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Discovery of Novel AKT2 Inhibitors for The Treatment of Breast Cancer: A Computational Approach

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ABSTRACT

Cancer is second leading cause for increasing in mortality, around 10 million deaths were reported globally. Akt, also called Protein Kinase B [PKB], is a serine/threonine kinase that assumes a major characteristic in the regulation of the PI3K/Akt pathway. One potential target for cancer treatment is Akt2. A ligand-based pharmacophore model was created and validated in order to discovering new Akt2 inhibitors using various scaffolds. Pharmacophore modelling was carried out for the marketed drugs using Pharmagist. Through the ZINCPharmer, 1000 most comparable pharmacophoric ligands were identified which were further filtered using Data Warrior tool based on their physicochemical properties. 21 hits with different scaffolds were picked out for docking studies using AutoDock vina. Based on the binding affinity and amino acid interaction 10 ligands are subsequently evaluated for ADMET properties using PKCSM webserver. ZINC02798447 have high binding affinity with better ADMET properties, which can act as novel leads for Akt2 inhibitor.
Key words: *Breast cancer, Akt2, Pharmacophore Modelling; Molecular Docking*

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INTRODUCTION

The most common form of cancer among women is breast cancer. The phosphoinositide 3-kinase [PI3K]/AKT signalling pathway is frequently dysregulated genetically and epigenetically in breast cancer. Serine/threonine kinase Akt, commonly referred to as Protein Kinase B (PKB), is essential for regulating the PI3K/Akt pathway. As a target downstream of PI-3 kinase, Akt can trigger a variety of biological reactions. Unfortunately, even though there are numerous AKT inhibitors in clinical usage, they are all linked to serious side effects and poor pharmacokinetics. Therefore, new medications that block the AKT enzyme are required to treat breast cancer. Drug development time and expenses can be greatly cut with the use of drug repurposing methods [1].In this manner, the chemical's inhibition through tiny molecules may make cancer cells more susceptible to apoptosis. High-throughput screening has been used to uncover Akt inhibitors up to this point, however it was primarily used for Akt1. We developed a construction-based pharmacophore to examine high dynamic Akt2 inhibitors using diverse scaffolds. With the use of the acquired pharmacophore models, powerful Akt2 inhibitors' critical pharmacophore components are to be found [2].Following their combination, these two varieties of pharmacophore models were employed as 3D search queries for chemical compound databases. Using drug-like filters and ADMET analysis, the chosen compounds were further refined and examined after being taken out of the database. Finally, 21 hits that have distinctive scaffolds, high evaluated activity, and excellent ADMET characteristics were selected. Atomic docking was carried out to investigate the bind states of these acquired hits, which may serve as a focus on the tight spot interactions between these hits and Akt2. Each analysis demonstrates that the seven hits could serve as new leads for the development of Akt2 inhibitors [3].

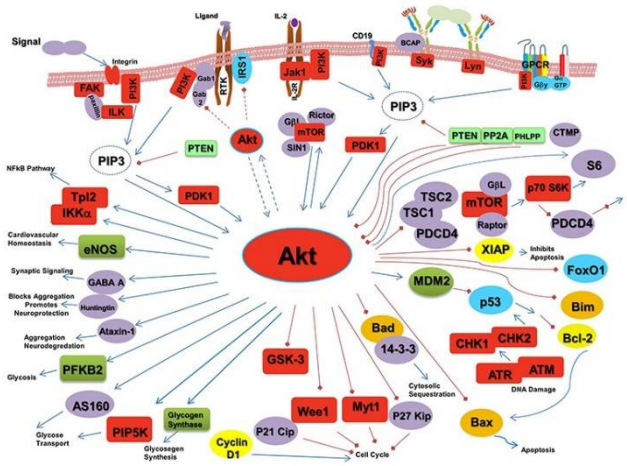


Fig1: The downstream effectors and the Akt pathway are shown schematically. Printed with permission from Cell Signalling Technologies, Inc., Beverly, Massachusetts, USA [www.cellsignal.com].

The greatest age-adjusted incidence rates were found in the male and female populations of the districts of Aizawl [269.4] and Papumpare[219.8], respectively. For the year 2020, there are expected to be 1,392,179 cancer patients in India, with the mouth, the cervix uteri, the breast, the lung, and the tongue being the common 5 top sites. In both sexes, the incidence of cancer has been steadily increasing, and it was especially high in Kamrup urban [annual percent change, 3.8%; P.05]. The most prevalent tumours discovered at an advanced stage were breast [57.0%], cervix uteri [60.0%], head and neck [66.6%], and stomach [50.8%], though lung cancer was more frequently seen in both men and women [44.0%] with distant metastases [47.6%][4].The pharmacological suppression of the PI3K/AKT pathway results in a a variety of toxicities, as is well recognised. Indeed, one of the major problems with the clinical development of drugs that inhibit AKT activity is safety. The three AKT isoforms' high homology also makes it difficult to create isoform-specific inhibitors, which could lessen their toxicity burden. PI3K inhibitors, such as idelalisib, have been linked to diarrhoea, which is probably due to an immune-mediated mechanism. Although the aetiology is still unknown, AKT suppression-induced diarrhoea was the most common adverse event (AE) of any grade observed with ATP-competitive inhibitors, peaking at 93% in the experimental arm of the LOTUS trial [ipatasertib + paclitaxel]. In fact, a few dose-finding experiments [DLT] found that diarrhoea was a frequent dose-limiting hazard. Nevertheless, it was mostly mild or moderate, with a prevalence of grade 3 or higher [G 3] episodes of 8 to 23% and infrequent G4 occurrences. With careful management using antidiarrheal medications, such as loperamide, these adverse effects typically had an early stage was curable. Although it wasn't intended in clinical trials, using antidiarrheal drugs as a preventative measure may increase the tolerability of AKT inhibitors and calls for more research in subsequent trials. Another issue with using AKT inhibitors is dermatological toxicity. Its exact aetiology likely depends on PI3K/role AKT's in keratinocyte development and survival [5-9].A well-known side effect of PI3K/AKT pathway inhibition is hyperglycaemia. It is caused by a breakdown of the insulin-mediated glucose homeostasis, which is primarily regulated by PI3K signalling via GSK3 and FOXO [Zhang et al., 2019]. The prevalence of hyperglycaemia of any grade varies substantially in clinical studies with AKT inhibitors, ranging from 92% with MK-2206 plus anastrozole to 4% with ipatasertib and paclitaxel.Ipatasertib and capivasertib phase I trials show a significant incidence of G3 hyperglycaemia but no signs of ketoacidosis or hyperosmolar coma. The varying occurrence of this toxicity throughout the studies may have been caused by appropriate patient selection Specifically, people with uncontrolled diabetes were excluded, and precise glucose blood level monitoring was used[10-13].

CADD's main goal is to screen, improve, and assess the compound's activity against the target. For both academic institutions and significant pharmaceutical firms, it creates a multidisciplinary strategy for higher efficacy with no/fewer side effects. Target identification, structure prediction, binding site/cavity, validation, understanding of protein-ligand interaction while screening numerous compounds, mastery of molecular dynamics simulations based on physiological conditions, and tallying with ADMET properties to the advancement of CADD are all examples of similarities [12-15].

Hence the present study aims to identify novel lead moieties to inhibit AKT pathway for the treatment of breast cancer through the utilization of computational tools.

MATERIAL AND METHODS

Drugs are identified form the clinical trial: The drug like capivasertib, iptasertib, uprosertib, miransertib, borusertib, afusertib, and somein preclinical trial drugs like A674563, A443654, AT7867, CCT128930, MK2206, GSK690693, API 1, PIT-1 which are determined for the PubChem database. These drugs are downloaded in the SDF format from PubChem[16].

Pharmacophore modelling: A pharmacophore is a three-dimensional framework created by mapping the physiologically powerful chemicals necessary for the ligand to attach to the appropriate target protein and interact with it. Molecule are subjected for pharmacophore modelling by uploading to free online tool called pharmagist in Sybyl Mol 2 format with valid mail ID. The uploaded molecules are combined in a variety of ways by the server to create the pharmacophore in Jmol format [17].

Collecting data from zinc database: Pharmgist produces the number of aligned molecules like 5 aligned molecule one up on another gives a cluster in the JMol format, where the molecule with maximum aligned 4 or 5 molecules with high scoring are selected and downloaded, further these molecules were uploaded in a tool for pharmacophore modelling I.e., ZINC pharma. Which will screen the zinc database and gives the molecules having same pharmacophoric features by submitting to query. After queries over zinc database give around more than a lakh hits of molecule, further obtained hits are filtered and get total of 1000 small molecules.Those above molecules are uploaded to the data warrior, where data warrior is free ware tool for doing pharmacophore modelling, these molecules are screened based on the standard drug physicochemical properties like molecular wight, log P, H donor, H acceptor, polar surface area, rotatable bond, and steric centre. For molecular docking analyses, all of these compounds were obtained as an SDF downloadable file [18].

Molecular Docking protocol: Docking studies were carried out to evaluate the different types of molecular interactions and ligand receptor binding affinities. The docking studies were carried out by means of Autodock vina, Biovia Discovery Studio 2020, PyRX, and PyMOL. The docking study was performed on crystal structure of complex of the catalytic portion of human AKT2 inhibitor[pdb id:7NH4] [19].

ADME/T studies: The substances that best suited the criteria of the structure-based pharmacophore as well as the best pharmacophore model were isolated, further filtered using Lipinski's rule, and then an ADMET [absorption, distribution, metabolism, excretion, toxicity] analysis was performed. The only compounds that can be considered hits are those that follow Lipinski's criteria, have strong expected activity, and have good ADMET characteristics. Also, all of the active compounds we gathered were included in the ADMET study to compare the ADMET attributes between the target proteins and the hits using PKCsM online free web server and SwissADME[http://www.swissadme.ch/] to predict their potential pharmacokinetic characteristics and toxicity[19].

RESULT AND DISCUSSION

Ligand-based pharmacophore modelling: Ligand-based pharmacophore modelling is a critical component of the drug development process when there is no macromolecular target structure. According to this method, the critical interactions between a ligand and a possible macromolecular target are retrieved from the 3D structures of numerous known ligands via ligand alignment**.** From PubChem, the 3D structures of 16 well-known AKT2 inhibitors were obtained. Water molecules and counter ions were taken out of these structures through cleaning. Each inhibitor was explicitly hydrogenated to make sure they were all all-atom structures, then the energy was minimised. Since the flexibility of the majority of ligands, it is essential to take into account a range of potential conformations for each molecule while developing a pharmacophore that closely reflects the experimental molecular orientation. Using the ZINCPharmer prepare ligands step of the create pharmacophore model process, these preparation steps were completed [16-19]. For each conformer of each ligand, different site points and pharmacophoric characteristics were established. The six built-in pharmacophore features that were used were the phase, hydrogen bond acceptor [A], hydrogen bond donor [D], hydrophobic group [H], negatively charged group [N], positively charged group [P], and aromatic ring [R]. A list of 34 variations was produced using the parameters 5 as the maximum number of sites, 4 as the lowest number of sites, and 6 as the minimum number of ligands that must match. This list included all 34 conceivable feature combinations that may result in typical pharmacophores. Then, all these variations were picked out to look for the AKT2 inhibitors' shared pharmacophore. Selecting the common pharmacophore allowed for the discovery of pertinent matches in the drug-like molecule.

Molecular docking studies: The molecular docking study of selected training set of drugs were carried out. To finding the amino acid interaction and binding affinity towards the protein of AKT2 inhibitors. Utilizing AutoDock Vina, molecular docking investigations were carried out. Capivasertib, iptasertib, uprosertib, miransertib, borusertib, afusertib, and some in preclinical trial drugs like A674563, A443654,

AT7867, CCT128930, MK2206, GSK690693, API 1, PIT-1. Table 1 shows the docking efficiency and binding interaction. With a docking score of -9.1 kcal/mol and conventional hydrogen bonds involving TYR229, LEU156, carbon hydrogen bonds with LEU295, ASN279, Pi-alkyl interactions with ASN279, and ASP274, CID25227436 was identified as one of the inhibitors. Via hydrogen bonds, Pi-Sigma interactions, and Pi-alkyl interactions, these substances adhere to the protein. The residues TYR229, LEU156, LEU295 ASN279, and ASP274 had a significant role in the interaction. So, the above-mentioned relationship may be substantial and provide an explanation for the inhibitory effects of molecules act as AKT2 inhibitors.

Table 1: Docking score and type of interaction of training set of molecules

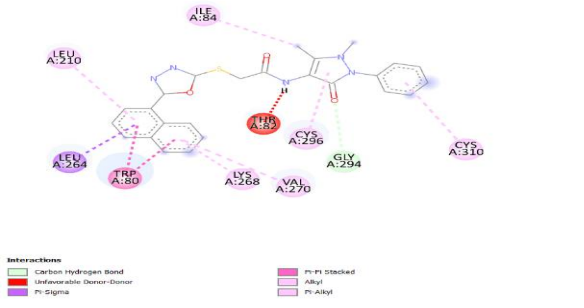
Compound	Docking score	Interacting residues	Type of Interaction
CID11175137	-7.5	LYS419 GLU228 ASN231 TYR176 TYR226 ARG174	Conventional hydrogen bond Carbon hydrogen bond Pi-Pi shaped Pi-alky
CID11314340	-8.5	ASP325 ILE36 LEU52 ARG328 ALA239 GLY327	Conventional Hydrogen, Pi-Cation, Alkyl, Pialkyl
CID130509	-7.9	MET445, ARG51, GLY67 TYR407, TYR444	Conventional hydrogen bond interaction, Pi-Pi stacked, Pi-Pi T Shaped, Pi-Alkyl interaction
CID17751819	-8.7	TYR176, ARG174, TYR175, ASN231, GLU228, LYS284, LYS289	Conventional hydrogen bond, Carbon hydrogen bond, Pi-Pi shaped, Pi-alkyl
CID24773090	-7.7	LYS284, TYR229, GLU228 ALA212, GLU418, HIS207 PRO208, GLU418	Conventional Hydrogen Bond, Carbon Hydrogen Bond, Unfavourable Acceptor, Amide Pi Staked, Alkyl, Pi-Alkyl
CID24788740	-7.9	LEU210, TYR18, LYS297 CYS296, ARG273, ILE84 GLU17	Conventional hydrogen bond, pi-cation, pi-anion, pi-donor hydrogen bond, alkyl, pi-alkyl
CID24905401	-7.1	PHE 293, TYR229, GLU234, PHE237	Conventional hydrogen bond halogen, pi-donor hydrogen bond, pi-pi t-shaped
CID25227436	-9.1	TYR229, LEU156, LEU295	Conventional hydrogen bond, carbon hydrogen
CID3664359	-7.5	ASN279, ASP274	bond, alkyl pi-alkyl
CID44240850	-7.6	TYR175, GLU175, LYS289 TYR176, ARG174	Conventional hydrogen bond, unfavourable donor, p- cation, alkyl, pi-alkyl
		GLY394, LYS389, ALA329 PRO388, ARG48, ILE36 LEU52	Conventional hydr0gen bond, unfavourable donor, p-sigma, alkyl, pi-alkyl

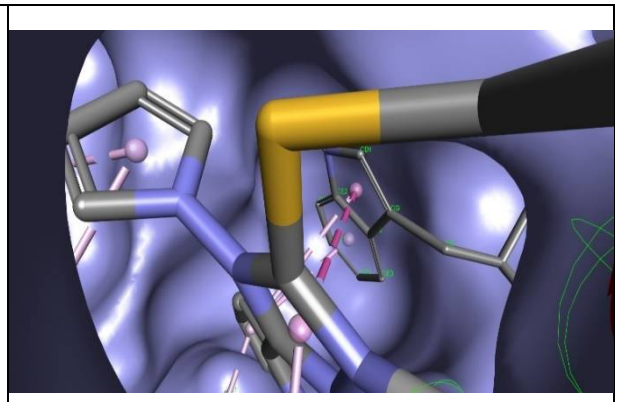
Following the pharmacophore-based chemical retrieval from the ZINC database using ZINCPharma, compounds underwent further docking. Here, 21 molecules were chosen for molecular docking with the target protein, PDB ID: 7NH4. In order to compare the binding interactions of compounds in training sets of drugs with those with better docking scores (higher the negative value), docking was conducted on the compounds. This comparison aids in identifying the hit substance as an AKT2 inhibitor. The identification of the compounds is based on the binding interaction. Between -10.8 to -12.5 kcal/mol is the docking score range. Molecular docking has discovered the molecule ZINC02798447, which exhibits Pi-Pi stacking interaction and has the best docking score of -12.5 kcal/mol with TRP80, Pi-alkyl interaction with LEU264, LEU210, LYS268, conventional hydrogen bond with binding interactions with ARG273, TYR18, CYS296. Comparetibly other good binding molecule ZINC09128777 with docking score of -11.8 kcal/mol showed carbon hydrogen bondinteraction with GLY294, unfavourable donor interaction with TYR18, Pi-Pi sacking interaction with TYR18, alkyl interaction with CYS296, GLY294, and Pi-alkyl interaction with GLY294, CYS310, LEU210. Using AutoDock tools and the Biovia Discovery Studio Visualizer, binding interactions between docked molecules and the proteins were discovered. The amino acid residues LEU210, TRP80, THR82, CYS296, GLY294, CYS310 that were implicated in the binding interaction were. These chosen compounds bind similarly to the compounds that are added to training sets of drugs, according to the molecular docking study. These 5 substances can thereby prevent AKT2 from activating. Table 3 illustrates the chemical structures of two docked compounds in both 2D and 3D.

Compound	Docking score	Interacting residues	Type of Interaction
ZINC02798447	-12.5	ARG273 TYR18 CYS296 LEU264 LEU210 LYS268 TRP80	Conventional hydrogen bond, unfavourable donor donor, pi-sulfur, pi-pi-staked, alkyl, pi-alkyl
ZINC09128777	-11.8	LEU210 TRP80 THR82 CYS296 GLY294 CYS310	Carbon hydrogen bond, unfavorablr donor, pi-sigma, pi-pi-stacked, alkyl, pi-alkyl
ZINC08901023	-11.6	TRP80 LEU210 TYR272 ASP274 THR82 ARG273 VAL270 GLN79	Conventional hydrogen bond, pi-anion, p-sigma, hydrogen bond, pi-pi t-staked, amide pi-staked,
ZINC13117684 ZINC11855556	-11.4 -10.8	LEU264 LYS268 LEU210 TYR2722 GLN79 CYS296 SER205 LEU264 LYS268 LEU210 TRP80 VAL270	Carbon hydrogen bond, unfavorablr donor, pi-sigma, pi-pi-stacked, alkyl, pi-alkyl Conventional hydrogen bond, carbon hydrogen bond, pi-sigma, pi-pi-staked, amide-pi-staked, alkyl, pi-alkyl

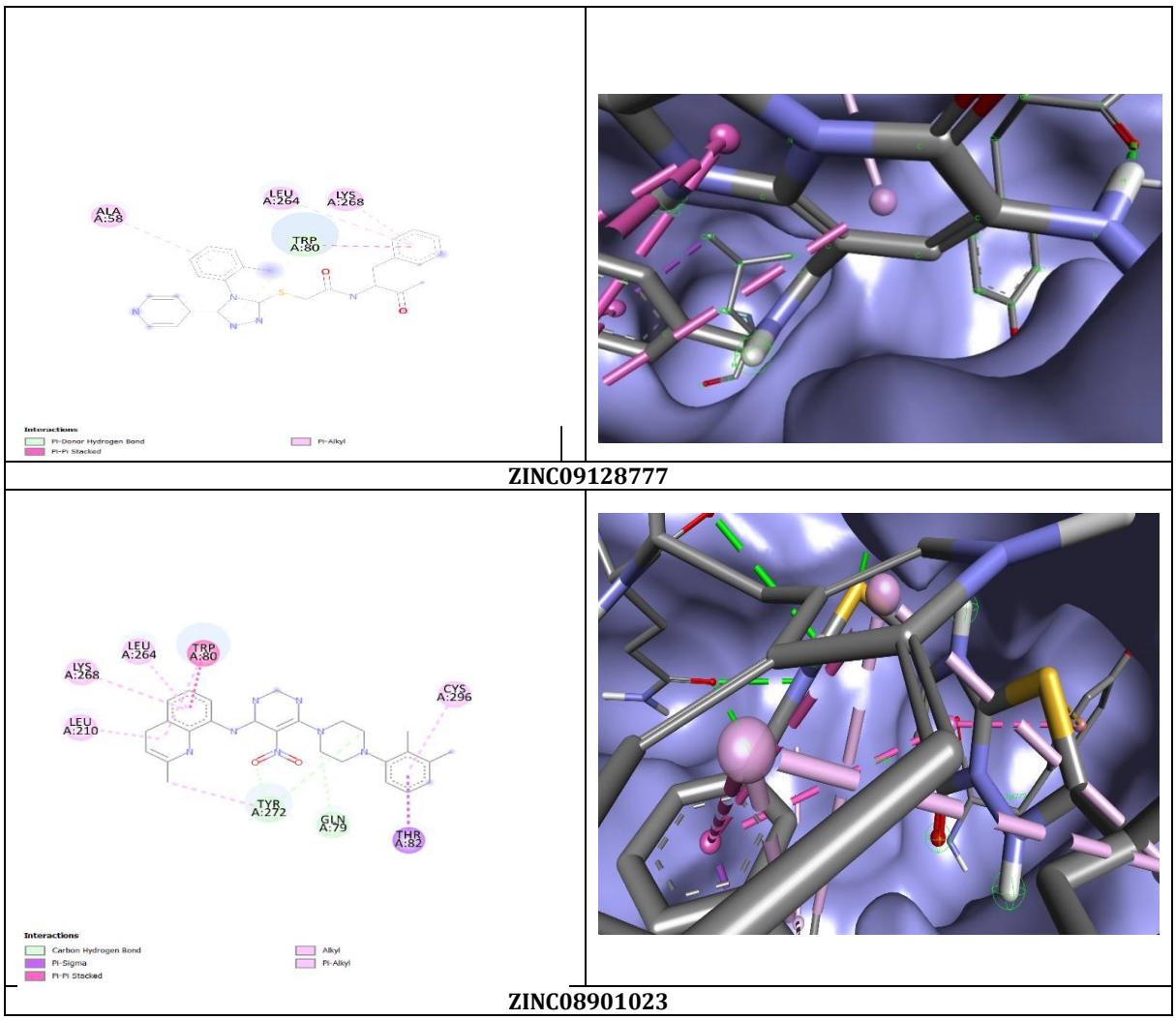
Table 2: Docking score of molecules obtained from ZINC database

Table 3: 2D and 3D interactions of top three molecules





ZINC02798447



ADMET studies: The ADMET properties with the zinc hits base drug I.e., ZINC02798447 has good properties like water solubility, $CaCo_2$ permeability, CNS permeability and it is non hepatotoxic molecule, while other molecules are showing hepatotoxicity. The ADME-T studies is carried out using PKSCM online software and swissadme. The zinc database molecule ZINC09128777will obey the Lipinski rule of five, by this the molecule can observer well by orally and it pass BBB and CNS to show its activity. The drug development process is sped up based on physicochemical characteristics by using drug-likeness filters. The drug-likeness rules/filters based on physicochemical features, however, have drawbacks, as demonstrated by several research.

CONCLUSION

Drug identification was carried out to identify to discovery of a novel akt2 inhibitors to treat breast cancer. Based on the preliminary studies 21 hits was found to be show high binding affinity with akt2. Capivasertib was selected as standard in this study. 10 ligands are subsequently evaluated for ADMET properties using PKCSM webserver. ZINC02798447 have high binding affinity with better ADMET properties, which can act as novel leads for Akt2 inhibitor.

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CONFLICT OF INTEREST

The authors declare that no conflict of interest.

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