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Research Article

Analyzing the Role of Phytochemicals in targeting Drug Transporter Protein ABCC6 using Molecular Docking and Molecular Dynamics Simulations

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ABSTRACT

Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype that lacks hormonal receptors. This reduces the therapeutic options for TNBC patients creating more focus on chemotherapy. Drug resistance has posed a major hurdle in treating TNBC patients. Deregulation of drug transporter proteins is one of the major factors that cause resistance to chemotherapeutic drugs. In this study, ABCC6, a drug transporter protein that is found dysregulated in several resistant cancer cells has been docked with natural compounds or phytochemicals with known anti-cancer activities. Subtrifloralactone G, a withanolide extracted from *Deprea subtriflora* is found to show highest binding energy with ABCC6 protein. Molecular dynamics simulations further prove the stability of the ABCC6 protein-subtrifloralactone G ligand complex. ADMET analysis shows that phytochemical subtrifloralactone G can be used as an anti-cancer therapeutic drug in treating resistant cancer cells. The study mainly focuses on the role of phytochemicals in treating resistant TNBC cells.

Introduction

Breast cancer (BC) is one of the most common malignancies amongst women across the globe. Of all molecular subtypes of breast cancer, TNBC is the most aggressive subtypes with increased rates of recurrence and metastasis. It accounts for 10 to 15% of all breast cancer types with high histologic grade.[1] This subtype of BC is characterized by a lack of hormonal receptor expression–estrogen (ER) negative, progesterone (PR) negative, and absent or reduced levels of HER2 protein. TNBC shows a strong correlation with BC gene (BRCA) 1/2 mutations and is commonly found in young and obese women. There are limited treatment options for TNBC patients. Hormonal and targeted therapies do not work for TNBC patients, as TNBC lacks hormone receptors and reduced HER2 levels.[2] Lack of hormonal receptors makes treatment of TNBC even more challenging. Chemotherapy and adjuvant chemotherapy are limited treatment options for TNBC patients.[1] Drug resistance is another major obstacle in the treatment of TNBC. Patients receiving chemotherapy often face drug resistance to a broad spectrum of chemotherapeutic agents. Of several factors that cause drug resistance in cancer, ATP-binding cassette (ABC) drug transporters are the major causative factor inducing drug resistance.[3]

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ATP-binding cassette (ABC) transporter superfamily members are involved in the transport of molecules across the cell membrane.[4] The structure of ABC transporters consists of two nucleotide-binding domains (NBD) and two transmembrane domains (TMD).[5] On the basis of sequence similarity and structure, the transporter family has been divided into seven subfamilies, designated as ABC A-G.[6] ABCC6, also known as multi-resistance protein (MRP) 6 belongs to ABC gene subfamily C and is encoded by the ABCC6 gene. ABCC6 is efflux transporter and effectively pumps NEM-GS (glutathione conjugate of N-ethlmaleimide and LTC4 (leukotriene C4).[7-9] Dysregulation of ABCC6 is observed in TNBC.[10-13] Studies show that MRP 6 is capable of inducing drug resistance in cancer for etoposide, doxorubicin, daunorubicin, cisplatin, and actinomycin D.[14,15]

For hundreds of years, humans have used herbs and plants for the treatment of several diseases.[16-19] Enough emphasis has been made on the relevance of phytochemicals (biologically active plant chemicals) in cancer prevention. Plants have been used as an alternative to chemotherapeutic drugs in cancer treatment, and more than 3,000 plants have been reported to exhibit anti-cancer properties.[20] Phytochemicals have shown cancer preventive properties, ability to restore sensitivity in resistant cancer cells,[21,22] and increase the efficiency of chemotherapeutic drugs.[23] Inhibitory action on NF-κB, a key player in tumorigenesis, has been observed by resveratrol, limonene, gingerol, genistein, apigenenin, and many others.[24]

Bioinformatics tools, like molecular docking, enable understanding of ligand interaction with small molecules, like substrate or regulators.[25] Molecular docking has gained immense importance in drug designing and discovery as they are fast, reliable, and economic alternative to the experimental approach. Molecular docking is used to find binding poses and binding affinity prediction. These predictions further suggest use of candidates as active compounds.[26] Molecular dynamics simulations study was developed in the late 1970s and has gained immense importance in studying atoms and molecules of biological importance. It enables the study of entire protein complexes in solvents, in proteins embedded in membranes or in nucleosomes or ribosomes complexes. Molecular dynamics study is a widely used simulation tool that allows understanding protein-ligand interactions at increased flexibility more effectively than molecular docking.[27] Molecular dynamics simulation allows evaluation of protein structures in equilibrium state. The binding ability and stability of protein-ligand complexes were evaluated using molecular dynamics simulations,[28,29] while molecular docking uses rigid structures obtained from protein data bank (PDB), molecular dynamics (MD) simulations work in more relevant dynamics.[30]

Resistant cancers have become a major problem in treating patients. In this study, 1,574 natural compounds were docked with ABCC6 protein to evaluate the binding stability of protein-ligand complex, and molecular dynamics simulations were performed of ABCC6 protein and docked phytochemical subtrifloralactone G. ADMET properties were also checked for ligand subtrifloralactone G.

Materials and Methods

ABCC6 Protein Preparation

The protein structure of ABCC6 was downloaded from Research Collaboratory for Structural Bioinformatics (RCSB) PDB (Fig. 1). PDB ID of ABCC6 protein is 6BZR, which is inbound with ADP ligand (adenosine-5'-diphosphate) ($C_{10}H_{15}N_5O_{10}P_2$) and citric acid ($C_6H_8O_7$). It is a membrane transport protein in *Homo sapiens.* It has two chains, A and B, with a 251 sequence length. The crystal structure of this protein is obtained through X-ray diffraction with resolution 2.79 Å. Water molecules were removed, and the energy minimization step was performed with the help of the standard steepest descent method in "yet another scientific artificial reality application" (YASARA).[31]

Preparation of Natural Compounds

In this study, the ligands were derived from naturally occurring plant-based anti-cancer compound activity target (NPACT) database (http://crdd.osdd.net/raghava/npact/). This database has approximately 1,500 natural compounds with known anti-cancer activities, and these compounds were compiled into a single dataset for docking purposes. For further use, ligands were cleaned, and hydrogens were added using the Marvin sketch tool.[32]

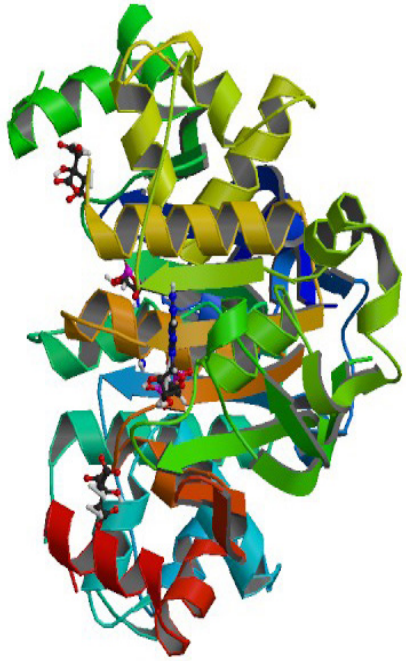

Fig. 1: ABCC6 protein structure downloaded from PDB (PDB ID: 6BZR)

Molecular Docking

It is an important tool in structural biology and assists in computational drug designing by presenting the interactions between two molecules and finds most feasible orientation that forms a reliable complex with minimum energy. The main objective of molecular docking is prediction of protein-ligand complex, wherein ligand binds to the cavity of protein. These cavities are called active sites as these sites become functionally active when acted by external molecules.[33] Docking is a crucial step to choose potential hits in virtual screening. It allows interaction between different atoms and molecules for a fixed period of time.[34] YASARA software was used to investigate the detailed interactions between hits and drug transporter proteins ABCC6. For molecular docking, YASARA uses AutoDock Vina 4.2 algorithm and AMBER03 force field. Removal of water molecules, small ions, and energy minimization was performed in YASARA. A profile was generated of all the hits and proteins. The protein-ligand interactions were further visualized in Discovery Studio. The free binding energy ΔG_{bind} was given by the following equation:

$$\Delta G = \Delta G_{vdW} + \Delta G_{Hbond} + \Delta G_{elec} + \Delta G_{tor} + \Delta G_{desolv}$$

where, ΔG_{vdW} = van der Waals term for docking energy; ΔG_{Hbond} = H bonding term for docking energy; ΔG_{elec} = electrostatic term for docking energy; ΔG_{tor} = torsional free energy term for ligand when the ligand transits from unbounded to bounded state; ΔG_{desolv} = desolvation term for docking energy.

The results of molecular docking are dependent on two factors: optimization search method, which detects docking complexes with minimum binding energies, and scoring function is used as benchmark to evaluate results obtained after docking.[35]

Molecular Dynamics Simulations

Molecular dynamics simulations allow understanding of binding stability of desired ligands to the proteins. A molecular dynamics simulation study was undertaken by using YASARA with AMBER03 force field for docked complex. Energy minimization was performed for protein-ligand complexes to remove unfavorable atoms. Steepest descent minimization was used, and simulation was continued at 10 ns. The molecular dynamics simulation was carried out within simulation box at following conditions: temperature 298 K, pressure 1 bar, coulomb electrostatics at cut-off 7.86, 0.9% NaCl, solvent density 0.997, pH 7.0, 1-fs time steps, periodic boundaries, and all atoms mobile.[34] The molecular complexes were stimulated at 10 ns with frame capture at every 2.5 ns (0, 2.5, 5, 7.5, and 10 ns). Different trajectories were analyzed through several quantities, including root mean squared deviation (RMSD).[35]

ADMET Analysis

To check different ADMET properties, admetSAR (http://www.admetexp.org) and Molinspiration tool (https://www.molinspiration.com/) was used.

Results and Discussion

Molecular Docking

In drug designing, molecular docking is the first approach to check feasibility of any biochemical reaction before carrying out experimental approach. This approach allows prediction of several different binding sites in target allowing development and selection of efficient and potent drug candidates. It also allows *in silico* evaluation of large databases to search for potent drug candidates.[36]

For ABCC6, the three best ligands are subtrifloralactone, helioxanthin, and 5-beta-spirostan-3-beta-ol 3-O-beta-D-glucopyranosyl-(1-2)-beta-D-glucopyranoside (Table 1). Subtrifloralactone G showed highest binding energy (9.053 kcal/mol). Positive YASARA score indicates stronger, stable, and efficient binding. Contacting residue receptors, hydrogen bond interactions, pi-sigma bond, alkyl bond, and pi-alkyl bond were noted. Fig. 2 shows docked results of ABCC6 protein and subtrifloralactone G in 3D and 2D format with different interactions at specified distances.

Fig. 2 shows 3D and 2D pose of ABCC6 protein-subtrifloralactone G ligand complex. The active compound, subtrifloralactone G showed hydrogen bond interactions with residues Ser-1306, Gly-1304, Ser-1307, and Lys-1305, van der Waals interactions with residues Tyr-1274, Thr-1301, Ile-1456, Val-1282, Ala-1303, Gly-1475, and Leu-1280, alkyl bonds with residues Ala-1281, Pro-1279, and pi-alkyl bonds with residues His-1458. Forces between

Table 1: Top three molecular docking results of phytochemicals with ABCC6

Ligand name	*Binding energy (kcal/mol)*	*Contacting residue receptors*
Subtrifloralactone G	9.053	Tyr 1274, Leu 1278, Pro 1279, Leu 1280 Ala 1281, Val 1282, Arg 1300, Thr 1301, Gly 1302, Ala 1303, Gly 1304, Lys 1305, Ser 1306, Ser 1307, Ile 1456, His 1458, Lys 1474, Gly A 1475
Helioxanthin	9.03	Tyr 1274, Leu 1278, Pro 1279, Leu 1280, Ala 1281, Thr 1301, Gly 1302, Ala 1303, Gly 1304, Lys 1305, Ser 1306, Ser 1307
5-beta-spirostan-3-beta-ol 3-O-beta-D-glucopyranosyl-(1-2)-beta-D-glucopyranoside	8.914	Tyr 1274, Leu 1278, Pro 1279, Leu 1280, Ala 1281, Arg 1300, Thr 1301, Gly 1302, Ala 1303, Gly 1304, Lys 1305, Ser 1306, Ser 1307, Pro 1346, Gln 1347, Asp 1348, Asp 1426, Ile 1456, His 1458, Lys 1474, Gly 1475

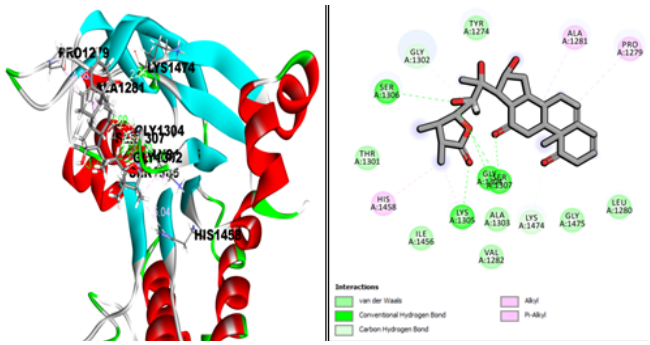

Fig. 2: 3D and 2D pose of ABCC6 and subtrifloralactone G

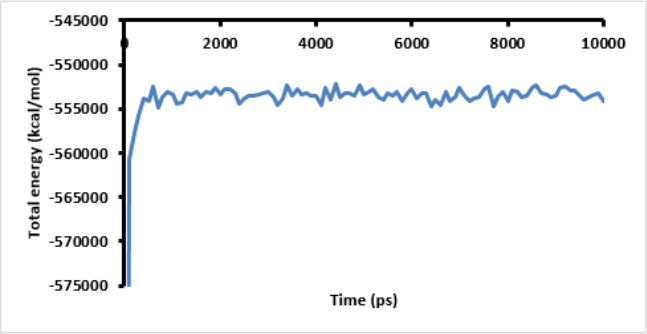

Fig. 3: Time (ps) *vs.* energy (kJ/mol) plot of ABCC6 protein-subtrifloralactone G ligand complex

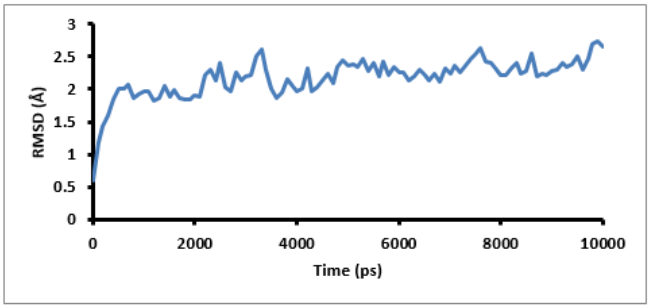

Fig. 4: Time (ps) *vs.* RMSD (Å) of ABCC6 protein-subtrifloralactone G ligand complex

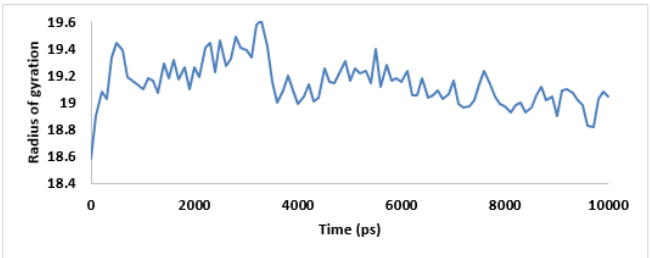

Fig. 5: Time (ps) *vs.* radius of gyration plot of ABCC6 protein-subtrifloralactone G ligand complex

molecules create interactions between the particles. There are four main types of interactions, *viz.*, electrostatic forces due to charges within the compound, electrodynamics forces, or van der Waals forces, steric forces that are generated due to proximity of different molecules and solvent related forces, like hydrophilic (hydrogen bonds) and hydrophobic interactions.[33]

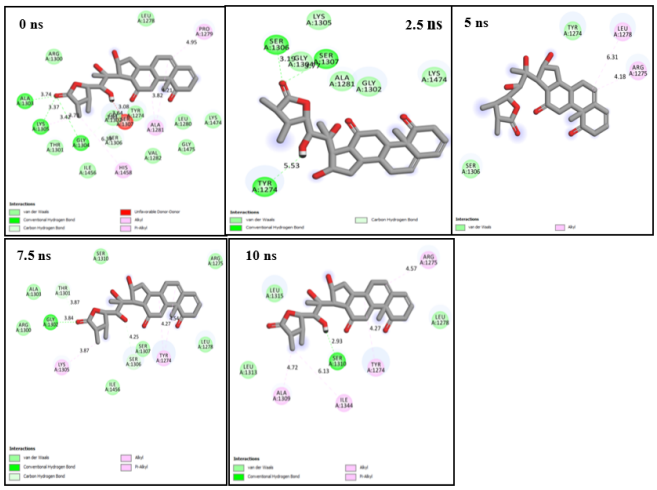

Fig. 6: Conformational changes in protein-ligand complex observed after every 2.5 ns of the molecular dynamics simulation (10 ns)

Table 2: ADMET properties of compound subtrifloralactone G

ADMET properties	*Subtrifloralactone G*	
Blood-brain barrier penetration	+	0.81
Human intestinal absorption	+	0.96
CYP2D6 inhibitor	-	0.94
Caco-2 cell permeability	-	0.79
Carcinogenicity	-	0.97
Human oral bioavailability	-	0.68

Table 3: Lipinski's rule of five

Molecular weight	*472.58*
AlogP	1.98
H-bond acceptor	7
H-bond donor	3
Rotatable bonds	3

The protein-ligand complex ABCC6 and subtrifloralactone G was further subjected to molecular dynamics simulations. Certain conditions were kept constant during simulations, like number of atoms, pressure, temperature, pH, and density. Fig. 3 shows time (in ps) *vs.* energy (kJ/mol) plot that depicts fluctuations of the complexes. Time (in ps) *vs.* RMSD plot (Fig. 4) shows stability of docked protein-ligand complex. Fig. 5 shows time (in ps) *vs.* radius of gyration plot, which shows spatial packing of amino acid residues, which shows protein stability. Higher value of radius of gyration indicates less compact structure. Further, the docked complexes were visualized in Discovery Studio 3 to display various interactions involved in the protein-ligand docking. Fig. 6 shows conformational changes in the protein-ligand complex when simulated at time period of 10 ns.

Multi-drug resistance proteins (MRP) are membrane glycoproteins that are ATP-dependent and facilitate the export of drugs from cells.[37] ABCC6 is an efflux drug transporter with a known role in inducing resistance to

Table 4: Drug-likeness of subtrifloralactone G ligand

miLogP	*TPSA*	*MW*	*natoms*	*nON*	*nOHNH*	*nviolations*	*nrotb*	*volume*
0.94	121.13	472.58	34	7	3	0	3	437.98

drugs, like etoposide, doxorubicin, daunorubicin, cisplatin, and actinomycin D.[38]

ADMET Analysis

ADMET analysis allows computational analysis of absorption, distribution, metabolism, excretion, and toxicity properties of drug candidate. In this study, we performed ADMET analysis of subtrifloralactone G compound to check whether it can be used as drug candidate or not. Several analyses like blood-brain barrier penetration, human intestinal absorption, CYP2D6 inhibitor, Caco-2 cell permeability, carcinogenicity, and biodegradation were calculated. The cytochrome P450 family plays a critical role in drug metabolism and excretion through liver. CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 are the most important isoforms. Dysregulation of these isoforms may lead to decreased drug metabolism. BBB is a highly selective membrane that prevents blood components from crossing the extracellular fluid in brain. Health impact assessment (HIA), Caco-2 cell permeability, and bioavailability are also important parameters that are considered for potential drug identification.[39] Table 2 shows blood-brain barrier penetration, human intestinal absorption, CYP2D6 inhibitor, Caco-2 cell permeability, carcinogenicity, and biodegradation. Subtrifloralactone G shows BBB penetration and HIA, but fails to show Caco-2 permeability. The compound also exhibits negative human oral bioavailability. The major drawback of natural compounds is reduced bioavailability. However, this can be solved by chemically modifying the structure or by using nanoparticle delivery of drugs in the body.[40]

Lipinski's rule of five states that a drug candidate should fulfill certain conditions to be used in humans–octanol-water partition coefficient (logP) should be < 5, molecular weight ≤ 500 kDa, number of H-bond donors should be ≤ 5; number of H-bond receptors should be ≤ 10.[39] Another rule was included: the number of rotatable bonds should be < 10 (Table 3).

Molinspiration Analysis

Molecular weight plays a key role in determining the therapeutic efficiency of the drug. Bulkier molecules cannot be easily transported, diffused, and absorbed when compared to lighter molecules. The molecular weight of subtrifloralactone G is 472.58, which is < 500. Oral bioavailability of drug molecules was characterized by lipophilicity (log P value) and topological polar surface area (TPSA) values. Log P value for subtrifloralactone G is 0.94. This value is within the acceptable limit for drug permeability. Table 4 shows that subtrifloralactone G ligand showed no violations of drug-likeness conditions.

The compound subtrifloralactone G is derived from *D. subtriflora* (Solanaceae family), a plant native to Peru.[41] Subtrifloralactone is a withanolide that are found exclusively in Solanaceae family.[42] Withanolides have shown potential in anti-tumor, anti-stress, anti-microbial, and anti-inflammatory activities. These withanolides have exhibited the potential to function as chemopreventive agents. 10 norwithanolides (a subclass of withanolides), subtrifloralactone A-J are isolated from *D. subtriflora.*[41] The helioxanthin is extracted from *Taiwania cryptomerioides* plant. This phytochemical exhibits a broad spectrum of activities. A study by Yueh-Min Lin showed anti-cancer activity of helioxanthin in oral squamous cell carcinoma.[43]

Drug resistance poses as one of the major obstacles in cancer treatment management. ABC transporter family plays an important factor in inducing resistance to drugs in cancer treatment. Up regulation of ABCC6, an efflux drug transporter is found in diverse cancer types.[44-46] Molecular docking was used to investigate ABCC6 protein-phytochemicals ligand affinity. ABCC6 protein has been observed to be interacting with subtrifloralactone G, helioxanthin, and 5-beta-spirostan-3-beta-ol 3-O-beta-D-glucopyranosyl-(1-2)-beta-D-glucopyranoside with binding energies 9.053, 9.03, and 8.914 kcal/mol, respectively. Molecular dynamics simulations showed that subtrifloralactone G shows stable configuration and has potential to be used as an anti-cancer agent. Molecular dynamics simulations results support the hypothesis that phytochemicals are reliable ligands that can be used as anti-cancer therapeutic agents. These results could be further used for designing phytochemicals derived drugs for resistant cancer types. ADMET analysis shows that subtrifloralactone G can be used as potential therapeutic candidate in targeting ABCC6 protein; however, further analysis is required to validate these results.

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