specificity of treatment and selectivity. According to molecular interaction, Osimertinib exhibits high affinity for mutant EGFR and HER2 [10, 11].

Osmertinib shows binding energy -107.23 kcal/mol with EGFR, while for HER2 complex it was -114.37 kcal/mol. Additionally, it has been observed that the interaction between the Osmertinib structure and R841 results in the formation of π-alkyl bond with indole group of the structure, H-bond and C-H-bond interactions and shows Van der waals forces of interaction. *Tupichinol E* shows binding energy -98.89 kcal/mol with EGFR, while for HER2 complex it was -99.25kcal/mol. Additionally, it has been observed that the interaction between the Tupichinol E structure and R841 results in the formation of π-alkyl bond with indole group of the structure, H-bond and C-H-bond interactions and shows Van der waals forces of interaction. *Osmertinib* has already received approval to treat a number of cancer kinds. *Tupichinol E* is a pharmacologically active compound which can be used against cancer because of their potent activity. The last 20% of the trajectory has an average of 8.5 interactions. Throughout the course, the occupancy of the various π-staking interactions (π-alkyl, π-amide, π-lone pair, and π-S) is 150% [16]. *Tupichinol E* therefore reacted hydrophobically (in a docking study) with at least 8 of the amino acids in the active site to achieve stability at the binding site. Since the occupancy value in this case is greater than 100%, any one of the eight amino acids can interact with *Tupichinol E* through more than one of the earlier mentioned non-covalent interactions [17, 18].

CONCLUSION: Due to efficiency and low cost, computational tools have made significant strides in the identification of novel therapeutic agents with potential pharmacological activity. This has made the difficult journey to the stage of clinical phase easier by providing arguments that are more convincingly in line with the theoretical results attained. Both the native and mutant EGFR and HER2 complexes had a substantial affinity for the drugs Osmertinib and *Tupichinol E*. As a result, it is promising for the treatment of breast cancer and its stages of progression, contributing to the issues of acquired resistance and safety profile with relation to current pharmacological treatment and constituting a development in precision medicine from the computational approach to the treatment of breast cancer.

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CONFLICTS OF INTEREST: No conflict of interest.

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