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Araştırma Makalesi / Research Article

Benzimidazolyum Tipi Moleküllerin Anti-Kanser Aktivitesinin VEGFR-2 ve TrxR Kristal Yapıları Kullanılarak Moleküler Doking Yoluyla Analizi

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Öz

N-heterosiklik karbenler biyoaktiviteleri dolayısıyla sıklıkla incelenen bir molekül ailesidir. Kanserin dünyada ölüme neden olan 2. hastalık olduğu için, N-heterosiklik karbenlerin anti-kanser aktivitesi de incelenen özellikler arasındadır. Bütün aday moleküllerin biyoaktivitesinin incelenmesinin zorlukları değerlendirildiğinde, in-siliko yöntemlerin öngörü elde etmek için kullanılması avantajlıdır. Teorik hesaplama yöntemleriyle, mevcut moleküllerin özellikleri incelenebildiği gibi daha uygun moleküllerin sentezlenmesi için önbilgiler elde edilebilir. Bu çalışmada daha önce karakterizasyonu tamamlanmış 1-(allil)-3-(2,4,6-trimetilbenzil)benzimidazolyum klorit (1) ve 1-(allil)-3-(2,3,4,5,6-pentametilbenzil)benzimidazolyum klorit (2) molekülleri DFT temelli hesap yöntemleri ile yapısal olarak incelenerek optimize edilmiştir. Optimize moleküllerin VEGFR-2 ve TrxR kristal yapıları ile etkileşimleri moleküler doking yöntemleri kullanılarak analiz edilmiştir.

Anahtar kelimeler: N-Heterosiklik Karbenler, Anti-Kanser, Moleküler Doking, DFT

Molecular Docking Analysis of Benzimidazolium Type Molecules with VEGFR-2 and TrxR Crystal Structures for Evaluating Anti-Cancer Activity

Abstract

N-heterocyclic carbenes is a molecule family that is frequently studied for their bioactivity. Since cancer is the second disease that causes death in the world, the anti-cancer properties of N heterocyclic carbenes is one of the analyzed activities. Considering the difficulties of studying the bioactivities of all candidate molecules, using in-silico methods for obtaining predictions is advantageous. With theoretical ways, both the properties of the existed molecules can be examined, and preliminary information can be obtained for designing of more suitable molecules. In this study, previously characterized of 1-(allyl)-3-(2,4,6-trimethylbenzyl)benzimidazolium chloride (1) and 1-(allyl)-3-(2,3,4,5,6-pentamethylbenzyl)benzimidazolium chloride (2) have been optimized by DFT-based calculation methods. In addition, the interactions of optimized molecules with VEGFR-2 and TrxR crystal structures were analyzed by using molecular docking methods.

Keywords: N-Heterocyclic Carbenes, Anti-Cancer, Molecular Docking, DFT

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1. Introduction

N-Heterocyclic Carbenes (NHCs) is a family of molecules that frequently used in organometallic chemistry due to their strong σ-donor and weak π-acceptor properties (Nair et al 2004, Serdaroğlu et al 2021). NHCs are popular since the first molecule was isolated by Arduengo in 1991 (Arduengo et al 1991). The catalytic activity and the diverse bioactivity of these molecules are still being studied recently (Vicin et al 2003; Frémont et al 2009, Serdaroğlu et al 2019).

According to the reports of the World Health Organization (WHO), cancer is the second mortal disease in world (https://www.who.int). Studies on the elucidation of formation of cancerous cell and metastasis mechanisms, which attracts the attention of scientists in many fields, are remarkable since very strong synthetic agents used in old cancer drugs cause damage in healthy cells as well as cancerous cells (Waks et al 2019; Miller et al 2019; Gupta and Massagué, 2006). This has led to the development of new inhibitor/activator agents for certain macromolecules in the certain cancer mechanisms (Dawson and Kouzaride, 2012; Nordling, 1953). For example, Vascular Endothelial Growth Factor (VEGF) is a homodimeric glycoprotein that is critical during angiogenesis that plays an important role in the formation and growth of tumor cells. VEGF Receptors (VEGFRs) are divided into two subgroups as VEGFR-1 and VEGFR-2. The studies about the increment of cervical, breast and lung cancer cases in overexpression of VEGFR-2 show that controlling the expression of VEGFR-2 is a suitable pathway for fighting against cancer (Sitohy et al 2012; Spannuth et al 2009; Rapisarda and Melillo, 2012; Tanno et al 2004). Similarly, it is known that free radicals and Reactive Oxygen Species (ROS) occur in oxygen metabolism, diseases, and inflammation in metabolically active cells. These harmful forms cause disruption of endogenous macromolecular structures such as DNA, proteins, and lipids. One of the defense mechanisms against these species is the activation of redox sensitive factors of the human body. The expression of Thioredoxin Reductase (TrxR), which is involved in these defense processes, increases significantly in some cancer types such as breast, thyroid, and prostate cancer. Thus, unlike conventional anti-cancer agents, TrxR inhibition can be used as a cancer-fighting mechanism. It is not experimentally possible to examine the interaction of each effective species in cancer mechanisms with possible anti-cancer agents (Lincoln et al 2003; Ganther 1999; Arnér and Holmgren, 2006; Nguyen et al 2006). Therefore, primarily analyzing candidate molecules by in-silico methods will provide foresight about both activity of existed molecules and synthesis of more active molecules. With this approach, in this study, 1-(allyl)-3-(2,4,6-trimethylbenzyl)benzimidazolium chloride (1) and 1-(allyl)-3-(2,3,4,5,6-pentamethylbenzyl) benzimidazolium chloride (2) have been optimized by DFT-based calculation methods (Şahin et al 2019). The interactions of optimized molecules with VEGFR-2 and TrxR crystal structures were analyzed by using molecular docking methods.

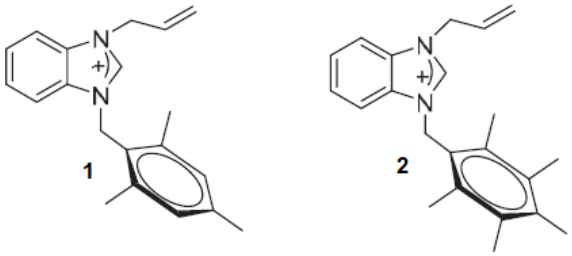

Figure 1. Molecular structure of **1** and **2**

2. Experimental

2.1. DFT/TDFFT Calculation Method

DFT calculations were performed with ORCA version 2.8 using the BP86 functional with a def2-SVP def2-SVP/J basis set, the tightscf, and KDIIS SOSCF options for geometry optimizations (Neese 2012; Neese et al 2020, Üstün et al 2019; Becke 1997; Lee et al 1988). TDDFT calculations were also carried out with nroots 45 and maxdim 225 options (Neese 2006). The quantum chemical parameters were calculated with the following Eq(1-4) (Parr et al 1999; Parr and Pearson 1983):

$$\eta = \frac{\mathrm{I} - \mathrm{A}}{2} = \frac{E_{HOMO} - E_{LUMO}}{2}$$

$$\delta = \frac{1}{\eta} = \frac{2}{E_{HOMO} - E_{LUMO}}$$

$$\chi = -\mu = \frac{I + A}{2} = \frac{(E_{HOMO} + E_{LUMO})}{2}$$

$$\omega = \frac{\mu^2}{2\delta}$$

The η, δ, χ, and ω symbols were defined as global hardness, global softness, absolute electronegativity, and electrophilicity index, respectively.

2.1. Molecular Docking Method

Molecular docking was carried out by using AutoDock 4.2. with crystal structure from RCSB protein data bank (PDB ID: 1ywn and 4cbq) (Miyazaki et al 2005; Parsonage et al 2016). Kollman charges and polar hydrogen atoms were evaluated for target molecules and the waters in proteins were removed in process. Randomized starting positions, Gasteiger charges, torsions have been evaluated for ligand molecules. The genetic algorithm population was used as 150 while applying Lamarkian genetic algorithms (Trott and Olson, 2010; Holt et al 2008).

organik madde içerikleri bakımından sıralamanın KT>OT-1>OT-3 şeklinde ve % 4.40>4.28>3.84 düzeyinde olduğu, organik tarım topraklarının orta seviyenin altında (<% 3) daha fazla oranda dağılım gösterdiği saptanmıştır (Çizelge 1, 2).

3. Result and Discussion

Structural properties of inorganic complexes provide useful information on reactivity. For example, the Highest Occupied Molecular Orbital (HOMO) of a molecule shows the active sites of electron-donor reactions; the Lowest Unoccupied Molecular Orbital (LUMO) indicates on which region the molecules accept electrons. In addition, calculated orbital energies that made with the same basis sets provide useful information about the electron donor/acceptor capacities of the molecules. In both of the molecules analyzed in this study, the HOMOs are located on the benzylic area. Therefore, the reactions in which the molecules act as a Lewis base will occur on benzyl part. Also, the molecules will accept electrons through benzimidazole since LUMO orbitals of both molecules are located on this residue.

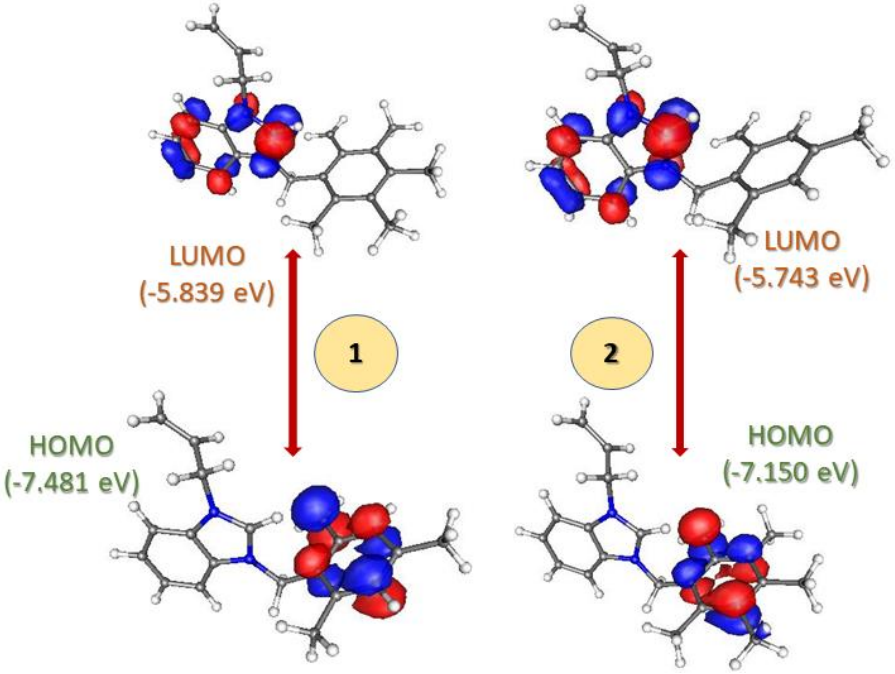

Figure 2. HOMO and LUMO orbitals of the **1** and **2**

It is common to obtain an idea about the reactivity with the relative energies of molecular orbitals. For example, it can be said that 1 with a lower calculated LUMO energy will be stronger electron-acceptor. However, the global reactivity descriptors that is developed by Koopmann are used to interpret the reactivity of molecules (Kaya et al 2016; Morell et al 2005). The ionization potential, electron affinity and electronegativity involve the direct evaluation of HOMO-LUMO energy gap of the molecules (Serdaroğlu and Şahin, 2019). The chemical hardness and softness can be evaluated as a measurement of the stability of molecules (Vijayaraj et al 2009). The softer molecule is embraced as more reactive. On the other hand, electrophilicity index is considered as a criterion of a molecule's activity against a nucleophile (Parthasarathi et al 2004). According to all the criteria, it could be confirmed that 1 is more reactive than 2.

Table 1. The calculated quantum chemical parameters* for the lower energy conformers each of **1** and **2** (in eV)

	IP	***EA***	***χ***	***η***	***δ***	***ω***
1	7.481	5.839	6.660	0.821	0.609	27.013
2	7.150	5.743	6.446	0.703	0.711	29.543

*IP: Ionization Potential ($-E_{HOMO}$); EA: Electron Affinity ($-E_{LUMO}$); χ: Electronegativity; η: Chemical Softness; δ: Chemical Hardness; ω: Electrophilicity Index

Table 2. Molecular Docking Results of **1** and **2** in the active site of certain receptors.

Compound	Bind. Aff.*	Amino Acids Residue
		1ywn
1	-4.51	Leu1033, Ala864, Val897, Cys917, Cys1043, Leu838, Phe916, Glu915, Val914, Asn921, Asp1044
2	-5.07	Leu1033, Ala864, Val897, Cys917, Arg1030, Cys1043, Val846, Leu838, Val914, Glu915, Phe916, Leu887, Gly920, Asn921, Glu883, Asn1031, Asp1044
		4cbq
1	-8.12	Thr117, Asn251, Ala38, Val41, Phe254, Ile10, Gly11, Ser12, Gly13, Pro14, Tyr33, Glu34, Gly35, Gly45, Gln46, Ile88, Thr87, Gly118, Ala119, Thr120
2	-8.18	Thr117, Asn251, Ile10, Ala38, Val41, Phe254, Gly11, Ser12, Gly13, Tyr33, Gly35, Glu34, Gly45, Gln46, Thr87, Ile88, Ala116, Gly118, Ala119, Thr120

* Binding Affinity in kcal/mol.

Molecular docking analysis has been recently accepted as an essential tool in drug design research (Ferreira et al 2015; Krovat et al 2005). This method provides useful data about the possible bioactivities. In addition, this method could give predictions on activities that cannot be analyzed by experimental procedures. With molecular docking method, it is possible to have an idea about not only the active sites of the studied macromolecules and also the magnitude of interaction with numerical results such as binding energy. In this study, interactions of both NHCs related to their anti-cancer activities with VEGFR-2 and TrxR crystal structures were investigated.

1 and **2** were interacted with approximately the same region of the VEGFR-2 crystal structure that involved Leu1033, Val897, Glu915 and Asp1044. The molecules preferred the alkyl/π-alkyl and Van der Waals interactions with the VEGFR-2 crystal. Both molecules have π-σ interaction with Leu1033 and also alkyl/π-alkyl interaction with Ala864, Val897, Cys917, Arg1030 and Cys1043. The details of the Van der Waals interactions of both molecules can be analyzed in Figure 3. The binding energies of all interactions were calculated -4.51 kcal/mol for **1** and -5.07 kcal/mol for **2** and these results can be interpreted as **2** has a stronger interaction with VEGFR-2.

The interactions of both molecules with TrxR are stronger than that of VEGFR-2 since the interactions with TrxR involved H-bonds with Thr117 and Asn251. **1** and **2** were interacted with approximately the same region of the TrxR crystal structure. As the details could be examined in Figure 4, **1** do not have any interaction except alkyl/π-alkyl and Van der Waals, but **2** have π-σ interaction with Ile10. The binding energy of **1** were calculated as -8.12 kcal/mol while it is calculated as -8.18 kcal/mol for **2**.

The cytotoxicity of **1** and **2** in DU-145 human prostate cancer cells, MCF-7, MDA-MB-231 breast cancer cells, and L-929 normal adipose cells were previously analyzed by MTT assay (Şahin et al 2019). The cytotoxicities of the molecules were indicated that these molecules have potent activity against the three cancer cell lines studied, and the efficacy of **2** is much higher than that of **1**. The experimental results are compatible with the molecular docking results of both VEGFR-2 and TrxR crystal structures.

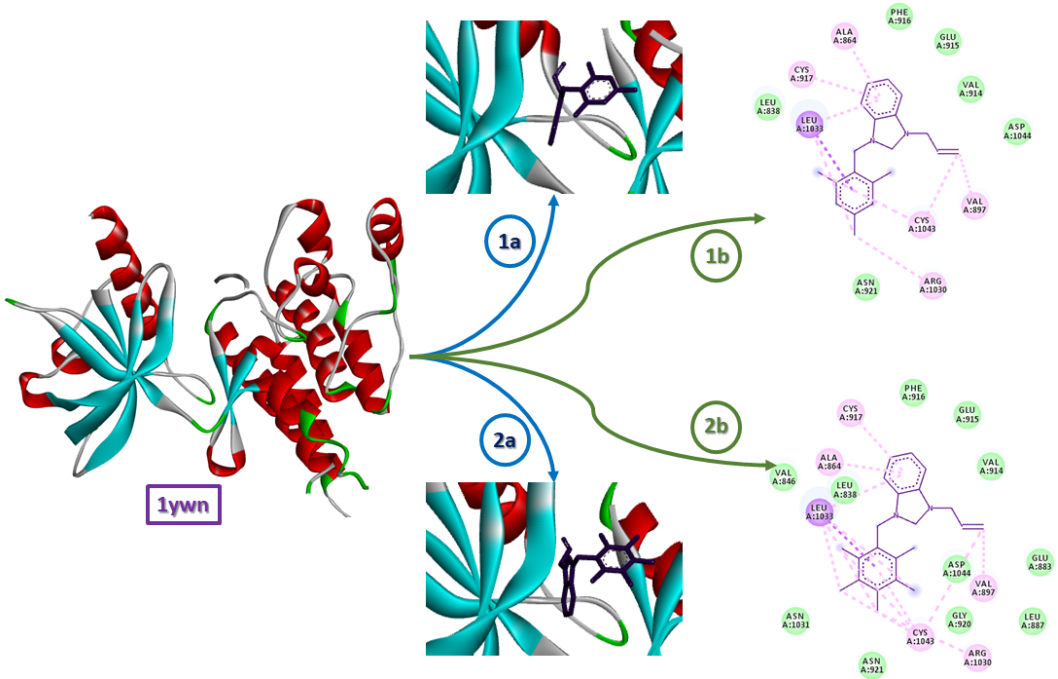

Figure 3. Molecular docking interaction of the molecules with VEGFR-2

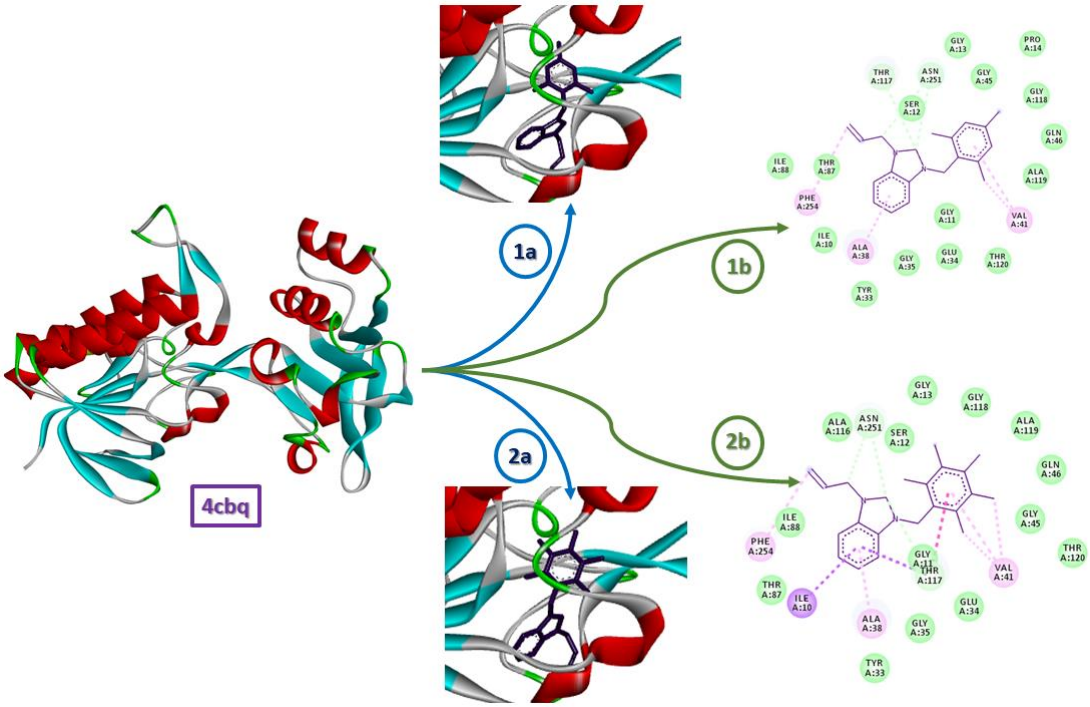

Figure 4. Molecular docking interaction of the molecules with TrxR

4. Conclusion

The anti-cancer activities have been performed for many candidate molecules since cancer is the second mortal disease all over the world. NHC-type molecules also have been analyzed for possible anti-cancer activity. It is not possible that the evaluation for anti-cancer activities of all candidate molecules because of the difficulties/constrictions of experimental anti-cancer procedures. Then, in-silico methods could be an alternative for analyzing the

bioactivities. The anti-cancer activity of the molecules that analyzed in this study were evaluated for Vascular Endothelial Growth Factor Receptor-2 and Thioredoxin Reductase crystal structures by molecular docking methods. Also, DFT-based calculation methods were used for structural analysis. All calculated results shows that anti-cancer activity of **2** could be better than **1**. Since this kind of in-silico methods have many options and constraints, the calculations of this kind of molecules must be improved for determining the best anti-cancer agent.

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