Inhibition Potency of Terpyridine Metal Complexes toward Penicillin-Binding Protein 1A

Svetlana Jeremić, Enisa Selimović, Milan Dekić, and Tanja Soldatović

***Abstract*—The potency of copper(II) and zinc(II) terpyridine complexes to inhibit penicillin-binding protein 1A (PBP1a) was investigated by *in silico* methods. The geometries of ligands are optimized using DFT calculations. In order to estimate the binding sites, inhibition constants and binding energies between ligands and PBP1a protein, molecular docking analysis is performed. The inhibition potency of examined terpyridine metal complexes is compared with the inhibition potency of lactivicin, an antibiotic already used in the treatment of Gram-negative and Gram-positive bacteria. Performed docking analysis indicated that investigated terpyridine metal complexes show higher inhibition potency toward PBP1a protein than lactivicin. The results identified these complexes as potential antimicrobial agents for further *in vitro* experiments.**

***Key words* — copper(II) terpyridine; zinc(II) terpyridine; penicillin-binding protein 1A; lactivicin; molecular docking analysis.**

I. Introduction

Antibiotics have transformed medicine by changing the outcome of bacterial infections. Since the discovery of penicillin by Sir Alexander Fleming in 1928, antibiotics extend expected life spans for almost 25 years in the USA and have had similar beneficial effects worldwide. Nevertheless, the emergence of resistant microorganisms endangering the efficacy of antibiotics - bacterial infections have again become a threat due to the resistance that has been seen to nearly all antibiotics that have been developed. This has been attributed to the overuse and misuse of antibiotics, as well as a lack of new drug development, urging to renew efforts in the research of new antimicrobials and investigation of their mechanism of action [1].

Epidemic antibiotic resistance has been described in numerous pathogens, including common respiratory pathogens such as *Streptococcus pneumoniae*. This pathogen causes pneumonia, otitis media, and sepsis, and has been responsible for over one million yearly deaths worldwide [2]. Presently, 25% of all invasive strains are resistant to penicillin, amoxicillin, and cephalosporins [3, 4].

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In the last few decades we have seen a dramatic increase in the number of β-lactam antibiotics and understanding of their mechanism in inhibition of peptidoglycan biosynthesis. Peptidoglycan component of bacterial cell wall consists of polymerized chains of repeating disaccharide subunits (N-acetylglucosamine and N-acetylmuramic acid cross-linked by stem pentapeptides), whose function is to provide cellular shape and maintain osmotic pressure [5]. Both the polymerization of disaccharide subunits and peptide cross-linking reactions are catalyzed by penicillin-binding proteins (PBPs), membrane-associated enzymes essential for cell division and daughter cell formation. PBPs possess the ability to covalently bind β-lactam antibiotics [6]. All enterobacteria appear to possess a similar spectrum of PBPs differing in molecular weight and affinity for β-lactams [7]. The high molecular-weight (HMW) PBPs (PBP1a/1b, PBP2, and PBP3) are physiologically important bifunctional enzymes that catalyze the final stages of peptidoglycan synthesis [6].

In Gram-positive bacteria, such as *S. pneumoniae*, the cell wall is composed mostly of peptidoglycan. *S. pneumoniae* has six PBPs, three of which are bifunctional - PBP1a, PBP2a, and PBP1b [8]. A PBP1a is essential for cell viability. This protein plays a key role in the formation of the cell septum during the bacterial division cycle and is involved in homologous DNA recombination mechanism, repair, and chromosome segregation [2]. To date, the mechanism used by bifunctional PBPs in the development of β-lactam resistance has remained unknown [2]. X-ray studies of soluble forms of pneumococcal PBP1a, both in apo (an inactive form, with no bounded ligand) as well as in antibiotic-bound forms, demonstrate that this protein contains three domains – the central transpeptidase domain flanked N-terminally by a GT/TP interdomain linker region, and C-terminally by a small, β-sheet rich unit. The active site of PBP1a is unusually narrow, suggesting that ligands must initially be threaded into the gorge to be recognized [2].

The search for effective inhibitors of PBP1a protein indicated that among the molecules with significant inhibitory affect there are different metal complexes of 2,2′:6′,2″-terpyridine molecule. The 2,2′:6′,2″-terpyridine (terpy) ligand is tridentate, nearly coplanar, N_3 donor ligand. It has been recognized as a useful ligand for transition metal and rare earth metal ions in inorganic chemistry. Square-planar, square-pyramidal and octahedral metal complexes with terpy ligands have been reported to be of great biological interest [9, 10, 11].

The biologically important metal ions copper(II) and zinc(II) have the ability to coordinate into different

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geometries depending on the specific arrangement of donor atoms in biomolecules, but strong π-acceptor ability of the tridentate chelate 2,2′:6′,2′′-terpyridine stabilizes the square-pyramidal geometry [10]. Different substituted terpyridene molecules generate complexes with copper(II) and zinc(II) ions, that exert anti-microbial, anti-bacterial, anti-fungal, anti-proliferative and inhibitory activity. For example, terpyridine complexes of Cu(II) and Zn(II), and especially complexes of Cu(TTP)Cl_2 and Zn(TTP)Cl_2 (TTP is 4 -[*p*-tolyl]- 2,2′:6′,2′′-terpyridine) are efficient inhibitors of furin. Furin, a human subtilisin-related proprotein convertase (SPC), is emerging as an important pharmaceutical target because it processes vital proteins of many aggressive pathogens. Inhibition is irreversible, competitive with substrate, and affected by substituents on the chelate. The free chelates are not inhibitors [12]. Complexes of terpyridine-based ligands with Zn(II) as a central metal ion has also shown significant inhibitory activity against B-DNA molecule and similar double helixes, as against penicillin binding protein 2a [9]. This fact indicated the importance of examining the possibility of complexes of terpyridine with Cu(II) and Zn(II) to inhibit PBPa1 protein, which is known to be a protein of great importance in the development of bacteriological infections.

II. Methodology Section

The inhibition potency of copper(II) and zinc(II) terpyridine complexes toward penicillin-binding protein 1A (PBP1a) is investigated using molecular docking analysis. The structures of terpyridine metal complexes are optimized using M06-2X functional [13] in combination with 6-311++G(d,p) basis set implemented in Gaussian 09 program package [14]. Molecular docking simulations were carried out using AutoDock 4.0 software [15]. The three-dimensional (3D) crystal structure of PBP1a protein was downloaded from the Protein Data Bank (PDB ID: 2C6W) [2]. The protein structure is released from the co-crystallized ligand, water molecules, and co-factors and prepared for docking simulations using Discovery Studio 4.0 [16]. The affinity maps of the target protein are established using AGFR (AutoGridFR) software [17]. According to AGFR, binding site with the lowest expected binding energy is grid box with dimensions 98.799Å x 35.214Å x 55.068Å in -x, -y, and -z directions. A grid point spacing of 0.375 Å was used for auto grid runs. The addition of polar hydrogen atoms and the calculation of Kollman charges are done by using AutoDockTools (ADT) graphical interface. The ligands are set to be flexible, while the structure of protein remains standing as rigid. The Lamarckian Genetic Algorithm (LGA) is used for protein-ligand flexible molecular docking simulations. Calculations are performed at a temperature of 298.15 K. Analysis of molecular docking simulation results and visualizations of predicted protein-ligand interactions are performed using BIOVIA Discovery Studio [16].

III. Results and Discussions

Molecular docking analysis is a useful and widely used method for predicting the inhibitory activity of biologically active compounds. Terpyridine metal complexes possess significant anti-bacterial, anti-fungal and anti-cancerous activity [9, 10]. Moreover, recent research has shown that complexes of terpyridine and some its derivatives with different metals show significant inhibitory activity against various protein molecules [18, 19]. Since terpyridine complexes of Cu(II) and Zn(II) are already selected as potential agents against different disease, here is investigated their potency to inhibit PBP1a protein. PBP1a protein is membrane-associated enzyme that plays essential roles in the peptidoglycan biosynthetic process [2]. In this way it enables the proliferation of bacteria *Streptococcus pneumoniae*. The infection caused by this pathogen have been treated with β-lactam antibiotics for almost a century, but the proliferation of strains that are highly resistant to such drugs is a problem of worldwide concern. Although *S. pneumoniae* has six penicillin-binding proteins, PBP1a is essential for the development of high-level resistance to penicillins and cephalosporins [2]. Therefore, the development of agents that enable the inhibition of this protein is of particular interest.

In order to be able to compare the inhibitory activity of terpyridine metal complexes with an activity of a compound already used for these purposes, the inhibitory potency of the drug lactivicin was also examined. Lactivicin is compound that shows moderate activity against Gram-negative and high activity against Gram-positive bacteria. The mechanism of its activity is based on the binding to PBPs and violation of its structure [20]. Structures of copper(II) terpyridine complex, zinc(II) terpyridine complex and lactivicin are shown in Fig. 1.

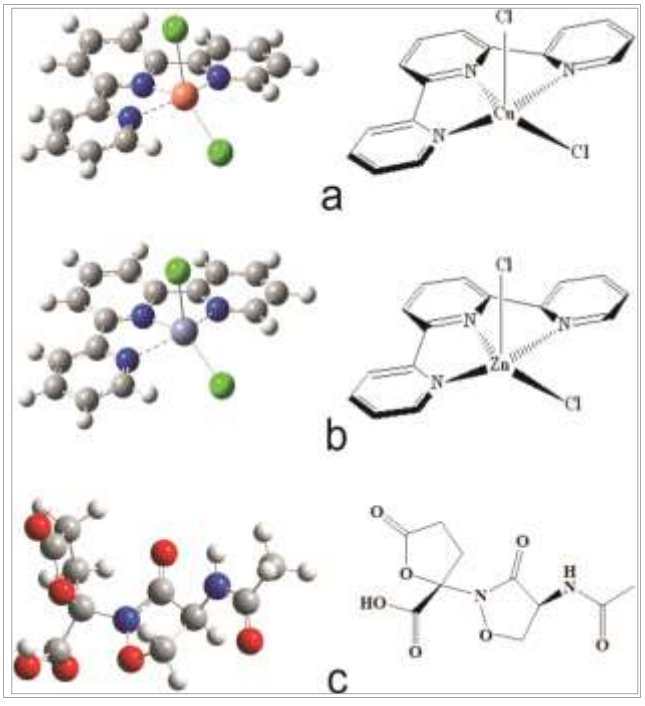

Fig. 1. 2D structures (right) and optimized structures (left) of copper(II) terpyridine complex (a), zinc(II) terpyridine complex (b) and lactivicin (c).

The evaluation of the inhibitory nature of ligand according to PBP1a was performed using molecular docking study. At the beginning of the research, the pockets and binding sites of the targeted protein were determined using the AGFR software [17]. All three estimated ligands showed

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the highest activity against protein at the same position. Grid box with dimensions 98.799Å x 35.214Å x 55.068Å in -x, -y, and -z directions, and with spacing of 0.375 Å is predicted. For all three estimated ligands it comprises the same pocket of the polypeptide chain (Fig.2).

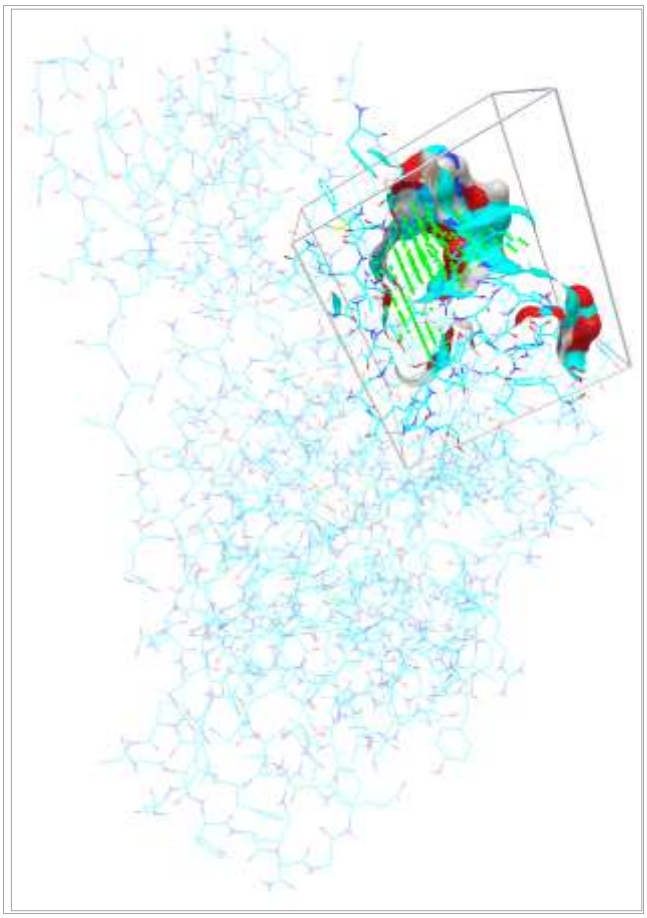

Fig. 2. The location of the most probable binding site of PBP1a for all three estimated ligands.

After configuring and computing affinity maps for a receptor-protein interactions, the one with the lowest binding energy was used for AutoDock4 calculations. Ten different conformations of protein-ligand complexes are set for molecular docking simulations. It should be emphasized that only one conformation has been accomplished when as ligand is used any of estimated terpyridine metal complexes. It can be explained by the complete rigidity of the ligand structure. On the other side, flexible structure of lactivicin allowed formation of ten protein-ligand complexes. The complex conformation that possesses the lowest binding energy was selected for further analysis.

TABLE I

THE IMPORTANT THERMODYNAMICAL PARAMETERS FROM MOLECULAR DOCKING SIMULATIONS BETWEEN PENICILLIN-BINDING PROTEIN 1A (PBP1A) (PDB ID: 2C6W) AND SELECTED COMPOUNDS

Ligand	ΔG_{bind} (kcal/mol)	Ki (μM)
$[CuCl_2(terpy)]$	-6.66	13.11
$[ZnCl_2(terpy)]$	-6.62	13.99
lactivicin	-5.48	95.46

The inhibitory potency of preferred compounds can be estimated based on the thermodynamical parameters obtained by statistical mechanical analysis. Values of the free energy of binding (ΔG_{bind}) expressed in kcal/mol and inhibition constant (Ki) expressed as micromolar concentration, are presented in Table 1. The lowest binding energy indicates to the easiest binding possibility of the investigated ligand. Low values of the inhibition constant indicate that a low concentration of inhibitor is required in order to inhibit the activity of the observed protein. Both ΔG_{bind} and Ki indicate that the highest inhibition potency shows Cu(II)-terpyridine complex. Somewhat lower inhibition potency has Zn(II)-terpyridine complex, while lactivicin shows the least.

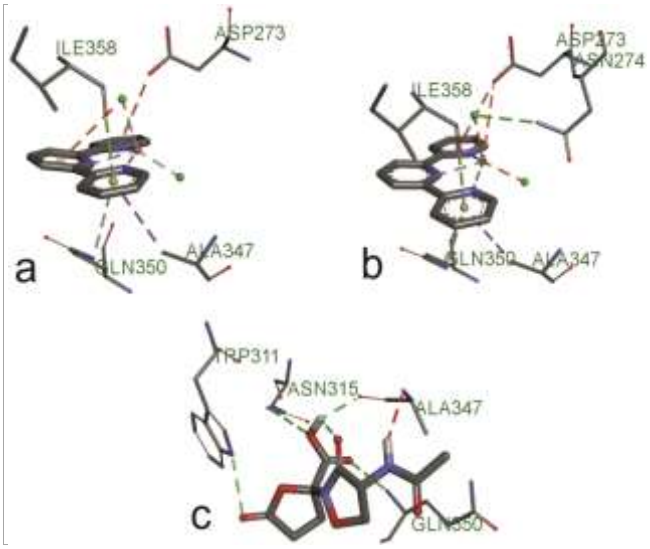

Fig. 3. Docking positions of the Cu(II)-terpyridine complex (a), Zn(II)-terpyridine complex (b) and lactivicin (c), with PBP1a protein.

Each of the three considered ligands achieves at least four protein-ligand interactions. PBP1a protein interact with Cu(II)-terpyridine complex forming π – anion electrostatic interaction via Asp273, then π – lone pair interaction via Ile358, π – donor hydrogen bond via Gln350, and π – σ interaction via Ala347. The interactions achieved by Zn(II)-terpyridine complex with PBP1a are the same as those achieved by the complex with copper, except that Zn-complex achieves another additional conventional hydrogen bond via Asn274 amino acid of PBP1a. Lactivicin generates with the protein four conventional hydrogen bonds, via the amino acids Trp311, Asn315, Ala347 and Gln350. It can be seen that all three ligands interact with PBP1a via Ala347 and Gln350 (Fig. 3). As it can be concluded, the number of ligand-protein interactions is not the only parameter that affects to the strength of inhibition, but it is also the type of interactions. Although conventional hydrogen bonds are among the most significant interactions in protein inhibition, the effect of interactions involving π–electrons derived from terpyridine should not be overlooked.

IV. CONCLUSIONS

Metal complexes of terpyridine are known as compounds with significant anti-bacterial, anti-fungal and anti-

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cancerous activity [9, 10]. On the other hand, there are numerous infections caused by bacteria *Streptococcus pneumonia*. Penicillin binding proteins (PBPs) play a key role in the proliferation of this bacterium [2]. Therefore, the device of molecules that would lead to the inhibition of this protein could significantly contribute to the treatment of infections caused by *S. pneumonia*.

The potency of Cu(II)-terpyridine complex and Zn(II)-terpyridine complex to inhibit penicillin-binding protein 1A (PBP1a) is investigated in this purpose. Due to it molecular docking simulations are performed. Inhibition potency of $[CuCl_2(terpy)]$ and $[ZnCl_2(terpy)]$ are compared with inhibition potency of lactivicin, molecule that is already used in the treatment of *S. pneumonia*.

The analysis of results obtained by molecular docking simulations indicated that the highest inhibition potency express Cu(II)-terpyridine complex, while Zn(II)-terpyridine complex shows somewhat lower inhibition potency. Both estimated complexes possess higher inhibition potency than lactivicin. All three investigated ligands react with PBP1a at the same reaction position, and in some cases, via the same amino acids of protein. All ligands generate at least four interactions with protein, among the most important are conventional hydrogen bonds, and interactions involving π–electrons from terpyridine.

Comparing inhibition potency of $[CuCl_2(terpy)]$ and $[ZnCl_2(terpy)]$ with inhibition potency of some other complexes of terpyridine and its derivatives with different metals [18, 19], it can be seen that here investigated complexes possess similar or higher inhibition potency toward here estimated protein.

The results of the docking analysis conducted in this study provide significant preliminary results related to the inhibitory activity of the complexes tested here. With all this in mind, the herein discussed metal complexes may be considered as potential agents in the treatment of bacterial infections. Among further studies of the biological activity of the terpyridine complex, *in vivo* studies are certainly the most important.

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