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Evaluation of metal-based antimicrobial compounds for the treatment of bacterial pathogens

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Abstract

Antimicrobial resistance (AMR) is one of the greatest global health challenges of modern times and its prevalence is rising worldwide. AMR within bacteria reduces the efficacy of antibiotics and increases both the morbidity and the mortality associated with bacterial infections. Despite this growing risk, few antibiotics with a novel mode of action are being produced, leading to a lack of antibiotics that can effectively treat bacterial infections with AMR. Metals have a history of antibacterial use but upon the discovery of antibiotics, often became overlooked as antibacterial agents. Meanwhile, metal-based complexes have been used as treatments for other diseases, such as the gold-containing drug auranofin, used to treat rheumatoid arthritis. Metal-based antibacterial compounds have novel modes of action that provide an advantage for the treatment of bacterial infections with resistance to conventional antibiotics. In this review, the antibacterial activity, mode of action, and potential for systemic use of a number of metal-based antibacterial complexes are discussed. The current limitations of these compounds are highlighted to determine if metal-based agents are a potential solution for the treatment of bacterial infections, especially those resistant to conventional antibiotics.

INTRODUCTION

Antimicrobial drug resistance (AMR) is one of the greatest global health challenges of modern times due to its growing prevalence and the increased risk of mortality for those with antimicrobial-resistant pathogens [1]. The World Health Organization and European Centre for Disease Prevention and Control have also highlighted AMR as a global issue [2, 3]. Part of the challenge posed by increasing AMR in bacteria is the need for novel agents with alternative modes of action to effectively treat bacteria with AMR [4, 5].

Annually, over 2 million infections and approximately 23000 deaths in the USA are caused by antibiotic-resistant *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* (ESKAPE) pathogen species [1]. Methicillin-resistant *S. aureus* (MRSA) was observed 1 year after the first clinical use of methicillin and genomic evidence suggests that resistance preceded the first clinical use of methicillin [6, 7]. Although current antibiotic classes have differing modes of action, some bacteria, such as *Mycobacterium tuberculosis* or *P. aeruginosa*, are resistant to multiple classes of antibiotics, diminishing their capability for effective treatment [1, 8]. There is increasing interest in the potential use of metal-based antimicrobial compounds for treating microbial infections as these have a distinct mode of action to conventional antibiotics and may have application in the treatment of recalcitrant microbial infections.

Mechanisms of AMR in bacteria

There are several mechanisms by which AMR is mediated in bacteria. Bacteria can promote the active efflux of antibiotics from the cell through the over-expression of efflux pumps or decrease the bacterial cell wall permeability to restrict antibiotic access to their target sites, ultimately reducing the concentration of the antibiotic within the cell and preventing its activity. Bacteria can also acquire alternative metabolic pathways to those inhibited by the drug, modify the antibiotic targets, or overproduce the target enzyme to circumvent the antibacterial activity of the drug. Finally, bacteria can degrade or modify the antibiotic via bacterial enzymes to inhibit its antibacterial activity [9–12]. Bacteria with AMR may possess

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Abbreviations: AMR, antimicrobial resistance; CF, cystic fibrosis; ESKAPE, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter*; MRSA, methicillin-resistant *Staphylococcus aureus*; NHC, *N*-heterocyclic carbene; ROS, reactive oxygen species; TB, tuberculosis; TrxR, thioredoxin reductase.
Two supplementary tables are available with the online version of this article.

one or more of these capabilities, depending on the extent of their resistance.

The formation of biofilms can also promote antibiotic resistance. Furthermore, if an antibiotic fails to fully penetrate the biofilm, it will not be able to target the microorganisms deep within the biofilm, leading to antibiotic resistance in these microorganisms [13].

AMR is particularly problematic in infections caused by Gram-negative bacteria due to their intrinsic resistance [14]. The structural difference between components of the bacterial cell wall, such as the presence of the outer membrane, can prevent antibacterial agents from entering or remaining within the cell [15]. These intrinsically resistant Gram-negative bacteria can also acquire other resistance mechanisms by increasing the expression of efflux systems, modifying outer membrane proteins or modifying drug targets, increasing their resistance to many broad-spectrum antibiotics [16, 17].

An ideal novel antibacterial compound must be able to combat both the acquired and the intrinsic resistance mechanisms of bacteria. By developing novel compounds with unique modes of action that can bypass the resistance mechanisms in place, it is likely that bacteria resistant to conventional antibiotics can be effectively treated. With the lack of antibacterial drug compounds with novel classes and modes of action currently in development [18], there is a great need for an alternative approach to the development of antibiotic compounds. Metal-based antibacterial compounds have been proposed as a candidate for the development of a novel class of antibiotics for the effective treatment of antibiotic-resistant bacteria.

Metal-based antibacterial compounds

In the past, metal ions have commonly been used for antibacterial purposes [19]. At the beginning of the 20th century, an arsenic-containing compound named salvarsan was discovered and served as the first effective treatment of syphilis, a bacterial infection caused by *Treponema pallidum* subspecies *pallidum* [20]. Antimony-based compounds have also been used as therapeutics for several centuries and pentavalent antimonials, or Sb(V), can be used for the treatment of leishmaniasis, a disease caused by the *Leishmania* parasite [21]. However, the discovery of penicillin and the antibiotics that followed caused a decline in the clinical use of metal-based antibacterial compounds. With the rise in AMR and the difficulties in producing antibiotics with novel modes of action, research into the use of metal-based pharmaceuticals as antibacterial agents has resurfaced.

For antibacterial purposes, metals can be complexed to a biomolecule, complexed to an antibiotic or used in conjunction with an antibiotic. As biomolecules are compounds commonly taken into a bacterial cell, complexing a metal with a biomolecule mediates entry of the metal into a certain area of the cell, to exert its antibacterial effect [22].

An advantage to the use of metal-based antibiotics is their diverse modes of action compared to conventional organic antibiotics [23]. Moreover, the addition of metals to organic antibiotics enables novel and additional modes of action compared to the organic drug alone [20]. Therefore, the use of these metal-based complexes alone or in combination with antibiotics holds promise as an effective treatment against resistant bacterial infections through these novel and additional modes of action.

Despite a long history of use for their antibacterial properties, metal-based compounds currently have few antibacterial applications compared to the large number of publications discussing metal-based anti-cancer compounds. This limited number of publications may be partly due to fear over the toxicity of metals when administered systemically. However, some metals are essential for life in small quantities. Without these metals, most enzymes would be incapable of conducting the transformations necessary to mediate their biological functions [24].

The purpose of this review is to discuss the different metal-based compounds currently proposed in the literature as antibacterial agents for treatment of patients, especially those with antibiotic-resistant bacterial infections. The main metals discussed in this review are silver, gold, gallium, copper and manganese. Although other metals have also demonstrated antibacterial activity (Table S1, available in the online version of this article), the metals mentioned above have been chosen as they show the most promising antibacterial activity and capability for safe topical or systemic administration as shown in the literature [25]. The strengths and limitations of each proposed antimicrobial metal are outlined to ultimately discuss if these metal-based compounds have the potential to be a solution for antibiotic resistance.

SILVER

Silver has an extensive history of antibacterial use. Herodotus noted the use of silver to carry water for Persian kings to keep the water fresh, particularly during military conflicts when fresh water from natural resources was scarce [19]. Avicenna described the use of silver filings in 980 C.E. as a blood purifier for heart-palpitations and offensive breath [26]. Colloidal silver was used in the 18th and 19th centuries as a wound antiseptic and silver nitrate for the treatment of burn wounds [19]. Silver nitrate was also used topically for the prevention of gonorrheal ophthalmia infections in newborns and was ingested for the treatment of stomach ulcers throughout the 1800s and into the 1940s [19, 27]. There is also a history of silver being used in coins and cutlery due to its antibacterial capabilities [28].

Currently, silver has many applications that make use of its antimicrobial properties. It is used in textiles and sprays to prevent the bad odours caused by sweat [28–30] and in cosmetics as an antimicrobial agent [31]. Silver is also a key component used in burn wound treatments [32]. An example is silver sulfadiazine, a broad-spectrum topical antibiotic approved by the Food and Drug Administration (FDA) to treat burn wounds [33, 34]. Silver has also been proposed for use in medical instruments, dental instruments and

implants for the prevention of infection [19, 35]. Currently, approximately 300 clinical trials examining silver-containing compounds for a range of different applications are ongoing or in the recruitment phase [36]. Although all of its current antimicrobial applications are for surface or topical use, they testify to the antimicrobial capabilities of silver and potential for its use as an antibacterial agent.

Antibacterial mode of action of silver

Although inert in its metallic form, silver releases small amounts of ions in an aqueous environment, which have antibacterial action at the metal surface [28]. These silver ions can target bacterial DNA, proteins and membranes to exert their antibacterial effects. Silver may also produce reactive oxygen species (ROS), although the literature is still unclear as silver is not redox-active [37].

Silver ions bind nucleic acids strongly, preferentially binding bases and probably leading to DNA modification [37]. Silver ions have been shown to form homo-base pairs with an increased affinity to guanine, which may lead to pyrimidine dimerization. Silver ions have also been shown to interact with adenine at a high concentration [38]. Microscopy analysis conducted on silver-treated bacteria has identified DNA condensation in the centre of the cells. This DNA modification leaves the bacterium prone to mutation and inhibits replication [37].

Silver ions have a strong affinity for sulfhydryl groups and will form an S-silver bond with them, leading to the inhibition of some bacterial proteins. These sulfhydryl groups belong to lateral chains of cysteine residues, which are often a ligand for a metal or cofactor in the metalloproteins involved in bacterial respiration, membrane structure, multiplication and metabolism [37, 39, 40]. Interference of silver ions with these cysteine residues can impair protein function, leading to bactericidal effects.

Observation of silver-treated *Escherichia coli* via transmission electron microscopy identified morphological and structural changes as well as enhanced permeability in the bacterial cell envelope [41]. Similarly, an enlargement of the periplasmic space in *Escherichia coli* treated with silver suggested the inner membrane shrank and detached from the cell wall. Upon silver administration to *S. aureus*, similar morphological changes were observed but to a lesser extent, suggesting a stronger resistance to silver ions [42].

Thirty-four unique proteins were identified as targets of silver ions in *Escherichia coli*, many of which are involved in glycolysis and the TCA [43]. The inhibitory activity of Ag(I) ions was demonstrated through extensive biochemical analysis in conjunction with X-ray crystallography of Ag(I) ions coordinated to a cysteine active site, resulting in the identification of the first molecular targets of silver in bacteria [43]. Novel silver-based therapeutics can take advantage of this knowledge by targeting these metabolic pathways. However, as the binding sites for GAPDH are conserved between humans and bacteria, silver ions must be targeted towards only bacteria, for them to serve as safe and effective antibiotics [44].

An additional advantage to silver is that it has an oligodynamic effect, with high microbicidal capacity at very low concentrations of silver ions in water (1 p.p.m.) [45]. The dissolved oxygen in the blood can further the oxidation of silver metals, enhancing the formation of complexes between released silver ions and organic molecules and increasing its antibiotic action [28]. Therefore, silver has promising antibacterial activity and is likely to be an effective candidate for the development of metal-based antibacterial compounds.

Silver-based antibacterial compounds

Recently, novel classes of silver complexes have gained attention due to their antibacterial properties, the most prominent being *N*-heterocyclic carbene (NHC) complexes of Ag(I) [33, 46–48]. NHCs are strong nucleophiles that bind metals with high stability, leading to increased bioavailability of the metal in physiological conditions [46, 49]. Consequently, NHC complexes with Ag(I) are often investigated in the current literature to examine their antibacterial efficacy and patient toxicity.

An NHC-Ag(I) complex that shows promise for development into an intravenous antibiotic to be used against antibiotic-resistant bacteria is 1,3-dibenzyl-4,5-diphenyl-imidazol-2-yli dene Ag(I) acetate (SBC3) (Fig. 1) [50]. SBC3 has shown MIC values between 3.13 and 20 μg ml^{-1} against MRSA, *Salmonella*, *Escherichia coli* and *P. aeruginosa* [51]. *In vivo* studies conducted in *Galleria mellonella* larvae observed that SBC3 administered at a concentration of 25 μg ml^{-1} inhibited the growth of *S. aureus* by 71.2% [52]. *G. mellonella* are a common infection model for these studies as the insect bears significant functional and structural similarities to that of the mammalian innate immune system [53, 54]. The study also demonstrated that SBC3 administration to *G. mellonella* larvae did not increase the density of circulating haemocytes (immune cells), and thus it is likely that SBC3 did not boost the insect immune response [52]. This observation indicates that the survival of the larvae was due to the antimicrobial activity of SBC3 and not a non-specific immune response induced by the compound. Therefore, the antibacterial activity of SBC3 has been demonstrated against several bacterial species both *in vitro* and *in vivo*, making it a promising antibacterial agent for use against these bacteria, pending a greater understanding of its safety upon systemic or topical administration in humans.

The antibacterial efficacy of eight novel silver complexes, four triphenylphosphino (Ph_3P)-Ag(I) benzoate complexes (**2**) to (**5**) and four NHC*-Ag(I) benzoate complexes (**6**) to (**9**) (Fig. 1) was assessed against *E. coli* and MRSA [55]. Both NHCs and phosphines can be used to increase the bioavailability of silver in physiological conditions for their use as antimicrobial agents [48, 56]. This study used Kirby–Bauer disc diffusion testing to determine the antibacterial activity of the eight novel Ag(I) complexes against both Gram-negative and Gram-positive bacteria.

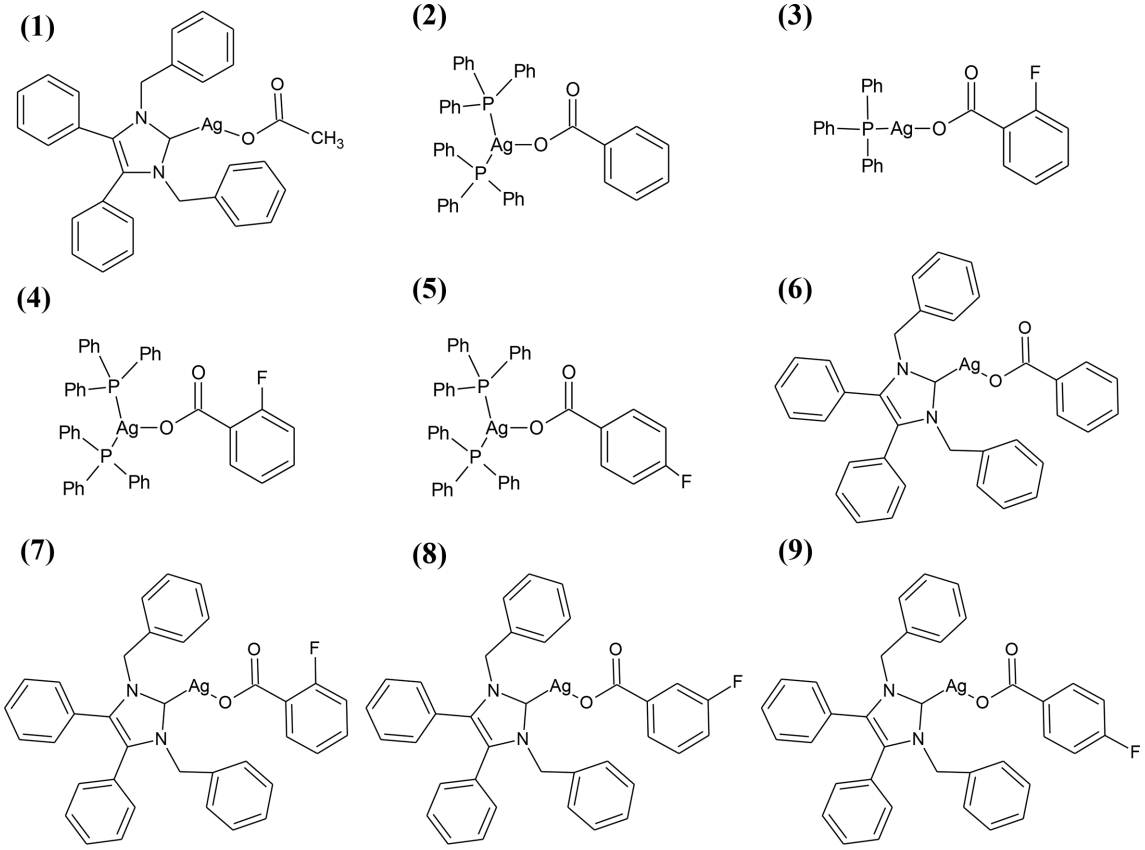

Fig. 1. Molecular structure of silver-based antibacterial compounds. (**1**) SBC3. (**2**)–(**5**) Ph_3P-Ag(I) benzoate complexes. (**6**)–(**9**) NHC*-Ag(I) benzoate complexes. Figure adapted from the literature [50, 55].

In MRSA, the NHC*-Ag(I) benzoate complexes (**7**) and (**9**) showed the highest activity, with zone of clearance radii of 4.2 and 5 mm, respectively [55]. These values were comparable to that observed for the SBC3 control. In comparison, the most active Ph_3P-Ag(I) benzoate complex, (**5**), had a zone of clearance radius of 4 mm. Although the activity of all compounds was lower than that of the antibiotic controls tetracycline and ciprofloxacin, the observed antibacterial activity of the NHC*-Ag(I) benzoate complexes in particular is high enough to warrant further research into their use as antibacterial agents.

In Gram-negative bacteria, NHC*-Ag(I) benzoate complexes also demonstrated higher activity than Ph_3P-Ag(I) benzoate complexes. In *Escherichia oli*, the NHC*-Ag(I) benzoate complexes (**6**), (**8**) and (**9**) exhibited the greatest activity with zones of clearance radii of 5–6 mm. In comparison, SBC3 displayed a zone of clearance radius of 4 mm and the Ph_3P-Ag(I) benzoate complexes demonstrated little to no inhibition [55]. The results of this study not only highlight the antibacterial activity of these novel NHC*-Ag(I) benzoate complexes, but also suggest that they may have higher antibacterial activity than those with phosphine ligands. This observation may provide insight into the further development of Ag(I)-based antibacterial complexes.

Systemic use of silver-based antibacterial compounds

In cases of clinical exposure to silver through ingestion, inhalation, dermal application, or haematogenous or urological entry, silver has shown low toxicity in humans with minimal risk [39]. Silver is absorbed into the body and enters the systemic circulation as a protein complex to be eliminated by the liver and kidneys. The metabolism of silver is modulated by its binding to metallothionines, which reduce its cellular toxicity and contribute to tissue repair [39]. Therefore, in low doses, silver does not have significant toxic side effects, as it is efficiently removed from the body. However, the toxicity of silver upon acute or chronic overexposure presents as a limitation to its potential for systemic use.

Acute symptoms of overexposure to silver include diarrhoea, hypotension, stomach irritation and bradypnoea. Chronic ingestion or inhalation of common clinical silver preparations such as colloidal silver or silver nitrate can lead to silver metal or silver sulphide deposits in the skin (argyria), eye (argyosis) or other organs. Although not life threatening, these deposits are cosmetically undesirable [39, 57]. It is important to note that soluble silver compounds, such as those proposed as antibacterial agents, have greater potential to produce adverse

effects in the human body because they are more readily absorbed than metallic or insoluble silver [58, 59].

Currently, silver-based FDA-approved drugs, such as silver sulfadiazine, are applied topically and not systemically [33, 34]. Due to the adverse effects of silver when administered systemically, silver-based compounds may only be safe for topical use. This limitation does reduce the applicability of silver-based antibacterial complexes, as they cannot be used for the treatment of systemic bacterial infections.

GOLD

The earliest recorded medical use of gold was in 2500 B.C.E. by the Chinese. In 17th century Europe, gold was used in treatments for ailments such as fainting, fevers, melancholy and falling sickness. In the 19th century, sodium tetrachloroaurate [$Na(AuCl_4)$] was used for the treatment of syphilis, the bacterial infection caused by *Treponema pallidum* subspecies *pallidum* [60]. Interest in the antimicrobial action of gold complexes originated in 1890 from the work of Robert Koch, which described the use of a gold-containing compound, potassium dicyanidooaurate (I) ($K[Au(CN)_2]$) against *Mycobacterium tuberculosis* [61].

Antibacterial mode of action of gold

Gold is an attractive metal for biological and medicinal applications, particularly Au(I) and Au(III). Au(I) is selective for enzymes bearing sulfhydryl or selenol groups whereas Au(III) is isoelectronic to Pt(II) in cisplatin and may exhibit similar properties [62–65]. These traits of Au(I) and Au(III) have been exploited for their anti-cancer effects, but Au(I) and Au(III) may also have antibacterial effects.

Although the exact antibacterial mode of action of gold is not fully understood, some studies have observed molecular targets that may be responsible [62]. Several studies strongly suggest that oxidative phosphorylation pathways in mitochondria are important intracellular targets of Au(I) and Au(III) [66, 67]. Moreover, a common mechanistic trait for the activity of Au(I) and Au(III) appears to be the inhibition of thioredoxin reductase (TrxR) [68]. Thioredoxin works with NADPH to form reduced disulfide bonds in cells among many other essential functions, such as DNA replication and responses against oxidative stress in some bacteria [69–73]. Consequently, strong inhibition of TrxR may lead to apoptosis of the bacterial cell [68].

Gold-based antibacterial compounds

Auranofin is an Au(I)-phosphine derivative (Fig. 2), currently approved as an antirheumatic drug, that has shown promising antibacterial activity by targeting TrxR [26, 74]. Recently, auranofin has demonstrated antibacterial activity against drug-resistant Gram-positive bacteria, including *S. aureus*, MRSA, *Enterococcus faecium*, and *Enterococcus faecalis*, as well as *M. tuberculosis* (MIC=0.5 μg ml^{-1}). Conversely, almost no activity was identified against *A. baumannii, P. aeruginosa* or *K. pneumoniae* (MIC≥16 μg ml^{-1}) [75]. This observed lack of antibacterial activity of auranofin against Gram-negative bacteria could be explained by the possibility that the glutathione system present in Gram-negative bacteria compensates for the lost reducing activity of TrxR caused by

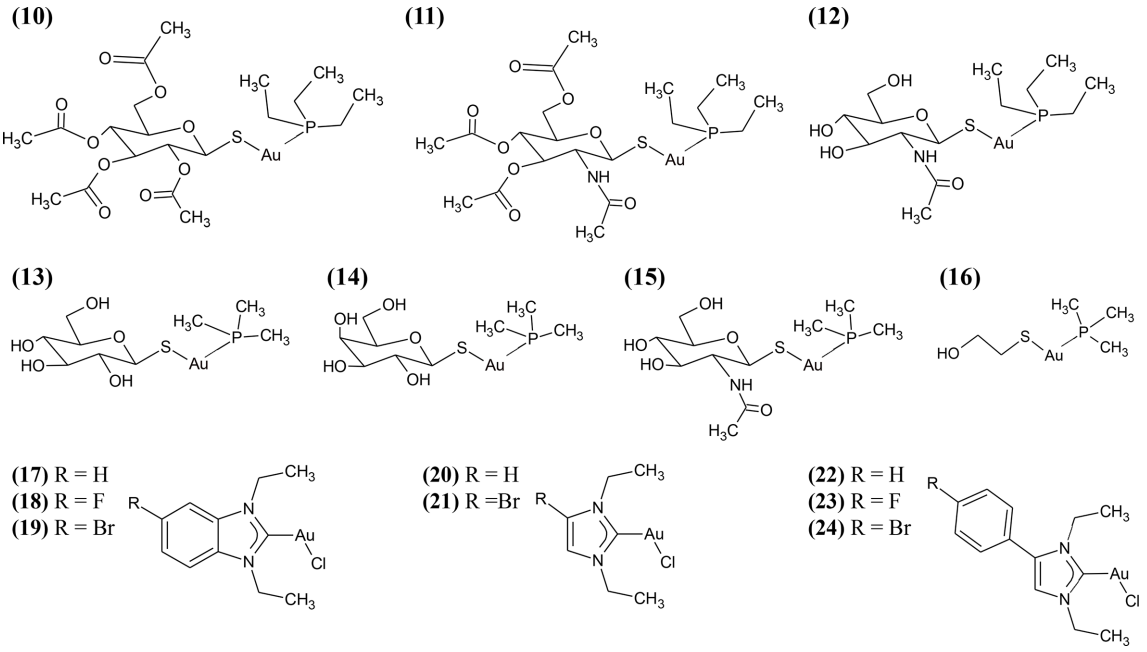

Fig. 2. Molecular structure of gold-based antibacterial compounds. (**10**) Auranofin. (**11**)–(**16**) Auranofin analogues. (**17**)–(**24**) NHC-Au(I) complexes. Figure adapted from the literature [78, 82, 92].

auranofin [76, 77]. Therefore, auranofin demonstrates promising antibacterial activity against Gram-positive bacteria in the ESKAPE pathogen species as well as *M. tuberculosis*. As antibiotic-resistant strains of these pathogens have high rates of morbidity and mortality [1], the ability of auranofin to target and kill these bacteria is a great asset.

The *in vivo* antibacterial efficacy of auranofin has also been demonstrated in a murine systemic MRSA infection model [77]. Auranofin was orally administered to mice 1 h post-infection at a dosage of either 0.125 or 0.25 mg kg^{-1}. It was observed that 80 % of mice receiving the higher dose of auranofin (0.25 mg kg^{-1}) survived for 5 days, significantly larger than the survival of the vehicle control group [77]. These results demonstrate that auranofin has antibacterial activity against the Gram-positive bacteria MRSA in a murine systemic infection model, thus increasing its potential as an effective antibacterial agent in humans.

To improve the efficacy and therapeutic index of auranofin, 40 auranofin analogues were prepared and then tested against Gram-positive and Gram-negative bacteria [78]. The study identified six different compounds, (**11**) to (**16**), that exhibited favourable MIC and minimum bactericidal activities up to 65-fold higher than that of auranofin (Fig. 2). Compounds (**13**) to (**16**) possessed a broader spectrum of activity, with bactericidal effects against both the Gram-positive and Gram-negative bacteria *S. aureus*, *A. baumannii*, *Enterobacter cloacae*, *Enterococcus faecium* and *Escherichia coli* (Table S2) [78].

The development of bacterial resistance is also of large concern when investigating potential antibacterial agents such as auranofin. A recent study found no detectable resistance in an *S. aureus* strain after 25 days of auranofin exposure [79]. Auranofin has also demonstrated potent activity against the biofilms of *Enterococcus faecalis* and *S. aureus*, as well as a synergistic antibacterial effect with the antibiotics fosfomycin, linezolid and chloramphenicol both *in vitro* and *in vivo* [80]. The observed lack of detectable resistance, potent activity against biofilms and synergistic microbicidal effect with antibiotics reduce the likelihood for the development of bacterial resistance to auranofin.

Although compounds containing phosphine ligands, such as auranofin, are the most studied in the medicinal applications of gold, interest is gaining in the stronger σ-donating NHCs [65]. NHCs have more stable and versatile steric, electronic and physical properties with thermodynamically stronger bonds to the metal in comparison to phosphine ligands [81]. Therefore, NHCs have gained more attention in medicinal chemistry, including their use as ligands for gold-based antibacterial agents [82].

Several proteins, enzymes and biochemical pathways have been identified as targets in the antibacterial mode of action of NHC-Au compounds. These targets include TrxR enzymes, G-quadruplexes, the zinc-finger enzyme PARP-1 and mitochondrial respiration [83–90]. The specific targets of each compound are dependent on the type of complex and nature of the coordinated NHC ligand. For instance, Au(III) complexes activate reduction through cellular sulfides [91], whereas Au(I)-containing cationic lipophilic complexes have enhanced effects against mitochondria [87]. Therefore, gold-based complexes with NHC ligands have similar antibacterial modes of action as gold, such as targeting TrxR and mitochondrial respiration, with slight differences in targets depending on the oxidation state of gold and nature of the NHC ligand.

Antibacterial Au(I)-based metallodrugs with NHC ligands have only recently been reported in the literature [82]. The antibacterial activity of NHC-Au(I) complexes (**17**)–(**24**) (Fig. 2) on several Gram-negative (*A. baumannii*, *Enterobacter cloacae*, *Escherichia coli*, *K. pneumoniae*, and *P. aeruginosa*) and Gram-positive (*Enterococcus faecium* and *S. aureus*) bacteria was compared to auranofin [92]. All complexes appeared to be highly effective against the Gram-positive strains of bacteria, with MIC values ranging from 0.64 to 12.51 μM, which were lower than those obtained for the control, auranofin. All complexes were effective inhibitors of bacterial TrxR, with half maximal inhibitory concentration (IC_{50}) values ranging from 0.1 to 0.5 μM [92]. The results of this study support the potential for gold-based metallodrugs with NHC ligands as antibacterial agents against specifically Gram-positive bacteria.

Systemic used of gold-based antibacterial compounds

Auranofin is currently FDA-approved for use as an antirheumatic drug and considered safe for systemic administration [75]. Although it demonstrates a low *in vitro* therapeutic index in eukaryotic cells, chronic exposure to auranofin over extended periods of time was found to be safe in patients with no cumulative toxicity observed over 5 years [93]. Auranofin has undergone clinical research for other applications as well, including a phase II clinical trial against chronic lymphocytic leukaemia (NCT01419691) [36]. Therefore, it is likely that auranofin could be administered as a systemic antibiotic to treat both acute and chronic bacterial infections without significant adverse effects. As auranofin is currently approved for clinical use as an antirheumatic drug, it is also likely that its approval for antibacterial uses could be accelerated compared to other proposed compounds.

GALLIUM

The antimicrobial properties of gallium were discovered almost a century ago for use against syphilis and trypanosomiasis [94], but until recently the discovery and use of conventional antibiotics left these observations overlooked. Gallium has been used as a diagnostic and therapeutic tool in clinical medicine for over three decades [95]. It was initially used in medical applications for the development of gallium-based radiopharmaceuticals to detect and monitor cancerous tissues. An example is radioactive ^{67}Ga, used for the localization of malignant cells and inflammatory or infective foci

[96–98]. Later, gallium uses were expanded to the development of gallium-based anticancer agents [99–101].

In 1971, the first evidence of the anticancer properties of Ga(III) was presented [95] and gallium now follows platinum as the second most-used metal in cancer treatment [102, 103]. The anticancer activity of gallium is believed to occur due to its ability to limit the availability of iron to malignant cells by binding transferrin, a blood iron transporter [104, 105]. This action stimulates calcium efflux from the mitochondria, compromising mitochondrial function, and consequently initiating apoptosis [106]. As iron is also required for bacterial function, focus has recently started to shift to the antibacterial properties of gallium.

Antibacterial mode of action of gallium

Iron is required by both the mammalian host and bacteria, which leads to competition for iron during a bacterial infection [107]. Within a mammalian host, iron is sequestered for use in the metabolic processes associated with respiration, redox homeostasis, and DNA synthesis and repair [108, 109]. Iron is also made unattainable to pathogens as a protective strategy against infection [110]. Some bacteria, such as *P. aeruginosa*, circumvent this protective mechanism by possessing multiple iron uptake systems. Examples of these iron uptake systems in *P. aeruginosa* are the two siderophores, pyochelin and pyoverdine, with low and high affinities to iron respectively [111]. As bacterial iron uptake systems cannot differentiate between iron and gallium, bacteria can incorporate gallium into the cell instead of iron, causing detrimental effects [112].

The antibacterial activity of gallium compounds is related to their ability to act as an iron mimetic [113]. Ga(III) ions are similar to Fe(III) ions and can thus target the iron metabolism of bacteria by replacing Fe(III). Normally, ferric reductases within the bacterium reduce Fe(III) to the more soluble ferrous ion Fe(II) for incorporation into iron-dependent enzymes [114]. Ga(III) incorporated into iron-dependent enzymes cannot be reduced to Ga(II), inhibiting the enzyme. Interference with iron metabolism in bacteria has proven effective at diminishing infection rates [113, 115, 116] as iron is an essential micronutrient for the growth, survival and virulence of many bacteria but is not easily accessible in a mammalian host [117, 118]. Thus, the use of gallium-based compounds that interfere with bacterial iron metabolism may have promising antibacterial properties.

Gallium-based antibacterial compounds

Gallium nitrate [$Ga(NO_3)_3$] is a gallium-based complex (Fig. 3) that has recently shown promising activity against several bacterial species including *P. aeruginosa* and *A. baumannii* [119]. Gallium nitrate has proven effective at inhibiting the growth of clinical *P. aeruginosa* strains, including multidrug-resistant and cystic fibrosis (CF) isolates (IC_{90}=1–40 μM). In a murine model of acute and chronic lung infections, gallium nitrate demonstrated a bactericidal effect, reducing lung injury and bacterial load [116]. Similarly, in *A. baumannii*, micromolar concentrations of gallium nitrate reduced bacterial growth in iron-poor media (IC_{90}=2–80 μM) and in human serum (IC_{90}=4–64 μM). Gallium nitrate administered to *G. mellonella* was shown to effectively suppress *A. baumannii* pathogenicity *in vivo* with a survival rate of ≥75 % [120, 121]. *P. aeruginosa* causes severe hospital infections and has high intrinsic antibiotic resistance [116] and *A. baumannii* is considered the most resistant pathogen among non-fermenting Gram-negative bacteria [119]. Therefore, there is great need for the development of novel antibacterial agents against these pathogens, and gallium nitrate may be an effective option.

The antibacterial activity of gallium nitrate was in part due to its ability to decrease iron uptake and to interfere with iron signalling by its iron-responsive transcriptional regulator *pvdS* [116]. *pvdS* is a key regulator of the iron starvation response in *P. aeruginosa* which regulates the expression of genes linked

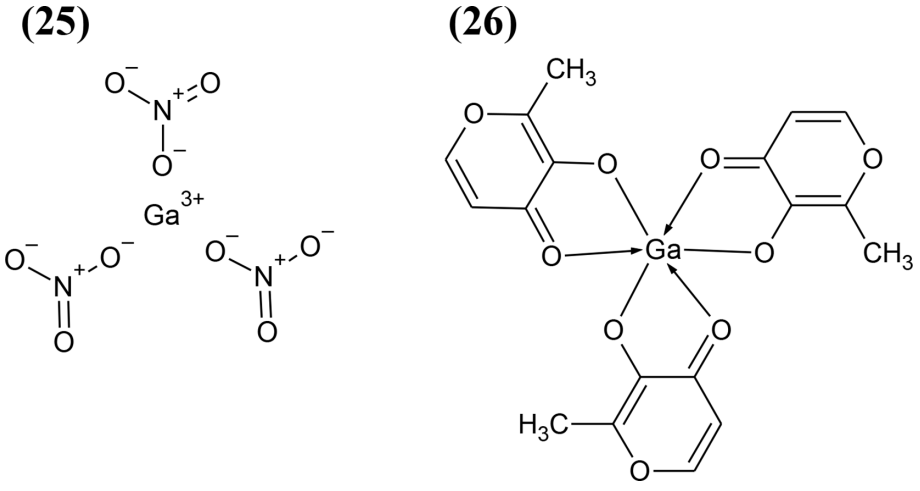

Fig. 3. Molecular structure of gallium-based antibacterial compounds. (**25**) Gallium nitrate [$Ga(NO_3)_3$]. (**26**) Gallium maltolate.

to the assembly and transport of the pyoverdine siderophore and the synthesis of extracellular factors such as protease PrpL and exotoxin A [122]. As the pyoverdine siderophore, protease PrpL, and exotoxin A are key virulence factors for *P. aeruginosa*, interference with *pvdS* leads to impaired iron uptake and reduced virulence in the bacterium.

Recently, the antibacterial activity of gallium nitrate was investigated in pre-clinical work and in a pilot phase Ib non-randomized clinical trial in individuals with CF and chronic *P. aeruginosa* airway infections [115]. In murine models of lung infection, systemic gallium nitrate treatment increased survival and reduced lung and blood *P. aeruginosa* counts. In patients with CF, it was observed that micromolar concentrations of gallium nitrate added to patient sputum samples *ex vivo* inhibited *P. aeruginosa* growth and increased its sensitivity to oxidants. Assays conducted *in vitro* also identified that the antibacterial activity of gallium was synergistic with the antibiotics colistin and piperacillin/tazobactam but antagonistic to tobramycin. In the phase I clinical trial in people with CF and chronic *P. aeruginosa* lung infections, systemic gallium nitrate treatment improved lung function; as indicated by statistically significant increases in forced expiratory volume in one second 14 and 28 days after a single infusion. Analysis of sputum samples from patients receiving gallium nitrate treatment also showed a reduction in sputum *P. aeruginosa* concentrations, although these data were not statistically significant [115]. A phase II, multi-centre, randomized, placebo-controlled study on the antibacterial action of gallium nitrate in CF patients with chronic *P. aeruginosa* infections was completed in 2018, with data analysis pending. The purpose of this study was to assess the safety and clinical efficacy of a 5 day infusion of intravenous gallium nitrate to improve pulmonary function over a 28 day period, as measured by an improvement in forced expiratory volume in 1 s (NCT02354859) [36]. As gallium nitrate is a source of free gallium, these results demonstrate that systemic gallium administration is effective in both a murine model of acute lung function and in a chronic model of infection in patients with CF. Furthermore, gallium treatment may work synergistically with certain antibiotics, although further research is required to test this *in vivo*.

The frequency at which *P. aeruginosa* develops resistance to gallium nitrate was compared to that of the conventional anti-pseudomonal antibiotics colistin, tobramycin and ciprofloxacin [115]. It was observed that spontaneous resistance to gallium nitrate was acquired at rates comparable to that of successful anti-pseudomonal antibiotics [115]. This frequency for the development of resistance suggests that gallium nitrate may be more effective against bacteria with high rates of resistance, such as *P. aeruginosa*.

A particular benefit to the antibacterial use of gallium-based compounds is their efficacy against biofilms, as biofilm-living bacteria are more resistant to antibiotics and are often responsible for chronic infections [123, 124]. While *P. aeruginosa* biofilms are relatively difficult to eradicate through the use of antibiotics [124, 125], they are highly sensitive to Ga(III). It has been shown that a sub-micromolar concentration of gallium nitrate (0.5 μM) strongly inhibited *P. aeruginosa* biofilm formation while higher concentrations (100 μM) were able to kill *P. aeruginosa* cells deeply embedded in the biofilm matrix, where most antibiotic efficacy is lost [116]. Other Ga(III) compounds have also shown activity against the formation of *P. aeruginosa* biofilms in the micromolar range, such as gallium trichloride ($GaCl_3$) and Ga(III)-citrate [126, 127]. As *P. aeruginosa* bacterial infections in CF commonly form biofilms, promoting antibiotic resistance [128–131], a therapy that can effectively treat *P. aeruginosa* biofilm formation would be particularly beneficial for CF patients.

Gallium maltolate (GaM) is another gallium-based compound that shows promising antibacterial activity against antibiotic-resistant bacteria with significant disease burdens, particularly staphylococci [132]. GaM is a water-soluble complex composed of a central gallium cation coordinated to three maltolate ligands with the formula $[Ga(Maltol_{-1H})_3]$ (Fig. 3). GaM has shown *in vitro* activity against *Rhodococcus equi* [133, 134], staphylococci [132], *P. aeruginosa* [112], *Salmonella* Newport [135] and *Mycobacterium avis* [136]. GaM was administered at concentrations of 50 to 200 μM to *S. aureus* and MRSA and was found to inhibit their growth *in vitro* at time points between 8 and 36 h after inoculation [137]. Topically administered GaM is also effective against *P. aeruginosa* in a murine burn/infection model [112]. Both *P. aeruginosa* and staphylococci are significant sources of human and veterinary morbidity and mortality due to their frequency of antibiotic resistance [1]. Thus, GaM demonstrates potential for the treatment of bacterial infections with a high need for novel antibiotics.

Systemic use of gallium-based antibacterial agents

Extensive data exist on the safety of the systemic administration of gallium nitrate. A phase I clinical trial conducted on patients with CF observed no adverse effects upon treatment with gallium nitrate. The study also monitored kidney function, electrolyte levels, blood counts and sputum levels, which all appeared unaffected, leading to the conclusion that gallium nitrate appears safe for systemic use [115].

Gallium nitrate is also the active component of the FDA-approved formulation of Ganite, a bone resorption inhibitor used to treat hypercalcaemia in cancer patients, and thus extensive data exist on its safety when administered systemically. From its use as a bone resorption inhibitor, data have been collected that show no cytotoxic effects on bone cells in drug-treated animals [138]. It is known that gallium is preferentially concentrated in inflamed tissues, macrophages, neutrophils and bacteria. Therefore, when administered systemically, gallium-based compounds have a potent, site-specific effect [138]. The only contraindication for the administration of Ganite is for patients with severe renal impairment (serum creatinine >2.5 mg dl^{-1}). Although discontinued by the manufacturer in 2012, the FDA determined that Ganite was not removed for any safety or efficacy reasons, and therefore it is still FDA-approved [139].

GaM has undergone investigation for oral and topical administration, demonstrating the potential for safe administration and enhanced bioavailability for both [140, 141]. GaM has been used safely in other mammals with no association with tissue toxicity and no side-effects upon oral administration, and has shown good bioavailability in humans [142]. Currently, GaM is used in the facial cream Gallixa, which is indicated as an anti-inflammatory agent and can be used to relieve the pain caused by many dermatological pathologies, including psoriasis, eczema and warts [143]. GaM has a neutral charge and pH, with moderate solubility in both water and lipids, making it well suited for systemic administration [141].

It is also important to note that most studies investigating the antibacterial activity of gallium-based compounds use iron-poor media, as high concentrations of iron have been shown to reduce the efficacy of gallium. Other studies show that gallium activity is further improved in human serum, however the addition of exogenous iron could severely reduce the antibacterial activity of gallium [119, 120].

COPPER

Copper has a long history of antibacterial use [144]. Around 400 BC the Greeks prescribed copper for pulmonary diseases and for the purification of drinking water. In Hinduism, Gangajal ('Holy water') is stored in copper utensils to keep the water clean [144]. Copper is currently a widely used antimicrobial agent for external purposes, such as in face masks and stain-resistant fabrics, for the prevention of bacterial growth [145, 146].

Copper has demonstrated high inhibitory potential for Gram-positive bacteria [147], is more chemically and physically stable than silver, has been shown to bind amine and carboxyl groups on the surface of Gram-positive bacteria more readily than silver particles, and is less expensive than silver [148]. These properties provide copper with higher antibacterial activity against Gram-positive bacteria, give it higher efficacy in physiological conditions, and make it a more cost-effective antibacterial agent. Moreover, copper has shown potential synergistic activity with drugs in the body [149, 150] allowing it to be used in combination with conventional antibiotics to increase their antibacterial activity against drug-resistant bacteria.

Antibacterial mode of action of copper

The antimicrobial activity of copper is not well understood, although a common hypothesis is through its generation of hydroxyl radicals [151, 152]. Copper releases metal ions when in an aqueous environment, such as Cu(I) and Cu(II). These ions directly facilitate hydrolysis through polarization of the target molecule followed by a nucleophilic attack by the newly formed hydroxyl radical. Copper ions can engage in nucleophilic attack of a bacterial cell membrane, nucleic acids or nucleic proteins, to alter their structure and impair their function, eventually leading to cell death [153].

When inside a bacterial cell, copper ions will also selectively bind the sulfhydryl groups of respiratory enzymes within the bacterial cell membrane and attack essential proteins, particularly in *Escherichia coli* [153–156]. Therefore, copper-based compounds may provide useful antibacterial activity for the treatment of antibiotic-resistant strains of bacteria such as *Escherichia coli*.

Copper-based antibacterial compounds

The development of copper-based antibacterial complexes is a current area of research interest. Like many agents [157], when administered in the form of metallic complexes with antibiotics, copper has demonstrated enhanced biological activity, compared to the parent compound alone [158, 159]. Cu(II) has been studied most extensively for the development of copper-based antibacterial complexes as it has proven effective against rheumatoid diseases, tuberculosis (TB), cancers and gastric ulcers [160–162].

Both the antimicrobial and anti-cancer properties of Cu(II)-based compounds were recently outlined [163]. The general formula for this newly developed family of metal-based antibiotics is [Cu(N,N)(CipA)Cl] where N,N represents a phenanthrene ligand to act as a photosensitizer, and CipA is ciprofloxacin (Fig. 4). The results of this study reported that these Cu(II)-antibiotic compounds appeared to specifically inhibit Gram-positive bacteria with greater potency than the free CipA antibiotic by an order of magnitude. Of these compounds, the most active against Gram-positive strains of bacteria were (**27**) and (**29**) with MIC_{95} values ranging from 7.81 to 31.25 μM. Of particular interest is the activity of (**29**) against MRSA as it had a low MIC_{95} value (15.2 μM) compared to CipA (125 μM). In Gram-negative strains, all three complexes exhibited moderate to poor antibacterial activity (MIC_{95}=31.25 to >125 μM), suggesting these Cu-N,N-CipA complexes selectively target Gram-positive bacteria [163].

Recently, the antibacterial activity of quinolone-based copper complexes has been investigated [164]. Quinolone-based transition metal complexes are known to interact with DNA gyrase enzymes, such as topoisomerases type II and IV, which participate in the under- or over-winding of DNA for replication [165]. These complexes bind the topoisomerases, preventing their proper function and converting them into DNA-damaging agents [166]. Therefore, the combination of quinolone-based antibiotics complexed with copper is a potential avenue for the creation of copper-based antibacterial complexes that are effective against antibiotic-resistant strains of bacteria.

The antibacterial effects of Cu(II) in a complex with nalidixic acid and 2,2′-bipyridine has been characterized (Fig. 4) [167]. Nalidixic acid is an orally administered antibiotic and is the first derivative within the quinolone antibiotic class [168]. The antibacterial activity of the nalidixic acid and Cu(II) complex, (**30**), was screened against the Gram-negative bacterium *Escherichia coli* and the Gram-positive bacterium *S. aureus*. These results were then compared to nalidixic acid,

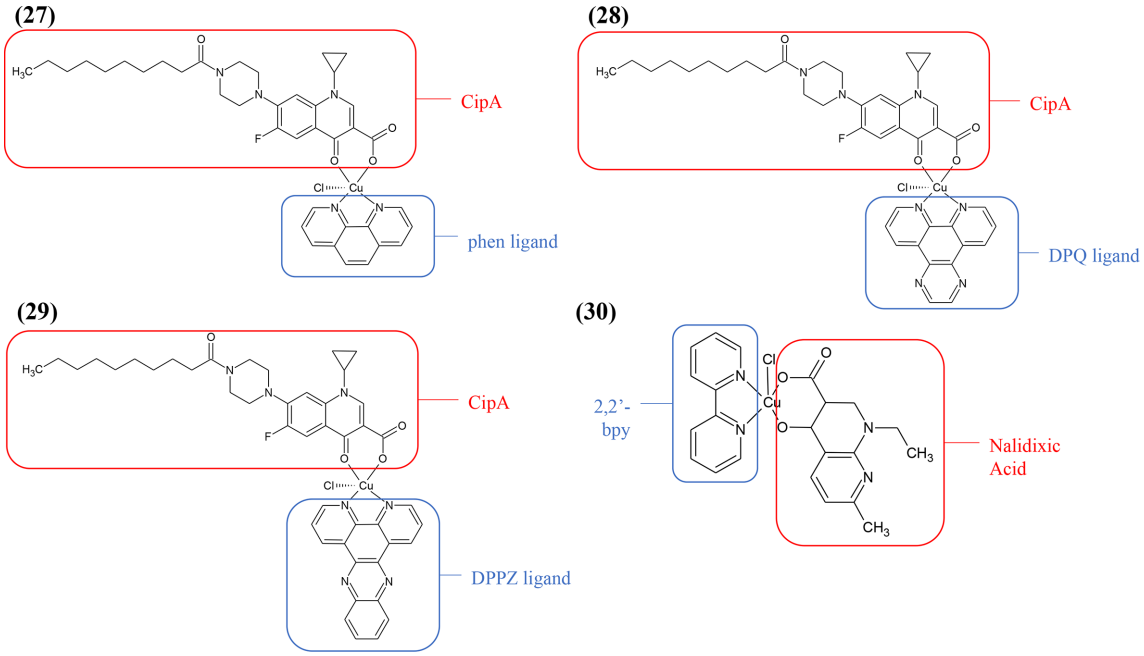

Fig. 4. Molecular structure of copper-based antibacterial compounds. The blue box indicates the ligand of each complex. The red box indicates antibiotic in the complex (CipA or nalidixic acid). Cu-N,N-(CipA$_{-H}$)Cl complexes include (**27**) Cu-phen-CipA, (**28**) Cu-DPQ-CipA and (**29**) Cu-DPPZ-CipA. (**30**) Cu(II) complex with nalidixic acid and 2,2′-bipyridine. phen=1,10-phenanthroline, DPQ=dipyridoquinoxaline, DPPZ=dipyridophenazine, bpy=bipyridine. Figure adapted from the literature [163, 167].

neomycin and streptomycin alone. The results of this *in vitro* study showed higher zones of clearance for (**30**) than nalidixic acid in both Gram-positive and Gram-negative bacteria, indicating that the complex had more broad-spectrum antibacterial activity than nalidixic acid alone. However, the activity of (**30**) was less than that of the neomycin and streptomycin drugs [167].

The enhanced antibacterial activity of the Cu(II) complex over nalidixic acid alone may be due to the high lipophilicity of the Cu(II) complex. According to chelation theory, the π electrons over the whole chelate ring become increasingly delocalized, causing the polarity of the ligand and central copper ion to decrease [169]. This reduced polarity allows the complex to penetrate the lipid layer of the cell membrane where the nalidixic acid can prevent bacterial cell growth [170]. Therefore, combination therapies of copper and quinolone antibiotics may provide a promising resource for the development of antibacterial complexes capable of treating antibiotic-resistant strains of bacteria.

To assess the risk for the development of bacterial resistance against copper, one study isolated *Bacillus subtilis* strains from soil samples containing differing concentrations of copper [147] and it was observed that the strains did not perform differently to the treatment regimen regardless of their originating microhabitat. Furthermore, when low dosages of the copper+antibiotic regimen were administered in an attempt to promote the development of tolerance, no adaptive evolution to the treatments was observed [147]. These findings promote the idea that a copper+antibiotic treatment may be less prone to the development of resistant mechanisms than antibiotics alone.

Systemic use of copper-based antibacterial compounds

Copper is the third most abundant trace element in human cells, after zinc and iron [171]. It is essential for regulating the strength of skin, the urinary tract, blood vessels and connective tissues within the body [172]. Its natural presence in the human body may indicate that copper is safer for systemic use than other metals.

The *in vivo* toxicity of the novel copper-based antibiotic compounds (**27**) to (**29**) was evaluated. The compounds were administered to *G. mellonella* larvae via direct injection into the haemocoel through the last pro-leg [163]. All tested complexes were well tolerated by the larvae and (**28**) appeared to be the least toxic (no death observed 72 h after exposure across the concentration range), although it also had the lowest antibacterial activity of the three compounds [163]. The low *in vivo* toxicity of these Cu-N,N-CipA complexes in *G. mellonella* indicates their potential for safe administration, although further research is required in more advanced models to determine the safety of these compounds in humans.

It is important to note that copper is known to cause toxicity at high levels [173, 174]. In addition, most studies investigating

the antibacterial activity of novel copper-based compounds have been conducted *in vitro* or in preliminary *in vivo* models. Further research is required in more advanced animal models and eventually humans to determine if copper-based compounds are safe for systemic or topical administration at therapeutic levels in humans to allow for their safe and effective use as antibiotics.

MANGANESE

Manganese does not have as strong of a history in antimicrobial use as other transition metals, although it has recently gained attention for use in metal-based antibacterial compounds [25]. Several manganese-based antibacterial complexes are currently under investigation for their antibacterial activity against antibiotic-resistant bacteria.

Antibacterial mode of action of manganese

Relatively small levels of transition metal ions, such as manganese, are essential micronutrients for the support of bacterial growth and homeostasis, whereas high levels of exposure can be toxic to bacteria. At toxic levels, transition metal ions bind and disable important biomolecules and promote oxidative stress [175, 176]. A lack of bacterial transporters to remove manganese allows for its concentration to build to toxic levels within the bacteria [177]. These antibacterial properties of manganese can be exploited for its use as a metal-based antibacterial compound, especially against bacterial strains resistant to conventional antibiotics.

Manganese-based antibacterial compounds

Mn(I), Mn(II) and Mn(III) have demonstrated *in vitro* antibacterial activity against varying strains of Gram-positive bacteria and *M. tuberculosis*, although their specificity for bacterial species and the ligands used in these metal complexes vary [177–179].

The efficacy of Mn(I) tricarbonyl complexes with ketoconazole, miconazole and clotrimazole ligands (Fig. 5) against the Gram-positive bacteria *S. aureus*, *S. epidermidis*, *Enterococcus faecalis* and *Enterococcus faecium* [178] was evaluated. Although commonly used as antifungal agents, ketoconazole, miconazole and clotrimazole also demonstrate antibacterial activity [180]. All metal complexes exhibited higher antibacterial activity than their pure antibiotic ligands alone, up to a factor of 30 in the case of clotrimazole. The activity among the metal complexes increased in the order of ketoconazole < miconazole < clotrimazole ligands. Compound (**35**) bearing the clotrimazole ligand had the highest antibacterial activity across all Gram-positive bacteria (MIC=0.625–2.5 μM). Furthermore, the addition of a methyl ester group in the 4- and 4′-positions of the 2,2′-bipyridine ligands in complexes (**31**) versus (**32**) and (**33**) versus (**34**) (Fig. 5) generally resulted in an increased MIC value and lower potency [178]. In contrast, no activity was observed against the Gram-negative bacterial

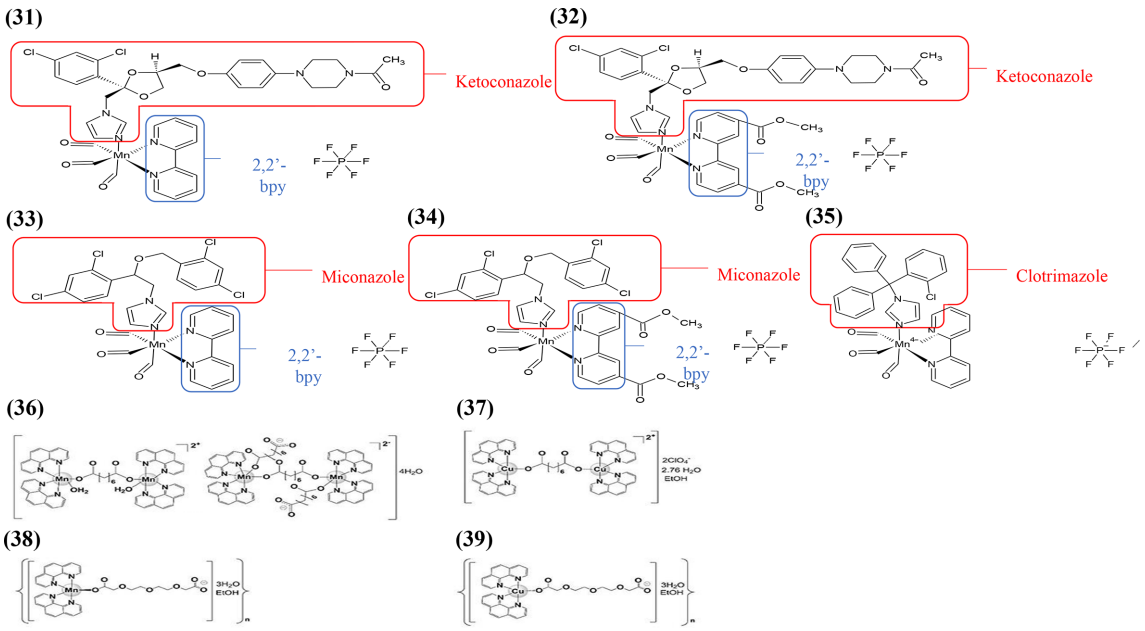

Fig. 5. Molecular structure of manganese-based antibacterial compounds. (**31**)–(**35**) Mn(I) tricarbonyl complexes with ketoconazole, miconazole and clotrimazole ligands. The general formula of the Mn(I) tricarbonyl compounds is $[Mn(CO)_3(bpy^{H,COOCH_3})(azole)]PF_6$ where azole=the antibiotic ligand and bpy=bipyridine. The red box highlights azole ligand. The blue box indicates 2,2'-bpy ligands. (**36**)–(**39**) Mn(II) and Cu(II) complexes containing 1,10-phenanthroline and dicarboxylate ligands. (**36**) and (**38**) use Mn(II), (**37**) and (**39**) are the Cu(II) counterparts, respectively. (**36**) $[Mn_2(oda)(phen_4)(H_2O)_2][Mn_2(oda)(phen)_4(oda_2]\cdot 4H_2O$, (**37**) $[Cu_2(oda)(phen)_4](ClO_4)_2\cdot 2.76H_2O\cdot EtOH$, (**38**) $[(Mn(3,6,9\text{-tdda})(phen)_2)\cdot 3H_2O\cdot EtOH]n$, (**39**) $[(Cu(3,6,9\text{-tdda})(phen)_2)\cdot 3H_2O\cdot EtOH]_n$. Figure adapted from the literature [177, 178].

strains of *Escherichia coli*, *P. aeruginosa*, *Yersinia pseudotuberculosis* and *Yersinia pestis* [178], which aligns with previous data [181, 182].

Manganese-based complexes containing Mn(II) have also shown promising antibacterial activity against resistant strains of bacteria. The effect of Mn(II) complexes containing 1,10-phenanthroline and dicarboxylate ligands (Fig. 5) against (multi)drug-resistant strains of *M. tuberculosis* [177] was assessed. The results of these water-soluble Mn(II) phen/dicarboxylate complexes were then compared to Cu(II) phen/dicarboxylate complexes to determine which metal-based antibiotic complex had the superior activity, selectivity and toxicity [177].

Of the nine complexes evaluated in this study, (**36**) and (**38**) were most effective against *M. tuberculosis* with MIC values of 0.47 and 0.76 µM, respectively. These MIC values are comparable to that of isoniazid (MIC=0.44 µM), an antibiotic used to treat TB. The copper counterparts (**37**) and (**39**) were less active against *M. tuberculosis* and more toxic towards VERO and A549 mammalian cell lines. These results show that Mn(II)-based complexes with phen/dicarboxylate ligands have high antibacterial activity against (multi)drug-resistant strains of *M. tuberculosis* and lower toxicity against mammalian cells than their copper counterparts. Therefore, these Mn(II)-based complexes may be an effective form of metal-based antibiotic for the treatment of (multi)drug-resistant *M. tuberculosis* infections.

Although Mn(III) has also been investigated for its antibacterial activity, less research exists, and the results are not as promising. The antibacterial activity of two new Mn(III) Schiff base complexes was evaluated and the compounds demonstrated some antibacterial activity. Compound 1 had an MIC value of 400 µg ml^{-1} against *Shigella dysenteriae* 1, *Vibrio cholerae non. 139*, *Escherichia coli* and *S. aureus*. Compound 2 had a MIC value of 500 µg ml^{-1} against *V. cholerae non. 0139*, *Escherichia coli* and *Streptococcus pneumoniae* [179]. The compounds were also tested on other human pathogenic strains but had no significant antibacterial effects. Therefore, Mn(I)- and Mn(II)-based antibacterial complexes show more promise as potential antibiotic candidates than Mn(III).

Systemic use of manganese-based antibacterial compounds

Manganese is a trace element in the human biological system. It is an essential cofactor for the activity of several enzymes including manganese superoxide dismutase, pyruvate carboxylase and arginase [183, 184]. The actions of these enzymes implicate manganese in amino acid, carbohydrate, glucose and cholesterol metabolism. Manganese is also involved in bone formation, the immune response, reproduction, scavenging for ROS, blood clotting and the haemostasis effect of vitamin K through these enzymes [185–189].

In most adults, it is safe to take manganese supplements without any side effects, unless there is an issue with clearing excessive manganese, such as in patients with liver disease [189]. Excessive levels of manganese in the body can have serious side effects for a patient, including poor bone health and neurological symptoms that resemble those of Parkinson's disease [189].

As many of the complexes discussed above are novel, limited data are available on their safety and toxicity for systemic use. Significant toxicity was observed when complexes (**31**) to (**35**) were tested on two mammalian cell lines (HEK293, J774.1) [178]. The toxicity of these compounds presents a low therapeutic index between antibacterial activity and toxicity towards the patient's cells.

The *in vitro* and *in vivo* toxicity of the Mn(II)-based antibacterial complexes demonstrated that (**36**) and (**38**) have promise for systemic use [177]. Complex (**36**) had IC_{50} values of 152.55 and >208.3 µM in VERO and A549 cells respectively, compared to an MIC value of 0.47 µM against *M. tuberculosis*. Complex (**38**) had IC_{50} values of 84.96 and 355.70 µM in VERO and A549 cells respectively, compared to an MIC value of 0.76 µM against *M. tuberculosis*. In comparison, the IC_{50} values for the control, isoniazid, were 2426 and 2325 µM in VERO and A549 cells respectively, compared to an MIC value of 0.44 µM against *M. tuberculosis* [177]. Although the compounds have a lower therapeutic index than conventional TB antibiotics such as isoniazid, the therapeutic index is high enough to warrant potential for the use of the Mn(II)-containing compounds (**36**) and (**38**) in humans.

Further *in vivo* studies in *G. mellonella* larvae showed further promise for the safe use of compounds (**36**) and (**38**) in humans. The data show 10% survival at the highest administered dose of 333 mg kg^{-1} and no fatalities when a 33 mg kg^{-1} dose was administered for complexes (**36**) to (**39**). In particular, complexes (**36**) and (**38**) showed a marked improvement in survival when the dose was reduced from 333 to 67 mg kg^{-1} [177]. These Mn(II)-based complexes demonstrate lower toxicity than their Cu(II) counterparts and the potential for systemic use against *M. tuberculosis*.

Clinical data are required to determine the safety and efficacy of manganese-based antibacterial compounds. Mn(I)- and Mn(II)-based complexes have demonstrated strong antibacterial activity against Gram-positive bacteria and *M. tuberculosis* respectively, warranting further research into their safety and efficacy. The Mn(II) complexes containing 1,10-phenanthroline and dicarboxylate ligands, (**36**) and (**38**), show low toxicity in mammalian cells and insect models [177, 178]. However, the therapeutic index of the Mn(I)-based complexes investigated by Simpson *et al.* is relatively low [178]. Furthermore, little research has been conducted to evaluate the safety of manganese-based antibacterial compounds *in vivo* in more advanced animal models. Therefore, further research is required to determine the safety and potential for systemic use of each manganese-based antibacterial compound in humans.

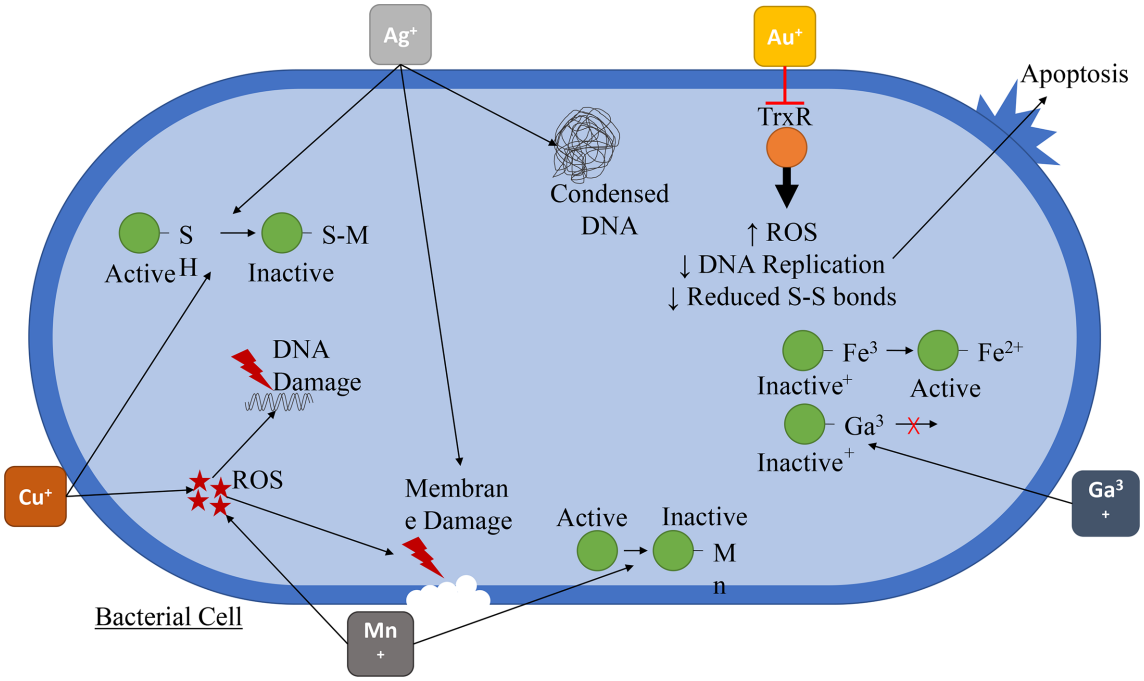

Fig. 6. Method of antibacterial action of all discussed metals. M=metal. Silver releases small amounts of ions which can bind to and condense DNA, rendering it inaccessible for replication and susceptible to mutations. Silver can also inactivate cysteine residues on enzymes through their sulfhydryl groups, inhibiting enzymatic activity, and can target bacterial membranes, increasing permeability and reducing their stability. Gold inhibits the reducing activity of TrxR, increasing ROS and decreasing DNA replication and the formation of reduced disulfide bonds. As Ga(III), gallium acts as an iron mimetic, incorporating itself into iron-dependent enzymes. As Ga(III) cannot be reduced to Ga(II), it inhibits the activity of the enzyme. Copper promotes the production of ROS, which then cause damage to DNA as well as the bacterial membrane. Manganese promotes the production of ROS and binds and inactivates important biomolecules.

CONCLUSIONS

Metal-based antibacterial complexes have several advantages over conventional antibiotics, making them an important candidate for the treatment of antibiotic-resistant bacteria. Metal complexes have access to multiple and novel modes of action, including ligand exchange reactions, the production of ROS, the release of bioactive molecules or interacting with nucleic acids [26]. The mode of action for each metal discussed in this review is summarized in Fig. 6. These multiple and novel modes of action may also prevent the development of antibiotic resistance, as bacteria must develop multiple mechanisms of resistance against the metal-based antibiotic. Relatively non-specific activity, such as the production of ROS, further increase the difficulty for the development of resistance. Furthermore, the rate at which resistance developed against metal complexes was comparable to that of conventional antibiotics [115], while others report no development of resistance, even after many rounds of treatment [190–192]. Although research is required to confidently determine the frequency at which bacteria develop resistance against specific metal-based complexes, the data thus far suggest that these compounds may be less likely to induce AMR.

Despite these advantages, there are some limitations to the use of metal-based antibiotics. First, the amount of research and knowledge amassed on the pharmacological and metabolic behaviour of organic compounds is vast, whereas little is still understood about metal complexes. Therefore, the toolkit for the development of organic compounds is much larger than that of metal-based antibiotics. The cost of metals is also an important factor to consider as more expensive metals will increase costs, particularly in the development of antibiotics where dosages can be high and there is significant market competition. Furthermore, although their multiple and novel modes of action may prevent the development of antibiotic resistance, they may also promote *in vivo* toxicity of metal-based antibacterial compounds, as mammalian cells contain some similar targets to bacteria. Finally, as some metals are not considered trace elements, they do not naturally exist within the human body. As a result, these metals may have an increased toxicity when used systemically or even topically in humans and further research is required to evaluate this risk.

It should be noted that although the above *in vitro* and *in vivo* experiments are indicative of the therapeutic applications of metal-based antibacterial compounds, some are not identical to treatment modalities in humans. For instance, the gallium study by Kaneko *et al.* used a different nasal aspiration treatment method than is used in humans [116] and it is possible the different treatment modalities could

have diverging effects. Moreover, in infection studies, if antibiotic treatment is administered relatively shortly after bacterial inoculation, the efficacy of the metal-based compound against established infections may be different. Therefore, more *in vivo* research is required to determine the antibacterial efficacy of each potential metal-based antibiotic in conditions similar to those seen clinically.

It is clear that for metal-based antibacterial agents to be effectively and safely used, more research is required. The number of metal-containing antibacterial compounds studied to date is vastly less than that of organic compounds, highlighting a gap in current research and knowledge that could provide a great benefit for the future of antibiotics. Moreover, some metals, such as nickel, cobalt, tungsten, molybdenum and osmium, have not been thoroughly investigated for their antibacterial activity. A greater understanding of the properties that give metal-based agents higher antibacterial activity and lower toxicity will provide a successful toolkit for the development of compounds that are safe and effective for systemic administration. It is possible that with further and more in-depth research, metal-based antibacterial compounds could become a solution for the treatment of bacterial infections resistant to antibiotics.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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