Investigation of The Binding Potential of Gadobutrol, Iohexol and Fluorescein Radiocontrast Agents to the TSH Receptor
Gadobutrol, Iohexol ve Floresein Radyokontrast Ajanlarının TSH Reseptörüne Bağlanma Potansiyelinin Araştırılması

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***Abstract*—Radiopaque agents can affect the human body in different ways. The resulting reactions are simple allergic and anaphylactic reactions. If the potential of these agents to bind to different receptors is determined beforehand, precautions can be taken against the side effects that may occur in the future. The aim of this study is to investigate the binding potential of the active substances Fluorescein, Gadobutrol, Iohexol, which are frequently used in routine, to the TSH receptor with molecular docking. The conformational analysis of 3 drugs on TSH receptor surfaces was performed by molecular docking using Autodocktools program. First, the atomic center of the receptor was determined at the grid stage, and the XYZ center of the grid box was set to 9.524, 48.24 and 25.257 Å, respectively, and the space gap was set to 0.5. Thus, a box was created in which the ligand can easily scan the entire surface. In the docking phase, a parameter file has been prepared and run for 100 conformation and 300 population size, accompanied by Lamarckian and Genetic Algorithms. The binding energies of fluorescein, gadobutrol and iohexol strengthen the possibility of spontaneous binding. However, when the inhibition concentrations are evaluated, it shows that fluorescein can more easily bind to the TSH receptor. Gadobutrol and iohexol are unlikely to reach these concentrations in the blood. This in vitro study demonstrates the potential for spontaneous binding of fluorescein, gadobutrol, and iohexol to the TSH receptor. Even if radiopaque drugs are used for diagnostic purposes, they may cause side effects by interacting with different receptors in the human body. Experimental studies are needed to confirm this possibility.**

***Keywords*—TSH receptor; molecular docking; binding energy; fluorescein; gadobutrol; iohexol**

***Özetçe*—Radyoopak ajanlar insan vücudunu farklı şekillerde etkileyebilir. Ortaya çıkan reaksiyonlar basit alerjik ve anafilaktik reaksiyonlardır. Bu ajanların farklı reseptörlere bağlanma potansiyeli önceden belirlenmesi durumunda ileride oluşabilecek yan etkilere karşı önlem alınabilir. Bu çalışmanın amacı rutinde sıklıkla kullanılan Fluorescein, Gadobutrol, Iohexol etken maddelerinin moleküler kenetlenme ile TSH reseptörüne bağlanma potansiyelinin araştırılmasıdır. 3 ilacın TSH reseptör yüzeyleri üzerindeki konformasyonel analizi, Autodocktools programı kullanılarak moleküler yerleştirme ile yapıldı. İlk olarak grid aşamasında reseptörün atom merkezi belirlendi ve grid kutusunun XYZ merkezi sırasıyla 9.524, 48.24 ve 25.257 Åolarak ayarlandı ve boşluk aralığı belirlendi. 0,5'e kadar. Böylece ligandın tüm yüzeyi kolayca tarayabileceği bir kutu oluşturuldu. Yerleştirme aşamasında, 100 konformasyon ve 300 popülasyon büyüklüğü için bir parametre dosyası hazırlanmış ve Lamarckian ve Genetik Algoritmalar eşliğinde çalıştırılmıştır. Floresein, gadobutrol ve ioheksolün bağlanma enerjileri, kendiliğinden bağlanma olasılığını güçlendirir. Ancak inhibisyon konsantrasyonları değerlendirildiğinde, floreseinin TSH reseptörüne daha kolay bağlanabildiğini göstermektedir. Gadobutrol ve ioheksolün kanda bu konsantrasyonlara ulaşması olası değildir. Bu in vitro çalışma, floresein, gadobutrol ve iohexol'ün TSH reseptörüne kendiliğinden bağlanma potansiyelini göstermektedir. Radyoopak ilaçlar tanı amaçlı kullanılsa bile insan vücudundaki farklı reseptörlerle etkileşerek yan etkilere neden olabilirler. Bu olasılığı doğrulamak için deneysel çalışmalara ihtiyaç vardır.**

***Anahtar Kelimeler*—TSH reseptörü; moleküler doking; bağlanma enerjisi; floresein; gadobutrol; iyoheksol**

I. INTRODUCTION

Contrast agents are diagnostic drugs that create contrast inside or around organs and tissues and make them visible [1], [2]. Radiopaque agents used in imaging techniques can affect the human body in different ways. Common side effects are allergic and anaphylactic reactions that occur by binding to histamine receptors [3], [4]. Contrast agents are known

to cause high secretion of catecholamines [5]. As it binds to sympathetic nervous system receptors, it can also bind to other receptors [6]. Binding of these agents to different receptors may result in different clinical symptoms [7]. The aim of this study is to investigate the binding potential of the active substances Fluorescein, Gadobutrol, Iohexol, which are frequently used in routine, to the TSH receptor with molecular docking.

II. Computational Setup

The conformational analysis of 3 drugs on TSH receptor surfaces was performed by molecular docking using Autodock-tools program [8]. Drugs and receptors are given in Fig. 1. First, the atomic center of the receptor was determined at the grid stage, and the XYZ center of the grid box was set to 9.524, 48.24 and 25.257 Å, respectively, and the space gap was set to 0.5. Thus, a box was created in which the ligand can easily scan the entire surface. In the docking phase, a parameter file has been prepared and run for 100 conformation and 300 population size, accompanied by Lamarckian and Genetic Algorithms [9], [10].

In Fig. 1, the TSH receptor is displayed in a rainbow style. This structure was prepared by downloading the 1XUM coded molecule from the PDB site. Ligand molecules were prepared from PubCHEM site. Since gadolinium, the ionic component of gadobutrol, is non-bond, it was not included in the docking studies, only the molecular interactions with the main parent component, butrol, were investigated.

The estimated free energy binding energy (BE) is the sum of (i) Final Intermolecular Energy = [vdW + Hbond + desolvation Energy] + Electrostatic Energy, (ii) Final Total Internal Energy, (iii) Torsional Free Energy, and (iv) Represents Unbound System's Energy = [(ii) Final Total Internal Energy].

III. Results and Discussion

The RMSD results obtained as a result of molecular docking are shown in Table I.

One of the most striking results in Table I is that fluorescein can easily dock the TSH receptor. Because its binding energy was calculated as -7.67 kcal/mol. In the literature, -6.0 kcal/mol and below of the ligand are accepted as good interaction indicators for rigid body docking [11]–[13]. Another important situation supporting our argument was that the energy of 100 conformations increased from -7.67 kcal/mol to -4.85 kcal/mol during docking. On the other hand, the negative lowest binding energy for all three ligands indicates that a stable ligand/receptor complex can be formed and that it is a good inhibitor [14]. Moreover, it was observed that the interaction that contributed most to these energies came from van der Waals (vdW) during the entire docking. For example, it was recorded as -9.25 kcal/mol for gadobutrol and -8.64 for iohexol. On the other hand, the electrostatic contribution remained at a minimal level and was calculated around zero. Another important parameter affecting the ligand/receptor interaction due to the binding energy was found to be H-bonds. Measurements recorded as 2.136 and 1.907 Åfor fluorescein, respectively, were measured as 2.076 and

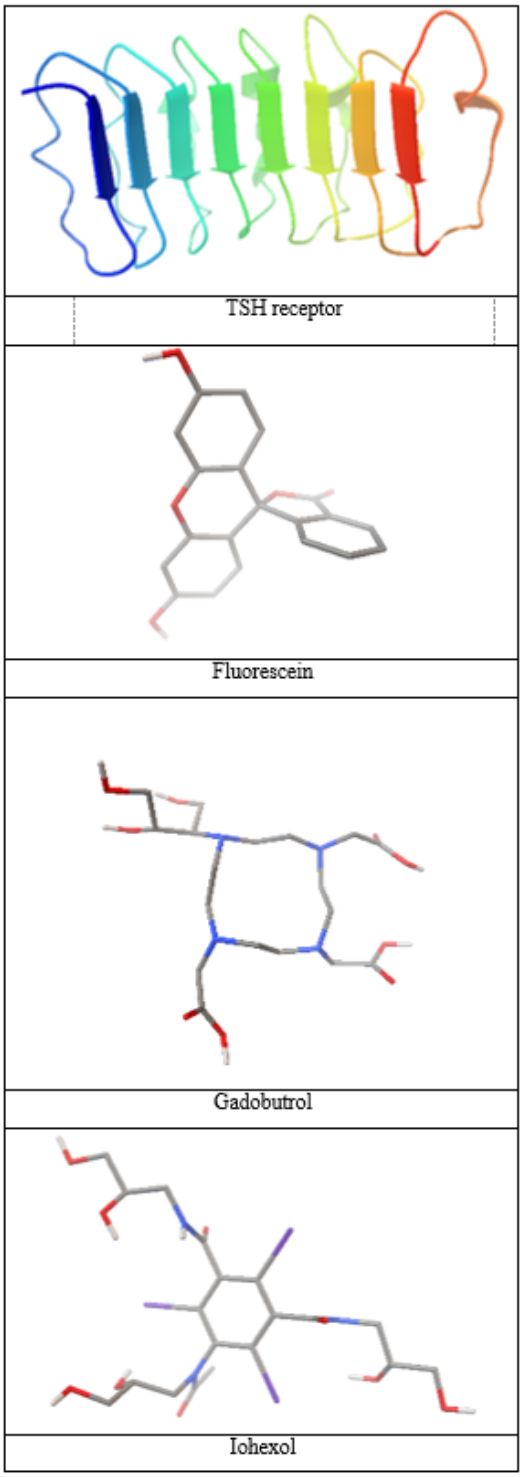

Figure 1: Elementary structures of receptor and ligand molecules. The receptor is given in rainbow style. Gray, red, white, blue and purple colors represent carbon (C), oxygen (O), hydrogen (H), nitrogen (N) and iodine (I) atoms, respectively.

Ligand molecules	Number of the lowest binding conformation	BE (kcal/mol)	van der Waals contribution [kcal/mol]	electrostatic contribution (kcal/mol)	IC (μM)	Detected H-bonds [Å]
Fluorescein	26	-7.67	-7.76	-0.51	2.37	TYR98(2.136); ASP118(1.907)
Gadobutrol	72	-4.97	-9.25	-0.49	226.78	TYR98(2.076); ASP120(2.044)
Iohexol	61	-3.39	-8.64	-0.12	3260	No H-bonds formed

Table I: RMSD results for the lowest binding conformations

2.044 Åfor gadobutrol (Fig. 2). For Iohexol, however, no H-bond formation was observed in the conformation with the lowest binding energy. During the docking studies, although the intermolecular interaction increased, it was observed that the inhibition decreased as expected.

In docking studies of 100 conformations, drug molecules found the lowest binding conformations at runes 26, 72 and 61, respectively (Table I). It was mentioned above that the interactions here are due to H-bonds. Other non-bond interactions that cause these drug molecules to dock on the TSH surface are depicted in Figures 3, 4, and 5, respectively.

All amino acids that are effective in the formation of conformation 26 of the TSH receptor with fluorescein, and vdW spheres and close contact wireframe spheres and H-bonds of the drug are given in Fig. 3A. For better understanding, the screenshot after removed close contacts and molecular surface were seen in Fig. 3B. In Fig. 3C, the vdW spheres and other close contact are neglected and only amino acids that cause hyrophobic interaction are given. These amino acids are TYR98, TYR116 and ILE117.

The same explanations in Figure 3 apply to Figures 4A, 4B and 4C, respectively. Again, as in the model in Figure 3, the amino acids that cause hydrophobic interactions in the gadobutrol/TSH model are the same. In addition, TYR98 amino acid formed a visible H-bond in these two drugs at the same time, as in H-bond interactions. According to these two results, we can say that this region is the most favorable site for both fluorescein and gadobutrol.

As in Figures 3 and 4, the order of expression in Figures 5A, 5B and 5C is also valid here. However, unlike the other two figures, the vdW spheres of iodine atoms from both are shown in purple (Figure 5A). Moreover, iohexol was docked at a different site from both. More amino acids than either affected hydrophobicity. These are the ones coded TYR195, PHE198, TYR212, VAL215 and ILE216. From this point of view, one of the reasons for the smallest increase in binding energy for this model is that docking the outer surface of the TSH receptor may be due to the longer and more complex chemical structure of iohexol. The other is that there may be more hydrophobic interactions occurring.

The fact that the contrast materials are not permeable to X-ray rays has made it important in diagnosis. These agents target receptors on the membranes of tumor cells and visualize tumors with a non-invasive optical imaging method.

In one study, the affinities of fluorescein and carbocyanin dyes for somatostatin and bombesin receptor-avid peptides were investigated. Receptor binding of fluorescein-somatostatin peptide conjugates was found to be highly sensitive to the type of linker and the fluorescein insertion site

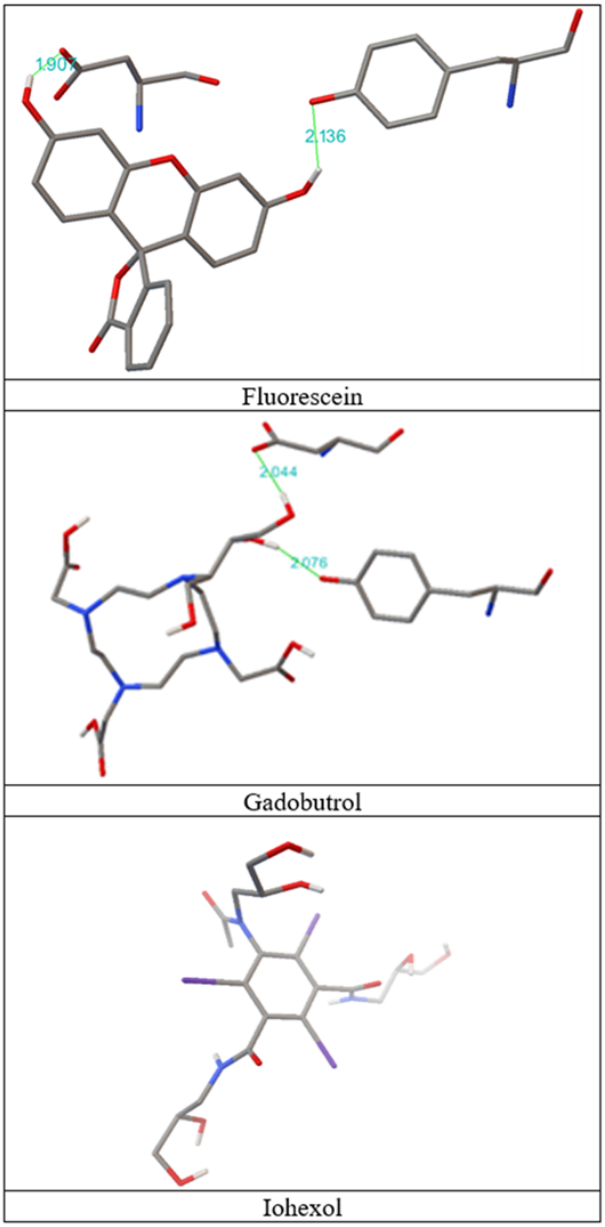

Figure 2: The lowest binding conformations of the ligand/receptor models

in the non-receptor linker region of the peptide. It shows that conjugation of dyes to truncated somatostatin and bombesin

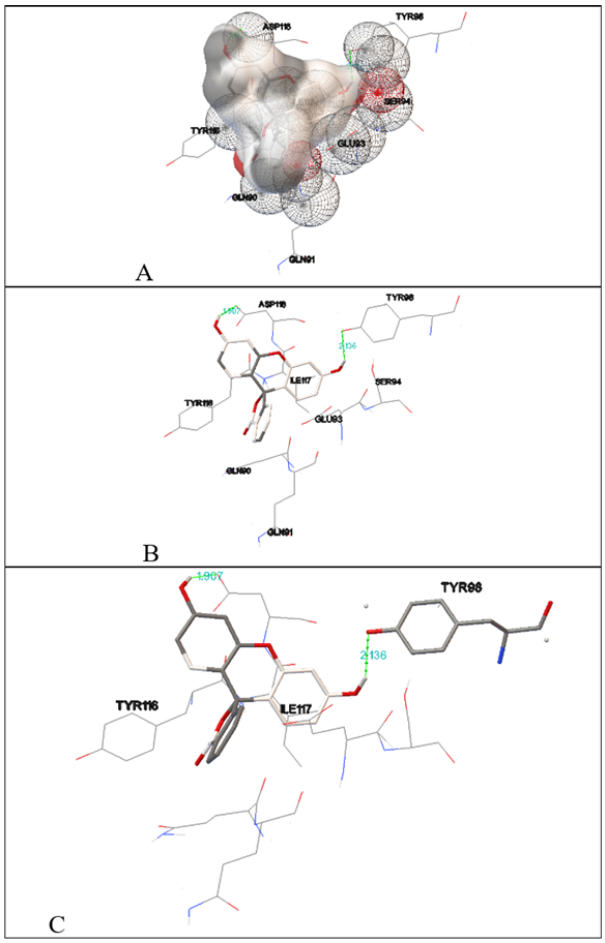

Figure 3: All interactions resulting from docking of the Fluorescein/TSH model

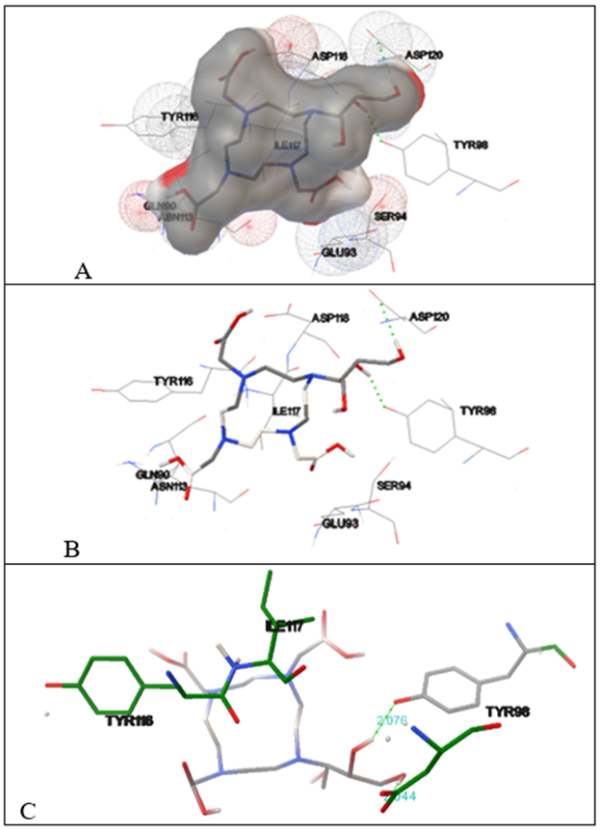

Figure 4: All interactions resulting from docking of the Gadobutrol/TSH model

peptide analogs results in promising diagnostic agents that retain high receptor-binding activity in vitro [15].

Gadobutrol (Gd-DO3A-butrol) is a paramagnetic radiocontrast agent used in magnetic resonance imaging (MRI). The chelation property of gadolinium element has made this molecule an agent worthy of investigation in terms of binding to different receptors.

Water-soluble paramagnetic contrast agents are usually metal chelates with unpaired electrons. With this feature, it has gained the ability to react with different biological molecules. Being soluble in water, it enables it to reach almost any localization. In this study, the binding energies show that this molecule tends to bind spontaneously with the TSH receptor. These agents, which are given intravenously, can bind to the TSH receptor in the first minutes. Depending on the elimination time from the blood, this binding may be short-lived. Because the inhibition concentrations are high. This binding state can either increase or decrease thyroid hormone secretion. This may vary depending on the effect of cellular secondary messengers. Caution may be required in the use of gadobutrol in patients with thyroid dysfunction. However, this data needs to be proven experimentally [16].

In a study in rats, it was shown that iohexol does not bind to serotonin, dopamine D2, muscarinic, noradrenaline, opiate, N-methyl-D-aspartate (NMDA) receptors. Even at high concentrations, iohexol did not cause neurotoxic side effects [17]. Nygaard et al. stated that the use of iohexol in patients with thyroid disease is safe [18]. Molen et al. stated that the use of iohexol increases the free iodine load and interferes with iodide uptake in the thyroid [19]. However, there are no molecular docking studies on its potential to bind to TSH receptors.

The binding energies of fluorescein, gadobutrol and iohexol strengthen the possibility of spontaneous binding. However, when the inhibition concentrations are evaluated, it shows that fluorescein can more easily bind to the TSH receptor. Gadobutrol and iohexol are unlikely to reach these concentrations in the blood.

IV. Conclusion

This is an *in silico* study that demonstrate the potential for spontaneous binding of fluorescein, gadobutrol, and iohexol to the TSH receptor. Even if radiopaque drugs are used for diagnostic purposes, they may cause side effects by interacting

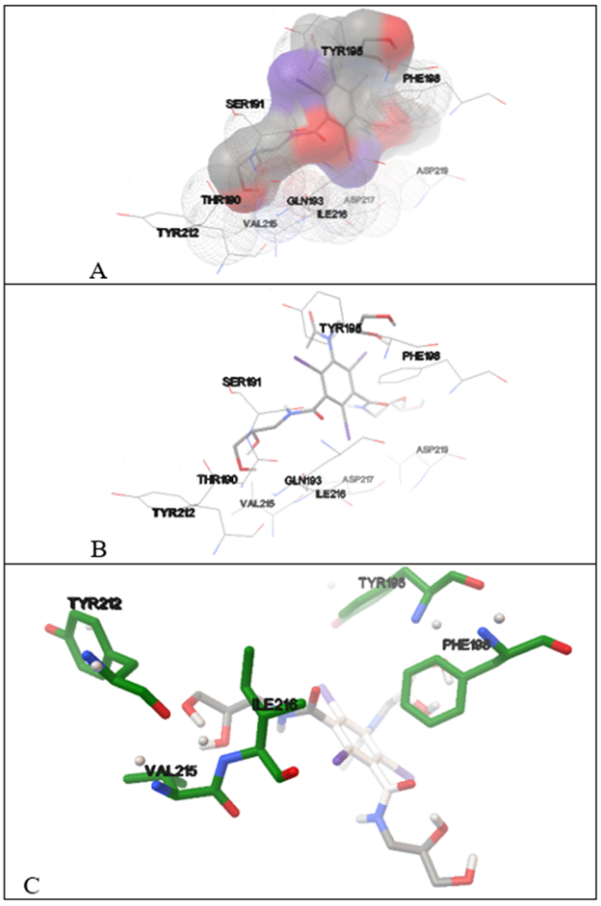

Figure 5: All interactions resulting from docking of the Iohexol/TSH model

with different receptors in the human body. Experimental studies are needed to confirm this possibility.

Author Contributions

D.K. is the corresponding author of the study and run molecular docking studies and reported the results. A.G., together with the corresponding author, prepared, edited and wrote the manuscript in the light of the literature.

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