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ATP-Binding Cassette Transporters Mediated Chemoresistance in MCF-7 Cells: Modulation by PhTAD-Substituted Dihydropyrrole Compounds

MCF-7 Hücrelerinde ATP-Bağlayıcı Kaset Taşıyıcıları Aracılı Kemorezistansın PhTAD-Sübstitüe Dihidropirol Bileşikleri ile Modülasyonu

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ÖZ

Amaç: ABC proteinleri, antibiyotikler ve ilaçlar gibi birçok substratı taşır. ABC'lerin artması kanserde kemorezistansa yol açmaktadır. Bu bilgiler ışığında, çalışmamızda hem PhTAD türevli dihidropirol bileşiklerinin MCF7 hücrelerinde ABC Transporterların gen ekspresyonları üzerindeki etkisini hem de bu bileşikler için insan ABCB1 yapısını hedef alan öngörücü moleküler bağlanma bölgelerini araştırmayı planladık.
Araçlar ve Yöntem: MCF-7 hücrelerindeki ABCB1, ABCC3, ABCC10, ABCC11 ve ABCG2'nin mRNA ekspresyon seviyeleri qPCR ile ölçülmüştür. Moleküler kenetlenme testleri hem AutoDock Tools 4.2 hem de PyMOL 2.4 programları ile gerçekleştirilmiştir. Ayrıca etkileşim analizi ProteinsPlus web servisi üzerinden yapılmıştır.
Tartışma: Bulgularımız, PhTAD ikameli dihidropirol içeren moleküllerin, kanser kemoresiztansının potansiyel bir düzenleyicisi olan ABC Transporterları etkilediğini göstermektedir.
Sonuç: Sonuçlarımız, bileşik (B) I, BII, BIII, BV, BVIII ve BXII'nin ABCB1'i artırdığını, BIV, BVI, BVII, BX, BIX, BXI, BXIII ve BXIV'ün ise ABCB1'i azalttığını ortaya koymuştur. Ayrıca, BI, BIV, BVI ve BVIII, ABCC3'ü yukarı regüle etmesine rağmen, BVII, BX, BXII, BXIII ve BXIV, ABCC3'ü aşağı regüle eder. Ayrıca, tüm bileşikler ABCC10 ekspresyonunu arttırmıştır. Tersine, ABCC11'in ekspresyonu ise tüm bileşikler tarafından azaltılmıştır. Ayrıca BII, BV ve BVI, ABCG2'yi artırırken, BI, BVII, BVIII, BIX, BX, BXI, BXII, BXIII ve BXIV, ABCG2'yi azaltmıştır. Bunun yanında ABCB1, ABCC3, ABCC11 ve ABCG2 miktarları, BVII, BIX, BX, BXI, BXIII ve BXIV ile paralel olarak azalmıştır. Ayrıca, yüksek bağlanma enerjisine sahip BXI ve BXIV'ün moleküler kenetlenme hesaplama sonuçları, ABCB1'in sıkı bir şekilde modüle edildiğini göstermiştir. Özellikle bu bileşikler, ABCB1 üzerindeki birçok hidrojen bağlama ve hidrofobik bölge ile etkileşime girmektedir.
Anahtar Kelimeler: ABCB1; ABCC3; kemorezistans; meme kanseri; PhTAD-dihidropirol

ABSTRACT

Purpose: ABC proteins transport many substrates such as antibiotics and drugs. Increase of ABCs lead chemoresistance in cancer. In view of this information, in our study, we planned to investigate both PhTAD-substituted dihydropyrrole compound's impact on gene expressions of ABC Transporters in the MCF7 cells, and predictive molecular binding sites target on human ABCB1 structure for these compounds.
Materials and Methods: The mRNA expression levels of ABCB1, ABCC3, ABCC10, ABCC11, and ABCG2 in the MCF-7 cell were measured by qPCR. Molecular docking assays were realized with both the AutoDock Tools 4.2 and PyMOL 2.4. Also, the interaction analysis was performed by ProteinsPlus web service.
Results: Our results revealed that CI, CII, CIII, CV, CVIII, and CXII increased ABCB1 while compound CIV, CVI, CVII, CX, CIX, CXI, CXIII, and CXIV decreased ABCB1. Besides, CI, CIV, CVI, and CVIII upregulate ABCC3, although CVII, CX, CXII, CXIII, and CXIV downregulate ABCC3. Moreover, ABCC10 expression is induced by all compounds. Conversely, ABCC11 expression is reduced by all compounds. Furthermore, CII, CV, and CVI increased ABCG2, while CI, CVII, CVIII, CIX, CX, CXI, CXII, CXIII, and CXIV decreased ABCG2. Also, ABCB1, ABCC3, ABCC11, and ABCG2 parallelly reduced by CVII, CIX, CX, CXI, CXIII, and CXIV. Also, the molecular docking calculation results of CXI and CXIV with high binding energy have shown that tightly modulated ABCB1. Especially, these compounds interact with many hydrogen bonding and hydrophobic site on ABCB1.
Conclusion: Our findings indicate that the PhTAD-substituted dihydropyrol containing molecules affect ABC transporters as a potential regulator of cancer chemoresistance.
Keywords: ABCB1; ABCC3; breast cancer; chemoresistance; PhTAD-dihydropyrrole

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INTRODUCTION

Cancer ranks second among the diseases that cause the most deaths in the world and it is expressed as unregulated DNA replicating itself and cell division.[1,2] Breast cancer is the leading cause of death in cancer cases in women.[3] Breast cancer is rare in men, but it is a common type of cancer in women.[4,5] Breast cancer has been shown to be caused by a variety of mechanisms caused by the multidrug resistance (MDR).[6] In previous studies, it was reported that MDR mechanisms in breast cancer caused multidrug resistance by decreasing drug concentration in the cell, and by detecting its up-expression in breast cancer resistance protein (BCRP), it decreased the effectiveness of drugs.[7,8]

MDR hypothesis is related to the development of pharmacokinetic resistance mechanism, the inability of the drug to reach sufficient concentration in the target area because of the overexpression and activities of drug transporters in the cancer chemotherapy resistance. This hypothesis was first explained and popularized in chemotherapy resistance in cancer.[9-11] Research continues in many areas to figure out MDR in cancer and to explore new drugs which can inhibit the improvement of drug resistance in cells. Many findings have demonstrated that the P glycoprotein, also known as multidrug delivery protein, is expressed in large amounts in many cancer cells because of genetic changes. This situation causes cancer cells to develop resistance to many anticancer drugs.[12-14]

The ATP-Binding Cassette (ABC) transporters gene family is identified according to the sequence and structure of ATP binding sites. They are responsible for the transport of many substrates such as hormones, lipids, sugars, amino acids, ions, polysaccharides, peptides, proteins, antibiotics, xenobiotics, drugs, and toxins, through intracellular and extracellular membranes.[15,16] ABC proteins use the energy from ATP hydrolysis and contain 49 different ABC genes that are divided into seven subfamilies (ABCA-ABCG).[17,18]

Genetic and molecular investigations have found that multidrug-resistant cancer cells raised MDR1 (Pgp / ABCB1) gene expression levels in humans. P-glycoprotein encoding by the MDR1 gene is produced at prominent amounts in many cancers.[19-22] Multidrug carriers are one of two important mechanisms defined in the flow of drugs pumped from cells. The other mechanism for drug flow involves the expression of a gene called multidrug resistance-associated protein (MRP). Both MDR1 and MRP genes are members of a superfamily of ATP-dependent carriers. Presumably, other members of this superfamily that have not yet been identified also play an effective role in drug resistance. MDR etiology can be multifactorial; however, standard drug resistance to cytotoxic drugs mentioned above is mostly on account of upregulation of P-glycoprotein that conducts as a drug exit pump and its overexpressed in human tumors.[12,23] In cancerous tissue, expression of P-glycoprotein results in the highest potential for resistance in tumors provided from tissues that normally express P-glycoprotein, such as epithelial cells of the pancreas, liver, adrenal, colon, and kidney. P-glycoprotein levels could be low at that case of determination in tumors before chemotherapy begins; however, they rise after exposure to chemotherapy agents, causing the exploration of MDR in these cells.[24] An important mechanism of cancer cell multidrug resistance is thought to involve apoptosis or suppression of other cell death pathways.[25,26]

Dihydropyrrole derivatives are important compounds that exhibit a variety of biological activities and are useful intermediates in the synthesis of natural products.[27] It has been stated in many studies by researchers that dihydropyrrole compounds have antitumor activity on various types of cancer and have been shown to have low toxicity effectively.[28] The resistance that occurs in cancer cells with the excretion of drugs from the cell can be overcome with dihydropyrrole compounds.[29] The number of pyrrole-based drugs is high, so pyrroles are among the most researched heterocyclics in drug discovery for therapeutic fields. There are many studies such as anticancer, antimicrobial and antiviral belonging to pyrrole compounds which are a specific target in biological activities.[30] However, related to pyrrole-derived compounds, sufficient studies have not been found for compounds' effect on the drug resistance mechanism.

In view of this information, we planned to investigate PhTAD-substituted dihydropyrrole compounds' impact on gene expressions of ATP-Binding Cassette Transporters in the MCF7 cells. Besides, we explored the compound I-XIV against the binding of human ABCB1 in the complex structure of PDB:7A69 (www.rcsb.org) using a molecular docking approach. Our findings indicate that the PhTAD-substituted dihydropyrrole containing molecules alternate ABC transporter gene levels as a potential regulator of cancer chemoresistance. Also, these molecules could be a likely inhibitor for ABCs.

MATERIALS and METHODS

Structure of Compounds

According to our previous study, we were synthesized PhTAD substituted dihydropyrrole compounds.[31] The structures of these compounds (C) were listed as shown in Figure 1.

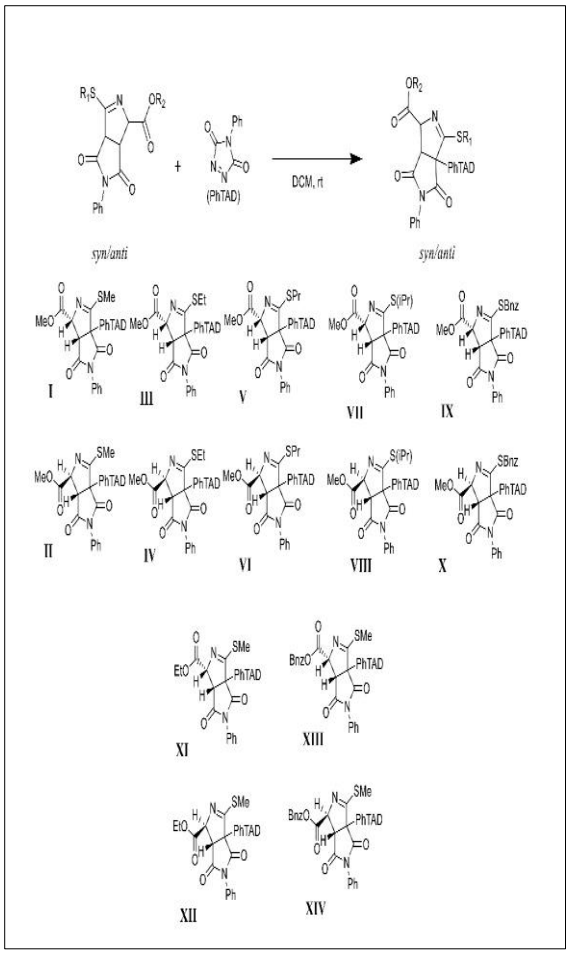

Figure 1. Structure of syn and anti PhTAD derivative compounds.

Cell Culture

The Human breast adenocarcinoma cell line (MCF-7, ATCC® HTB22 ™, Manassas, VA, USA) was used for cell culture. MCF-7 cells were cultured using media containing 25 g/100 mL sodium bicarbonate (Sigma–Aldrich), 10 % fetal bovine serum (Sigma–Aldrich), RPMI-1640 (Sigma–Aldrich), penicillin/streptomycin (Sigma–Aldrich), and cells were incubated condition properly which are humidified atmosphere of 5% CO_2 and at 37 °C in the incubator. After that, the cells had grown to 75% saturation, and the cells were washed with phosphate-buffered saline (PBS) and detached from flasks with 1X Trypsin (Sigma–Aldrich). Nearly 2×10^6 cells were seeded per well of a 6-well culture plate in 3 mL of growth medium. Cells were incubated at 37 °C and 5% CO2 and 90 % humidity for 24 h until applied for PhTAD substituted dihydropyrrole compounds.

Total RNA Isolation, Determination, and cDNA Synthesis

Properly amount of PhTAD substituted dihydropyrrole compounds were dissolved in Dimethyl sulfoxide (DMSO) and diluted in a culture medium. In our previous study, the effect of PhTAD substituted dihydropyrrole compounds on cell viability was measured by the methyl thiazolyl diphenyl tetrazolium bromide (MTT) assay. Briefly, the cells were seeded in a 96-well plate at a density of $1x10^4$ cells/well in a final volume of 100 µL and incubated for 24 h. The final concentrations of compounds were applied on cells as 1.56, 3.125, 6.25, 12.5, 25, 50, 100 µM incubated for 24 h. MTT was added to the plate. After two hours, the medium was removed and then formazan crystals were dissolved in 100 µL DMSO. The absorbance was measured at 570 nm via a microplate reader (Thermo Multiskan Go). The cell viability percentage was determined with the help of the Microsoft Excel program and 50% suppressive concentration (IC_{50}) was calculated by logarithmic slope graph.

Cell viability %=(compound treatment absorbance)/(negative control absorbance value×100)

According to the IC_{50} results we obtained in our previous study applied appropriate doses on MCF-7 as shown in Table 1.[32] These compounds were applied to the $2x10^6$ cells at the specified doses; after that, cells were incubated at 37 ^oC for 24 h. Total RNA was isolated with the GeneJET RNA Purification Kit (Cat No: K0731, Thermo Scientific). Both the concentration and purity of total RNA in the samples were measured at 260 and 280 nm via spectrophotometer (Multiskan Go µDrop, Thermo Scientific). Total RNA concentrations and purity values were shown in Table 2. cDNA was synthesized from 100 ng total RNA using the Maxima First Strand cDNA Synthesis Kit (Cat No: K1671, Thermo Scientific) according to the manufacturer's instructions.

Table 1. Applied cytotoxic doses of PhTAD derivative compounds on MCF7 cells.

Compounds Name	IC_{50} Dose (µM)
I	25
II	25
III	25
IV	25
V	50
VI	12.5
VII	12.5
VIII	12.5
IX	6.25
X	12.5
XI	12.5
XII	50
XIII	25
XIV	25
Negative Control	-

Table 2. Total RNA concentrations and A260/280 values.

Compounds	Total RNA (ng/µL)	$A_{260/280}$ value
I	95.69	2.002
II	373.2	1.997
III	102.1	2.001
IV	242.5	2.058
V	162	2.022
VI	400	2.058
VII	466	2.068
VIII	334	2.066
IX	198	2.059
X	321	2.058
XI	174	2.052
XII	238.6	2.054
XIII	110	2.099
XIV	353.7	2.054
Negative Control	219.5	2.109

qPCR

The cDNA was used for quantitative real-time Polymerase Chain Reaction (qPCR) analysis using the Maxima SYBR Green/ROX qPCR Master Mix (2X) (Cat No: K0221, Thermo Scientific), and the threshold cycle (CT) was measured by Real-Time PCR System (PikoReal™, Thermo Scientific). Relative gene expression levels of ABCB1, ABCC3, ABCC10, ABCC11, and ABCG2 were calculated as a fold change using the $2^{-\Delta\Delta CT}$ method. According this calculation, ΔCT = CT (target gene)-CT (β-actin) and Δ(ΔCT) = ΔCT (negative control)-ΔCT (treatment of compound). The primer pairs used for qPCR are shown in Table 3. Estimated target gene specificity and PCR product sizes were confirmed using NCBI Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/).

Table 3. Nucleotide sequences of forward (F) and reverse (R) primers, size of the products and primer specific annealing temperatures (Ta) for the real-time PCR detection.

Gene	Primer Sequences (5'-3')	Product Length (bp)	Ta (°C)
β-Actin F	TGACGTGGACATCCGCAAAG	205	51
β-Actin R	CTGGAAGGTGGACAGCGAGG		
ABCB1 F	GTTCAGGTGGCTCTGGATAAG	93	55
ABCB1 R	AGCGATGACGTCAGCATTAC		
ABCC3 F	TACTCCAAGACAGAGACAGAGG	111	53
ABCC3 R	CCGGTAGCGCACAGAATAAT		
ABCC10 F	TCACCCTGTCTCCACTGTAT	133	49
ABCC10 R	AACTGGCACCTCTGGTTTAG		
ABCC11 F	GTGGTGCTGATCGTCTTCTT	106	53
ABCC11 R	CCATGGTTCCATTGCTCTCT		
ABCG2 F	TCGTACTGGGACTGGTTATAGG	101	53
ABCG2 R	GTTGGTCGTCAGGAAGAAGAG		

Molecular Docking Simulation

The multidrug resistance protein to the human ABCB1 in the complex structure of PDB:7A69 (www.rcsb.org) was selected for molecular docking assay and modeled using AutoDock4.2 software (Ref M1). The binding site analysis was performed by PyMOL2.4 (refM2) and Protein-Ligand Interaction Profiler (PLIP) tools (RefM3).[33-35]

RESULTS

Effects of PhTAD Substituted Dihydropyrrole Compounds Modulate ABCB1 Gene Expression In MCF-7 Cells

The mRNA expression of ABCB1 upregulated by CI (0.25-fold), CII (0.65-fold), CIII (2.5-fold), CV (1.5-fold), CVIII (0.4-fold), and CXII (0.7-fold) treatment compared to negative control in MCF-7 cells (Figure 2). However, CIV, CVI, CIX, and CXI treatment compared to negative

control approximately downregulated 50% of the mRNA expression of ABCB1. CVII and CXIII downregulated 70% of the mRNA expression of ABCB1. Also, CX and CXIV treatment compared to negative control in MCF-7 cells markedly downregulated 6-fold and 13-fold ABCB1 gene expression levels, respectively.

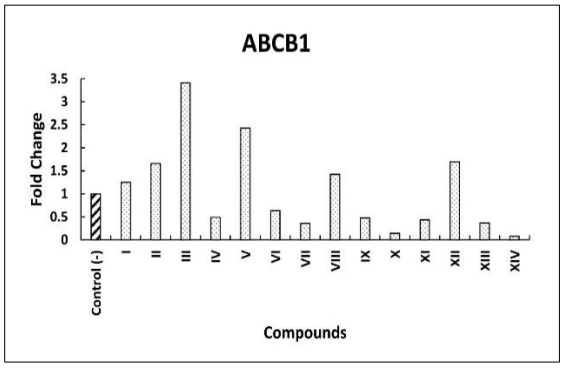

Figure 2. Effects of PhTAD substituted dihydropyrrole compounds on ABCB1 gene expression in MCF-7 cells.

Effects of PhTAD Substituted Dihydropyrrole Compounds on ABCC3 Gene Expression in MCF-7 Cells

The mRNA expression of ABCC3 increased by CI (~0.3-fold), CIV (~0.4-fold), CVI (~1.4-fold), and CVIII (~1.5-fold) treatment compared to negative control in MCF-7 cells (Figure 3). But CVII and CXII treatment compared to negative control decreased 30% the mRNA expression of ABCC3. Besides, ABCC3 gene expression nearly decreased 50% by CX, and CXIV. Especially, CXIII treatment compared to negative control seriously decreased ~75% ABCC3 gene expression level.

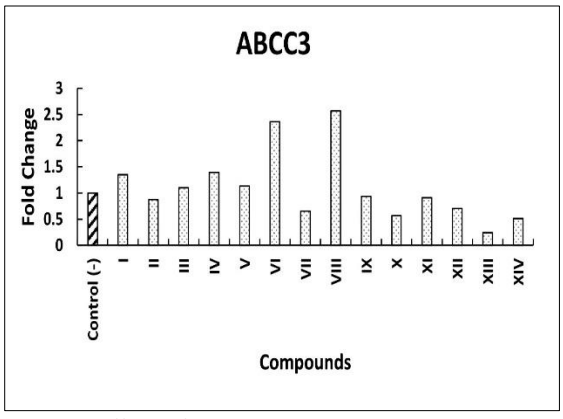

Figure 3. Effects of PhTAD substituted dihydropyrrole compounds on ABCC3 gene expression in MCF-7 cells.

Effects of PhTAD Substituted Dihydropyrrole Compounds Induce ABCC10 Gene Expression in MCF-7 Cells

The mRNA expression levels of ABCC10 considerably increased by CI (4-fold), CII (2.25-fold), CIII (7-fold), CIV (3.5-fold), CV (5.75-fold), CVI (2-fold), CVII (3.2-fold), CVIII (2.2-fold), CIX (4.5-fold), CX (4-fold), CXI (7.2-fold)**,** CXII (6.2-fold), CXIII (0.25-fold) and CXIV (2.2-fold) treatment compared to negative control in MCF-7 cells (Figure 4).

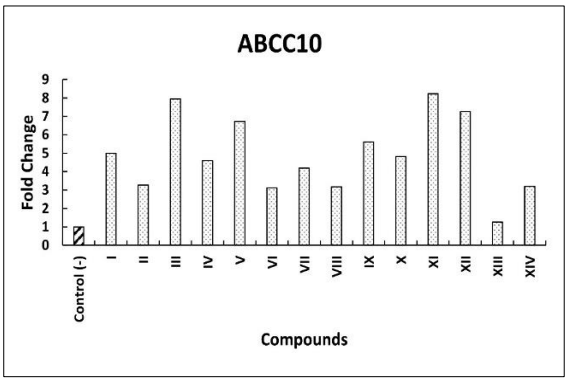

Figure 4. Effects of PhTAD substituted dihydropyrrole compounds on ABCC10 gene expression in MCF-7 cells.

PhTAD Substituted Dihydropyrrole Compounds Reduce ABCC11 Gene Expression in MCF-7 Cells

The mRNA expression levels of ABCC11 considerably downregulated by CI (4-fold), CII (1.3-fold), CIII (0.2-fold), CIV (5.5-fold), CV (1.2-fold), CVI (15-fold), CVII (1.2-fold), CVIII (16-fold), CIX (4-fold), CX (2.5-fold), CXI (26-fold)**,** CXII (1-fold), CXIII (4.5-fold) and CXIV (13-fold) treatment compared to negative control in MCF-7 cells (Figure 5). Especially, CVI, CVIII, and CXI are extremely suppressed on ABCC11 gene expression.

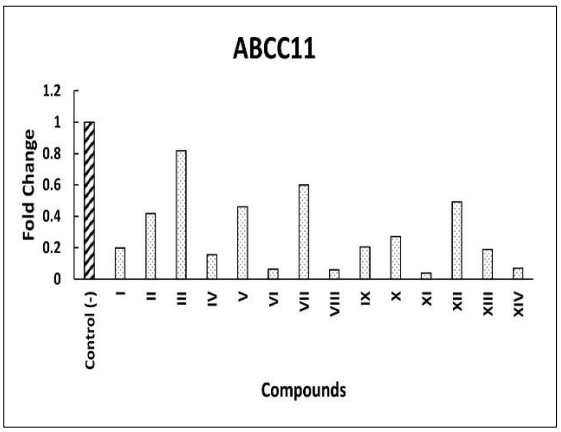

Figure 5. Effects of PhTAD substituted dihydropyrrole compounds reduce ABCC11 gene expression in MCF-7 cells.

PhTAD Substituted Dihydropyrrole Compounds on ABCG2 Gene Expression in MCF-7 Cells

CII, CV, and CVI treatment compared to negative control approximately increased 1.5-fold ABCG2 gene expression levels in MCF-7 cells (Figure 6) even though ABCG2 expression decreased by CI (2-fold), CVII (2.4-fold), CVIII (1.5-fold), CX (2.2-fold), CXII (1.2-fold), CXIII (3-fold), and CXIV (0.3-fold). Interestingly, CIX and CXI treatment compared to negative control markedly downregulated 220-fold and 31-fold the mRNA expression level of ABCG2, respectively.

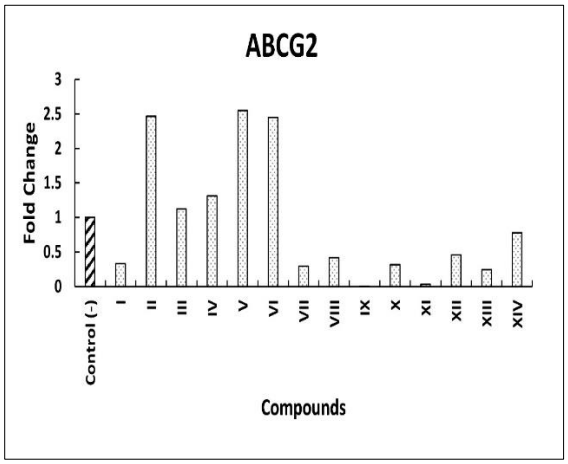

Figure 6. Effects of PhTAD substituted dihydropyrrole compounds regulate on ABCG2 gene expression in MCF-7 cells.

PhTAD Substituted Dihydropyrrole Compounds Target on ABCB1

Molecular docking study was performed on the compounds I-XIV to assay the mode of their interaction in the active site of the multi drug resistance protein on ABCB1 (PDB:7A69) using AutoDock Tools. Table 4 summarizes the binding depiction and binding energy values. From the docking assay, obtained poses were selected depending on the strong binding energy.

Furthermore, 3D interactions of compounds XI, and XIV, also interaction of the surface with corresponding amino acids were evaluated by molecular docking methods. Conventional hydrogen bonds were found between the GLN990, GLN725 and N-phenylmaleimide parts of the compounds. All compounds embedded into the hydrophobic pocket of the inhibitor pocked were occupied by PhTAD, phenyl ring, or N-maleimide part which interacted with Ile306, Phe303, Trp232, Tyr307. The highest dock score was found as -10.38, -9.99, -9.28, -9.20, -9.19, -9.13, -8.91, -8.72 kcal/mol for compounds XI, II, XIV, X, IX, VI, VII, IV, respectively (Figure 7).

Table 4. Data from the molecular docking of the PhTAD derivatives compounds on 7A69.

Compounds	Binding İnteraction	Binding Energy	No of H Bond
I	Imide-Ph(Phe728A, Tyr307A) Esterside (Gln990A) Imide C=O(Gln725A) PhTAD-Ph(Phe770A, Gln990A) NtriazoC=O(Gln725A, Phe303A)	-8.39	3
II	Imide-Ph (Phe303A, Trp232A) PhTAD-Ph(Phe994A) PhTAD-C=O(Gln838A)	-9.99	2
III	Imide-Ph (Phe728A, Tyr307A) Imide-C=O(Gln725A) PhTAD-Ph(Phe303A) PhTAD-C=O(Gln990A)	-9.00	2
IV	Imide-Ph (Phe728A, Tyr307A) Imide-C=O(Gln725A) PhTAD-Ph (Phe994A, Gln990A, Val991, Phe303A) PhTAD-C=O(Gln990A)	-8.72	2
V	PhTAD-Ph(Phe303A) Imide-C=0 (Tyr310A) Imid-Ph(Phe983A, Phe336A) PhTAD-C=O(Gln725A)	-8.53	2
VI	Esterside (Gln990A) Imide-Ph (Phe738A, Tyr307A) Imide-C=O(Gln725A) PhTAD-Ph(Tyr307A, Ala987A, Val991) PhTAD-C=O(Ala987A)	-9.13	4
VII	PhTAD-Ph(Phe303A, Ile306A) Imide-C=0 (Trp232A) Imide-Ph (Met994A)	-8.91	3
VIII	Imide-Ph (Met986A,Phe303A, Trp232A, Phe983A) PhTAD-Ph (Phe303A,Trp232A), Ala302A,Ile299A) PhTAD-C=O(Gln990A)	-8.40	3

IX	PhTAD-Ph(Phe728A,Trp232A) Imide-Ph (Phe343A,Phe303A, Trp232A, Ala302) Imide-C=0 (Gln990A) S-side (Gln725A)	-9.19	2
X	Imide-Ph (Ile340A) PhTAD-Ph (Trp232A, Phe303A, Phe343A)	-9.20	1
XI	PhTAD-Ph(Phe303A,Phe343A)	-10.38	2
XII	Imide-Ph (Phe728A,Tyr307A) Imide-C=O(Phe303A) PhTAD-Ph (Ile299A,Trp232A) PhTAD-C=O (Gln990A) Esterside (Phe303A)	-8.38	2
XIII	Imide-Ph (Ile306A,Tyr307A) İmide C=O(Gln725A) Esterside (Phe343A, Leu339A)	-9.04	2
XIV	Imide-Ph (Phe303A,Trp232A) PhTAD-Ph(Phe343A) PhTAD-C=O(Ile340A,Phe343A) Triazol H bond(Gln347A) Esterside (Leu339A) Imideco(Trp232A,Gln990A, Ile306A)	-9.28	3

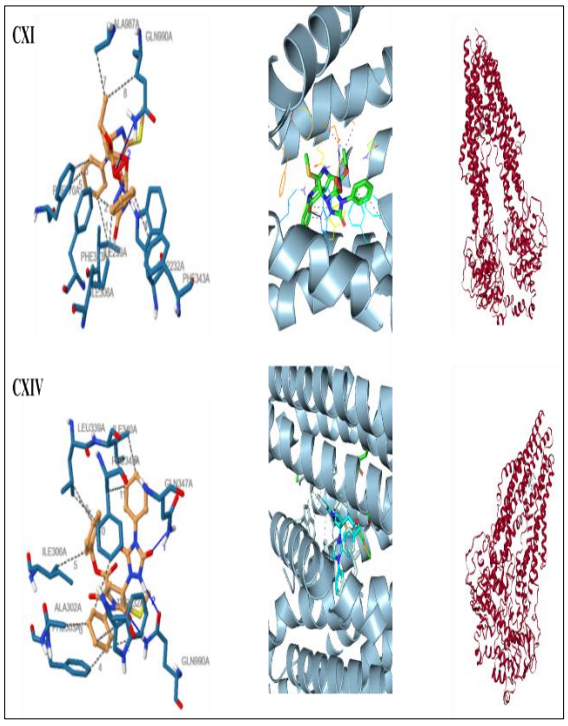

Figure 7. Compound XI and XIV superimposed in the human ABCB1 multidrug resistance protein active side and 3D predicted binding mode of XI and XIV (H-bond: blue, hydrophobic interaction: green).

DISCUSSION

It is known that the structures and mechanisms of various cancer cells develop resistance to drugs, so the cells continue to grow and multiply abnormally. Multidrug resistance detected in cancer cells is overexpression of ABC transporters. Due to the mechanism caused by MDR, drugs are excreted from cancer cells, and the effectiveness of drugs is significantly weakened. ABC transporter modulators are an important strategy for overcoming drug resistance due to their potential to increase the effectiveness of anticancer drugs.[9,18] Pyrrole core stands out in many new drug discoveries by medical chemists due to its pharmaceutical and pharmacological properties.[27]

Boger et al. showed that the cytotoxic activity of the pyrrole compounds was determined and reversed sensitization of the multi-drug resistant in human colon cancer cell line (HCT116 / VM46).[36] Besides, according to another study, pyrrole-derived compounds exhibited significant cytotoxicity and reactivated multidrug-resistant cancer cell lines at non-toxic concentrations.[37] Furthermore, a study related to TRPV1 agonistic and anticancer activities of pyrrole derived compounds have shown that these compounds have significant antiproliferative potency, and the compounds were found to be sensitive to those showing the MDR phenotype in apoptosis-resistant many cancer cells such as human glioblastoma (GBM, U373), human non-small cell lung cancer (A549), human melanoma (SKMEL-28), anaplastic oligodendroglioma (Hs683) and breast cancer (MCF-7).[38] In addition, Finiuk et al. demonstrate that pyrrole-derived compounds showed multidrug-resistant transporters effects in human pancreatic, hepatocarcinoma, and colon carcinoma cells. Also, these compounds have shown the most pronounced effect against human cervical carcinoma cells (KB3-1 and KBC-1).[39] As a result of the literature review of pyrrole-derived compounds, sufficient resources have not been found for the drug resistance mechanisms of the compounds. Therefore, the study has an important and original value.

In view of this information, in our study, we planned to investigate both PhTAD-substituted dihydropyrrole compounds' impact on gene expressions of ABC Transporters in the MCF7 cells, and predictive molecular binding sites target on human ABCB1 structure for these compounds.

Our findings indicate that CI, CII, CIII, CV, CVIII, and CXII treatment compared to negative control in MCF-7 cells increased ABCB1 gene expression levels, while CIV, CVI, CVII, CX, CIX, CXI, CXIII, and CXIV decreased ABCB1 gene expression levels (Figure 2). Especially, CX and CXIV markedly downregulated 6-fold and 13-fold ABCB1 gene expression levels, respectively.

In addition, CI, CIV, CVI, and CVIII treatment compared to negative control in MCF-7 cells upregulate the mRNA expression of ABCC3, although CVII, CX, CXII, CXIII, and CXIV downregulate the mRNA expression of ABCC3 (Figure 3). Moreover, ABCC10 gene expression levels induced by CI, CII, CIII, CIV, CV, CVI, CVII, CVIII, CIX, CX, CXI**,** CXII, CXIII, and CXIV treatment compared to negative control in MCF-7 cells (Figure 4). Conversely, the mRNA expression levels of ABCC11 reduced by CI, CII, CIII, CIV, CV, CVI, CVII, CVII, CIX, CX, CXI, CXII, CXIII, and CXIV treatment compared to negative control in MCF-7 cells (Figure 5). Especially, CVI, CVIII, and CXI are extremely suppressed on ABCC11 gene expression.

Furthermore, CII, CV, and CVI treatment compared to negative control in MCF-7 cells increased ABCG2 gene expression levels, while CI, CVII, CVIII, CIX, CX, CXI, CXII, CXIII, and CXIV decreased ABCG2 gene expression (Figure 6). Interestingly, CIX and CXI treatment markedly downregulated 220-fold and 31-fold the mRNA expression level of ABCG2, respectively.

Our results revealed that gene expression levels of ABCB1, ABCC3, ABCC11 and ABCG2 were parallelly reduced by CVII, CIX, CX, CXI, CXIII, and CXIV. These findings indicate that PhTAD substituted dihydropyrrole compounds deactivated chemoresistance mechanisms of cancer cells.

In addition to gene expression analysis, we tested potential targets on ABCB1 for these molecules (Table 4). Moreover, our Docking assay revealed that compounds I-XIV could interact with key amino acid residues at the active site of the ABCB1-gene MDR protein on side. CXI, CII, CXIV, CX, CIX, CVI, CVII, and CIV have shown markedly highest dock scores for ABCB1 protein, respectively. Especially, CXI and CXIV have important binding sites on ABCB1 and could be potential inhibitors (Figure 7).

In conclusion, our findings indicate that the PhTAD-substituted dihydropyrrole containing molecules alterate ABC transporter gene levels as a potential regulator of cancer chemoresistance. Besides, these molecules may have a considerable inhibitor effect on ABCB1. These compounds might be used as a potential molecule for cancer drug design. However, further studies on these compounds are needed.

Conflict of Interest

The authors declare that there is not any conflict of interest regarding the publication of this manuscript.

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Authors' Contributions

Concept/Design: BY, SM, MA, AA, MG, TY. Data Collection and/or Processing: BY, SM, MA, AA, MG, TY. Data analysis and interpretation: BY, SM, AA, MG, TY. Literature Search: BY, SM, MG, TY. Drafting manuscript: BY, SM, MA, AA, MG, TY. Critical revision of manuscript: BY, SM, MA, AA, MG, TY. Supervision: BY.

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