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COMPUTERISED DESIGNING OF DOXORUBICIN WITH BREAST CANCER CELLS

DOKSORUBİSİNİN MEME KANSERİ HÜCRELERİ İLE BİLGİSAYARLI TASARIMI

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ABSTRACT

Objective: Breast cancer is the type of cancer that starts in breast cells and has the highest incidence in the world after lung cancer. Doxorubicin is widely used in the treatment of some leukemia and Hodgkin's lymphoma, as well as in the treatment of bladder, breast, stomach, lung, ovary, thyroid, soft tissue sarcoma, multiple myeloma and other cancers. We want to determine the binding interaction of Doxorubicin in the active site of the Galectin-3 with ASN-48, ARG-32, ASN-62 and GLU-72 residues, conformation and docking score energies.

Material and Method: We used docking methods to detect the efficiency of Doxorubicin at breast cancer cells, clarify the role of Galectin-3 and elucidate the interaction between Galectin-3 and Doxorubicin.

Results: The docking score obtained for Doxorubicin in the active side of the Galectin-3 protein was -5.32 kcal/mol. In to the active site of protein, Doxorubicin was bound with strong hydrogen bond by the residue ASN-48, ARG-32, ASN-62 and GLU-72, and a salt bridge with the same amino acid residue were established and stability was achieved.

Conclusion: The development of specific therapies targeting cancer stem cells may provide hope for prolonging the life span and improving quality.

Keywords: Docking, breast cancer, Galectin-3, Doxorubicin

ÖZET

Amaç: Meme kanseri, meme hücrelerinde başlayan ve akciğer kanserinden sonra dünyada en yüksek insidansa sahip olan kanser türüdür. Doksorubisin, bazı lösemi ve Hodgkin lenfomasının yanı sıra mesane, meme, mide, akciğer, yumurtalık, tiroid, yumuşak doku sarkomu, multipl miyeloma ve diğer kanserlerin tedavisinde yaygın olarak kullanılır. Çalışmamızda; Doksorubisin'in Galektin 3'ün aktif bölgesinde yer alan, ASN-48, ARG-32, ASN-62 ve GLU-72 rezidüleri ile yaptığı bağlanma etkileşimlerini, konformasyonlarını ve doking skor enerjilerini belirlemek istedik.

Gereç ve Yöntem: Meme kanseri hücrelerinde Doxorubicin etkinliğini tespit etmek, Galectin-3 rolünü netleştirmek ve Galektin-3 ve Doksorubisin arasındaki etkileşimi aydınlatmak için doking yöntemi kullanılmıştır.

Bulgular: Tüm konformerler arasında Doksorubisin Galektin-3 proteini ile en iyi kenetlenme sonucunu -5,32 kcal/mol docking skoruna sahip konformer vermiştir. Doksorubisin güçlü hidrojen bağları ile ASN-48, ARG-32, ASN-62 ve GLU-72 rezidülerine bağlanmış ve aynı amino asit rezidüleri ile bir tuz köprüsü de kurarak, kararlılık sağlanmıştır.

Sonuç: Kanser kök hücrelerini hedef alan spesifik tedavilerin geliştirilmesi açısından, yaşam süresinin uzatılması ve kalitenin arttırılması için bu çalışmanın umut sağlayabilir olduğunu düşünmekteyiz.

Anahtar Kelimeler: Doking, meme kanseri, Galektin-3, Doksorubisin

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INTRODUCTION

The highest incidence and mortality rate of all cancers in the female population is breast cancer (1, 2). It is estimated that there are approximately 2.5 million survivors of breast cancer in the US (3). This figure will expand to 3.4 million in 2015, representing an increase of 31% (4). The millions more worldwide are probably grossly underestimated because of the poor or inefficient reporting systems and the lack of reliable cancer registries in third-world countries (5, 6).

Molecular characteristics of the tumor are important for breast cancer treatment (6, 7). Anthracyline drugs such as doxorubicin are first extracted from Streptomyces peucetius var. caesius. Doxorubicin is used in the treatment of several cancers including breast, lung, gastric, ovarian, thyroid, non-Hodgkin's and Hodgkin's lymphoma, multiple myeloma, sarcoma, and pediatric cancers (8–10). The biggest side effect of doxorubicin is cardiotoxicity (11-15).

Galectin-3 is an endogenous carbohydrate-binding protein that has been shown to have a variety of cellular functions (16). Galectin-3 may increase or decrease apoptosis depending on cell type and stimulus. Overexpression of galectin-3 in breast carcinoma cells makes cells resistant to chemotherapeutic drugs (17, 18).

Various in vitro assays and theoretical calculation methods have been used to detect the efficiency of Doxorubicin in various breast cancer cells, clarify the role of Galectin-3 and elucidate the interaction between Galectin-3 and Doxorubicin.

Molecular docking is one of this methods to detect interaction of proteins. This method has become an increasingly important tool for new effective drug discovery. The molecular docking can be used to make model for the interaction between a small molecule (ligand) and a protein (receptor) at an atomic level; which allows us to understand the basic biochemical processes, enabling us to characterize the behavior of small molecules in the binding site of target proteins (19, 20).

MATERIALS AND METHODS

Molecular Docking

Using the Glide SP (standard precision) module of the Maestro version 11.4 in the Schrodinger Software program (Schrödinger Release 2017-4: Maestro, Schrödinger, LLC, New York, NY, 2017) (21-23) the docking calculations were performed. To prepare ligand for docking calculations, first, the most stable conformation of Doxorubicin was generated from the result of the molecular dynamic calculation, and then was prepared to optimization by the Lig Prep tool in the Maestro 11.4 version of the Schrödinger Software program using the OPLS force field (24). After selecting the ionization states at pH 7.0 ± 2.0, possible stereoisomers were produced for the ligand. Because of Doxorubicin induces the expression of galectin-3 in breast cancer cell line and in primary tumors from breast cancer patients, we choose galectin-3 (25) (PDB code: 2XG3) which could be a potential target to prevent Doxorubicin induced chemo resistance in breast cancer. By using SWISS-MODEL server, the crystal structure obtained from the protein data bank was arranged to get a better protein homology model (26). All water was removed, polar hydrogens were added, bond orders were assigned, charges were defined using PROPKA (27) at pH 7.0, and galectin-3 was optimized by using the Protein Preparation Wizard tool (28). Energy minimization was carried out by preferring 0.3A˚ RMSD and the OPLS3 force field to converge heavy atoms. To generate a grid, receptor grid generation tool was used; a cubic box was formed that centered on the centroid of the ligand with specific dimensions. By defining routable groups of an active site of protein, ligand-receptor docking was occurred. Absorption, distribution, metabolism, and excretion (ADME) properties were also calculated using the Qik-Prop module of the Schrodinger software to specify the physicochemical and biological functional properties such as the molecular weight (MW), percent human oral absorption, predicted octanol/water partition coefficient (QPlogPo/w), polar surface area (PSA), and number of violations of Lipinski's rule of five (29), which is important for generating an effective drug in new drug development.

RESULTS

Molecular Docking Results

The docking score obtained for Doxorubicin in the active side of the Galectin-3 protein was -5.32 kcal/mol, as shown in Table 1 and Figure 1. The binding pocket of the Galectin-3 (2XG3.pdb) protein has a hydrophobic, polar, positive charged and negative charged regions as indicated by the green, blue, dark blue and orange respectively. The most likely binding position between Galectin-3

Table 1. The conformation and docking score energies

Ligand	Docking Score (kcal/mol)
1	-5.32
2	-5.19
3	-4.75
4	-4.72
5	-4.59
6	-4.57
7	-4.55
8	-4.50

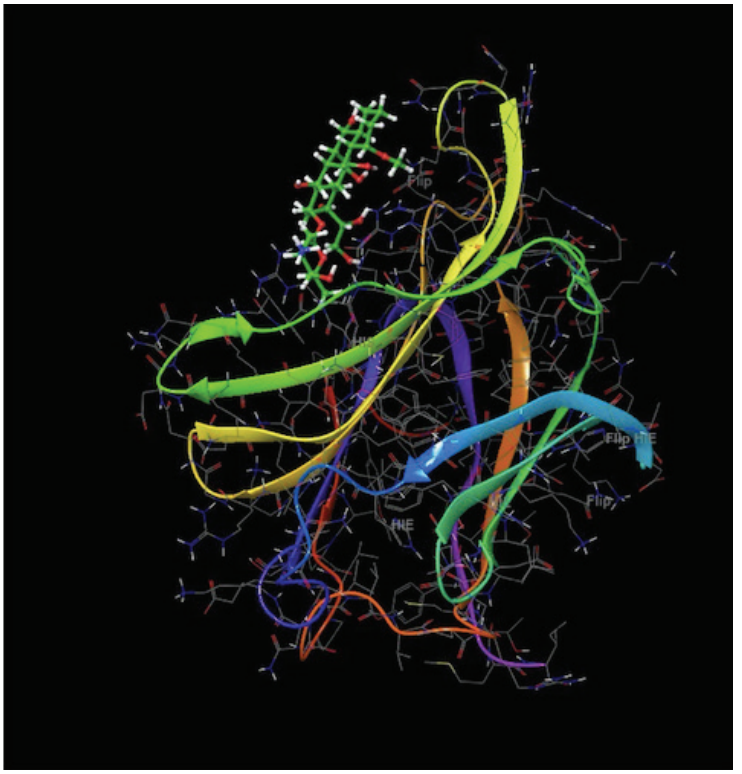

Figure 1: The docked molecular structure of Doxorubicin and Galectin-3

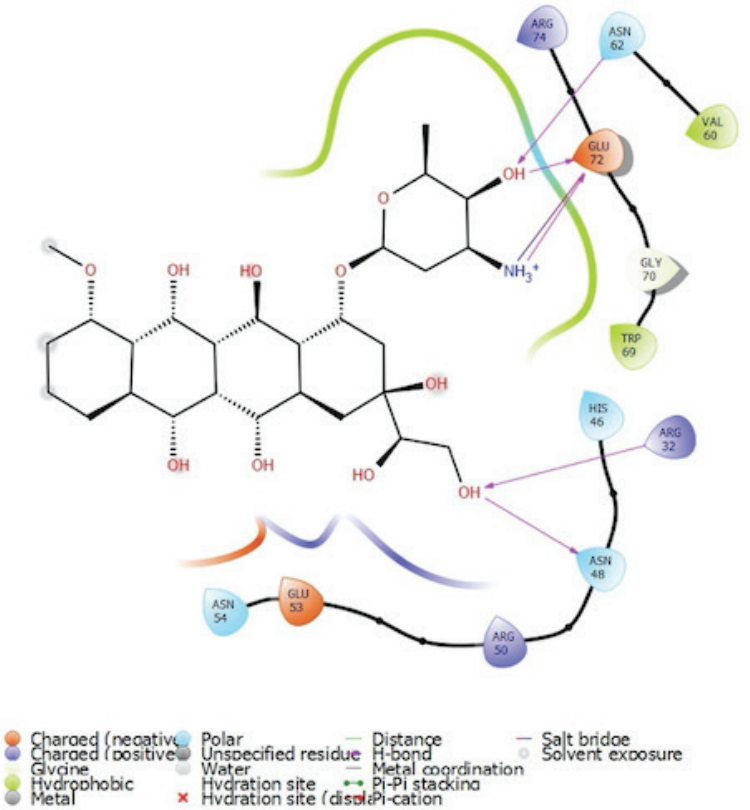

Figure 2: 2D ligand interaction of Doxorubucin in the active side of Galectin-3

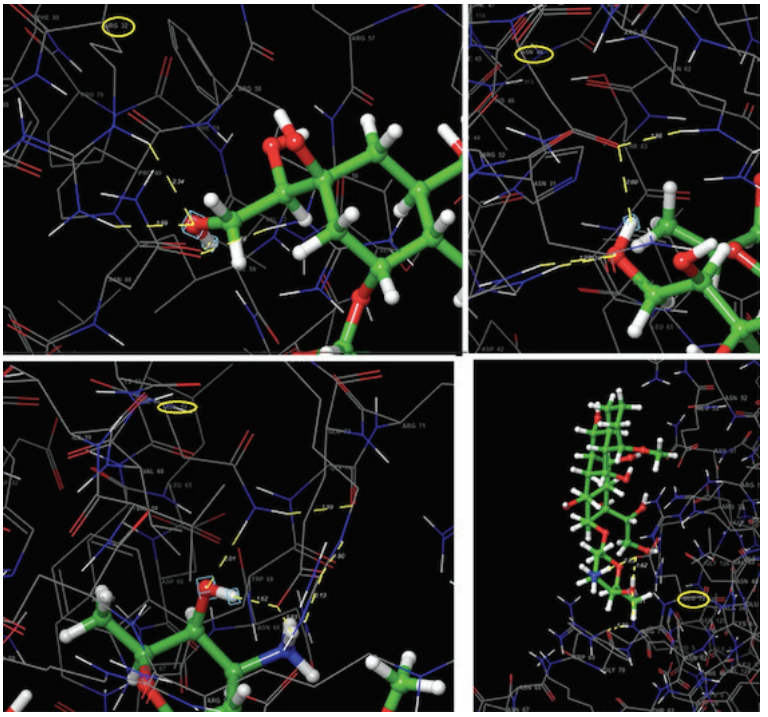

Figure 3: The binding interaction of Doxorubucin in the active side of the Galectin-3 with ASN-48, ARG-32, ASN-62 and GLU-72 residues

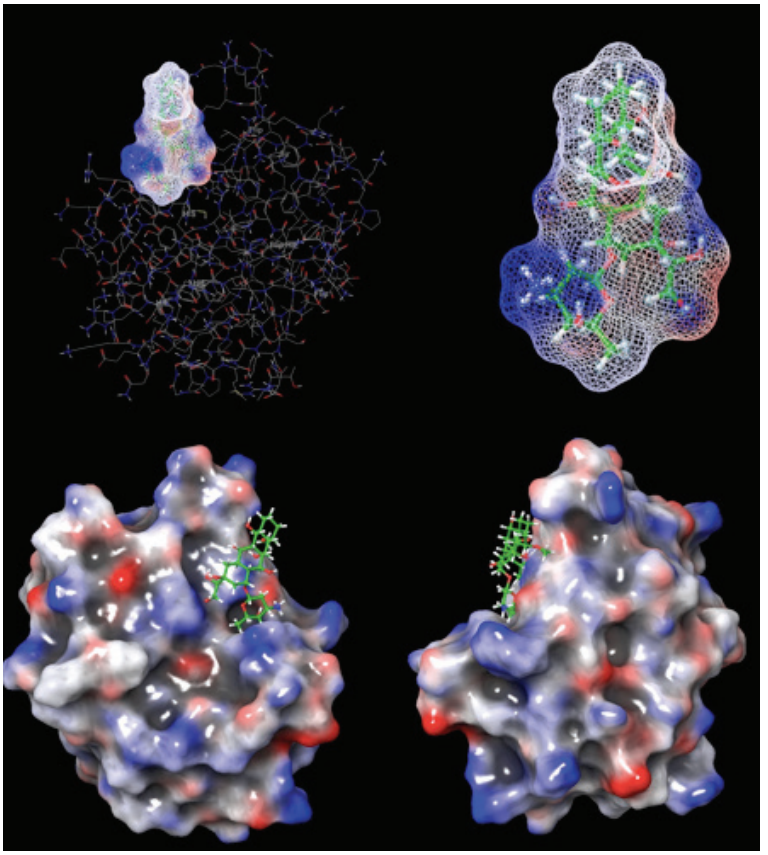

Figure 4: The electrostatic potential of Galectin-3 and Doxorubicin

protein and Doxorubicin was determined by hydrogen bonds represented by the purple line and were picturized in the 2D ligand interaction graph in Figure 2. In to the active site of protein, Doxorubicin was bound with strong hydrogen bond by the residue ASN-48 (2 Å), ARG-32 (2.34 Å and 1.99 Å), ASN-62 (2.01 Å) and GLU-72, (1.83 Å and 1.62 Å) and a salt bridge with the same amino acid residue were established and stability was achieved. The binding interactions of Doxorubicin with ASN-48, ARG-32, ASN-62 and GLU-72 residues were also shown in Figure 3.

The electrostatic potential map surfaces of the Doxorubicin and Galectin-3 protein were also constituted to define the regions that were electron-rich and electron-poor. The lowest potential (electron-rich) regions were expressed in red; while those with the highest potential (electron poor) were shown in blue. The electrostatic potential of Galectin-3 and Doxorubicin were also shown in Figure 4.

The pharmacokinetic parameters of drugs such as their permeability towards QPlogP for octanol/gas, QPlogP for octanol/water, PlogS for aqueous solubility, P log K hsa Serum Protein Binding, Predicted CNS Activity, Caco-2 Permeability, skin permeability, the blood–brain barrier, percentage oral absorption were also calculated and tabulated using the Qik Prop application of the Maestro software package in Table 2.

Table 2. The ADME properties of Doxorubicin

Property			**Value**	**Recommended**
Solute	Molecular Weight	=	561.668	(130.0 / 725.0)
Solute	Dipole Moment (D)	=	8.379	(1.0 / 12.5)
Solute	Total SASA	=	810.135	(300.0 /1000.0)
Solute	Hydrophobic SASA	=	511.383	(0.0 / 750.0)
Solute	Hydrophilic SASA	=	298.752	(7.0 / 330.0)
Solute	Carbon Pi SASA	=	0.000	(0.0 / 450.0)
Solute	Weakly Polar SASA	=	0.000	(0.0 / 175.0)
Solute	Molecular Volume (A^3)	=	1.589.762	(500.0 /2000.0)
Solute	vdW Polar SA (PSA)	=	200.070	(7.0 / 200.0)*
Solute	No. of Rotatable Bonds	=	14.000	(0.0 / 15.0)
Solute as Donor - Hydrogen Bonds		=	10.000	(0.0 / 6.0)*
Solute as Acceptor - Hydrogen Bonds		=	18.750	(2.0 / 20.0)
Solute Globularity (Sphere = 1)		=	0.813	(0.75 / 0.95)
Solute Ionization Potential (eV)		=	9.913	(7.9 / 10.5)
Solute Electron Affinity (eV)		=	-1.778	(-0.9 / 1.7)*
Predictions for Properties:				
QP Polarizability (Angstroms^3)		=	48.479M	(13.0 / 70.0)
QP log P for hexadecane/gas		=	17.269M	(4.0 / 18.0)
QP log P for octanol/gas		=	41.651M	(8.0 / 35.0)*
QP log P for water/gas		=	33.727M	(4.0 / 45.0)
QP log P for octanol/water		=	-1.812	(-2.0 / 6.5)
QP log S for aqueous solubility		=	-1.553	(-6.5 / 0.5)
QP log S - conformation independent		=	-1.734	(-6.5 / 0.5)
QP log K hsa Serum Protein Binding		=	-1.050	(-1.5 / 1.5)
QP log BB for brain/blood		=	-2.894	(-3.0 / 1.2)
No. of Primary Metabolites		=	9	(1.0 / 8.0)*
Predicted CNS Activity (-- to ++)		=	--	
HERG K+ Channel Blockage: log IC50		=	-5.282	(concern below -5)
Apparent Caco-2 Permeability (nm/sec)		=	3	(<25 poor
Apparent MDCK Permeability (nm/sec)		=	1M	(<25 poor
QP log Kp for skin permeability Jm		=	-7.744	(Kp in cm/hr)
Lipinski Rule of 5 Violations		=	3	(maximum is 4)
Jorgensen Rule of 3 Violations		=	2	(maximum is 3)
% Human Oral Absorption in GI (+-20%)		=	0	(<25% is poor)
Qual. Model for Human Oral Absorption		=	low	(>80% is high)

CONCLUSIONS

The molecular docking approach can be used to model the interaction between a small molecule (ligand) and a protein (receptor) at an atomic level; which allows us to understand the basic biochemical processes, enabling us to characterize the behavior of small molecules in the binding site of target proteins.

In this study; by examining the interaction between doxorubicin and Galectin-3, the most stable binding exposure between all binding poses were determined. Characterization of the interaction between ligand and protein and ADME profiles were also obtained as a result of molecular docking calculations.

When this tables are examined; The octanol / water value is -1.812, and this value (suggested values-2, 6.5) appears to be within the acceptable range, although the molecular weight of doxorubicin is acceptable at 561,668 (recommended values 130-725). The development of specific therapies targeting cancer stem cells may provide hope for prolonging the life span and improving quality.

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