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Molecular Docking and Dynamics Simulations for *In -Silico* Evaluation of 2,4-Di-tert-butylphenol (2,4-DTBP) as a Potential Breast Cancer Inhibitor

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Abstract

BREAST CANCER (BC) is still one of the major causes of death in females, mainly because of the uncontrollable proliferation of breast cells. In this study, we analyzed the potency of 2,4-Di-tert-butylphenol (2,4-DTBP), a natural compound, against cancer through computer programs like molecular docking and molecular dynamics (MD) simulations. Molecular docking was conducted with AutoDockTool to evaluate 2,4-DTBP's interaction with critical breast cancer proteins, specifically PIK3CA, ESR1, and PTEN. Binding affinities (kcal/mol) and inhibition constants were evaluated to determine notable protein-ligand interactions. Molecular dynamics (MD) simulations were performed using the CABS-flex webserver to assess the stability of these interactions via RMSF and binding free energy analyses. The study utilized multiple methodologies, including PASS prediction, ADME assessment, toxicity profiling, Lipinski's Rule of Five, and evaluation of anticancer activity. Strong binding affinities of 2,4-DTBP towards important breast cancer proteins were found by the docking results; the binding energy values ranged from -5.25 to -7.86 kcal/mol. Meaningful binding energies of -7.86, -7.68, and -7.36 kcal/mol have been detected for PIK3CA, ESR1, and PTEN, respectively. Protein-ligand complex stability was validated by molecular dynamics simulations, and PTEN exhibited the most stable association because of its higher structural stiffness and lower RMSF values. The 2,4-DTBP demonstrated acceptable standards in every evaluated metric. According to the *in-silico* results, 2,4-DTBP exhibits encouraging potential as a strong inhibitor of significant breast cancer targets, specifically PTEN, PIK3CA, and ESR1, which calls for additional in-vitro and in-vivo studies to further investigate its efficacy in treating cancer.

Keywords: *In-silico* studies, Molecular docking, Molecular dynamics simulation, Breast cancer, Natural product, Anti-cancer lead, ADMET.

Introduction

Breast cancer is a major public health issue globally [1]. It is the second most common cancer and cause of death in women globally [2]. The same goes for Saudi Arabia. In 2020, breast cancer was responsible for 14.2% of all cancers and 29% of cancers in Saudi Arabia, as reported by the Saudi Cancer Registry [3]. This disease has emerged as a significant health burden, with increasingly heterogeneous sets of risk factors [4]. Both endogenous and exogenous factors are implicated in its pathogenesis, rendering it even more challenging to treat clinically [5]. Moreover, conventional therapy regimes, such as chemotherapy [6] and radiotherapy, are also associated with side effects, adding to the treatment burden and compounding additional complications in patient care.

One such complication of conventional breast cancer treatment is the development of multidrug resistance (MDR), which dramatically compromises treatment efficacy and attenuates patient survival rates [7].

Due to these limitations, much research is being directed towards identifying novel therapeutic alternatives. These therapies are intended as primary treatments, adjunct therapies, or methods of prevention of breast cancer to improve patient outcomes [8,9]. The etiology and progression of breast cancer include complex biological processes and systems [10, 11], and numerous biomarkers are

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utilized for diagnosis [12-15]. Targeting these processes with natural bioactive compounds offers a promising method for developing novel therapies [16-18]. Interestingly, more than 50% of the drugs in use today are natural product-derived, highlighting their continued relevance in contemporary drug discovery and therapeutic development [19].

Fungi are rich sources of natural products, and they account for about 47% (33,500 of 70,000) of the bioactive secondary metabolites of microbial origin [20]. About one-third of such fungal compounds are from the genus *Aspergillus and Penicillium* [21]. Among the species of *Aspergillus*, *Aspergillus niger* is a widely researched species comprising many biosynthetic gene clusters (BGCs) with high potential for the production of secondary metabolites like pyranones, alkaloids, cyclopentapeptides, polyketides, and sterols. These compounds exhibit efficacy in an enormous spectrum of activities like antibacterial, anticancer, antioxidant, and antiviral activities [22]. Virtual screening is a popular and potent method for bioactive compound discovery, especially new drug discovery. Virtual screening entails the assessment of large libraries of chemical structures for determining optimal ligand-receptor binding, thus dramatically lowering the temporal and fiscal investments in drug discovery [23]. Several studies have employed virtual screening, more so docking approaches, to investigate different chemical databases. Such endeavors above routinely emphasize the role of physicochemical properties in the identification of new biomolecules [24]. For example, a recent report stressed the application of structural similarity between natural products for the discovery of potential anti-cancer inhibitors based on molecular docking strategies [25]. In the study, molecular docking coupled with molecular dynamics simulations (MDS) was employed to assess the natural molecule 2, 4-DTBP as a good candidate for the design of efficient breast cancer inhibitors.

Material and Methods

Data mining

From previously published work, fifteen bioactive components of *A. niger* strain AK-6 were extracted [26]. The target biologically active compound (2, 4-DTBP)'s 3D structure and canonical SMILES were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/). The protein data bank (https://www.rcsb.org/) provided the 3D structure of breast cancer proteins. The process is depicted in (Fig. 1).

Molecular docking

Ligand preparation

The three-dimensional structures of ligand, and control compound (standard drug) (Fig. 2) were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in SDF format. Open Babel was used to convert the structures from SDF to AutoDock Tools readable PDB file format. AutoDock Tools was used to add hydrogen atoms to the ligand molecules and set root and torsions. The ligands were saved in PDBQT file format.

Proteins collection and preparation

Protein crystal structures were obtained and retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) for the proteins ESR1 (PDB ID: 6V8T) (Fig. 3a), PTEN (PDBID: 1D5R) (Fig. 3b), and PIK3CA (PDBID: 3HIZ) (Fig. 3c). The proteins were prepared for docking using AutoDockTools (v.1.5.7). SwissModeller was used to help remodel proteins that had missing residues [27]. GalaxyRefine was used to further improve the models. Before docking, PyMOL (version 1.7.4.5) was used to eliminate all water molecules and heteroatoms from the proteins [28]. Both the drug and protein structures are eventually transformed to PDBQT format in docking research [29].

Molecular docking

In computer science, molecular docking is a key approach to the prediction of drug candidate pharmacodynamic activity through the determination of their binding orientations and assessing their interactions against receptor sites [30]. The molecular docking process was carried out based on a predefined docking protocol through AutoDock Tools. Proteins were processed as rigid receptors, while the bioactive molecule was designated the ligand [31]. The target search area for molecular docking is given in Table 1. The protein and bioactive compound interactions were then visualized with the aid of Discovery Studio 2021 [32]. The docking protocol validity was ensured by replicate processes for the compound 100 docking poses were generated.

Molecular dynamics simulation

The cabs-flex web server (http://biocomp.chem.uw.edu.pl/CABSflex2) was utilized to assess the molecular dynamics simulation and make sure that the docking results had the greatest possible potential for acting as inhibitors. Water molecules were one of the key elements to take into account in molecular dynamics simulations, which provide better binding confirmations for the docked complex and closely acknowledge the physiological environment conditions, in order to assess the stability of the protein-ligand complexes in comparison to molecular docking. Based on the previously reported docking results, the complexes were chosen for molecular dynamics simulations. Determining how the docked complexes interacted was essential to figuring out how they worked as inhibitors; this was accomplished by looking at the root mean square fluctuation (RMSF) for the selected complexes,

which then offers details about the diversity, which subsequently provides information regarding the diversity and mobility of every amino acid residue in the protein. The RMSF analysis results were downloaded from the web server and subsequently visualized using Microsoft Excel.

In-silico prediction of activity spectra for substances (PASS)

Anticancer potential of the natural compound was predicted through PASS (Prediction of Activity Spectra for Substances) software. The software estimates the probable activity spectrum of the compound against various activities on the basis of its probable activity (Pa) and probable inactivity (Pi) [33]. Pa and Pi values lie in the range of 0.000 to 1.000, and only the activity where Pa > Pi is treated as possible for the compound. A Pa value above 0.7 means a high probability of experimental pharmacological activity, while a value of 0.5 to 0.7 indicates a moderate possibility. But if Pa is below 0.5, the probability of experimentally confirming the activity is low; although it can be an indicator of the potential to discover a new compound [34].

Lipinski's Rule of Five (RO5)

The designed compound drawn through ChemDraw software were subjected to initial screening under Lipinski's rule of 5 by Molinspiration server (https://www.molinspiration.com/). This rule is used to determine whether a chemical compound affects certain biological activities or pharmacological effects that may possibly render it a viable oral drug in humans [35].

Drug-likeness and pharmacokinetics prediction

We employed SwissADME (http://www.swissadme.ch/) [36] and pkCSM (http://structure.bioc.cam.ac.uk/pkcsm) [37] to predict drug-likeness and pharmacokinetic properties. The predictions were for describing the physicochemical properties and metabolic activities of the bioactive compound.

Anticancer sensitivity prediction: The anticancer potential of the filtered compounds was evaluated using the PaccMann webserver, a machine learning-based compound sensitivity prediction platform (https://huggingface.co/spaces/jannisborn/paccmann)

The webserver utilizes molecular structure-activity relationships and transcriptomic data of cancer cell lines in order to forecast the hypothetical activity of compounds.

The chemical structures under investigation were compared against a broad panel of cancer cell lines, and sensitivity metrics like IC50 values were established to evaluate their drug like activity. The ensuing data were examined by utilizing the CCLE dataset. The Cancer Cell Line Encyclopedia (CCLE) is an extensive catalog of human cancer cell lines representing diverse tumor types [38].

Results

Molecular docking

Binding energy of the ligand-protein complex.

Docking studies play a key role in predicting high-affinity binders as well as virtual screening of compound libraries [39]. The compound (2,4-DTBP) possessed consistently good binding energies for all the target proteins ranging from -5.25 to -7.86 kcal/mol. Significantly, the highest binding affinities of the compound against ESR1 (6V8T), PIK3CA (3HIZ), and PTEN (1D5R) were observed when compared to the other proteins that were studied. Among all the compounds screened, 2,4-DTBP is found to have the highest binding energies, with significant values of -7.86 kcal/mol for PIK3CA (3HIZ), -7.68 kcal/mol for ESR1 (6V8T), and -7.36 kcal/mol for PTEN (1D5R) as listed in Table 2.

*Interaction and binding affinity of compounds towards ESR1(6V8T) protein***.** In this study, the compound 2,4-DTBP and Tamoxifen, as a control, exhibited binding affinities of -7.68 and -11.53 kcal/mol, respectively, as shown in Table 3. Among the two compounds, the highest binding energy was displayed by Tamoxifen, followed by 2,4-DTBP, suggesting strong interactions with the ESR1 protein. The assessment of noncovalent interactions, as carried out using Discovery Studio Software, indicated that all compounds displayed hydrogen and hydrophobic bonds, which not only enhanced the binding affinity but also improved binding specificity. The compound, 2,4-DTBP exerted one hydrogen bond with Leu346, several hydrophobic interactions withLeu525,Leu428,Ile424,Met388, Leu384, Glu353, Ala350, Leu391,Leu387,Arg394, Leu349, and an aromatic interaction with Phe404; and for Tamoxifen no hydrogen bond founded. These hydrogen bonds promoted the nonbond interactions. Besides hydrogen bonding interactions, hydrophobic interactions frequently play a significant role in facilitating the stable and efficient binding of ligands to their target counterparts. In the present study, we recognized several hydrophobic interactions and specific residues such as Gly521, Met421, Ile424, Met388, Leu384, Leu428, Leu391, Phe404, Leu387, Glu353, Trp383, Met343, Thr347, Val533, Lys531, Met528, Cys530, and Ala350; and aromatic interactions with Leu346 and Leu349; and carbon-hydrogen bonds with Leu525 interacting with Tamoxifen (see Table 3). The 2D and 3D representations of nonbond interactions are shown in Fig. 4.

The inhibition constant is a reflection of the binding affinity of a protein and a ligand (inhibitor). The lower the value, the stronger the binding interaction and the more inhibition of protein

activity. A higher value reflects a weaker binding affinity and less efficient inhibition.

The inhibition constants of '2,4-DTBP- ESR1' complex and 'Tamoxifen - ESR1' complex were determined as 2.33 μM and 3.55 nM, respectively (see Table 3). Both Tamoxifen and 2,4-DTBP have high affinities to bind with ESR1, suggesting high inhibitory power, with a comparatively higher binding affinity in the case of Tamoxifen.

Interaction and binding affinity of compounds towards PIK3CA(3HIZ)protein

The binding energies of 2,4-DTBP and Alpelisib (Control) are -7.86and -12.77kcal/mol (Table 4), respectively. Compared with the ESR1 protein, the compound showed a slightly better binding against PIK3CA(3HIZ).

The analysis identified two hydrogen bonds (Table 4) with amino residue GLN859 in the 2,4-DTBPcompound; and four hydrogen bonds with Phe934, Asp933, Val851, and Gln859 in Alpelisib. In 2,4-DTBP, some hydrophobic interactions such as pi-alkyl, alkyl, van der waals, and pi-sigma bonds were observed with Phe930, Tyr836, Leu834, Ile848, Ile932, Val851, His931, Tyr904, Ile817, Thr813, Gly935, Asp810, Asp933, and Leu814.

On the other hand, Alpelisib created hydrophobic bonds with Met772, Leu814, Asn853, Cys838, Ile932, His931, Ile848, Lys802, Phe930, Ile800, Arg852, Trp780, Met922, Val850, Ser854, and Thr856 residues; and carbon-hydrogen bond with Asp933,His855,and Val851. The 2D and 3D structures of nonbond interactions are shown in (Fig.5).

Inhibition constants of '2,4-DTBP-PIK3CA' and 'Alpelisib-PIK3CA' complexes were found to be 1.73 μM and 433.90 pM, respectively (Table 4). Among them, the complex with inhibition constant 433.90 pM has the strongest binding affinity, and the complex with inhibition constant 1.73 μM has the weakest binding affinity.

Interaction and binding affinity of compounds towards PTEN (1D5R) protein. The binding affinities of the compounds being researched, i.e., 2,4-DTBP and Capivasertib (Control), are measured at -7.36 and -12.04 kcal/mol, respectively (refer to Table 5). Notably, the Control's binding energy was more favorable in comparison to the binding energy measured for 2,4-DTBP against the ESR1 protein. In comparison to the above-mentioned proteins, the 2,4-DTBP compound exhibited the worst binding affinity toward PTEN, with a measure of -7.36 kcal/mol. In this study, the nonbonded interactions were conducted with the Discovery Studio Software. Our result indicated that both drugs exhibited effective interactions with amino acid residues, and both of them possessed hydrogen bonding (see Table 5) with amino residues (see Fig. 6), with one instance of 2,4-DTBP with Gln171, and six instances with Asp162, Gln171, Asp92, and Lys125 for Capivasertib. Hydrogen bonds facilitated the nonbonded interactions.

Besides the H-bond interactions, hydrophobic interactions generally play an important role in ensuring the ligand binds effectively and stably to its target, and in this study, we observed some hydrophobic interactions (Table 5) and the residues like Lys128, His93, Arg130, and Ala126 with 2,4-DTBP; and van der waals bond with Val166, Thr167, Gly165,Ile168,Gly129,Cys124, Asp92, Lys125 and Gly127; and hydrophobic interactions with Lys164,His93,Ala126,and Val45; and van der waals bond with Val166,Ile168,Gly129,Gly127,Arg130, and Cys124 for Capivasertib. The 2D and 3D representations of nonbond interactions are given in Fig. 6.

Inhibition constants of complexes '2,4-DTBP - PTEN' and 'Capivasertib - PTEN' were 4.05 μM and 1.49 nM, respectively (Table 5). Though the maximum binding affinity towards PTEN was observed for Capivasertib, a high degree of interaction was also displayed by compound 2,4-DTBP

A computerized technology for predicting the shape of ligand-receptor complexes, molecular docking is becoming more and more acknowledged as a useful tool in drug research [40]. Stronger binding of the inhibitor to its target protein is indicated by a greater negative binding energy (BE) value, which quantifies the degree of interaction between the ligand and the protein complex [41, 42]. As a result, a reduced rate of ligand detachment from the target protein is expected, indicating that the ligand may have a longer half-life [43]. In light of this, the hits (2,4-DTBP) in this investigation showed high binding (lower BE) to all target proteins, particularly ESR1, PIK3CA, and PTEN, indicating that they may be able to inhibit these target proteins and function as promising and potent anticancer medicines.

Molecular dynamics simulation

The docking results were subsequently validated and supplemented by Molecular Dynamics (MD) simulations, which shed light on the stability and dynamic behavior of the ligand-protein interactions across time. The compound-complex with the ESR1 protein shows good amino acid residue fluctuation values, average below 3 Å, which are similar to those seen in the protein-drug complex, according to the results of the Molecular Dynamics analysis. The 2,4-DTBP compound exhibits good stability when binding to ESR1, but there is an increase in fluctuation at residues 325–345. A high RMSF value (above 5 Å) indicates that this region is especially vulnerable to fluctuations, which could have an impact on the dynamics of the interaction. Consistent

binding stability is shown by the following reduction and stabilization seen spanning residues 360–530 indicating consistent binding stability. A similar pattern is observed with Tamoxifen, which displays a stability profile comparable to the protein-drug complex but with minor fluctuations at residues 453-467 and 530-544. These findings suggest that both compounds exhibit stable binding to ESR1, with localized flexibility influencing specific interaction dynamics (Fig. 7).

For the PTEN protein, the RMSF plot indicates that the 2,4-DTBP-PTEN complex exhibits similar fluctuation patterns when compared to Capivasertib (positive control for PTEN). Both complexes predominantly show RMSF values below 3 Å, suggesting stable interactions with the PTEN protein. However, residues in the range of 280-295 display higher fluctuations in both complexes, with slightly elevated RMSF values observed in the 2,4-DTBP complex. This indicates a localized reduction in stability in that region for 2,4-DTBP compared to Capivasertib. Despite these differences, the overall RMSF analysis demonstrates that 2,4-DTBP forms interactions with PTEN that are largely comparable to those of the control, Capivasertib, with some variations in specific residue regions (Fig.8).

For the PI3KCA protein, the PI3KCA-2,4-DTBP complex demonstrates favorable fluctuations compared to the PI3KCA-Alpelisib complex. The RMSF plot indicates that 2,4-DTBP maintains overall stability with lower fluctuations across most residues. Notable fluctuations are observed in the PI3KCA-2,4-DTBP complex at residues 343, 421, and 975, but the RMSF values remain below 4 Å, suggesting strong potential for stable interactions between PI3KCA and 2,4-DTBP.

Conversely, the PI3KCA-Alpelisib complex exhibits increased fluctuations at multiple residues, with several exceeding 4 Å, including at residues 341 and 761. These higher fluctuations imply reduced stability in the interaction of PI3KCA with Alpelisib when compared to 2,4-DTBP. Overall, the data suggests that the PI3KCA-2,4-DTBP complex demonstrates better stability and lower flexibility, reinforcing the favorable binding potential of 2,4-DTBP with PI3KCA (Fig. 9).

Important first clues regarding the stability of the molecule as a ligand while interacting with the target protein's amino acid residues can be obtained by evaluating molecular docking and then running molecular dynamics simulations and monitoring RMSF values. To provide a more thorough understanding of whether bioactive compounds from natural sources can form more stable interactions, especially at the active site, thereby establishing their potential to act as effective drug candidates, a targeted analysis of the amino acid residues at the active site may be carried out.

Estimation of activity spectra for substances (PASS)

The Pass Online webserver was used to evaluate the possible anticancer effects of (2,4-DTBP). This is a potent instrument that can forecast hundreds of biological processes, including a compound's possible anticancer effects. For a particular compound, only actions with Pa > Pi were deemed possible. A high chance of experimental pharmacological effect was indicated by Pa > 0.7, whilst a moderate probability of experimental pharmacological action was suggested by Pa between 0.5 and 0.7. The likelihood of pharmacological activity was minimal if Pa was less than 0.5 [44]. The results of the anticipated actions for 2,4-DTBP are displayed in Table 6. With Pa values of 0.525 and 0.449, respectively, exceeding Pi, the compound demonstrated a moderate likelihood of antioxidant and antimutagenic activity, suggesting a plausible chance of pharmacological action.

Additionally, the chemical demonstrated promise as a caspase 8 stimulant (Pa = 0.394) and TP53 expression enhancer (Pa = 0.506), indicating potential anticancer processes through apoptosis regulation. Additional activities showed moderate probabilities, including apoptosis agonist (Pa = 0.377) and anticancer-related activities such anticarcinogenic (Pa = 0.315) and cancer-associated diseases therapy (Pa = 0.317). However, there was limited potential in areas such as antileukemic, antimetastatic, and antineoplastic activity for particular cancer types, as evidenced by lower probability (Pa < 0.5).

Evaluation of Lipinski's Rule of Five (RO5)

Lipinski's rule of five was used to make the medication easily accessible. According to Lipinski's rule, which describes the requirements for oral activity, a drug must have a molecular mass of less than 500 Da, high lipophilicity (LogP) of less than 5, hydrogen bond donors of less than 5, hydrogen bond acceptors of less than 10, and molar refractivity between 40 and 130.

Table 7 displays the findings of the molecular characteristics and drug-likeness of 2,4-DTBP. 2,4-DTBP meets all of the requirements outlined in Lipinski's rule of five, indicating that, given its physicochemical characteristics, this compound would be appropriate for use as an effective drug in for human consumption.

Drug-likeness and pharmacokinetics parameters of the bioactive compound

Table 8 displays the ADMET prediction result from the SwissADME webserver and pkCSM. The anticipated ADME outcomes will help create leads with better drug-like properties. With a value of -3.924, the pharmacokinetics profile of (2,4-DTBP) indicates restricted solubility in aqueous settings, as can be shown in Table 8. In contrast, 2,4-DTBP

exhibits a comparatively high intestinal absorption rate in humans (92.034%), indicating good bioavailability when taken orally. 2,4-DTBP has a high intestinal absorption rate, but its poor central nervous system (CNS) penetration (log PS of -0.848) and blood-brain barrier (BBB) permeability (log BB of 0.478) suggest that it is doubtful to reach therapeutic dosages in the central nervous system.

Its low brain permeability is further supported by the fact that the chemical is neither a substrate nor an inhibitor of P-glycoproteins. With a proportion unbound in humans of 0.044, the volume of distribution (Vdss) is 0.611 log L/kg, suggesting a limited systemic distribution. There is little chance of drug-drug interactions via cytochrome P450 pathways because 2,4-DTBP does not function as a substrate or inhibitor for CYP2D6, CYP3A4, CYP1A2, or CYP2C9 in terms of metabolism. Despite not being a substrate for renal organic cation transporter 2 (OCT2), its excretion profile reveals a moderate overall removal rate of 0.759 log mL/min/kg, suggesting that alternative pathways might be involved in its renal excretion. 2,4-DTBP exhibits no hepatotoxicity, AMES toxicity, or hERG inhibition (both I and II), indicating a good safety profile. It does, however, show some degree of toxicity in *T. Pyriformis* (1.572 log µg/L) and Minnow toxicity (log mM of 0.006), as well as the ability to cause skin sensitization. The potential efficacy of 2,4-DTBP for therapeutic uses outside the central nervous system is indicated by its overall low water solubility, strong intestine absorption, restricted CNS permeability, and relatively modest distribution volume. Its appropriateness as a therapeutic candidate with controllable toxicity concerns is further supported by its advantageous metabolism and excretion profiles and low probability of critical interactions between medications.

Cell line studies

In order to predict anticancer activity, the PaccMann database was utilized by implementing the structure-activity relationship algorithm. The analogs were placed to screen over a panel of breast cancer cell lines to determine their anticancer activity [45]. The most effective was the analog against the cell line MCF7 with an IC_{50} of 2.579, followed by BT474 with an IC_{50} of 2.929 and MDAMB23 with an IC_{50} of 2.844. They were more effective compared to other cell lines such as MDAMB45, where the IC_{50} was 3.462, and HCC1937, where the IC_{50} was 3.868. Remarkably, all the analogs obtained exhibited improved activity over the control compounds, as indicated by their lower IC_{50} values. In addition, both the epistemic and aleatoric confidence metrics were in line with the validity of the observations, with confidence levels all above 0.93.More importantly, BT549 and BT474 cell lines possessed the highest confidence levels in the CCLE dataset, with epistemic and aleatoric confidence levels of up to 0.975 and 0.957, respectively. This study recognizes the improved anticancer activity of the derived analogs, especially in the breast cancer cell lines, as supported by their heightened activity and reproducible predictive parameters.

Discussion

Breast cancer is a major and life-threatening disease among women globally [46]. Various chemotherapeutic agents have been developed and used since the past to treat the advancement of breast cancer by affecting various molecular targets of tumor advancement and metastasis. Although the agents have been seen to exhibit remarkable efficacy, their administration has been accompanied by harmful side effects and drug resistance [47]. In the current investigation, we have investigated various treatment approaches with the natural compounds isolated from *A. niger* using *insilico* techniques to analyze their anticancer activity. Thus, for the identification of the putative anticancer molecules, we utilized the natural compound isolated from *A. niger* with literature support [26]. Then, virtual screening was performed with target receptors of breast cancer. Various researches have emphasized the capability of receptor-based virtual screening to lead to the discovery of novel anticancer drugs [48]. For instance, Muthiah et al. utilized computational methods to discover therapeutic drugs that act against specific receptors on cancer cells [49].

In the same way, Hart et al. utilized molecular docking and dynamic simulation techniques to screen potential inhibitors of cancer treatment and illustrated the consistency of computational tools in drug discovery [50].

These structure-based virtual screening and molecular docking approaches have been efficient in the discovery of highly active molecules that may act as leads in the design and development of anticancer molecules [51].

Molecular docking and simulation studies were used for the prediction of the binding efficacy of ligands with target macromolecules in breast cancer mechanisms. Molecular docking and molecular dynamic results based on which we suggest that the bioactive compound of *A. niger*, can display a potential anticancer activity and found that this compound was showing better activity against the target PIK3CA, ESR1, and PTEN proteins. Our results showed high binding affinities of 2,4-DTBP with key breast cancer proteins with score ranging from -5.25 to -7.86 kcal/mol, suggesting its potential as a candidate therapeutic molecule.

Particularly, PIK3CA, ESR1, and PTEN binding energies were -7.86, -7.68, and -7.36 kcal/mol, respectively, indicating strong interaction with these key targets implicated in cancerogenesis.

The high binding affinities generated indicate that 2,4-DTBP possesses the potency to significantly inhibit cancer-related pathways via binding interactions with key residues of these protein targets. These results warrant its therapeutic application as a potent breast cancer inhibitor, particularly given its competitive binding to other reported bioactive molecules.

Furthermore, the molecular interactions such as hydrogen bonding, hydrophobic interactions, and van der Waals forces reflect the stability and specificity of 2,4-DTBP binding, upholding its therapeutic potential. To gain insight into the ligand and complex structural alterations, we have performed Molecular Dynamics (MD) simulations.

We concluded in our research that our compound (2,4-DTBP) was more stable with the PTEN protein. This suggests that this compound can be used as effective modulators of PTEN activity, thereby targeting the PI3K/AKT signalling pathway. A series of in vitro studies have confirmed our *in-silico* findings, which reveal that (2,4-DTBP) possesses anticancer activity[52-57].These reports have shown that 2,4-DTBP has anticancer activity against a broad panel of cancer cell lines. In the present study, we are reporting for the first time that (2,4-DTBP) possesses significant anticancer activity against breast cancer by effectively interacting with key breast cancer proteins such as PIK3CA, ESR1, and PTEN, through binding energy and stability analysis via dynamic simulation. Thus, our results suggest that these compounds can also be utilized as potential candidates for anticancer therapy. In this analysis, the PTEN-2,4-DTBP complex was the most stably interacting with the least fluctuations between residues, pointing to rigid and strong binding. ESR1-2,4-DTBP was moderately stable with some flexibility, while PI3KCA-2,4-DTBP was the most flexible, pointing to the least stable interaction. Regardless of these differences, the complexes all had favorable binding energy, pointing to therapeutic potential. Aside from the study above, we also verified drug-likeness using Lipinski's rule of 5. Rule of five (RO5) is a rule of thumb used to determine drug-likeness or to verify if a chemical compound having a specific pharmacological or biological activity possesses chemical properties that would make it a likely orally active drug in humans. Based on the parameters that most "drug-like" molecules possess LogP ≤ 5, molecular weight ≤ 500, hydrogen bond acceptors ≤ 10, and hydrogen bond donor's ≤ 5. Additional parameters are filters such as Molar Refractivity (70 – 110). Our results indicate that 2,4-DTBP satisfies all the requirements stipulated by Lipinski's rule of 5, which means that this compound can be used as an active drug in humans based on its physicochemical properties.

According to the PASS prediction results, 2,4-DTBP has a moderate activity towards anticancer activity with possible pharmacological effects in antioxidant, anti-mutagenic activities, and apoptosis regulation, implying its potential as a promising candidate for cancer-related applications. We have also made a comparison of the ADMET properties of the hit ligand. We have compared these properties because these compounds can be drug candidates for diseases like cancer.

The ADMET prediction profile for 2,4-DTBP indicates that, even though the compound is poorly water-soluble and has poor CNS permeability, it is highly intestinal absorptive and has a good metabolism profile, making it a good drug candidate with acceptable toxicity and adequate bioavailability for non-CNS indications. Augmenting these observations, cell line investigations highlight the outstanding anticancer potential of its derived analogs, with remarkable activity against MCF7, BT474, and MDAMB23 breast cancer cell lines, wherein low IC_{50} values and high confidence parameters reflect their higher efficacy and reliability compared to control compounds.

Therefore, based on these properties, this compound can be considered a lead compound for further optimization and development. Moreover, its notable properties provide promising potential in drug development versus breast cancer. On this basis, further research can continue to optimize this compound to create potent natural drugs versus this disease.

Conclusions

Prior to experimental phase, *insilico* experiments can be time and cost-saving. Molecular docking and molecular dynamics simulation were performed in this research to analyze the potential of natural product obtained from *Aspergillus sp*. as a source of potential anticancer compounds. Further, the most active complexes of docking results were submitted for simulation to confirm the stability of complexes. Based on the molecular docking result, it is concluded that compound 2,4-DTBP was docked successfully with all target proteins with docking scores in the range of (-5.25 to -7.86 kcal/mol). The highest among these docking scores were obtained with 2,4-DTBP against PIK3CA, ESR1, and PTEN proteins, respectively. Based on RMSF analysis, the PTEN-2,4-DTBP complex showed the most stable interaction with fewer fluctuations among residues and indicating a strong and rigid binding. Complex ESR1-2,4-DTBP was quite stable with flexibility, indicated by the presence of a single notable peak and generally stable regions. Complex PI3KCA-2,4-DTBP was the most flexible with drastic fluctuations in numerous regions, representing the least stable of the three complexes. Docking studies showed high binding affinity for PTEN-2,4-DTBP complex and molecular dynamics simulation confirmed its stability with insignificant residue fluctuations in the

RMSF study. Following the evaluation of molecular docking and molecular dynamics simulations (MDS), the overall pharmacological profile, which includes PASS predictions, ADMET properties, cell line studies, and related parameters, was thoroughly evaluated and served as a platform for the design of promising drug candidates. These findings of this study underscore the tremendous therapeutic promise of the hit compound identified for treating breast cancer, and they form a great basis for continued research. Additional in vitro confirmations would be needed to establish its effectiveness, while in vivo and associated studies will be important to advance it as a drug candidate.

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Conflicts of interest: All Authors declare that there is no conflict of interest.

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Author`s contributions: All authors shared equally in conceptualization, study design, sample collection, and Ultrasonography, Data analyses, Manuscript drafting, and Manuscript finalization.

TABLE 1. The search space used in this study

Protein ID	Spacing	Grid Box Center (Axis)		
		X	Y	Z
ESR1(6V8T)	0.475	77.8651	4.3972	121.5798
PTEN(1D5R)	0.481	33.1987	87.4018	25.8243
PIK3CA(3HIZ)	0.503	59.122	56.417	111.425

TABLE 2. Docking results for the bioactive compound (2,4-DTBP) against target proteins

S. No.	Target Proteins	Total Free Binding Energies (kcal/mol) for (2,4-DTBP)
1	BRCA1 (1T15)	-5.40
2	BRCA2 (3EU7)	-6.67
3	CYP19A1 (3S79)	-7.04
4	CHEK2 (2CN5)	-5.99
5	PTEN (1D5R)	-7.36
6	PIK3CA (3HIZ)	-7.86
7	ESR1 (6V8T)	-7.68
8	ABCG2 (6FFC)	-6.55
9	PDPK1 (3H9O)	-7.17
10	TP53 (3dcy)	-6.46
11	TP53 (4mzi)	-5.25

TABLE 3. Docking results for the best binding affinity with ESR1 protein

Potential drug	Binding affinity (kcal/mol)	No. of H-bond	Inhibition constant, Ki (µM/ nM/ pM)	Residues
2,4-DTBP	-7.68	1	2.33 uM	LEU525,LEU428,ILE424,MET388.LEU384,GLU 353,ALA350,PHE404,LEU391,LEU387,ARG394, LEU346,LEU349
Tamoxifen*	-11.53	0	3.55 nM	GLY521,MET421,ILE424,MET388,LEU384,LEU 428,LEU391,LEU349,PHE404,LEU387,GLU353, TRP383,LEU525,MET343,THR347,VAL533,LYS 531,MET528,CYS530,ALA350,LEU346

Estimated Inhibition Constant, Ki = nM; nanomolar, uM; micromolar, pM; picomolar.
* Positive control for ESR1 proteins

TABLE 4. Docking results for the best binding affinity with PIK3CA (3HIZ) protein.

Potential drug	Binding affinity (kcal/mol)	No. of H-bond	Inhibition constant, Ki (µM/ nM/ pM)	Residues
(2,4-DTBP)	-7.86	1	1.73 uM	PHE930,TYR836,LEU834,ILE848,ILE932,VAL85 1,HIS931,TYR904,ILE817,THR813,PHE934,GLY9 35,ASP810,ASP933,LEU814
Alpelisib*	-12.77	4	433.90 pM	MET772,LEU814,ASN853,GLY837,ASP933,CYS 838,ILE932,HIS931,ILE848,LYS802,TYR836,GL U849,PHE930,ILE800,ARG852,TRP780 MET922,VAL850,HIS855,SER854,GLN859,VAL8 51,THR856

* Positive control for PIK3CA protein

TABLE 5. Docking results for the best binding affinity with PTEN (1D5R) protein

Potential drug	Binding affinity (kcal/mol)	No. of H-bond	Inhibition constant, Ki (μM/ nM/ pM)	Residues
(2,4-DTBP)	-7.36	1	4.05 uM	GLN171, LYS128, HIS93, ARG130,ALA126,VAL166, THR167, GLY165,ILE168,GLY 129,CYS124, ASP92, LYS125, GLY127
Capivaserti*	-12 .04	6	1.49 nM	ASP162,LYS164,VAL166,LYS128,THR167,ILE168,GLN171,LYS125,GLY129,GLY127,ASP92,GLY165,HIS93,ALA126,VAL45,ARG130,CYS124

* Positive control for PTENproteins

TABLE 6. The predicted probabilities of biological Activity for the bioactive compound by PASS online server

Bioactive compound	Biological Activities	Pa	Pi
(2,4-DTBP)	Antioxidant	0,525	0,006
	Antimutagenic	0,449	0,018
	TP53 expression enhancer	0,506	0,087
	Caspase 8 stimulant	0,394	0,056
	Apoptosis agonist	0,377	0,084
	Apoptosis antagonist	0,213	0,102
	Anticarcinogenic	0,315	0,053
	Cancer associated disorders treatment	0,317	0,074
	Myc inhibitor	0,288	0,106
	Antileukemic	0,130	0,123
	Antimetastatic	0,213	0,143
	Antimutagenic	0,449	0,018
	Antineoplastic (bladder cancer)	0,165	0,097
	Antineoplastic (bone cancer)	0,199	0,160
	Antineoplastic (cervical cancer)	0,103	0,095
	Antineoplastic (squamous cell carcinoma)	0,133	0,043
	Aromatase inhibitor	0,065	0,025
	Bcl2 antagonist	0,138	0,023
	Breast cancer-resistant protein inhibitor	0,083	0,032

Pa = probability of being active; Pi = probability of being inactive.

TABLE 7. Lipinski's rule of 5 of the identified ligand

Ligands	Mass	Hydrogen bond donor	Hydrogen bond acceptors	LOGP	Molar Refractivity
(2,4-DTBP)	185.000000	1	1	0.581060	47.620800

TABLE 8. Predicted pharmacokinetics parameters of 2,4-DTBP

Properties	Parameters	(2,4-DTBP)
Absorption	Water solubility (log mol/L)	-3.924
	Caco2 permeability (log Papp in 10^{-6} cm/s)	1.666
	Intestinal absorption (human) (% Absorbed)	92.034
	(skin permeation) (Log Kp)	-2.301
	P-glycoprotein substrate	No
	P-glycoprotein I inhibitor	No
	P-glycoprotein II inhibitor	No
Distribution	BBB permeability (log BB)	0.478
	CNS permeation (Log PS)	-0.848
	VDss (human) (log L/kg)	0.611
	Fraction unbound (human) (Fu)	0.044
Metabolism	CYP2D6 substrate	No
	CYP3A4 substrate	Yes
	CYP1A2 inhibitor	Yes
	CYP2C19 inhibitor	No
	CYP2C9 inhibitor	No
	CYP2D6 inhibitor	No
	CYP3A4 inhibitor	No
Excretion	Total Clearance (log mL/min/kg)	0.759
	Renal OCT2 substrate	No
Toxicity	AMES toxicity	No
	Max. tolerated dose (human) (log mg/kg/day)	0.42
	hERG I inhibitor	No
	hERG II inhibitor	No
	Oral Rat Acute Toxicity (LD50)(mol/kg)	2.351
	Oral Rat Chronic Toxicity (LOAEL)(log mg/kg_bw/day)	1.696
	Hepatotoxicity	No
	Skin Sensitization	Yes
	T. Pyriformis toxicity (log ug/L)	1.572
	Minnow toxicity(log mM)	0.006

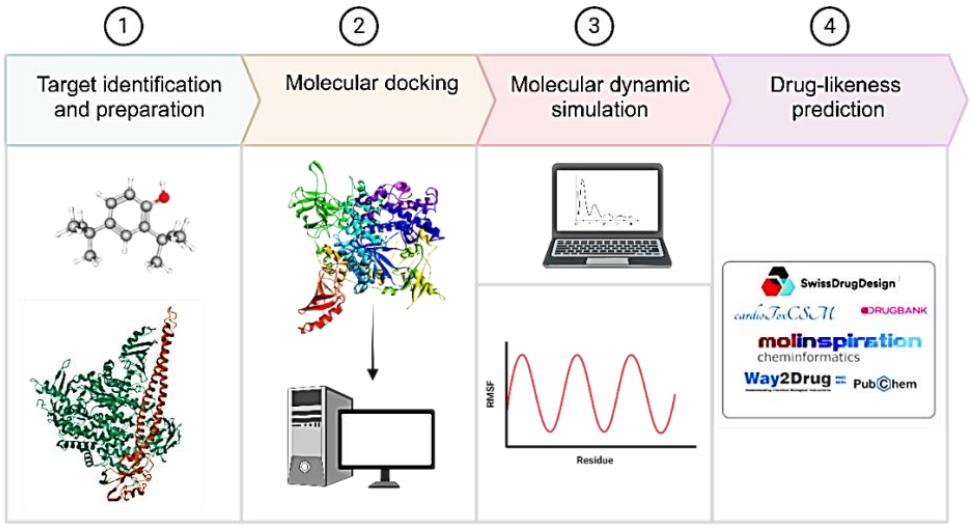
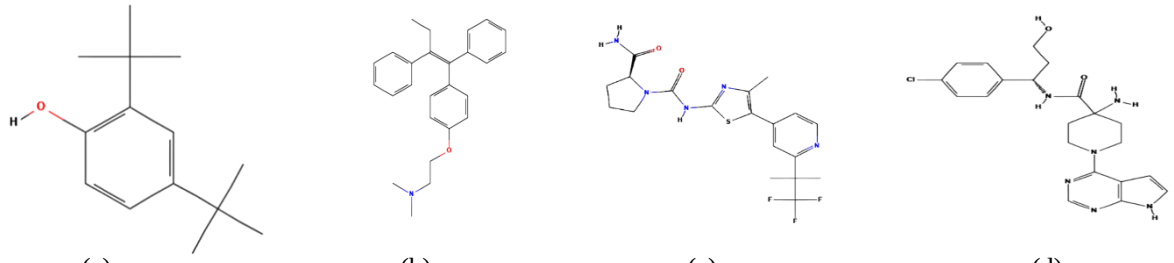


Fig. 1. The workflow



(a) (b) (c) (d)

Fig. 2. The chemical structure of the screened compounds (a) 2,4-DTBP, (b) Tamoxifen, (c) Alpelisib, and (d) Capivasertib.

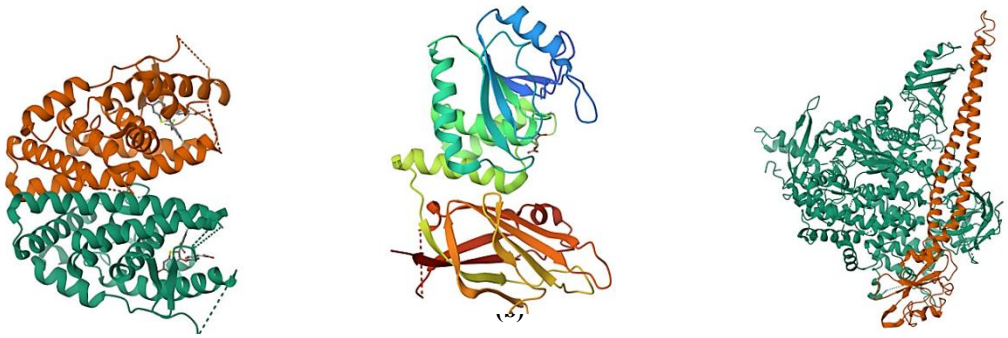
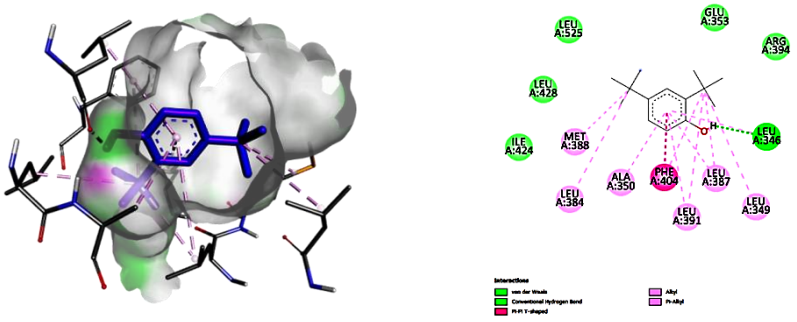
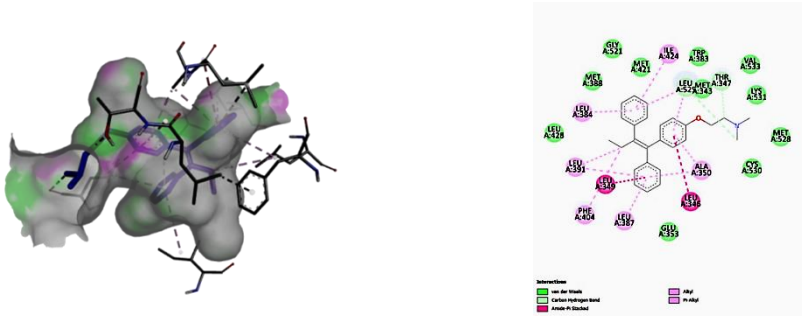


Fig. 3. The three-dimensional structure of (a) ESR1 (6V8T), (b) PTEN (1D5R), and (c) PIK3CA (3HIZ).

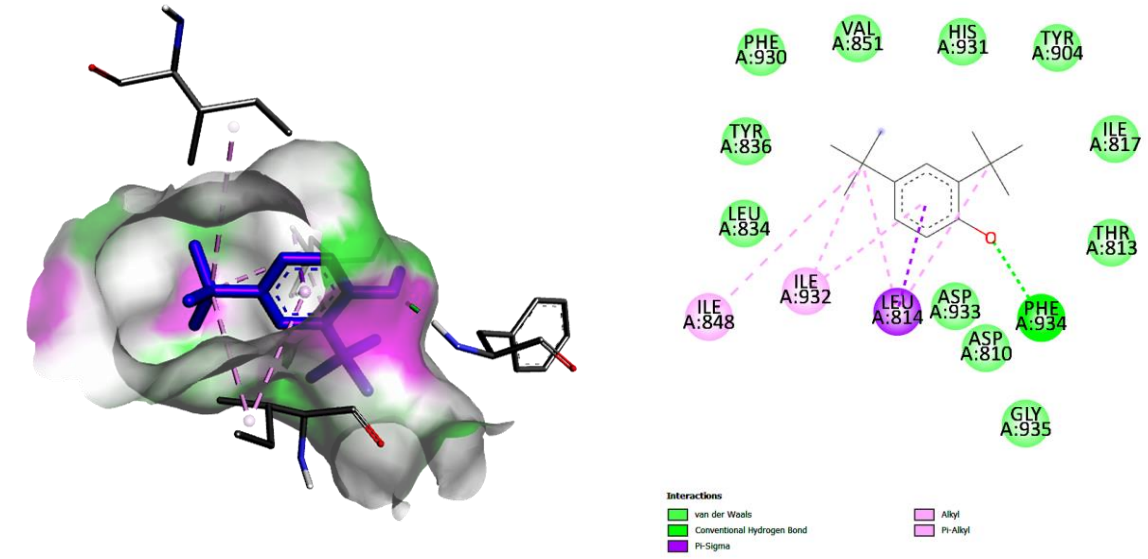


(a)

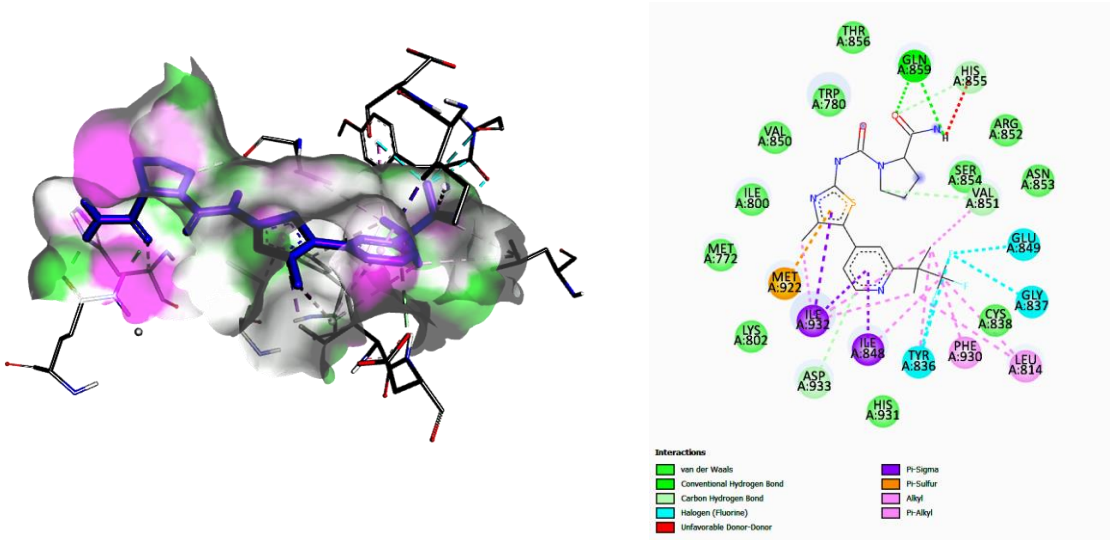


(b)

Fig. 4. The docking results for the screened compounds, including (a) 2,4-DTBPand (b) Tamoxifen, with the ESR1 (PDB: 6V8T) protein. Different color codes represent the types of residual interactions.

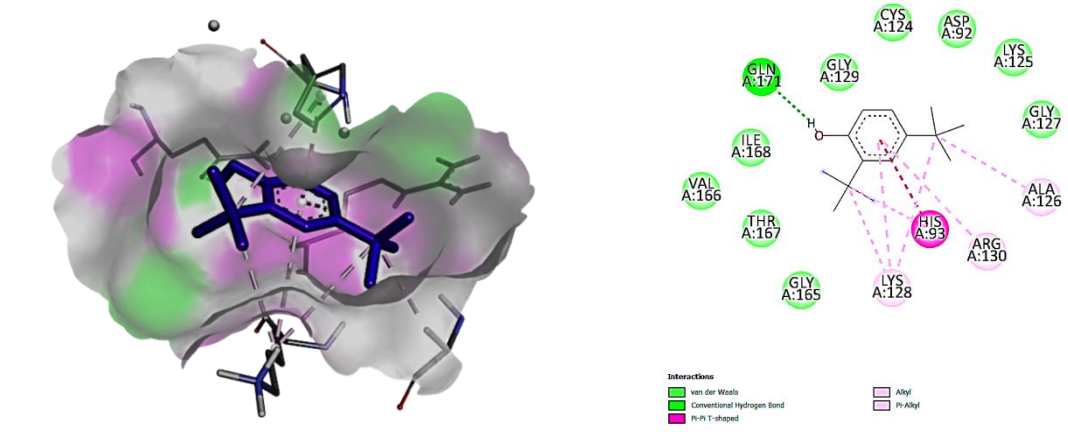


(a)

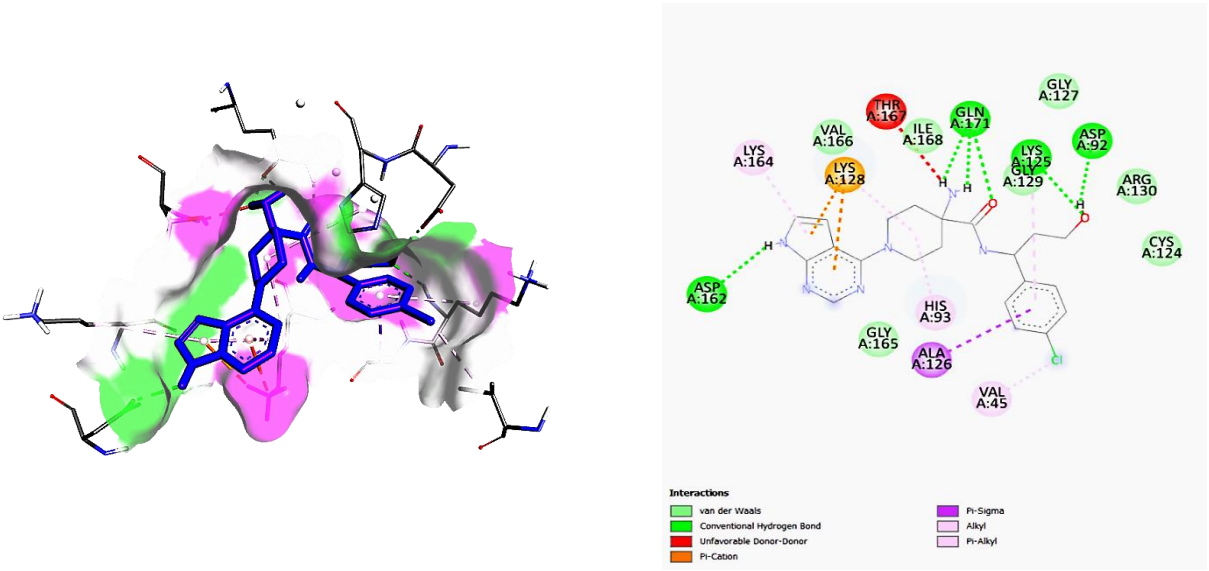


(b)

Fig. 5. The docking results for screened compounds, including (a) 2,4-DTBPand (b) Alpelisib, with the PIK3CA (PDB: 3HIZ) protein.



(a)



(b)

Fig. 6. The docking results for screened compounds, including (a) 2,4-DTBPand (b) Capivasertib, with the PTEN (PDB: 1D5R) protein.

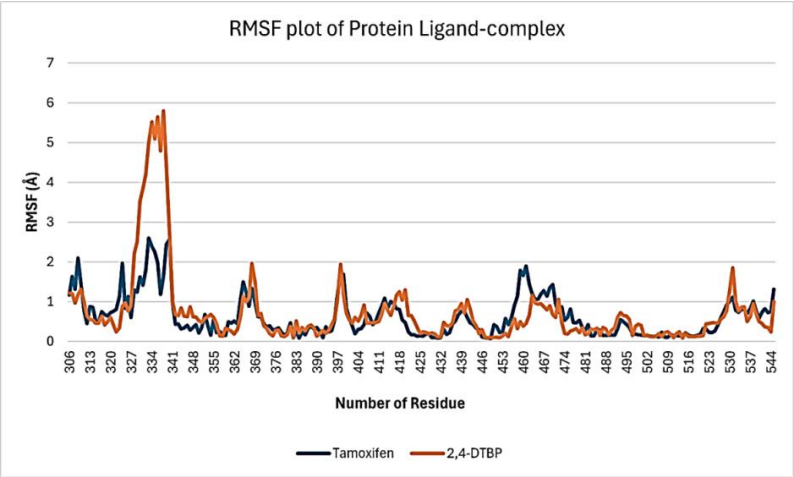


Fig. 7. RMSF plot of ESR1 residues in complex with 2,4-DTBP. The plot represents the Root Mean Square Fluctuation (RMSF) values across the protein residues, reflecting the flexibility and stability of ESR1 in response to ligand binding. Regions with higher RMSF values indicate greater flexibility, while lower RMSF values suggest increased rigidity.

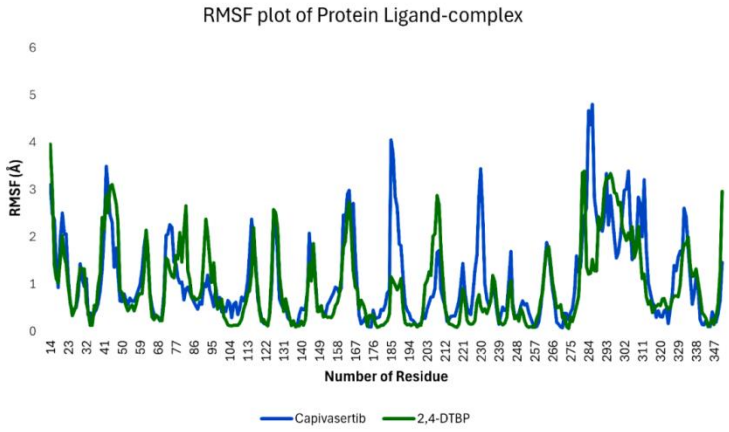


Fig. 8. RMSF plot of PTEN residues in complex with 2,4-DTBP.

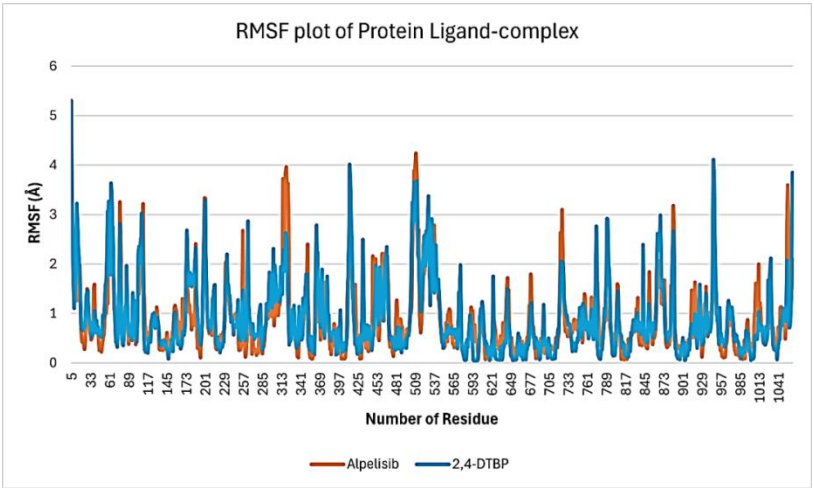


Fig. 9. RMSF plot of PI3KCA residues in complex with 2,4-DTBP.

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الالتحام الجزيئي ومحاكاة الديناميكيات للتقييم داخل السيليكون لـ ٢ ، ٤- دي-ثلاثي-بوتيل فينول (٢ ، ٤- دي تي بي بي) كمثبط محتمل لسرطان الثدي

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الملخص

سرطان الثدي (قبل الميلاد) لا يزال واحدا من الأسباب الرئيسية للوفاة في الإناث ، ويرجع ذلك أساسا إلى انتشار لا يمكن السيطرة عليها من خلايا الثدي. في هذه الدراسة ، قمنا بتحليل قوة ٢ ، ٤-دي-ثلاثي-بوتيل فينول (٢ ، ٤-دتب) ، وهو مركب طبيعي ، ضد السرطان من خلال برامج الكمبيوتر مثل الالتحام الجزيئي والديناميات الجزيئية (مد) المحاكاة. تم إجراء الالتحام الجزيئي مع أوتودوكتول لتقييم التفاعل ٢ ، ٤-دتب مع البروتينات سرطان الثدي الحرجة ، وتحديدا بيك ٣كا ، إسر ١ ، و بتن. تم تقييم الانتماءات الملزمة (كيلو كالوري/مول) وثوابت التثبيط لتحديد تفاعلات البروتين-يجند البارزة. تم إجراء عمليات محاكاة الديناميكيات الجزيئية باستخدام خادم ويب كابس-فليكس لتقييم استقرار هذه التفاعلات عبر رمسف وتحليلات الطاقة الحرة الملزمة. استخدمت الدراسة منهجيات متعددة ، بما في ذلك التنبؤ بالمرور ، وتقييم أدم ، وتوصيف السمية ، وقاعدة ليبينسكي الخمسة ، وتقييم النشاط المضاد للسرطان . تم العثور على صلات قوية ملزمة من ٢ ، ٤-دتبب نحو البروتينات سرطان الثدي الهامة من خلال نتائج الالتحام ؛ تراوحت قيم الطاقة ملزمة من -٥,٢٥ إلى -٧,٨٦ كيلو كالوري / مول. وقد تم الكشف عن طاقات ملزمة ذات مغزى من -٧,٨٦ ، -٧,٦٨ ، و -٧,٣٦ كيلو كالوري/مول ل بيك٣كا ، إسر ١ ، و بتن ، على التوالي. تم التحقق من استقرار مركب البروتين-يجند من خلال محاكاة الديناميكيات الجزيئية ، وأظهرت بتن الارتباط الأكثر استقرارا بسبب صلابة هيكلية أعلى وانخفاض قيم رمسف. أظهرت ٢ ، ٤-دتبب معايير مقبولة في كل مقياس تقييمها.وفقا لنتائج في سيليكون ، ٢ ، ٤-دتبب المعارض تشجيع إمكانات كمثبط قوي لأهداف سرطان الثدي كبيرة ، وتحديدا بتن ، بيك ٣كا ، و إسر ١ ، الذي يدعو إلى دراسات إضافية في المختبر وفي الجسم الحي لمزيد من التحقيق في فعاليته في علاج السرطان.

الكلمات الدالة: دراسات في السيليكون، الالتحام الجزيئي، محاكاة الديناميكيات الجزيئية، سرطان الثدي، منتج طبيعي، الرصاص المضاد للسرطان، أدميت.