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Molecular docking analysis of N-heterocyclic carbene and silver N-heterocyclic carbene complexes with thioredoxin reductase

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Molecular docking is a frequently used method in computer-supported drug design studies. It is possible to obtain useful information about the magnitude and character of the interaction between known targets and bioactive molecules thanks to docking methods. Besides the well-known antibacterial activity of NHC molecules and their Ag-complexes, anti-cancer, and anti-proliferative activity of them have been studied frequently. In anti-cancer studies, the inhibition effects of drug-candidate molecules on the growth and proliferation of cancer cell are generally examined. In this study, thioredoxin reductase was selected as a cancer target molecule and interaction of 1-allyl-3-benzylbenzimidazolium, 1-allyl-3-(naphthylmethyl)-benzimidazolium, 1-allyl-3-(anthracen-9-yl-methyl)benzimidazolium, Cl[1-allyl-3-benzylbenzimidazolium-2-ylidene]Ag(I), Cl[1-allyl-3-(naphthylmethyl)benzimidazolium-2-ylidene]Ag(I), Cl[1-allyl-3-(anthracen-9-yl-methyl)benzimidazolium-2-ylidene]Ag(I) with thioredoxin reductase were analyzed by molecular docking methods.

Keywords: NHCs, Ag-NHC complexes, thioredoxin reductase, molecular docking.

Introduction

Metabolically active cells cause free radicals and reactive oxygen species (ROS) during many intracellular processes such as oxygen metabolism, inflammation, and also diseases[1]. Free radicals also cause the formation of hydrogen peroxide and organic hydroperoxide in the presence of O_2. These ROS induce oxidative damage of biomolecules such as DNA, protein and lipids[2]. Although there are many protective mechanisms that fight against ROS and free radicals, these mechanisms could be insufficient. When the amount of ROS is higher than the intracellular antioxidant capacity, oxidative stress occurs in the tissue. When a cell senses oxidative stress, prevention measures are taken by the body, such as reducing ROS production, increasing metabolic antioxidant capacity, or activating signal pathways required for repairing the biomolecule[3]. One of these response mechanisms for regulating intracellular stress is to activate or inactivate redox-sensitive factors. NADPH activates thioredoxin peroxidase/thioredoxin reductase signal cascades against oxidative stress. Thioredoxin reductase is involved in many intracellular processes such as induction of cell growth, oxidative stress and apoptosis[4]. Therefore, overexpression of thioredoxin reductase is considered to have effects on many types of breast cancer, thyroid, and prostate cancer. It has also been noted that increasing amounts of thioredoxin reductase have action in colorectal tumors compared to normal cells[5]. Due to above mentioned facts, thioredoxin reductase has the potential to be analyzed as a cancer agent.

N-heterocyclic carbenes (NHCs) are electron-rich and neutral sigma-donor ligands. In addition to their well-known catalytic activities, biological activities of NHCs are among the important research areas in organometallic chemistry[6]. The antibacterial activity of ruthenium- and rhodium-complexes, which are considered as the first examples of metal-NHC complexes, has been determined. Since the use of metal-based drugs in cancer and infectious diseases, many metal-NHC complexes have become the subject of bioactivity researches[7]. Au-NHC type complex has been reported as the pioneering metal-NHC anti-cancer molecule. Although Ag-NHC complexes have been generally studied for anti-infective activity, there are many studies for investigating their

cytotoxic effects against cancer cells[8]. For the silver complexes, the results are close to the gold- and platinum-NHC complexes and comparable with the established cytotoxic "5-fluorouracil"[9].

Molecular docking can be considered as a key tool in structural molecular biology and computer-assisted drug-design studies[10]. Ligand-protein docks give useful results for predicting predominant binding modes. In this study, the interaction of 1-allyl-3-benzylbenzimidazolium [**1a**], 1-allyl-3-(naphthylmethyl)benzimidazolium [**1b**], 1-allyl-3-(anthracen-9-yl-methyl)benzimidazolium [**1c**], Cl[1-allyl-3-benzylbenzimidazolium-2-ylidene]Ag(I) [**2a**], Cl[1-allyl-3-(naphthylmethyl)benzimidazolium-2-ylidene]Ag(I) [**2b**], Cl[1-allyl-3-(anthracen-9-yl-methyl)benzimidazolium-2-ylidene]Ag(I) [**2c**] molecules for which synthesis and characterization studies have been completed and anti-proliferative effects have been investigated, with thioredoxin reductase were analyzed by molecular docking methods.

Results and discussion

Molecular docking was performed for *in silico* determination of the interactions between NHC derivative ligands and their silver complexes. The best conformations with optimum binding energy are shown in Fig. 1. Different types of inter-

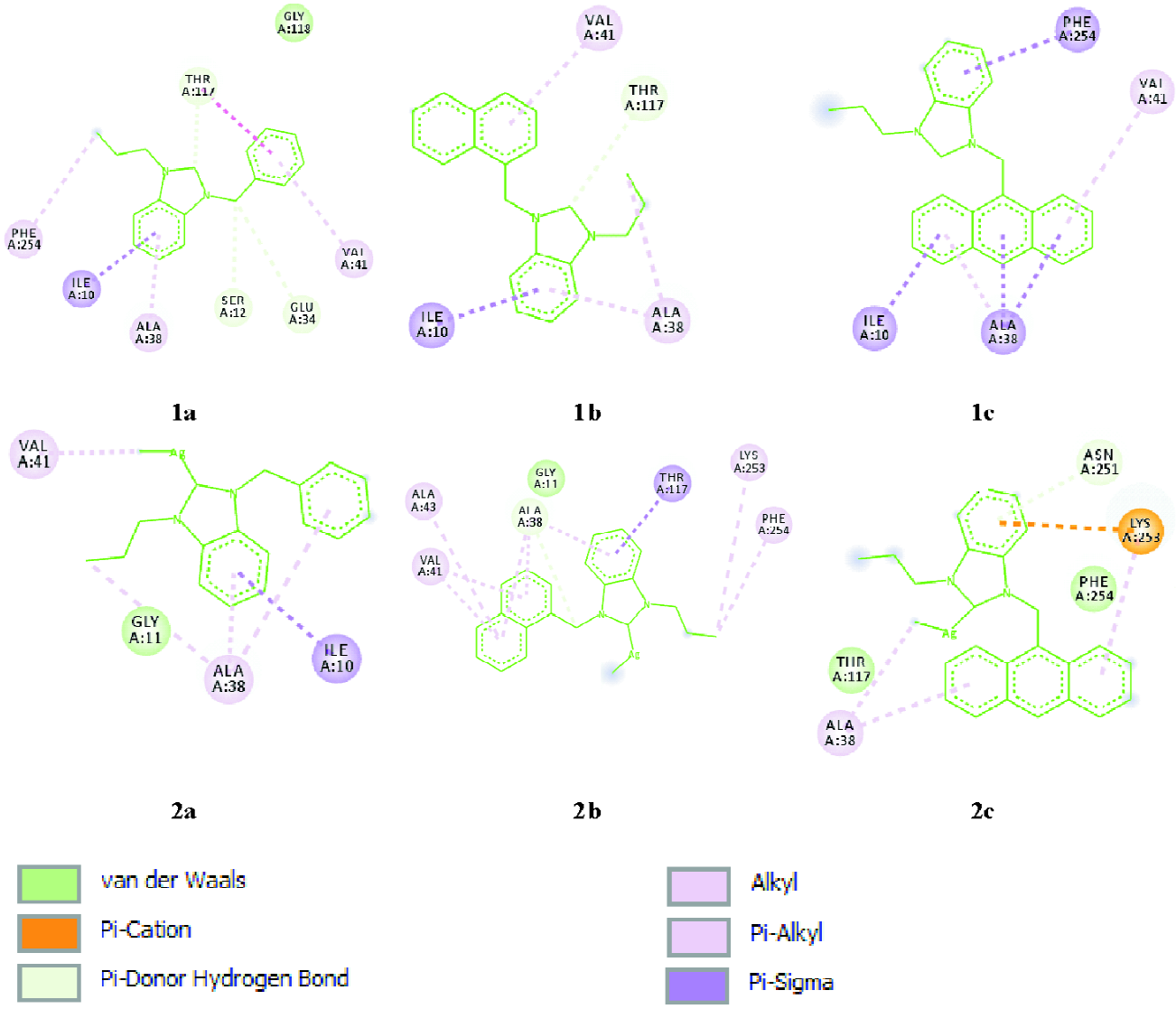

Fig. 1. Most important interactions of molecules with thioredoxin reductase.

actions have been detected between molecules and thioredoxine reductase target molecule. The interaction of **1a** molecule with a binding energy of 7.91 kcal/mol was determined with the region formed by Ile10, Ser12, Glu34, Ala38 Val41, Thr117 and Phe254 amino acids. Among these interactions, the most effective one is a pi-donor hydrogen bond between Thr117 and the NCHN part of the benzimidazole region of the molecule. Furthermore, the pi-donor hydrogen bonding between Ser12 and Glu34 and benzyl-benzimidazole linkage was determined. The pi-sigma interactions, which are observed in all molecules, due to the conjugated pi-electrons of the benzene ring in the benzimidazole region of the ligand, occurred with Ile10 in this molecule. The alkyl and pi-alkyl interactions between the **1a** molecule and Ala38, Val41 and Phe254 are also noteworthy. **1b** has weaker interactions with the same region of the enzyme compared to **1a** with 9.0 kcal/mol binding energy. Only one pi-donor hydrogen bond with Thr117 was observed for **1b**. In addition, pi-sigma interaction with Ile10 and pi-alkyl interactions with Val41 and Ala38 were detected in **1b** molecule. Hydrogen bond was not recorded for **1c** probably due to the large anthracene molecule which may be hindering the bonding. However, the binding energy of 8.79 kcal/mol was determined due to the pi-sigma interactions with Ile10, Ala38 and Phe254, which occurred more than the other molecules. In experimental studies, the lowest activity was determined for **1b** molecule which is agreement with the molecular docking results. The experimental anticancer activities of complex molecules were higher than the ligands, and more effective interactions were displayed with molecular docking for complex molecules as expected. Since the experimental antiproliferative activity does not change regularly depending on cell type and time, it is difficult to make a comparison with molecular docking results. **2a** has interactions in the area formed by Ile10, Gly11, Ala38 and Val41 amino acids with a binding energy of 7.71 kcal/mol. The reason for not having an effective hydrogen bond could be that the NCHN region of benzimidazole, which is active for H-bond in ligands, is closed with Ag-C bond in these complexes. The pi-sigma interactions observed with Ile10 in all ligands is also effective for the complexes. **2b** has the lowest binding energy among the complexes with 7.53 kcal/mol. The pi-donor H-bond with Ala38 and also pi-alkyl interactions with Ala38, Val41, Ala43, Lys253 and Phe254 are effective. The pi-sigma interaction of Thr117 contributes to the binding energy. Finally, it can be said that **2c** interacts with approximately the same site of the target molecule. It also has the highest binding energy with 7.89 kcal/mol among the complexes. The two most remarkable interactions of the molecule are the pi-donor H-bond with Asn251 and the pi-cation interaction with Lys 253.

Calculation and docking method

Molecular docking was performed using AutoDock 4.2. with crystal structure of thioredoxin reductase from RCSB protein data bank (PDB code: 4CBQ). Water in the proteins were removed and polar hydrogen atoms and Kollman charges were evaluated for target molecules in the docking process. Gasteiger charges[11], 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[12].

Conclusions

Thioredoxin reductase can be used as a target molecule in anti-cancer studies. Examining the interactions of this enzyme with molecular docking methods, which have recently been considered as an important tool for drug design studies *in silico*, can provide useful information. Determined interactions of thioredoxin reductase with NHC ligands and their Ag complexes is consistent with experimental results. In this study, how the molecules interact to what extent and in which region of the thioredoxin reductase was examined. Forthcoming studies are expected to be important in obtaining useful information on the activity of both NHC molecules and Ag-NHC complexes.

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