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The Therapeutic Potential of Berberine and Resveratrol in Type 2 Diabetes Treatment: Pharmacokinetic and Bioactivity Properties, and Molecular Docking Analysis

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ABSTRACT

Type 2 diabetes (T2D), typically characterized by insulin resistance, is a metabolic disorder that occurs when the body cannot use insulin effectively or does not produce enough insulin. In the treatment of T2D, insulin, metformin, and sulfonylureas are commonly used. Given the limitations of current treatment options, there is a strong need for intensive efforts in the discovery of new drugs. Berberine exhibits antidiabetic effects and possesses anti-inflammatory and antioxidant properties. Resveratrol is another natural compound that has been extensively researched due to its antioxidant and anti-inflammatory characteristics. This study aimed to investigate the interactions between berberine and resveratrol with proteins related to or causing T2D, including ADIPOR1 (PDB-ID: 6ks1), ADIPOR2 (PDB-ID: 5lxg), TNF-α (PDB-ID: 7kpb), PTP1B (PDB-ID: 4i8n), GLUT1 (PDB-ID: 4pyp), IGF-IR (PDB-ID: 8eyr), IGF1 (PDB-ID: 6pyh), ADAMTS9 (PDB-ID: 3ppv), and SPHK2 (PDB ID: 4v24). SwissADME was used to assess the pharmacokinetic properties of berberine and resveratrol. Molecular docking was performed to analyze the interactions between these ligands and the specified proteins. Additionally, the potential bioactivity features of compounds were determined. Protein-protein interactions were obtained from the STRING database. The study data indicated that both compounds have high blood-brain barrier (BBB) penetration and gastrointestinal absorption ability (HIA). Besides, berberine exhibited the highest binding affinity with GLUT4 (-10.1 Kcal/mol), GLUT1 (-9.3 Kcal/mol), and SPHK2 (-9.3 Kcal/mol), while resveratrol showed strong binding with SPHK2 (-9.0 Kcal/mol) and TNF-α (-8.7 Kcal/mol) and. All proteins displayed binding energies of more than -7 Kcal/mol, suggesting that both berberine and resveratrol hold promise as potential drug candidates for T2D.

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Berberin ve Resveratrolün Tip 2 Diyabet Tedavisindeki Terapötik Potansiyeli: Farmakokinetik ve Biyoyararlılık Özellikleri ile Moleküler Docking Analizi

ÖZET

Tip 2 diyabet (T2D), tipik olarak insülin direnci ile karakterize edilen ve vücudun insülini etkili bir şekilde kullanamadığı veya yeterli insülin üretemediği bir metabolik bozukluktur. T2D tedavisinde insülin, metformin ve sülfonilüreler yaygın olarak kullanılmaktadır. Mevcut tedavi seçeneklerinin sınırlamaları göz önüne alındığında, yeni ilaçların keşfinde yoğun çabalara ihtiyaç vardır. Berberin, antidiyabetik, anti-inflamatuvar ve antioksidan özelliklere sahiptir. Resveratrol, antioksidan ve anti-enflamatuvar özellikleri nedeniyle kapsamlı bir şekilde araştırılan bir başka doğal bileşiktir. Bu çalışmanın amacı, berberin ve resveratrolün ADIPOR1 (PDB-ID: 6ks1), ADIPOR2 (PDB-ID: 5lxg), TNF-α (PDB-ID: 7kpb), PTP1B (PDB-ID: 4i8n), GLUT1 (PDB-ID: 4pyp), IGF-IR (PDB-ID: 8eyr), IGF1 (PDB-ID: 6pyh), ADAMTS9 (PDB-ID: 3ppv) ve SPHK2 (PDB ID: 4v24) dahil olmak üzere T2D ile ilişkili veya T2D'ye neden olan proteinlerle olan etkileşimlerini araştırmaktır. Berberin ve resveratrolün farmakokinetik özelliklerini değerlendirmek

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için SwissADME kullanılmıştır. Bu ligandlar ile belirtilen proteinler arasındaki etkileşimleri analiz etmek için moleküler yerleştirme yapılmıştır. Ayrıca, bileşiklerin potansiyel biyoyararlılık özellikleri belirlenmiştir. Protein-protein etkileşimleri STRING veri tabanından elde edilmiştir. Çalışma verileri, her iki bileşiğin de yüksek kan-beyin bariyeri (BBB) penetrasyonu ve gastrointestinal emilim yeteneğine (HIA) sahip olduğunu göstermiştir. Bunun yanında, berberin GLUT4 (-10.1 Kcal/mol), GLUT1 (-9.3 Kcal/mol) ve SPHK2 (-9.3 Kcal/mol) ile en yüksek bağlanma afinitesini gösterirken, resveratrol SPHK2 (-9.0 Kcal/mol) ve TNFR1 (-8.7 Kcal/mol) ile güçlü bağlanma göstermiştir. Tüm proteinler, -7 Kcal/mol'den daha yüksek bağlanma enerjileri sergilemiş, bu da berberin ve resveratrolün T2D için potansiyel ilaç adayları olarak umut verici olduğunu göstermektedir.

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INTRODUCTION

Type 2 diabetes (T2D) is a metabolic disorder primarily distinguished by insulin resistance, signifying a condition where the body's ability to utilize insulin effectively is compromised, often accompanied by insufficient insulin production (American Diabetes Association, 2020). It is estimated that by the year 2045, approximately 783 million adults worldwide will have T2D (Magliano et al., 2021). Genetic, epigenetic, and environmental factors (such as obesity, unhealthy eating habits, lack of physical activity, etc.) are associated with the etiology of the disease (Prasad and Groop, 2015; Ghosh et al., 2022). In the treatment of T2D, insulin, metformin, and sulfonylureas are commonly used, and in some cases, surgical options may be considered (Davies et al., 2018; Gebrie et al., 2021). Additionally, maintaining healthy eating habits and regular physical activity are important for keeping blood sugar levels under control. It is known that multiple signaling pathways are involved in the development of T2D. Some of these include glucose transporter 4 (GLUT4), c-Jun N-terminal kinase (JNK), AMP-activated protein kinase (AMPK), and peroxisome proliferator-activated receptor gamma (PPAR-gamma) (Wen et al., 2022; Sanches et al., 2023).

Berberine is a natural compound derived from plants and has been used in traditional medicine for centuries. It has anti-diabetic, anti-inflammatory, antioxidant, and regulatory effects on lipid metabolism (Utami et al., 2023). It can exhibit antidiabetic activity by increasing insulin sensitivity and reducing glucose production. Molecular studies related to berberine's antidiabetic activity have suggested that berberine may activate an enzyme called AMPK, which can enhance insulin sensitivity and improve glucose metabolism (Xu et al., 2014).

Resveratrol is a natural polyphenol compound found primarily in grapes and red wine. Numerous studies have shown that resveratrol is a potent antioxidant capable of combating free radicals in the body, preventing cellular damage, and reducing the aging process (Meng et al., 2021; Rudrapal et al, 2022). Additionally, it has been reported to have beneficial effects on cardiovascular health, including vasodilation, blood pressure regulation, and cholesterol level management (Rui et al., 2021). Furthermore, research has explored its potential in slowing down the development of neurological diseases (Andrade et al., 2018). Finally, there is a hypothesis that resveratrol may help regulate blood sugar levels, increase insulin sensitivity, and thereby reduce the risk of T2D (Zhu et al., 2017).

Molecular docking studies are a computer-based computational and modeling approach aimed at predicting how a drug candidate or a chemical compound can interact with a target protein or molecule, specifically identifying the binding site or interaction region (Meng et al., 2011). These studies are crucial for exploring drug-target interaction mechanisms in the design and optimization of new drugs. Also, it can be used to assess the potential repurposing of existing drugs by examining their interactions with different target proteins.

In this context, the aim of this study is not only to analyze the potential antidiabetic activity of berberine using a molecular docking approach but also to evaluate the antidiabetic, pharmacokinetic, and bioactivity effectiveness of resveratrol in comparison to berberine. Understanding the interactions between berberine and resveratrol with proteins associated with T2D ((adiponectin receptor 1 (ADIPOR1) and adiponectin receptor 2 (ADIPOR2), tumor necrosis factor-alpha (TNF-α), protein tyrosine phosphatase 1B (PTP1B), glucose transporter 1 (GLUT1), glucose

transporter 4 (GLUT4), insulin-like growth factor 1 receptor (IGF-IR), insulin-like growth factor 1 (IGF1), a disintegrin and metalloproteinase with thrombospondin motifs 9 (ADAMTS9), sphingosine kinase 2 (SPHK2)) is crucial for uncovering the drug potentials of these compounds for antidiabetic activity. Molecular docking studies play a significant role in elucidating how these compounds interact with specific proteins, providing valuable insights into their potential therapeutic applications in the context of T2D.

MATERIAL and METHOD

Prediction of Druglikeness

To assess the drug likeness of berberine and resveratrol, their SMILES format was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and the SwissADME website (https://www.swissadme.ch/) was utilized to analyze their absorption, bioavailability, distribution, metabolism, and excretion properties. Furthermore, the compounds' passage through the gastrointestinal wall and blood-brain barrier (BBB) was evaluated based on the BOILED-Egg data (Daina et al., 2017).

Selection of Targets Related to Berberine and Resveratrol

Berberine and resveratrol underwent target prediction through the SwissTargetPre-diction database, which can be accessed at https://www.swisstargetprediction.ch/. This tool utilizes computational methods to anticipate probable protein targets with which these substances could potentially interact. To ensure accuracy and uniformity, the standardized gene names linked to these anticipated targets were then obtained through the UniProt platform, yielding an exhaustive compilation of proteins associated with berberine and resveratrol.

Molecular Docking

In this research, molecular docking served as a validation technique, employing computer simulations to forecast the binding affinity between receptors and ligands. Docking analyses were performed involving berberine and resveratrol, as well as target proteins whose 3D structures were retrieved from the PubChem database. The 3D structures of the selected target proteins associated with T2D were sourced from the Protein Data Bank (RCSB PDB) database (https://www.rcsb.org). These chosen targets encompassed ADIPOR1 (PDB-ID: 6ks1), ADIPOR2 (PDB-ID: 5lxg), TNF-α (PDB-ID: 7kpb), PTP1B (PDB-ID: 4i8n), GLUT1 (PDB-ID: 4pyp), GLUT4 (PDB ID: 7wsn), IGF-IR (PDB-ID: 8eyr), IGF1 (PDB-ID: 6pyh), ADAMTS9 (PDB-ID: 3ppv), and SPHK2 (PDB ID: 4v24). Optimal ligand binding conformations within the target proteins were determined using the AutoDock tool. This tool assessed the binding conformations based on their free binding energy, employing a scoring function. Subsequently, additional optimization for molecular docking of the target protein structures was conducted using AutoDock 4.2 software (https://cadd.labshare.cn/cb-dock2/php/blinddock.php). Data regarding berberine and resveratrol were acquired through the online platform DeepDataSource (molinstincts.com).

Protein-Protein Interaction (PPI) Network

Target interactions were determined through the String platform (https://www.string-db.org/). In order to pinpoint central nodes and crucial proteins within the PPI network, calculations for centrality degrees, encompassing betweenness, closeness, and subgraph centrality, were executed utilizing Cytoscape without any external intervention.

Suggested Bioactivity

A thorough evaluation of the possible bioactive characteristics of berberine and resveratrol is crucial for understanding their unique impacts and mechanisms of operation. To predict their potential bioactivity, we utilized the PASS platform (http://www.way2drug.com/passonline/index.php). This platform is renowned for its exceptional predictive capabilities, encompassing 3678 different activity types, and maintaining an average accuracy rate of around 95%. These predictions are derived solely from the structural formulas of the compounds.

RESULTS

SWISS Adme Property

The SMILES format of berberine (https://pubchem.ncbi.nlm.nih.gov/compound/2353) and resveratrol (https://pubchem.ncbi.nlm.nih.gov/compound/445154) was obtained by transferring it to the SwissADME platform (http://www.swissadme.ch/). The canonical SMILES of berberine were COC1=C(C2=C[N+]3=C(C=C2C=C1)C4=CC5=C(C=C4CC3)OCO5)OC and resveratrol C1=CC(=CC=C1C=CC2=CC(=CC(=C2)O)O)O. The three-dimensional configuration of compounds can be observed in Figure 1A and 1B. Berberine's and resveratrol's drug-likeness was comprehensively assessed using filters based on Lipinski (Pfizer), Ghose (Amgen), Veber (GSK), Egan (Pharmacia), and Muegge (Bayer). In terms of drug-like properties, it was found that both compounds exhibit favorable drug-like properties according to all filters (Figure 1A and 1B).

The Swiss ADME bioavailability radar assesses six

different physicochemical properties. These properties are LIPO (lipophilicity), SIZE, POLAR (polarity), INSOLU (insolubility), INSATU (insaturation), and FLEX (flexibility). Berberine is situated within a favorable range for bioavailability, complying with all of these properties. However, resveratrol complies with all of these properties except for INSATU. This analysis could assist in evaluating berberine and resveratrol as a potential drug candidate.

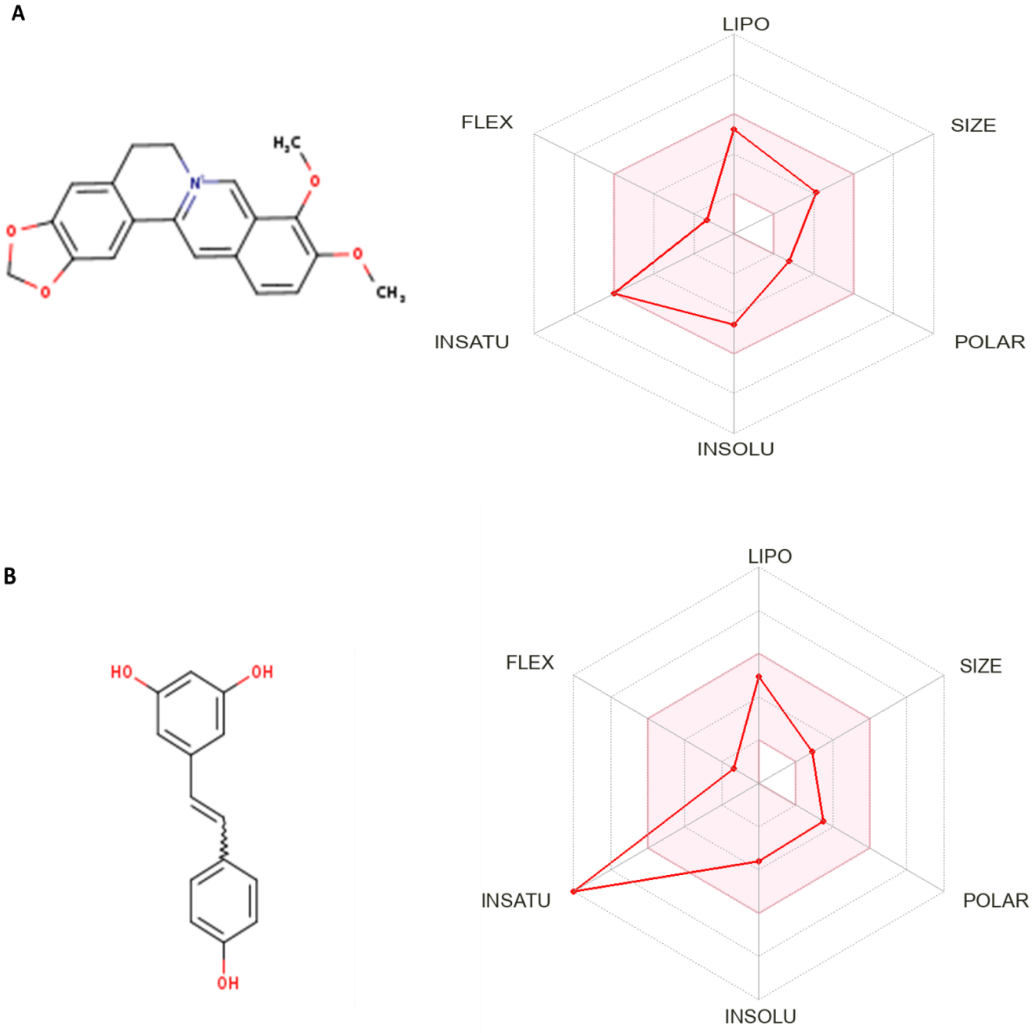


Figure 1. (A) Berberine 3D conformer and Swiss ADME properties (B) Resveratrol 3D conformer and SwissADME properties (right side of the figure) (http://www.swissadme.ch/index.php#). The oral bioavailability radar takes into account six physicochemical properties. Within this radar, the optimal range for each property is highlighted in pink, indicating the most favorable conditions. These properties include lipophilicity (XLOGP3) falling between -0.7 and +5.0, molecular weight (MW) ranging from 150 to 500 g/mol, polarity measured by the topological polar surface area (TPSA) between 20 and 130 Å2, solubility with a log S not exceeding 6, saturation levels with a sp3 hybridization fraction of at least 0.25, and flexibility restricted to no more than 9 rotatable bonds.

Şekil 1. (A) Berberin ve resveratrol 3D konformerleri ve (B) SwissADME özellikleri (şeklin sağ tarafı) (http://www.swissadme.ch/index.php#). Oral biyoyararlanım radarı, altı fizikokimyasal özelliği dikkate alır. Bu radarda, her bir özellik için optimal aralık pembe renkte vurgulanmış olup en uygun koşulları belirtir. Bu özellikler arasında -0.7 ile +5.0 arasındaki lipofilite (XLOGP3), 150 ile 500 g/mol arasındaki molekül ağırlık (MW), 20 ile 130 Å2 arasında ölçülen tepe polar yüzey alanı (TPSA) ile polarite, çözünürlük log S'nin 6'yı geçmemesi, en az 0.25 sp3 hibridizasyon fraksiyonu doygunluk seviyeleri ve 9'dan fazla dönebilen bağla sınırlı olmayan esneklik yer alır.

According to the BOILED-Egg diagram, both compounds have the high BBB penetration and gastrointestinal (GI) tract. However, berberine possesses the substructure of P-glycoprotein (PGP), while resveratrol does not have this substructure. The details are explained in Figure 2.

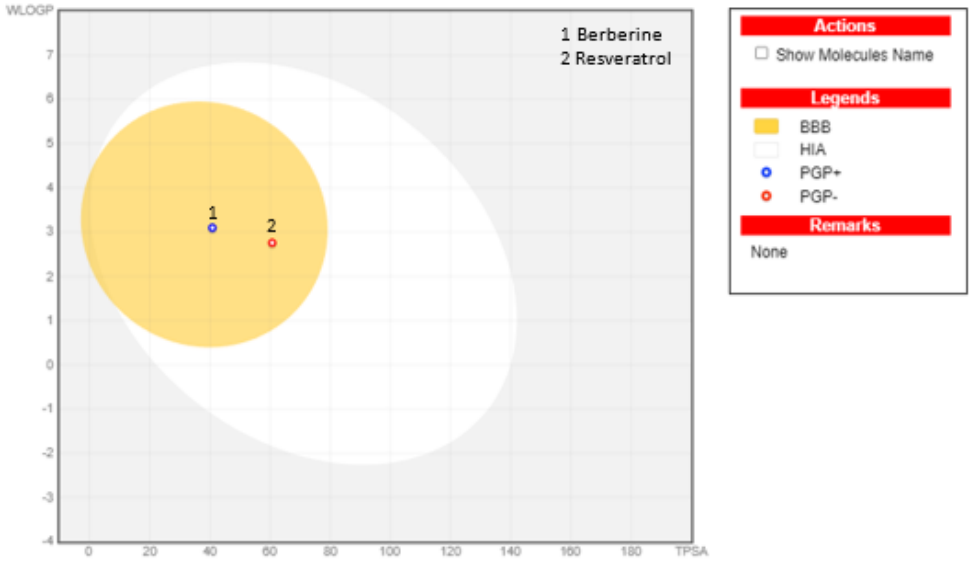


Figure 2. The BOILED-Egg diagram. The compounds within the white ellipse are likely to be efficiently absorbed by the GI tract. Those within the yellow ellipse, resembling the yolk, are expected to have a high likelihood of crossing the BBB and reaching the central nervous system. The blue points indicate compounds that are anticipated to be substrates of PGP+ and may be actively transported out of the brain or GI (1). On the other hand, the red points represent compounds predicted not to be substrates of PGP- (2).

Şekil 2. BOILED-Egg diyagramı. Beyaz elips içindeki bileşiklerin sindirim sistemi tarafından etkili bir şekilde emilmesi muhtemeldir. Sarı elips içindeki, yumurtanın sarısına benzeyen bileşiklerin, kan-beyin bariyerini geçme ve merkezi sinir sistemine ulaşma olasılığı yüksektir. Mavi noktalar, PGP+ substratı olması beklenen ve beyin veya sindirim sistemi dışına aktif olarak taşınabilecek bileşikleri gösterir (sol taraf). Diğer yandan, kırmızı noktalar, PGP- substratı olmayan bileşikleri temsil eder (sağ taraf).

It was observed that berberine differs from resveratrol in terms of molecular weight, H-bond donor and acceptor count, lipophilicity (LogP), topological polar surface area (TPSA), rotatable bond count, solubility (Log S), van der Waals volume, and P-gp substrate properties. Although both compounds show high gastrointestinal absorption, berberine cannot cross the blood-brain barrier (BBB). Additionally, it was observed that berberine also differs from resveratrol in terms of P-gp substrate properties. While berberine can be expelled by P-glycoprotein, resveratrol does not exhibit this characteristic (Table 1).

Table 1. Physicochemical Properties of Berberine and Resveratrol Based on Bioavailability and Drug Similarity Analyses

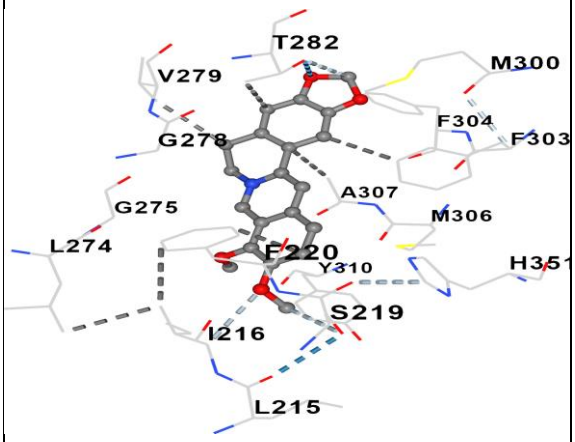
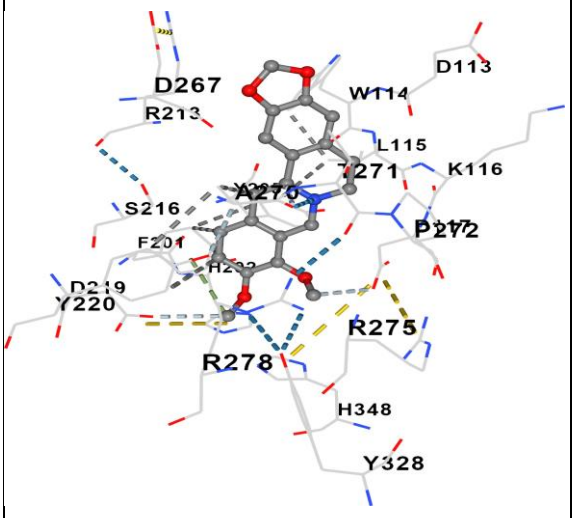
Çizelge 1. Biyoyararlanım ve İlaç Benzerliği Analizlerine Dayalı Berberin ve Resveratrol'ün Fizikokimyasal Özellikleri

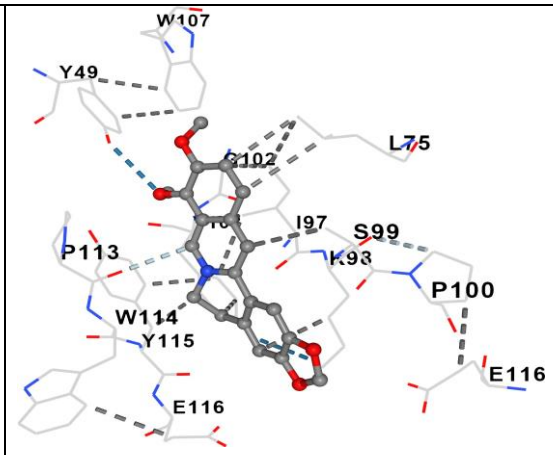
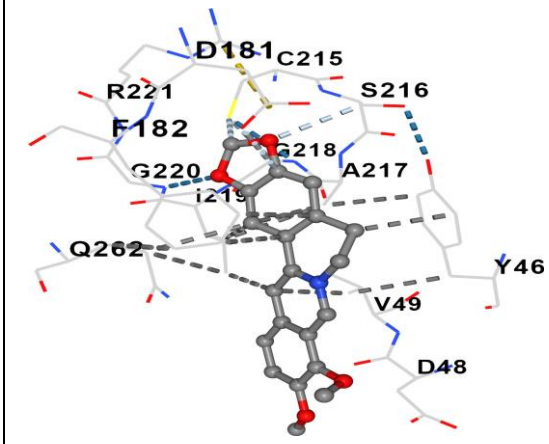
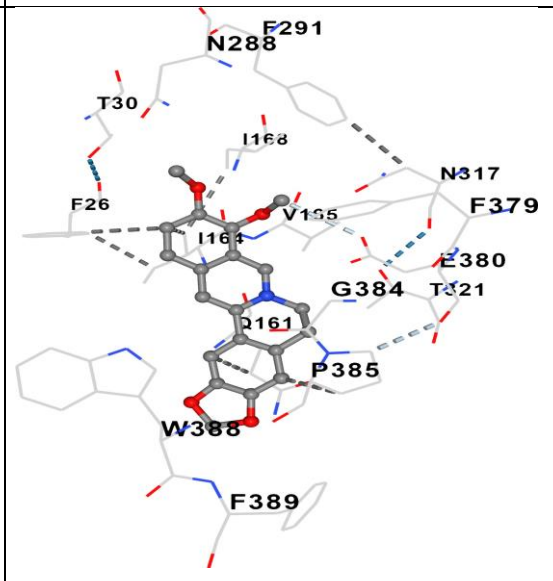
Physicochemical Property	**Berberine**	**Resveratrol**
Molecular Weight (g/mol)	336.38	228.25
H-Bond Donors	4	3
H-Bond Acceptors	7	3
LogP (lipophilicity)	1.91	2.78
TPSA (Topological Polar Surface Area) Å²	127.59	60.69
Rotatable Bonds (Flexibility)	5	1
Log S (Solubility)	-4.95	-3.51
VdW Volume Å³	299.70	207.24
BBB Permeability	No	Yes
Gastrointestinal Absorption	High	High
P-gp Substrate	Yes	No
CYP1A2 Inhibitor	No	No
CYP2C19 Inhibitor	Yes	No
CYP2C9 Inhibitor	Yes	No
CYP2D6 Inhibitor	No	No
CYP3A4 Inhibitor	No	No
Synthetic Accessibility Score	3.29	2.84

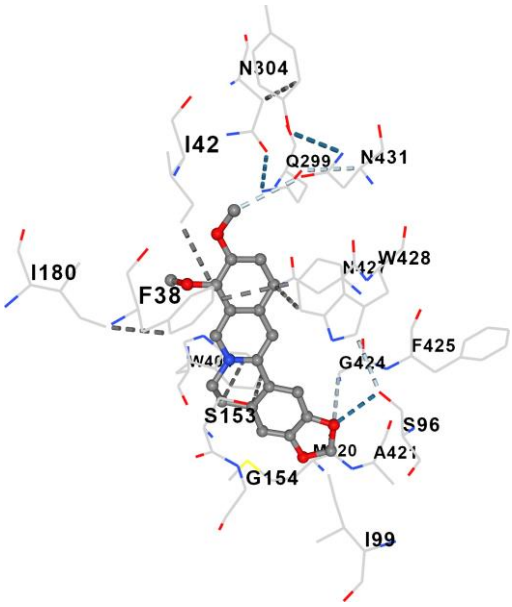
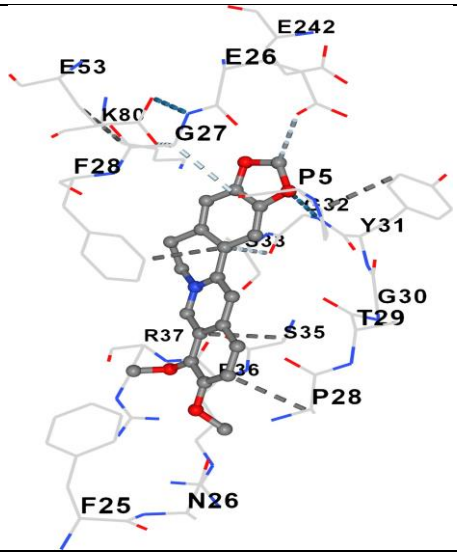
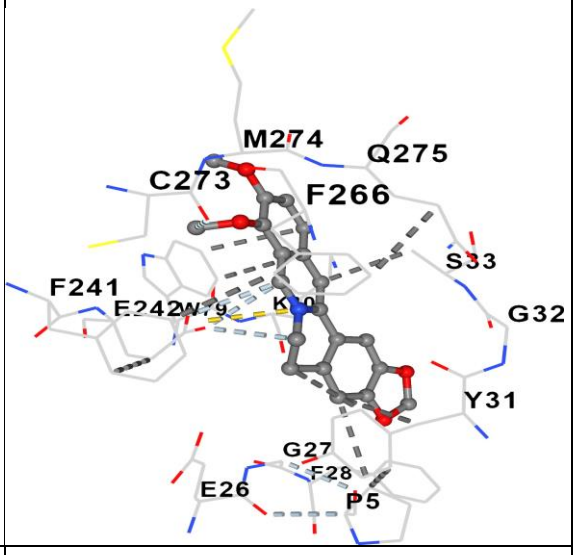
Molecular docking

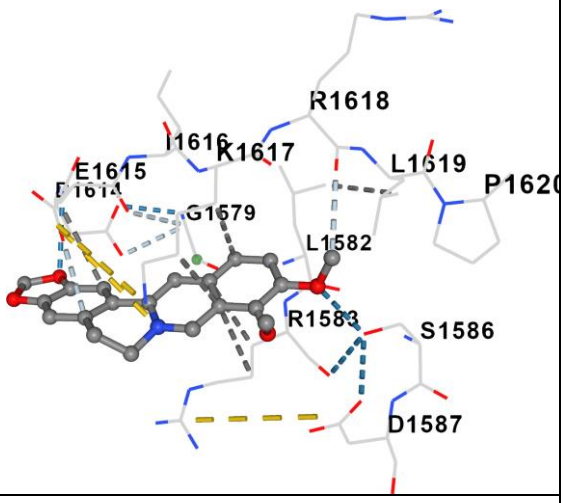
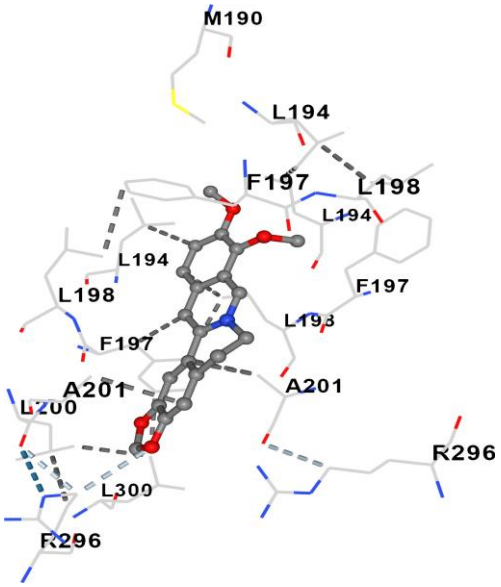
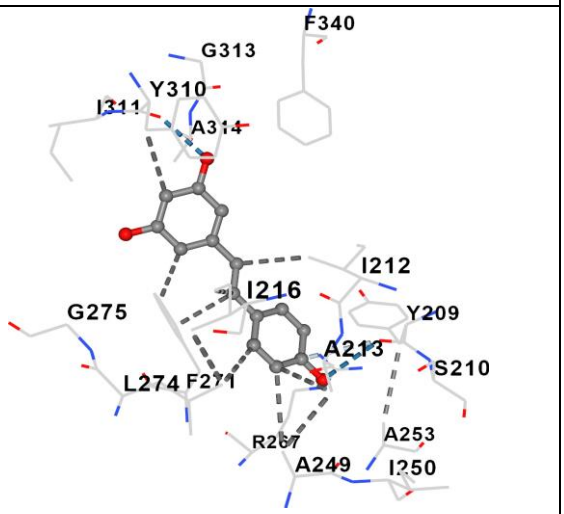
Nine common targets were selected as potential targets of berberine and resveratrol in the treatment of T2D. Their binding affinity with these ligands is provided in Table 1. Among the selected targets, it was determined that the GLUT1, GLUT4, and SPHK2 proteins had the best binding energy values of -10.1, -9.3, and -9.3 Kcal/mol for berberine, respectively. Additionally, ADIPOR1 (-9.0 Kkal/mol), ADIPOR2 (-8.1 Kkal/mol), TNF-α (-9.0 Kkal/mol), PTP1B (-8.5 Kkal/mol), IGF-IR (-8.6 Kkal/mol), IGF1 (-8.3 Kkal/mol) andADAMTS9 (-7.4 Kcal/molalso exhibited significantly high binding energies. However, it was determined that TNF-α and SPHK2 protein had the best binding energy, with a value of -8.7 and -9.0 Kcal/mol for resveratrol. In addition, ADIPOR1 (-7.1 Kkal/mol), ADIPOR2 (-7.7 Kkal/mol), GLUT4 (-8.4 Kkal/mol), PTP1B (-7.2 Kkal/mol), GLUT1 (-7.3 Kkal/mol), IGF-IR (-7.5 Kkal/mol), IGF1 (-7.3 Kkal/mol) and ADAMTS9 (-7.1 Kcal/mol) also exhibited significantly high binding energies (Table 2).

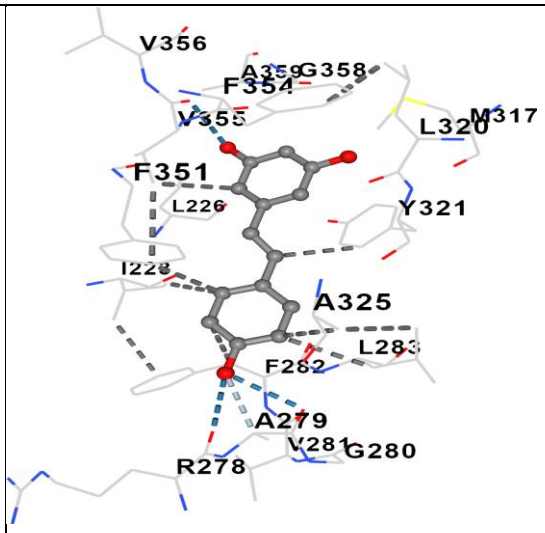
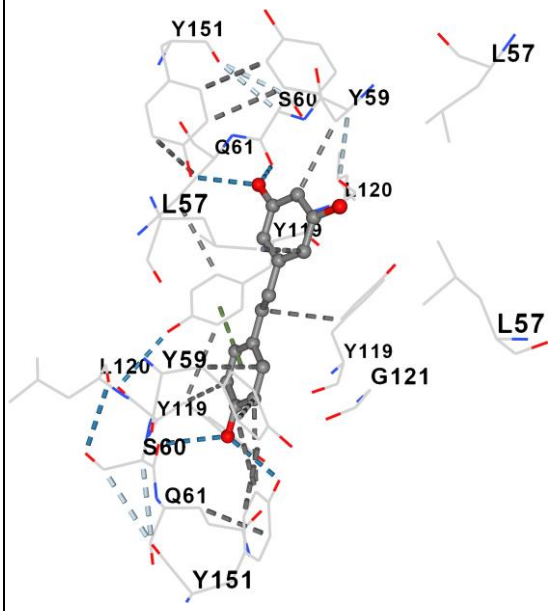
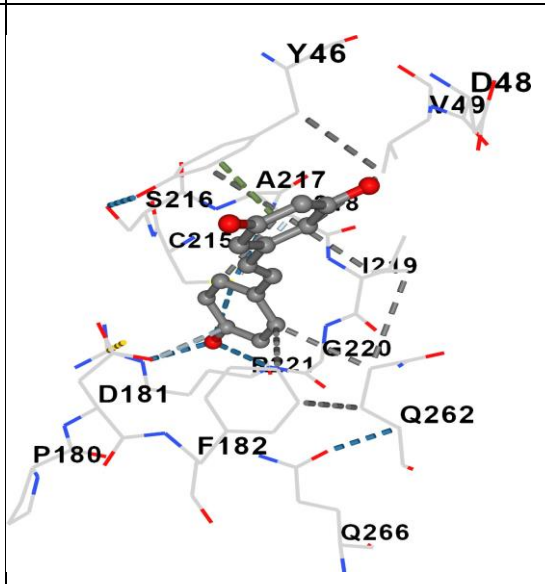
Table 2. Interaction of berberine and resveratrol with target proteins
Çizelge 2. Hedef proteinler ile berberine ve resveratrolün interaksiyonu

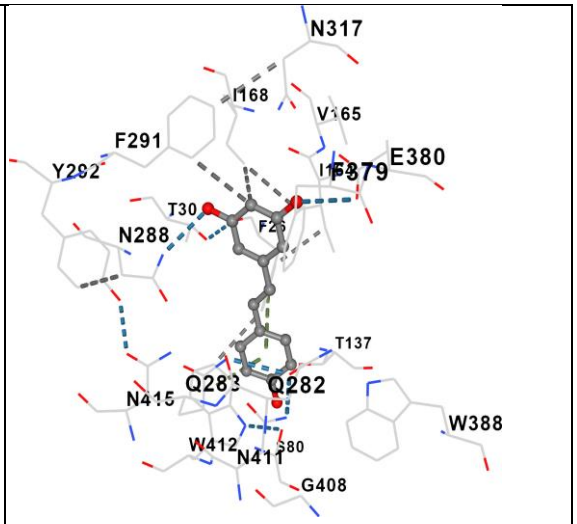
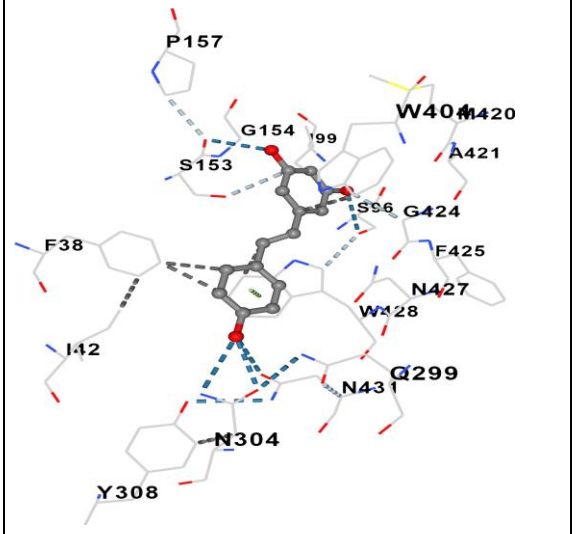
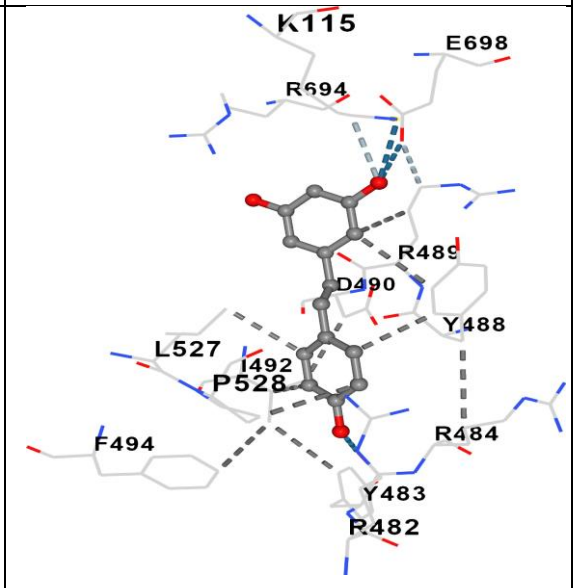
Target Protein	Binding Affinity (Kcal/mol)	Cavity volume (Å³)	Contact residues	Ligand-Molecule Interaction
Berberine				
ADIPOR1	-9.0	4297	Chain A: LEU215 ILE216 SER219 PHE220 LEU274 GLY275 GLY278 VAL279 THR282 MET300 PHE303 PHE304 MET306 ALA307 TYR310 HIS351	
ADIPOR2	-8.1	440	Chain H: GLN39 SER40 PRO41 GLY42 LYS43 SER44 VAL93 TYR95 Chain L: ALA9 GLN38 LYS39 GLN40 GLY41 SER85 TYR87 GLY100 GLY101 THR102 LYS103	

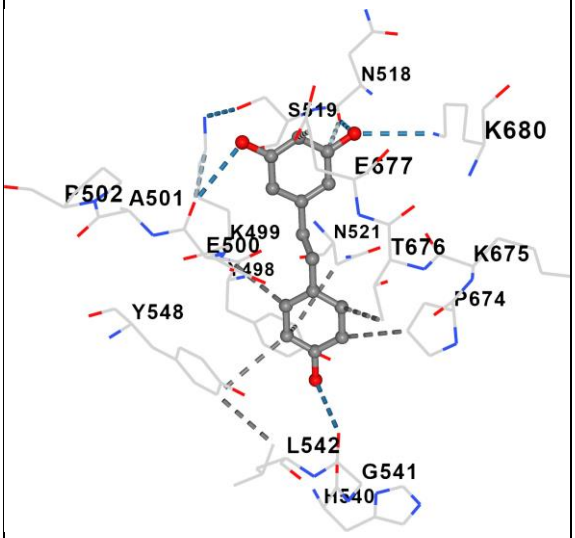
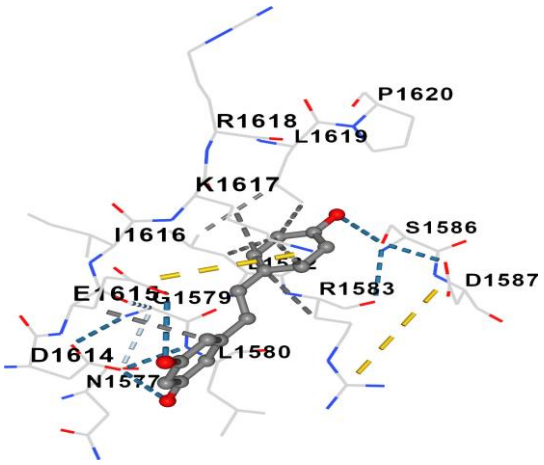
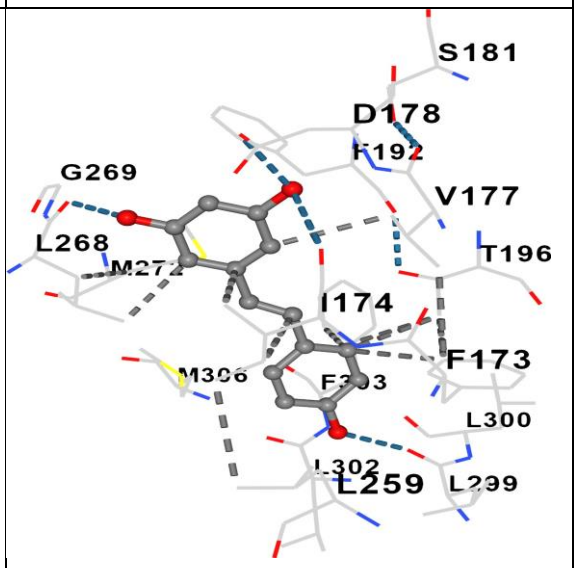
TNF-α	-9.0	25422	Chain A: LEU75 ILE97 LYS98 SER99 PRO100 GLU116 Chain C: PRO113 TRP114 TYR115 GLU116 Chain L: TYR49 Chain H: GLY102 TYR103 TRP107	
PTP1B	-8.5	234	Chain A: TYR46 ASP48 VAL49 ASP181 PHE182 CYS215 SER216 ALA217 GLY218 ILE219 GLY220 ARG221 GLN262	
GLUT1	-9.3	1429	Chain A: PHE26 THR30 GLN161 ILE164 VAL165 ILE168 GLN282 GLN283 ILE287 ASN288 PHE291 ASN317 THR321 SER324 LEU325 VAL328 PHE379 GLU380 GLY384 PRO385 TRP388 PHE389 ASN411	

GLUT4	-10.1	3458	Chain A: PHE38 ILE42 SER96 ILE99 SER153 GLY154 ILE180 GLN299 ASN304 TYR308 TRP404 MET420 ALA421 GLY424 PHE425 ASN427 TRP428 ASN431	
IGF-IR	-8.6	9791	Chain B: PHE700 SER704 Chain A: PRO5 GLU26 PHE28 HIS30 GLU53 TYR54 LYS80 LEU81 PHE82 THR316 Chain D: THR29 GLY30 TYR31 GLY32 SER35 ARG37 ALA38	
IGF1	-8.3	3062	Chain B: TYR31 GLY32 SER33 Chain D: PRO5 GLU26 GLY27 PHE28 TRP79 LYS80 PHE241 GLU242 PHE266 CYS273 MET274 GLN275	
ADAMTS9	-7.4	127	Chain A: GLY1579 LEU1582 ARG1583 SER1586 ASP1587	

			ASP1614 GLU1615 ILE1616 LYS1617 ARG1618 LEU1619 PRO1620	
SPHK2	-9.3	10611	Chain A: LEU194 PHE197 LEU198 LEU200 ALA201 ALA202 ARG296 LEU300 Chain B: MET190 LEU194 PHE197 LEU198 LEU200 ALA201 ARG296 ALA297 LEU300 Chain C: LEU194 PHE197 LEU198 LEU200 ALA201 ARG296 ALA297 LEU300	
Resveratrol				
ADIPOR1	-7.1	4297	Chain A: TYR209 SER210 ILE212 ALA213 ILE216 ALA249 ALA253 ARG267 PHE271 LEU274 GLY275 TYR310 ILE311 GLY313 ALA314 PHE340 VAL344	

ADIPOR2	-7.7	4654	Chain A: TRP114 ASP117 PHE201 HIS202 TYR205 GLU209 SER212 ARG213 SER216 ASP219 TYR220 ALA270 ARG275 ARG278 TYR328 HIS348	
TNF-α	-8.7	25422	Chain A: LEU75 LEU76 THR77 ILE97 ASN137 ARG138 Chain L: LEU46 TYR49 LEU54 ALA55 ASP56 Chain H: ALA31 TYR32 TYR33 TYR53 GLU99 ALA100 TYR101 TRP107	
PTP1B	-7.2	234	Chain A: TYR46 ARG47 ASP48 VAL49 ASP181 PHE182 CYS215 SER216 ALA217 ILE219 GLY220 ARG221 GLN262 GLN266	

GLUT1	-7.3	1429	Chain A: PHE26 THR30 SER80 THR137 VAL165 ILE168 GLN282 GLN283 ASN288 PHE291 ASN317 PHE379 GLU380 TRP388 GLY408 ASN411 TRP412 ASN415	
GLUT4	-8.4	3458	Chain A: PHE38 ILE42 SER95 SER96 ILE99 GLY150 SER153 GLY154 PRO157 GLN299 ASN304 TYR308 TRP404 MET420 ALA421 GLY424 PHE425 ASN427 TRP428 ASN431	
IGF-IR	-7.5	1571	Chain B: LYS115 Chain A: ARG482 TYR483 ARG484 TYR488 ARG489 ASP490 ILE492 PHE494 LEU527 PRO528 ALA691 ARG694 GLU698 Chain C: ALA8	

IGF1	-7.3	2118	Chain A: TYR498 LYS499 GLU500 ASN518 SER519 ASN521 HIS540 GLY541 LEU542 TYR548 Chain D: PRO674 LYS675 THR676 GLU677 LYS680	
ADAMTS9	-7.1	127	Chain A: ASN1577 GLY1579 LEU1580 LEU1582 ARG1583 SER1586 ASP1587 ASP1614 GLU1615 ILE1616 LYS1617 ARG1618 LEU1619 PRO1620	
SPHK2	-8.7	26771	Chain A: ARG24 GLY26 LYS27 GLY111 SER112 ASN131 PHE173 ILE174 VAL177 ASP178 GLU180 SER181 LYS183 TYR184 ARG185 ARG186 GLY188 GLU189 PHE192 THR196 LEU198 LEU200 LEU259 LEU268 GLY269 MET272 VAL290 LEU299 LEU300 LEU302 PHE303 MET306 LEU319 Chain B: MET190 PHE192 THR193 THR196 PHE197 GLU271 MET272 PHE303 LEU304 ALA305 MET306 GLU307 Chain C: ARG186	

The interactions between berberine and target proteins were assessed using the AutoDock tool, which utilizes a scoring function based on binding free energies to evaluate binding conformations. Subsequently, the structures of target proteins were subjected to molecular docking optimization, and the analysis was conducted using AutoDock 4.2 software (https://cadd.labshare.cn/cb-dock2/php/blinddock.php). The cavity volume (Å^3) value, which represents the size of the binding pockets in the protein, along with protein-ligand interactions from the molecular docking analysis, is provided in Table 2. The contact points between residues and bonds show the amino acids and binding configurations between ligands and target proteins. The blue dashed lines represent hydrogen bond interactions, the yellow dashed lines represent hydrophobic interactions, and the red dashed lines represent electrostatic interactions (Table 2).

Constructing a Protein-Protein Interaction Network (PPI)

To explore protein-protein interactions and identify 9 shared targets, we utilized the String database (https://www.string-db.org/). These interactions were instrumental in uncovering the functional connections and associations among the proteins. Next, the data was imported into Cytoscape v3.7.2 to construct a PPI network composed of 7 nodes and 10 edges (Figure 3).

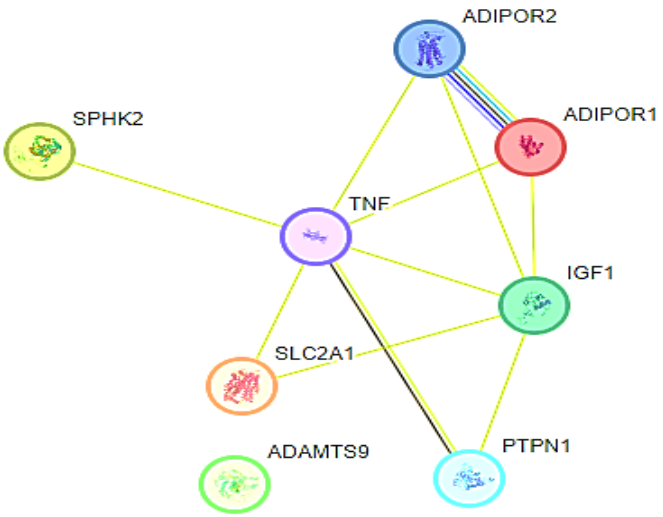


Figure 3. Protein interaction network analysis
Şekil 3. Protein etkileşim ağı analizi

Succession of bioactivities

Bioactivity prediction was performed using the platform accessible at "http://www.way2drug.com/passonline/predict.php." The predictive model for the bioactivity spectrum is founded on a Bayesian approach. Within this tool, predictions are generated based on the Pa:Pi (active to inactive ratio) at three distinct threshold levels: Pa > 30%, Pa > 50%, and Pa > 70%. The results of these forecasts are presented in terms of Pa (probability of activity) and Pi (probability of inactivity). Compounds exceeding a Pa value of 0.7 are considered promising candidates for the specified biological activity (Table 3).

DISCUSSION

In the present study, the therapeutic effect of berberine and resveratrol was evaluated in situ for type 2 diabetes (T2D) using a molecular docking approach. Molecular docking studies are employed to understand how a molecule affects a target protein. In this study, molecular docking was performed to assess the binding status of berberine and resveratrol with the 3D structures of proteins associated with the T2D, including ADIPOR1 and ADIPOR2, TNF-α, PTP1B, GLUT1, GLUT4, IGF-IR, IGF1, ADAMTS9, SPHK2. These proteins can influence various processes in T2D, including insulin signaling, cell death, inflammation, and cell growth. Therefore, understanding the roles of these proteins in the pathogenesis and treatment of T2D is crucial. In the realm of molecular docking studies, the term "low-energy binding" typically signifies a robust and stable interaction between a ligand and a protein, indicating a strong affinity.

Understanding and regulating the function of GLUT1 and especially GLUT4 proteins can play a significant role in the treatment and prevention of T2D. GLUT1 is responsible for the uptake of glucose into cells and can also transport some lipids besides glucose (Pragallapati and Manyam, 2019). Considering that

lipid metabolism disorders are common in individuals with T2D and can increase the risk of cardiovascular diseases, controlling them is essential (Martín-Timón et al., 2014; Rubino et al., 2016; Petersen and Shulman, 2018). In addition to GLUT1, GLUT4 is also a carrier protein located on the cell membrane that regulates the entry of glucose into the cell. In the case of T2D, glucose cannot be taken up by GLUT4 proteins, and the body develops insulin resistance. Overcoming insulin resistance and ensuring the effective functioning of GLUT4 proteins are important for effective treatment. Molecular binding analysis showed that berberine exhibited the best binding to the GLUT4 protein (-10.1 Kcal/mol), followed by the GLUT1 protein (-9.3 Kcal/mol). However, resveratrol, although less tightly bound compared to berberine, has binding energies above -7 kcal/mol for both GLUT1 and GLUT4. Additionally, another significant protein in the pathogenesis of T2D is SPHK2. SPHK2 regulates cellular responses by modulating lipid signal pathways in cells, which can affect inflammation and oxidative stress levels, potentially contributing to the development of T2D (Qi et al., 2021). The data from this study showed that berberine and resveratrol exhibit a high binding affinity with the SPHK2 enzyme (-9.3 and -9.0 Kcal/mol, respectively). The high binding affinities of these compounds with this enzyme demonstrated that they should be considered target enzymes in advanced studies related to T2D.

Table 3. The prospective biological activity spectrum associated with berberine and resveratrol
Çizelge 3. Berberin ve resveratrol ile ilişkilendirilen olası biyolojik aktivite spektrumu

Activity	Pa	Pi
Berberine		
P-glycoprotein substrate	0.408	0.040
GABA aminotransferase inhibitor	0.424	0.039
Resveratrol		
APOA1 expression enhancer	0.923	0.002
JAK2 expression inhibitor	0.912	0.003
Sugar-phosphatase inhibitor	0.835	0.011
Aldehyde oxidase inhibitor	0.831	0.007
Fatty-acyl-CoA synthase inhibitor	0.823	0.004
Glucan endo-1,6-beta-glucosidase inhibitor	0.816	0.006
Glucose oxidase inhibitor	0.799	0.012
Beta glucuronidase inhibitor	0.752	0.003
GABA aminotransferase inhibitor	0.719	0.004
Insulysin inhibitor	0.707	0.008
Gluconate 2-dehydrogenase (acceptor) inhibitor	0.737	0.038

Adiponectin is a hormone secreted from adipose tissue known for its ability to increase insulin sensitivity in the body (Khoramipour et al., 2021). In other words, sufficient production of adiponectin and the effective functioning of adiponectin receptors can help cells use insulin more effectively. Adiponectin can reverse this condition by reducing insulin resistance. Adiponectin binds to cells through two different receptors, namely ADIPOR1 and ADIPOR2 (Thundyil et al., 2012). These receptors facilitate the uptake of adiponectin hormone into cells and initiate signaling pathways that convey its effects. The function of ADIPOR1 and ADIPOR2 includes regulating intracellular signal transduction and contributing to metabolic processes, including increasing insulin sensitivity (Li et al., 2022). They have been investigated as potential drug targets for the treatment and prevention of T2D (Deng et al., 2023). Medications or therapeutic approaches aimed at enhancing the activity of these receptors can help increase insulin sensitivity, thereby assisting in the management of T2D. The present study showed that berberine exhibits binding energies of -9.0 Kcal/mol and -8.1 Kcal/mol with ADIPOR1 and ADIPOR2, respectively. On the other hand, resveratrol has binding energies of -7.1 Kcal/mol with ADIPOR1 and -7.7 Kcal/mol with ADIPOR2. Berberine binds to these receptors with lower energy values compared to resveratrol.

TNF-α can potentially harm insulin-producing beta cells in the pancreas, leading to a reduction in insulin production and the progression of T2D (Rehman and Akash, 2016). Furthermore, TNF-α can negatively affect insulin receptors on the cell surface, causing cells to perceive insulin less effectively. Additionally, TNF-α can disrupt glucose metabolism, contributing to elevated blood sugar levels (Wondmkun, 2020). In the treatment of T2D, drugs that aim to reduce the effects of TNF-α work towards enhancing insulin sensitivity by controlling inflammation (Li et al., 2023). In this study, both drug candidates showed nearly identical binding energies with TNF-α, with berberine at -9.0 Kkcal/mol and resveratrol at -8.7, indicating that both drug candidates have the potential to modulate inflammation associated with T2D.

Druglikeness is a crucial concept in drug research and development, determining a molecule's potential as a drug and predicting its pharmacokinetic profile (Bickerton et al., 2012). Pharmaceutical companies use various filters, including Lipinski's Rule of Five, the Ghose filter, the Veber filter, and the Mudgee filter, to establish drug-likeness criteria for compounds like berberine and resveratrol. According to Lipinski's Rule of Five, compounds must meet specific criteria, including a molecular weight below 500 daltons, limited hydrogen bond donors and acceptors, and a suitable log P (octanol-water partition coefficient). The Ghose filter assesses criteria such as molecular weight, LogP, atom count, and molar refractivity. The Veber filter considers rotatable bonds and polar surface area, while the Mudgee filter examines ring and bond counts (Kralj et al., 2023). According to SwissADME properties, both berberine and resveratrol exhibited drug-like characteristics according to Lipinski's five rules, Ghose, Veber, Egan, and Muegge filters. Also, both have high GI absorption and BBB permeability. Additionally, significant bioactivity analysis indicated that resveratrol exhibited characteristics such as enhancing APOA1 expression at 0.923 Pa. Apolipoprotein A1 (APOA1) is an apolipoprotein associated with High-Density Lipoprotein (HDL), a lipoprotein. APOA1 plays a crucial role as a structural component of HDL, contributing to HDL's functions in cholesterol transport and metabolism (Mangaraj et al., 2016). Due to its ability to raise HDL levels and potentially improve metabolic health and reduce cardiovascular risk, it is believed that APOA1 may have the potential to reduce the risk of T2D (Zvintzou et al., 2023). Higher HDL levels are generally associated with a lower risk of T2D. Furthermore, bioactivities with Pa values greater than 0.7, which may be associated with T2D, are listed in Table 3. When examining the predicted bioactivity properties of resveratrol, it is evident that it has a significantly greater number of activities related to T2D compared to berberine. Additionally, berberine's bioactivities had Pa values below 0.7. While the binding energy of resveratrol to proteins associated with T2D was like that of berberine in molecular docking analysis, their predicted bioactivities are quite distinct. Furthermore, resveratrol has been observed to possess characteristics such as a sugar-phosphate inhibitor (Pa 0.8305), glucose oxidase inhibitor (Pa 0.799), beta-glucuronidase inhibitor (Pa 0.752), and insulysin inhibitor (Pa 0.707).

The results of this study indicate that both berberine and resveratrol should be considered as potential drug candidates for T2D treatment. Molecular docking studies revealed that berberine and resveratrol have high binding affinities to critical proteins associated with T2D. Particularly, it is strong binding to GLUT1, GLUT4, TNF-α, and SPHK2 protein, suggesting that berberine and resveratrol modulate inflammation, glucose intake, and insulin resistance. These findings emphasize the importance of considering berberine and resveratrol as drug candidates with multiple targets for T2D treatment.

CONCLUSION

Swiss ADME data showed that berberine aligns with favorable bioavailability characteristics, while resveratrol lacks INSATU compliance. According to the BOILED-Egg diagram, both compounds demonstrate high BBB permeability and effective GI absorption. Molecular docking studies have demonstrated the high binding affinities of berberine and resveratrol to key proteins associated with T2D. These results underscore the significance of considering these compounds as promising drug candidates with multiple targets for the treatment of T2D. Future research should further investigate the efficacy of berberine and resveratrol in T2D treatment through more comprehensive experiments supported by clinical studies. Additionally, the pharmacokinetics and safety of berberine and resveratrol should be explored in greater detail, especially regarding their potential roles in early T2D diagnosis, treatment response monitoring, and risk reduction factors.

Contribution of Authors

SÇ: Designed, performed, analyzed, wrote, reviewed and edited.

Conflict of Interest

The author declares no conflict of interest.

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