Antimicrobial Activity of Rhenium Di- and Tricarbonyl Diimine Complexes: Insights on Membrane-Bound S. aureus Protein Binding

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Abstract: Antimicrobial resistance is one of the major human health threats, with significant impacts on the global economy. Antibiotics are becoming increasingly ineffective as drug-resistance spreads, imposing an urgent need for new and innovative antimicrobial agents. Metal complexes are an untapped source of antimicrobial potential. Rhenium complexes, amongst others, are particularly attractive due to their low in vivo toxicity and high antimicrobial activity, but little is known about their targets and mechanism of action. In this study, a series of rhenium di- and tricarbonyl diimine complexes were prepared and evaluated for their antimicrobial potential against eight different microorganisms comprising Gram-negative and -positive bacteria. Our data showed that none of the Re dicarbonyl or neutral tricarbonyl species have either bactericidal or bacteriostatic potential. In order to identify possible targets of the molecules, and thus possibly understand the observed differences in the antimicrobial efficacy of the molecules, we computationally evaluated the binding affinity of active and inactive complexes against structurally characterized membrane-bound S. aureus proteins. The computational analysis indicates two possible major targets for this class of compounds, namely lipoteichoic acids flippase (LtaA) and lipoprotein signal peptidase II (LspA). Our results, consistent with the published in vitro studies, will be useful for the future design of rhenium tricarbonyl diimine-based antibiotics.

Keywords: rhenium; tricarbonyl; antimicrobial; S. aureus; MRSA; AutoDock; membrane; proteins; LspA; LtaA

1. Introduction

The expansion of resistance to conventional antibiotics has become a notable health threat and necessitated the development of alternative treatment options for battling such a global problem [1]. Amongst the six nosocomial pathogens that exhibit multidrug resistance and virulence, the methicillin-resistant S. aureus (MRSA) is a major cause of community- and hospital-acquired infections worldwide, ranging from superficial skin and soft tissue infections [2] to invasive infections and sepsis [3]. This pathogen represents the most common and the second most common cause of healthcare-associated and bloodstream infections (BSI), as well as the most important cause of BSI death [4]. Since the bacterium is increasingly showing resistance to multiple antibiotics, in 2017 the World Health Organization listed it among the high priority group of human pathogens. Indeed, the same year, the Centers for Disease Control and Prevention (CDC) reported that more than 119,000 people suffered from S. aureus bloodstream infections in the United States, with nearly 20,000 of them (>16%) eventually dying.
What has exacerbated the problem of antimicrobial resistance (AMR) is the fact that fewer new antibiotics are reaching the market, with the last entirely original class of antibiotics discovered in the late 1980s. This is because large pharmaceutical companies have left the market due to a lack of financial incentives [5]. Consequently, in the last few years, academic research groups at universities around the globe have taken up the challenge to prepare and discover new antibiotic drugs that may serve as lead compounds for new structurally viable drugs. In our era, strategies for the discovery and development of new drugs combine computational and experimental approaches. This is true in virtually all medicinal discovery areas, including the design and discovery of molecules as appropriate candidates for treatment of *Staphylococcus aureus* infection. Computer-aided drug design (CADD) methods are computational approaches to guiding and expediting the experimental findings for new drug design processes [6–10]. CADD can be used in a qualitative and quantitative mode to improve the biovalidity and prediction rates for ligand binding affinity, as well as specificity, in a manner that can lead to the identification of biological targets of known drugs and the design process of new agents in a simpler, more efficient, and less expensive manner. In a modern drug design process, typically hundreds of compounds can be tested in a short time. The existing methodologies such as, e.g., site-identification by ligand competitive saturation (SILCS) [8,11], have become versatile tools for ligand–protein binding prediction. The foundation of the CADD technique is based on molecular docking [12–14] and molecular dynamics simulations [15,16].

Within the specific context of this article, CADD has been used, e.g., to evaluate medicinal plant-derived active compounds that could be used as therapeutic alternatives for MRSA infection [17–20]. The study of receptor–ligand interactions in the framework of molecular docking has drawn attention to the importance of probing the efficiency of these plant-derived antimicrobial agents [17,18] and testing antimicrobial activity using screened lead compounds, focusing on the role of computational screening methods [20–23] in tackling the problem. However, a major strategy still pursued in the field is that of modifying already approved antibiotics [24]. As may be expected, all of these molecules are purely organic compounds. While some of these new derivatives (some are currently in preclinical or clinical development) will provide feasible short-term solutions, it is probable that the pathogens will rapidly adapt and develop resistance to these molecules as well [24].

As an alternative to organic compounds, there is an increasing awareness in academia of the potential of metal complexes to act as the new class of molecules for this purpose. Indeed, the unique chemistry and larger variety of 3D geometries of metal compounds can address targets and modes of action that are unavailable to organic molecules. Over the last decade, complexes of virtually all the transition metals have been evaluated as antimicrobial agents [25–27], with rhenium (Re), among others [28,29], showing promising potential for new antibiotic development [30–33]. While other transition metal complexes [34–47] act against Gram-negative bacteria, carbonyl rhenium complexes have demonstrated a very potent activity towards Gram-positive pathogens, particularly towards *Staphylococcus aureus*, involving both the methicillin-resistant (MRSA) and methicillin-sensitive (MSSA) strains [31–33,48–51].

Our group is principally interested in the development of the chemistry of carbonyl rhenium complexes for their use in different medicinal applications, including their evaluation as anticancer [52–54] and antibacterial agents [49,50]. Due to their very low in vivo toxicity [55–58], tricarbonyl complexes of rhenium are particularly attractive. The same type of molecules (i.e., those of the $\text{fac-}[\text{Re(CO)}_3]^+$ core) are also the ones most widely investigated, showing the highest anticancer and antimicrobial effectiveness against *S. aureus* strains [59]. It is still unclear what molecular features of carbonyl rhenium complexes make them such promising medicinal agents. In a study that we have recently reported [49], we concluded that, unlike anticancer complexes, positively charged rhenium species are most effective against the microbes, and we hypothesized that charged compounds may interact more effectively with phosphatidylglycerol and cardiolipin anionic membrane lipids. Later, however, we found that, by substituting a single neutral carbonyl with a
nitrosonium cation, the compounds lose their antimicrobial effect [60]. Thus, in terms of their antibacterial effectiveness, both the required molecular features and mechanism of action of these agents remain largely unknown.

In order to advance knowledge of the issues mentioned above, we evaluated the antimicrobial activity of dicarbonyl rhenium diimine complexes (i.e., of the cis-[Re(CO)2]+/2+ core) and compared it to that of structurally similar fac-[Re(CO)3]+ species. This part of the study was performed because (a) no antimicrobial data is available on the carbonyl complexes of the cis-[Re(CO)2]+/2+ core lacking other π-acid ligands, and (b) a comparison of the activity of cis-[Re(CO)2]+/2+ and structurally similar fac-[Re(CO)3]+ species may provide information about the key molecular features required for the design of an effective Re-based antibiotic agent. Furthermore, we computationally evaluated the binding affinity of all the compounds (both active and inactive molecules) against structurally characterized membrane-bound S. aureus proteins. We performed this study principally to (a) identify possible biological targets of the active complexes; (b) possibly understand the underlying reasons for the observed differences in the antimicrobial efficacy of Re complexes; and (c) offer support for the rational design of rhenium complexes based on the computational protocol for CADD.

2. Results and Discussion
2.1. Synthesis and Characterization of the Metal Complexes

Rhenium carbonyl complexes investigated in this study were prepared according to the procedures illustrated in Scheme 1. Tricarbonyl species 6–10 (Figure 1) were obtained in a high yield and purity, according to established routes generally used in the preparation of these compounds. The fac-[Re(CO)3(NN)Br] complexes (6–8, 9a, and 10, where NN = relevant bidentate diimine ligand) may be obtained in one step from [Re(CO)5Br] by boiling this precursor in toluene in the presence of one equivalent of NN. The resulting yellow product, isolated by filtration, is generally of a high purity (>96% by NMR or HPLC) and can be used for further modification by the substitution reaction of the coordinated bromide atom by other monodentate ligands, as in the case of species 9b and 9c. For this reaction, we found that the best conditions consist in the treatment of a fac-[Re(CO)3(NN)Br] complex with trifluoromethanesulfonic acid to produce the intermediate fac-[Re(CO)3(NN)(CF3SO3)] molecule, followed by the addition of L (where L = pyridine: py or N-methyl imidazole: Melm). The reaction is also high yielding, but the desired fac-[Re(CO)3(NN)L]CF3SO3 salt requires purification on alumina or via HPLC.

Scheme 1. Synthetic scheme for the preparation of the complexes investigated in this study. NN = relevant bidentate diamine ligand; L = pyridine (py) or N-methyl imidazole (Melm). General conditions: i: Et4NBr, diglyme; ii: NN, ethanol/water, toluene, or CH2Cl2; iii: L, methanol or neat L; iv: Br2, CH2Cl2; v: tetrakis(dimethylamino)ethylene, acetonitrile, under N2; vi: tetrakis(dimethylamino)ethylene, CH2Cl2, under N2. For more details, refer to Section 4.
The preparation of dicarbonyl cis-[Re(CO)\textsubscript{2}(NN)X\textsubscript{2}] species (1–5 and 11, where X = Br or L, Figure 1) is more demanding and requires several steps, starting from the common [Re(CO)\textsubscript{3}Br] precursor. We have recently published the details of this chemistry [61], showing that the synthetic route is favorable if X is a halide or an aromatic heterocycle (or a combination of both). However, yields of cis-[Re(CO)\textsubscript{2}(NN)X\textsubscript{2}] species are much lower than the comparable fac-[Re(CO)\textsubscript{3}(NN)Br] complexes. Briefly, cis-[Re(CO)\textsubscript{2}(NN)X\textsubscript{2}] species may be prepared following the sequential two-electron oxidation of fac-[Re(CO)\textsubscript{3}Br\textsubscript{3}]\textsuperscript{2−} to cis-[Re(CO)\textsubscript{2}Br\textsubscript{4}]\textsuperscript{−} [62], its one-electron reduction to cis-[Re(CO)\textsubscript{2}Br\textsubscript{4}]\textsuperscript{2−}, the complexation of NN to cis-[Re(CO)\textsubscript{2}(NN)Br\textsubscript{2}], its one-electron reduction to cis-[Re(CO)\textsubscript{2}(NN)Br\textsubscript{2}]\textsuperscript{−}, and, finally, the stepwise substitution of Br with L to obtain cis-[Re(CO)\textsubscript{2}(NN)Br\textsubscript{L\textsubscript{2}}] and cis-[Re(CO)\textsubscript{2}(NN)L\textsubscript{2}]\textsuperscript{+}. It is interesting to point out here that, contrary to other similar complexes, the presence of NN in the coordination sphere of the 17-electron Re\textsuperscript{II} complexes (1a–c and 11) imparts stability to the molecules, which are stable in solution and do not decompose by releasing CO [63,64].

New complexes were characterized by standard techniques, including X-ray crystallography for the dicarbonyl species 4a and 11 (Figure 2) and tricarbonyl complexes 6–8 and 10 (Figure 3, with relevant bond distances and angles for the solid-state molecular structures in the Supplementary Materials). Within the series of dicarbonyl cis-[Re(CO)\textsubscript{2}(NN)Br\textsubscript{2}] species, the preparation of compound 11 (where NN = bathophenanthroline: batho-phen) was particularly challenging. Indeed, the reaction of either cis-[Re\textsuperscript{III}(CO)\textsubscript{2}Br\textsubscript{4}]\textsuperscript{−} or cis-[Re\textsuperscript{II}(CO)\textsubscript{3}Br\textsubscript{4}]\textsuperscript{2−} with bathophen leads to a mixture of products which are very difficult to separate. Normally, cis-[Re(CO)\textsubscript{2}(NN)Br\textsubscript{2}] complexes are obtained as cis-cis-trans species (with the two Br atoms in the trans position to each other). Only when one of the bromides is substituted for L, the intermediate penta-coordinated complexes undergo Berry pseudorotation, which establishes an equilibrium between the cis-cis-trans and cis-cis-cis isomers [61]. These can be separated by column chromatography and crystallized separately (as in the case of 3a, Figure 2). In the preparation of 11, we found not only that the reaction leads to disproportionation, giving 10, but also that the cis-cis-cis isomer of 11 (cis-11) and the mono carbonyl mer-[Re(CO)(NN)Br\textsubscript{3}] complex (mer-12, Figure 2) are formed. Complex 11 can be separated from the mixture, but despite our efforts, the other
complexes formed could not be eluted separately in our chromatographic purification procedures. We should underline here that we were able to identify the products obtained in the reaction only by co-crystallizing them in a mixture. We also note that, to our knowledge, mer-[Re(CO)(NN)Br$_3$] (mer-12) is a unique example of a diimine rhenium mono carbonyl complex that is structurally characterized.

Figure 2. ORTEP representations of the crystal structures of Re dicarbonyl complexes. Thermal ellipsoids are at 30% probability. Hydrogen atoms are omitted for clarity. Note: the compounds cis-11 and mer-12 co-crystallize in a mixture, where 10 and 11 are also present.

Figure 3. ORTEP representations of the crystal structures of Re tricarbonyl complexes 6–8 and 10. Thermal ellipsoids are at 30% probability. Hydrogen atoms are omitted for clarity.
2.2. Antimicrobial Properties of the Complexes

The antimicrobial activity of complexes 1–11 (15 neutral, 6 cationic) was determined against eight different microorganisms, including four Gram-negative bacteria (Enterobacter cloacae ATCC 3047, Klebsiella pneumonia ATCC 13803, Acinetobacter baumannii ATCC 19606, and Pseudomonas aeruginosa PAO1 NCTC10332), two Gram-positive bacteria (methicillin-resistant Staphylococcus aureus MRSA43300 and methicillin-sensitive S. aureus ATCC25923), and two fungi (Candida albicans SC5314 and C. auris, a clinical strain). The species of these two genera are responsible for the majority of hospital-acquired infections and are challenging to treat, especially in the case of their co-infections [65]. The results of our study are given in Table 1. We found that none of the dicarbonyl complexes showed antimicrobial potential. Only compounds 4b, 5b, and 11 were weakly active against the S. aureus strains, but their MIC values (25 and 50 µM, respectively) were much higher than those of the active rhenium complexes 13–19 (Figure 4) [31,32,48–50].

Table 1. Antimicrobial activity, assessed by determining the minimal inhibitory concentrations (MIC, µM, mean of n = 3 independent experiments) of different Re-bearing complexes.

| Compound | A. baumannii | P. aeruginosa | K. pneumoniae | S. aureus MRSA | S. aureus MSSA | E. cloacae | C. albicans | C. auris |
|----------|--------------|---------------|---------------|----------------|----------------|------------|-------------|----------|
| 1a–5a    | >100         | >100          | >100          | >100           | >100           | >100       | >100        | >100     |
| 1b–3b    | >100         | >100          | >100          | >100           | >100           | >100       | >100        | >100     |
| 4b        | >100         | >100          | >100          | 25             | 25             | >100       | >100        | >100     |
| 5b        | >100         | >100          | >100          | 50             | 50             | >100       | >100        | >100     |
| 1c–3c     | >100         | >100          | >100          | >100           | >100           | >100       | >100        | >100     |
| 6–8, 9a–c, 10 | >50         | >50           | >50           | >50            | >50            | >50        | >50         | >50     |
| 11        | >100         | >100          | >100          | 50             | 50             | >100       | >100        | >100     |
| 13        | n.a.         | n.a.          | n.a.          | 0.7            | 22.8           | n.a.       | n.a.        | n.a.    |
| 14        | n.a.         | n.a.          | n.a.          | 1.6            | 1.6            | n.a.       | n.a.        | n.a.    |
| 15        | n.a.         | n.a.          | n.a.          | 0.4            | 0.6            | n.a.       | 6.2         | 50       |
| 16        | n.a.         | n.a.          | n.a.          | 0.8            | 0.8            | n.a.       | 6.2         | 50       |
| 17        | 8            | 32            | 32            | 0.25           | 0.25           | n.a.       | n.a.        | n.a.    |
| 18        | n.a.         | n.a.          | n.a.          | 0.8            | 3.1            | n.a.       | 3.1         | 50       |
| 19        | n.a.         | n.a.          | n.a.          | 1.6            | 6.2            | n.a.       | 50          | 50       |
| Meropenem | >100         | 1.25          | >100          | 6.25           | >100           | 50         | -           | -        |
| Vankomycin | >100       | >100          | >100          | 6.25           | 6.25           | -          | -           | -        |
| Fluconazole | -         | -             | -             | -              | -              | -          | 1           | >64     |
| Amphotericin | B       | -             | -             | -              | -              | 0.3        | 1           |         |

Note: n.a. = not available.

2.3. Molecular Docking Study: Membrane-Bound S. aureus Proteins

The results obtained from our in vitro antimicrobial investigation raised a fundamental question, namely: “what sets apart cationic fac-[Re(CO)3]+ complexes from other structurally similar neutral complexes or compounds lacking the tricarbonyl core?” Or, in other words, “why are complexes 13–19 (Figure 4) active antimicrobial agents while other rhenium complexes are not?” Compounds 13–19 are different molecules, but they share some common features (e.g., the same charge, with a lipophilic diimine or polydentate ligand with a pyridine in the coordination sphere). In addition, Table 2 presents the predictability rates of the drug-likeness properties for these rhenium complexes. The descriptor values were retrieved using the AlvaDesc v.2 software (Milano, Italy) [66].
Figure 4. Structures of previously published active fac-[Re(CO)_3]^+ complexes. Complex 13 [32]; complex 14 [31]; complexes 15, 16, 18, and 19 [49,50]; complex 17 [48].

Table 2. Drug-likeness properties of active antimicrobial rhenium complexes 13–19.

| Compound | MW    | RBN | TPSA(Tot) | HBA | HBD | LOGP99 | BLTF96 | BLTA96 | BLTD48 | ESOL  | cRo5 | Ro5 |
|----------|-------|-----|-----------|-----|-----|--------|--------|--------|--------|-------|------|-----|
| 13       | 700.827 | 3   | 81.79     | 6   | 1   | 6.8    | -3.09  | -3.22  | -3.23  | -7.19 | 1    | 0   |
| 14       | 1072.267 | 16  | 187.36    | 14  | 2   | 4.8    | 1.13   | 1.49   | 1.73   | -5.67 | 0    | 1   |
| 15       | 681.767  | 3   | 66        | 0   | 6   | 6.7    | -2.99  | -3.11  | -3.11  | -7.02 | 1    | 0   |
| 16       | 647.827  | 7   | 72.48     | 0   | 8   | 4.1    | -2.03  | -2.04  | -1.98  | -4.94 | 1    | 0   |
| 17       | 771.297  | 5   | 70.93     | 0   | 8   | 7.1    | -3.94  | -4.17  | -4.23  | -7.85 | 1    | 0   |
| 18       | 867.067  | 3   | 108.24    | 2   | 1   | 8.5    | -4.13  | -4.38  | -4.45  | -8.74 | 1    | 0   |
| 19       | 1021.367 | 8   | 136.15    | 0   | 12  | 8.0    | -3.84  | -4.06  | -4.11  | -9.29 | 0    | 1   |

Labels: MW—molecular weight, RBN—rotatable bond number, TPSA—total polar surface area in A^2, HBA—number of H bond acceptors, HBD—number of H bond donors, LOGP99—Wildman–Crippen octanol–water partition coeff., BLTF96—Verhaar Fish base-line toxicity from MLOGP (mmol/L), BLTD48—Verhaar Daphnia base-line toxicity from MLOGP (mmol/L), BLTA96—Verhaar Algae base-line toxicity from MLOGP (mmol/L), ESOL—estimated solubility (logS) for aqueous solubility using LOGPcons., cRo5—complementary Lipinski alert index, Ro5—Lipinski rule of 5.

At this early stage of investigation, to aid in our search for an answer to the question, a purely experimental approach focused on, e.g., microbial gene expression analysis and transcriptomic data would be costly and time consuming. We thus decided to adopt an
in silico approach in order to guide future synthetic, SAR, and mechanistic studies. There are, fortunately, some experimental facts that helped us to focus our attention on specific enzymes that may be considered as possible targets for one or more of the compounds 13–19. Although mechanistic studies are limited and specific biological targets are still unknown, the effective antimicrobial $\text{fac-[Re(CO)}_3\text{]}^+$ complexes appear to act predominately on the membrane of the bacteria. The complex of Metzler-Nolte and Bandow, i.e., compound 14 in Figure 4, targets the cytoplasmic membrane of *Bacillus subtilis*, affecting its architecture and disrupting essential cellular processes taking place at the membrane, such as respiration, as well as cell wall biosynthesis and integrity [30]. Similarly, Mendes et al. have shown that the mechanism of action of the $\text{fac-[Re(CO)}_3(\text{bpy})(\text{ctz})\text{]}^+$ complex (17 in Figure 4, where ctz = the drug clotrimazole) involves a sequence of events initiated by membrane insertion, followed by membrane disorganization, the inhibition of peptidoglycan biosynthesis, and the breakdown of the membrane potential [48].

Based on these data, in order to possibly understand the differences in the antimicrobial effects of the previously published active $\text{fac-[Re(CO)}_3\text{]}^+$ complexes (13–19, Figure 4) and inactive $\text{fac-[Re(CO)}_3\text{]}^+$ and $\text{cis-[Re(CO)}_3\text{]}^+$ complexes, we decided to investigate the binding affinity of all the above compounds against membrane-bound *S. aureus* proteins. The in silico docking studies were also performed in order to gain insights on the possible targets of the molecules by the careful analysis of the data. A PDB search revealed that nine structurally characterized membrane-bound *S. aureus* MRSA proteins are available on the database. Of these, we selected eight, comprising four penicillin-binding proteins (PBPs) [67–70] and the following enzymes: lipoteichoic acid synthase [71] (specifically its extracellular catalytic domain, eLtaS), type-I signal peptidase (SpsB) [72], lipoprotein signal peptidase II (LspA) [73], and lipoteichoic acid flippase (LtaA) [74]. The pre-screening of the binding affinities (b.a.) was performed with the AutoDock Vina software [14]. The calculated b.a. were recorded as docking scores in kilocalories per mole (kcal/mol), and the results are given in the Supplementary Materials (Table S1a,b). In the initial screening, the metal complexes were first docked at the known inhibitor-binding site of the specific protein, and the b.a. was compared to that of the same inhibitor. At this stage, only proteins where complexes showed a b.a. of ca. $-9.0$ kcal/mol that was greater than the corresponding inhibitor’s b.a. ($\Delta$ values in Table S1a,b), or a b.a. of ca. $-10.0$ kcal/mol that was comparable to the corresponding inhibitor’s b.a., were considered as possible targets for the complexes.

Within the abovementioned constrains, in general terms, our initial analysis revealed the following (detailed values are in the Supplementary Materials, Table S1a,b):

1. With the exception of the $\text{cis-[Re(CO)}_3\text{]}^+$ complexes 1b–3b and the $\text{fac-[Re(CO)}_3\text{]}^+$ complexes 6, 7, and 10, none of the inactive rhenium di- or tricarbonyl compounds showed any b.a. for the enzyme evaluated.

2. The inactive molecules 1b–3b, 6, 7, and 10 showed an affinity for the penicillin-binding protein 4 (PBP4), with b.a. values ranging from $-8.9$ (1b) to $-12.3$ (10) kcal/mol.

3. Compound 10 also showed a strong affinity for lipoteichoic acid flippase (LtaA), with a b.a. of $-10.3$ kcal/mol.

4. Amongst the active antimicrobial rhenium complexes (i.e., molecules 13–19, Figure 4), complexes 16 and 17 showed the lowest b.a. values for the selected enzymes. These were higher than those of the inactive compounds but lower than those of the known inhibitors.

5. With variations within the series, the other active antimicrobial rhenium complexes (13–15 and 18–19) showed good b.a. values for five enzymes. These were the penicillin-binding protein 4 (PBP4, b.a. ranging from $-9.1$ (13) to $-10.7$ (19) kcal/mol); type-I signal peptidase (SpsB, all complexes except 14, b.a. ranging from $-9.1$ (13) to $-10.4$ (19) kcal/mol); lipoteichoic acid synthase (LtaS, only 15, 18, and 19, b.a. ranging from $-9.4$ (15) to $-10.9$ (19) kcal/mol); lipoteichoic acid flippase (LtaA, only 15, 18, and 19, b.a. ranging from $-10.4$ (15) to $-11.3$ (19) kcal/mol); and lipoprotein signal peptidase II (LspA, all complexes except 13, b.a. ranging from $-8.7$ (15) to $-10.6$ (18) kcal/mol).
Interestingly, PBP4, LtaS, and LtaA are all involved in bacterial wall biosynthesis [70,72,74–78]. PBP4 is a transpeptidase that performs the crosslinking reaction in the synthesis of the peptidoglycan backbone [78]. LtaS catalyzes the polymerization of lipoteichoic acid (LTA) polyglycerol phosphate, a reaction that presumably uses phosphatidylglycerol as a substrate [71]. This enzyme is required for staphylococcal growth and the cell division process [79,80]. LtaA acts upstream of LtaS [81], and it is presumed to catalyze the translocation reaction of anchor lipid-linked disaccharide gemfibrozil-diacylglycerol from the cytoplasmic leaflet of the membrane to the extracellular side of the plasma membrane, where lipoteichoic acids are assembled [74–77]. A flippase with a similar structure (MurJ) [82,83] is also involved in the translocation of disaccharide-pentapeptide building blocks, attached to a polyisoprene lipid carrier (called lipid II), across the cytoplasmic membrane, where peptidoglycan polymerization (i.e., the polysaccharide matrix that protects bacteria from osmotic lysis) takes place [77]. The remaining two proteins are SpsB and LspA. SpsB is a proteolytic enzyme that plays a crucial role in bacterial viability by processing proteins that are translocated across the membrane [72,84], while LspA is involved in bacterial lipoprotein post-translational processing [85] and is essential for the survival and virulence of Gram-positive bacteria [86,87]. This latter enzyme is considered as one of the major targets for the development of new antibiotics [88]. The calculated binding affinities of active Re complexes for these possible targets (b.a. ranging from ca. −9 to −11 kcal/mol) are fully consistent with the experimental results reported by Wenzel et al. [30] and Mendes et al. [48], in that the inhibition of these proteins leads to membrane disorganization and affects peptidoglycan/wall biosynthesis [70,74–78].

Following this initial screening, the active complexes 13–19 were more comprehensively analyzed for their binding affinities for the selected receptors. Extensive semi-flexible docking was performed, introducing flexibility into the amino acid side chains and complex rotatable bonds of the binding pockets of the receptors. The number of modes was set to 200, and the exhaustiveness was set to 40. Each docked complex was calculated in triplicate mode. The triplication test detects whether there is a variation in the obtained cluster compactness of the poses and changes in the top-ranked compounds from the previous run; thus, one avoids bias in the scoring. If a bias in the scoring is present, the solution for such a case, along with the control experiment, is a reduction in the chemical space search (e.g., narrowing of the search box). The performed protocol provides information as to whether the best selected molecules remain amongst the highest scored compounds in the rank-ordered docking list. After the calibration procedure for the docking, the molecules were virtually screened against the eight target proteins. The localization of the active pocket amino acid residues was predicted according to Jendele et al. [89]. The results are summarized in Table 3, while the detailed ranking of the obtained pockets is in the Supplementary Materials (Table S2).

Accordingly, the computational results of this library of compounds are shown in Table 4. For the PBP receptors, the docking protocol identified 15 and 19 as having the greatest b.a. for these enzymes, particularly for PBP2a and PBP4 (Table 4). As other non-active rhenium complexes showed a b.a. for PBP4, we posit that this protein is not a probable target for active complexes. Conversely, the b.a. of 15 and 19 for PBP2a is of interest (b.a. values of −9.2 and −9.8 kcal/mol respectively, Table 4). The expression of the penicillin-binding protein 2a (PBP2a) is responsible in methicillin-resistant S. aureus (MRSA) for the high-level resistance of the bacteria to β-lactam antibiotics [78]. PBP2a is a unique transpeptidase, as it is capable of catalyzing cell wall crosslinking despite the presence of β-lactam antibiotics. The inhibition of PBP2a by 15 and 19 may thus possibly additionally account for the strong antimicrobial activity of these complexes against MRSA [49,50]. Computationally, in the case of 19, the stabilization of the protein–drug complex is based on the detected H-bonds between the compound and the surrounding amino acid environment (Ser, Thr, and Gln residues). A detailed distribution of the amino acids of the best complexes is given in the Supplementary Materials (Table S3). In this case, the intramolecular backbone H-bonds stabilize the β-turn structure with the ligand position.
Table 3. Predicted binding sites.

| PBD ID | Area (Å^2) | Volume (Å^3) | Pocket Residues ID/Flexible Chains |
|--------|------------|--------------|-----------------------------------|
| 2OLV (PBP2) | 3500.5 | 7715.2 | ALA_112, VAL_367, GLY_339, LYS_127, LYS_135, THR_150, VAL_153, THR_148, GLU_171, LYS_194, PRO_231, ASN_193, GLY_229 |
| 4DKI (PBP2a) | 5537.8 | 9122.9 | THR_398, PRO_401, VAL_443, THR_444, SER_461, TYR_519, GLY_520, THR_582, ALA_601, ARG_612, ASP_638 |
| 3VSL (PBP3) | 9921.9 | 13845.0 | GLY_424, VAL_390, LEU_425, THR_426, MET_453, LEU_518, ASP_519, LYS_618, TYR_636 |
| 5TXI (PBP4) | 4258.4 | 4521.5 | SER_75, ALA_74, THR_77, LYS_78, SER_137, SER_185, SER_262, PHE_241, THR_260, GLY_261, PRO_113, LEU_115, GLU_114 |
| 2W5Q (LtaS) | 132.1 | 103.4 | LEU_254, GLU_255, GLN_297, GLY_298, LYS_299, THR_300, SER_301, HIS_347, PHE_353, TRP_354, ASN_355, LYS_397, HIS_416 |
| 4WVJ (SpsB) | 1922.1 | 3375.8 | TRP_236, GLU_117, GLU_159, TYR_161, ASN_18, ASP_20, LYS_21, LEU_268, SER_343, TRP_346, TYR_347, ARG_350, LYS_48 |
| 6S7V (LtaA) | 1758.2 | 2257.5 | LEU_219, PRO_221, LEU_225, ALA_229, ILE_230, ALA_230, VAL_234 |
| 6RYP (LspA) | 8452.7 | 1485.4 | ALA_103, VAL_367, GLY_339, LYS_127, LYS_135, THR_150, VAL_153, THR_148, GLU_171, LYS_194, PRO_231, ASN_193, GLY_229 |

For the second group of receptors (namely LtaS, SpsB, LtaA, and LspA), the docking protocol identified complexes 14, 15, 18, and 19 as having a high b.a. for lipoteichoic acid flippase (LtaA, all complexes except 14) and lipoprotein signal peptidase II (LspA, see Table 4). As mentioned above, flippases such as LtaA catalyze the translocation reactions of anchor lipid-linked disaccharide gentiobiosyl-diacylglycerol and lipid II across the cytoplasmic membrane, where essential cell wall polymers (i.e., lipoteichoic acid and peptidoglycan) are assembled (Figure 5) [74–77,81–83]. LspA, on the other hand, is involved in bacterial lipoprotein post-translational processing [85] and is essential for the survival and virulence of Gram-positive bacteria (Figure 6) [86,87]. The possible inhibition of these enzymes by active antibiotic rhenium complexes would disrupt essential cellular processes taking place at the membrane and ultimately lead to cell death. It should be mentioned that our computational analysis did not identify possible targets of complexes 13, 16, and 17. If, for the former complexes, this indicates that the compounds may exert their antibiotic activity against MRSA via mechanisms not involving membrane-bound proteins, for 17, the results appear to support the experimental evidence of Mendes et al. [48]. Indeed, the authors reported that 17 interferes with the cycling of the undecaprenyl precursor in peptidoglycan biosynthesis ("lipid II cycle"), leading to the accumulation of UDP-MurNAc-pentapeptide (i.e., lipid I, the ultimate cytoplasmic peptidoglycan precursor) in the cytoplasm of treated cells. Thus, 17 inhibits the MurG-mediated conversion of lipid I to lipid II [31]. The X-ray structure of MRSA MurG is not available in the PDB database; thus, we could not computationally confirm the experimental data of Mendes et al. [48].
Table 4. Molecular docking scores and related properties.

| Receptor      | Drug     | Affinity * | H-Bonds | Receptor’s Rgyr (nm) | System’s Rgyr (nm) | Receptor’s SASA (nm²) | Receptor’s Prob. Drugability | Ligand’s SASA (nm²) | System’s SASA (nm²) | Contact Area (nm²) | Detected H-Bonds with AA Residue |
|---------------|----------|------------|---------|----------------------|-------------------|----------------------|-------------------------------|----------------------|-------------------|-------------------|---------------------------------|
| 2OLV (PBP2)   | 13       | −6.9       | 3       | 3.29                 | 3.29              | 295.26               | 0.82                          | 7.60                 | 295.24            | 3.80              | ASP 156, LYS 194, PRO 231       |
|               | 14       | −7.4       | 3       | 3.29                 | 3.29              | 295.26               | 0.82                          | 12.14                | 294.91            | 6.24              | ASP 156, LYS 194, PRO 231       |
|               | 15       | −8.1       | 2       | 3.29                 | 3.28              | 295.26               | 0.82                          | 7.87                 | 294.87            | 4.13              | ASP 89                         |
|               | 16       | −5.7       | 4       | 3.29                 | 3.28              | 295.26               | 0.82                          | 8.05                 | 295.15            | 4.08              | THR 87, GLN 92, HIS 94, GLU 95 |
|               | 17       | −7.8       | 1       | 3.29                 | 3.32              | 295.26               | 0.82                          | 7.90                 | 299.57            | 3.13              | ASP 156                         |
|               | 18       | −7.9       | 1       | 3.29                 | 3.29              | 295.26               | 0.82                          | 10.77                | 295.17            | 5.43              | PRO 72                          |
|               | 19       | −7.2       | 1       | 3.29                 | 3.30              | 295.26               | 0.82                          | 9.35                 | 295.14            | 4.73              | ASP 237                         |
| 4DKI * (PBP2a)| 13       | −6.7       | 1       | 3.66                 | 3.66              | 317.73               | 0.76                          | 7.09                 | 316.08            | 4.10              | THR 398                        |
|               | 14       | −7.2       | 3       | 3.66                 | 3.65              | 317.73               | 0.76                          | 11.21                | 316.08            | 6.43              | THR 398, GLY 520                |
|               | 15       | −9.2       | 1       | 3.66                 | 3.66              | 317.73               | 0.76                          | 7.55                 | 315.86            | 4.71              | THR 600, LEU 603, MER 605       |
|               | 16       | −5.9       | 4       | 3.66                 | 3.66              | 317.73               | 0.76                          | 7.87                 | 316.27            | 4.66              | ASP 516, GLN 521, MET            |
|               | 17       | −6.7       | 4       | 3.66                 | 3.67              | 317.73               | 0.76                          | 7.7                  | 317.2             | 3.9               | ASP 403, GLN 521, MET            |
| 3VSL (PBP3)   | 13       | −7.0       | 3       | 3.11                 | 3.11              | 301.97               | 0.81                          | 7.25                 | 300.48            | 4.37              | THR 525, GLU 623, GLN 626       |
|               | 14       | −7.0       | 3       | 3.11                 | 3.11              | 301.97               | 0.81                          | 12.00                | 299.12            | 7.42              | GLY 525, ASP 519, GLU 623       |
|               | 15       | −8.6       | 3       | 3.11                 | 3.11              | 301.97               | 0.81                          | 7.80                 | 300.81            | 4.48              | GLY 623                         |
|               | 16       | −5.3       | 1       | 3.11                 | 3.11              | 301.97               | 0.81                          | 8.01                 | 301.13            | 4.42              | GLN 626                         |
|               | 17       | −6.9       | 0       | 3.11                 | 3.11              | 301.97               | 0.81                          | 7.69                 | 300.78            | 4.44              | GLU 263                         |
|               | 18       | −7.6       | 2       | 3.11                 | 3.11              | 301.97               | 0.81                          | 11.22                | 302.30            | 5.44              | GLU 263                         |
|               | 19       | −6.7       | 3       | 3.11                 | 3.11              | 301.97               | 0.81                          | 11.88                | 301.39            | 6.23              | GLU 263                         |
| 5TXI * (PBP4) | 13       | −6.3       | 0       | 2.16                 | 2.17              | 151.84               | 0.8                           | 7.7212               | 155.88            | 1.83              | GLU 114, SER 262, TYR 268, TYR 291, GLU 297 |
|               | 14       | −9.1       | 5       | 2.16                 | 2.16              | 151.84               | 0.8                           | 11.821               | 150.85            | 6.40              | GLU 114, SER 262, TYR 268, TYR 291, GLU 297 |
|               | 15       | −7.0       | 0       | 2.16                 | 2.17              | 151.84               | 0.8                           | 7.951                | 156.74            | 1.5               | GLY 247                         |
|               | 16       | −5.6       | 0       | 2.16                 | 2.16              | 151.84               | 0.8                           | 7.9606               | 150.89            | 4.45              | GLU 114, SER 262, TYR 268, TYR 291, GLU 297 |
|               | 17       | −7.1       | 2       | 2.16                 | 2.17              | 151.84               | 0.8                           | 7.6                  | 155.2             | 2.6               | THR 240, GLY 247                |
|               | 18       | −8.1       | 3       | 2.16                 | 2.16              | 151.84               | 0.8                           | 10.5309              | 151.17            | 5.60              | GLU 114, SER 262, TYR 268, TYR 291, GLU 297 |
|               | 19       | −10.02     | 3       | 2.16                 | 2.16              | 151.84               | 0.8                           | 12.6803              | 151.04            | 6.73              | SER 116                        |
### Table 4. Cont.

| Receptor | Drug    | Affinity | H-Bonds | Receptor’s Rgyr (nm) | System’s Rgyr (nm) | Receptor’s SASA (nm$^2$) | Receptor’s Prob. Drugability | Ligand’s SASA (nm$^2$) | System’s SASA (nm$^2$) | Contact Area (nm$^2$) | Detected H-Bonds with AA Residue |
|----------|---------|----------|---------|----------------------|---------------------|--------------------------|-------------------------------|-------------------------|------------------------|------------------------|-------------------------------|
| 2W5Q (LtaS) | 13     | −6       | 1       | 2.07                 | 2.06                | 177.84                   | 0.81                          | 7.25                    | 178.03                 | 3.53                   | ASP 502                        |
|          | 14     | −6.2     | 0       | 2.07                 | 2.07                | 177.84                   | 0.81                          | 10.73                   | 177.85                 | 5.36                   | -                              |
|          | 15     | −7.8     | 1       | 2.07                 | 2.07                | 177.84                   | 0.81                          | 7.99                    | 178.05                 | 3.89                   | ASP 366                        |
|          | 16     | −5.7     | 0       | 2.07                 | 2.06                | 177.84                   | 0.81                          | 7.94                    | 176.11                 | 4.83                   | -                              |
|          | 17     | −7.5     | 1       | 2.07                 | 2.07                | 177.84                   | 0.81                          | 7.72                    | 184                    | 0.7                    | ASP 521                        |
|          | 18     | −8.9     | 2       | 2.07                 | 2.06                | 177.84                   | 0.81                          | 10.98                   | 176.25                 | 6.28                   | GLY 296, GLY 478              |
|          | 19     | −7.5     | 0       | 2.07                 | 2.06                | 177.84                   | 0.81                          | 9.2                     | 177.15                 | 4.98                   | -                              |
| 4WV J* (SpsB) | 13    | −7.3     | 2       | 2.77                 | 2.75                | 239.62                   | 0.82                          | 7.26                    | 238.84                 | 4.02                   | SER 343                        |
|          | 14     | −8.3     | 2       | 2.77                 | 2.75                | 239.62                   | 0.82                          | 12.90                   | 237.89                 | 7.31                   | TYR 182, ALA 330             |
|          | 15     | −9.5     | 2       | 2.77                 | 2.76                | 239.62                   | 0.82                          | 7.76                    | 238.10                 | 4.64                   | ASP 20                         |
|          | 16     | −6.1     | 0       | 2.77                 | 2.75                | 239.62                   | 0.82                          | 8.09                    | 237.67                 | 5.02                   | -                              |
|          | 17     | −7.1     | 0       | 2.77                 | 2.76                | 239.62                   | 0.82                          | 7.70                    | 238.36                 | 4.1                    | -                              |
|          | 18     | −7.5     | 2       | 2.77                 | 2.75                | 239.62                   | 0.82                          | 8.89                    | 238.49                 | 5.01                   | GLU 51, PRO 340              |
|          | 19     | −8.9     | 2       | 2.77                 | 2.74                | 239.62                   | 0.82                          | 10.14                   | 238.36                 | 5.70                   | GLU 50, VAL 378              |
| 6S7V * (LtaA) | 13    | −8.3     | 1       | 2.13                 | 2.12                | 192.79                   | 0.81                          | 7.55                    | 190.60                 | 4.87                   | GLY 259                        |
|          | 14     | −8.6     | 1       | 2.13                 | 2.12                | 192.79                   | 0.81                          | 11.06                   | 187.8256               | 8.01                   | ILE 256                        |
|          | 15     | −10.0    | 1       | 2.13                 | 2.12                | 192.79                   | 0.81                          | 7.91                    | 190.39                 | 5.15                   | TYR 377                        |
|          | 16     | −6.2     | 0       | 2.13                 | 2.12                | 192.79                   | 0.81                          | 7.84                    | 190.33                 | 5.15                   | -                              |
|          | 17     | −8.0     | 0       | 2.13                 | 2.12                | 192.79                   | 0.81                          | 7.8                     | 189.5                  | 3.8                    | -                              |
|          | 18     | −9.7     | 0       | 2.13                 | 2.12                | 192.79                   | 0.81                          | 11.06                   | 189.55                 | 7.15                   | -                              |
|          | 19     | −10.2    | 2       | 2.13                 | 2.12                | 192.79                   | 0.81                          | 9.71                    | 189.48                 | 6.51                   | ILE 230, TYR 377              |
| 6RYP * (LspA) | 13    | −7.4     | 1       | 1.86                 | 1.84                | 108.41                   | 0.82                          | 7.31                    | 107.33                 | 4.19                   | GLY 54                         |
|          | 14     | −10.0    | 2       | 1.86                 | 1.83                | 108.41                   | 0.82                          | 12.55                   | 105.94                 | 7.51                   | ASP 136                        |
|          | 15     | −10.5    | 0       | 1.86                 | 1.84                | 108.41                   | 0.82                          | 7.91                    | 106.88                 | 4.71                   | -                              |
|          | 16     | −7       | 0       | 1.86                 | 1.84                | 108.41                   | 0.82                          | 7.82                    | 105.99                 | 5.15                   | -                              |
|          | 17     | −8.1     | 2       | 1.86                 | 1.85                | 108.41                   | 0.82                          | 7.5                     | 107.3                  | 4.05                   | ILE 120, THR 140              |
|          | 18     | −9.2     | 2       | 1.86                 | 1.83                | 108.41                   | 0.82                          | 9.76                    | 106.50                 | 5.83                   | GLY 54, THR 140              |
|          | 19     | −11.5    | 1       | 1.86                 | 1.83                | 108.41                   | 0.82                          | 10.58                   | 106.47                 | 6.26                   | THR 140                        |

* Framed values indicate relative binding affinities < −9.0 kcal/mol.
Figure 5. (A) Schematic diagram of the lipoteichoic acid synthetic machinery in MRSA with the possible target of the active antimicrobial rhenium complexes. For more details about scheme (A), see [75]. Computer-generated lowest energy pose of the selected complex 19 in the hydrophobic C-terminal pocket of lipoteichoic acid flippase (LtaA): (B) side view; (C) top view; (D) detail of the binding region. In (C,D), the two amino acid residues most likely involved in the H-bonding interactions with 19 are shown in green.

Finally, in Figures 7 and 8, the hydrophobic gaussian surface was used for the graphical representation of the binding pockets of the ligands. The hydrophobicity scales of Wimley and White were used for defining the hydrophobicity of the amino acid residues [90]. This prediction assumes that apolar sites are preferentially disposed to the molecular interior, forming a hydrophobic core, whereas polar sites are disposed outside the molecular interior. In the Supplementary Materials (Figure S8), the representation of the protein surface of the non-polar polar ratio (NPP) [91] and the patch analysis of the electrostatic surface potential are depicted [92]. To analyze the effect on the distortion of the receptor and the conformation changes when binding the complex, the results of the Rg for the receptors and the complexes are presented in Table 2. As can be understood from the values, the Rgs of the explored systems did not change significantly for any of the shown complexes. The solvent accessible surface area (SASA) was also assessed for all cases. We did not observe intrinsic flexibility changes of the receptor SASA and system SASA, which can also be seen from the data in the Table 2. We found, in most cases, that the interfaces gain accessibility in order to promote stable interactions. The localization of the complexes preserves the SASA, which is an indication of the protein stability in the presence and absence of the complexes (i.e., ligands). With this property, we have a clearer picture of the existing changes in the protein conformation. The available surface area is maintained before and after the docking, and, as intuitively predicted, the rhenium complexes prefer localizing in the hydrophobic pockets of the possible target enzymes (Figures 7 and 8).
Figure 5. (A) Schematic diagram of the lipoteichoic acid synthetic machinery in MRSA with the possible target of the active antimicrobial rhenium complexes. For more details about scheme (A), see [75]. Computer-generated lowest energy pose of the selected complex in the hydrophobic C-terminal pocket of lipoteichoic acid flippase (LtaA): (B) side view; (C) top view; (D) detail of the binding region. In (C, D), the two amino acid residues most likely involved in the H-bonding interactions with the complex are shown in green.

Figure 6. (A) Schematic diagram of the lipoprotein post-translational processing pathway with the possible target of the active antimicrobial rhenium complexes. For more details about scheme (A), see [73]. Computer-generated lowest energy pose of selected complex in lipoprotein signal peptidase II (LspA): (B) side view; (C) top view; (D) detail of the binding region. In (C, D), the amino acid residue most likely involved in the H-bonding interactions with the complex is shown in green.

Finally, in Figures 7 and 8, the hydrophobic gaussian surface was used for the graphical representation of the binding pockets of the ligands. The hydrophobicity scales of Wimley and White were used for defining the hydrophobicity of the amino acid residues [90]. This prediction assumes that apolar sites are preferentially disposed to the molecular interior, forming a hydrophobic core, whereas polar sites are disposed outside the molecular interior. In the Supplementary Materials (Figure S8), the representation of the protein surface of the non-polar polar ratio (NPP) [91] and the patch analysis of the electrostatic surface potential are depicted [92]. To analyze the effect on the distortion of the receptor and the conformation changes when binding the complex, the results of the $R_g$ for the receptors and the complexes are presented in Table 2. As can be understood from the values, the $R_g$s of the explored systems did not change significantly for any of the shown complexes. The solvent accessible surface area (SASA) was also assessed for all cases. We did not observe intrinsic flexibility changes of the receptor SASA and system SASA, which can also be seen from the data in the Table 2. We found, in most cases, that the interfaces gain accessibility in order to promote stable interactions. The localization of the complexes preserves the SASA, which is an indication of the protein stability in the presence and absence of the complexes (i.e., ligands). With this property, we have a clearer picture of the existing changes in the protein conformation. The available surface area is maintained before and after the docking, and, as intuitively predicted, the rhenium complexes prefer localizing in the hydrophobic pockets of the possible target enzymes (Figures 7 and 8).

Figure 7. Gaussian surface representation of the hydrophobicity of (A) 15 and PBP2 (2OLV); (B) 19 and PBP2a (4DKI); (C) 15 and PBP3 (3VSL); and (D) 19 and PBP4 (5TXI). The red-blue color palette changes from hydrophilic blue to hydrophobic red.
Figure 8. Gaussian surface representation of the hydrophobicity of (A) 18 and Lipoteichoic acid synthase (LtaS; 2W5Q); (B) 15 and type-I signal peptidase (SpsB; 4WVJ); (C) 19 and Lipoteichoic acid flippase (LtaA), top view (6S7V); and (D) 19 and Lipoprotein signal peptidase II (LspA; 6RYP). The red-blue color palette changes from hydrophilic blue to hydrophobic red.

3. Conclusions

In this study, we reported the synthesis, characterization, and antimicrobial effects of a series of rhenium di- and tricarbonyl diimine complexes. Due to the lack of activity of the tested species, in an effort to identify the possible targets of the active complexes (and thus possibly understand the underlying reasons for the observed differences in the antimicrobial efficacy of Re complexes), we computationally evaluated the binding affinity of the active and inactive molecules against structurally characterized membrane-bound *S. aureus* proteins. Whereas the inactive compounds did not show an affinity for the enzymes, our docking protocol identified two possible major targets for some molecules of this class of compounds, namely lipoteichoic acid flippase (LtaA) and lipoprotein signal peptidase II (LspA). To our knowledge, our study is the first reported attempt to computationally identify MRSA biological targets of antibiotic rhenium complexes [93–97]. Experimental data are required in the future to confirm the in silico results, but our data are in line with the limited mechanistic studies that have previously been published on microbicidal rhenium species. Indeed, if the complexes inhibit the catalytic activity of LtaA and LspA, essential cell wall polymers cannot be assembled, leading to microbial death. We emphasize that LtaA and LspA may be targets for a fraction of known active antimicrobial Re complexes (namely 14, 15, 18, and 19 in this study). The penicillin-binding protein 2a (PBP2a) might also be targeted by 15 and 19, while MurG may be inhibited by 17. We were not able to identify possible targets for compounds 14 and 16; thus, their mechanism of action and targets remain unknown. We also showed that active rhenium complexes tend to localize in the hydrophobic pockets of target enzymes. In terms of the key molecular features common to active rhenium carbonyl complexes, our data support the notion that active diimine species are only cationic complexes of the \( \text{fac-}[\text{Re(CO)}_3]^+ \) core. If a CO ligand is substituted, leading to dicarbonyl \( \text{cis-}[\text{Re(CO)}_2]^+ \), regardless of the overall charge of the compounds, the molecules are devoid of any antimicrobial activity. Arguably, the most significant outcome of our study, i.e., the identification of LtaA and LspA as possible targets for this class of antibiotics, is that it offers the scientific community involved in this research.
support for the rational design of rhenium complexes based on the computational protocol for computer-aided drug design.

4. Materials and Methods

4.1. Reagents and Chemicals

All reagents and solvents were purchased from standard sources and used without further purification. The compound [Re(CO)3Br] was purchased from Sigma-Aldrich. Complexes (Et4N)[Re(CO)3Br] [62], 1a-2a [60], 1b–5b [61], 1c [98], 9a [99], 9b [100], 9c [101], and 10 [49] were synthesized according to published procedures. Unless otherwise noted, the solvents used in the preparation of all molecules were dry and O2-free.

4.2. Instruments and Analysis

A Bruker Advance III 400 MHz spectrometer was used to record the NMR spectra. The 1H chemical shifts of molecules are reported relative to the residual solvent protons. A Bruker FTMS 4.7-T Apex II spectrometer was used to perform the mass analysis in the positive mode. UV-vis spectra were measured on a Thermo Fisher Scientific Jasco V730 spectrophotometer. A Bruker TENSOR II spectrometer was used to record the IR spectra, with the following parameters: 16 scans for the background, and 32 scans for a sample, with a resolution of 4 cm⁻¹ in the 4000–600 cm⁻¹ region. Single crystals were measured on a Stoe IPDS2 diffractometer (CuKα1 (λ = 1.5406 Å)) equipped with a cryostat from Oxford Cryosystems. The ShelXT structure solution program was used to solve the crystal structures. We used the Intrinsic Phasing refined with the ShelXL refinement package, using least squares minimization [102,103]. Data were deposited in the Cambridge Crystallographic Data Centre. The CCDC numbers are 2184717–2184724. The elemental analysis was performed using a LECO CHNS-932 elemental analyzer.

4.3. Synthetic Procedures

(TDAE)[Re(CO)3(bpy)Br]2 (1′) was synthesized according to a similar published procedure [61]. Briefly, [Re(CO)3(bpy)Br]2 (1a, 63.5 mg, 114.0 µmol) was dissolved in dry CH2Cl2 (DCM) (20 mL) in a glove box. Tetrakis(dimethylamino)ethylene (TDAE, 13.24 µL, 57.0 µmol, 0.5 eq.) was dissolved in dry DCM (1 mL). The latter solution was added dropwise to the solution of 1a. The mixture was stirred under inert conditions for 15 min. The solvent was removed under reduced pressure, giving compound 1′ as a purple solid. Yield: 71.9 mg, 55.0 µmol, 96%. IR (cm⁻¹): 1861, 1775. UV-vis (DMF), λmax [nm]: 593, 426, 308, 300. The results of the elemental analysis are as follows: calculated for C34H36BrN6O3Re: C, 31.01%; H, 3.06%; N, 8.51%; found: C, 30.71%; H, 2.94%; N, 7.89%.

[Re(CO)3(bpy)(Melm)Br] (3a). Degassed complex 1′ (35.6 mg, 27.0 µmol) was dissolved in dry toluene (20 mL). Anhydrous N-methyl imidazole (Melm, 4.32 µL, 54 µmol, 2 eq.) was added, and the mixture was stirred at 100 °C for 48 h. The mixture was cooled to room temperature and the brown precipitate was isolated by centrifugation, giving compound 3a. Yield: 15.8 mg, 28.1 µmol, 52%. Single crystals suitable for X-ray diffraction were grown by layering pentane on a CH2Cl2 solution of the compound, giving dark brown crystals. IR (cm⁻¹): νCO: 1877, 1779. UV-vis (DMSO), λmax [nm]: 528, 375, 310, 303. 1H NMR (400MHz, CD2Cl2, ppm): δ: 9.06 (dd, J = 5.5, 1.1 Hz, 2 H), 8.70 (d, J = 8.2 Hz, 2 H), 8.30 (td, J = 7.9, 1.5 Hz, 2 H), 7.69 (ddd, J = 7.6, 5.6, 0.9 Hz, 2 H), 7.28 (s, 1 H), 4.79 (t, J = 1.4 Hz, 1 H), 6.59 (t, J = 1.5 Hz, 1 H), 3.57 (s, 3 H). ESI-MS (MeOH): m/z: 582.9 [M + Na]+. The results of the elemental analysis are as follows: calculated for C16H14BrN4O2Re: C, 34.29%; H, 2.52%; N, 10.00%; found: C, 34.70%; H, 2.40%; N, 9.52%.

[Re(CO)3(bpy)(py)]PF6 (4a) was synthesized according to a similar published procedure [61]. Briefly, complex 2a (54 mg, 97 µmol) and pyridine (py, 1 mL, ca. 100 eq.) were dissolved in MeOH (20 mL), and the mixture was stirred at 70 °C overnight. The solvent was removed under reduced pressure. The residue was dissolved in water (75 mL), and a solution of KPF6 (36 mg, 194 µmol, 2 eq.) in water (5 mL) was added dropwise to the rhenium. The precipitate was isolated by centrifugation, giving compound 4a as a brown-
orange solid. Yield: 45 mg, 64.1 µmol, 66%. Single crystals suitable for X-ray diffraction were grown by the diffusion of pentane into an acetone solution of the compound, giving dark orange crystals. IR (cm\(^{-1}\)), \(\nu_{\text{CO}}\): 1901, 1823. UV-vis (DMF), \(\lambda_{\text{max}}\) [nm]: 481, 357, 302.

\(^1\)H NMR (400 MHz, CD\(_2\)Cl\(_2\), ppm) \(\delta\): 9.32 (dd, \(J = 0.73, 1.56, 5.41\) Hz, 2H), 8.36–8.40 (m, 4H), 8.34 (d, \(J = 8.19\) Hz, 2H), 8.18 (dt, \(J = 1.59, 7.95\) Hz, 2H), 7.74 (dd, \(J = 1.28, 5.47, 7.67\) Hz, 2H), 7.59–7.65 (m, 2H), 7.05–7.11 (m, 4H). \(^{13}\)C NMR (101 MHz, CD\(_2\)Cl\(_2\), ppm) \(\delta\): 205.9 (2C), 156.6 (2C), 155.3 (4C), 152.5 (2C), 141.1 (2C), 137.6 (2C), 129.3 (2C), 126.5 (4C), 125.1 (2C). ESI-MS (MeOH): \(m/z\) (2C), 567.6 [M]+. The results of the elemental analysis are as follows: calculated for C\(_{13}\)H\(_8\)F\(_6\)N\(_4\)O\(_2\)PRe: C, 33.76%; H, 2.59%; N, 7.99%; found: C, 33.75%; H, 2.72%; N, 7.67%.

\([\text{Re} \left(\text{CO}\right)\text{2(bpy)} \left(\text{Melm}\right)] \left(\text{PF}_{6}\right)\) (5a). Compound 1′ (132 mg, 100 µmol) was dissolved in anhydrous Melm (8 mL) and the mixture was stirred at 110 °C for 60 min. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (eluent: EtOAc, then DCM/MeOH 100:0, increased to 98:2). The first fraction, compound 3a, was collected with the first gradient (100% EtOAc) as a yellow solid (amount: traces). The second fraction was collected with the last gradient as mobile phase. Once dried, the counter ion was exchanged with KPF\(_6\) (17.2 mg, 93.4 µmol) in H\(_2\)O (15 mL). Compound 5a was isolated by centrifugation as a violet solid. Yield: 24 mg, 33.9 µmol, 17%. IR (cm\(^{-1}\)), \(\nu_{\text{CO}}\): 1885, 1802. UV-vis (DMF), \(\lambda_{\text{max}}\) [nm]: 500, 363, 307, 300.

\(^1\)H NMR (400 MHz, CD\(_2\)Cl\(_2\), ppm) \(\delta\): 9.21–9.26 (m, 2H), 8.32 (dd, \(J = 0.86, 8.19\) Hz, 2H), 8.12 (dt, \(J = 1.59, 7.95\) Hz, 2H), 7.62 (ddd, \(J = 1.22, 5.44, 7.64\) Hz, 2H), 7.29 (s, 2H), 6.63–6.67 (t, 2H), 6.44–6.51 (t, 2H), 3.53 (s, 6H). \(^{13}\)C NMR (101 MHz, CD\(_2\)Cl\(_2\), ppm) \(\delta\): 190.6 (2C), 157.6 (2C), 152.5 (2C), 141.2 (2C), 140.4 (2C), 134.0 (2C), 128.6 (2C), 124.5 (2C), 122.3 (2C), 34.7 (2C). ESI-MS (MeOH): \(m/z\) 567.6 [M]+. The results of the elemental analysis are as follows: calculated for C\(_{20}\)H\(_{18}\)F\(_6\)N\(_4\)O\(_2\)PRe: C, 33.95%; H, 2.85%; N, 11.88%; found: C, 34.29%; H, 2.74%; N, 11.52%.

\((\text{TDAE})[\text{Re} \left(\text{CO}\right)\text{2(phen)} \left(\text{Br}\right)]\) (1′′) was synthesized according to a similar published procedure [61]. Briefly, cis-[Re\((\text{CO})\text{2(phen)} \left(\text{Br}\right)]\) (62.8 mg, 107.9 µmol) was dissolved in dry DCM (17 mL) in a glove box. TDAE (12.56 µL, 53.9 µmol, 0.5 eq.) was dissolved in dry DCM (1.5 mL). The latter solution was added dropwise to the rhenium in the glove box, and the mixture was stirred for 15 min at room temperature. The solvent was removed under reduced pressure, giving 1′′ as a brown-purple solid. Yield: 67.7 mg, 49.6 µmol, 92%. IR (cm\(^{-1}\)), \(\nu_{\text{CO}}\): 1856, 1771.

\([\text{Re} \left(\text{CO}\right)\text{2(phen)(py)} \left(\text{Br}\right)]\) (2c). Degassed complex 1′′ (20 mg, 14.7 µmol) was dissolved in degassed pyridine (2 mL) and the mixture was stirred at 100 °C for 20 min. The reaction mixture was cooled down to room temperature and extracted in DCM (50 mL) with HCL 0.1 M (3 × 50 mL). The organic phase was dried over sodium sulfate, and the solvent was removed under reduced pressure. The crude was purified by flash column chromatography (stationary phase: aluminum oxide, mobile phase: pentane/EtOAc/MeOH 1:2:0, increased to 0:1 and finally 0:99:1), giving compound 2c as a brown solid. Yield: 0.9 mg, 1.6 µmol, 5%. IR (cm\(^{-1}\)), \(\nu_{\text{CO}}\): 1864, 1778. UV-vis (DMSO), \(\lambda_{\text{max}}\) [nm]: 511, 380, 269. \(^1\)H NMR (400MHz, CD\(_3\)CN, ppm) \(\delta\): 9.61 (dd, \(J = 5.1, 1.2\) Hz, 2H), 8.84 (dd, \(J = 8.3, 1.3\) Hz, 2H), 8.25–8.29 (m, 2H), 8.17 (s, 2H), 8.11 (dd, \(J = 8.3, 5.1\) Hz, 2H), 7.71–7.78 (m, 1H), 7.19 (s, 2H). The results of the elemental analysis are as follows: calculated for C\(_{10}\)H\(_{13}\)BrN\(_3\)O\(_2\)Re: C, 39.25%; H, 2.25%; N, 7.23%; found: C, 39.42%; H, 2.15%; N, 6.99%.

\([\text{Re} \left(\text{CO}\right)\text{2(phen)(MeIm)} \left(\text{Br}\right)]\) (3c). Degassed complex 1′′ (17 mg, 12.5 µmol) was dissolved in anhydrous Melm (2 mL) and the mixture was stirred at 110 °C for 20 min. The reaction mixture was cooled down to room temperature and extracted in DCM (50 mL) with HCl 0.1 M (3 × 50 mL). The organic phase was dried over sodium sulfate, and the solvent was removed under reduced pressure. The crude was purified by flash column chromatography (stationary phase: aluminum oxide, mobile phase: pentane/EtOAc 1:1, increased to 0:1), giving compound 3c as a violet solid. Yield: 1.8 mg, 31.1 µmol, 12%. IR (cm\(^{-1}\)), \(\nu_{\text{CO}}\): 1876, 1773. UV-vis (DMSO), \(\lambda_{\text{max}}\) [nm]: 528, 275. \(^1\)H NMR (400MHz, (CD\(_3\))\(_2\)SO, ppm) \(\delta\): 9.46–9.50 (m, 2H), 8.73–8.78 (m, 2H), 8.21 (s, 2H), 8.01–8.07 (m, 2H),
7.64–7.67 (m, 1H), 6.81–6.84 (m, 1H), 6.46–6.49 (m, 1H), 3.41 (s, 3H). The results of the elemental analysis are as follows: calculated for C_{18}H_{34}BrN_{4}O_{2}Re: C, 36.99%; H, 2.41%; N, 9.59%; found: C, 37.38%; H, 2.73%; N, 10.05%.

The following general procedure was applied for the synthesis of complexes 6–8 [50]. To a solution of [Re(CO)_{5}]Br (1.0 equiv.) in hot toluene, the appropriate bipyridine (bpy) ligand (1.0 equiv.) was added, and the mixture was refluxed for 7–9 h. After the solution had cooled to the room temperature, the reaction mixture was filtered and washed with cold toluene (2×), yielding fac-[Re(CO)_{3}(bpy)Br] as a bright fluorescent yellow powder. The solid was then dried in vacuo for 24 h. The complexes were found to be pure (≥ 96%) by NMR and HPLC.

fac-[Re(CO)_{3}(4′,4′-di-tert-butyl-2,2′-bipyridine)] (6), where 4′-bpy is 4,4′-di-tert-butyl-2,2′-bipyridine. Pale yellow solid, yield 92%. IR (solid, cm^{-1}): νCO: 2016, 1999, 1849. UV-vis (DMF), λ_{max} [nm]: 368, 292. \textsuperscript{1}H-NMR (400 MHz, CD_{3}CN, ppm): 8.96 (d, J = 5.99 Hz, 2H) 8.10 (d, J = 1.89 Hz, 2H) 7.51 (dd, J = 5.87, 1.96 Hz, 2H) 1.45 (s, 1H). ESI\textsuperscript{-}MS (MeOH): m/z, 576.9 [Re(CO)_{3}(C_{18}H_{34}N_{2}) (H_{2}O)]\textsuperscript{+}, [M-Br + H_{2}O]\textsuperscript{+}. Single crystals suitable for X-ray diffraction were grown by the diffusion of pentane into a DCM solution of the compound, giving yellow needles. The results of the elemental analysis are as follows: calculated for C_{21}H_{32}BrN_{2}O_{2}Re: C, 40.78%; H, 3.91%; N, 4.53%; found: C, 40.43%; H, 4.08%; N, 4.44%.

fac-[Re(CO)_{3}(CF_{3}-bpy)Br] (7), where CF_{3}-bpy is 4,4′-trifluoromethyl-2,2′-bipyridine. Orange solid, yield 87%. IR (solid, cm\textsuperscript{-1}): νCO: 2015, 1932, 1897. UV-vis (DMF), λ_{max} [nm]: 417, 304. \textsuperscript{1}H-NMR (400 MHz, CD_{3}CN, ppm): 9.33 (d, J = 5.75 Hz, 2H) 8.46 (d, J = 5.75, 1.22 Hz, 2H). ESI\textsuperscript{-}MS (MeOH): m/z, 580.7 [Re(CO)_{3}(C_{12}H_{14}F_{6}N_{2})(H_{2}O)]\textsuperscript{+}, [M-Br + H_{2}O]\textsuperscript{+}. Single crystals suitable for X-ray diffraction were grown by the diffusion of pentane into a DCM solution of the compound, giving orange needles. The results of the elemental analysis are as follows: calculated for C_{13}H_{9}BrF_{3}N_{2}O_{2}Re: C, 28.05%; H, 0.94%; N, 4.36%; found: C, 27.59%; H, 1.12%; N, 4.23%.

fac-[Re(CO)_{5}(Et_{2}N-bpy)Br] (8), where (Et_{2}N-bpy is N4,N4,N4,N4-tetraethyl-[2,2′-bipyridine]-4,4′-diimine. Pale yellow solid, yield 92%. IR (solid, cm\textsuperscript{-1}): νCO: 2008, 1886, 1866. UV-vis (DMF), λ_{max} [nm]: 367, 373. \textsuperscript{1}H-NMR (400 MHz, CD_{3}CN, ppm): 8.49 (d, J = 6.60 Hz, 2H) 7.04 (d, J = 2.81 Hz, 2H) 6.54 (dd, J = 6.72, 2.69 Hz, 2H) 3.49 (q, J = 7.21 Hz, 8H) 1.28 (t, J = 7.21 Hz, 12 H). ESI\textsuperscript{-}MS (MeOH): m/z, 568.9 [Re(CO)_{5}(C_{18}H_{26}N_{4})\textsuperscript{+}, [M-Br]\textsuperscript{+}. Single crystals suitable for X-ray diffraction were grown by the diffusion of pentane into a DCM solution of the compound, giving yellow needles. The results of the elemental analysis are as follows: calculated for C_{26}H_{26}BrN_{4}O_{2}Re: C, 38.89%; H, 4.20%; N, 8.64%; found: C, 38.45%; H, 4.17%; N, 8.47%.

cis-[Re(CO)_{2}(batho-phen)Br]_{2} (11). Degassed (Et_{4}N) [Re(CO)_{2}Br] (500 mg, 722 \textmu mol) and batho-phen (240 mg, 722 \textmu mol) were dissolved in dry DCM (80 mL). The mixture was stirred under inert conditions at room temperature for 72 h. The solvent was removed under reduced pressure, and the crude was purified by flash column chromatography on silica (Eluent: DCM / Pentane 1:9), giving complex 11 as an orange-red solid. Yield: 82 mg, 112 \textmu mol, 15%. Single crystals suitable for X-ray diffraction were grown by the slow evaporation of DCM solution of the compound, giving dark brown needles. IR (cm\textsuperscript{-1}), νCO: 1999, 1849. UV-Vis (DMF), λ_{max} [nm]: 429, 288. The results of the elemental analysis are as follows: calculated for C_{26}H_{18}Br_{2}N_{5}O_{2}Re: C, 42.52%; H, 2.20%; N, 3.81%; found: C, 42.00%; H, 2.42%; N, 3.84%.

4.4. Biological Tests

The culture conditions and in vitro antimicrobial activity determination were performed exactly as previously reported [60]. We thank Dr Aleksandra Barac (University Clinical Center of Serbia) and Prof. Cornelia Lass-Floerl (University of Innsbruck) for kindly providing the clinical C. auris strain 7. All other microbial strains (Enterobacter cloacae ATCC 3047, Klebsiella pneumoniae ATCC 13803, Acinetobacter baumannii ATCC 19606, Pseudomonas aeruginosa PAO1 NCTC10332, Staphylococcus aureus MRSA43300 (methicillin-resistant) and S. aureus ATCC25923 (methicillin-sensitive), and Candida albicans SC5314) were obtained...
from the American Type Culture Collection (ATCC) and the National Collection of Type Cultures (NCTC).

4.5. In Silico Calculations

4.5.1. Preparation of the Ligand Database and Ligands: Receptors Complexes

The docking calculations were performed with AutoDock Vina version 1.2.0 (The Scripps Research Institute, La Jolla, San Diego, CA, USA) [14] and AutoDock4 version 4.2.6 (AD4, The Scripps Research Institute, La Jolla, San Diego, CA, USA) [104]. The receptor/protein PDBQT files were prepared, and the grid box size was determined using the AutoDock Tools version 1.5.7 (ADT, Scripps Research Institute, La Jolla, San Diego, CA, USA) [104]. Biovia Discovery Studio Visualizer 2021, version 21.1.0.20298 (Dassault Systèmes, San Diego, California, CA, USA), was used to visualize the receptor and ligand interactions. Figures were prepared with the ADT software. The structures of complexes 5–11 and 15 were obtained by the determined X-ray structures. Chemical structures as .CIF files were converted to .MOL2 files using the Mercury (Build RC1) software, version 3.7 (CCDC 2001–2015). All complexes (ligands) were optimized with the hybrid meta-GGA functional wB97XD [105–109], designed to account for dispersion, which was used in combination with the standard SDD basis sets [110]. The optimized structures were subject to frequency analysis to verify that they represented minima on the potential energy surface. All calculations were performed with Gaussian 09 software (version 5.0.9, Carnegie Mellon University, Gaussian, Inc., Wallingford, CT, USA).

The ADT software was then used to investigate the complexes’ structures in terms of their combinations with nonpolar hydrogens, additions of Gasteiger changes, and rotatable bonds. The rhenium atom is not parametrized in AD4 and AutoDock Vina; thus, AutoDock Vina calculations were performed using Mn instead of Re. The resulting binding poses of the Mn complexes were then cross-checked with the corresponding Re complexes using AD4, where the following line was added to the AD4 atom parameters file: “atom_par Re 2.95 0.066 12.000 -0.00110 0.0 0 -1 -1 1 # Non-H-bonding”. The binding poses of the Mn and Re complexes were found to be the same. Moreover, due to the fact that ADT failed to assign a Gasteiger change to the metal ion, a charge of 0.320 (to either Mn or Re) was assigned to the atom by editing the corresponding .PDBQT file [111].

The crystal structure of *S. aureus* proteins were obtained from the RCSB protein data bank (http://www.rcsb.org). Only the structures of membrane protein annotation (PDBTM, MemProtMD, OPM or mpstruc) were considered and selected. All water molecules were removed, and the required files for AutoDock Vina and AD4 were prepared by assigning hydrogens and Kollman charges to the protein structures, and finally converting them from the .PDB file format to .PDBQT file format.

4.5.2. Molecular Docking

The docking calculations were conducted using the AutoDock Vina software (https://vina.scripps.edu/) with adapted parameters for the rhenium complexes. The extended version of the Vina code was used via the integrated platform SAMSON [https://www.samson-connect.net] as a SAMSON extension [112]. This provides additional functionality when preparing receptors and ligands, docking libraries, and analyzing and exporting docking results. Both the number of flexible side chains and the size of the search domain were different for all the cases because of the receptor’s conformation (i.e., the chain orientation and position of residues). On average, there were about 30 flexible side chains with unlocked rotatable bonds. The search space was defined by a docking box wrapper of the space around the receptors. The scaling of the box depended on the defined pocket score. The number of modes was set to 200 with an energy range = 3 kcal/mol (default value). The energy range is the maximum energy difference between the best binding mode and the unfavorable one displayed (kcal/mol). Energy (affinity) values that differed by more than 3 kcal/mol from the best mode were not saved among the results. In the configuration file, the parameter called “exhaustiveness” was set to 40. This parameter
controls how comprehensive the search space is. In AutoDock Vina, the electrostatic interactions were handled with the hydrophobic and the hydrogen bonding terms. The post-docking analysis of the favorable ligand–receptor complexes was performed via the Protein–Ligand Interaction Analyzer Extension in SAMSON [112]. With the help of the Protein–Ligand Interaction Analyzer, it was possible to calculate the radius of gyration, hydrogen bonds, residues surrounding the ligand, and solvent-accessible surface area (SASA) of the receptor and ligand and of the form complexes. The multistep validation protocol was considered in this study, and the ability of the combined methodology was examined independently through initial screening and extensive semi-flexible docking.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ph15091107/s1, Figures S1–S8: 1H-NMR spectra of compounds; Figure S9: IR spectra (solid state) of compounds; Figure S10: UV-vis spectra (in DMF) of compounds; Figure S11: Visualization of the surface protein surface polarity: (A) non-polar to polar SASA color-coded from low NPP ratio (purple) to high NPP ratio (green); and (B) color-coded from negative charge (red) to positive charge (blue). Regions of high hydrophobicity are colored green, low hydrophobicity are colored purple; Figure S12a: Binding orientation of the compounds with hydrogen–acceptor and hydrogen–donor distances: A. 15 and PB2P: 2OLV; B. 19 and PB2P2a: 4DKI; C. 15 and PB3P: 3VSL; D. 19 and PB4P: 5TXI; Figure S12b: Binding orientation of the compounds with hydrogen–acceptor and hydrogen–donor distances: A. 18 and lipoteichoic acid synthase (LtaS): 2WSQ; B. 15 and type-I signal peptidase (SpsB): 4WVJ; C. 19 and lipoteichoic acid flippase (LtaA): 6S7V; D. 19 and lipoprotein signal peptidase II (LspA): 6RYP; Table S1a: In silico pre-screening of binding affinities (b.a.; docking scores, kcal/mol) of rhenium complexes against structurally characterized membrane-bound S. aureus proteins: penicillin-binding proteins (PBPs); Table S1b: In silico pre-screening of binding affinities (b.a.; docking scores, kcal/mol) of non-toxic complexes against other structurally characterized membrane-bound S. aureus proteins; Table S2: Pockets prediction: mapping the ranking with residue environment distribution; Table S3: Percentage distribution of the surrounding residue types for the two groups of proteins; Table S4a–S4i: Selected bond lengths of the complexes; Table S4a’–S4i’: Selected bond angles of the complexes.

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