

A Repurposed Drug Combination Overcomes Paclitaxel Resistance and Minimizes Toxicity via Dual Metabolic and pH Modulation in Breast Cancer

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Abstract

Background: Paclitaxel (PTX) remains a cornerstone in breast cancer treatment, but its effectiveness is limited by both chemoresistance and significant side effects. A promising strategy to overcome these challenges is to repurpose existing drugs. The present investigation sought to explore the combined anticancer efficacy of a new triple-combination treatment, including paclitaxel, dapagliflozin, and pantoprazole, specifically targeting MCF-7 breast cancer cells, and evaluated the safety profile of this combination in normal human foreskin fibroblasts (HFF). **Materials and Methods:** Cytotoxicity was assessed using the MTT assay. The degree of synergy was assessed using the Combination Index (CI) and the Dose Reduction Index (DRI). Furthermore, the underlying molecular mechanisms were investigated using ELISA-based quantification of relevant biomarkers, including HIF-1 α , p-mTOR, Cathepsin D, LC3B-II, Cleaved Caspase-3, NF- κ B, HO-1, Bcl-2, and Bax. These findings were subsequently corroborated by in silico molecular docking studies targeting PDK1, SGLT2, Cathepsin D, MMP9, TRPV1, and COX-2. **Results:** The triple combination exhibited potent, synergistic cytotoxic effects on MCF-7 cells, as evidenced by a significantly lower IC₅₀ value (4.1 μ g/mL at 72 hours) and a high selective toxicity index (>243.9). Synergism was further substantiated by CI values. This combination treatment downregulated HIF-1 α and p-mTOR, inhibited Cathepsin D, activated autophagy (LC3B-II), and induced apoptosis (cleaved caspase-3). Furthermore, in HFF cells, it alleviated paclitaxel-induced stress and inflammation. Docking analyses showed favorable binding affinities of dapagliflozin to PDK1, SGLT2, and TRPV1, with docking scores of -8.4, -8.3, and -8.4 kcal/mol, respectively. Pantoprazole showed favorable binding affinities to cathepsin D1, MMP9, and COX-2, with docking scores of -8.3, -9.0, and -8.8 kcal/mol, respectively. **Conclusion:** The triple mixture exhibits potent, targeted anticancer effects by disrupting pathways that support cancer cell survival while safeguarding normal cells. This strategy offers a promising avenue to overcome paclitaxel resistance and minimize associated side effects.

Keywords: cancer, Paclitaxel chemoresistance, drug repurposing, Dapagliflozin, Pantoprazole, metabolic reprogramming

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1. Introduction

Worldwide, breast cancer remains one of the most common and dangerous types of cancer, which puts a significant burden on healthcare systems globally [1]. Despite advances, breast cancer remains a leading cause of death due to resistance and spread. Paclitaxel is a common treatment working by stabilizing microtubules and

inducing cell death in fast-dividing cells [2]. However, Its clinical usefulness is greatly limited by two main issues: frequent chemoresistance development and dose-limiting toxicities like neuropathy and myelosuppression [3]. These constraints diminish treatment effectiveness and detract from patient well-being, underscoring the critical

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need for novel approaches to overcome resistance while minimizing adverse consequences [4]. Recent studies have focused on drug repurposing and combination therapies. These approaches aim to improve the precision of treatments and overcome resistance by using strategies that target multiple pathways [5- 7].

Given the high costs and lengthy timelines of new drug creation, drug repurposing has become a practical, effective strategy in cancer treatment. It involves using approved drugs for new purposes, capitalizing on their safety data, pharmacokinetics, and faster development [8, 9]. This strategy is especially relevant in cancer treatment to quickly address resistance and side effects, bypassing the lengthy process of developing new drugs. Combining repurposed compounds with established chemotherapy agents like paclitaxel can produce synergistic effects, improve resistance, and reduce dose-related toxicities, thereby expanding therapeutic options [10]. In light of this reasoning, several medications exhibited these properties, including dapagliflozin and pantoprazole. Dapagliflozin, an SGLT2 inhibitor used in the treatment of diabetes, has exhibited off-target anticancer effects, including suppression of glycolytic metabolism and induction of metabolic stress in cancer cells [11]. At the same time, pantoprazole, a proton pump inhibitor (PPI), has shown potential to change the acidic environment of tumors. It also inhibits proton pumps in cancer cells (V-ATPase) and increases tumor sensitivity to chemotherapy [12, 13].

Cancer cells resist chemotherapy through a complex adaptive process involving metabolic changes and a protective tumor environment. Central to this is metabolic reprogramming, where cells switch from efficient oxidative phosphorylation to aerobic glycolysis the Warburg effect even with ample oxygen. This shift not only generates energy but also supplies building blocks for rapid growth and activates survival pathways [14]. Pyruvate Dehydrogenase Kinase 1 (PDK1) and mTOR are key players in this metabolic change. PDK1 blocks pyruvate from entering mitochondria, while mTOR, a vital kinase, interprets signals from nutrients and growth factors to control protein production and cell growth. Overactivation of this pathway often leads to chemoresistance by boosting cancer cell survival and reducing apoptosis [15].

The tumor microenvironment (TME) serves a dual role, functioning both as a shield and a facilitator of therapeutic resistance. Hypoxia, a condition frequently observed in solid tumors, induces stabilization of Hypoxia-Inducible Factor-1 α (HIF-1 α), which subsequently drives glycolysis, angiogenesis, and cellular survival. Furthermore, the TME undergoes protease-mediated modification, including by Cathepsin D and Matrix Metalloproteinase-9 (MMP9) [16- 18]. Cathepsin D, a lysosomal protease, is often overexpressed in aggressive cancers. This overexpression facilitates cancer spread and metastasis and also enables resistance to chemotherapy. It does this by breaking down components of the extracellular matrix and by blocking signals that would normally cause programmed cell death [19]. Likewise, MMP9 promotes tumor invasion and metastasis by degrading the basement

membrane; furthermore, it contributes to therapeutic resistance by modulating the availability of growth factors and the infiltration of immune cells [20].

This hostile microenvironment not only physically shields tumor cells but also creates biochemical barriers that hinder drug absorption and effectiveness. Addressing chemoresistance requires a multifaceted approach. One promising strategy is dual targeting, which involves simultaneously attacking the cancer cell's internal metabolism and disrupting its external protective microenvironment to induce synthetic lethality. Inhibiting metabolic centers like PDK1 can cut off energy and biosynthetic support, while targeting factors like Cathepsin D and MMP9 can block invasion, restore apoptosis, and improve drug delivery. This combined approach aims to overcome redundant survival pathways, potentially re-sensitizing tumors to traditional chemotherapy such as paclitaxel.

Paclitaxel is a key chemotherapy drug used to treat breast cancer. It primarily acts by disrupting microtubule dynamics, thereby preventing cell division [21]. Despite its effectiveness, the clinical success of this treatment is often reduced by the development of resistance to chemotherapy and serious side effects. These side effects, such as neuropathy, significantly affect patients' quality of life [22]. In conjunction with this concept, multiple studies were conducted to mitigate the adverse effects of paclitaxel and to reduce the development of chemoresistance. One such study demonstrated that targeting the glycolytic pathway, specifically, the use of dichloroacetate, a PDK1 inhibitor, can effectively re-sensitize resistant cancer cells by reversing the Warburg effect and inducing apoptosis [23]. Concurrently, Clinical trials assessing the combined use of esomeprazole, a proton pump inhibitor, have demonstrated enhanced chemotherapeutic effectiveness and a reduction in neuropathy, possibly through mechanisms involving P-glycoprotein blockade and tumor pH modulation [24].

Despite this trial, the study gap remains regarding the use of triple-combination therapy. We propose a mixture of paclitaxel, dapagliflozin, and pantoprazole as a potentially effective therapeutic approach, achieved through a multi-targeted mechanism. Paclitaxel directly disrupts the cytoskeleton, whereas dapagliflozin, an SGLT2 inhibitor, exploits the metabolic vulnerability of neoplastic cells by inhibiting glucose uptake, a critical process for their survival and treatment resistance [25]. At the same time, Pantoprazole, a proton pump inhibitor, is believed to alter the tumor microenvironment. This modification may impede key proteases involved in tumor invasion and reduce the inflammatory responses associated with chemotherapy [26].

This research aims to clarify the mechanistic interactions among paclitaxel, dapagliflozin, and pantoprazole. It thoroughly investigates their underlying mechanisms to enhance understanding of their anticancer effects, potential synergy, selectivity, and safety in breast cancer. Furthermore, the study examines the potential cytoprotective roles of dapagliflozin and pantoprazole in reducing complications associated with paclitaxel and in

preventing the development of chemoresistance.

2. Materials and Methods

2.1. MTT assay

2.1.1 Study Drugs and Tissue Culture

The study medications, including dapagliflozin, pantoprazole, and paclitaxel, were obtained as raw materials from the Samarra Pharmaceutical Factory in Iraq. Each drug, individually, and a mixture of these drugs in equal parts (1:1:1) were prepared and diluted in Minimum Essential Medium (MEM) to achieve concentrations ranging from 0.1 to 1000 µg/mL for each drug and for the triple mixture.

The cytotoxic effects of the compounds were assessed in the MCF7 breast cancer cell line to explore their potential anticancer activity and in normal human foreskin fibroblasts (HFF cells) as a healthy-tissue control to establish safety profiles and evaluate the cytotoxicity of the triple combination in normal healthy cells. To identify any possible harmful drug interactions within the mixture that might occur due to pharmaceutical interactions.

The main goal of this study is to investigate the combined effects of three compounds as anticancer agents by using lower concentrations of each and comparing their overall impact to that of each substance alone. Additionally, the research aims to evaluate the safety of the triple mixture on healthy cells by examining its potential to mitigate paclitaxel's side effects at reduced concentrations. Paclitaxel's role here was twofold: it served as a positive control for comparison with the mixture's impact and as an ingredient of the mix.

The Tissue Culture Unit at the Iraqi Centre for Cancer and Medical Genetics Research at Al-Mustansiriyah University provides these cell lines, which are authorized for supply.

2.1.2. Cell Culture Models and Maintenance

The study used two cell lines, including the MCF7 breast cancer cell line at passage 21 [27, 28]. The healthy, normal cell line (HFF) is derived from human foreskin fibroblasts, with passage (No 10) [29]. MEM medium from US Biological (USA) was employed for cell culture, supplemented with 10% (v/v) fetal bovine serum (FBS) sourced from Capricorn Scientific (Germany), and containing 100 IU/mL penicillin alongside 100 µg/mL streptomycin to inhibit bacterial contamination. The cells were maintained in a humidified incubator at 37°C during exponential growth [30].

2.1.3. Cell Viability and Cytotoxicity Assay

The MTT assay is frequently used as a colourimetric method to assess cellular metabolic viability. The underlying principle involves metabolically active cells reducing the tetrazolium salt MTT to insoluble purple formazan crystals, predominantly through mitochondrial dehydrogenase activity. Typically, cells are cultured in 96-well plates and exposed to varying concentrations of test compounds. Following incubation periods of 24 or 72

hours, MTT reagent is added, and incubation is continued to facilitate reduction in viable cells. The resulting formazan is solubilized, and its absorbance is quantified spectrophotometrically at a specific wavelength, serving as an objective indicator of cell viability [31].

This assay demonstrates a direct relationship between viable cell count and formazan production: application of the pharmacological agent decreases formazan levels, indicating cytotoxic effects, as evidenced by reduced absorbance. The generated dose-response curve enables calculation of the IC₅₀ value, defined as the drug concentration that reduces cell viability by 50%. This analysis was performed using GraphPad Prism software (version 9.5.0, build) [32, 33].

Cells were seeded at 10,000 cells per well in 96-well plates and incubated at 37°C for 24 hours to achieve cellular confluence. To assess the cytotoxic effects of paclitaxel, dapagliflozin, pantoprazole, and their triple combination, an MTT assay was used. Each concentration was tested in six replicate wells, with cells exposed to 0.1, 1, 10, 100, and 1000 µg/mL of each drug or the mixture. A solvent control containing 0.1% DMSO (the same concentration present in every drug-treated well) was incorporated into all experimental runs. This 0.1% DMSO carrier control did not significantly affect cellular viability in either the MCF-7 or HFF cell lines relative to untreated control groups (no statistically significant difference, $p > 0.05$; as shown in Supplementary Table 1. Untreated controls (culture medium only) were also included. After 24 and 72 hours of incubation, MTT reagent was added, and formazan crystals were dissolved. Absorbance was measured at 570 nm using a microplate reader. Detailed experimental parameters (volumes, incubation times, and solubilization steps) are provided in Supplementary Table 2. Growth inhibition was calculated using the following equation [34].

Growth inhibition % = (optical density of control wells - optical density of treated wells) / (optical density of control wells) * 100%

2.2. Evaluation of the Selective Toxicity Index

The selective toxicity index quantified the extent to which the triple mixture targeted cancer cells relative to paclitaxel. It evaluated the effects on cancer cell lines at 24 and 72 hours post-treatment. The IC₅₀ values for both treatments were calculated, and the selective cytotoxicity index was derived from growth curves of MCF7 cancer cells and normal human foreskin fibroblasts (HFF) [35], as demonstrated by the following mathematical expression.

Selective toxicity Index (SI) = (IC₅₀ of normal cell lines) / (IC₅₀ of cancer cell lines)

An SI score greater than 1.0 indicates that the drug targets tumor cells more effectively than normal cells.

2.3. Analysis of Drug Synergism: Combination Index and Dose Reduction Index

Compusyn, a computational simulation tool, was employed to determine the combination index

(CI) and dose-reduction index (DRI). The primary purpose of calculating the CI was to assess whether the mixture's components worked together synergistically, additively, or antagonistically. This was evaluated using concentration-effect curves, which showed the percentage of cell growth inhibition at various drug concentrations at 24 and 72 hours. A CI below 1 indicates synergy, exactly 1 indicates an additive effect, and above 1 suggests antagonism. All analyses were conducted using Compusyn software (Biosoft, Ferguson, MO) [36, 37].

The Dose Reduction Index (DRI) is a key measure for evaluating the effectiveness of drug combinations. It indicates the maximum drop in each drug's concentration that still achieves the desired cell-killing effect. A DRI value above 1 indicates a favorable reduction in dosage, whereas a value below 1 indicates an undesirable decrease. These DRI values were obtained using Compusyn software (Biosoft, Ferguson, MO) [36, 37].

2.4. Molecular Validation of Anticancer Mechanisms, Synergy, and Selective Safety

To investigate the anticancer mechanisms of the triple mixture, assess whether the constituent medications exhibit synergistic effects, and evaluate the safety profile of the triple mixture, a triple-molecular validation approach was employed (ELISA, in silico molecular docking, and in silico prediction of pharmacokinetic drug-drug interactions).

2.4.1. ELISA Biomarker Quantification

To evaluate the anticancer mechanism of the triple mixture and determine whether the drug combination acts synergistically to more effectively kill cancer cells while sparing normal cells, we selected two distinct panels of molecular targets. This choice was based on the hypothesis that the triple mixture can overcome cancer cells' resistance through metabolic reprogramming and modulation of the tumor microenvironment, while also protecting normal healthy cells.

The molecular targets in panel one, detected in the MCF-7 cell line, were used to illustrate the potential underlying mechanism of synergy among mixture ingredients and to enhance chemosensitization in MCF-7 cells. This panel targets pathways known to drive breast cancer progression, therapy resistance, and survival under metabolic stress. Included are Hypoxia-Inducible Factor 1-alpha (HIF-1 α), Phosphorylated Mammalian Target of Rapamycin (p-mTOR), Cathepsin D, Microtubule-associated protein 1A/1B-light chain 3B-II (LC3B-II), and Cleaved Caspase-3, the activated executioner caspase, providing a direct readout of apoptotic cell death.

The molecular targets in panel two, detected in the HFF cell line, were used to illustrate the mixture's cytoprotective ability. These molecular targets were used to assess markers of cellular stress, inflammation, and apoptosis to evaluate the protective effect of the combination against paclitaxel-induced toxicity in normal cells, including Nuclear Factor Kappa B p65 (NF- κ B p65), Heme Oxygenase-1 (HO-1), and (Bax/Bcl-2 ratio).

a. Cell Treatment and Lysate Preparation

MCF-7 and HFF cells were seeded at 5×10^5 cells per flask and allowed to adhere overnight before being subjected to various treatment concentrations for 72 hours, aligning with the time point of maximal synergistic effect observed in cytotoxicity assays. The experimental conditions included an untreated control in MEM medium containing 0.1% DMSO (vehicle) and paclitaxel monotherapy at its IC₅₀ concentration for MCF-7 cells (212.5 μ g/mL), corresponding to its 24-hour IC₅₀ in MCF-7 cells. This concentration was selected to represent the maximal cytotoxic potency of paclitaxel before the onset of the marked resistance observed at 72h. a triple combination at a synergistic concentration of 1 μ g/mL for the mixture (each drug at 0.33 μ g/mL), and monotherapies with dapagliflozin (0.33 μ g/mL) and pantoprazole (0.33 μ g/mL) as single-agent controls.

Following incubation, cells were washed twice with cold phosphate-buffered saline (PBS) and lysed on ice for 30 minutes in RIPA lysis buffer (Thermo Fisher Scientific, USA) supplemented with 1% protease and phosphatase inhibitors (Sigma-Aldrich, USA). The lysates were then centrifuged at $14,000 \times g$ for 15 minutes at 4°C, and the supernatants were collected. The total protein concentration of each sample was quantified using a bicinchoninic acid (BCA) assay kit (Pierce, USA) in accordance with the manufacturer's instructions, to facilitate normalization in subsequent ELISA procedures [38, 39].

b. Enzyme-Linked Immunosorbent Assay (ELISA) Procedure

Commercially available high-sensitivity sandwich ELISA kits were used to quantitatively determine each target protein. The specific kits employed included: Human HIF-1 α ELISA Kit (ABCam, ab234579), Human Phospho-mTOR (Ser2448) ELISA Kit (R&D Systems, DY3887), Human Cathepsin D ELISA Kit (Invitrogen, EHCDD), Human LC3B II ELISA Kit (MyBioSource, MBS2601325), Human Cleaved Caspase-3 (Asp175) ELISA Kit (Cell Signaling Technology, #7231), Human NF- κ B p65 ELISA Kit (Thermo Fisher Scientific, BMS228INST), Human HO-1 ELISA Kit (Enzo Life Sciences, ADI-960-071), and Human Bcl-2/Bax ELISA Kit (Sigma-Aldrich, RAB0270). All procedures were performed strictly in accordance with the manufacturer's guidelines. Protein lysates for each treatment condition were prepared from three independent biological replicates each derived from a distinct cell culture passage and drug treatment and analyzed in technical duplicates within the ELISA assays.

Briefly, 100 μ L of each standardized protein lysate (or provided standard) was aliquoted in duplicate into the designated antibody-coated 96-well microplates and incubated accordingly. Following comprehensive washing steps to eliminate unbound components, a horseradish peroxidase (HRP)-linked detection antibody specific to the target was introduced. After an additional wash, a tetramethylbenzidine (TMB) substrate was added, producing a blue coloration proportional to the target

protein quantity. The reaction was subsequently halted with sulfuric acid, resulting in a yellow coloration. The absorbance of each well was immediately recorded at 450 nm using a Synergy HTX multi-mode microplate reader (BioTek, USA). A standard curve derived from reference standards on each plate facilitated interpolation of sample concentrations using Gen5 software (BioTek, USA). Final protein concentrations were expressed in picograms (pg) or nanograms (ng) per milligram of total cellular protein [40, 41].

2.4.2. *In silico* molecular docking study

In line with the purpose of the ELIZA molecular validation, and to further support it, *in silico* molecular docking was conducted.

A. Study design

The structural models of dapagliflozin, pantoprazole, SB-705498, dichloroacetate, Marimastat, odanacatib, and celecoxib were developed and refined using ChemDraw (Cambridge Soft, USA) and Chem3D. The last five medications served as controls for comparison. These models aimed to elucidate the molecular basis of the proposed triple-mixture anticancer effect, assess the synergy among the mixture components, evaluate the potential of dapagliflozin and pantoprazole to reduce paclitaxel chemoresistance, and explore how these agents could mitigate paclitaxel-associated adverse effects.

Guided by the overarching hypothesis that the combined use of paclitaxel, dapagliflozin, and pantoprazole can overcome chemoresistance through dual metabolic reprogramming and modulation of the tumor microenvironment, the selection of molecular docking targets was carefully considered. The primary objective was to computationally validate the mechanisms of synergy and selective safety. Accordingly, targets were selected to represent the fundamental pathways underlying paclitaxel resistance, metabolic adaptation, and protection of normal cells.

The selection of targets for the *in silico* molecular docking study was carefully aligned with the investigation of the potential underlying mechanisms of the triple-mixture anticancer impact, synergistic effects among the mixture ingredients, enhanced chemosensitivity, and an improved safety profile. In the case of dapagliflozin, targets were chosen to validate its hypothesized functions in metabolic reprogramming and cytoprotection. Docking assays against SGLT2 and PDK1 were conducted to evaluate the potential of these compounds to inhibit glucose uptake and alter cancer cell metabolism, thereby reversing glycolysis-driven chemoresistance and fostering a synergistic, synthetic lethal environment [23, 42]. Concurrently, the study also examined predicted binding to the Transient Receptor Potential Vanilloid 1 (TRPV1) receptor, which provided a mechanistic rationale for the observed modulating effect of dapagliflozin on paclitaxel, via a reduction in paclitaxel's neuropathic adverse effects [43].

For pantoprazole, specific targets were selected to investigate its role in disrupting the tumor

microenvironment and reducing toxicity. Docking studies against Cathepsin D were conducted to assess its potential to inhibit this critical lysosomal protease involved in invasion and evasion of apoptosis, thereby enhancing the effectiveness of paclitaxel-induced cell death. Analysis of MMP9 was conducted to explore its potential role in overcoming microenvironment-mediated resistance by suppressing matrix metalloproteinase activity, a known contributor to tumor progression and therapy resistance [44, 45]. Furthermore, the interaction with COX-2 was examined to establish a molecular basis for the drug's capacity to mitigate paclitaxel-related adverse effects, including inflammation and neuropathic pain, via cyclooxygenase-2 inhibition in normal tissues [46]. This targeted computational methodology explicitly linked docking simulations to fundamental biological hypotheses regarding efficacy and safety, thereby advancing the development of combination therapy.

The three-dimensional structures of these critical oncological targets were obtained from the Protein Data Bank (PDB codes: 5IRX for TRPV1, 7VSI for SGLT2, 4RRW for PDK1, 1GKC for MMP9, 1LYB for Cathepsin D, and 5KIR for COX-2). These structures were chosen for docking studies because they represent constitutively active, disease-associated conformations that are considered primary therapeutic targets in cancer research.

AutoDock Tools was used to examine key dependencies in oncogenesis. The process involved identifying optimal ligand conformations and generating PDBQT files for subsequent analysis. Molecular structures of the following ligands (dapagliflozin, pantoprazole, SB-705498, dichloroacetate, Marimastat, odanacatib, and celecoxib) and of the following receptors (TRPV1, SGLT2, PDK1, MMP9, Cathepsin D, and COX-2) were loaded into AutoDock Tools for docking. The resulting data, including binding energies and interaction profiles, were analyzed with BIOVIA Discovery Studio, UCSF Chimera, and AutoDock [47, 48].

B. Computational Evaluation of Binding Affinity and Therapeutic Potential

To assess the *in-silico* docking results in a clinically relevant setting, the binding affinities of each investigational drug (dapagliflozin and pantoprazole) were compared to those of a clinically validated reference inhibitor targeting the same protein. This comparison was conducted using an affinity analysis ratio [48]. Calculated as follows:

$$\text{Affinity Ratio} = |\Delta G_{\text{test}}| / |\Delta G_{\text{ref}}|$$

Where (ΔG_{test} and ΔG_{ref}) denote the computed Gibbs free energy of binding (in kcal/mol) for the test compound and its reference inhibitor, respectively, an affinity ratio above 1.0 signifies that the test compound is predicted to bind more strongly than the reference inhibitor.

A qualitative Synergistic Impact Score (SIS) was devised as an exploratory composite metric aimed at enabling comparative analysis of docking outcomes within the specialized mechanistic framework of this investigation. This score consolidates three principal

dimensions into a weighted system [48, 49].

$$\text{Synergistic Impact Score (SIS)} = \alpha \cdot S_{\text{affinity}} + \beta \cdot S_{\text{interactions}} + \gamma \cdot S_{\text{relevance}}$$

The component scores, each on a 1–10 scale, are defined as follows: S_{affinity} is based on the magnitude of the calculated binding energy (ΔG in kcal/mol), with more negative ΔG values resulting in higher scores; $S_{\text{interactions}}$ represents the complexity and stability of the molecular interaction profile, including factors such as the number and type of hydrogen bonds, hydrophobic contacts, and π -interactions; and $S_{\text{relevance}}$ reflects the presumed functional importance of the drug's target within the proposed triple-drug anticancer mechanism, which involves metabolic reprogramming, microenvironment modulation, or cytoprotection.

The weighting coefficients ($\alpha=0.5$, $\beta=0.3$, $\gamma=0.2$) were selected based on a considered rationale: binding affinity (α) is given priority as the primary physicochemical factor determining target engagement. Interaction complexity (β) is assigned a secondary weight, reflecting its influence on binding stability and specificity. Target relevance (γ) is allocated a lower weight to incorporate mechanistic context, recognizing that in silico docking alone cannot definitively establish functional biological effects. This specific weighting scheme was established a priori by the authors for this analysis and represents one of several possible interpretative frameworks. A higher final SIS score indicates a predicted greater potential contribution of the compound to overall synergistic effect, as modeled within this scheme.

The Synergistic Impact Score (SIS) represents an unverified, newly proposed index introduced exclusively for generating hypotheses and for the exploratory ordering of computational docking forecasts within the scope of this research. This metric lacks empirical substantiation and must not be regarded as a quantitative indicator of genuine biological cooperativity nor as a replacement for laboratory verification. The index serves solely as a means to rank computational hypotheses in preparation for subsequent empirical testing. Accordingly, readers should exercise due prudence when evaluating SIS outcomes.

C. In Silico Prediction of Pharmacokinetic Drug-Drug Interactions

The potential pharmacokinetic drug-drug interactions (DDIs) associated with the triple combination of paclitaxel, dapagliflozin, and pantoprazole were systematically examined through a consensus methodology that integrated literature mining with computational prediction tools. Emphasis was placed on interactions involving cytochrome P450 (CYP) enzymes (namely CYP2C8, CYP3A4, and CYP2C19) and the ATP-binding cassette efflux transporter P-glycoprotein (P-gp/ABCB1), given their critical roles in the metabolism and transport processes of the drugs under investigation.

An extensive review of the primary literature and prescribing information for each drug was conducted to determine their roles as substrates, inhibitors, or

inducers of metabolic enzymes and transporters. These profiles were then cross-referenced and analyzed with the Liverpool Drug Interaction Group database (University of Liverpool, UK), a respected online resource for predicting drug-drug interactions in oncology and other therapeutic areas [50]. For each potential drug pair in the combination, the database was queried to assess the direction (inhibition or induction), magnitude, and clinical significance of the interaction.

The probability of an interaction was classified as Probable, Possible, or Unlikely based on the strength of in vitro evidence, established mechanistic pathways, and existing clinical data. Subsequently, the predicted clinical outcome, such as increased drug exposure or increased risk of toxicity, was inferred. This in silico analysis aimed to establish a pharmacokinetic context for the observed in vitro synergy and to identify potential safety concerns.

2.5. Analysis of Drug-Induced Morphological Changes

The morphological changes in MCF7 and HFF cells were documented after 72 hours of exposure to the test compounds, using inverted light microscopy.

2.6. Ethical approval

This study was conducted exclusively using in vitro cell line models and did not involve human participants or laboratory animals. Nonetheless, all procedures adhered strictly to our institution's ethical standards for laboratory research.

2.7. Statistical Analysis

All experiments were performed with the appropriate number of biological and technical replicates as specified in each method section. Quantitative data from cytotoxicity assays (MTT) and ELISAs are expressed as the mean $\pm$ standard deviation (SD). One-way ANOVA was used to assess significant differences among multiple treatment groups, such as different drug concentrations or comparisons between single agents and their combinations. Post hoc analysis using the Least Significant Difference (LSD) test was conducted for pairwise comparisons, particularly in dose-response studies. For ELISA measurements of biomarker levels across various treatment conditions, one-way ANOVA followed by Tukey's post hoc test was employed for multiple comparisons. A p-value of less than 0.05 was regarded as statistically significant. All statistical analyses and graphical representations of dose-response curves, including the determination of half-maximal inhibitory concentration (IC_{50}) values, were conducted using GraphPad Prism software (version 9.5.0) [33, 51].

This study uses uppercase and lowercase letters in data tables to differentiate between statistical groups and significance levels. The same letters mean no significant difference, while different letters indicate significant differences. Specifically, capital letters compare column means, and lowercase letters compare row means. This method provides a clear and efficient way to present complex statistical data, allowing readers to interpret the results quickly without needing detailed explanations.

3. Results

3.1. Cytotoxic assay

3.1.1. Cytotoxic Efficacy of (paclitaxel, dapagliflozin, and pantoprazole) mixture

The cytotoxic effect of the mixture was assessed against MCF-7 breast cancer cells and HFF normal fibroblasts. The mixture exhibited a concentration- and time-dependent inhibition of cellular proliferation in both cell lines. After 24 hours of exposure, the MCF7 cell line showed significant growth inhibition, with values increasing from $13 \pm 1.2\%$ at $0.1 \mu\text{g/ml}$ to $76 \pm 4.8\%$ at $1000 \mu\text{g/ml}$. This effect was more pronounced after 72 hours, with inhibition increasing from $27 \pm 2.1\%$ to $86 \pm 5.1\%$ across the same concentration range.

The estimated IC_{50} value for MCF7 cells decreased from $42.8 \mu\text{g/ml}$ at 24 hours to $4.1 \mu\text{g/ml}$ at 72 hours, indicating a significant increase in mixture potency with increasing incubation time. On the other hand, HFF cells were significantly less sensitive to the mixture, with only $24 \pm 2.5\%$ inhibition at 72 hours at the highest concentration, and IC_{50} values remained $> 1000 \mu\text{g/ml}$ at both time points (Table 1 and 2).

3.1.2. Cytotoxicity of individual mixture medications

The study thoroughly assesses the cytotoxicity of paclitaxel, dapagliflozin, and pantoprazole, as well as their interactions when combined. It also explores whether their combined effects are synergistic, antagonistic, or simply additive in enhancing anticancer activity.

A. Cytotoxicity of paclitaxel

The effect of paclitaxel on cellular proliferation was evaluated across MCF7 and HFF cell lines, exhibiting a response that is specific to cell type. In MCF7 cells, the inhibitory effect was concentration-dependent but did not persist over the observed time course. An initial significant inhibition at 24 hours (e.g., 62% at $1000 \mu\text{g/ml}$) diminished by 72 hours (39% at the same concentration), indicating the emergence of resistance to paclitaxel. Consequently, the IC_{50} increased from $212.5 \mu\text{g/ml}$ at 24 hours to greater than $1000 \mu\text{g/ml}$ at 72 hours. Conversely,

HFF cells demonstrated a classical pattern of time- and concentration-dependent cytotoxicity, with inhibition levels increasing over time (e.g., from 62% to 76% at $1000 \mu\text{g/ml}$) and the IC_{50} decreasing from $253.3 \mu\text{g/ml}$ to $72.6 \mu\text{g/ml}$. Statistical analysis confirmed significance ($p < 0.05$) across all concentrations within each time point, implying a narrow therapeutic window for paclitaxel (Table 3).

B. dapagliflozin Cytotoxicity

The SGLT2 inhibitor dapagliflozin, when tested for cytotoxicity alone, displayed moderate cytotoxicity against MCF7 cells. Its effects were both concentration- and time-dependent, with maximal inhibition reaching $49 \pm 3.8\%$ and $59 \pm 4.2\%$ after 24 and 72 hours at $1000 \mu\text{g/ml}$, respectively. The IC_{50} values were high, exceeding $1000 \mu\text{g/ml}$ at 24 hours and decreasing to $357.1 \mu\text{g/ml}$ at 72 hours. This pattern of inhibition suggests that, while dapagliflozin exhibits intrinsic anti-proliferative properties against breast cancer cells, yet, its potency as a standalone agent is relatively low compared with conventional chemotherapy (Table 4).

C. pantoprazole cytotoxicity

Study outcomes revealed that pantoprazole exhibited a dose- and time-dependent cytotoxic effect on MCF7 cells. Its growth-inhibitory effect was slightly less than that of dapagliflozin at comparable concentrations and durations, with a higher inhibition of $53 \pm 4.1\%$ at $1000 \mu\text{g/ml}$ after 72 hours. The IC_{50} values were the highest among the individual components, remaining above $1000 \mu\text{g/ml}$ at 24 hours and declining to $812.5 \mu\text{g/ml}$ after 72 hours (Table 5).

3.1.3. Comparison of cytotoxicity among paclitaxel, dapagliflozin, pantoprazole, and the triple mixture

The outcomes of the comparison among the mixture and each of its ingredients revealed that the triple mixture demonstrated superior cytotoxic efficacy against MCF-7 breast cancer cells compared with individual monotherapies at both 24 and 72 hours. At 24 hours, inhibition ranged from 13% to 76% across various concentrations, significantly exceeding the effects of

Table 1. The Cytotoxic Impact of the Mixture on Survival Rates of MCF7 and HFF Cell Lines at 24 and 72 Hours.

Con. ($\mu\text{g/ml}$)	Cellular proliferation inhibition (mean $\pm$ SD)					
	MCF7 cell line			HFF cell line		
	24 hr.	72 hr.	P- value	24 hr.	72 hr.	P- value
0.1	E 13 ± 1.2	E 27 ± 2.1	0.012*	D 0 ± 0.5	E 2 ± 0.8	0.102
1	D 28 ± 2.3	D 46 ± 3.1	0.005*	D 3 ± 0.7	D 8 ± 1.2	0.024*
10	C 41 ± 3.1	C 56 ± 3.8	0.008*	C 7 ± 1.1	C 13 ± 1.6	0.015*
100	B 62 ± 4.2	B 72 ± 4.5	0.048*	B 12 ± 1.8	B 19 ± 2.1	0.019*
1000	A 76 ± 4.8	A 86 ± 5.1	0.025*	A 17 ± 2.0	A 24 ± 2.5	0.011*
IC 50	$42.8 \mu\text{g/ml}$	$4.1 \mu\text{g/ml}$	-	$>1000 \mu\text{g/ml}$	$>1000 \mu\text{g/ml}$	-
LSD value	4.1	5.2	-	2.8	3.4	-

Data are presented as the average with standard deviation (SD) for cellular proliferation inhibition (%). Within each column, different uppercase letters (e.g., A, B, C) indicate statistically significant differences ($p < 0.05$) among concentrations for each cell line and time point, as determined by one-way ANOVA followed by LSD post-hoc testing. IC_{50} values were calculated from dose-response curves. An asterisk (*) signifies statistical significance at $p < 0.05$.

Table 2. A Comparison of the Cytotoxic Effects of the Mixture on MCF7 and HFF Cells.

Con. (µg/ml)	Cellular proliferation inhibition (mean ± SD)					
	24 hr.			72 hr.		
	MCF7 cell line	HFF cell line	P- value	MCF7 cell line	HFF cell line	P- value
0.1	E 13 ± 1.2	D 0 ± 0.5	< 0.001*	E 27 ± 2.1	E 2 ± 0.8	< 0.001*
1	D 28 ± 2.3	D 3 ± 0.7	< 0.001*	D 46 ± 3.1	D 8 ± 1.2	< 0.001*
10	C 41 ± 3.1	C 7 ± 1.1	< 0.001*	C 56 ± 3.8	C 13 ± 1.6	< 0.001*
100	B 62 ± 4.2	B 12 ± 1.8	< 0.001*	B 72 ± 4.5	B 19 ± 2.1	< 0.001*
1000	A 76 ± 4.8	A 17 ± 2.0	< 0.001*	A 86 ± 5.1	A 24 ± 2.5	< 0.001*
IC 50	42.8 µg/ml	>1000 µg/ml	-	4.1 µg/ml	>1000 µg/ml	-
LSD value	4.1	2.8	-	5.2	3.4	-

Data are presented as the average with standard deviation (SD) for cellular proliferation inhibition (%). Within each column, different uppercase letters (e.g., A, B, C) indicate statistically significant differences ($p < 0.05$) among concentrations for each cell line and time point, as determined by one-way ANOVA followed by LSD post-hoc testing. IC₅₀ values were calculated from dose-response curves. An asterisk (*) signifies statistical significance at $p < 0.05$.

paclitaxel, dapagliflozin, and pantoprazole at equivalent doses, with an IC₅₀ of 42.8 µg/mL. By 72 hours, its potency markedly increased, achieving 86% inhibition at 1000 µg/mL and a substantially lower IC₅₀ of 4.1 µg/mL. In contrast, the individual agents showed diminished efficacy, with paclitaxel's IC₅₀ exceeding 1000 µg/mL at 72 hours, suggesting potential resistance. Statistical analysis confirmed that the mixture's effects were significantly superior ($p < 0.05$) at all concentrations tested compared with each drug alone (Supplementary Table 3 and 4).

3.2. Selective toxicity assay

The selective toxicity index (SI) outcomes revealed a highly favorable profile for the triple-drug combination. Specifically, in MCF7 breast cancer cells, the IC₅₀ decreased from 42.8 µg/mL at 24 hours to 4.1 µg/mL at 72 hours, whereas it remained above 1000 µg/mL in normal HFF cells. This resulted in an exceptionally high SI exceeding 243.9 at 72 hours, demonstrating a strong and selective cytotoxic effect on cancer cells. In contrast, paclitaxel alone exhibited poor selectivity, with an SI of only 0.341 at 72 hours, indicating greater toxicity to normal cells (Supplementary Table 5).

3.3. Evaluation of Drug Synergism: Estimating the Combination Index and Dose Reduction Index

The synergistic potential of the triple-drug combination comprising paclitaxel, dapagliflozin, and pantoprazole was quantitatively assessed by employing the Chou-Talalay method, as implemented in Compusyn. The Combination Index (CI) and Dose Reduction Index (DRI) were estimated at 24 and 72 hours of incubation across the concentration range. A CI value less than 1 revealed synergism, with lower values corresponding to stronger synergistic effects. A DRI value greater than 1 indicates a favourable reduction in the dose required to achieve a given cytotoxic effect, thereby suggesting a potential decrease in drug-related toxicity.

After 24 hours of incubation, the combination exhibited a concentration-dependent synergistic profile (Supplementary Table 6). At the lowest tested mixture concentration (0.1 µg/ml), a CI of 0.00705 was estimated, indicating a very strong synergism, particularly at mixture concentrations of 1 µg/ml and 10 µg/ml. At 100 µg/ml, the interaction remained strongly synergistic, whereas at the highest concentration (1000 µg/ml), the CI value of 0.58446 indicated a synergistic combination. The corresponding DRI values were substantial across all drugs at 24 hours, with particularly high values observed

Table 3. The Effect of Paclitaxel on the Survival Rates of MCF7 and HFF Cell Lines at 24 and 72 Hours.

Con. (µg/ml)	Cellular proliferation inhibition (mean ± SD)					
	MCF7 cell line			HFF cell line		
	24 hr.	72 hr.	P- value	24 hr.	72 hr.	P- value
0.1	E 11 ± 1.5	E 2 ± 0.8	0.018*	E 7 ± 1.1	E 14 ± 1.8	0.021*
1	D 18 ± 2.1*	D 7 ± 1.2	0.009*	D 16 ± 1.9	D 26 ± 2.3*	0.011*
10	C 24 ± 2.8*	C 16 ± 2.1*	0.023*	C 25 ± 2.4*	C 32 ± 3.0*	0.034*
100	B 47 ± 3.9*	B 33 ± 3.2*	0.015*	B 46 ± 3.5*	B 57 ± 4.1*	0.027*
1000	A 62 ± 4.5*	A 39 ± 3.8*	0.002*	A 62 ± 4.2*	A 76 ± 4.8*	0.014*
IC 50	212.5 µg/ml	>1000 µg/ml	-	253.3 µg/ml	72.6 µg/ml	-
LSD value	5.1	3.9	-	4.2	5.3	-

Data are presented as the average with standard deviation (SD) for cellular proliferation inhibition (%). Within each column, different uppercase letters (e.g., A, B, C) indicate statistically significant differences ($p < 0.05$) among concentrations for each cell line and time point, as determined by one-way ANOVA followed by LSD post-hoc testing. IC₅₀ values were calculated from dose-response curves. An asterisk (*) signifies statistical significance at $p < 0.05$.

Table 4. The Effect of Dapagliflozin on the Survival Rates of Breast Cancer Cells at 24 and 72 Hours.

Concentration ($\mu\text{g/ml}$)	Cellular proliferation inhibition (mean $\pm$ SD)		P- value
	24 hr.	72 hr.	
0.1	E 0 $\pm$ 0.5	E 4 $\pm$ 1.1	0.052
1	D 9 $\pm$ 1.4	D 17 $\pm$ 2.0	0.011*
10	C 15 $\pm$ 2.1	C 29 $\pm$ 2.8	0.006*
100	B 37 $\pm$ 3.2	B 45 $\pm$ 3.6	0.037*
1000	A 49 $\pm$ 3.8	A 59 $\pm$ 4.2	0.028*
IC 50	>1000 $\mu\text{g/ml}$	357.1 $\mu\text{g/ml}$	—
LSD value	2.35	3.25	-

Data are presented as the average with standard deviation (SD) for cellular proliferation inhibition (%). Within each column, different uppercase letters (e.g., A, B, C) indicate statistically significant differences ($p < 0.05$) among concentrations for each cell line and time point, as determined by one-way ANOVA followed by LSD post-hoc testing. IC₅₀ values were calculated from dose-response curves. An asterisk (*) signifies statistical significance at $p < 0.05$.

for dapagliflozin (e.g., 1041.41 at a 0.1 $\mu\text{g/ml}$ mixture) and pantoprazole (e.g., 416.871 at a 0.1 $\mu\text{g/ml}$ mixture), indicating a significant potential to reduce individual drug concentrations.

The synergistic effect was significantly enhanced and persisted longer. After 72 hours of incubation, the triple combination exhibited very strong synergy throughout all tested concentrations. The CI values ranged from 0.00611 to 0.02148, indicating that the synergistic interaction became more powerful and consistent with extended exposure. Additionally, the DRI values at 72 hours remained notably high, particularly for pantoprazole and paclitaxel, highlighting the sustained dose-reduction benefits (Supplementary Table 6, Figure 1).

3.4. Molecular Validation of Anticancer Mechanisms, Synergy, chemosensitivity, and cytoprotective impact

3.4.1. ELISA Biomarker Quantification

To elucidate the molecular mechanisms underlying the synergistic anticancer activity, chemosensitivity, and cytoprotective effects of the triple mixture, a panel of key protein biomarkers was assessed in MCF-7 and HFF cells using ELISA.

A. In MCF-7 Cells

In MCF-7 breast cancer cells, the triple mixture produced a pronounced modulatory effect compared with individual treatments. The outcome showed that paclitaxel alone significantly increased HIF-1 α and p-mTOR levels compared with untreated control cells. On the other hand, the triple mixture minimized these pro-survival signals, reducing HIF-1 α to 280.6 ± 18.9 pg/mg and p-mTOR to 185.4 ± 15.2 pg/mg, significantly lower than in all other treatment groups.

Furthermore, markers associated with tumor progression and therapy resistance exhibited a similar pattern. The lysosomal protease Cathepsin D, associated with invasion and evasion of apoptosis, was highest with paclitaxel monotherapy. The triple mixture reduced expression by approximately 50% relative to paclitaxel alone. In addition, the triple mixture uniquely enhanced autophagy by significantly elevating the autophagosome marker LC3B-II (210.5 ± 12.6 ng/mg), which was more than double that in the paclitaxel-treated group.

Most importantly, the triple mixture potently induced apoptosis. The level of the executioner caspase, Cleaved Caspase-3, was markedly elevated in cells treated with the triple mixture (110.8 ± 9.4 pg/mg), significantly exceeding that induced by paclitaxel monotherapy (45.6 ± 4.3 pg/mg). Monotherapies with low-dose dapagliflozin or pantoprazole alone showed biomarker profiles largely

Table 5. The Impact of Pantoprazole on the Survival of Breast Cancer Cells After 24 and 72 Hours.

Concentration ($\mu\text{g/ml}$)	Cellular proliferation inhibition (mean $\pm$ SD)		P- value
	24 hr.	72 hr.	
0.1	D 2 $\pm$ 0.8	E 5 $\pm$ 1.2	0.072
1	D 4 $\pm$ 1.0	D 11 $\pm$ 1.8	0.019*
10	C 16 $\pm$ 2.2	C 20 $\pm$ 2.5	0.124
100	B 22 $\pm$ 2.8	B 37 $\pm$ 3.3	0.009*
1000	A 46 $\pm$ 3.8	A 53 $\pm$ 4.1	0.044*
IC 50	>1000 $\mu\text{g/ml}$	812.5 $\mu\text{g/ml}$	—
^b LSD value	2.85	3.15	-

Data are presented as the average with standard deviation (SD) for cellular proliferation inhibition (%). Within each column, different uppercase letters (e.g., A, B, C) indicate statistically significant differences ($p < 0.05$) among concentrations for each cell line and time point, as determined by one-way ANOVA followed by LSD post-hoc testing. IC₅₀ values were calculated from dose-response curves. An asterisk (*) signifies statistical significance at $p < 0.05$.

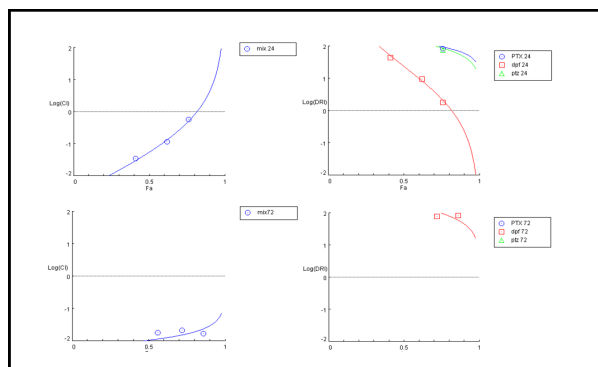


Figure 1. The Left Panel Shows the Log of the Combination Index (CI), and the Right Panel Shows the Log of the Dose Reduction Index (DRI) for the Triple Mixture at 24 Hours (above) and 72 Hours (below) of Incubation. PTX: Paclitaxel, dpf: Dapagliflozin, ptz: Pantoprazole.

similar to those of the untreated control.

Collectively, these results indicate that the triple mixture concurrently disrupts oncogenic signaling (HIF-1 α , p-mTOR), inhibits invasion-associated proteins (Cathepsin D), activates autophagy (LC3B-II), and induces apoptotic cell death (Cleaved Caspase-3) compared with other treatment groups in MCF-7 cells. (Supplementary Table 7).

B. in HFF Cells

The safety assessment of the treatments was conducted using human foreskin fibroblasts (HFF) by evaluating indicators of inflammation, oxidative stress, and cell death. Findings indicated that Paclitaxel alone significantly induces cellular stress in normal HFF cells, as demonstrated by a marked increase in the inflammatory marker NF- κ B p65, a notable elevation in the antioxidant enzyme HO-1 (65.8 ± 5.9 ng/mg), and alterations in mitochondrial function associated with apoptosis. Notably,

the Bax/Bcl-2 ratio, an indicator of cellular susceptibility to apoptosis, increased sharply in cells treated with paclitaxel.

Remarkably, normal cells exposed to the triple mixture exhibited a biomarker profile that was relatively similar to that of untreated, healthy cells. Levels of NF- κ B p65, HO-1, Bcl-2, and Bax in the triple mixture group were not significantly different from those in the control, dapagliflozin-only, and pantoprazole-only groups. Consequently, the Bax/Bcl-2 ratio remained low, comparable to the baseline ratio in untreated cells (Supplementary Table 8).

3.5. Molecular docking studies

In addition to the ELISA findings, the study provided further insights into the potential synergistic anticancer effects among the ingredients in the triple mixture. It also examined how dapagliflozin and pantoprazole may reduce the risk of paclitaxel chemoresistance and mitigate its associated adverse effects. This was proved by investigating the interactions of dapagliflozin and pantoprazole with six key molecular targets implicated in cancer: (TRPV1, SGLT2, PDK1, MMP9, Cathepsin D, and COX-2). These targets were selected for their structural stability and their importance in disease pathways, underscoring their potential as therapeutic targets. The analysis employed established computational tools, including AutoDock Tools 1.5.7, BIOVIA Discovery Studio, UCSF Chimera, and AutoDock, to provide the methodological basis [53].

3.5.1. Docking analysis to determine the synergy within the triple mixture

To evaluate the ability of dapagliflozin and pantoprazole to enhance the anticancer impact of paclitaxel synergistically, their binding affinities for PDK1 and cathepsin D, respectively, were assessed. Dapagliflozin

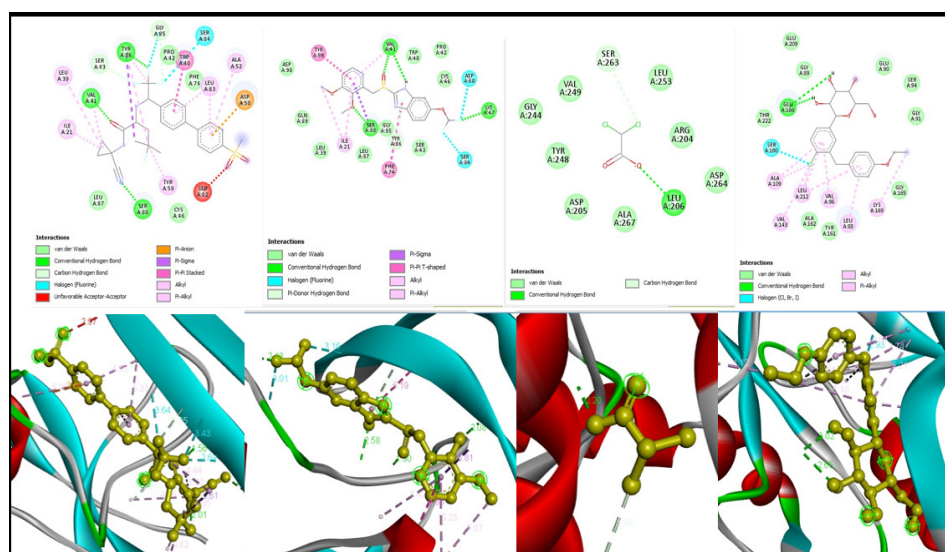


Figure 2. The Structural Configurations of the PDK1 and Cathepsin D Binding Sites are Presented in Both Two-dimensional (Top Image) and Three-dimensional (Bottom image) Perspectives. These illustrations depict the binding interactions of dapagliflozin (first image on the right) and dichloroacetate (second image on the right) with the PDK1 subunit, and of pantoprazole (third image on the right) and odanacatib (fourth image on the right) with the cathepsin D subunit.

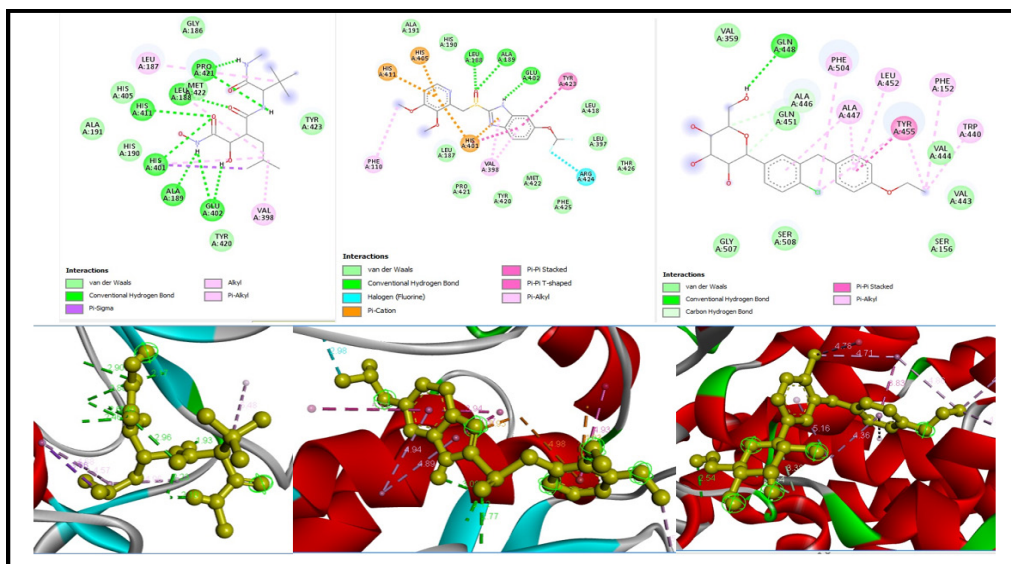


Figure 3. The Structural Configurations of the SGLT2 and MMP9 Binding Sites are Shown in Both Two-dimensional (Top Image) and Three-dimensional (Bottom Image) Views. These illustrations depict the binding interactions of dapagliflozin (1st image on the right) with the SGLT2 subunit and of pantoprazole (2nd image on the right) and Marimastat (3rd image on the right) with the MMP9 subunit.

exhibited the highest affinity for PDK1, with a docking score of -8.4 kcal/mol. The interaction profile reveals two conventional hydrogen bonds, one with GLU A:166 at 2.65 Å and another at 3.01 Å. A halogen bond interaction is observed with SER A:160 at a distance of 2.93 Å. Four alkyl bonds are observed involving residues ALA A:109, VAL A:143, LEU A:212, and LYS A:169 at distances of 3.74 Å, 4.75 Å, 4.74 Å, and 4.11 Å, respectively. Finally, five pi-alkyl bonds are observed involving residues VAL A:96, ALA A:109, LEU A:188, and two residues of LEU A:212 at distances of 4.15 Å, 5.20 Å, 4.54 Å, 5.10 Å, and 5.29 Å, respectively (Figure 2).

For comparison, a molecular docking study was performed with Dichloroacetate, a PDK1 inhibitor [54]. The molecular docking simulation with PDK1 yielded a binding affinity of -4.2 kcal/mol. Analysis of the interaction profile revealed one carbon-hydrogen bond involving residue LEU A:206 at a distance of 2.20 Å. Furthermore, one carbon-hydrogen bond was observed with residue SER A:263 at a distance of 3.30 (Figure 2).

Consistent with this concept, another component, pantoprazole, exhibited the highest affinity for cathepsin D, with a docking score of -8.3 kcal/mol. The interaction profile reveals three conventional hydrogen bonds, with LYS A:47 at 2.77 Å, SER A:88 at 2.07 Å, and two residues of VAL A:41 at 1.89 Å and 2.57 Å. Two fluorine atoms are bound to residues of ASP A:50 at 3.01 Å and SER A:84 at 3.15 Å. One hydrogen bond is present with the residue of TYR A:86 at 3.24 Å. Finally, six hydrophobic bonds are present with residues of SER A:88, TYR A:59, PHE A:74, VAL A:41, and two residues of ILE A:21 at distances of 3.80 Å, 4.85 Å, 5.18 Å, 5.42 Å, 5.06 Å, and 5.23 Å, respectively (Figure 2).

For comparison, a molecular docking study was performed using Odanacatib, a cathepsin D inhibitor [55]. The molecular docking simulation with cathepsin D yielded a binding affinity of -8.8 kcal/mol. Analysis of the interaction profile revealed an

8-carbon-hydrogen bond involving the following residues, VAL A:41 at a distance of 2.28 Å, TYR A:86 at a distance of 1.55 Å, SER A:88 at a distance of 2.42 Å, SER A:43 at a distance of 2.38 Å, GLY A:25 at a distance of 2.85 Å, SER A:84 at a distance of 3.64 Å, GLY A:85 at a distance of 3.42 Å, and TYR A:86 at a distance of 2.68 Å. One pi-anion is bound to the residue APS A:50 at a distance of 3.68 Å. One pi-sigma bond is bound to the residue TYR A:86 at a distance of 3.60 Å. One pi-pi stacked bond is bound to the residue TRP A:40 at a distance of 5.46 Å. Three alkyl bonds are present with the residues VAL A:41 at a distance of 4.79 Å, ILE A:21 at a distance of 5.11 Å, and LEU A:39 at a distance of 5.23 Å. Finally, there are 6 π -alkyl bonds, with one residue of TYR A:59 at a distance of 4.82 Å, one residue of ALA A:52 at a distance of 5.05 Å, two residues of TYR A:86 at distances of 4.43 Å and 4.84 Å, and two residues of LEU A:83 at distances of 4.65 Å and 5.32 (Figure 2).

3.5.2. Docking analysis to identify the mechanisms that overcome chemoresistance

To evaluate the capacity of dapagliflozin and pantoprazole to inhibit the development of paclitaxel chemoresistance in combination, their binding affinities for SGLT2 and MMP9, respectively, were assessed. Dapagliflozin exhibited the highest affinity for SGLT2, with a docking score of -8.3 kcal/mol. The interaction profile reveals one conventional hydrogen bond to GLN A:448 at 2.53 Å. A two-carbon hydrogen bond interaction is observed with two residues of ALA A:446 at distances of 3.37 Å and 3.53 Å. One pi-pi stacked bond is observed to TYR A:455 at 3.82 Å. Finally, eight pi-alkyl bonds are observed involving residues of PHE A:152, TRP A:440, PHE A:504, LEU A:452, two residues of TYR A:455, and two residues of ALA A:447 at distances of 4.93 Å, 4.72 Å, 4.78 Å, 5.44 Å, 4.70 Å, 4.86 Å, 5.15 Å, and 4.36 Å, respectively (Figure 3).

Consistent with this concept, another component,

pantoprazole, exhibited the highest affinity for MMP9, with a docking score of -9.0 kcal/mol. The interaction profile reveals three conventional hydrogen bonds, with LEU A:188 at 2.12 Å, ALA A:189 at 2.76 Å, and GLU A:402 at 3.00 Å. One fluorine atom is bound to residues of ARG A:424 at 2.98 Å. Four pi-cation bonds are present, with one residue of HIS A:411 at 4.03 Å and three residues of HIS A:401 at 3.94 Å, 4.97 Å, and 4.13 Å. Four pi-pi-stacked bonds are present, with one residue of HIS A:411 at 4.03 Å and three residues of HIS A:401 at 3.94 Å, 4.97 Å, and 4.13 Å. Three pi-alkyl bonds are present, with PHE A:110 and two residues of VAL A:398 at 5.02 Å, 4.88 Å, and 4.95 Å, respectively (Figure 3).

For comparison, a molecular docking study was performed with Marimastat, an MMP9 inhibitor [56]. The molecular docking simulation with MMP9 yielded a binding affinity of -7.3 kcal/mol. Analysis of the interaction profile revealed nine conventional hydrogen bonds involving the following residues, LEU A:188 at a distance of 1.93 Å, HIS A:401 at a distance of 2.46 Å, HIS A:411 at a distance of 2.39 Å, ALA A:189 at a distance of 2.16 Å, two residues of PRO A:421 at distances of 2.23 Å and 2.10 Å, and three residues of GLU A:402 at distances of 2.68 Å, 2.89 Å, and 2.87 Å, respectively. One pi-sigma bond is present to HIS A:401 at 3.65 Å. Finally, three alkyl bonds are present to residues VAL A:398 at 4.56 Å, LEU A:188 at 4.95 Å, and LEU A:187 at 5.48 Å (Figure 3).

3.5.3. Docking analysis to identify the mechanisms that overcome paclitaxel's adverse effect

To evaluate the ability of dapagliflozin and pantoprazole to mitigate the adverse effects of paclitaxel, their binding affinities for TRPV1 and COX-2, respectively, were assessed. Dapagliflozin exhibited the highest affinity for TRPV1, with a docking score of -8.4 kcal/mol. The interaction profile reveals one conventional hydrogen bond with ALA A:400 at a distance of 3.01 Å. One pi-pi T-shaped bond is observed with the residues of TYR A:495

at a distance of 5.15 Å. One alkyl bond is observed with the residues of ARG A:499 at a distance of 4.27 Å. Finally, three pi-alkyl bonds are observed, involving one residue of TYR A:495 and two residues of ILE A:703, at distances of 4.58 Å, 5.14 Å, and 5.04 Å, respectively (Figure 4).

For comparison, a molecular docking study was performed with (Sb-705498), an approved TRPV1 inhibitor drug [57]. The molecular docking simulation with TRPV1 yielded a binding affinity of -8.0 kcal/mol. Analysis of the interaction profile revealed one carbon-hydrogen bond with the GLU residue A:513 at a distance of 3.26 Å. Two halogen (fluorine) bonds with the GLN residue A:498 at distances of 3.33 Å and 3.48 Å. One pi-sigma bond with the ASP residue A:707 at a distance of 3.86 Å. One pi-pi T-shaped bond with the TYR residue A:495 at a distance of 4.79 Å. Finally, three alkyl bonds with one residue of LEU A:699 at a distance of 4.04 Å, and two residues of ILE A:703 at distances of 4.59 Å and 4.09 Å (Figure 4).

Consistent with this concept, another component, pantoprazole, exhibited the highest affinity for COX-2, with a docking score of -8.8 kcal/mol. The interaction profile reveals three conventional hydrogen bonds, one with HIS A:39 at 2.14 Å, and two with GLN A:461 at 2.97 Å and 2.56 Å, respectively. Two carbon-hydrogen bonds are observed, with PRO A:153 at 3.78 Å and with GLY A:45 at 3.61 Å. One halogen-fluorine bond is observed with GLY A:135 at 3.30 Å. Finally, eight alkyl bonds are observed, with TYR A:130 at 5.11 Å, CYSA:36 at 4.88 Å, PRO A:156 at 5.10 Å, LEU A:152 at 5.12 Å, two residues of CYS A:47 at 4.99 Å and 5.14 Å, and two residues of PRO A:153 at 4.29 Å and 4.50 Å (Figure 4).

For comparison, a molecular docking study was performed with celecoxib, a COX-2 inhibitor [58]. The molecular docking simulation with COX-2 yielded a binding affinity of -8.1 kcal/mol. Analysis of the interaction profile revealed four conventional hydrogen bonds involving the following residues: one residue from

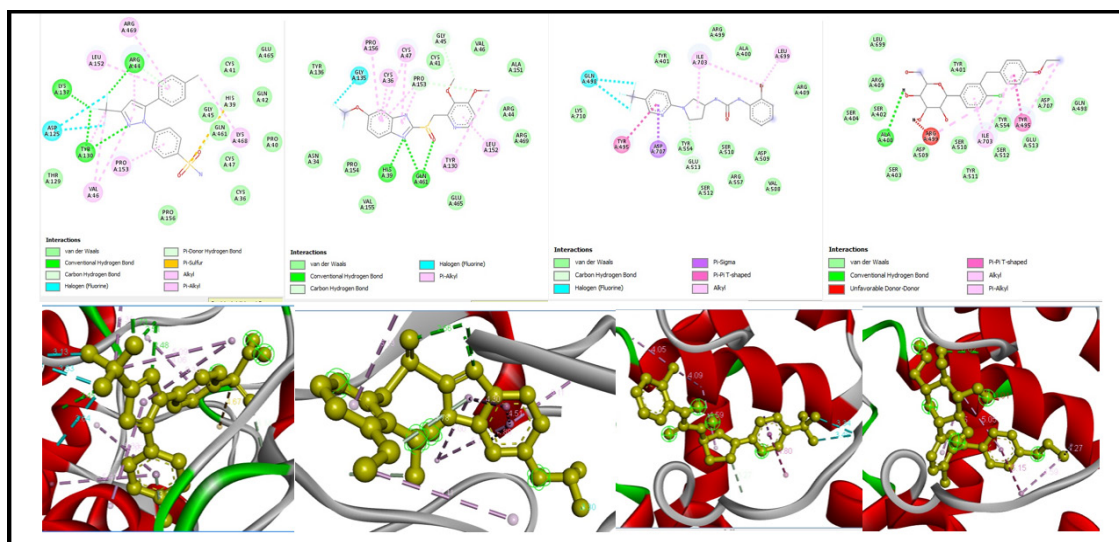


Figure 4. The Structural Configurations of the TRPV1 and COX-2 Binding Sites are Presented in Both Two-dimensional (Top Image) and Three-dimensional (Bottom Image) Perspectives. These illustrations depict the binding interactions of dapagliflozin (first image from the right) and SB-705498 (second image from the right) with the TRPV1 subunit, and of pantoprazole (third image from the right) and celecoxib (fourth image from the right) with the COX-2 subunit.

each of ARG A:44 and LYS A:137, and two residues from TYR A:130, with distances of 2.44 Å, 1.73 Å, 2.47 Å, and 2.50 Å, respectively. One carbon hydrogen is bound to the residue HIS A:39 at a distance of 3.13 Å. Three halogen (fluorine) bonds are present with the residues ARG A:44 at a distance of 3.63 Å, ASP A:125 at a distance of 3.13 Å, and ASP A:125 at a distance of 3.62 Å. One (pi-donor hydrogen bound) is bound with the residue of ARG A:44 at a distance of 3.13 Å. One (pi-sulfur bound) is bound with the residue of HIS A:39 at a distance of 4.67 Å. Two alkyl bonds are observed, with LYS A:468 at 4.15 Å and with VAL A:46 at 5.08 Å. Finally, 6 π -alkyl bonds, with residue of TYR A:130 at a distance of 5.35 Å, ARG A:44 at a distance of 5.14 Å, VAL A:46 at a distance of 5.33 Å, PRO A:153 at a distance of 4.05 Å, LEU A:152 at a distance of 5.33 Å, and ARG A:469 at a distance of 5.47 Å (Figure 4).

3.5.4. Comparative Analysis of Molecular Docking with Reference Inhibitors

To evaluate the therapeutic potential of the triple-drug combination of dapagliflozin and pantoprazole, their binding affinities were compared with those of well-established reference inhibitors for each target. The affinity ratio was determined by dividing the absolute value of the binding free energy of the test compounds ($|\Delta G_{\text{test}}|$) by that of the reference inhibitors ($|\Delta G_{\text{ref}}|$). An affinity ratio exceeding 1.0 suggests that the test compound has a higher predicted binding affinity than the clinically or experimentally validated reference.

The comparative findings indicate that both dapagliflozin and pantoprazole exhibit strong binding profiles across multiple targets implicated in cancer metabolism, invasion, and chemoprotection. As the following

Dapagliflozin demonstrated a notably strong affinity for PDK1, with a binding free energy of -8.4 kcal/mol. Its affinity ratio of 2.00 relative to dichloroacetate ($\Delta G = -4.2$ kcal/mol) suggests that it may possess a more effective inhibitory impact on glycolytic flux.

Pantoprazole demonstrated a high affinity for Cathepsin D, with a ΔG of -8.3 kcal/mol. This closely aligns with the reference inhibitor odanacatib, which has a ΔG of -8.8 kcal/mol. The resulting affinity ratio of 0.94 indicates that pantoprazole has comparable protease-inhibitory potential.

Both compounds demonstrated notable affinity for targets implicated in overcoming chemoresistance, such as SGLT2 and MMP9, and for reducing paclitaxel-induced toxicity, including TRPV1 and COX-2. Their affinity ratios often exceeded 1.0, indicating their capacity for multi-target engagement.

These computational results corroborate the proposed dual mechanisms (metabolic reprogramming and microenvironmental modulation) and provide a molecular explanation for the synergistic and protective effects (Supplementary Table 9).

3.6. Hypothesis-Generating Observations Regarding Pharmacokinetic Drug-Drug Interactions

In addition to the pharmacodynamic mechanisms discussed above, an exploratory *in silico* analysis was conducted to investigate whether favorable pharmacokinetic drug-drug interactions (DDIs) might theoretically contribute to the observed synergistic cytotoxicity. These findings, originally presented in subsection 3.7, are summarized here as hypothesis-generating observations requiring further validation.

As detailed in subsection 3.7 and Supplementary Table 10, our computational analysis predicted that pantoprazole might weakly inhibit CYP3A4 and P-glycoprotein (P-gp), both of which are involved in paclitaxel metabolism and efflux. Nevertheless, several critical caveats must be emphasized. First, pantoprazole is recognized clinically as a weak inhibitor of these pathways, with suppressive efficacy substantially lower than that of dedicated CYP3A4 or P-gp blocking agents. Second, the concentrations of pantoprazole employed in our *in vitro* experiments (0.33–333 $\mu\text{g/mL}$) are markedly higher than typical human plasma levels achieved with standard oral dosing (~5–10 $\mu\text{g/mL}$). Third, our predictions were generated using computational tools and literature-derived data, rather than direct experimental measurements of enzyme activity or transporter function in our cellular system.

Therefore, while the predicted interactions offer a hypothesis-generating rationale for the enhanced intracellular accumulation of paclitaxel and dapagliflozin observed in our MCF-7 cells, these findings should not be interpreted as conclusive evidence of clinically relevant pharmacokinetic synergy. Instead, we propose that the observed synergistic cytotoxicity is primarily driven by the pharmacodynamic mechanisms confirmed by our ELISA and molecular docking data (disruption of HIF-1 α , p-mTOR, Cathepsin D, and induction of apoptosis), with any potential pharmacokinetic contribution remaining speculative at this stage. Future

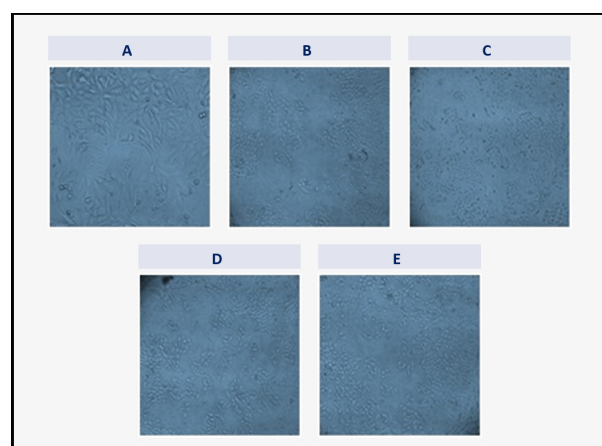


Figure 5. The Morphological Features of MCF7 Cells were Examined Utilizing an Inverted Microscope. The experimental groups consisted of: (A) untreated MCF7 cells used as controls; (B) MCF7 cells treated with 1000 $\mu\text{g/mL}$ of a triple mixture for 72 hours; (C) MCF7 cells exposed to 1000 $\mu\text{g/mL}$ of dapagliflozin for 72 hours; (D) MCF7 cells subjected to 1000 $\mu\text{g/mL}$ of pantoprazole for 72 hours; and (E) MCF7 cells treated with 1000 $\mu\text{g/mL}$ of paclitaxel for 72 hours.

studies using physiologically relevant drug concentrations, human plasma-level matched dosing, and in vivo models are necessary to validate whether pharmacokinetic interactions meaningfully contribute to the triple combination's efficacy (Supplementary Table 10).

3.7. Morphological Evaluation of Cytotoxicity

To substantiate the cytotoxic effects observed in viability assays, morphological alterations in MCF-7 breast cancer cells and human foreskin fibroblasts (HFF) were examined using inverted light microscopy after 72 hours of exposure to the test compounds.

In MCF-7 cells, untreated control cells (Panel A) displayed typical epithelial morphology, characterized by adherent, confluent monolayers with clear, well-defined borders. Conversely, exposure to the triple combination at 1000 $\mu\text{g/mL}$ (Panel B) induced significant morphological changes indicative of cytotoxicity, including extensive cell shrinkage, rounding, detachment from the substrate, and the presence of cellular debris. Treatment with individual agents at 1000 $\mu\text{g/mL}$, dapagliflozin (Panel C), pantoprazole (Panel D), and paclitaxel (Panel E), also caused cytotoxic effects, including decreased confluence and increased cell rounding; however, these effects were less pronounced than those observed with the triple combination, which caused the most substantial disruption (Figure 5).

In untreated HFF normal fibroblasts (Panel A), cells exhibited the characteristic elongated, spindle-shaped morphology typical of healthy fibroblasts, forming a confluent and adherent monolayer. Notably, exposure to the triple combination at a high concentration of 1000 $\mu\text{g/mL}$ (Panel B) caused minimal morphological changes. The HFF cells maintained their spindle shape, adherence, and monolayer integrity, with no significant signs of shrinkage, rounding, or detachment. These observations suggest a pronounced selective toxicity of the triple mixture towards cancer cells, while sparing normal fibroblasts (Figure 6).

These visual observations are consistent with the elevated Selective Toxicity Index (SI), supporting the conclusion that the triple-drug combination demonstrates significant and selective cytotoxic effects against MCF-7 breast cancer cells while maintaining the viability and morphology of normal HFF cells.

4. Discussion

Despite extensive research, cancer treatment continues to face two primary challenges: resistance of cancer cells to therapy and adverse effects associated with current treatments. Developing highly selective therapies to overcome resistance remains costly, prompting exploration of alternative strategies. Drug repurposing, defined as the use of existing medications for new therapeutic indications, offers significant advantages in oncology, including reduced development costs and established safety profiles. Alongside this concept, combining repurposed drugs with traditional therapies may enhance efficacy through synergy or by improving the safety profile, which can be achieved by using a reduced

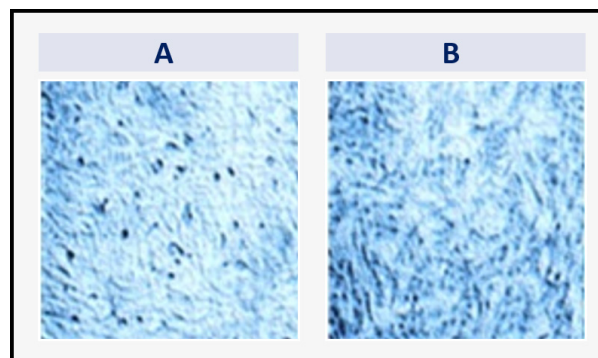


Figure 6. The Morphological Features of Human Foreskin Fibroblast (HFF) Cells were Observed Using an Inverted Microscopy Technique. Panel A depicts the untreated HFF cell line, whereas Panel B illustrates the cells following a 72-hour exposure to a triple mixture at a concentration of 1000 $\mu\text{g/mL}$.

chemotherapy dose. This approach holds promise for more effective and safer cancer treatments.

Along with this concept, this study evaluated a triple combination of paclitaxel, dapagliflozin, and pantoprazole for anticancer activity against breast cancer. Paclitaxel was selected in accordance with breast cancer standard treatment protocols, whereas dapagliflozin and pantoprazole were selected for their previously demonstrated anticancer properties. In this study, numerous laboratory validations and simulations were conducted to elucidate the mechanisms underlying the triple combination's anticancer, synergistic, and selective toxicities, as well as its safety.

MTT assay results showed that the triple mixture (paclitaxel, dapagliflozin, and pantoprazole) exhibited potent, time- and concentration-dependent cytotoxicity against MCF-7 breast cancer cells, with a dramatic decline in IC_{50} from 42.8 $\mu\text{g/mL}$ at 24 hours to 4.1 $\mu\text{g/mL}$ at 72 hours. In contrast, normal human foreskin fibroblasts (HFF) were largely unaffected, with IC_{50} values above 1000 $\mu\text{g/mL}$ at each incubation period. This differential effect yielded a highly favorable selective toxicity index (SI) of >243.9 at 72 hours, indicating preferential targeting of cancer cells. In contrast, the Paclitaxel monotherapy had a narrow therapeutic window, with an SI of only 0.341, highlighting its non-selective toxicity. Its cytotoxicity outcomes revealed the development of resistance, as evidenced by a decline in cytotoxicity with prolonged incubation.

One of the primary objectives in formulating this triple mixture was to demonstrate the potential for synergistic anticancer effects among its constituent ingredients and to mitigate paclitaxel resistance in cancer cells, which was achieved by including dapagliflozin and pantoprazole in the formulation.

The study results support this hypothesis, as the MTT cytotoxicity assay shows that the triple mixture is more effective than each individual component, indicating synergistic interaction. Additionally, the combination index analysis confirmed a strong synergism among the components of the triple mixture. Moreover, by comparing the mixture's cytotoxic effects with those of paclitaxel alone. The results indicated the development of paclitaxel

resistance, particularly with prolonged exposure. In contrast, no such resistance was observed with the triple mixture, suggesting that the combined ingredients may inhibit the emergence of paclitaxel resistance in cancer cells.

To gain a more comprehensive understanding of the synergy mechanism and enhance chemosensitivity, the ELISA data provide a molecular foundation, demonstrating that the triple combination uniquely downregulates critical cancer-cell survival pathways and resistance pathways. Specifically, it markedly suppresses HIF-1 α and p-mTOR. By inhibiting HIF-1 α , the combination disrupts hypoxia-driven glycolytic metabolism and angiogenesis, thereby depriving cancer cells of adaptive survival signals under paclitaxel stress [59]. Concurrent suppression of p-mTOR impedes protein synthesis and proliferation, sensitizing cells to paclitaxel-induced mitotic arrest [15, 21]. In addition to this depression, the ELISA outcomes showed that the triple mixture inhibited Cathepsin D, a lysosomal protease overexpressed in aggressive cancers. Inhibition of Cathepsin D by blocking extracellular matrix degradation and apoptosis evasion enhances paclitaxel's cytotoxic effect [60]. Furthermore, ELISA results showed a robustly activated pro-death pathway, as evidenced by increased LC3B-II (an autophagy marker) and Cleaved Caspase-3. Upregulation of LC3B-II indicates autophagy induction, which can promote cell death under metabolic stress, whereas elevated cleaved caspase-3 confirms the execution of apoptosis. Together, these coordinated actions dismantle key resistance mechanisms metabolic flexibility, prosurvival signaling, and invasion capacity while amplifying paclitaxel's microtubule-stabilizing lethality, thereby overcoming chemoresistance and restoring therapeutic efficacy [61, 62].

Building upon previous findings, molecular docking analyses provided a structural explanation, as follows. high predicted binding affinity ($\Delta G = -8.4$ kcal/mol) for Dapagliflozin with PDK1, with an affinity ratio of 2.00 relative to the reference inhibitor, Dichloroacetate. PDK1 is a key glycolytic gatekeeper. By inhibiting PDK1, dapagliflozin forces a metabolic shift from glycolysis to oxidative phosphorylation, reversing the Warburg effect and depriving cancer cells of the rapid ATP and biosynthetic precursors needed to survive paclitaxel-induced stress [63]. High predicted binding affinity of pantoprazole ($\Delta G = -8.3$ kcal/mol) for Cathepsin D, with an affinity ratio of 0.94 relative to the reference inhibitor Odanacatib. Cathepsin D, a lysosomal protease, plays a significant role in facilitating cellular invasion and evading apoptosis. Its inhibition has been shown to impede extracellular matrix degradation and to reestablish apoptotic sensitivity, thereby blocking a critical pathway of resistance to paclitaxel-induced cytotoxicity [64]. By targeting these molecular targets, a molecular basis for the synergistic mechanism was proven. Moreover, dapagliflozin exhibited high affinity for SGLT2 ($\Delta G = -8.3$ kcal/mol) and, by targeting it, reduced the development of resistance by inhibiting glucose uptake, depriving cancer cells of glycolytic energy, and reversing

metabolic chemoresistance to paclitaxel [65]. Finally, the High predicted binding affinity of pantoprazole to MMP9 ($\Delta G = -9.0$ kcal/mol) and an affinity ratio of 1.23 relative to the reference, Marimastat. Inhibition of MMP9 reduces invasion and metastasis by suppressing matrix metalloproteinase activity and counteracting microenvironment-mediated paclitaxel resistance [66]. By targeting these molecular targets, a molecular basis for overcoming the development of chemoresistance was demonstrated.

Finally, the in silico pharmacokinetic analysis predicts that pantoprazole inhibits CYP3A4, which is responsible for paclitaxel metabolism [67] and P-glycoprotein (P-gp), key players in paclitaxel metabolism and efflux [68]. This interaction is likely to increase intracellular paclitaxel levels in tumor cells, thereby significantly decreasing the IC₅₀. Consequently, the observed synergistic effect arises from multi-target pharmacodynamic disruption of cancer cell survival pathways, combined with favorable pharmacokinetic interactions that enhance drug delivery and overcome the development of resistance. These computational interactions support the proposed dual mechanism and the triple mixture's ability to overcome resistance.

Another objective in formulating this triple mixture was to demonstrate its potential to enhance the safety profile of paclitaxel through the modulatory effects of the other two ingredients, pantoprazole and dapagliflozin. Selectivity index (SI) outcomes supporting this hypothesis showed a highly favorable SI across all triple-mixture test concentrations and incubation periods, peaking at 243.9 at 72 hours. This indicates preferential targeting of cancer cells. In contrast, paclitaxel alone exhibited a narrow therapeutic window, with an SI of only 0.341, reflecting its non-selective toxicity. In addition to the combination's selectivity index, the dose-reduction index further supports its potential. Both indices exceeded 1 for all drugs tested, with particularly high values for pantoprazole and paclitaxel after 72 hours (e.g., DRI values of 1123.84 and 388.362, respectively), suggesting a substantial capacity to reduce individual cytotoxic concentrations while maintaining therapeutic efficacy.

To deepen the understanding, the results from ELISA and molecular docking offer a clear molecular explanation for the significantly improved safety profile observed with the triple-drug combination. This enhancement is primarily attributed to the cytoprotective effects of dapagliflozin and pantoprazole. While paclitaxel monotherapy caused notable cellular stress in normal human foreskin fibroblasts (HFF), as indicated by a marked increase in the inflammatory marker NF- κ B p65, which is a central mediator of chemotherapy-induced inflammatory responses, driving adverse effects like neuropathy, mucositis, and tissue damage [69]. elevated oxidative stress response (HO-1), which is a critical inducible antioxidant enzyme that protects normal tissues from chemotherapy-induced oxidative stress and inflammation, thereby mitigating drug-related adverse effects [70]. and a pro-apoptotic Bax/Bcl-2 ratio of 1.46, which is a crucial determinant of mitochondrial apoptosis;

a high ratio favors cell death, while a low ratio promotes survival, directly influencing a cell's susceptibility to chemotherapy-induced toxicity [71]. The triple combination preserved biomarker levels similar to those of untreated, healthy cells. This normalization suggests that the repurposed agents effectively safeguard normal tissues from the off-target cytotoxic effects of paclitaxel.

Molecular docking simulations offer insight into the specific protective mechanisms involved. Dapagliflozin demonstrated a high predicted binding affinity ($\Delta G = -8.4$ kcal/mol) for TRPV1, with an affinity ratio of 1.05 relative to the reference inhibitor SB-705498. The TRPV1 channel is known as a crucial regulator of pain signaling and neuroinflammation. Dapigliflozin may decrease or desensitize TRPV1, which could reduce overactive pro-nociceptive pathways and thereby lessen paclitaxel-induced neuropathic pain [72, 73]. Simultaneously, pantoprazole exhibited a strong binding affinity to COX-2 ($\Delta G = -8.8$ kcal/mol), a key enzyme involved in inflammation and neuropathic pain, with an affinity comparable to that of the selective inhibitor celecoxib (affinity ratio 1.09) [74]. This interaction provides a molecular explanation for pantoprazole's potential to inhibit the inflammatory cascade implicated in paclitaxel toxicity, including neuropathy and tissue damage. By targeting these molecular targets, the underlying mechanisms for overcoming the adverse effects of paclitaxel were elucidated.

The scope of the present study is constrained by its reliance on in vitro methodologies. Although these methods offer valuable insights into mechanistic interactions, they do not capture the intricate pharmacokinetics, systemic toxicity profiles, and tumor-stroma dynamics observed in vivo. Specifically, as noted above, the concentrations of pantoprazole used in our experiments exceed typical human plasma levels, and our pharmacokinetic predictions remain speculative. Consequently, these preclinical findings offer a strong rationale and underscore the need for further rigorous in vivo investigation to support the potential translational application of this repurposed triple therapy.

In conclusion, Despite paclitaxel's established role in treating breast cancer, its clinical use is limited by two main problems: the development of resistance to chemotherapy and significant side effects. This study aimed to address these issues by exploring a new triple-combination therapy. This therapy uses dapagliflozin and pantoprazole together to improve paclitaxel's effectiveness while reducing its side effects. Our laboratory tests showed that this combination is highly effective, synergistically killing MCF-7 breast cancer cells, while remaining highly selective and not harming normal human fibroblasts.

The observed synergistic effect arises from dual reprogramming of metabolic processes and the surrounding microenvironment. This combined approach successfully inhibited key survival pathways, including HIF-1 α and p-mTOR, as well as resistance markers such as Cathepsin D and MMP9, while simultaneously triggering pro-apoptotic signals, including LC3B-II and Cleaved Caspase-3. Structural validation via molecular docking

confirmed the high-affinity binding of the repurposed drugs to relevant targets, including PDK1 and Cathepsin D. Moreover, this combination exhibited a protective effect in normal cells, normalizing stress and inflammatory markers that were elevated by paclitaxel treatment alone, and this effect was mediated by blocking the action of TRPV1 and COX-2.

The current investigation ultimately highlights the potential of the combined application of paclitaxel, dapagliflozin, and pantoprazole as a viable approach to surmount paclitaxel resistance and alleviate its associated toxicities. This strategy, which simultaneously targets cancer cell metabolism, alters the tumor microenvironment, and offers cytoprotection, serves to re-sensitize resistant cells and expand the therapeutic window. Consequently, these results necessitate additional preclinical and clinical assessment to facilitate the translation of this repurposed triple therapy into a more efficacious and better-tolerated treatment protocol for individuals diagnosed with breast cancer.

Author Contributions

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors state that they did not use any generative AI or AI-assisted tools during their research.

Data Availability Statement

The information underpinning the conclusions of this investigation can be obtained from the corresponding

author upon justifiable inquiry. This encompasses raw absorbance measurements from MTT assays, ELISA-based protein quantitation results, molecular docking output files (comprising binding energies and interaction patterns), and unprocessed microscopy images. Limitations are placed on the accessibility of certain information owing to institutional regulations and the ongoing character of associated research endeavors; nevertheless, all data presented in the manuscript will be provided by the corresponding author upon reasonable request.

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