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Original Article

Drug Repurposing Revealed Abemaciclib and Palbociclib as Inhibitor of Phosphodiesterase-5 (PDE5) in Cancer-Associated Fibroblasts Paving the Way for the Treatment of Breast Cancer

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ABSTRACT

Background: A common element of the tumor microenvironment, cancer-associated fibroblasts play a major role in the development of breast cancer via a variety of pathways. Studies show that cancer-associated fibroblasts over express phosphodiesterase-5 in comparison to normal fibroblasts and that this overexpression is linked to increased fibroblast activation and a more aggressive CAF phenotype. High PDE5 levels in CAFs contribute to the remodeling of the extracellular matrix, increased secretion of pro-tumorigenic factors and suppression of anti-tumor immune responses. **Materials and Methods:** Targeting PDE5 in CAFs thus offers a convincing method to interfere with these cells' pro-tumorigenic activities. The potential to inhibit PDE5 was examined for four FDA-approved CDK4/6 inhibitors: Abemaciclib, Palbociclib, Alvocidib and Ribociclib. **Results:** These drugs, with established safety profiles, were screened and docked against PDE5, showing binding affinities of -7.8, -7.8, -7.1 and -6.8 kcal/mol, respectively, compared to Sildenafil's -7.0 kcal/mol, a known inhibitor of PDE5. ADMET predictions, PASS analysis and Swiss Target Prediction affirmed their drug-like properties and potential for PDE5 inhibition. **Conclusion:** Based on these results, Abemaciclib and Palbociclib were chosen for *in vitro* experiments with cancer-associated fibroblasts. The 2 drugs significantly decreased the expression of PDE5 mRNA, indicating their potential as therapeutic agents for the treatment of breast cancer.

Keywords: Breast cancer, Cancer-associated fibroblasts, CDK4/6 inhibitors, Inhibitor, Phosphodiesterase-5 (PDE5).

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INTRODUCTION

Breast cancer remains one of the leading causes of cancer-related deaths among women worldwide.[1] Despite advancements in early detection and treatment, there is a growing need for innovative therapeutic strategies. The Tumor Microenvironment (TME) plays a critical role in cancer progression and among the various cellular components of the TME, Cancer-Associated Fibroblasts (CAFs) have garnered significant attention.[2,3] These cells support tumor growth, invasion and metastasis.[3] Recent research has highlighted the role of Phosphodiesterase 5 (PDE5) in CAFs, suggesting that its inhibition could offer a new avenue for breast cancer therapy.[4]

CAFs are a prominent component of the TME and contribute to breast cancer progression through multiple mechanisms.[4,5] They secrete growth factors, cytokines and Extracellular Matrix (ECM) components that facilitate tumor cell proliferation, invasion and immune evasion.[6] CAFs also promote angiogenesis, thereby ensuring a steady supply of nutrients and oxygen to the growing tumor.[7] By modulating the ECM, CAFs create a physical barrier

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that hinders the penetration of therapeutic agents, leading to drug resistance.[8]

PDE5 is an enzyme that hydrolyzes cyclic Guanosine Monophosphate (cGMP) to 5'-GMP, thereby regulating intracellular levels of cGMP.[9] This regulation is crucial for various physiological processes, including smooth muscle relaxation, platelet function and neural signalling.[10] PDE5 inhibitors are well-known for their use in treating erectile dysfunction and pulmonary hypertension.[11,12] However, recent studies have uncovered a novel role of PDE5 in cancer biology, particularly within the TME.[13]

Research indicates that PDE5 is highly expressed in CAFs compared to normal fibroblasts.[4,14] This overexpression is associated with enhanced fibroblast activation and a more aggressive CAF phenotype. High PDE5 levels in CAFs contribute to the remodelling of the ECM.[6,8] increased secretion of pro-tumorigenic factors,[4] and suppression of anti-tumor immune responses.[13,15] Therefore, targeting PDE5 in CAFs presents a compelling strategy to disrupt the pro-tumorigenic functions of these cells.[16-18] PDE5 inhibition in CAFs can alter the TME in several ways. By reducing ECM stiffness and remodelling, it can enhance the penetration and efficacy of chemotherapeutic agents.[19] Furthermore, inhibiting PDE5 decreases the secretion of pro-tumorigenic cytokines and growth factors, thereby attenuating the supportive role of CAFs in tumor progression.[4] CAFs are major contributors to tumor angiogenesis through the secretion of Vascular Endothelial Growth Factor (VEGF) and other pro-angiogenic factors.[7] PDE5 inhibitors can reduce the expression of these factors, leading to decreased angiogenesis and subsequent tumor growth inhibition.

CAFs play a role in creating an immunosuppressive TME by secreting immunosuppressive cytokines and recruiting regulatory T cells (Tregs).[20] Inhibition of PDE5 in CAFs can mitigate these immunosuppressive effects, thereby enhancing the anti-tumor immune response.[13] This could potentially improve the efficacy of immunotherapies in cancer.

Preclinical studies have demonstrated that PDE5 inhibitors can effectively reduce tumor growth and metastasis in breast cancer models.[4] For instance, sildenafil has been shown to inhibit the proliferation and invasion of breast cancer cells *in vitro* and *in vivo*.[4,15] These effects are partly mediated by the modulation of the TME, including the suppression of CAF activity.

Targeting CAFs has emerged as a promising approach in cancer therapy due to their critical role in the TME. Recent studies have highlighted the overexpression of Phosphodiesterase 5 (PDE5) in CAFs compared to normal fibroblasts, correlating with a more aggressive CAF phenotype in cancer.[4,14] Inhibiting PDE5 in CAFs can disrupt their pro-tumorigenic functions by altering the TME, reducing Extracellular Matrix (ECM) stiffness and decreasing the secretion of growth factors and cytokines that facilitate tumor progression.[4,19] Further, this strategy can enhance the penetration and efficacy of chemotherapeutic agents, suppress tumor angiogenesis and mitigate immunosuppressive effects, thereby improving the overall anti-tumor immune response.[7,13] Preclinical evidence supports the efficacy of PDE5 inhibitors in reducing tumor growth and metastasis, demonstrating their potential in breast cancer therapy.[4,15] Targeting PDE5 in CAFs offers a compelling new avenue for breast cancer treatment, promising to enhance current therapeutic strategies and improve patient outcomes.

MATERIALS AND METHODS

Retrieval and preparation of drug molecules

Four FDA approved drug molecules namely Abemaciclib, Palbociclib, Alvocidib and Ribociclib were selected. The structures of all the 4 drug molecules are represented in Figure 1. The primary justification for selecting this set of small molecules is that these drug molecules have substantial capacity to inhibit the activity of crucial enzymes such as CDK4/6.[21] These drugs have established safety profiles and are readily available for further investigation. Furthermore, it is noteworthy that in last two decades, these drug molecules has got recognition as potential therapeutic agents for clinical conditions related CDK4/6 activity.[22] The geometry of these drug molecules was optimized using a molecular mechanic's force field. This ensures that the molecule is in a stable conformation for docking. Further, these drug molecules were assigned atom types and partial charges using a molecular mechanics force field "AMBER." Hydrogen atoms were added to the drug molecules and checked for any missing or incorrect valence states. Energy of the drug molecules was minimized using a molecular mechanic's force field to remove any unfavourable interactions or clashes between atoms. Drug molecules were converted to PDBQT format, the appropriate file format for molecular docking software AutoDock Vina. 3D structures of these ligands were docked against target protein PED5.

Retrieval and Refinement of Receptor Protein

The crystal structure (Figure 2) of human PDE5 was downloaded from the RCSB PDB (https://www.rcsb.org/) bearing ID: 2H42.[23] The target protein's crystal structure was then refined using a standard receptor preparation protocol.[24,25] The inhibitor's structural coordinates were completely removed from the protein-inhibitor complex. The Swiss-PDB Viewer was used to minimize the energy by moving atoms to release local constraints on the target protein's stability. The protein wizard was prepared in the AutoDock Vina tool by removing water molecules, adding missing atoms and optimizing its geometry. Kollman United Atom Charges were then assigned to the target protein. Finally, the target protein was prepared in PDBQT format for molecular docking studies.

Molecular Docking Protocol

All four drug molecules were screened against PDE5. Molecular docking of the protein-ligand complex was carried out using AutoDock Vina. The grid parameters were calculated using the "Grid" feature of AutoDock. The center and size of the grid box around the ligand were large enough to accommodate the entire ligand. The conformation of the ligand with the minimum binding energy was considered the most stable drug molecule. Using Discovery Studio, the interactions between the proteins and drug molecules were further examined for the docked poses of the drug molecules at the target binding sites of PDE5.

ADME and Toxicity properties of drug molecules

The evaluation of the Absorption, Distribution, Metabolism, Excretion and Toxicity (ADME and Toxicity) properties of drug molecules were determined by using the pkCSM online tool (https://biosig.lab.uq.edu.au/pkcsm/). SMILES notations of drug molecules obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) were uploaded on the pkCSM server to determine their ADME and Toxicity properties.

Prediction of Activity Spectra of drug molecules using PASS online

To determine the Prediction of Activity Spectra for Substances (PASS), smiles notation of drug molecules were uploaded on PASS online server (https://www.way2drug.com/passonline/). This server predicts the potential biological activities associated with drug molecules.

Prediction of Activity Spectra of ligand molecules using SwissTargetPrediction tool

To explore the biological activity spectrum associated with drug molecules, we utilized the Swiss Target Prediction tool, accessible at http://www.swisstargetprediction.ch/. Swiss Target Prediction is an online platform specifically developed to predict potential targets for small molecules or drugs. SMILES notation of all four drug molecules were uploaded to prediction tool to determine potential targets based on structural similarities and known ligand-protein interaction patterns.

Relative mRNA expression of PDE5 in Abemaciclib and Palbociclib treated Cancer Associated Fibroblasts (CAFs)

To investigate the effect of 2 predicted drug molecules namely Abemaciclib and Palbociclib on PED5 gene expression, Cancer Associated Fibroblasts (CAFs) were treated with IC_{50} concentration (Abemaciclib -168 nmol and Palbociclib-306 nmol/) of both drugs for the duration of 24 hr. The IC_{50} concentrations for both drugs were chosen from.[26] The RNA was extracted and cDNA was synthesised using QIAwave RNA Mini Kit and cDNA Synthesis Kit, Quigen. The relative fold change in PED5 expression in treated and untreated cells was calculated using the 2-ΔΔCT method, SYBR™ Green Master Mix (Thermo Fisher Scientific, USA) on an Applied Biosystems RT-PCR System. The primer sequences for PDE5 were as follows 5'-TTCCATGTGCTAGCCAGGTAAA-3' (sense) and 5'GGTCCAAAACCATGCACAATTT-3' (antisense) and for GAPD was as follows 5'-GGCCTCCAAGGAGTAAGACC-3' sense and 5'-CTGTGAGGAGGGGAGATTCA-3' antisense.[27,28]

Statistical Analysis

Data was analysed by GraphPad Prism. *t*-test was employed to compare the mean values between treated and untreated group. Mean difference at $p<0.05$ was considered as statistically significant.

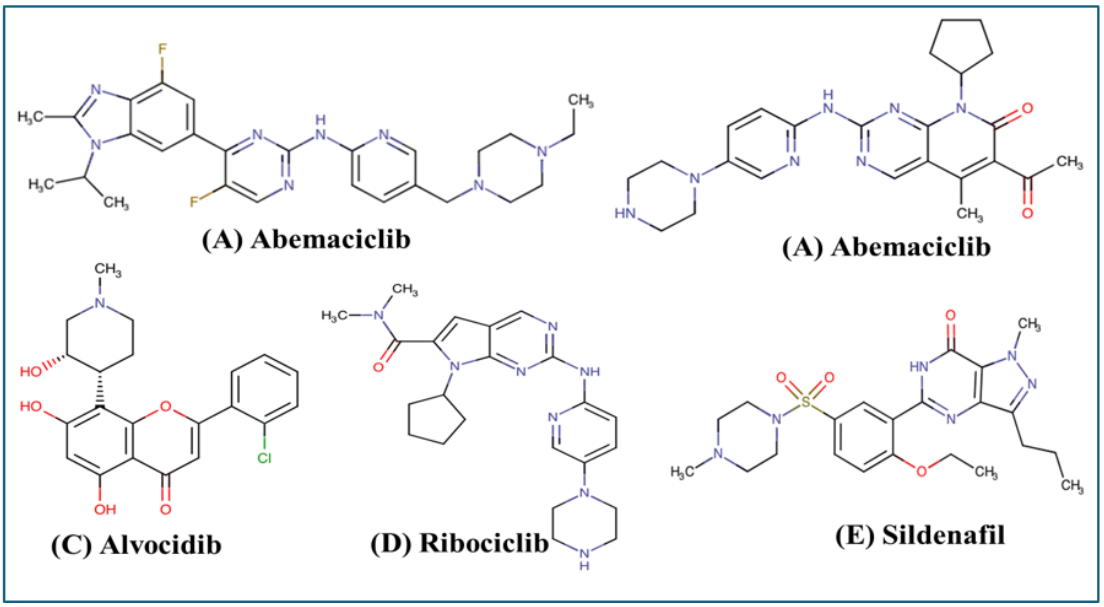

Figure 1: Structure of drug molecules. (A) Abemaciclib; (B) Palbociclib; (C) Alvocidib; (D) Ribociclib; and (E) Sildenafil.

RESULTS

Four FDA approved CDK4/6 inhibiting drug molecules namely Abemaciclib, Palbociclib, Alvocidib and Ribociclib were selected. These drugs have established safety profiles and are readily available for further investigation. All four drug molecules were screened and docked against PDE5. Their binding affinities and their pattern of interactions with PDE5 are represented in Table 1 and Figure 3, respectively. Palbociclib showed binding affinity of -7.8 kcal/mol, Abemaciclib -7.8 kcal/mol, Alvocidib -7.1 kcal/mol and Ribociclib -6.8 kcal/mol. Figure 2a-d represents a 2D interaction of the protein-ligand complexes. All drug molecules formed significant hydrogen as well as pi-alkyl, alkyl and van der Waals interactions were with PDE5 (Table 2). In addition, we also conducted the redocking of Sildenafil which showed a binding of -7.0 kcal/mol, which is less than all four drug molecules (Table 1).

ADMET predictions, PASS analysis and SwissTargetPrediction

ADMET predictions, PASS analysis and Swiss Target Prediction collectively affirm the drug-properties of all 4 drug molecules for the inhibition of PDE5. The summarized outcomes of ADMET predictions for all 4 drug molecules are presented in Table 3A-D, indicating that it complies with crucial pharmacokinetic criteria.

Table 1: Binding affinities and inhibition constant (Ki) of drug molecules with target protein PDE5.

Name of the ligand	Binding Energy (kcal/mol)	pKi
Abemaciclib	-7.8	5.72
Palbociclib	-7.8	5.72
Alvocidib	-7.1	5.21
Ribociclib	-6.8	4.99
Sildenafil	-7.0	5.13

This positions all four drug molecules as a promising candidate for therapeutic development targeting PDE5 inhibition in breast cancer. PASS Analysis results, as detailed in Table 4A-C; underscore favourable biological properties associated with three of four drug molecules, highlighting their relevance in various significant biological processes. We could not get any PASS results associated with Ribociclib. Additionally, Swiss Target Prediction analysis as elucidated in Figure 4A-D not only confirms but also elaborates on the diverse biological properties linked to all four drug molecules. These findings collectively suggest that all four drug molecules hold promise as potential therapeutic candidates, especially in their ability to target the activity of enzymes and other cytosolic proteins.

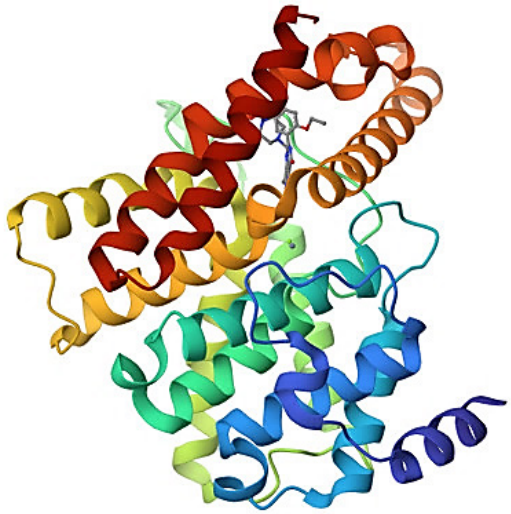

Figure 2: Crystal structure of PDE5 in complex with Sildenafil (adapted from PDB; Wang *et al.*, 2006).

Table 2: Interacting amino acid residues of target protein PDE5 with drug molecules as its potent inhibitors.

Ligand molecules	Target protein	Amino acid residues of target protein interacting with ligand molecules	
		Conventional hydrogen bonds	Other bonds (van der Walls, Alkyl, Pi-Alkyl, Pi-Cation, Pi-Sigma).
Abemaciclib	PDE5	-	LYS174, LYS178, LEU177, PHE150, LYS181, LEU154, LEU168, TYR173, SER169, LEU165, SER166, ASN158, GLN163, ASN158.
Palbociclib		THR233	PRO237, LYS316, TRP236, ASN313, LYS234, LYS312, PRO235, GLY309, PRO304, SER24, PHE23, ASP22.
Alvocidib		LEU268	THR266, SER127, TYR128, GLY283, ALA287, ILE288, PHE284, SER279, MET280.
Ribociclib		THR248	ARG271, PHE251, LYS274, ASN275, GLN323, GLN234, PRO278, LEU320, ALN240, TRP317, ILE277, GLU244, ALA247.
Sildenafil		ARG196	ARG131, ILE203, ALA295, GLU294, PHE200, ARG204, GLN291, GLU201, GLY197, SER132, HIS134.

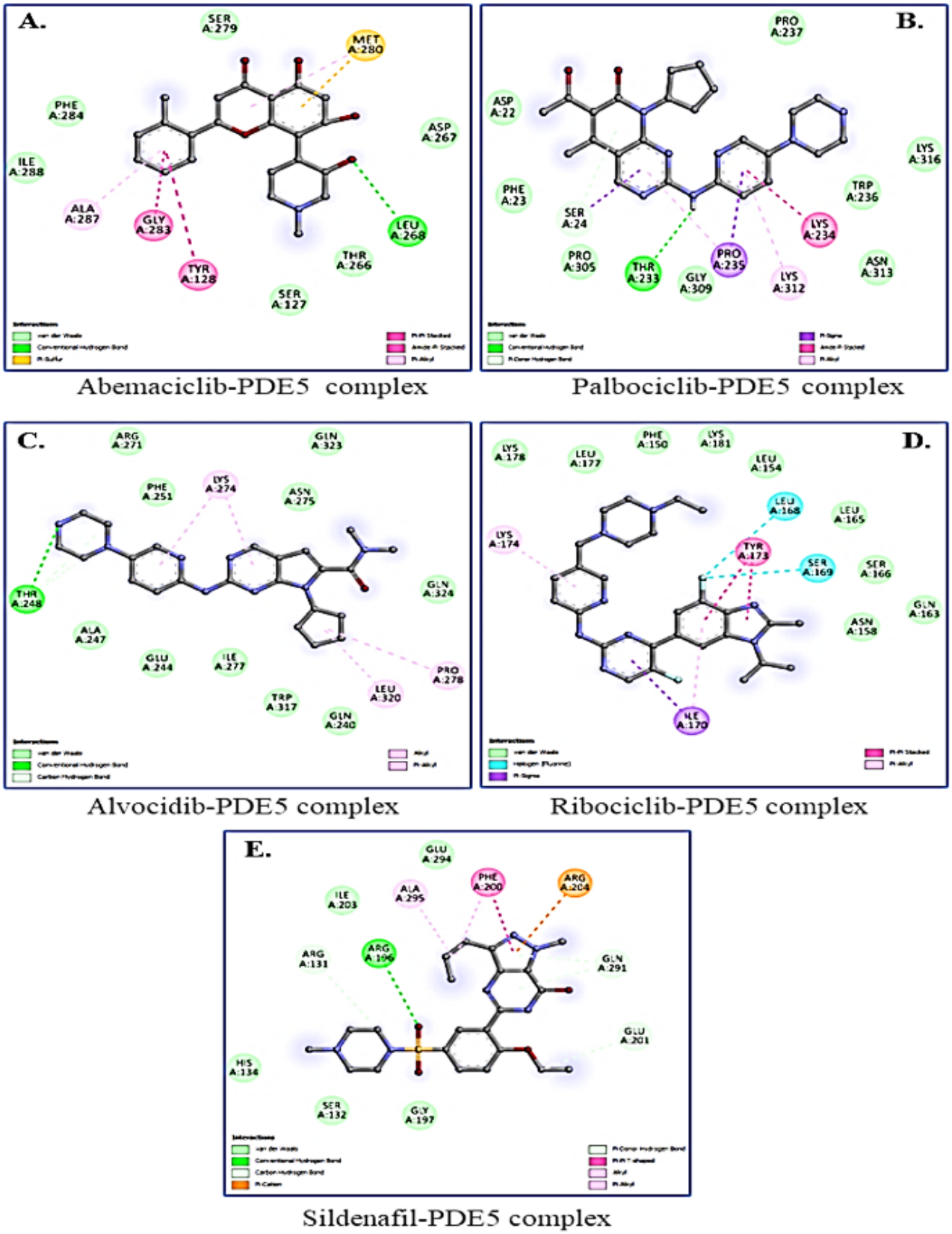

Figure 3: Pattern of interactions of drug molecules with PDE5. (A) Abemaciclib-PDE5 complex; (B) Palbociclib-PDE5 complex; (C) Alvocidib-PDE5 complex; (D) Ribociclib-PDE5 complex; and (E) Sildenafil-PDE5 complex.

Relative mRNA expression of PDE5 in untreated and treated Cancer Associated Fibroblasts (CAFs)

Based on the binding affinity, ligand protein interactions and ADMET predictions, PASS analysis and Swiss Target Prediction we selected two drug molecules namely Abemaciclib and Palbociclib for *in vitro* studies using CAFs. Figure 5 provides a visual representation of the effect of IC_{50} concentrations of Abemaciclib (168 nmol) and Palbociclib (306 nmol) on PED5 gene expression. Results indicated that treatment of CAFs with both drugs led to a significant decrease ($p<0.01$) in the mRNA expression of PDE5 compared to untreated cells. This significant reduction in PDE5 mRNA expression underline the inhibitory effect of Abemaciclib and Palbociclib on PDE5, highlighting their potential as a therapeutic agent for targeting PDE5 in breast cancer treatment.

DISCUSSION

The discovery of CDK4/6 inhibitors has shown significant impact on impeding cancer progression.[29] The success of CDK4/6 inhibitors in clinical trials highlights their potential as

Table 3: *In silico* prediction of ADME and Toxicity properties for all four drugs namely Abemaciclib, Palbociclib, Alvocidib and Ribociclib.

Property	Model name	Predicted value			
		Abemaciclib	Palbociclib	Alvocidib	Ribociclib
Absorption					
	Water solubility (Numeric (log mol/L).	-2.909	-2.817	-3.717	-2.899
	Intestinal absorption (human) (Numeric (% Absorbed).	83.946	83.374	89.526	95.751
Distribution					
	VDss (human) (Numeric (log L/kg).	-0.165	1.537	0.502	0.778
	BBB permeability (Numeric (log BB).	-1.572	-0.486	-1.059	-1.234
	CNS permeability (Numeric (log PS).	-3.215	-3.147	-2.221	-2.237
Metabolism					
	CYP2D6 substrate (Categorical (Yes/No)	No	No	No	No
	CYP3A4 substrate (Categorical (Yes/No)	Yes	Yes	Yes	Yes
	CYP1A2 inhibitor (Categorical (Yes/No)	No	No	Yes	No
	CYP2C19 inhibitor (Categorical (Yes/No)	Yes	No	Yes	No
	CYP2C9 inhibitor (Categorical (Yes/No)	No	No	No	No
	CYP2D6 inhibitor (Categorical (Yes/No)	No	No	No	No
	CYP3A4 inhibitor (Categorical (Yes/No)	Yes	No	Yes	No
Excretion					
	Total Clearance (Numeric (log mL/min/kg).	0.284	0.763	0.332	0.698
Toxicity					
	AMES toxicity (Categorical (Yes/No).	No	No	No	No
	Hepatotoxicity (Categorical (Yes/No).	Yes	Yes	Yes	Yes

a key component in the treatment of various cancers, offering a targeted approach to disrupt the cell cycle and inhibit tumor growth.[30-32] The investigation into the inhibitory effects of FDA approved small drug molecules on PDE5 provides a possibility for developing novel therapeutic strategies for breast cancer treatment.

Molecular docking of ligand molecules against target proteins is crucial for developing novel cancer therapeutics.[33-35] It predicts how drugs bind to specific proteins, helping identify potent inhibitors.[25,33] This accelerates drug discovery, optimizes lead compounds and enhances our understanding of drug-target interactions, ultimately leading to more effective cancer treatments.[36] FDA-approved drugs namely Abemaciclib, Palbociclib, Alvocidib and Ribociclib, were selected based on their established safety profiles and availability. The binding affinities of the four drug molecules, as determined through molecular docking, are indicative of their potential to inhibit PDE5 effectively. Both Abemaciclib and Palbociclib showed the highest predicted binding affinities of -7.8 kcal/mol, followed by Alvocidib at -7.1 kcal/mol and Ribociclib at -6.8 kcal/mol. For comparison, Sildenafil, a known PDE5 inhibitor, showed a binding affinity of -7.0 kcal/mol. These results suggest that Abemaciclib and Palbociclib are particularly strong candidates for PDE5 inhibition. Interaction patterns of these drug molecules with PDE5, including significant hydrogen bonds, pi-alkyl, alkyl and van der Waals interactions, further support their inhibitory potential. These interactions are crucial as they stabilize the drug-protein complex, enhancing the inhibitory effect on PDE5.[37] The detailed 2D interaction provided a comprehensive view of these interactions, highlighting the specific residues involved and the nature of the interactions.

Determining ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties of ligand molecules is vital for cancer therapeutics and ensures drug efficacy and safety by evaluating how a drug behaves in the body.[38] Understanding ADMET properties helps optimize drug design, reduce adverse effects and improve the success rate of clinical trials.[39] The ADMET predictions underscore the pharmacokinetic viability of

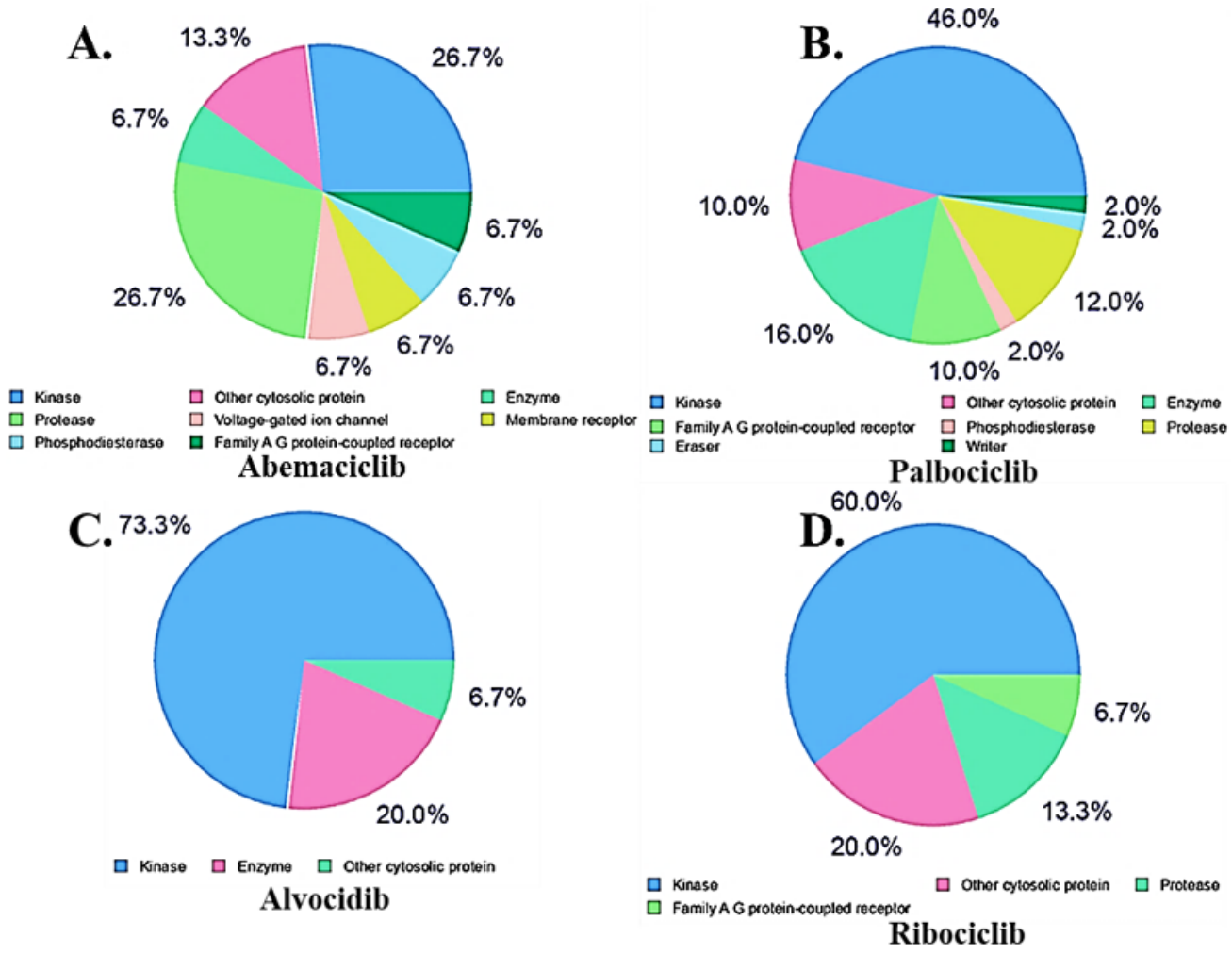

Figure 4: SwissTargetPrediction analysis showing diverse biological properties linked to all four drug molecules drug molecules. (A) Abemaciclib; (B) Palbociclib; (C) Alvocidib; and (D) Ribociclib.

Table 4A: PASS (Prediction of Activity Spectra for Substances) analysis of Abemaciclib. Probability "to be active" was set at Pa>0, 7.

Pa	Pi	Activity
0.757	0.009	Signal transduction pathways inhibitor.
0.586	0.048	Antineoplastic.
0.542	0,004	Cyclin-dependent kinase inhibitor.
0.449	0.004	Cyclin-dependent kinase 1 inhibitor.
0.428	0.024	Protein kinase inhibitor.
0.407	0.009	HERG channel blocker.
0.392	0.013	HIV attachment inhibitor.
0.428	0.111	Proteasome ATPase inhibitor.
0.336	0.027	Tyrosine kinase inhibitor.
0.319	0.010	Janus tyrosine kinase inhibitor.
0.321	0.013	p21-activated kinase inhibitor.
0.324	0.019	Potassium channel blocker.
0.368	0.064	CDK9/cyclin T1 inhibitor.
0.307	0.028	TRKB antagonist.
0.348	0.073	Rhinitis treatment.
0.308	0.034	Antineoplastic (colorectal cancer).
0.345	0.077	Autoimmune disorders treatment.
0.351	0.099	Neurodegenerative diseases treatment.
0.316	0.202	Antineoplastic (non-Hodgkin's lymphoma).

Table 4B: PASS (Prediction of Activity Spectra for Substances) analysis of Palbociclib. Probability "to be active" was set at Pa>0, 7.

Pa	Pi	Activity
0.933	0.002	Cyclin-dependent kinase 4 inhibitor.
0.881	0.003	Cyclin-dependent kinase 2 inhibitor.
0.825	0.003	Cyclin-dependent kinase inhibitor.
0.604	0.044	Antineoplastic.
0.539	0.004	Cyclin-dependent kinase 1 inhibitor.
0.537	0.015	Antineoplastic (breast cancer).
0.513	0.028	Signal transduction pathways inhibitor.
0.346	0.003	Fibroblast growth factor 3 antagonist.
0.350	0.010	TRKB antagonist.
0.356	0.030	Platelet aggregation inhibitor.
0.327	0.003	Fibroblast growth factor 2 antagonist.
0.336	0.036	Protein kinase inhibitor.
0.301	0.005	Fibroblast growth factor antagonist.
0.316	0.036	HIV attachment inhibitor.
0.316	0.110	CDK9/cyclin T1 inhibitor.
0.377	0.175	Nicotinic alpha4beta4 receptor agonist.
0,303	0,141	Kinase inhibitor.
0.322	0.238	General pump inhibitor.

all four drug molecules, confirming their potential as therapeutic agents.[40] The compliance with crucial pharmacokinetic criteria is particularly important for the development of effective drugs with minimal adverse effects.[41-43]

PASS (Prediction of Activity Spectra for Substances) analysis is significant for cancer therapeutics as it predicts the biological activity of ligand molecules. This computational tool helps identify potential anti-cancer properties, guides drug design and reduces experimental costs and time by prioritizing compounds with the highest likelihood of success in treating cancer. The PASS analysis results highlight favourable biological properties associated with Abemaciclib, Palbociclib and Alvocidib, further emphasizing their therapeutic potential. The absence of PASS results for Ribociclib may require additional investigations to fully elucidate its biological relevance.[44,45]

Swiss Target Prediction analysis is crucial for cancer therapeutics as it predicts potential protein targets of ligand molecules.[46,47] This tool aids in understanding drug-target interactions, optimizing drug design and identifying off-target effects, thereby enhancing the development of effective and safe cancer treatments by focusing on the most promising therapeutic targets.[44] Swiss Target Prediction analysis provided further validation of the diverse biological properties linked to these drug molecules. This

Table 4C: PASS (Prediction of Activity Spectra for Substances) analysis of Alvocidib. Probability "to be active" was set at Pa>0, 7.

Pa	Pi	Activity
0.915	0.003	Membrane permeability inhibitor.
0.859	0.006	Anaphylatoxin receptor antagonist.
0.809	0.011	HIF1A expression inhibitor.
0.772	0.042	Membrane integrity agonist.
0.733	0.006	Histidine kinase inhibitor.
0.707	0.010	CYP1A substrate.

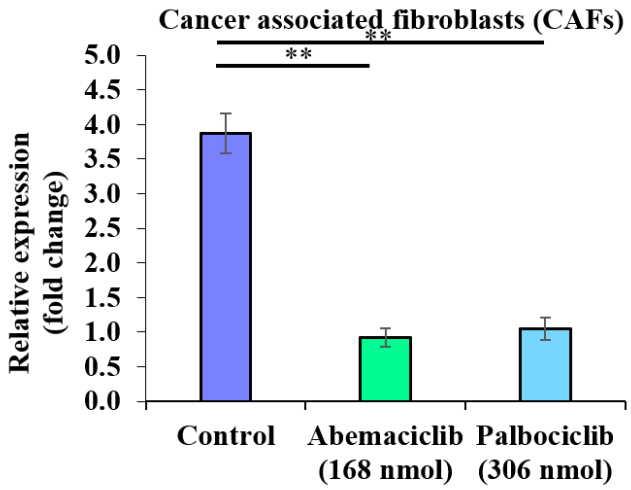

Figure 5: Effect of IC_{50} dose of Abemaciclib and Palbociclib on the mRNA expression of PDE5 in Cancer Associated Fibroblasts (CAFs).

comprehensive analysis confirmed the potential of these drugs to target not only PDE5 but also other enzymes and cytosolic proteins, broadening their scope of therapeutic applications.

Based on the binding affinity and other data, Abemaciclib and Palbociclib were selected for *in vitro* studies using the CAFs. The IC_{50} concentrations of Abemaciclib were effective in reducing PDE5 expression, highlighting their potential as therapeutic agents for targeting PDE5 in breast cancer treatment. This significant reduction in PDE5 mRNA expression highlights the inhibitory effect of Abemaciclib and Palbociclib on PDE5.

The findings from this study suggest that Abemaciclib and Palbociclib, in particular, may be developed as therapeutic agents for breast cancer treatment through their inhibition of PDE5. The significant reduction in PDE5 mRNA expression in treated CAFs indicates a potential mechanism through which these drugs exert their anti-cancer effects.

CONCLUSION

These study things to see the probable of CDK4/6 inhibitors, principally Abemaciclib and Palbociclib, as current inhibitors of PDE5. The combination of molecular docking studies, ADMET predictions, PASS analysis, and *in vitro* studies make available a comprehensive considerate of their possible as therapeutic agents for breast cancer treatment. The momentous reduction in PDE5 mRNA expression in treated CAFs reflects their potential to improve therapeutic outcomes. Additional research and clinical investigations are necessary to fully elucidate their therapeutic potential and optimize their use in breast cancer therapy.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

PDE5: Phosphodiesterase-5; **TME:** Tumor microenvironment; **CAFs:** Cancer-associated fibroblasts; **ECM:** Extracellular matrix; **cGMP:** Cyclic guanosine monophosphate; **VEGF:** Vascular endothelial growth factor.

SUMMARY

The study explores the potential of CDK4/6 inhibitors, primarily Abemaciclib & Palbociclib, as PDE5 inhibitors in breast cancer treatment. Results show a reduction in PDE5 mRNA expression, but further research is needed.

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