SOCIETY FOR NATURAL SCIENCES STUDENT JOURNAL

Beyond Penicillin: Metal Complexes as the Next Generation Antimicrobial Agents

A Chemical Approach to the Antibiotic Crisis

Elizabeth Kálusová

School of Natural Sciences, Durham University, Durham, DH1 3LE, UK

Supervisor: Dr John M. Sanderson

1. Introduction

1.1 The Rise of Antibiotic Resistance

In 2024, the World Health Organisation (WHO) recognised 15 pathogens with significant resistance to multiple antibiotics. Alarmingly, some exhibited nearly complete resistance, posing a severe threat to human health.[1] Consequently, there is an ongoing search for new antibiotic classes, with metal complexes playing an important role in this pursuit of novel treatments.

Paul Ehrlich's discovery of synthetic prodrugs arsphenamine, also known as Salvarsan, and Neosalvarsan to treat syphilis caused by *Treponema pallidum*, and the derivation of the first antibiotic penicillin from mould *Penicillium rubens* in 1928 by Sir Alexander Fleming, mark the beginning of the most prolific era of antibiotic discovery.[2] In 1940, the same year penicillin became a commercialised antibiotic, the first resistant strain of *Escherichia coli* was identified. This resistance was due to the ability of this strain to inactivate penicillin by penicillinase production.[3] By the late 1960s *Staphylococcus aureus* and *Staphylococcus pneumoniae* followed in gaining resistance, later continued by high rates of penicillin resistance in *Enterobacteriaceae*.[3] Similar to penicillin, a rapid succession between antibiotic discovery and the emergence of resistance was observed with other antibiotics.[2] For methicillin, introduced in 1959, resistance of *Staphylococcus* was reported in 1960.[2] Following its discovery in 1953, vancomycin-resistant coagulase-negative *Staphylococci* were reported in 1979, and vancomycin resistance in *Enterococci* was described in 1989.[2]

In 2022, the Global Antimicrobial resistance and Use Surveillance System (GLASS) report revealed evidence of concerningly high resistance in pathogens causing hospital-acquired infections (HAIs).[4] Strain resistance at rates as high as 57% in *Klebsiella pneumoniae*, and over 56% in *Acinetobacter* species were documented.[4] *K. pneumoniae* has been identified as the top priority pathogen in the 2024 WHO Bacterial Priority Pathogen List (BPPL) rising from 5th place in 2017 (Table 1), with *Acinetobacter baumannii* shortly following in third place.[1] These gram-negative bacterial pathogens lead to various infection syndromes, including bloodstream and urinary tract infections, and are particularly problematic among intensive care patients, causing severe HAIs.[4]

Table 1: *Top 10 Pathogens Prioritised in the 2024 WHO Bacterial Priority Pathogens List.*[1]

Priority	Pathogen	Gram Type	Antibiotic Class Resistance	Infection(s)†
1	*Klebsiella pneumoniae*	gram-negative	Carbapenem	Bloodstream, respiratory tract, urinary tract, intra abdominal, hospital-acquired
2	*Escherichia coli* (3rd generation*)	gram-negative	Cephalosporin	Bloodstream, respiratory tract, urinary tract, intra abdominal
3	*Acinetobacter baumannii*	gram-negative	Carbapenem	Blood, urinary tract, lungs, wounds, hospital-acquired
4	*Mycobacterium tuberculosis*	variable	Rifampicin	Tuberculosis
5	*Escherichia coli*	gram-negative	Carbapenem	Device-associated, urinary tract, pneumonia
6	*Klebsiella pneumoniae* (3rd gen.)	gram-negative	Cephalosporin	Pneumonia, bloodstream, urinary tract
7	*Salmonella* Typhi	gram-negative	Fluoroquinolone	Typhoid fever
8	*Shigella* species	gram-negative	Fluoroquinolone	Shigellosis, gastrointestinal
9	*Enterococcus faecium*	gram-positive	Vancomycin	Abscesses, wounds, peritonitis, urinary tract
10	*Pseudomonas aeruginosa*	gram-negative	Carbapenem	Surgical site, urinary tract, bloodstream, hospital-acquired

* 3rd generation refers to resistance to third-generation cephalosporin antibiotics, which are broad-spectrum antibiotics effective against a wide range of gram-negative pathogens.[4]
† Infection refers to the specific type of illness or diseases that the listed pathogen can cause.

1.2 Bacterial Classification

Bacteria are classified by structural characteristics and appearance after Gram staining. Gram-positive organisms retain the crystal violet-iodine stain due to a thick peptidoglycan layer composed of alternating N-acetyl glucosamine and N-acetylmuramic acid, cross-linked to peptides (Figure 1).[5] This layer, embedded with teichoic and lipoteichoic acids, provides rigidity and allows for pathogenicity and ion transport.

Gram-negative bacteria contain cytoplasmic and outer membranes, with the outer membrane comprising a lipopolysaccharide outer leaflet and a phospholipid inner leaflet (Figure 1).[6] Their thinner peptidoglycan layer fails to retain the crystal violet-iodine complex, therefore cells appear red from the safranin counterstain.

Multi-drug-resistant (MDR) gram-negative bacteria pose significant challenges to global health. Resistance is acquired through several mechanisms, including mutation, horizontal gene transfer, enzymatic degradation of antibiotics, efflux pumps, reduced membrane permeability, and adaptive survival strategies such as biofilm formation, where microorganisms form a network encased in a protective polymeric matrix.[7] The global impact of MDR underscores the urgent need for non-antibiotic strategies to manage and prevent gram-negative infections.[7]

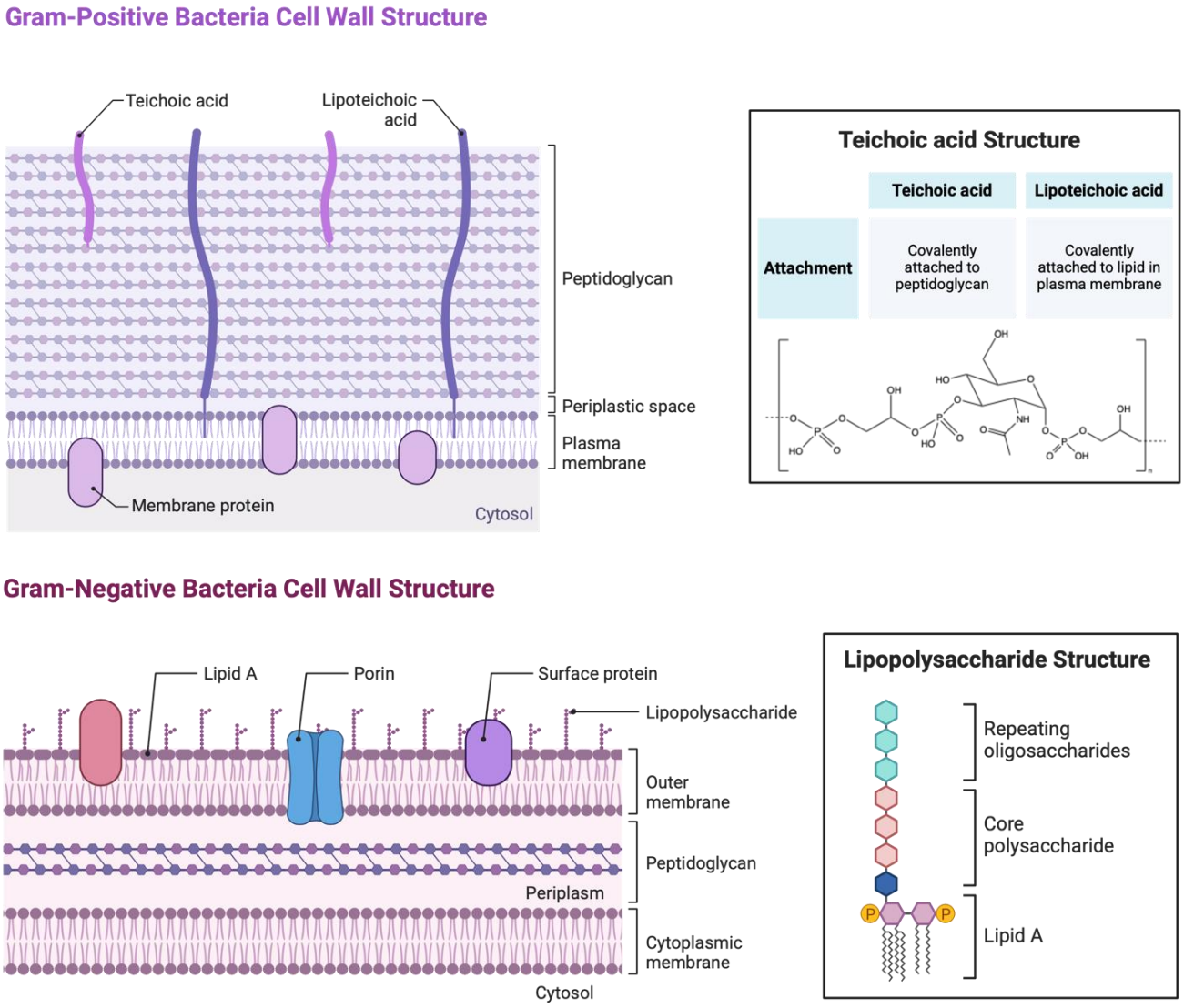
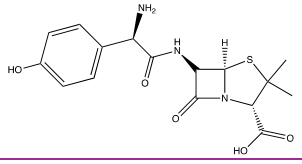
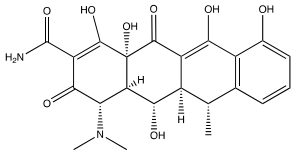
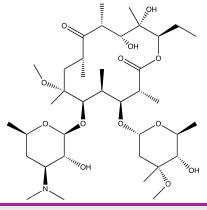
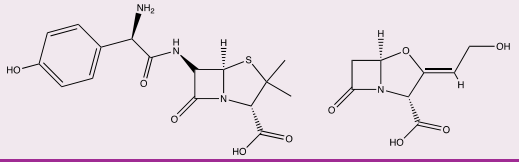
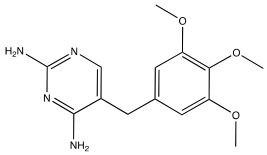
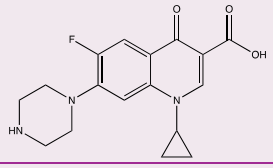
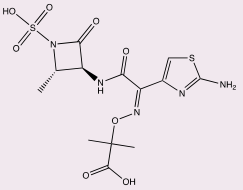
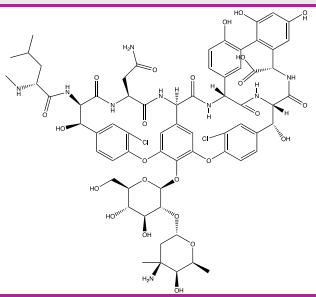
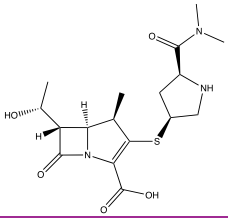


Figure 1: *Structures of bacterial gram-positive (top) and gram-negative (bottom) cell wall structures. Created with BioRender.com.*

Table 2: *Prescribing frequency of antibiotic classes and associated drug examples according to the English Surveillance Programme for Antimicrobial Utilisation and Resistance (2023-2024), adapted from ref. 19.*

Antibiotic Class	Prescribing Frequency /%	Drug Example	Structure
Penicillin*	32.7	amoxicillin	
Tetracyclines	25.8	doxycycline	
Macrolides, Lincosamides, Streptogramins	13.3	clarithromycin	
Beta-lactam/ beta-lactamase-inhibitor combinations	8.2	co-amoxiclav	
Sulfonamides and Trimethoprim	7.2	trimethoprim	
Fluoroquinolones	2.5	ciprofloxacin	
Cephalosporins and Monobactams	2.0	monobactams: aztreonam	
Other (eg. Glycopeptides, Carbapenems)	8.3	glycopeptides: vancomycin	
		carbapenems: meropenem	

* Excluding beta-lactam/beta-lactamase-inhibitor combinations

Decrease in usage reported due to resistance concerns.

1.3 Factors contributing to Antimicrobial Resistance (AMR)

The discovery of novel antibiotics rapidly slowed down after the 1980s, however, drug-resistant pathogen continued to threaten worldwide public health. Drug-resistant pathogens develop resistance to multiple or all antimicrobial treatments through natural selection and genetic adaptation. This natural process is named antimicrobial resistance, and it presents an enormous risk to modern medicine.[4] Murray *et al.* (2022) estimated 4.95 million deaths related to bacterial AMR in 2019, with 1.27 million directly attributable to AMR, whereas Lord Jim O'Neill's review estimates deaths attributable to AMR to rise to 10 million by 2050 exceeding cancer mortality by 1.8 million.[8-10] While this prediction does have limitations in the form of errors, biases, and limited reporting of AMR data before 2000, the projected 10 million deaths demand action to protect humanity from this growing global health threat.[10] AMR requires a well-coordinated global response, but addressing it is problematic due to a lack of alternative options to antibiotics.

AMR arises due to various complex contributing factors, with excessive and incorrect use of antibiotics, along with improper prescription practices, significantly accelerating resistance development. This includes instances where patients receive antibiotics without adequate bacterial isolation and susceptibility testing, or the incorrect type, dosage, or duration of treatment.[2] Table 2 presents the most frequently prescribed antibiotic classes and their usage patterns.

Overuse of antibiotics in livestock results in antibiotic residues in animal-derived products, and this, coupled with the ease of modern transportation, increases the risk of transmitting resistant bacteria and contributes to AMR.[2,11] AMR directly threatens medical treatment effectiveness and indirectly affects procedures such as surgery or chemotherapy, where infection management is a priority. Furthermore, AMR not only affects the global and individual health and healthcare systems, but also the economy and agriculture.

The European Environment Agency reports a 28.3% decrease in sales of antimicrobials for farmed animals and aquaculture between 2018 and 2022 in the EU-27 due to stewardship efforts.[12] The implementation of antimicrobial stewardship programmes in human medicine could help alleviate some of the demanding pressure to develop novel antibiotics.[1] However, alarmingly, these efforts are yet to yield significant successes. Many pharmaceutical companies withdrew investments in infectious disease drug discovery due to the rapid development of resistance and profitability concerns.[1]

1.4 Metal Complexes as Antimicrobials

Historically, metals and metal complexes have been used as antimicrobials despite limited understanding of their mechanisms of action.[10] Examples include earlier mentioned Salvarsan which utilised arsenic, a gold compound Auranofin used for treatment of rheumatoid arthritis, or chemotherapeutic platinum-based drug Cisplatin discovered in 1978 and still used today.[14] While various metal-based complexes reached human clinical trials for treatment of cancer (palladium-based for prostate cancer), malaria, and neurodegenerative disease, a limited number of studies have focused on antimicrobial applications of metal complexes.[13]

The broad mechanisms of action of metal complexes, discussed further in section 2, make it more challenging for bacterial resistance to develop in comparison to antibiotics. Further, the customisable properties, such as ligand design or chelation, allow for selective targeting of bacterial cells while sparing human cells, positioning metal complexes as a plausible source for novel antimicrobials.[15] Similarly, metal complexes show potential in eradicating biofilm cultures, known to enhance bacterial resistance to antibiotics. Harrison *et al.* (2004) demonstrated that biofilm-embedded bacteria did not exhibit additional resistance to metal toxicity compared to planktonic cells, suggesting their effectiveness.[16]

While their application seems promising, there are issues associated with the use of metal complexes as antimicrobials. Toxicity is a crucial consideration as

high toxicity in mammalian cells can result in severe side effects, a strong immune response or apoptosis. Frei *et al.* (2020) identified 88 non-toxic metal-based compounds of hit-rate 9.9% in addition to recognising that the ligand environment plays a significant role in modulating toxicity.[14] This study agreed with Morrison *et al.* (2020) on employing metal complexes for drug development, however addressing toxicity remains challenging.[17] With estimated antibiotic consumption of 40 billion doses per day in 2018 proposed by Browne *et al.* (2021), the environmental consequences of using metals as antimicrobials need to be closely considered.[18] Mining of metals, transportation cost, potential resistance development as part of a natural selection process, and the unpredictable effects on microbiome are negative consequences which would require further research and regulation.

Although significant challenges remain, metalloantimicrobials offer the most promising avenue for combating antimicrobial resistance and the threat it poses on global health.[13] This review examines the mechanisms of action of metal complexes exerting antimicrobial effects, compares antimicrobial efficacy of various metal complexes, and analyses the demonstrated synergistic effects of metal complexes.

2. Characteristics of Metal Complexes as Antimicrobial Agents

Metal-based compounds demonstrated an ability to interact with biological molecules via unique mechanisms, not readily accessed by organic molecules.[23] While traditional antibiotics can only target a single bacterial pathway, metal complexes can utilise a multi-target approach, interacting with multiple cellular components, including deoxyribonucleic acid (DNA) or a cell membrane, which can reduce likelihood of resistance development.[22,23] Their combination of atypical electronic properties with respect to the chemistry of living systems, reactivity, and stereochemistry are attractive features for utilising coordination complexes for antibiotic development (Figure 2).[23,24] Modification of the coordination sphere with a range of ligands allows for fine-tuning of properties such as solubility, charge, and lipophilicity.[23] Additionally, the three-dimensional structure can be tailored for interactions with specific bacterial target, further highlighting the structural versatility and high target selectivity of metal complexes (Figure 2).[22-24]

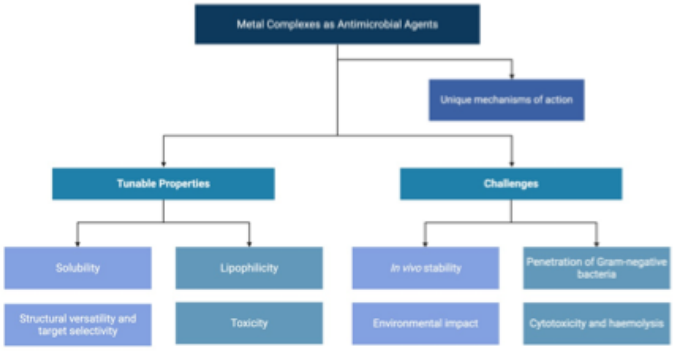


Figure 2: *An overview of the key characteristics that contribute to the potential of metal complexes as antimicrobial agents, and the challenges associated with their development and implementation. Created with BioRender.com.*

Despite the versatile features of metal complexes, substantial challenges remain. *In vivo* stability is crucial for the effective action of a metal complex.[25] Lekhan *et al.* (2022) proposed co-crystallisation of metal compounds and organic antimicrobials as an effective way to combat *in vivo* stability.[25] Similarly, Shemchuk *et al.* (2020) also observed enhanced antimicrobial activity following co-crystallisation of antimicrobial agents.[26] The enhanced stability might be due to reduced likelihood of ligand dissociation because of rigid crystal lattice formation, or protection from proteins or buffers that might otherwise displace ligands or alter metal oxidation state.[25,26]

Toxicity is a significant challenge in the development of metalloantibiotics. Many metals possess inherent toxicity towards bacteria, therefore contributing to

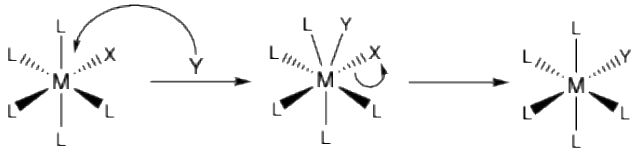


Figure 3: *General mechanism of ligand exchange reaction in an octahedral complex, showing the replacement of ligand X by a different ligand, Y.*

the overall antimicrobial activity of a complex by disrupting cellular processes.[14] In contrast,

cytotoxicity and haemolysis caused by metal-based compounds require fine-tuning of complex properties to prevent harm to the host. Frei *et al.*'s (2020) study, which analysed 906 metal-containing compounds for their antimicrobial activity, provides valuable insights into activity and toxicity of various metals.[14] This analysis can be used to determine suitable candidates for the development of safe and effective metalloantibiotics.[14]

Further significant challenges include high costs, scarcity of metals, and the environmental pollution associated with mining of metals.[13] Additionally, the structure of gram-negative bacteria poses a barrier to cellular entry of metal complexes into gram-negative bacteria, as exemplified by the limited number of metal complexes demonstrating activity against gram-negative species.[6,7] Despite these challenges, numerous metal complexes were found to be active against gram-positive and negative bacteria, with low-toxicity and low haemolysis.[14]

This chapter examines the mechanisms of action of metal complexes, including ligand exchange reactions, reactive oxygen species (ROS) generation, membrane disruption, and metal ion sequestration, highlighting the importance of understanding these mechanisms for the design of new metal-based drugs.

2.1 Mechanisms of Action

2.1.1. Ligand Exchange

Ligand exchange or substitution reactions involve the replacement of one or more ligands in a complex ion by a different ligand(s) (Figure 3). This reaction is a key mechanism for many metal complexes to exert antimicrobial activity, allowing them to interact with biomolecules such as nucleic acids, proteins, or enzymes - for complexes with labile ligands.[22,23]

DNA encodes the genetic information that determines a bacterium's virulence factors, which are crucial in host invasion, disease progression and evasion of host defences.[27] Metal complexes can target DNA, potentially disrupting gene expression and the synthesis of virulence factors.[27] By targeting crucial biological molecules, metal complexes can prevent bacteria from successfully establishing infection in the host.[29]

Ligand exchange occurs upon the binding of a metal complex to a biological target. For example, in the case of cisplatin, chloride ligands are replaced by water molecules upon entering the cancer cell (Figure 4).[22,23] While the ligand exchange is crucial for cisplatin's activity, it is the subsequent binding of cisplatin to DNA, creating platinum-DNA adduct, that leads to the inhibition of replication and transcription causing cell death.[22,23]

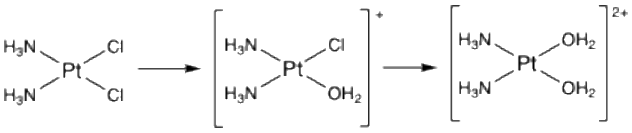


Figure 4: *Ligand exchange reaction of cisplatin upon cell entry, showing the replacement of chloride ligands with water molecules.*

In other cases, the release of a ligand itself can have a therapeutic or antimicrobial effect. For example, the carbon monoxide (CO)-releasing manganese complex MnG9MeBeim has demonstrated antibacterial activity comparable to standard care (Figure 5).[22,23,28]

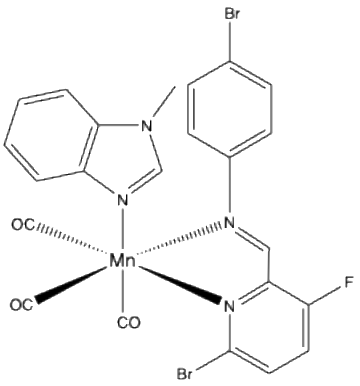


Figure 5: *Structure of MnG9MeBeim complex, adapted from ref. 28.*

While DNA targeting is a powerful antimicrobial strategy, bacteria can develop resistance mechanisms. Metals in the environment can act as co-selective agents, meaning their presence, even at sub-lethal concentration, exerts selective pressure that *indirectly* favours the spread of both metal resistance and AMR genes, as they are often located on the same mobile genetic element such as plasmid.[29] This indirect selection for bacteria that

already carry AMR genes highlights the challenge of developing effective and safe metal-based antimicrobials. Optimisation is essential to minimise co-selection pressure and ensure long-term efficacy.

2.1.2. Redox and Reactive oxygen species (ROS)

The metal oxidation state influences ligand exchange kinetics, coordination geometry and ligands proprties.[22,23,30] While low-toxicity species are preferred for patient administration, redox reactions can be strategically employed as a tuneable characteristic to activate the metal-based compounds at the desired site of action, therefore reducing side effects.[22,23,30]

Reduction-based activation exploits a difference in redox potential between the disease environment and surrounding tissues.[22,23] This mechanism is common in anticancer metal-based drugs containing platinum or ruthenium.[23] For example, platinum (IV) prodrug can be used as redox trigger.[23] In this process Pt(IV) is reduced to Pt(II) at the tumour site, triggering greater bioactivity (Figure 6).[14,22,23]

Conversely, oxidation-based activation involves ROS, such as superoxide anion (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), peroxide (O_2^{-2}), hydroxyl radicals (OH•) and hydroxyl anions (OH^-), inducing oxidative stress.[31,32] This results in the antioxidant defences being overwhelmed, leading to disruption of essential cellular processes and ultimately bacterial cell death.[31,32]

Although ROS show antimicrobial activity against gram-positive and gram-negative bacteria, uncontrolled ROS can cause host cell damage.[31] Therefore, controlled ROS generation is crucial when designing metal-based antimicrobials relying on oxidative activation.

2.1.3. Membrane disruption

The cell membrane is selectively permeable to ions and organic molecules, regulating the movement of substances across it.[33] This permeability is a crucial factor in determining a drug's effectiveness within a bacterium.[33] Lipophilicity is a key parameter influencing membrane permeability and uptake; therefore, increasing lipophilicity typically enhances a metal complex's ability to penetrate the lipid

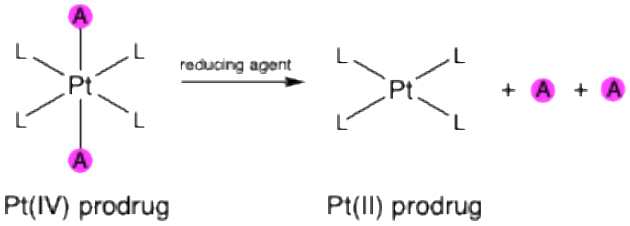


Figure 6: *Reduction of platinum(IV) prodrug to platinum(II) resulting in the activation of its bioactivity. Label A represents any axial ligand.*

bilayer.[33-35] Chelation can further increase lipophilicity by promoting electron delocalisation.[35] While higher lipophilicity facilitates metal complex uptake into the cell, particularly in gram-negative bacteria, factors such as solubility, bioavailability, and accumulation must also be considered when assessing the efficacy of metal-based compound.[34-36]

Metal complexes can interact with the bacterial membrane in various ways, including pore formation and lipid peroxidation.[37,38] These interactions disrupt the membrane's integrity, compromising its ability to regulate the movement of substances in and out of the bacterium.[37] Similar to how oxidised phospholipids can destabilise membranes of cancer cells and facilitate the entry of ROS, metal complexes can induce pores in the bacterial membranes, leading to microbial death.[37,38]

2.1.4. Metal ion sequestration

Metal sequestration is a bacterial strategy involving the secretion of metal-binding molecules, such as siderophores, to capture and transport essential metals from the environment (Figure 7).[39,40] This process is critical for bacterial survival and growth, particularly under nutrient-deficient conditions, such as during infection.[39,40] Siderophores, high-affinity iron-binding molecules, are secreted by many pathogenic bacteria during infections to assimilate iron from host proteins like transferrin.[39,40]

Recently, this process has been exploited for the delivery of antimicrobial agents into bacteria. In a "Trojan horse" mechanism, antibiotics or antimicrobial agents are conjugated to siderophores (Figure 7).[39-41] This strategy tricks bacteria into taking up the drug, potentially leading to cell death.[40] It offers a solution to the challenge of delivering antimicrobial agents into gram-negative bacteria. For example, Gallium (III) demonstrates antimicrobial activity against gram-negative *Pseudomonas aeruginosa* by exploiting siderophore uptake.[41] Due to its similarity in chemical properties, gallium mimics iron and is mistakenly taken up by bacteria. Once inside the cell, gallium replaces iron in metalloproteins, disrupting bacterial function.[41] This "Trojan horse" approach enables gallium to bypass the cell's defence mechanisms and inhibit bacterial growth and survival by rendering iron-dependent enzymes.[41]

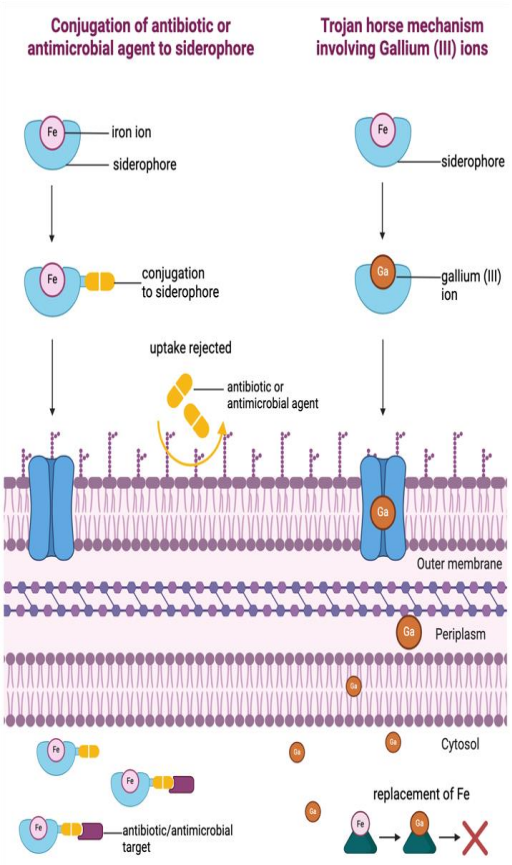


Figure 7: *Mechanisms of Metal ion Sequestration.* ***Left:*** *Illustration of an antibiotic or antimicrobial agent conjugated to a siderophore for active transport into gram-negative bacterium.* ***Right:*** *The 'Trojan horse' mechanism where gallium(III) mimics iron, allowing its entry into gram-negative bacterium via iron uptake system. Created with BioRender.com.*

3. Metal Complexes Active Against Bacterial Species

This chapter examines the antimicrobial potential of metal complexes incorporating antimony, manganese, and gallium. While other metals, such as silver, copper, and zinc are well-established for their antimicrobial properties, these selected metals offer distinct advantages in addressing antimicrobial resistance through novel mechanisms that could complement or surpass conventional treatments.

Antimony, historically used against parasitic infections, was chosen due to the promising membrane-disrupting mechanism of its organoantimony compounds. Manganese, with its carbon monoxide-releasing capabilities, inhibits bacterial respiration and stress response, providing a distinct antimicrobial approach. Gallium, known for its ability to mimic iron in a 'Trojan horse' strategy, shows significant activity against prioritised pathogens, making it a compelling metal-based antibiotic alternative.

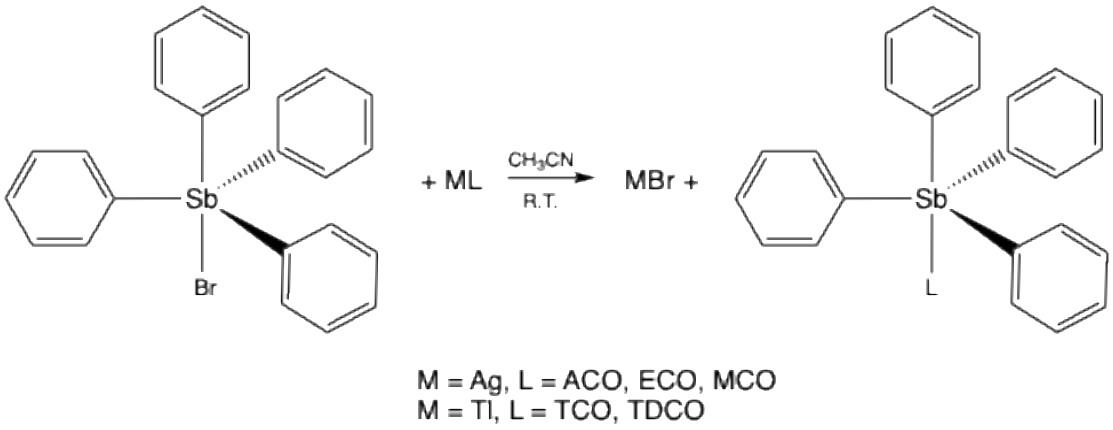


Figure 8: *Synthetic route to organoantimony cyanoximate complexes with formula $SbPh_4L$, adapted from ref. 45.*

Beyond their mechanisms, these metals also present adaptable ligand frameworks, which can be strategically modified to enhance their antimicrobial efficacy and therapeutic potential, particularly against pathogens prioritised in the WHO's BPPL.[1]

3.1 Antimony

The pioneering application of a group 5 element in medicine dates to 1910, when Paul Erlich's arsenic-derived compound code-named Compound 606 was commercially introduced as Salvarsan for syphilis treatment.[2,42] Another group 5 element, antimony, has been utilised as a treatment for Leishmaniasis caused by parasites of the genus *Leishmania*.[43,44] While the exact mechanism of antiparasitic action of Sb(III) is not fully understood, Sb(III) is believed to disrupt redox balance, leading to increased oxidative stress and parasite death.[43]

Building upon the success of the antimonial treatment of Leishmaniasis, a novel group of organoantimony oximate compounds was designed for the assessment of anticancer, and later antimicrobial activity.[42,44-46] Organoantimony cyanoximates were synthesised, since oximes have previously demonstrated biological activity and low toxicity, laying the groundwork for the assessment of their antimicrobial activity.[42,45] $Sb(C_6H_5)_4L$ (L= cyanoxime) compounds contain lipophilic fragment that aids intake of tetraphenyl-antimony cyanoximates into the bacterium (Figure 8).[45]

Five selected compounds, never previously exposed to pathogens, were tested against *E. coli* (gram-negative), *P. aeruginosa* (gram-negative), and *S. aureus* (gram-positive) in a disc diffusion assay, measuring zones of inhibition.[45] $SbPh_4$(ACO) and $SbPh_4$(ECO) demonstrated antimicrobial activity against all three pathogens, suggesting that organoantimony compounds possess strong potential as both broad- and narrow-spectrum antimicrobials and that the presence or absence of outer membrane in bacterium does not impact their activity (Figure 9).[45]

The activity of $SbPh_4$(MCO) agrees with ACO and

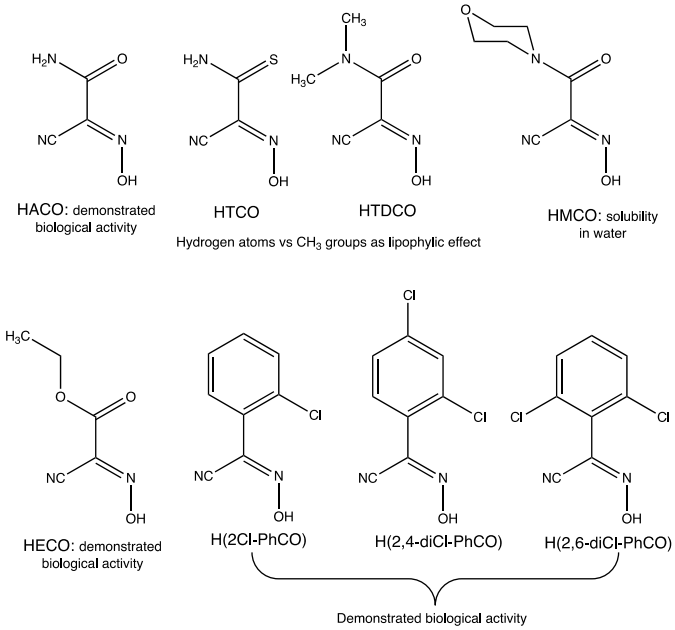


Figure 9: *Chemical structures and biological activity of ligands coordinated to $SbPh_4L$ and $SbMe_3L_2$, adapted from ref. 44-46.*

ECO, as it was effective against *P. aeruginosa* and *S. aureus*.[45,46] This study revealed the efficacy of

organoantimony cyanoximates in combatting priority list pathogens and provided insights into the significance of $SbPh_4$ for antimicrobial activity, since cyanoximates alone exhibited no antimicrobial activity.[45]

3.1.1. $SbPh_4$(ACO)

Further quantitative analysis of $SbPh_4$(ACO) showed minimum inhibitory concentration*[1] (MIC) values ranging from 50-100 $\mu g/mL$ for the three bacterial pathogens, confirming potential as a broad-spectrum antimicrobial (Figure 8).[44] $SbPh_4$(ACO), with its bactericidal properties and membrane-disrupting mechanism of action, is an attractive candidate for a metal-based antimicrobial (Figure 9).[44] The membrane-disrupting mechanism of $SbPh_4$(ACO), affecting the permeability of outer membranes of gram-negative pathogens, offers a promising strategy to combat AMR.[44-46] It exhibited synergistic effects with polymyxin-B and cefoxitin antibiotics against antibiotic-resistant strains of *P. aeruginosa* and *S. aureus*, suggesting $SbPh_4$(ACO) could be used to enhance the efficacy of current treatments and reduce the dosage required.[44] At a synergistic MIC of 12.5 $\mu g/mL$, $SbPh_4$(ACO)'s

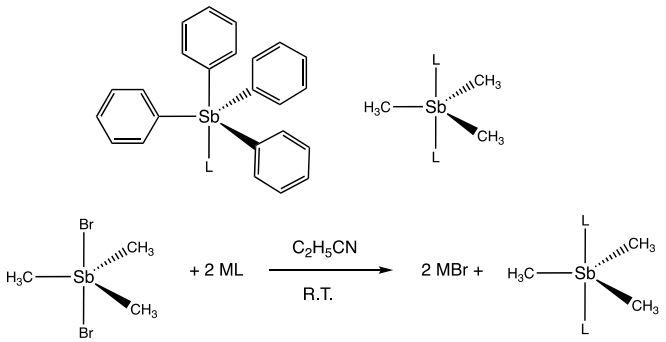


Figure 10: *Structural comparison of $SbPh_4L$ and $SbMe_3L_2$ complexes (top), and the synthetic route to $SbMe_3L_2$ complexes (bottom).*

cytotoxicity was below harmful threshold against mammalian cells.[44]

3.1.2. SbMe3(MCO)2

Building on Salpadoru *et al.* (2024), the tetraphenyl backbone was replaced by trimethyl groups to assess the chemical stability and antimicrobial activity of $SbMe_3L_2$ compounds (Figure 10).[44,46]

The same ligands were used, in addition to new anionic cyanoxime ligand groups 2,4-diCl-$PhCO^-$, 2,6-diCl-$PhCO^-$, and 2Cl-$PhCO^-$ (Figure 9, 10).[44,46] While $SbPh_4$(MCO) inhibited *P. aeruginosa* with relatively high MIC 200 $\mu g/mL$, the four-fold reduction in the MIC (50 $\mu g/mL$) for $SbMe_3(MCO)_2$ indicates that the trimethyl backbone enhances its antimicrobial activity.[46] Similarly to $SbPh_4$(ACO), $SbMe_3(MCO)_2$ caused disruption in *P. aeruginosa* membranes, suggesting that both complexes utilise the same mechanism of action.[44-46] While the cytotoxicity and synergistic effects of $SbMe_3(MCO)_2$ require further research, the two organoantimony complexes underscore the therapeutic potential of antimony-based compounds in addressing the growing challenge of AMR.[44,46]

In conclusion, the two promising compounds should be tested against a broader range of pathogens and evaluated for toxicity and efficacy *in vivo* using the *Galleria mellonella* (wax moth) model.[47] Further clinical assessment will validate the antimicrobial potential of $SbPh_4$(ACO) and $SbMe_3(MCO)_2$, providing insights into their mechanism of action and potential synergy with existing antibiotics or each other, paving a way for innovative metal-based treatments.[6]

3.2 Manganese

Manganese has historically been used in medicine to treat psoriasis, an autoimmune condition causing inflammation to the skin.[48] It is a naturally occurring element with an essential role in biochemical processes including immune regulation, bone growth, and defence against oxidative stress.[49] Its biocompatibility makes it a good candidate for therapeutic applications, but poor stability has

* MIC is the lowest concentration of a substance that visibly inhibits the growth of a microorganism. The lower the MIC value, the lower the concentration of antimicrobial required to inhibit growth, therefore the more effective the antimicrobial.

limited the use of early metal complexes in effective medical treatments.[49]

3.2.1. $Mn(CO)_3$(2,2-bipyridyl)(azole)

Repurposing well-established drugs for antimicrobial applications by coordinating them with organometallic fragments offers a strategic approach to combatting rising AMR.[50] Coordination of antifungal azole ligands – ketoconazole, miconazole, and clotrimazole – to manganese(I) tricarbonyl complexes led to formation of $Mn(CO)_3$(2,2-bipyridyl)(azole) complexes (Figure 11).[50] An early study of five complexes showed no significant antimicrobial activity against gram-negative bacteria but demonstrated activity against *S. aureus.* It also revealed enhanced activity compared to the azole ligands alone and greater stability of $Mn(CO)_3$-azole complexes in biological media, as indicated by the lack of ligand exchange.[50] Mendes *et al.*'s (2022) findings enhanced the understanding of the mechanism of action of manganese(I) tricarbonyl complexes.[51] Mn(I)-clotrimazole complex demonstrated activity against gram-positive *S. aureus* (MIC: 2 $\mu g/mL$) without the requirement of light irradiation, which is often required for the release of CO with carbon monoxide-releasing molecules.[51]

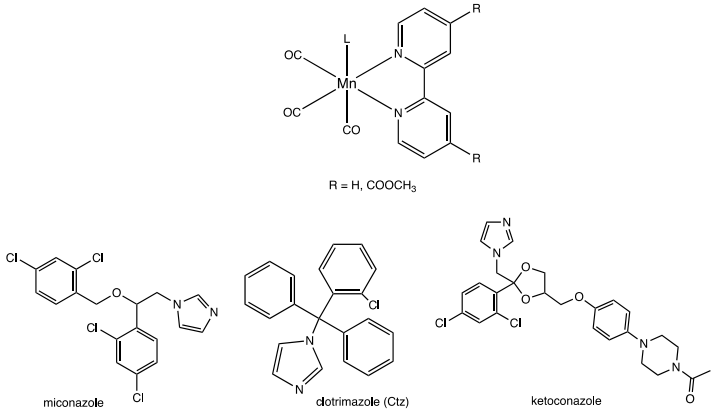


Figure 11*: General structure of manganese(I) tricarbonyl complexes (top), and chemical structures of the repurposed antifungal azole ligands (bottom).*

3.2.2. Manganese(I) complexes with triazole-derived ligands

The dual action mechanism where the Mn(I) centre contributes to CO release and a clotrimazole ligand provides membrane-targeting properties motivated investigation of manganese(I) complexes with 1,2,3-triazole-derived ligands (Figure 12) and their effectiveness against gram-positive and gram-negative bacteria.[51,52] The 1,2,3-triazole-based complexes have previously shown antimicrobial properties, and offer ease of tunability through cycloaddition reactions.[52,53] Complexes showed activity against *S. aureus* with MIC values of 1.89 $\mu g/mL$ for di-triazole ligands, 1.93 $\mu g/mL$ for N-ethyl triazole-pyridine ligand, and 1.79 $\mu g/mL$ for p-totyl triazole-pyridine substituents (Figure 12).[52] The significant difference in MIC values of complexes and the clotrimazole alone (MIC: 1411.33 $\mu g/mL$) indicates that the presence of clotrimazole is essential for complex activity.[52]

The three complexes demonstrated good activity against three gram-negative pathogens leading in the BPPL - *E. coli, P. aeruginosa,* and, in particular, *A. baumannii* (MIC comparable with *S. aureus*).[1,52] Similarly, good activity was detected with two other gram-negative bacteria *Salmonella enterica,* and *K. pneumoniae*.[52] The increased antimicrobial activity of complexes against gram-negative bacteria following replacement of bipyridyl ligand with 1,2,3-triazole ligands shows an excellent potential for exploitation of these complexes as metal-based

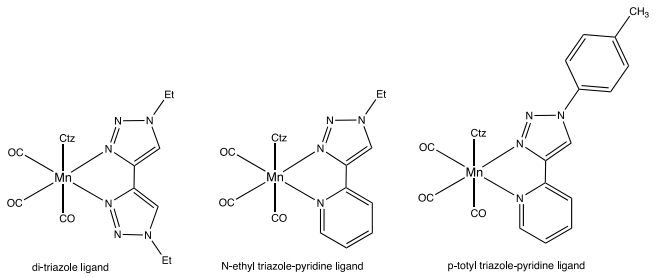


Figure 12: *Structures of the three tested manganese(I) complexes with three different triazole-derived ligands.*

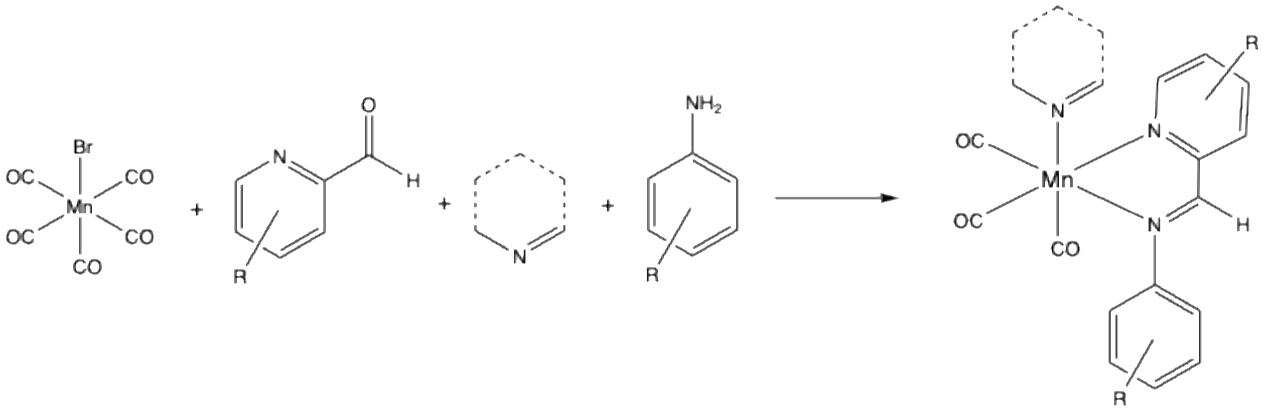


Figure 13: *General reaction scheme of the combinatorial synthesis of manganese(I) complexes[28].*

antibiotic alternatives (Figure 12). Two out of the three tested complexes (di-triazole ligand, N-ethyl triazole-pyridine ligand) (Figure 12) also demonstrated minimal adverse effects on human monocyte cells, showing viability greater or equal to 75% at concentration 7.57 μM and 7.74 μM, respectively.[52] The activity against both gram-positive, and especially gram-negative bacteria, coupled with relatively low cytotoxicity makes manganese(I) tricarbonyl complexes good candidates for further development.[52] It would be interesting to explore the synergistic effects with antibiotics to increase the efficacy of existing treatments and reduce resistance development, as well as their antimicrobial effects with other repurposed antimicrobial or antifungal agents.

3.2.3. MnG9MeBeim and combinatorial chemistry of manganese(I) tricarbonyl complexes

Building on the promising antimicrobial properties of manganese(I) tricarbonyl complexes, Scaccaglia *et al.* (2024) utilised a combinatorial chemistry approach to rapidly synthesise 420 novel manganese tricarbonyl compounds (Figure 13).[28,52] Of the 420, 10 lead compounds were fully characterised based on their antimicrobial activity.[28] One compound, MnG9MeBeim, exhibited antimicrobial activity against gram-positive *Staphylococcus epidermidis, Bacillus subtilis,* and, more importantly, activity against methicillin resistant (MRSA) and methicillin susceptible *S. aureus* (MSSA) (Figure 5).[28]

The MIC values ranged from 0.78 $\mu g/mL$ with the resistant/susceptible strains to 1.56 $\mu g/mL$, comparable with standard care of gram-positive infections with vancomycin.[7] MnG9MeBeim inhibits the bacterial respiratory chain by releasing carbon monoxide ligands proximally to the membrane, causing bacterial cell death by disruption of the adenosine triphosphate production.[28] With low haemolysis of red blood cells, minimal toxicity to human cells, a high therapeutic index (>100[2†]), and a novel mechanism of action, MnG9MeBeim is a promising antimicrobial agent. [28] Its activity against both bacterial and resistant species offers an advantage over existing antibiotics.[28] This study also demonstrates the significance of combinatorial chemistry in accelerating the drug discovery process by elucidating structure-activity relationships by systematically varying the components of complexes.[28]

3.3 Gallium

Though gallium does not have a known function in biological systems, its use in biomedicine dates to the 1900s, when it was employed for imaging and diagnosis.[55] Gallium nitrate ($Ga(NO_3)_3$), marketed as Ganite, is approved by the U.S. Food and Drug Administration to treat malignancy-associated hypercalcemia, elevated calcium levels in patients diagnosed with cancer.[56] In 2024, gallium maltolate

† Quantitative measurement indicative of the relative safety of a drug. The larger the therapeutic index, the safer the drug, the larger the difference between the therapeutic and toxic doses. Vancomycin has a narrow therapeutic index.[54]

entered a phase 1 clinical trial for treating relapsed and refractory glioblastoma, an aggressive primary brain cancer.[57] This semi-metallic element, in its +3-oxidation state, also exhibits antimicrobial activity against various pathogens, likely due to a multi-target mechanism of action.[58]

3.3.1. Gallium nitrate and gallium protoporphyrin

Gallium(III) complexes, namely gallium nitrate ($Ga(NO_3)_3$) and gallium protoporphyrin (GaPP), have gained significant attention for their antimicrobial activity against gram-positive, gram-negative, and resistant bacterial strains (Figure 14).[60-65]

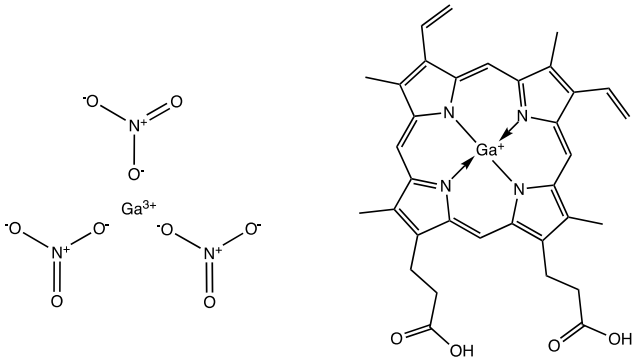


Figure 14: *Structures of gallium nitrate (left), and gallium protoporphyrin (right).*

In chemical properties, Ga(III) closely resembles iron(III).[58,59] This similarity in ionisation potential, ionic radius, and electron configuration enables iron mimicry by exploiting iron transport mechanisms and binding to iron-binding protein sites.[58,59] An example of iron mimicry is the binding of gallium to iron-scavenging siderophores (Figure 7). This unique antagonist mechanism makes resistance development to gallium more challenging, as reduction of iron uptake (effectively gallium) would be detrimental to bacterial growth and survival.[58,59]

Gallium nitrate inhibits the bacterial iron mechanisms and iron uptake pathways.[60,61] Pathogen-specific targets of gallium nitrate were identified, including enzymes such as ribonucleotide reductase, catalase, and superoxide dismutase (SOD).[65] GaPP inhibits haem uptake, which is a crucial for the function of haem-containing proteins, such as haemoglobin, involved in oxygen transport.[58,60-63] Similar to gallium nitrate, GaPP targets catalase and SOD.[58,60-63] $Ga(NO_3)_3$ exhibited antimicrobial activity against *E. coli* and *Enterococcus faecalis* at MIC of 256 μg/mL, and against *A. baumannii, K. pneumoniae, P. aeruginosa,* and *S. aureus* at MIC of 512 μg/mL.[65]

While significant for its potential as a novel antimicrobial agent, these MIC values might be considered high in comparison to the antimicrobial activity of other metals.[65] Lower MIC values for both $Ga(NO_3)_3$ and GaPP were observed against *P. aeruginosa,* colistin-resistant *P. aeruginosa*, *K. pneumoniae,* carbapenem-resistant *K. pneumoniae, A. baumannii,* and *S. aureus* (both MRSA and MSSA) in follow up studies.[60-64]

3.3.2. Synergistic effects of GaPP/$Ga(NO_3)_3$

Building upon the exceptional individual antimicrobial activity of these two gallium complexes, their synergistic effects became of interest.[60,61,63] The inhibition of different iron-sourcing pathways suggests that combined treatment would theoretically enhance antimicrobial activity.[58,60-63] GaPP/$Ga(NO_3)_3$ demonstrated antimicrobial activity against various pathogens, with the strongest activity observed against *K. pneumoniae* and the best growth inhibition of *P. aeruginosa*.[60] Time-kill assays demonstrated bactericidal activity of the dual treatment against *P. aeruginosa, K. pneumoniae*, and MRSA, and that lower concentrations of the compounds and antibiotic are required in combination, reducing the likelihood of resistance development.[60]

These findings motivated further *in vivo* investigation of the synergistic activity of GaPP/ $Ga(NO_3)_3$ against carbapenem-resistant *K. pneumoniae*[61], *P. aeruginosa*[63], and methicillin-resistant *S. aureus*[64], with results presented in Table 3.

Gallium demonstrates significant antimicrobial activity against both gram-positive and gram-negative infections. Notably, GaPP/Ga(NO3)3 synergy resulted in remarkably low *in vivo* survival rates (Table 3) of pathogens prioritised on the WHO's BPPL[1,61,63,65]. This synergy allows for simultaneously targeting multiple bacterial

Table 3: *In vivo evaluation of the synergistic effects of GaPP/Ga(NO$_3$)$_3$ ag ainst bacterial pathogens.*[61,63,64]

Treatment	Bacteria	FICI /µg mL^{-1} *	Synergy explanation	*In vivo* evaluation
GaPP/ Ga(NO$_3$)$_3$	Carbapenem-resistant *K. pneumoniae*[61]	Combined: 0.26 Ga(NO$_3$)$_3$: 1 GaPP: 0.125	Synergy, combination highly promising for a treatment	Pulmonary murine model: 100% survival with combined treatment, singly/untreated mortality within 48h of inoculation
	P. aeruginosa[63]	Combined: 0.5	Synergy, also observed with siderophore mutants	Pulmonary murine model: 100% survival at low and high doses, singly/untreated mortality within 48h of inoculation
	Methicillin-resistant *S. aureus*[64]	Combined: not explicitly stated Ga(NO$_3$)$_3$: 12.5 GaPP: 64	Graphs of growth recovery of haemin and ferric ammonium citrate indicate synergy in inhibiting MRSA growth	Murine MRSA infection model: 100% survival rate, compared to 100% mortality in control group, no observable drug-related toxicity

* Fractional Inhibitory Concentration Index (FICI) evaluates the effectiveness of combination of antimicrobial treatments and quantifies the degree of

pathways, reducing the likelihood of resistance development compared to single-target antibiotics.

Further understanding the specific mechanism of GaPP/Ga(NO3)3 synergy could inform the development of effective synergistic strategies using other metals, antibiotics, or diverse ligands, potentially extending the lifespan of existing antimicrobials.

4. Conclusion and Future work

Metal-based antimicrobials represent a promising alternative to antibiotics, addressing the global challenge of AMR through multi-target mechanisms. The characterisation of novel metal complexes, the repurposing of anticancer or antifungal compounds, and the strategic exploitation of synergistic effects may reduce dependence on conventional antibiotics and mitigate resistance development.

4.1 Key findings

Antimony, manganese, and gallium demonstrate strong activity against gram-positive, gram-negative, and antibiotic-resistant bacterial species prioritised by the WHO BPPL[1], with efficacy comparable to standard treatments.

Antimony effectively penetrates the bacterial membranes of gram-negative bacteria, and this, in synergy with conventional antibiotics, could enhance antibiotic uptake by disrupting membrane integrity or increasing permeability. Organoantimony complexes do not rely on enzyme inhibition, making it more challenging for bacteria to develop resistance.

Manganese complexes, as CO-releasing molecules, inhibit bacterial oxidative metabolism, leading to energy depletion and cell death, thereby weakening bacterial defences without causing DNA or protein damage.

Gallium exploits the iron uptake mechanism through iron mimicry. Once inside, it replaces Fe^{3+} in metalloproteins, resulting in non-functional proteins, disruption of iron-dependent processes, and bacterial starvation. As iron uptake is essential for survival, it is highly challenging for bacteria to develop resistance against gallium.

4.2 Challenges

Toxicity, solubility, lipophilicity, and *in vivo* stability present significant challenges that must be addressed to enable therapeutic applications. While cytotoxicity towards bacterial cells is desired, mechanisms such as ROS generation can also harm mammalian cells, contributing to off-target toxicity.[31,32] This toxicity can be mitigated by ligand modification, specifically by attaching biocompatible organic moieties to shield the metal ion. Selectivity can be improved by designing ligands that preferentially bind bacterial membranes, such as cationic ligands for negatively charged lipid bilayers.

Building on Scaccaglia *et al.* (2024), combinatorial chemistry computational methods can be employed to screen libraries of metal-ligand combinations, identifying complexes with low toxicity and high activity. [28] Stability is closely related to toxicity; if ligands dissociate rapidly prematurely, potentially forming toxic byproducts, ligand optimisation through chelation or prodrug approaches can be implemented. Prodrugs, such as the Pt(IV) prodrugs discussed in section 2.1.2, selectively activate in the bacterial environment, further optimising dissociation. [14,22,23]

Poor solubility of many metal complexes can limit bioavailability and lead to aggregation, diminishing antimicrobial action. Solubility can be enhanced without compromising complex activity through co-crystallisation, as demonstrated by Lekhan *et al.* (2022), or by attaching hydrophilic groups.[25]

4.3 Future Research

Combinatorial chemistry, computational methods, synergistic effects, and biological evaluations could address the existing challenges of AMR and drive the development of metal-based formulations for therapeutic applications.[28,47,63-65]

The antimicrobial synergy between metal complexes and antibiotics, such as GaPP/$Ga(NO_3)_3$ or $SbPh_4$(ACO)/polymyxin-B, is critical for reducing resistance emergence.[44,63-65] Exploring new synergistic combinations of other metals, complexes, and antibiotics could yield novel treatments that enhance antibiotic efficacy while extending their lifespan by preventing resistance.

Integrating computational methods, synergy studies, biological evaluations, and imaging techniques will provide a comprehensive approach to drug discovery. Computational models can predict the interactions between metal-antibiotic combinations, while imaging techniques can visualise the biological effects, aiding further optimisation.[65]

Combinatorial chemistry accelerates drug discovery by generating large libraries of antimicrobial candidates.[28] High-throughput screening enables rapid identification of active compounds, which can be evaluated for their ADMET (absorption, distribution, metabolism, excretion, toxicity) properties and assessed in time-kill assays and *in vivo* models.[28,65]

With continued innovation, thanks to their broad mechanisms of action and adaptability, metal-based complexes have the potential to reshape the landscape of antimicrobial treatment, offering new hope for patients affected by drug-resistant infections.

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