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Investigation of Potential Antibacterial Properties of Some Azo Compounds by Molecular Docking Method

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Abstract: In this study, molecular docking studies were applied to three azo dyes, 2-[(3,5-diamino-1H-pyrazol-4-yl)diazenyl]-5-nitrobenzoic acid (A), 2-[(3,5-dimethyl-1H-pyrazol-4-yl)diazenyl]-5-nitrobenzoic acid (B) and 2-[(5-amino-3-methyl-1H-pyrazol-4-yl)diazenyl]-5-nitrobenzoic acid (C), which synthesized in our previous studies, to investigate their potential antibacterial properties. Modelling was performed on SwissDock web server using EADock DSS algorithm. Docking simulations of ligands (A, B and C) were performed into the *E*. coli beta-ketoacyl-acyl carrier protein synthase III (KAS III) active site to determine the probable binding conformations and inhibitory effects. Docking results were also compared with triclosan used as a commercial antibacterial agent and it was found that compound B had the best antibacterial property.

***Key words**:* Molecular docking, Azo compound, Antibacterial properties, Protein

Moleküler Docking Yöntemi ile Bazı Azo Bileşiklerinin Potansiyel Antibakteriyel Özelliklerinin İncelenmesi

Özet: Bu çalışmada, önceki çalışmalarımızda sentezlediğimiz üç azo boyarmaddenin, 2 - [(3,5-diamino-1H-pirazol-4-il)diazenil]-5-nitrobenzoik asit (A), 2-[(3,5)-dimetil-1H-pirazol-4-il) diazenil]-5-nitrobenzoik asit (B) ve 2-[(5-amino-3-metil-1H-pirazol-4-il) diazenil]-5-nitrobenzoik asit (C), potansiyel antibakteriyel özelliklerini araştırmak için moleküler doking çalışmaları yapıldı. Modelleme, SwissDock web sunucusunda EADock DSS algoritması kullanılarak gerçekleştirildi. Olası bağlanma konformasyonları ve inhibe edici etkileri belirlemek için *E*. coli beta-ketoaçil-açil taşıyıcı protein sentaz III (KAS III) aktif bölgesine ligandların (A, B ve C) bağlanma simülasyonları yapıldı. Doking sonuçları ayrıca ticari bir antibakteriyel madde olarak kullanılan triklosan ile karşılaştırıldı ve B bileşiğinin en iyi antibakteriyel özelliğe sahip olduğu bulundu.

***Anahtar kelimeler**:* Moleküler kenetlenme, Azo bileşik, Antibakteriyel özellikler, Protein

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1. Introduction

Bacteria are single celled, fast reproducing microorganisms that have the ability to adapt quickly to the changes in their environment. Antibiotics, on the other hand, are very clinically important drugs which are used in the treatment of infectious diseases caused by microorganisms which prevent the growth of bacteria [1]. However, new antibiotic discoveries have recently slowed down considerably, while the incidence of multiple antibiotic-resistant microorganisms has increased dramatically and a future in which antibiotics have lost their influence has begun to appear soon [2]. Antibiotic resistance indicates that the antibiotic cannot kill or prevent the growth of resistant bacteria at the treatment dose. In recent years, this problem caused by the inability to develop new antibiotic species brings along other problems [3, 4]. But, the discovery of new antibiotics includes a long and costly process [5]. For this reason, before starting the drug design, the molecular interaction of the ligand and receptor by molecular docking can be examined on the computer and the molecules that are part of the drug design can be examined without requiring much cost [6].

Docking is a method used to predict the preferred positions of atoms in space as a molecule forms a stable complex with another molecule and it examines the nature of the binding of possible drugs to target molecules. Key-lock analogy can be used to understand the docking process and it is desired to find the position where the key must stop to unlock. The protein and ligand, which can move continuously, will stop at their lowest potential energy position as a result of these movements. An optimization process is used to calculate this. By changing the position of the ligand relative to the protein, its energy is calculated and this process is continued until the lowest energy is found. In other words, the aim is to find the conformation where the free energy of the protein-ligand system is the lowest. Docking is often used to find the conformation of small molecules, such as drug-active substance candidates, to their binding to protein-based targets. Therefore, the docking process plays an important role in rational drug design [7,9].

In this study, the interaction of previously synthesized [10] azo compounds (A, B, C) with E.coli beta-ketoacyl-acyl carrier protein synthase III (KAS III) was investigated by molecular docking study. The results were then compared with triclosan, a substance which is involved in antibacterial soaps, toothpastes, deodorants, mouthwashes, shaving lotions, cosmetics and many other products to prevent or reduce germ contamination.

2. Material and Method

Due to their structure and functional groups, compounds 2-[(3,5-Diamino-1H-pyrazol-4-yl)diazenyl]-5-nitrobenzoic acid (A), 2-[(3,5-dimethyl-1H-pyrazol-4-yl)diazenyl]-5-nitrobenzoic acid (B) and 2-[(5-amino-3-methyl-1H-pyrazol-4-yl)diazenyl]-5-nitrobenzoic acid (C) that are expected to have potentially high antibacterial properties have been selected which were synthesized firstly in our previous work [10]. Molecular docking studies of the mono azo molecules were carried out by SwissDock web server using EADock DSS algorithm [11]. High resolution crystal structure of the KAS III (PDB ID: 1HNJ) was obtained from the protein data bank website [12]. Mono azo molecules were prepared for docking by energy minimized using DFT/B3LYP/6-311G(d,p) level of theory with Gaussian 09 program package [13]. All images in molecular docking studies were obtained using UCSF Chimera program [14].

3. Results

The fully optimized azo compounds (A, B, C) and triclosan (T) were shown in Figure 1 and Figure 2, respectively.

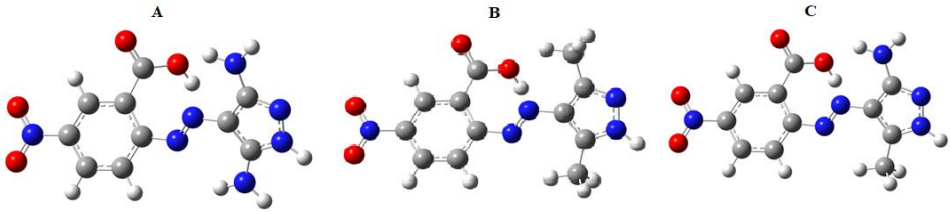

Figure 1. The fully optimized azo compounds at DFT/B3LYP/6-311G(d,p) level

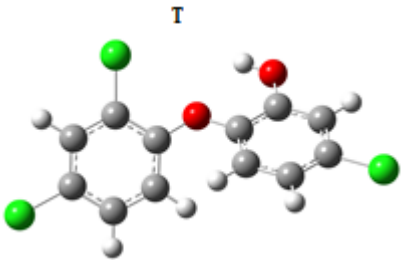

Figure 2. The optimized structure of triclosan

Figure 3 shows the interactions between A, B, C ligands and KAS III enzyme.

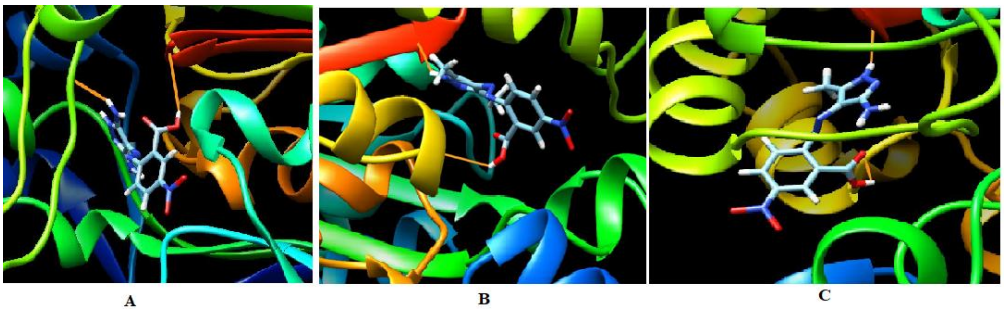

Figure 3. Interactions between ligand and protein

Figure 4 shows the binding of triclosan in the cavity of KAS III enzyme and the interaction between triclosan-protein couple.

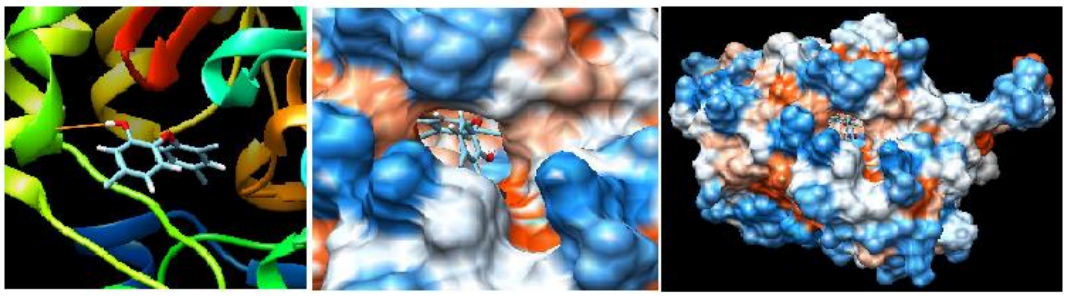

Figure 4. Interactions between triclosan and protein

Figure 5 shows the binding of A, B and C ligand in the cavity of KAS III enzyme.

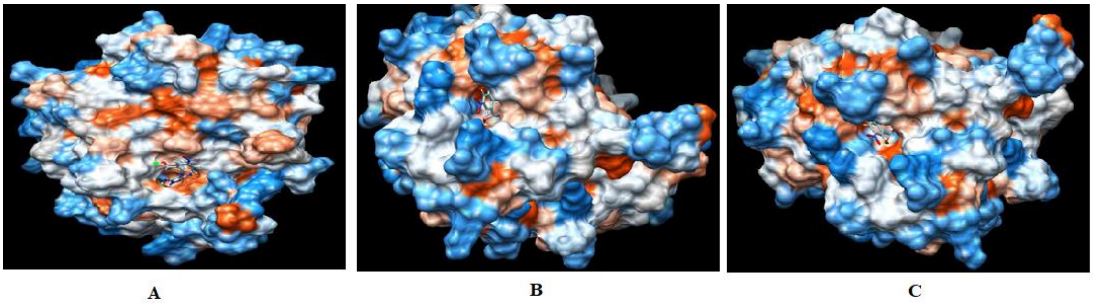

Figure 5. The binding of the ligand in the cavity of protein

Table 1. Calculated values of the ligand-protein couple

Ligand	ΔG (kcal/mol)	Full Fitness Score	Hydrogen Bond Location (Length)
A	-7.33	1412.34	-NH_2 grp. H & Leu189 aa O (1.889 Å) -NH_2 grp. H & Leu189 aa O (1.889 Å)
B	-7.87	1439.27	Arom. -NH grp. H & Phe304 aa O (1.795 Å) -COOH grp. H & Ala246 aa O (1.803 Å)
C	-7.76	1420.80	Arom. -NH grp. H & Phe304 aa O (1.792Å) -COOH grp. H & Ala246 aa O (1.955 Å)
T (Triklosan)	-7.52	1419.75	-OH grp. H & Gly186 aa O (2.419 Å)

4. Conclusion and Comment

Optimized geometries of the studied molecules (A, B, C, T) were used in docking studies. Geometry optimization of the compounds was done using Becke-3-Lee-Yang-Parr (B3LYP) functional with 6-311G(d,p) basis set in gas phase [15,16]. The fully optimized geometries of the mono azo molecules and triclosan are shown in Figure 1 and Figure 2.

Molecular docking studies were performed to investigate the KAS III inhibitory activity of the mono azo molecules (Figures 3 and 5). In addition, the binding properties of a commercial antibacterial agent triclosan with KAS III were also investigated by molecular docking studies. All the studied compounds showed significant binding affinity to the KAS III (Table 1). The most stable poses of the studied molecules with KAS III were determined based on Gibbs free energies and full fitness scores. Moreover, the hydrogen bonds in the binding site were also analysed.

The docking energies of A-KAS III, B-KAS III, C-KAS III and T-KAS III couples were found to be -7.33, -7.87, -7.76 and -7.52 kcal/mol, respectively. The highest energy released from the B-KAS III interaction showed that the B-KAS III was the most stable couple among the studied molecules. The B-KAS III couple showed two hydrogen bonding interactions. One of them was between N-H proton of aromatic group and oxygen atom of Phe304 amino acid with 1.795 Å in length. The second hydrogen bond was formed between proton of -COOH group and oxygen atom of Ala246 amino acid with a bond length of 1.803 Å.

The hydrogen bonds appeared between C-KAS III and B-KAS III were built by the same atoms of the ligands and target molecules at the same parts of the couples. However, the bond length between the proton of -COOH group and the oxygen atom of Ala246 amino acid (1.955 Å) was slightly longer than the B-KAS III couple. This result showed that the interaction between C-KAS III was slightly weaker than B-KAS III couple. As shown in Figure 3, two hydrogen bonds were observed between A and KAS III. These bonds were formed between the proton of -NH_2 group and the oxygen atom of Leu189 amino acid and the proton of -COOH group and the oxygen atom of Gly306 amino acid had 1.889 Å and 2.238 Å bond lengths, respectively. Docked pose in the Figure 4 involved hydrogen bonding which indicated an interaction between T and KAS III protein. The hydrogen bonding observed between the proton of -OH group and the oxygen atom of Gly186 amino acid which is 2.419 Å in length. The longest hydrogen bond formed was between triclosan and KAS III enzyme among the molecules studied, showing that T-KAS III binding was relatively weaker than other couples.

According to the molecular docking studies, the full fitness scores between A-KAS III, B-KAS III, C-KAS III and T-KAS III were found to be 1412.34, 1439.27, 1420.80 and 1419.75, respectively. The highest score was calculated for the interaction between B and KAS III among all studied complexes. The observation of the highest full fitness score

between B-KAS III complexes showed that the best orientation and proximity were between these couples.

From these results we can conclude that, the B and C mono azo molecules have higher affinity towards *E.* coli beta-ketoacyl-acyl carrier protein synthase III (KAS III) rather than A and triclosan. According to the binding energies, full fitness scores, number and length of hydrogen bonds formed, B and C showed better binding affinity than the studied a commercial antibacterial agent and can be used in the development of antibacterial agents and drugs.

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