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Molecular docking studies of N-Heterocyclic Carbene molecules with Thioredoxin Reductase and DNA

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Abstract

Thioredoxin which is induced by thioredoxin reductase causes the proliferation of cancerous cells and metastasis due to its effects on cell growth, besides its regulatory effects on the amount of reactive oxygen species. One of the procedures recently used in cancer treatment is thioredoxin reductase inhibition. Different types of bioactivities of NHC and metal-NHC complexes have been studied and anti-cancer is one of these activities. In addition to in-vitro anticancer activity, molecular docking methods are also one of the important methods used in drug design. This method achieves foresight about future studies and the mechanisms that are difficult to analyze experimentally. In this study, previously synthesized and characterized [1-(2-methyl-2-propenyl)-3-(4-methylbenzyl) benzimidazolium]$^+$ (1a) and [1-(2-methyl-2-propenyl)-3-(4-isopropylbenzyl) benzimidazolium]$^+$ (1b) molecules and their Ag(I)-NHC complexes (2a and 2b) were investigated using molecular docking method for thioredoxin reductase. In addition, the interaction of these molecules with DNA was evaluated. 2b has the best binding energy of -8.95 kcal/mol with the region that comprised Ile10, Phe254, Ala38, Val41 of thioredoxin reductase. Also, ligands interacted with Cyt11, Gua10, Cyt9, and Thy8 while complexes interacted with Ade5, Ade6, Thy7, and Thy8 part of DNA.

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1. Introduction

Cancer is one of the highly fatal diseases in the world [1]. One of the most important causes of cancer is oxidative stress [2]. It is known that cellular metabolisms produce Reactive Oxygen Species (ROS). The amount of ROS is important in many cellular processes such as gene expression and cell proliferation [3]. High ROS level lead degradation in some components such as proteins, lipids, nucleic acids, and even cell death in later stages. The formation of ROS and their scavenging by intracellular antioxidant systems must be in balanced for healthy cells. In cancerous cells, a ROS level increment is observed due to abrupt proliferation and high metabolic rate [4]. The conditions in which the ROS level increases dramatically is called oxidative stress [5].

The Thioredoxin (Trx) system contains the redox-active protein Thioredoxin, Thioredoxin Reductase (TrxR) enzyme, and NADPH [6]. The Trx system plays an important role in many cellular functions such as redox control of transcription factors, synthesis of deoxyribonucleotides, cell growth, and protection against oxidative stress [7]. Trxs are small proteins conserved in all species, from bacteria to humans. All Trxs contain redox-active sites that reduce disulfides in proteins and peptides [8]. Reduced Trx catalyzes the reduction of ROS in many intracellular and extracellular proteins and oxidizes in this process. TrxR is required for oxidation of Trx [9]. Since cancer cells generally lead to high oxidative stress, the expression of antioxidant proteins such as Trx is increased [10]. It has been noted that Trx expression increases in many cancer types such as lung, pancreatic, colorectal, and breast cancer [11]. However, overexpression of thioredoxin due to oxidative stress gives rise to the growth of cancer cells, metastasis, and resistance to chemotherapeutic agents [12]. So Trx can be considered a potential target in cancer and the inhibition of TrxR could be a good strategy for treatment. Because Trx system can be regulated by the inhibition of TrxR, some synthetic TrxR inhibitors have reached the clinical test stage while some of them have received FDA approval for cancer treatment. For example, arsenic trioxide is used in the treatment of leukemia. This molecule irreversibly inhibits TrxR, and it is also recorded that

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this inhibition is efficient in MCF-7 breast cancer [13]. Curcumin is a recent well-known anti-cancer agent, and it has been determined that the detected activity of this molecule is emerged from TrxR inhibition [14]. Further studies are still needed to resolve the effects of the thioredoxin system and TrxR inhibition in cancer treatment. The studies must be focused on both elucidating the thioredoxin system and synthesizing more effective TrxR inhibitors [15].

NHCs are known as easily synthesized and modified molecules [16]. These molecules which are known for their catalytic activity have remarkable results in many bioactivity researches [17]. Many metal-NHC complexes have been synthesized and analyzed after the usage of metal complexes in treatment procedures [18]. Besides the many known activities of metal-NHC complexes, good results have been obtained from anticancer studies especially for Au-NHC and also Ag-NHC [19,20]. Therefore, evaluation of the interaction of Ag(I)-NHC complexes with TrxR for analysis of the anticancer activity seems as a reasonable strategy. In this study, previously synthesized and characterized [1-(2-methyl-2-propenyl)-3-(4-methylbenzyl)benzimidazolium]$^+$ (**1a**) and [1-(2-methyl-2-propenyl)-3-(4-isopropylbenzyl) benzimidazolium]$^+$ (**1b**) molecules [21] and their Ag(I) complexes (**2a** and **2b**) (Figure 1) were investigated using molecular docking method for TrxR. In addition, the interaction of these molecules with DNA was evaluated.

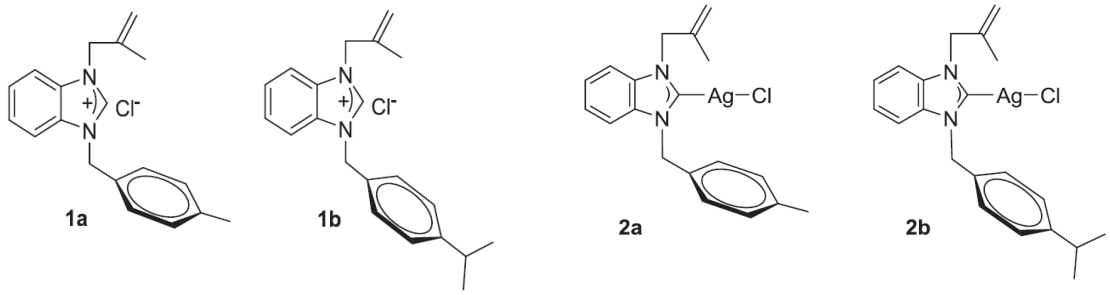


Figure 1. Alkyl substituted allyl benzimidazolium molecules and their Ag(I)-NHC complexes

2. DFT Optimization and Molecular Docking Method

Before the molecular docking process, molecules are optimized with ORCA package program. ORCA version 2.8 using the BP86 functional were used for DFT-based calculations, with a def2-SVP def2-SVP/j basis set for ligand and complexes. Also, grid4, and tightscf options were used for geometry optimizations [22,23]. Molecular docking was performed using AutoDock 4.2. with the crystal structure of thioredoxin reductase (PDB id: 4CBQ) [24] and DNA dodecamer (PDB id: 1BNA) [25] from RCSB protein data bank [26]. Water in the proteins was removed and polar hydrogen atoms and Kollman charges were evaluated for target molecules in the docking process. Gasteiger charges, randomized starting positions, optimizations, and torsions have been evaluated for ligand molecules. The genetic algorithm population was used as 150 while applying Lamarkian genetic algorithms [27]. The spacing values of the **1a** and **2a** were used as 0.375 and npts are 68-58-54 against for TrxR docking. Additionally, the molecular dockings were performed with 0.375 spacing and 46-46-54 npts values for **2a** and **2b**. In DNA docking performance all the crystal structure were scanned.

3. Results and Discussion

In a healthy person, the body has a perfect balance, and the disease could actually be considered as an imbalance in the body. The type of imbalance determines the type of the disease. The body has mechanisms that work to reconstruct the balance [28-30]. Cancer is still one of the most fatal diseases in the world. The most important cause of cancer is the imperfection of the balance of ROS in the body [31]. The increasing amount of ROS leads to decay of basic components such as lipids, proteins. Various mechanisms are induced in order to rearrange the deteriorated ROS balance. Trx system is one of the systems that works for the regulation of ROS balance as well as having important functions in many processes such as cell growth and synthesis of deoxyribonucleotides [32]. Cancer increases ROS levels in the body and Trx system is induced. This causes overexpression of Trx, and this overexpression

also causes cancer cell growth, metastasis, and resistance to the chemotherapeutic agent [33]. Trx system consists of Trx, TrxR, and NADPH and TrxR induces Trx. Inhibition of TrxR for restriction of Trx over-expression is considered as a possible cancer treatment procedure.

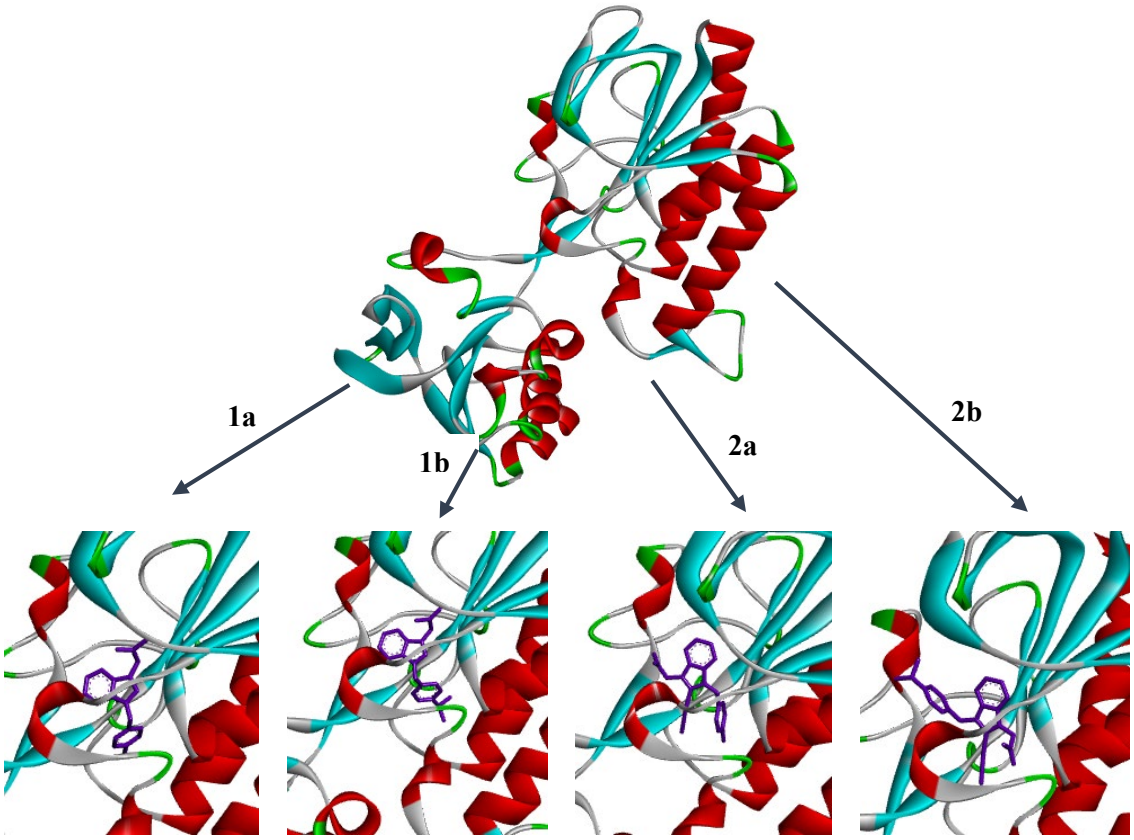


Figure 2. Ribbon style crystallographic structure of thioredoxin reductase (upper). Docked residues of the molecules in thioredoxin reductase (down)

The basic principle in standard in vitro anticancer studies is to examine the effect of molecules on specific cancer cells [34,35]. It is clear that the in vitro analysis of the effects is important. However, it is very difficult (sometimes impossible) to examine the mechanism of action of molecules in treatment. Therefore, molecular docking methods have recently become an essential tool in activity and drug design studies. Many experimental studies are supported by molecular docking studies with suitable target molecules [36-38]. In this study, the interactions of NHC ligands and their Ag(I) complexes were investigated by molecular docking with TrxR (Figure 2 and Figure 3) and DNA (Figure 4).

1a interacted with the region which comprised Ala38, Ile88, Ile10, Gly35, Glu34, Thr117, Gly11, Ser12, and Val41 in TrxR with -7.13 kcal/mol binding energy. Van der Waals interactions occur most intensely, and amino acids in these interactions can be analyzed in Figure 3. The molecule made alkyl and pi-alkyl interactions with Ile88, Ile10, Val41, and Ala38. Ala38 also shows pi-sigma interactions with the conjugate electrons of the benzimidazole ring of the molecule. Carbon hydrogen bonding with Glu34 and Ser12 also contribute to the binding energy. 2a, the Ag(I) complex of 1a, has a binding energy of -7.37 kcal/mol with approximately the same region of TrxR. Van der Waals interactions are more remarkable in 2a, like 1a. Pi-sigma interaction with Thr117, and the alkyl/pi-alkyl interactions with Val41, Phe254, and Ala38 also are also noteworthy. 1b was docked in a region where Ala38, Il10, Ile88, Pro14, Thr117 were located in TrxR. Pi-sigma interaction between Ala38 and benzimidazole, alkyl interactions with Ile10, Ile88, and Pro14, and many van der Waals interactions contribute to the binding energy of -8.58 kcal/mol. 2b, the Ag(I) complex of 1b, was docked with a binding energy of -8.95 kcal/mol to approximately the same region as the

other molecules. In this molecule, van der Waals interactions are conspicuous, and the amino acids that make up these interactions can be studied in Figure 3. Pi-sigma interaction with Ile10 and the alkyl/pi-alkyl interactions with Phe254, Ala38, Val41 are also noteworthy.

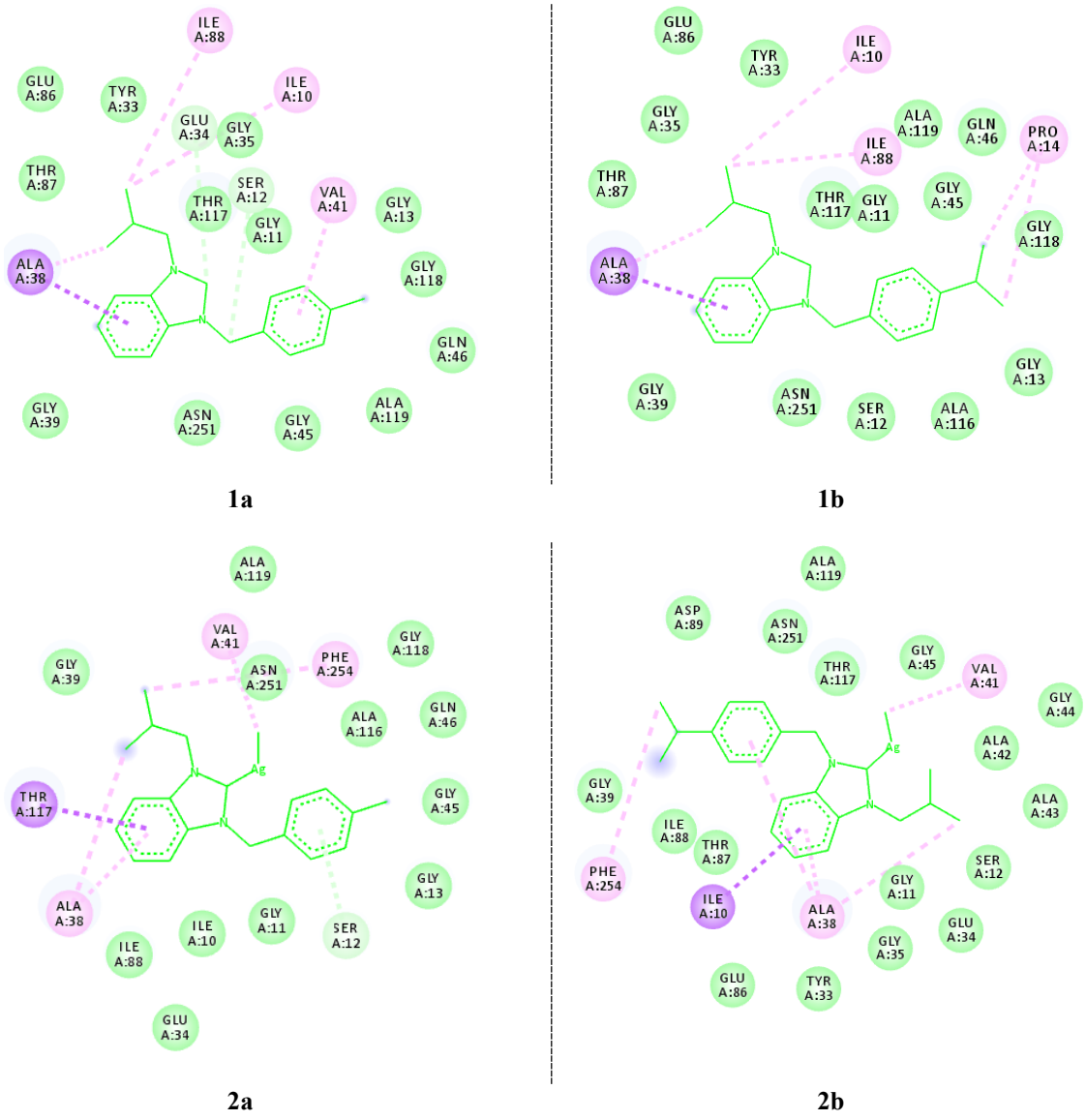
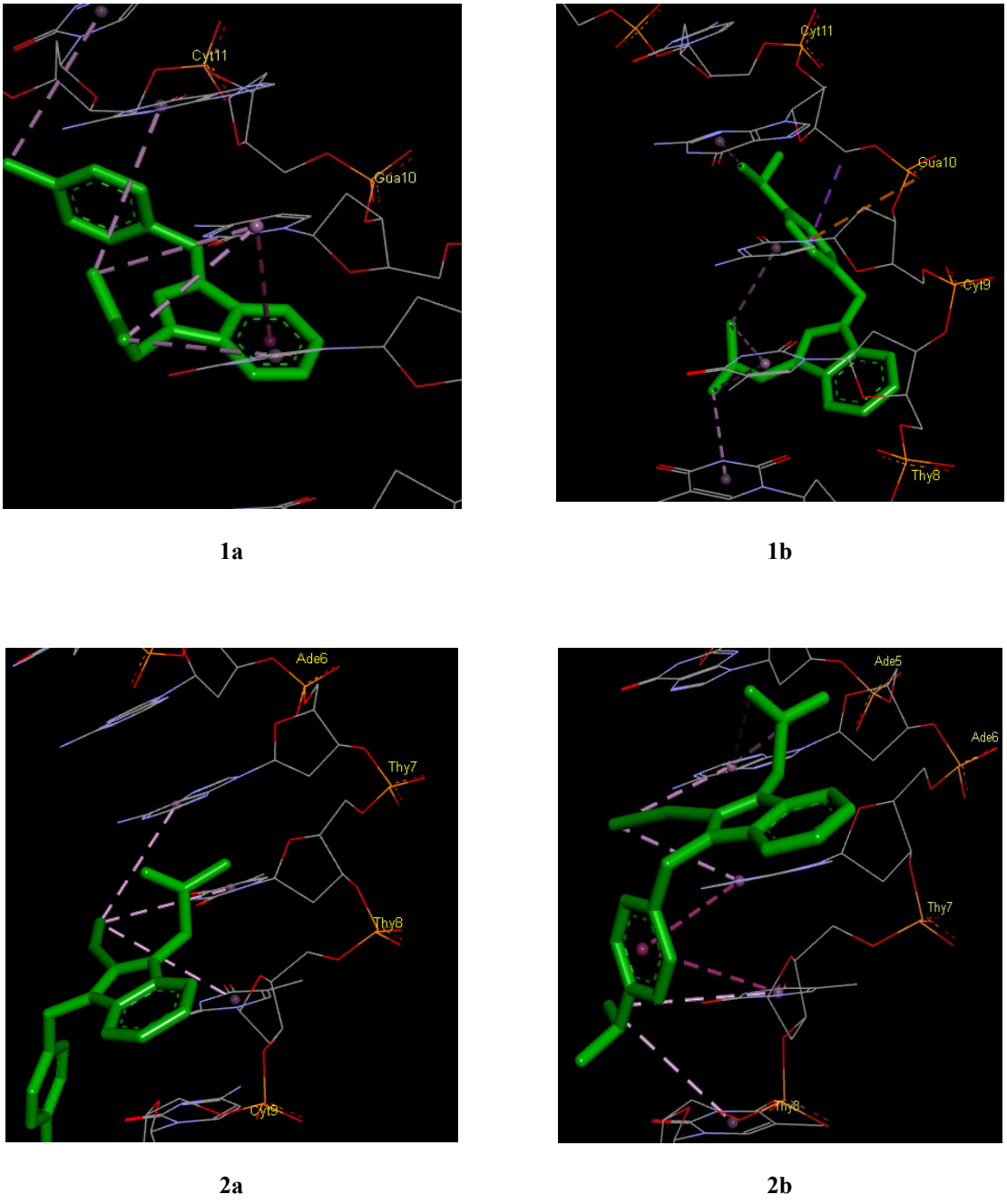


Figure 3. Graphical illustration of the interactions between TrxR and the molecules

In previous anti-cancer studies of the molecules [21] analyzed in this study, it has been determined that complex molecules have higher anti-cancer activity than ligand molecules. In addition, it had been stated that the isopropyl substituted molecule had higher anti-cancer activity than the methyl-substituted molecule. In molecular docking studies, the binding energy is accepted as a suitable criterion for comparing the activity. Accordingly, the binding energies obtained within this study are in accordance with the experimental results.

It is important to get information about the interaction between molecules and DNA in many activity studies, especially in anti-cancer studies. The obtained results will also be important in new drug design studies. For this reason, the interactions of the molecules in this study with DNA were made by using DNA dodecamer (pdb id: 1BNA). **1a** and **1b** ligands interacted with Cyt11, Gua10, Cyt9, and Thy8 (Figure 4). However, **2a** and **2b** complexes interacted with Ade5, Ade6, Thy7, and Thy8. The interactions are mostly pi-pi (pink) and pi-alkyl (purple) interactions (Figure 4).



1a **1b**

2a **2b**

Figure 4. Graphical illustration of the interactions between DNA dodecamer and the molecules

4. Conclusion

New developments have been provided in cancer research in every day. Since the disease is wide in terms of diversity and mechanism of action, unfortunately, the studies have not been able to catch up with the pace of the disease. In addition to in vitro anti-cancer studies, all kinds of methods to understand the mechanism of action are important in these studies. The Trx system is one of the mechanisms examined in recent cancer studies. The studies about the synthesis and analysis of molecules for restricting Trx expression by TrxR inhibition are continuing. However, due to the difficulty of conducting these studies, it is reasonable to carry out these studies in silico. In silico studies both give an idea about the mechanism of action of the molecule and provide foresight in designing new studies. In this study, the interactions of molecules with thioredoxin reductase were examined and the binding energy results obtained with TrxR agree with the experimental results, but it is clear that much more studies must be done. In the next

studies, it is planned to diversify the studies with different substituted ligands and molecules.

Conflicts of interest

There is no conflict of interest between the authors.

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