Contribution to Elucidating the Mechanism of Antimicrobial Action of a Dinuclear Pt/Cu Complex Against *Enterococcus faecalis* – *In Silico* Approach

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Abstract: A previously conducted experimental study demonstrated that the dinuclear Pt/Cu complex examined here can very effectively inhibit the growth and development of *Enterococcus faecalis*, a bacterium whose infections can lead to serious consequences and which is resistant to numerous antibiotics. The structure of the investigated complex consists of [Pt(DMEAIm^{iPr})Cl$_2$] on one side and [CuCl$_2$(terpy)], connected by 4,4′-bipyridine as a bridging ligand. As the aforementioned study revealed that this complex does not inhibit the gyrase of *E. faecalis*, the present work investigates the mechanism of bacterial growth inhibition in the presence of this dinuclear complex by applying the molecular method. It was found that the compound effectively inhibits the hydrolases of *E. faecalis*, even more efficiently than Doxycycline, and that both ligands bind to these proteins at the same active sites. Therefore, it is reasonably assumed that the mechanism of *E. faecalis* growth inhibition by the tested complex primarily involves the inhibition of the bacterium's hydrolases.

Keywords: Dinuclear Pt/Cu complex, Molecular docking, Mechanism of *E. faecalis* growth, Hydrolases.

1. Introduction

The bacterium *Enterococcus faecalis* is a Gram-positive bacterial species that is naturally found in the human intestinal tract and can be used as a probiotic [1]. Although it can grow and develop in the presence of oxygen, it is very resistant to unfavorable conditions such as lack of oxygen, high temperatures, acidity of the environment, and others. This bacterium can lead to numerous unwanted infections such as urogenital infections, bacteremia, endocarditis, meningitis, etc., and can also be the cause of colon cancer. Especially dangerous and frequent are infections that occur in

patients with weakened immunity, such as oncology patients, transplant patients, or patients in intensive care.

Treatment of infections caused by *E. faecalis* bacteria is difficult due to resistance to numerous antibiotics. Therefore, a combination of antibiotics is often used for this purpose, and the tendency in drug development is to find effective inhibitors of the development of this bacterium.

During the recently conducted analysis of the antibacterial activity of a series of binuclear newly synthesized Pt(II) complexes, it was observed that the compound comprising [Pt(DMEAIm^{iPr})Cl_2] on one side and [$CuCl_2$(terpy)], connected by 4,4′-bipyridine as a bridging ligand (here labeled as D2d), inhibits the growth of *E. faecalis* very well under experimental conditions [2]. On the other hand, the docking analysis, which investigated the possibility of inhibition of the gyrase of this bacterium by the D2d complex, indicated that this enzyme cannot be inhibited by the examined compound. Therefore, the main task of this research is to investigate the mechanism of inhibition of the growth and development of the bacterium *E. faecalis*. It is necessary to establish which enzymes that the tested complex can inhibit are of vital importance for the growth and development of this bacterium, and whose inactivation can prevent the spread of the infection caused by this bacterium.

2. Methodology

The structure of the ligands was optimized using the B3LYP level of theory in combination with the 6–311 + G(d,p) basis set for C, H, N, and Cl atoms and with the aug-cc-pVTZ-PP basis set for metal ions [3]. For this purpose, the Gaussian 09 software was utilized [4]. As target proteins are used methionine aminopeptidase (PDB ID: 3TB5) and bile salt hydrolase (PDB ID: 4WL3), both hydrolases determinated for the *E. faecalis* [5,6]. The Discovery Studio 4.0 (BIOVIA Discovery Studio 2017) was used for protein structure preparation [7], while for molecular docking analysis was used AutoDock 4.0 software package [8]. During the molecular docking analysis, the maximum grid box size (126 x 126 x 126 npts) was used for both proteins. The grid box center for the 3TB5 protein was positioned at the point with coordinates -10.595 × 28.547 × 23.481 along the x, y, and z axes, respectively, while the center of the grid box for the 4WL3 protein was located at the point with coordinates -13.016 × 63.683 × 48.744.

3. Results and Discussion

In a previously conducted *in vivo* study, it was demonstrated that the D2d compound examined in this study exhibits significant inhibitory potential against the bacterium *E. faecalis* ($MIC_{\mu g/ml}$=7.03, $MBC_{\mu g/ml}$=56.25) [2]. However, molecular docking analysis indicated that this compound is not capable of inhibiting the gyrase of this bacterium. The molecular docking analysis applied here investigates the potential of the D2d complex to inhibit two selected hydrolases (PDB ID: 3TB5 and PDB ID: 4WL3) [5,6].

Table 1. Thermodynamic parameters of the stability of the corresponding protein-ligand complexes. The inhibition constant values (K_i) are presented in nM. Energy values are presented in kcal/mol.

Complex	ΔG_{bind}	K_i	ΔG_{inter}	$\Delta G_{vdw+hbond+desolv}$
3TB5-D2d	**-10.51**	**19.66**	-12.16	-9.49
3TB5-DX	-9.28	157.16	-11.37	-9.96
4WL3-D2d	**-9.56**	**98.37**	-11.21	-9.09
4WL3-DX	-8.20	969.73	-10.29	-9.32

Based on the values of the Gibbs free energy of binding (ΔG_{bind}) and inhibition constant (K_i) as the most important thermodynamical parameters of the inhibition potency of the investigated compound (Table 1), it can be concluded that the here investigated complex can very successfully inhibit selected hydrolases. Comparing the inhibition potency of the D2d molecule with the inhibition potency of Doxycycline (DX), an antibiotic already used in the medicinal antibacterial treatment [9], it can be seen that D2d possesses better inhibition potency than the mentioned antibiotic. In a previously conducted *in vivo* study, DX exhibited $MIC_{\mu g/ml}$=7.81 and $MBC_{\mu g/ml}$=62.50 against *E. faecalis* [2]. Both D2d and DX interact with 3TB5 through the amino acid His175, while DX also forms a bond via the amino acid Tyr60, which is adjacent to Glu61, the residue through which D2d binds to this protein. When forming a complex with the 4WL3 protein, D2d binds to the amino acid Pro137, whereas DX binds via Leu138.

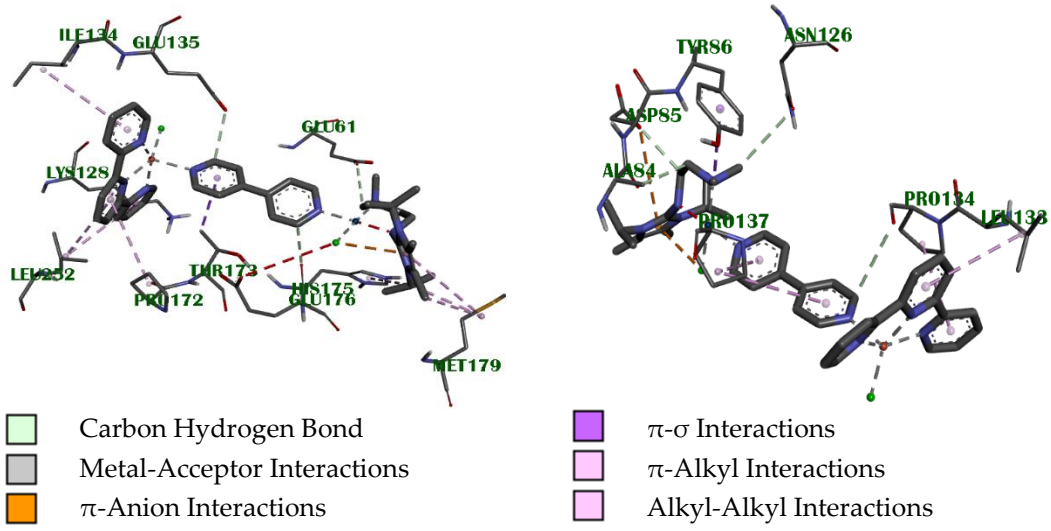

Figure 1. Docking positions for the most stable 3TB5-D2d (left) and 4WL3-D2d (right) complexes with the presented amino acids over which are formed interactions

4. Conclusions

A previously conducted *in vivo* study established that the dinuclear Pt/Cu complex examined here very effectively inhibits the growth of *E. faecalis*, even more successfully than Doxycycline, which is used as an antibiotic. On the other hand, molecular docking

analysis revealed that this complex is not capable of inhibiting the gyrase of the mentioned bacterium.

In this study, molecular docking analysis confirmed that the investigated dinuclear complex more effectively inhibits the hydrolases of *E. faecalis*, even more than Doxycycline. Both examined ligands bind to the selected hydrolases via the same or neighboring amino acid residues, which confirms that they inhibit the protein by targeting the same reactive sites. The small difference in the inhibitory activity values of the D2d and DX ligands, obtained both in this study and in previously conducted experiments, suggests that the mechanism by which the D2d complex inhibits the growth of *E. faecalis* probably involves the inhibition of this bacterium's hydrolases.

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