Investigation of the anticancer activity of 2-amino-6-methylbenzothiazole and corresponding Pd(II) complex using molecular docking simulations

Sandra S. Jovičić Milić[1*], Marko Antonijević[1], Đorđe S. Petrović[2], Verica V. Jevtić[2], Danijela Lj. Stojković[1]

1 University of Kragujevac, Institute for Information Technologies, Department of Science, Kragujevac, Jovana Cvijića bb, 34000, Kragujevac; e-mail: sandra.jovicic@pmf.kg.ac.rs, mantonijevic@uni.kg.ac.rs, danijela.stojkovic@kg.ac.rs

2 University of Kragujevac, Faculty of Science, Radoja Domanovića 12, 34000 Kragujevac, Republic of Serbia; e-mail: djordje.petrovic@pmf.kg.ac.rs, verica.jevtic@pmf.kg.ac.rs

* *Corresponding author*

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Abstract: In our prior investigations, it has been established that compound di(2-amino-6-methylbenzothiazole)dichloridopalladate(II) (**C1**) exhibits promising efficacy in inhibiting the growth of colon carcinoma, thereby demonstrating potential as an anticancer agent. To elucidate the underlying mechanism of action against cancer, a comprehensive investigation involving DNA binding analysis and a series of assays to evaluate the inhibitory potential of compound **C1** against key proteins involved in cancer metabolism were conducted. The significant inhibitory potential of **C1** towards Bcl-2, Ki-67, and CDK-4 was determined. In order to investigate the underlying mechanism behind the anticancer properties and to assess the inhibition of various proteins involved in different metabolic pathways of **C1**, molecular docking simulations were conducted. The investigation revealed that the observed lack of similarity between the experimental outcomes and the inhibition of Bcl-2 and CDK-4 by **C1** and 2-amino-6-methylbenzothiazole (**L1**) suggests that the metabolic pathways involving these proteins do not contribute to the anticancer properties of **C1**. The observed correlation between the inhibition of Ki-67 and the experimental outcomes was found to be significant. The inhibition of Ki-67 in cell cycle regulation is a promising approach to the development of anticancer drugs. Further research is required to explore the potential application of **C1** as a Ki-67 inhibitor.

Keywords: Bcl-2, Ki-67, CDK-4, Anticancer agents, Pd(II) complexes

1. Introduction

Thiazoles, as a biologically significant class of compounds, have become very interesting for investigation of their anticancer potential. They represent heterocyclic compounds containing both nitrogen and sulfur atoms as part of an aromatic ring. Because of their structure, derivatives of thiazole are able to bind with transition metals (soft Lewis

acids) such as palladium(II) ion. The thiazole ring is an integral part of penicillin and vitamin B1-thiamine, as well as a large number of already used drugs. Some of them are, ritonavir (medicine for HIV/AIDS) [1], tiazofurin (antitumor effect) [2], bacitracin (antibiotic effect) [3] and many others.

The aim of this paper is to present the results of molecular docking simulations of 2-amino-6-methylbenzothiazole (**L1**) and previously synthesized palladium(II) complex (**C1**) [5] regarding the inhibitory potential towards Bcl-2, Ki-67, and CDK-4. All three examined proteins are important parts of the cell cycle in tumor cells. Ki-67 is an important cancer biomarker, but, according to recent papers, it also has a significant role in cell cycle regulation [4].

2. Methodology

According to the aforementioned paper [5] di(2-amino-6-methylbenzothiazole)dichloroidopalladate(II) (**C1**) is expressing its anticancer activity towards colorectal carcinoma by inducing apoptosis. According to the Flow cytometry method, **C1** induced apoptosis via Bcl-2 (B-cell lymphoma 2) inhibition. It was also concluded that **C1** decelerated proliferation of cancer cells by decreasing Cyclin-D, and growth by inhibiting Ki-67. To further investigate the binding potential of 2-amino-6-methylbenzothiazole **L1** and **C1** towards the aforementioned proteins molecular docking studies were performed. Structures of investigated proteins were obtained through RCSB Protein Data Bank with 1G5M (Bcl-2), 1R21 (Ki-67), and 2W96 (Cyclin-depended kinase-4). Structures of investigated compounds were obtained through the optimization via the Gaussian 16 software package, while molecular docking simulations were done by AMDock platform implementing AutoDock4.2, following the methodology given in our previous work [6].

3. Results and discussion

According to the results of our previous endeavors, [5] **C1** shows good binding potential when it comes to the binding to (circulating tumor DNA) CT-DNA molecules, which indicates that its inhibitory potential towards the specific carcinoma could come as a consequence of CT-DNA binding. However, another investigated complex - di(2-amino-6-chlorobenzothiazole)dichloridopalladate(II) (**C2**) showed better binding affinity towards CT-DNA and at the same time lower cytotoxic activity indicating the possibility of another mechanism of expressing cytotoxic potential. Because of that a series of mechanistic pathways included in cancer expression were investigated. It was determined that Caspase 3 is not involved in the mechanism of cytotoxic activity. However, changes in the expression of Bcl-2, Ki-67, and Cyclin D, were recorded. These proteins were selected according to their role in apoptosis protection (Bcl-2), proliferation (Ki-67), and cell cycle regulation from the G1 to S phase of mitosis (Cyclin D). All three proteins were found to be inhibited by **C1.** To further investigate

interactions with these proteins, molecular docking simulations were performed. Thermodynamic parameters describing these interactions are given in Table 1.

Table 1. Thermodynamic parameters describing interactions between Bcl-2, Ki-67, and Cyclin-depended kinase-4 (CDK-4) with **C1** and **L1.**

	Bcl-2	**Ki-67**	**CDK-4**
		C1	
ΔG_{bind} (kcal mol^{-1})	-6.45	-6.43	-5.89
k_i (μM)	18.71	22.53	48.15
		L1	
ΔG_{bind} (kcal mol^{-1})	-6.16	-5.09	-5.34
k_i (μM)	30.53	190.00	120.00

As can be seen from the molecular docking simulations, parameters describing inhibition of Bcl-2 and CDK-4 indicate similar anticancer potential of **C1** and **L1**, which is not in accordance with the experimental results. However, inhibition of Ki-67 follows the trend set by IC_{50} values (**C1**=12.34 ± 0.74 and **L1**= 389.45 ± 5.14) which is in correlation to binding energies (ΔG_{bind}) and inhibitory constants (k_i) obtained through molecular docking simulations. These findings indicate that out of three investigated proteins, inhibition of Ki-67 is the most probable mechanistic pathway of the anticancer activity of **C1**.

To further explain this phenomenon, the interactions between Ki-67 and **C1/L1** were investigated (Figure 1).

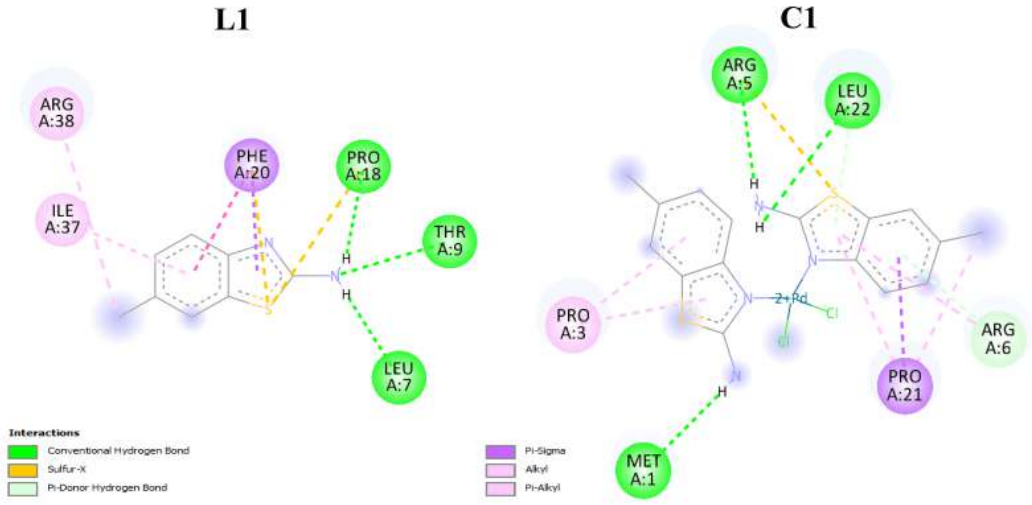

Figure 1. Interactions between Ki-67 and **L1** (left) and **C1** (right).

According to Figure 1, interactions between Ki-67 and **C1** and **L1**, and overall number of hydrogen bonds and other non-covalent interactions do not have an impact on the differences between binding energies, and consequently inhibitory constants, of **C1** and **L1.** However, the layout of the hydrogen bonds and other non-covalent interactions allows for the better stabilization of **C1** in comparison to **L1**, regardless of the higher voluminosity of the **C1**.

4. Conclusions

In our previous endeavors, we have determined that compound **C1** shows good anticancer activity toward colon carcinoma. To determine the mechanistic pathway for anticancer activity also performed the DNA binding analysis, as well as a series of essays investigating inhibitory potential of **C1** towards various proteins important for cancer metabolism. It was found that **C1** shows good inhibitory potential towards Bcl-2, Ki-67, and CDK-4. To further examine inhibition of which of these proteins (describing different metabolic routes) is responsible for the anticancer activity of **C1**, molecular docking simulations were performed. It was found that inhibition of Bcl-2 and CDK-4 by **C1** and **L1** shows no resemblance to the experimental results, indicating that metabolic routes containing these proteins were not responsible for the anticancer potential of **C1**. However, inhibition of Ki-67, showed a good correlation with the experimental results. Preventing the Ki-67 from being involved in cell cycle regulation is one of the novel tactics in the anticancer drug design and development and further investigations are needed for the application of **C1** as a Ki-67 inhibitor.

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