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Evaluation of Some Sulfonamide Derivatives as a Potential Inhibitors of The Carbonic Anhydrase IX/XII by ADME and Molecular Docking Method

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Abstract

Molecular docking is a simulation technique that calculates the binding score of the molecules of interest to protein structures and visualizes the bond structure. It is a widely used technique for foresight because it helps to determine bond relationships between molecular structures before laboratory applications in the development of new drugs. ADME studies also provide clues for the determination of the molecules analyzed by the molecular docking method to be drug candidates. In this study, inhibition of CA IX and CA XII enzymes by sulfonamide derivatives synthesized in our previous study was investigated using molecular docking method. The CA enzyme family has an important role in the survival and spread of cancer cells. Therefore, we aimed to find new drug candidates that inhibit these enzymes. We found that the sulfonamide derivative named **6** binds to the active sites of both CA IX and CA XII enzymes with -7.44 kcal/mol and -6.39 kcal/mol energy, respectively, closest to the binding of the reference molecule acetazolamide. In addition, the compatibility of all compounds used in the study with drug-like properties was investigated using the ADME method. In conclusion, it can be said that the

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sulfonamide derivatives named 1-9 generally have the characteristic features of a drug (physicochemical and structural properties) and oral bioavailability.

Keywords: Molecular docking; ADME; CA IX; CA XII; Sulfonamide derivatives.

Karbonik Anhidraz IX/XII'nin Potansiyel İnhibitörleri Olarak Bazı Sülfonamit Türevlerinin ADME ve Moleküler Yerleştirme Metodu ile Değerlendirilmesi

Öz

Moleküler yerleştirme, ilgili moleküllerin protein yapılarına bağlanma enerjilerini hesaplayan ve bağ yapısını görselleştiren bir simülasyon tekniğidir. Çeşitli hastalıklara ilişkin yeni ilaçların keşfi ve geliştirilmesinde, laboratuvar uygulamalarından önce moleküler yapılar arasındaki bağlanmaları belirlemeye yardımcı olması sebebiyle, yaygın olarak kullanılan bir tekniktir. ADME çalışmaları da moleküler yerleştirme yöntemiyle analiz edilen moleküllerin ilaç adayı olma özelliklerinin belirlenmesinde ipuçları sağlamaktadır. Bu çalışmada, daha önceki çalışmamızda sentezlenen sülfonamit türevleri tarafından CA IX ve CA XII enzimlerinin inhibisyonu, moleküler yerleştirme yöntemi kullanılarak araştırılmıştır. Karbonik anhidraz (CA) enzim ailesi, kanser hücrelerinin hayatta kalmasında ve yayılmasında önemli bir role sahiptir. Bu nedenle bu enzimleri inhibe eden yeni ilaç adayları bulmayı amaçladık. 6 numaralı sülfonamit türevinin hem CA IX hem de CA XII enzimlerinin aktif bölgelerine sırasıyla -7,44 kcal/mol ve -6,39 kcal/mol enerji ile referans molekül asetazolamitin (AZM) bağlanmasına en yakın şekilde bağlandığını bulduk. Ayrıca çalışmada kullanılan tüm bileşiklerin ilaç benzeri özelliklerle uyumluluğu ADME yöntemi kullanılarak araştırılmıştır. Sonuç olarak, 1-9 olarak adlandırılmış sülfonamit türevlerinin genel olarak bir ilacın karakteristik özelliklerine (fizikokimyasal ve yapısal özellikler) ve oral biyoyararlanıma sahip olduğu söylenebilir.

Anahtar Kelimeler: Moleküler yerleştirme; ADME; CA IX; CA XII; Sülfonamit türevleri.

1. Introduction

The microenvironment is a key player in cancer cell survival and spread, in cancer research. Hypoxia and acidosis in the tumor microenvironment affect cancer biology. Therefore, the carbonic anhydrase family of enzymes, which maintains intracellular pH, has attracted the attention from cancer research [1].

Carbonic anhydrase (CA) enzymes are a family of metalloenzymes that are found in all living things and contain metal ions in their structure. CA enzymes catalyze the reversible conversion of carbon dioxide (CO_2) to bicarbonate (HCO_3^-) and hydrogen ions (H^+) (Eqn. (1)).

$$CO_2 + H_2O \overset{CAE}{\longleftrightarrow} H_2CO_3 \overset{CAE}{\longleftrightarrow} HCO_3^- + H^+ \quad (1)$$

This protein chain consists of 260 amino acids and varies according to the metal ion they contain in their structure [2, 3]. The active site of the molecule is in the form of a centrally diffused cavity, with the zinc ion adjacent to the bottom of this cavity [4]. CA isoenzymes are encoded by seven distinct gene families, the α-carbonic anhydrase family is the species found in humans, and 16 different isoenzymes of this species have been found so far. Of these, five (CA I, II, III, VII, and XIII) are cytosolic, four (CA IV, IX, XII, and XIV) are membrane-bound, two (CA VA and VB) are mitochondrial, and one (CA VI) is in salivary and milk [5, 6].

The ability of sulfonamides to readily acquire an ionic form is essential for inhibiting an enzyme. The interaction of sulfonamides with the enzyme is as follows; First, the N atom in the R-SO2-NH- compound must form an ionic bond with Zn^{2+} in the active site of the carbonic anhydrase enzyme. Second, it must form two hydrogen bonds with the THR-199 amino acid. This basic interaction mechanism is given in Fig. 1 [7, 8].

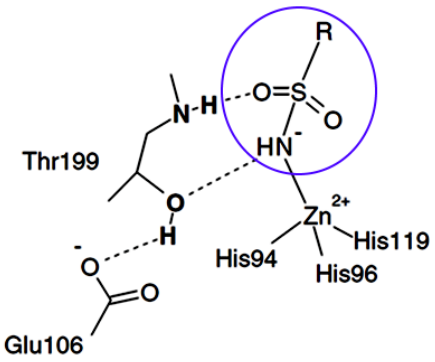


Figure 1: The mechanism of interaction between carbonic anhydrase enzyme and a sulfonamide derivative

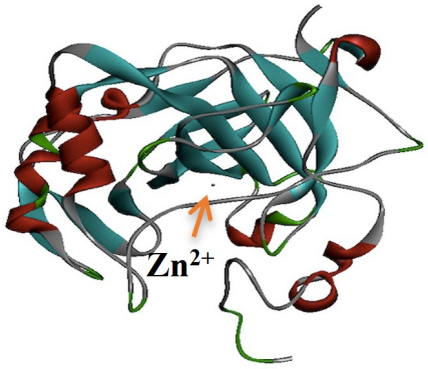
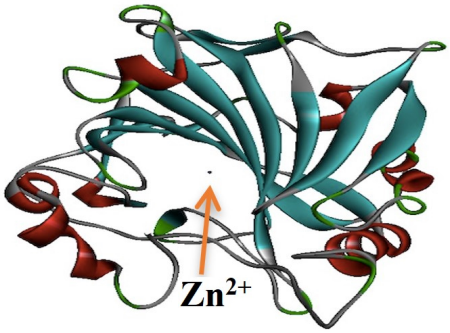
CA enzyme plays important roles in pH regulation, bone development/resorption, electrolyte secretion, calcification, lipogenesis, urea cycle, bicarbonate synthesis and many other physiological events apart from carboxylation reactions [9, 10]. In addition, CA IX and XII are overexpressed in some cancers due to their induction by the hypoxic environment in solid tumor cells. Overexpression of these enzymes in tumor cells supports survival in hypoxia and cell migration [11-13]. For this reason, besides cancer treatments such as chemotherapy and radiotherapy, drug studies that provide inhibition of related enzymes are important research areas for cancer treatment.

Sulfonamides are effective molecules in drug studies that have been used as anti-microbial for many years. Their broad biological activities such as anti-inflammatory, anti-tumor and antibacterial have made sulfonamides popular for drug studies [14-17]. Especially sulfonamide derivatives such as acetazolamide, which are used as CA enzyme inhibitors, are synthesized and their effectiveness in the treatment of various diseases is being investigated. In this study, we investigated the inhibition of CA IX and CA XII enzymes by some sulfonamide derivatives that we synthesized in our previous study [14, 18], using molecular docking method.

2. Materials and Methods

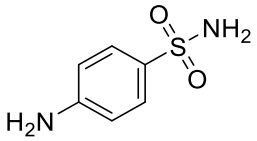
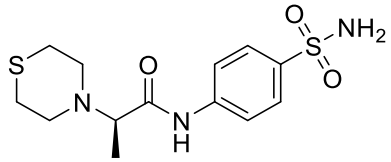
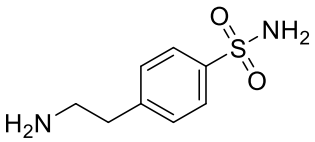
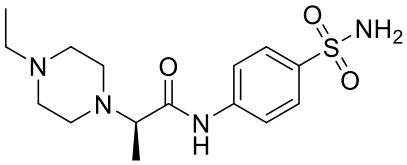
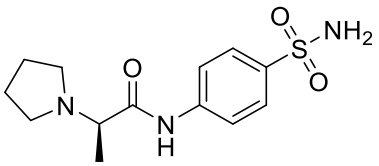
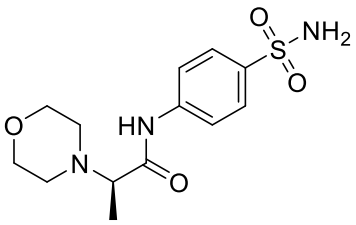
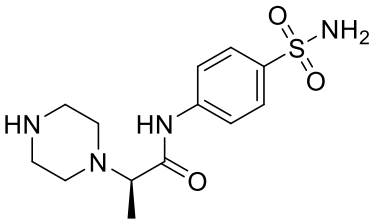
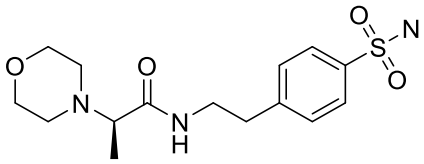
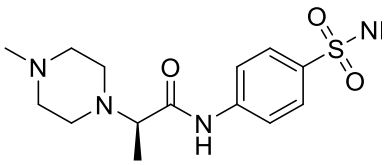
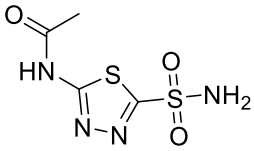
The crystal structures of both, first the extracellular domain of human CA XII and the other catalytic domain of tumor-associated human CA IX, were retrieved from the Protein Data Bank. The X-ray structures of both proteins are in complex with a classical, clinically used sulfonamide inhibitor, acetazolamide. PDB information of CA IX and CA XII are given in the Table 1. Before docking, the acetazolamide molecule and all heteroatoms were removed from the protein structures, polar hydrogens were added for a good imitation of the physiological environment.

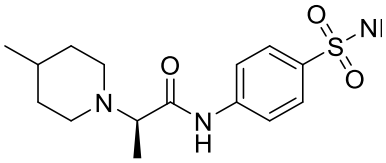
Table 1: The properties of CA IX and CA XII protein structures

PDB ID	Protein Structure	Resolution (Å)	Method
3IAI		**2.2**	**XRD**
1JD0		**1.5**	**XRD**

The molecular structures of the sulfonamide derivatives, which is binding to the active sites of CA IX and CA XII were examined, are shown in Table 2. Synthesized sulfonamides were evaluated experimentally for antimicrobial and cytotoxicity [14,18]. For the sulfonamide derivatives synthesized in our previous study, 1 and 2 were named for the starting materials, 3-10 for the derivatives. In addition, acetazolamide (AZM) was used as a reference molecule.

Table 2: Molecular structures of sulfonamide derivatives and reference molecule

Molecule Name	Molecule Structure	Molecule Name	Molecule Structure
1		**7**	
2		**8**	
3		**9**	
4		**10**	
5		**AZM**	

6 

The docking studies of the molecules to CA IX and CA XII proteins was done using Autodock 4.2.6 software [19]. Ligand energy was optimized by Avogadro version 1.2 with Force Field type MMFF9, and saved in .mol2 format [20]. AutoDockTools was used to prepare molecules and receptors for docking. Docking studies were performed by Lamarckian genetic algorithm, with 50 as the total number of runs for each binding site. In each respective run, a population of 150 individuals with 27×10^3 generations and 25×10^6 energy evaluations were employed. In accordance with the mechanism described in Fig. 1, a different narrow region was chosen as the grid around Zn^{2+}, taking into account the size of each compound.

The binding of each sulfonamide derivative to the protein structures was determined by comparison with that of the reference molecule acetazolamide. The protein-ligand complexes were visualized and analyzed using AutoDockTools and Biovia Discovery Studio Visualizer 2020. It was also done using the SwissADME website to determine the physicochemical, pharmacokinetic and solubility (ADME) properties of the synthesized compounds [21].

3. Results and Discussion

3.1. Physicochemical properties and drug likeness screening

Chemical synthesis, biological screening (in vitro and in vivo) and investigation of pharmacokinetic properties are the basic steps in determining the drug potential of a chemical compound. First of all, it is necessary to determine the pharmacokinetic properties, metabolism, excretion (ADME) and toxicity data of newly synthesized compounds from in vivo test applications, which is a very costly task. Thus, weak drug candidate compounds that would lead to clinical failure can be eliminated [22]. SwissADME is a free web tool that offers applications for this purpose. It provides information on the size, solubility, lipophilicity, saturation, skin permeability, and intestinal absorption of compounds. The ADME results of synthesized compounds are given in Table 3.

All compounds synthesized are suitable based on the Lipinxi, Veber and Ghose rules. The molecular weight of sulfonamide derivatives is small equal to 500 daltons, a hydrogen bond

acceptor number ≤10, and a donor number ≤5. Also, lipophilicity or logP value was calculated as ≤5. The calculated hydrogen bond donor number for the compounds is less than 5 and the hydrogen bond acceptor number is less than 10. Based on all these rules, as given in Table 4, it can be said that the sulfonamide derivatives synthesized have the characteristic features of a good drug (physicochemical and structural properties) and have oral bioavailability (Fig. 2). Solubility is an important criterion in drug absorption, and when all compounds are evaluated on this scale, they are determined to have high solubility. All the pharmacokinetic properties of the compounds, including gastrointestinal absorption, blood-brain barrier crossing, skin permeability, and drug excretion, were investigated using the Boiled-Egg model and showed in Fig. 3. All sulfonamide derivatives in the study exhibited high GI and did not penetrate the blood-brain barrier. Skin permeability parameters, another pharmacokinetic property, were calculated in accordance with the criteria defined by Potts and Guy. These criteria are that skin permeability will decrease as the negativity of the LogK value increases, and Table 4 shows the values of the synthesized compounds. Finally, the synthesized compounds did not inhibit 5 important enzymes of the cytochrome P450 (CYP) system, which have an important role in the degradation of drug candidates for elimination from the body.

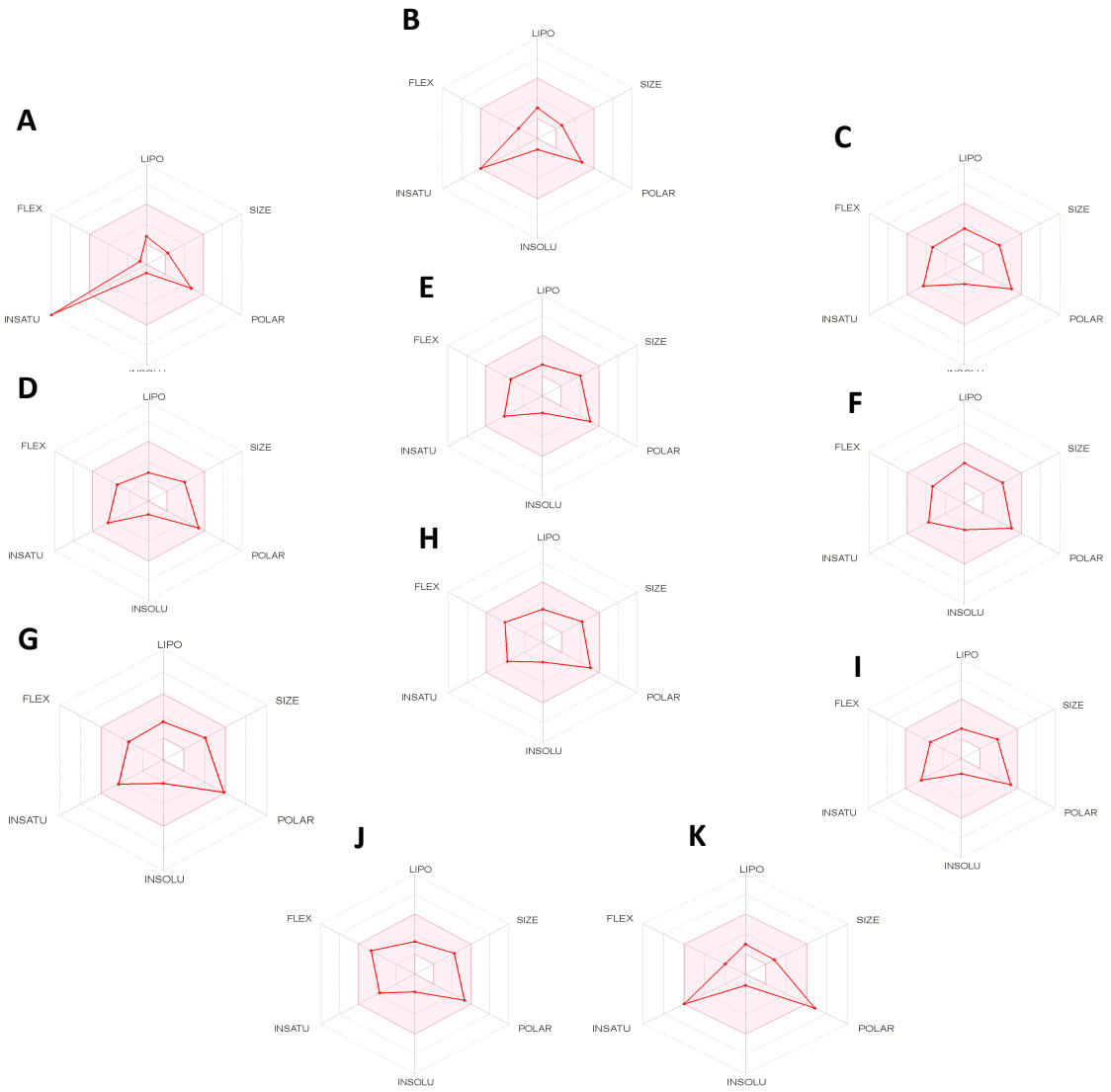


Figure 2: Bioavailability radar plot shows optimal physicochemical area for drug candidates as A)1 B)2 C)3 D)4 E)5 F)6 G)7 H)8 I)9 J)10 K)AZM (The pink area defines the optimal range for each properties)

Table 3: In-silico physic-chemical properties of sulfonamide derivatives.

ADME Properties	Molecule ID										
	Acetazolamide	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
					Physicochemical-properties						
Molecular weight (g/mol)	222.25	172.20	200.26	297.37	312.39	326.41	325.43	329.44	340.44	313.37	341.43
No. of heavy atoms	13	11	13	20	21	22	22	21	23	21	23
No. of rotatable bonds	3	1	3	5	5	5	5	5	6	5	7
No. of H-bond acceptors	6	3	4	5	6	6	5	5	6	6	6
No. of H-bond donors	2	2	2	2	3	2	2	2	2	2	2
Molar refractivity	45.22	41.84	49.92	80.48	87.20	92.10	90.10	88.07	96.91	81.57	89.64
TPSA ($Å^2$)	151.66	95.56	94.56	100.88	112.91	104.12	100.88	126.18	104.12	110.11	110.11
					Lipophilicity						
M LOGP	-2.34	-0.30	0.12	0.14	-0.66	-0.40	0.66	0.14	-0.14	-0.66	-0.41
					Water solubility						
LogS (ESOL)	-1.14	-0.85	-1.12	-1.97	-1.33	-1.70	-2.62	-2.12	-1.94	-1.51	-1.79
Solubility	Very soluble	Very soluble	Very soluble	Very soluble	Very soluble	Very soluble	Soluble	Soluble	Very soluble	Very soluble	Very soluble
					Pharmacokinetics						
Absorption	Low	High	High	High	High	High	High	High	High	High	High
BBB permeant	No	No	No	No	No	No	No	No	No	No	No
CYP1A2 inhibitor	No	No	No	No	No	No	No	No	No	No	No
CYP2C19 inhibitor	No	No	No	No	No	No	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No	No	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No	No	No	No	No	No	No
Log K_p (skin permeation-cm/s)	-7.84	-7.79	-7.64	-7.67	-8.57	-8.33	-7.28	-7.91	-8.16	-8.38	-8.27
					Druglikeness						
Lipinski violation	Yes:0	Yes:0	Yes:0	Yes:0	Yes:0	Yes:0	Yes:0	Yes:0	Yes:0	Yes:0	Yes:0
Ghose violation	No:1 violation	No:1 violation	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Veber violation	No:1 violation	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Bioavailability score	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55

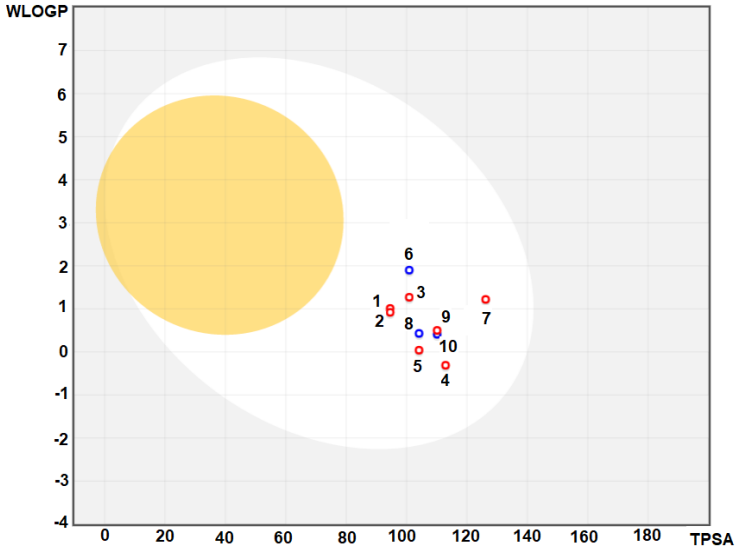


Figure 3: The Boiled-Egg model represents an intuitive evaluation of synthesized compound 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10

3.2. Molecular Docking Study

Molecular docking results for both CA IX and CA XII were presented based on comparison of all molecules with the reference molecule. Acetazolamide, used as a reference, is a heterocyclic sulfonamide. It is a diuretic and carbonic anhydrase inhibitor medication that is used to treat several illnesses [23]. CA IX contains four domains [24, 25]: an N-terminal proteoglycan-like (PG) domain, a CA catalytic domain, a transmembrane segment (TM), and an intracytoplasmic (IC) portion. Similarly, CA XII includes these domains except the Pg domain. It is known that for the inhibition of these enzymes, they form a semi-covalent tetrahedral bond between the Zn ion in the active site and the inhibitor [26]. In particular, sulfonamide derivative inhibitors bond with Zn through nitrogen with consequent substitution of the zinc-bound water molecule, and by two H-bonds of the sulfonamide moiety with residue Thr199 [27-29]. An X-ray crystal structure of the catalytic domain of CA IX in complex with acetazolamide was determined [4]. In the structure with 3IAI ID from PDB, 2 hydrogen bonds are established between the native ligand acetazolamide and Thr199, and also formed a hydrogen bond with GLN92. The reference molecule was redocked to control the binding site and we observed the same bindings with the same amino acids (Fig. 4). Similarly, the binding properties of other compounds with the CA IX enzyme are given in Table 4.

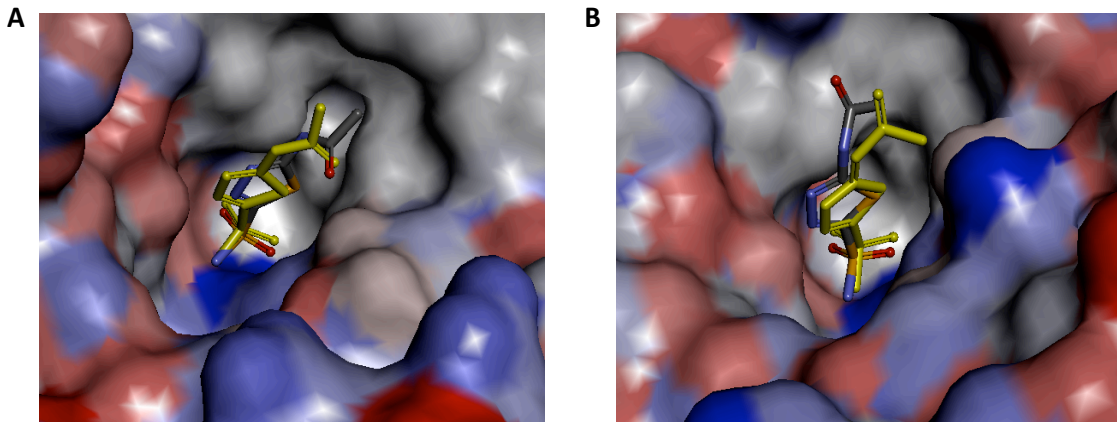


Figure 4: A) Redock image for the CA IX-Acetazolamide complex. The yellow colored molecule represents acetazolamide in complex with the enzyme in the original PDB file. RMSD: 1.0857 Å. B) Redock image for CA XII- Acetazolamide complex. The yellow colored molecule represents acetazolamide in complex with the enzyme in the original PDB file. RMSD: 1.1292 Å

Figure 5 shows that the hydrogen bonds of the reference molecule with CA IX are between THR199 and N, between THR199 and O, and between GLN92 and O. In addition, acetazolamide establishes several van der Waals interactions with residues HIS119, VAL143, VAL131, LEU91, LEU141 and THR200.

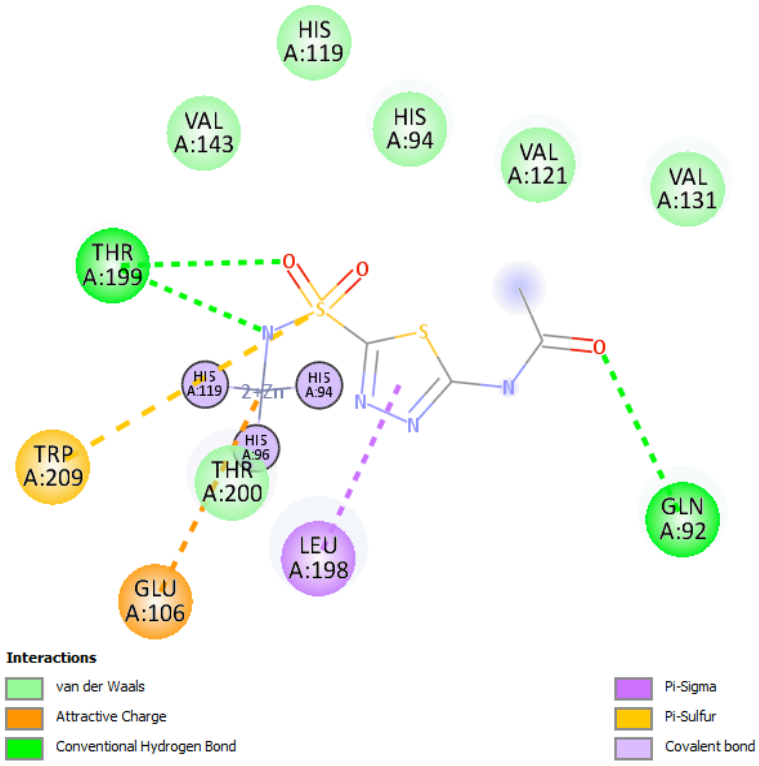


Figure 5: 2D plots of interactions of Acetazolamide with CA IX

Table 4: The docking scores for CA IX

Receptor	Molecule ID	Binding Energy (kcal/mol)	Inhibition Constant, Ki	Hydrogen Bonds (Donor - Acceptor)	The distance of hydrogen bonding (Å)
CA IX	1	-4.67	374.87 µM	H18 – Glu106:OE2	3.05
				H18 – THR199:OG1	1.62
				O10 - THR199:N	2.94
	2	-5.65	71.95 µM	H20 – Glu106:OE2	3.01
				H20 – THR199:OG1	1.75
				O9 – THR199:N	2.86
				H19 – Pro201:O	2.10
				H18 – Pro201:O	2.11
	3	-5.77	58.62 µM	H30 – Glu106:OE2	2.91
				H30 – THR199:OG1	1.70
				O14 - THR199:N	2.90
	4	-6.19	28.85 µM	H31 – Glu106:OE2	3.10
				H31 – THR199:OG1	1.65
				O12 - THR199:N	2.95
	5	-6.38	21.15 µM	H31 – THR199:OG1	1.65
				O12 - THR199:HN	1.97
	6	-7.44	3.52 µM	H31 – Glu106:OE2	2.98
				H31 – THR199:OG1	1.65
				O12 - THR199:N	2.79
	7	-6.00	40.23 µM	H31 – THR199:OG1	1.69
				O12 - THR199:N	2.92
	8	-6.30	23.97 µM	H31 – THR199:OG1	1.79
				O12 - THR199:HN	1.87
	9	-6.29	24.51 µM	H31 – THR199:OG1	1.69
				O12 - THR199:N	3.04
				O16 – GLN92:NE2	2.81
	10	-5.99	40.68 µM	H33 – Glu106:OE2	2.86
				H33 – THR199:OG1	1.82
				O12 – THR199:OG1	3.39
				O12 - THR199:N	2.88
				O16 – GLN92:NE2	2.83
	Reference-Acetazolamide	-5.25	141.79 µM	N1 – THR199:OG1	2.81
				O1 – THR199:OG1	3.01
				O1 - THR199:N	2.90
				O3 – GLN92:NE2	2.89
	Native Ligand Acetazolamide			N1 – THR199:OG1	2.75
				O1 – THR199:OG1	3.28
				O1 - THR199:N	2.85
				O3 – GLN92:NE2	3.25

In almost all adducts used in this study, the interaction of sulfonamide derivatives with enzyme active site is quite similar. All derivative molecules established hydrogen bonds with Thr199 of CA IX both through the oxygen and the hydrogen. Some of them also formed hydrogen bonds with GLU106. It is known that GLU106 is involved in the catalytic mechanism of the CA enzyme [30]. It has been observed that sulfonamide derivatives named 1, 2, 3, 4, 6 and 10 form H bond with this amino acid. The binding score of the sulfonamide named **6** is the highest, -7.44

kcal/mol. Moreover, when the binding diagrams were examined, it was determined that only one of them formed hydrogen bonds with GLN92, just like acetazolamide. This molecule, named **9**, also interacted with the enzyme structure with similar van der Waals interactions (Fig. 6).

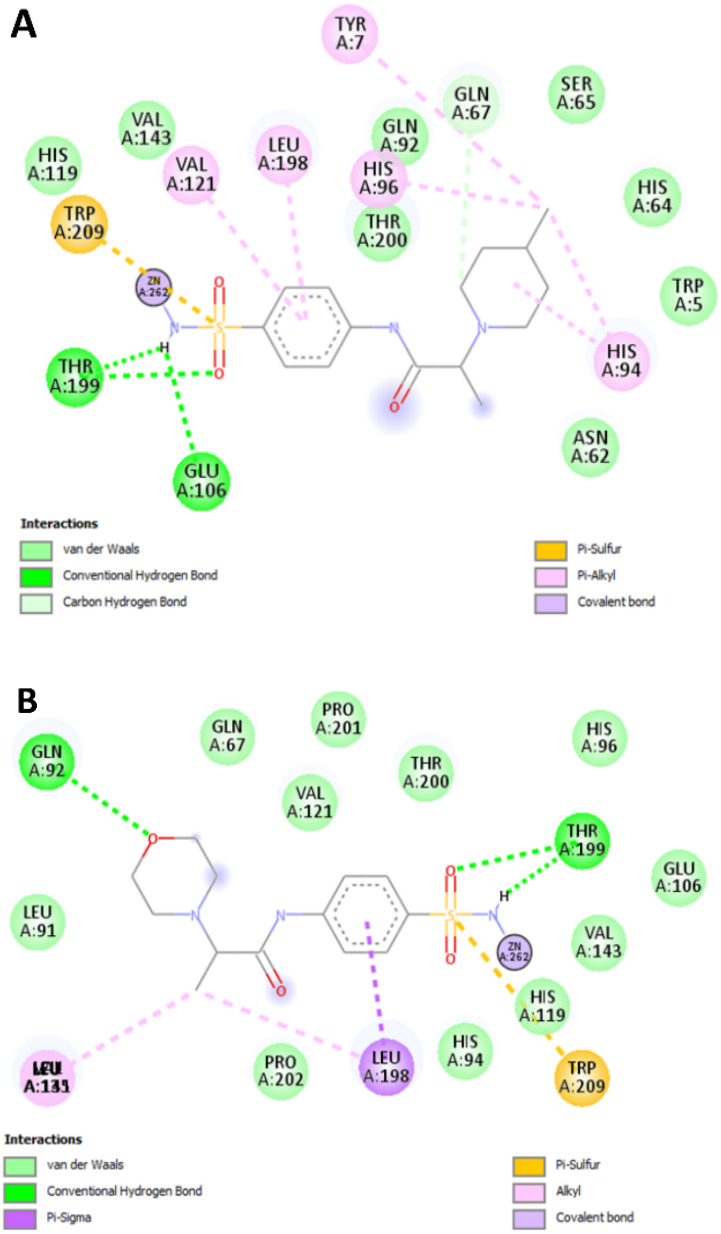


Figure 6: 2D plots of interaction of sulfonamide with enzyme. A) **6** with CA IX and B) **9** with CA IX

The docking results of all molecules with CA XII (PDB ID: 1JD0) are shared in Table 5. It was determined that 2 different hydrogen bonds were formed between acetazolamide and CA XII with the Thr199 residue. There were also van der Waals interactions with HIS199, VAL143, HIS94, THR200, VAL121 and LEU141.

Table 5: The docking scores for CA XII

Reseptör	Molecule ID	Binding Energy (kcal/mol)	Inhibition Constant, Ki	Hydrogen Bonds (Donor - Acceptor)	The distance of hydrogen bonding (Å)
CA XII	1	-3.92	1.33 mM	N11 – THR199:OG1 O10 – THR199:OG1 O10 - THR199:N	2.91 3.14 2.60
	2	-4.70	357.36 µM	H20 – Glu106:OE1 H20 – THR199:OG1 O9 - THR199:N H19 – THR200:OG1 H18 – PRO201:O	2.89 1.99 2.92 2.06 2.58
	3	-5.88	48.91 µM	H30 – THR199:OG1 O14 – THR199:OG1 O14 - THR199:N	2.24 3.39 2.94
	4	-5.29	132.08 µM	H31 – THR199:OG1 O12 - THR199:N	1.64 2.91
	5	-6.34	22.45 µM	H31 – THR199:OG1 O12 - THR199:N	1.91 2.94
	6	-6.39	20.75 µM	H31 – THR199:OG1 O12 - THR199:N	2.03 2.87
	7	-6.44	18.88 µM	H31 – THR199:OG1 O12 - THR199:N H30 – GLN92:OE1 S16 – TRP5:NE1	1.75 2.72 2.13 3.78
	8	-5.29	132.85 µM	H31 – THR199:OG1 O12 - THR199:N O4 – GLN92:NE2 N16 – SER135:OG	1.77 2.78 3.15 2.97
	9	-6.10	33.59 µM	H31 – THR199:OG1 O12 - THR199:N	1.82 2.85
	10	-5.10	183.52 µM	H33 – THR199:OG1 O12 – THR199:OG1 O12 - THR199:N O16 – SER135:OG	1.99 3.15 2.63 2.83
	Reference-Acetazolamide	-3.53	2.60 mM	N1 – THR199:OG1 O2 – THR199:OG1 O2 - THR199:N	2.76 3.00 2.80
	Native Ligand Acetazolamide			N1 – THR199:OG1 O2 - THR199:N	2.87 2.96

In silico studies revealed that all synthesized molecules showed average binding energies towards the target protein ranging from -6.44 to -3.32 kJ mol^{-1}. All sulfonamide derivatives formed hydrogen bonds with the key amino acid Thr199. Some are also linked by hydrogen bonds with different amino acids (**2**, **7**, **8**, and **10**). In addition, van der Waals interactions, which are important for enzyme inhibition, were examined. All derivatives have van der Waals interaction with HIS119 and VAL143 in common.

In this context, based on the binding between acetazolamide and CA XII, the molecules with the most similar and common bonds with CA XII are **6** and **9**, shown in Fig. 7.

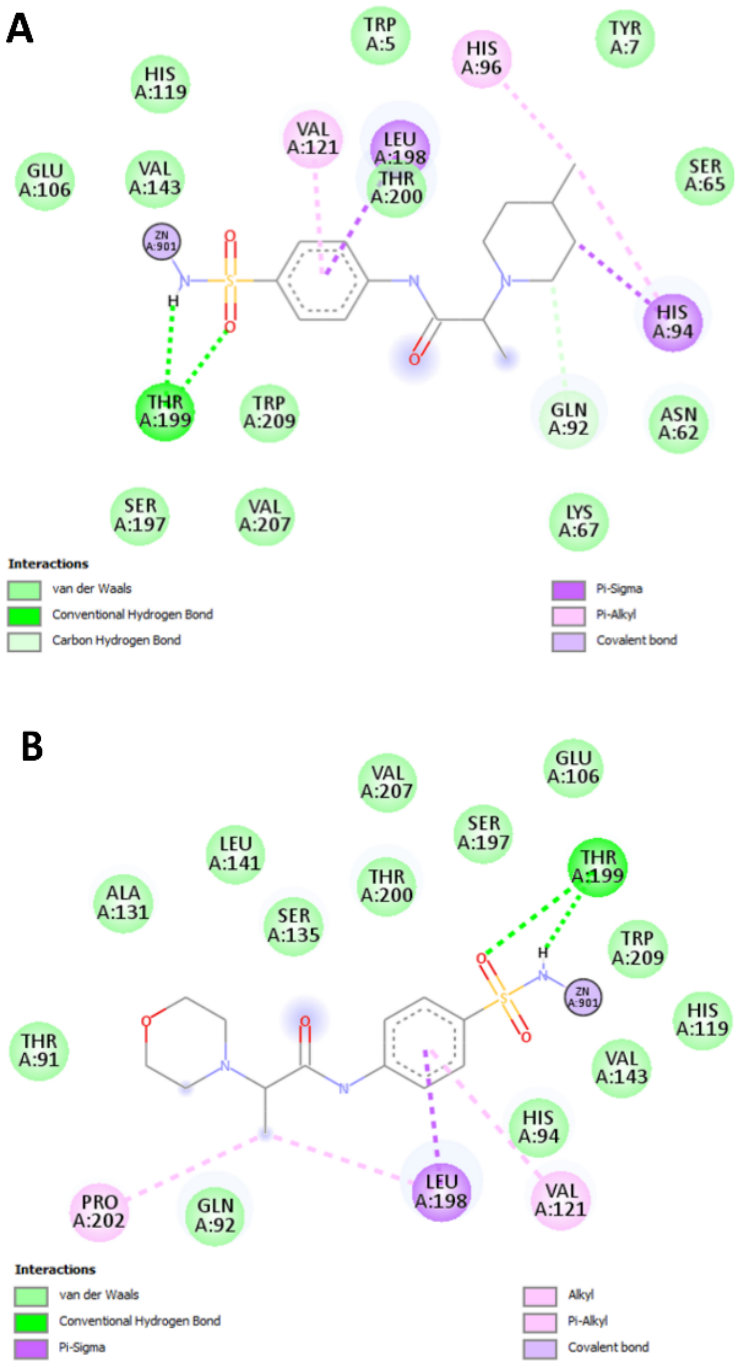


Figure 7: 2D plots interactions of sulfonamide with enzyme. A) **6** with CA XII and B) **9** with CA XII

4. Conclusion

In summary, overexpression of CA IX is associated with tumor hypoxia and is therefore being investigated as a diagnostic and therapeutic marker. Sulfonamides, which have efficacy in many different diseases, have the potential to be used as antitumor drugs. The availability of simulations to design selective CA enzyme inhibitors also contributes to this. In particular, CA

IX-targeted therapeutic agents have been developed and clinical trials have also yielded positive results [31]. In this study, we aimed to determine the anti-cancer properties of sulfonamide derivatives, which we synthesized and determined many properties in our previous study, and especially their contribution to CA IX and CA XII enzyme inhibition. In conclusion, we found good binding energies for both CA IX and CA XII of the sulfonamide derivative named **6** and **9**. In addition, compared to acetazolamide, we think that they may be candidates as an anti-tumor drug due to the similarity of the bond types and attachment to the active site of the enzyme (Fig. 8).

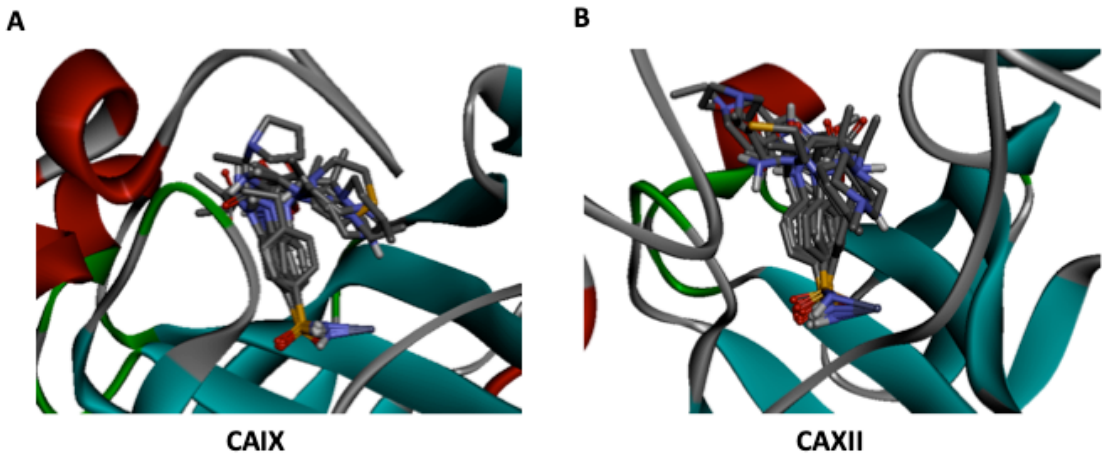


Figure 8: Superimposition of sulfonamide derivatives at the active site of the A) CA IX and B) CA XII

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