Articles

Materials Synthesis and Applications

2017

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Recommended Citation

FINAL

REVIEW ARTICLE

The Antibacterial Activity of Metal Complexes Containing 1,10-phenanthroline: Potential as Alternative Therapeutics in the Era of Antibiotic Resistance

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Abstract: The "antibiotic era", characterized by the overuse and misuse of antibiotics, over the last half-century has culminated in the present critical "era of resistance". The treatment of bacterial infections is challenging because of a decline in the current arsenal of useful antibiotics and the slow rate of new drug development. The discovery of a new gene (*mcr-1*) in 2015, which enables bacteria to be highly resistant to polymyxins (such as colistin), the last line of antibiotic defence left, heralds a new level of concern as this gene is susceptible to horizontal gene transfer, with alarming potential to be spread between different bacterial populations, suggesting that the progression from "extensive drug resistance" to "pan-drug resistance" may be inevitable. Clearly there is a need for the development of novel classes of anti-bacterial agents capable of killing bacteria through mechanisms that are different to those of the known classes of antibiotics. 1,10-phenanthroline (phen) is a heterocyclic organic compound which exerts *in vitro* antimicrobial activity against a broad-spectrum of bacteria. The antimicrobial activity of phen can be significantly modulated by modifying its structure. The development of metal-phen complexes offers the medicinal chemist an opportunity to expand such structural diversity by controlling the geometry and varying the oxidation states of the metal centre, with the inclusion of appropriate auxiliary ligands in the structure, offering the opportunity to target different biochemical pathways in bacteria. In this review, we summarize what is currently known about the antibacterial capability of metal-phen complexes and their mechanisms of action.

Keywords: 1,10-phenanthroline, metal complexes, antibacterial activity, antibiotic resistance, alternative therapeutics.

INTRODUCTION

Antibiotic Resistance

The discovery of antibiotics in 1939 and 1940 was an important historical advance in medicine for establishing a pivotal role in the control of untreatable and fatal infectious diseases [1]. The "antibiotic era" has been marked by the introduction of a myriad of new antimicrobials with the almost immediate subsequent emergence of resistance to those drugs [2]. The sequence of events, characterized by the overuse and misuse of antibiotics, over the last half-century has led to the present critical "era of resistance" [3]. Bacterial resistance to antibiotics has evolved and now impacts significantly on public health, responsible for high rates of mortality and morbidity globally [4]. Over 13 million deaths occur worldwide as a result of the emergence of new

infectious diseases and the re-emergence of diseases caused by multidrug resistant (MDR) strains of bacteria [3]. The development and spread of antimicrobial resistance is related to (i) a lack of public knowledge about antibiotics causing human overuse/misuse of antibiotics and unnatural selective pressure on bacteria, (ii) misuse of antibiotics in animal feed stocks associated with food production, (iii) natural process of evolution of bacterial resistance to antibiotics and (iv) a diminished interest in the development of antibiotics within the pharmaceutical industry [1, 5]. The onset of the "era of resistance" has seen antibacterial drugs become less effective or even ineffective [6]. Furthermore, therapeutic options for the treatment of infections have become limited, leading frequently to recurrent infections, treatment failure and increase of morbidity and mortality, resulting in a global health emergency [1].

The big challenge for public health is the development and/or implementation of effective strategies to decrease the emergence and spread of antimicrobial resistance [1]. The magnitude of the problem worldwide and the impact on hu-

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man health and on the costs for the health-care sector are worrying. Infection by drug-resistant bacteria requires the administration of high doses of antibiotics, resulting in higher drug toxicity, longer hospital stays and higher mortality [7]. Because of this, the annual impact of resistant infections is estimated to be US$ 21 to 34 billion in excess health care costs and US$ 8 million additional hospital days in the United States and over €1.6 to 2.5 billion additional hospital days in the European Union [5, 6]. Bacteria are responsible for approximately 90% of all hospital acquired infections, with immuno-compromised patients being more susceptible to serious infections, with higher mortality rates than people with healthy immune systems. The risk of fatality associated with infections caused by resistant bacteria as compared to antibiotic sensitive bacteria is much higher. Furthermore, in most cases of acquired infections involving resistant bacteria the risk of fatality is exacerbated by prolonged bacterial exposure as a result of delayed or a lack of an appropriate therapy, and not just because of issues associated with any increased in the virulence of the organism [1].

The microorganisms that are mainly involved in the resistance process are called the ESKAPE pathogens - *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa* and *enterobacteriaceae* - emphasizing their capacity to "escape" from common antibacterial treatments [4]. The pathogens that present the greatest challenges are the multidrug resistance (MDR) and extensively drug resistant (XDR) strains. MDR strains are defined when they are non-susceptible to three or more antimicrobial classes, while XDR strains are non-susceptible to all antimicrobials [4]. Of particular concern are multi- and methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), *P. aeruginosa*, *Escherichia coli*, *Mycobacterium tuberculosis* and *K. pneumoniae* producing extended spectrum *β*-lactamases (ESBL) and carbapenemases [1].

Bacteria can be intrinsically resistant to therapeutic agents and it happens through inherent (natural) resistant. Otherwise, bacteria can be multidrug resistant and it occurs when they are transformed through the acquisition of new genetic material from other resistant organisms. Normally this process is called horizontal gene transfer (HGT) and it is performed by bacterial conjugation, transduction, transformation or biofilm formation to spread drug resistance [7]. Transposons in bacteria can facilitate the direct or indirect transfer and incorporation of drug resistance genes into the host's genome or plasmids. HGT has recently been reported as multidrug resistance in bacteria can also be caused by chromosomal mutations and the regulation of resistant genes. Mechanisms of this type can be classified into: (i) inactivation or modification of drugs; (ii) alteration of a target site of antibiotics (it is found that many bacteria are resistant to antibiotics through this mechanism); (iii) acquisition of alternative metabolic pathways to those inhibited by antimicrobials; (iv) decreasing drug permeability of drugs across the cell membrane before they can reach their target sites and exert their effects on bacteria; (v) enzymatic modification or degradation of the antimicrobial agents; (vi) over-expression of the drug target; and (vii) increasing the active efflux pumps that pump out or extrude antibiotics from the cell [1, 7, 8].

Implications of Biofilms

It is a particular concern when bacteria become resistant to various antimicrobial agents simultaneously and mainly when they form biofilms [1]. An estimated 80% of bacterial infections in humans are caused by biofilms, and the Centers for Disease Control have declared biofilms to be the most pressing clinical impediment of the century [9]. Biofilm formation is particularly problematic with implantable devices such as prosthetic hips, which often require surgical removal to eliminate the infection. Biofilm formation is a complex process involving multiple bacterial signaling systems including quorum sensing (QS), nutrient and chemical signal response, and extra-cellular matrix formation [9]. A bacterial biofilm is a cooperative community of unicellular organisms attached to a solid surface or encased in a hydrated matrix of polysaccharide, protein and nucleic acids. The extracellular polysaccharides (EPS) are an insoluble and slimy secretion that is released by bacterial cells, encapsulating adjoining cells in a well-organized and structured matrix capable of assisting in dissemination of nutrients that are necessary for cell growth, to bind external nutrient molecules required for cell sustenance and growth, to provide protection from external environmental stresses that includes protection from antibiotics, disinfectants and from dynamic environments [10].

Biofilms are usually formed through several steps (i) the initial step in biofilm formation is the adherence of bacteria to a foreign body or biomaterial; (ii) the transformation from reversible to irreversible attachment is a relatively rapid process, taking place within a few minutes or less; (iii) bacterial adhesion is mediated by fimbriae, pili, flagella and extracellular polymeric substance, which form a communication bridge between bacteria and the conditioning films; (iv) the aggregation and accumulation of adherent bacteria lead to the formation of multiple cell layers as the biofilm matures, and (v) the last step is the detachment of bacteria from the biofilm into a planktonic state, which allows them to initiate a new cycle of biofilm formation [7, 10]. Some hypotheses for drug resistance in biofilms have been reported in the literature: (i) in biofilms, the penetration of antibiotics is slow and incomplete resulting in an in-effective response to antibacterial agent which must diffuse and penetrate into the bacterial cells; (ii) a concentration gradient of a metabolic substrate or product leads to zones of slow-growing or non-growing bacteria with less uptake of antimicrobial agents than in planktonic cells; (iii) an adaptive stress response is expressed by some bacteria to cope with environmental fluctuations, such as temperature change, oxidative stress or DNA damage; and (iv) a small fraction of bacteria differentiate into a highly protected persistent state that reduces the susceptibility of their biofilm to antibiotics [11, 12].

The Limitations of Current Therapeutic Strategies

The treatment of bacterial infections is becoming more difficult because of a decline in the current arsenal of useful antibiotics, the development of antibiotic resistance and the slow rate of new drug development [1, 13]. Recent review articles in Chemistry World by King are highly critical of the serious underinvestment globally by governments and

β-lactams   Macrolides   Fluoroquinolones

Penicillin   Erythromycin   Ciprofloxacin
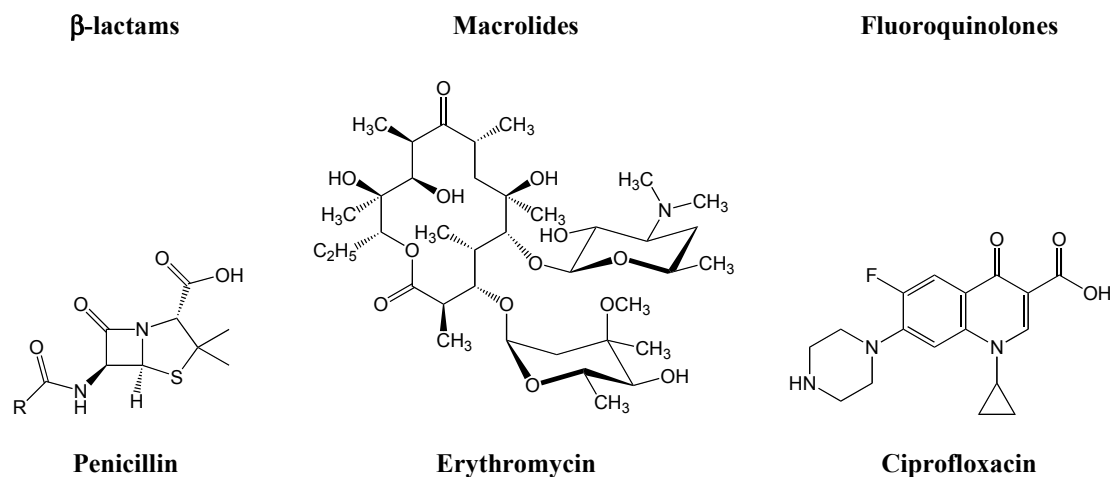
Fig. (1). Typical structures of the β-lactams (penicillin), Macrolides (erythromycin) and fluoroquinolones (ciprofloxacin).

pharmaceutical companies for the development of new antimicrobial drugs, even though predictions are that the cost of antimicrobial resistance (AMR) will be 300 million premature deaths and up to $100 trillion lost to the global economy by 2050 [14, 15]. The US market for antibacterials is dominated by six antibacterials which fall into just three structural classes, the β-lactams (Rocephin®, Augmentin®), macrolides (Zithromax®, Biaxin®), and fluoroquinolones (Cipro®, Levoquin®) (Fig. **1**) [16]. This limited number of structural classes, in combination with ineffective management of drug usage, is at the heart of the cause of the current "era of resistance" crisis.

There is also a lack of diversity in the cellular target of the known antibiotics. Almost all clinically used antibiotics inhibit DNA, RNA, protein, or cell wall synthesis, and there are less than twenty-five molecular targets that account for their activity. The gravity of the crisis is appreciated as we witness such developments as the resistance against glycopeptide antibiotics, like vancomycin or teicoplanin, being observed with increasing frequency and resistance emerging within one year for the recently introduced oxazolidinones. The discovery of a new gene (*mcr-1*) in 2015, which enables bacteria to be highly resistant to polymyxins, such as colistin (Fig. **2**), the last line of antibiotic defence left, heralds a new level of concern for the "era of resistance" as this gene is susceptible to horizontal gene transfer, with alarming potential to be spread between different bacterial populations. This discovery is very significant as it suggests that the progression from extensive drug resistance to pan-drug resistance may be inevitable [17].

The resistance problem is exacerbated by the statistic that bacteria in biofilm communities can be up to 1000-fold more resistant to eradication compared with their planktonic counterparts. Indeed, very few chemical scaffolds have been identified that can inhibit or disperse bacterial biofilms. An illustration of the lack of development of novel antimicrobials is the fact that only five new compounds have been approved over the last twelve years [18]. Both, persistence and spread of antibiotic resistance, in combination with decreased effectiveness and increased toxicity of current antibiotics have emphasized the urgent need to search alternative sources of antibacterial substances [1].

There is clearly a requirement for the development of new antibacterial agents and given the mechanisms associated with resistance in bacteria, there is a need to ensure that new approaches to drug development involve compounds that can target bacterial cells in a completely different way to those associated with the known antibiotics. In this review, we explore the potential of inorganic medicinal chemistry as an alternative approach to developing antibacterial therapeutics, with a specific focus on the mechanisms of action of metal complexes containing 1,10-phenanthroline ligands.

INORGANIC MEDICINAL CHEMISTRY

Inorganic medicinal chemistry has a relatively short history as a specific field, beginning in the 1970's with the discovery and clinical development of the successful anticancer drug cisplatin [19]. The design and synthesis of coordination complexes with novel structures and exhibiting biological activity, such as antimicrobial, anti-inflammatory, antioxidant and anticancer, establishes this exciting field as offering great potential to improve the quality of life [20]. Inorganic medicinal chemistry offers an alternative approach to organic drug development through opportunities for the design of therapeutics with the ability to target different biochemical pathways [21]. The development of drugs that incorporate metal ions into their molecular structure offers the medicinal chemist an opportunity to exploit structural diversity, vary oxidation states of the metal and also offer the possibility of improving the activity of an established organic drug through its coordination to the metal centre [22, 23]. The antimicrobial capabilities of metals have been known for thousands of years, with historical applications in water and food preservation (Cu and Ag), agriculture (Cu) and medicine (Ag, Cu, Hg, Te, Mg and As) [24]. The medicinal use of metals as antimicrobials remained prevalent until the discovery of the "cillin" antibiotics in the 1920's which then saw their applications decline in popularity. Today, the burgeoning threat of multidrug resistant microbes to public health and the dearth of new clinically efficacious antibiotics have resulted in a renaissance in the use of antimicrobially-active metal ions and their complexes. Interdisciplinary research in the field of inorganic medicinal chemistry is advancing our knowledge of metal toxicity and facilitating the design of metal-containing compounds as effective and targeted antimicrobials

Colistin
(polymyxin E)
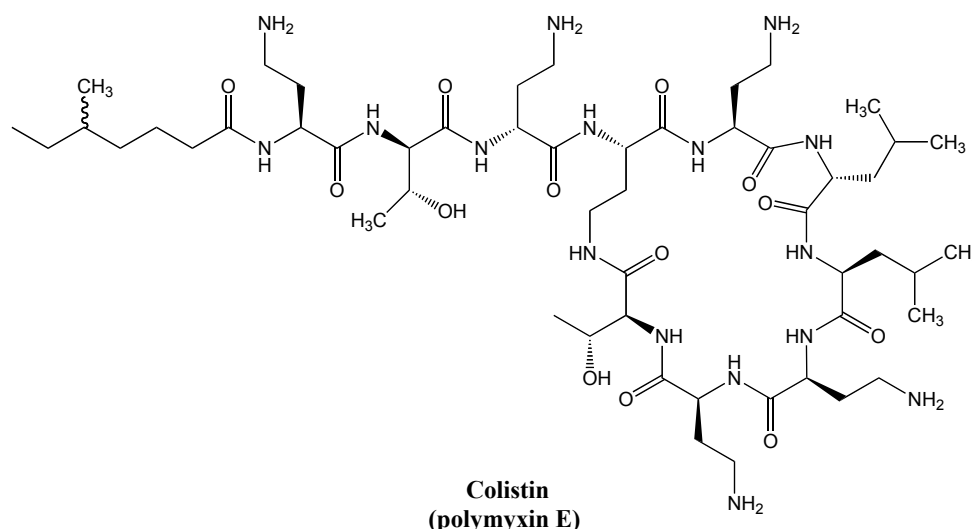
Fig. (2). Structure of the polymyxin, colistin.

offering a realistic alternative to antibiotics [25]. We do not associate the activity of most conventional antibiotics with the presence of metal ions. However, a number of antibiotics are known to require coordinated metal ions to function properly [25]. These include bleomycin (BLM), streptonigrin (SN), bacitracin and albomycin which depend on coordinated metal ions to maintain proper structure and/or biological function. The term 'metalloantibiotic' has been coined to describe such metal ion dependent antibiotics and they are known to exert their bioactivities through interactions with a variety of biomolecules including DNA, RNA, proteins lipids and receptors. More recently, the term 'metalloantibiotic' has been applied, more generally, to metal complexes that exhibit antibacterial capability.

BIOINORGANIC CHEMISTRY OF BACTERIA

An estimated 25% of all proteins in cells require at least one transition metal ion to ensure function [26], and for the known structurally characterised enzymes, the transition elements (as proportions) that have been identified as cofactors are, Zn (9%), Fe (8%), Mn (6%), Cu (1%), Co (1%), Ni (0.5%), V (<1%), Mo(<1%), W (<1%) [27]. Many metalloenzymes are involved in the regulation of the metabolic and physiological processes essential for microbial cell growth and homeostasis [26, 28, 29]. For example, iron is required for important cellular processes including DNA replication, central metabolism and respiration, whilst enzymes containing Zn(II) and Mn(II) centres are crucial for detoxifying reactive oxygen species (ROS) and reactive nitrogen species (RNS) [30]. Stringent control of the homeostasis of transition metal ions is essential for all life forms. Limiting the availability of these metal ions to the pathogen, for example through the imposition of nutritional immunity by infected mammalian host cells, will disrupt homeostasis, compromise cellular vitality and ultimately kill the organism [31]. Furthermore, the use of selected chelators to sequester metal ions has been shown to inhibit the biological function of metal-dependent proteins in microbes interfering with microbial nutrition, growth and development, cellular differentiation, adhesion to biotic and abiotic structures as well as controlling the progression of the *in vivo* infection [32, 33].

On the other hand, the ability of microbes to scavenge metals from vital metalloenzymes in host cells is considered an important virulence attribute [34]. Indeed, an important facet of infectious disease is the relentless contest between host and pathogen for transition metal ions.

Whilst relatively small quantities of metal ions are required to sustain microbial growth and reproduction, exposure to high concentrations can be devastating. Not surprisingly, the host cell immune system attempts to exploit this vulnerability by overloading the bacterial cells with excess metals in their mission to destroy the pathogen [34]. Under such duress, the microbes deploy a range of resistance strategies, which includes restricting uptake, extra- and intracellular sequestration, enzymatic detoxification and efflux, in their defence against metal poisoning [34, 35]. Lemire *et al.* [24] categorised the antimicrobial mechanisms of metal toxicity into 5 specific mechanisms fully recognising that these categories were not confined to specific microbial cells or metals. An emphasis was also made on the importance of the molecular interactions and functions for metal toxicity. The 5 proposed mechanisms were a) Protein dysfunction and impaired enzyme activity due to oxidative protein damage or exchange of a structural or catalytic metal; b) Production of reactive oxygen species (ROS) and antioxidant depletion demonstrated in numerous metal toxicity studies particularly for iron [36] and copper [37]; c) Impaired cell membrane function and loss of membrane potential; d) Interference with nutrient uptake and assimilation which can directly affect gene expression and the microbial signalling mechanisms of quorum sensing and e) Genotoxicity that was demonstrated by catalytic Fenton-type reactions in Fe which was linked to DNA damage and cell death [38]. The authors further reported that microbiological studies on genotoxicity have demonstrated toxicity with Mn(II), Cr(VI), Co(II), Cd(II), Mo(IV), Sb(III) and As(III) but not Ni(II), Cu(II), Te(IV), Pb(II), Ag(I) and Al(III) [1]. This highlights the fact that metals have their own unique biochemical mechanisms of toxicity in microbial cells and they may trigger several of these proposed mechanisms simultaneously, but further studies on the association between each of these mechanisms and microbial cellular death processes as the result of metal tox-

icity are needed at a molecular level. Moreover, further investigation into the precise role of these mechanisms in metal resistance and biofilm formation is important to understand the potential mode of action of novel inorganic drugs.

1,10-PHENANTHROLINE

1,10-phenanthroline (phen) (Fig. **3**) is a heterocyclic organic compound which exerts excellent *in vitro* antimicrobial activity against a broad-spectrum of bacterial and fungal pathogens [39-42]. The *in vitro* antibacterial action of phen has been demonstrated on a range of bacterial species [40, 41, 43-45]. Ostensibly, the antimicrobial activity of phen can be significantly modulated by modifying its structure by either extending its backbone at the -5,6- position or by the substitution of the aromatic protons by suitable substituents (Fig. **4**) [44]. Phen, as a π donor, is also known to form 'charge-transfer' derivatives with organic and inorganic groups to yield organic complexes and quaternary salts that also exhibit significant *in-vitro* antibacterial activity [29, 46, 47].
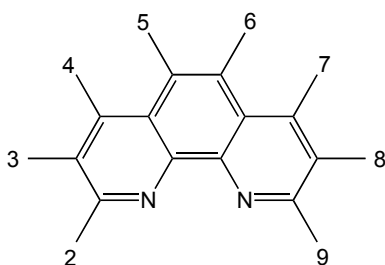

Fig. (3). Structure of 1,10-phenanthroline (phen) with numbering for substituents.

The antimicrobial capability of phen is associated with its properties as a chelator and its ability to sequester metal ions in biological systems [32]. Chelation activity is the combination of a metal ion with a chemical compound that coordinates the formation of a chelating ring, often used to remove heavy metals ions. For many microorganisms, phen has the capability of inhibiting the biological role of metal-dependent proteins, interfering with metal acquisition, bioavailability and metabolism for crucial reactions, disturbing the microbial cell homeostasis and culminating in the blockage of microbial nutrition, growth, development, cellu-

lar differentiation, adhesion to biotic and abiotic structures as well as playing an important role in the *in vivo* infection progression [48, 49]. The iron chelating proteins, transferrin and lactoferrin, have evolved in mammalian cells for the acquisition of iron *via* specific pathways. These naturally occurring chelators have been shown to have potential to prevent the growth of microbial pathogens and their proliferation in host cells [50, 51]. Therefore, synthetic metal chelators, such as phen, have received substantial attention as therapeutic agents because of their ability to alter the metabolism and homeostasis of essential metals such as iron, copper and zinc. Furthermore, upon sequestration of metal ions the resulting metal-phen complexes are themselves capable of exerting a biological response in their own right [32]. MacLeod and his co-workers demonstrated the importance of the chelating ability of phen in 1952 when they showed that both its 1,7- and 4,7-phenanthroline isomers were inactive against lactic acid bacteria [52]. Furthermore, the interaction of phen with DNA appears to be dependent on the availability of metal cations, such as Cu^{2+} [32].

In 2008, Soares *et al.* demonstrated that the cell surface and secreted molecules of group B *Streptococcus* (GBS) are often essential virulence factors involved in the adherence of the bacteria to host cells or that they are required to suppress the defense mechanism of hosts. Cleavage of the host extracellular matrix by GBS may be a relevant factor in the process of bacterial dissemination and/or invasion. These workers demonstrated that phen, acting as a metallopeptidase inhibitor, completely inhibited this cleavage. Notably, phen (0.1 mM) strongly blocked GBS growth as well as its interaction or invasion in human cell lineages. Herein, GBS strain 90356 adhered to and invaded efficiently to the Human Umbilical Vein Endothelial Cells (HUVECs) model. But the adhesive property of this strain 90356 was significantly inhibited by ~93% and also its intracellular viability (invasion) in HUVECs was strongly reduced, by ~82%. The authors presumed that the metallopeptidases assist GBS in deriving essential nutrients from human proteins in maintaining GBS metabolic machinery and physiological processes such as cellular growth [53].

In 2015, Tay *et al.* tested 1,10-phenanthroline-5,6-dione (phendione) (Fig. **4**), as a potentially novel antimicrobial agent against *Enterococcus* and it effectively eradicated *E. faecalis* biofilms. It was proposed that the antimicrobial ac-

1,10-phenanthroline-5,6-dione
(Phendione)

2,9-dimethyl-1,10-phenanthroline
(Neocuproine)

dipyridophenazine
(DPPZ)
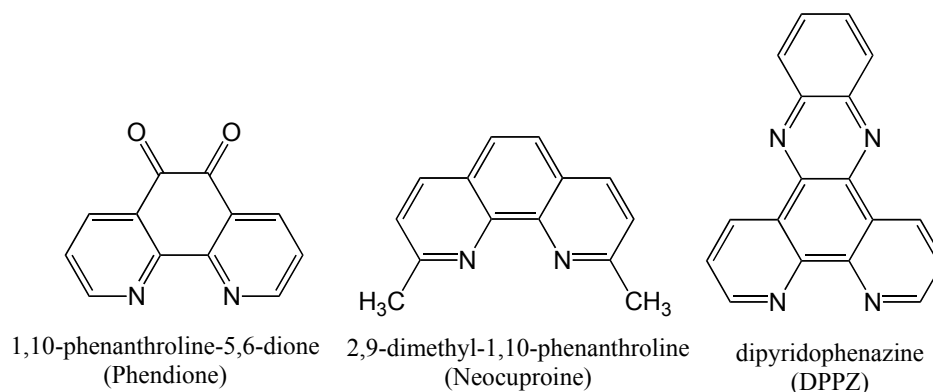
Fig. (4). Examples of modified phen structures.

tivity of phendione appears to be related to its ability to selectively disturb essential metals (Zn^{2+}, Fe^{2+}, Ca^{2+}, Cu^{2+}, Co^{2+}, Mn^{2+} and Ni^{2+}) required for bacterial metabolism and physiological processes. Therefore, chelation of these metal ions is responsible for the disruption of cellular vitality and subsequent cell death. In this study, they demonstrated the ability of phendione to inhibit the growth of *E. faecalis* in the presence of excess Zn^{2+} ions, suggesting that phendione exerts its antimicrobial effects through its ability to chelate Zn^{2+}[54].

METAL COMPLEXES OF 1,10-PHENANTHROLINE

Historical Perspective

Phen is an avid chelating agent which forms complexes with a range of metals [55]. Indeed, the biological activity of phen has been attributed to its ability to sequester trace metals and the resulting metal complexes are believed to be the actual active species [56]. Metal complexes containing phen have shown significant potential as broad-spectrum agents capable of eliciting cytotoxicity towards diseases and infections manifested by cancer, [42, 57] viruses, [58] bacteria [59] and fungi [60, 61]. Phen, its related organic compounds and their corresponding metal complexes are known to inhibit the growth of fungal pathogens by damaging their mitochondrial function, uncoupling respiration, causing non-specific DNA cleavage, disrupting cell division and inducing gross distortions in fungal cell morphology [61, 62]. In 1969, Dwyer *et al.* published their comprehensive, landmark treatise on the *in vitro* and *in vivo* antibacterial activities of dicationic Mn(II) Fe(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Ru(II) chelates containing phen and substituted phen (R-phen) ligands [43, 44]. The results demonstrated that the metal chelates were more active than the 'metal-free' phen ligand, against *M. tuberculosis, S. aureus, S. pneumonia, C. Perfringens, E. coli and P. Vulgaris*. With the exception of the anti-tubercular profiles, the antibacterial activity was largely independent of the type of metal present. These phen chelates were also shown to be useful clinically as topical antimicrobials but selected compounds were found to be ineffective against mice infected with *M. tuberculosis, S. aureus* and *S. pneumonia*, when administered parenterally. Whereas cationic metal-phen complexes can be bacteriostatic [43] and bactericidal [39] towards many Gram-positive bacteria they are less effective against Gram-negative organisms. This activity trend has been explained in terms of the high polar lipid content of the cell walls of Gram-negative bacteria and the consequent impact on the ability of the complexes to reach vital membrane or intracellular sites [43]. In 1970, Cade *et al.* reported that, *in-vitro*, metal complexes of 3,5,6,8- or 3,4,7,8-tetramethyl-phen (metal = Ni(II), Fe(II), Co(II), Cu(II), Zn(II), Cd(II), Mn(II), Ru(II)), the metal-free phen hydrochlorides and their quaternary salts were bactericidal to veterinary samples of *Erysipelothrix rhusiopathiae* and *Fusiformis nodosus* [63]. Resistant variants of the bacterium *E. rhusiopathiae* were not produced after repeated subculture in the presence of the complexes, [Cu(3,4,7,8-tetramethyl-phen)$_2$]X$_2$ (X = benzoate or acetate). A similar result had been observed for *Staphylococcus aureus, M. tuberculosis, E. coli, C. albicans* and *Trichophyton mentagrophytes* in that they develop little resistance to this type of complex [40, 64]. Based on these results it

was suggested that the complexes offered potential as therapeutics to treat erysipelas in pigs, and that they may also be beneficial in the treatment of other topical bacterial infections. Furthermore, [Cu(3,4,7,8-tetramethyl-phen)$_2$]X$_2$ (X = benzoate or acetate) was also shown to be at least as effective as solutions of formalin and dioxyethyl laural ammonium chloride in addressing foot-rot in sheep. Extensive microbiological and pharmacological studies of this class of phen-chelate led to the clinical investigation of the highly stable Ni(II) and Fe(II) complexes of the 3,4,7,8-tetramethyl-phen ligand [40, 64]. These complexes were shown to exhibit a wide spectrum of antimicrobial actions and to produce negligible toxic responses in skin, subcutaneous tissues and mucous membranes [64]. Preliminary studies had shown that the complexes were effective in controlling infections due to clinical isolates of *S. aureus.* The Ni(II) complex was as effective as hexachlorophene, when deployed as a preoperative skin preparation, in reducing the incidence of postoperative staphylococcal wound infection. Furthermore, the Ni(II) complex was also as effective as hexachlorophene in the prophylaxis of staphylococcal infection in patients undergoing elective obstetric or gynaecological surgery [65]. This complex also controlled secondary infection in adolescents with persistent acne vulgaris.

In 1970, Cade *et al.* also examined the clinical applications of Mn(II) complexes of 3,4,7,8-tetramethyl-phen as topical treatments for a variety of skin conditions, including chronic dermatological infections due to dermatophytes (*e.g. Malassezia furfur*, *Trichophyton rubrum*) or *Candida* species [66]. The complexes induced a significant reduction in the microbial infection in approximately 50% of cases, with infection due to Gram-positive bacteria generally more responsive to treatment than that due to Gram-negative bacteria. No noteworthy irritation or sensitization of the underlying dermatosis or dermatomycosis was observed, and, furthermore, significant microbial resistance did not develop.

These early results clearly demonstrated the antibacterial chemotherapeutic potential of metal complexes of 1,10-phenanthroline. The research was performed at a times when the antibiotic era was at its height and consequently the significance of the results was not embraced by the pharmaceutical sector. More recently there has been a surge in interest in the antimicrobial applications of metal-based compounds, with metal complexes incorporating phen ligands prominent, and it is imperative that we strive to fully understand how these complexes exert their biological activity and how they differ from the resistance-prone antibiotics.

BACTERIAL CELL WALL PERMEABILITY

Like all cells bacteria have a cytoplasmic membrane, consisting of a phospholipid bilayer and proteins, which performs all of the general functions of a cell membrane (eg. cytoplasm protection, permeability barrier and selective transport of molecules into and from the cell) but differs from the cell membrane in eukaryotes as it does not contain sterols and it comprises a wider variety of fatty acids and a higher content of phospholipids [67, 68]. Bacteria also differ from eukaryotes in that they must be able to survive in much harsher environments and therefore they possess a robust cell wall which protects their cytoplasmic membrane and pre-

vents chemical, biological and physical damage to the organism [67]. Gram-positive cells have cell walls composed of a thick layer of peptidoglycan and also of two types of teichoic acids inlaid in their structure: wall teichoic acids, which are coupled to peptidoglycan, and lipoteichoic acids, which are anchored to the cell membrane. The peptidoglycan layer is made up of repeating units of the disaccharide N-acetyl glucosamine-N-actyl muramic acid cross-linked by pentapeptide side chains that play an important role in physical strength and bacterial shape as well as in cell division, morphological differentiation and adaptive responses [68, 69]. Teichoic acids are long anionic polymers that bind cations playing a role in cation homeostasis. Additionally, networks of metal cations between wall teichoic acids also influence the rigidity and porosity of the cell wall and profoundly affect the interactions of bacteria with other cells or molecules [68]. The cell wall in Gram-negative cells does not contain teichoic acid and they typically have a much thinner peptidoglycan layer covered by an outer membrane [70]. The outer membrane is a lipid bilayer, a distinguishing feature of Gram-negative bacteria, as Gram-positive bacteria lack this structure. It is composed of glycolipids - mainly lipopolysaccharides (LPS), also known as endotoxins responsible for much of the toxicity of Gram-negative bacteria, and by two classes of proteins: lipoproteins and β-barrel transmembrane proteins. LPS molecules bind each other avidly, especially if cations like Mg^{++} are present to neutralize the negative charge of phosphate groups present on the molecule, and form a non-fluid continuum barrier very effective for hydrophobic molecules. The transmembrane proteins are β sheets wrapped into cylinders and some of them, such as the porins (OmpF and OmpC), allow the passive diffusion of small hydrophilic molecules (*i.e.* mono- and disaccharides, amino acids, molecules ~700 Daltons) across the outer membrane [68, 71, 72]. Due to the limited diameters of passive diffusion channels, bulky molecules (*i.e.* iron–siderophores and complex oligosaccharides) are assumed to be taken up exclusively by active transporters [72].

Several classes of molecules can pass across the outer membrane without accessing the channels of porins, these include hydrophobic substances such as detergents, uncharged antibiotics, enzymes like nucleases, phosphatases, kinases, proteases, *etc.*) [73]. But, in general, porins are the major outer membrane proteins with important permeability barrier properties, which define the size exclusion limit for hydrophilic molecules by limiting the size and the rate of the passage of the molecules through these channels, restricting molecules of similar sizes to the diameters of the porin channels [73]. The conception of "exclusion" reflects the probability that these substances are not taken up across the outer membrane sufficiently to cause physiological effects on cells, involving semi-selective exclusion of potentially harmful molecules and affording Gram-negative bacteria an advantage in surviving in lethal environments [73]. The size exclusion molecular properties of the pores in the outer membrane are typical of Gram-negative bacteria. The Gram-positive cell wall is porous and has a high molecular exclusion limit of >100000 g.mol^{-1}. It means that peptidoglycan structure provides rigidity and protection to the cell, but is unlikely to be a contributing factor to any limiting of uptake of molecules [71, 74, 75]. For example, *E. coli* and *S. typhir-*

nuriurn outer membranes are permeable for hydrophilic solutes up to 600 - 800 g.mol^{-1}. However, solutes up to 3000 - 9000 g.mol^{-1} can penetrate the outer membrane of *P. aeruginosa*. And still there is some evidence that the exclusion molecular weight of certain "exotic" Gram-negative bacteria would be as large as 20000 g.mol^{-1} [75, 77]. But even the molecular exclusion limit of *P. aeruginosa* being larger than that of *E. coli*, the outer membrane permeability of *P. aeruginosa* has been determined to be approximately 8% of that of *E. coli* [75, 78]. This is because most of the *P. aeruginosa* pores appear to be nonfunctional, restricting the rate of uptake. And low outer membrane permeability along with effective efflux mechanisms is responsible for the high intrinsic antibiotic resistance of *P. aeruginosa* [71, 75, 78]. Therefore, the presence of the outer membrane, as a very effective and selective permeability barrier, greatly decreases the permeability to antibacterials, and it is regarded as a key mechanism of drug resistance (or sensitivity) in many Gram-negative bacteria [70, 79]. For compounds to act as antibacterials it is generally accepted that they must be lipophilic and be capable of permeating the cell wall and cytoplasmic membrane of bacteria [80]. Ironically, ionic silver (Ag^+) is a well-known antimicrobial, in its own right, and its activity has been linked to its ability to bind to bacterial cell surfaces and interact with proteins involved in cell wall synthesis [81, 82].

CELL WALL PERMEABILITY AND ACTIVITY OF METAL-PHEN COMPLEXES

Cationic metal ions do not easily cross the cell membrane of bacteria and in nature uptake of essential metal ions is achieved by transporter proteins which effectively wrap the cations up in a hydrophobic blanket. The same effect can be achieved by complexation with hydrophobic chelating ligands such as phen. The ability of such phen-based complexes to cross the cell membrane is explained in the context of Overtone's concept of cell permeability [83] and Tweedy's chelation theory [84]. According to Overtone's concept the lipid membrane surrounding the bacterial cell favors the passage of lipid soluble compounds and such lipid solubility is considered to be an important factor controlling antibacterial activity. Tweedy's chelation theory explains how, upon complexation, involving membrane-permeable ligands such as phen, the polarity of the metal ion is reduced to a significant extent due to (i) overlap of the ligand orbitals and partial sharing of the positive charge of the metal ion with donor groups, (ii) delocalisation of π-electrons over the chelate ring as a whole, leading to an increase in the lipophilicity and lipid membrane penetration capability of the metal complex. This process can allow phen-based complexes to penetrate the cell membrane of bacterial cells and once embedded in the cell membrane or inside the cell they can interact with relevant biomolecules in the microorganisms, leading to inhibition of the cell growth and cell death [85, 86].

Cell membrane permeability is a particular problem associated with Gram-negative bacteria. Whereas there has been some modest success in the development of new drugs to treat Gram-positive bacteria, the situation obtaining for the development of drugs for Gram-negative species involves far less success [87, 88]. This situation is particularly concern-

ing as there are very few drugs for Gram-negative bacteria in the development pipeline. In addressing this challenge it has recently been demonstrated that pre-exposure to silver Ag^{+1} ions can potentiate the activity of a broad range of antibiotics against drug-resistant, Gram-negative bacteria by increasing their cell membrane permeability and thus restoring their antibiotic susceptibility [89].

Sixty years ago Dwyer and his co-workers demonstrated the relationship between lipophilicity and antibacterial activity, for phen-complexes, when they demonstrated that the activity of $[Ru(phen)_3]^{2+}$ against a range of Gram-positive and Gram-negative bacteria was significantly improved through the introduction of methyl substituents on the phen ligands (Fig. **5**), with the methylated complexes even displaying moderate activity against Gram-negative bacteria [90]. The antimicrobial activity of ruthenium(II) polypyridyl complexes containing phen ligands has attracted significant attention more recently [91]. In a series of papers Keene and Grant, *et al.* have reported the antibacterial activities of a range of lipophilic mono-, bi-, tri- and tetra-nuclear Ru-phen complexes (Fig. **6**) [92-97]. Based on their own experience and the evidence in the literature they concluded that the development of mononuclear polypyridylruthenium(II) complexes as antimicrobials was limited due to their comparatively high MIC values when compared with that of state-of-the-art antibiotics [91]. Although the mode of anti-bacterial action of these ruthenium(II) complexes is not fully understood, DNA is considered to be their main target for biocidal activity.

With the aim to develop better antimicrobials, Keene and Grant, *et al.* elected to design dinuclear and higher nuclearity, enantiomeric, $Rubb_n$, $Rubb_n$-tri, $Rubb_n$-tetra complexes, incorporating the lipophilic bb_n ligands (bb_n = bis[4(4'-methyl-2,2'-bipyridyl)]-1, *n*-alkane; n = 2,5,7,10,12,14 or 16) (Fig. **6**) [92]. Interestingly, the dinuclear complexes containing the short linking chains (bb_2 and bb_5) displayed very poor activity against the range of bacterial strains tested [92]. The more lipophilic $Rubb_n$ complexes (where *n* >5) exhibited very good activity against Gram-positive and Gram-negative strains (MIC = 1-16 µg/ml), and preliminary toxicity studies indicated that the complexes were significantly less toxic to eukaryotic cells (IC_{50}= >78 µg/ml). Further-

more, these $Rubb_n$ complexes were also very active against drug-resistant strains such as MRSA and VRE, with the $Rubb_n$ complexes with the more lipophilic longer linking aliphatic chains ($Rubb_{12}$, $Rubb_{14}$ and $Rubb_{16}$) exhibiting the greatest activity (MIC = 1-4 µg/ml) [92]. Only slight differences in the anti-bacterial activity were observed between the enantiomeric forms of the complexes. The results of cellular uptake studies correlated well with the activity profiles of these complexes, with the uptake following a similar trend ($Rubb_{12}$ < $Rubb_{14}$ < $Rubb_{16}$; with Log$P$ = -3.4, -2.7 and -1.9, respectively), a trend not observed for the mononuclear complexes, -$[Ru(Phen)(bb_7)]^{2+}$ and -$[Ru(Me_4Phen)_3]^{2+}$ (Fig. **6**) [95]. Furthermore, the uptake in Gram-negative bacteria was significantly less when compared to that of the Gram-positive species [95]. The dinuclear $Rubb_n$ complexes enter bacteria in an energy-dependent manner and they significantly permeabilise the cell membranes [96], and interestingly the most active compound, $Rubb_{16}$, preferentially binds RNA and accumulates at the ribosomes in the bacteria [97]. These workers also showed that cellular uptake of the $Rubb_n$ complexes could be significantly increased by including labile chloride groups on each metal centre but the resulting Cl- $Rubb_n$ complexes were found to be less active than their $Rubb_n$ counterparts [93]. Furthermore, the activity of the dinuclear complexes against eukaryotic cells varies depending on the lipophilic nature of the $Rubb_n$ ligand offering the possibility of making this class of complexes selective for bacteria [98].

These workers also examined the anti-bacterial activity of the tri- and tetra-nuclear complexes, $Rubb_n$-tri and $Rubb_n$-tetra (Fig. **7**) [94]. All of the tri- and tetra-nuclear complexes exhibited good antibacterial activity with the highly lipophilic linear complexes $Rubb_{12}$-tri, $Rubb_{16}$-tri, $Rubb_{12}$-tetra and $Rubb_{16}$-tetra displaying the most activity, up to four times more active than their dinuclear counterparts. Ironically, the trinuclear complexes were the most lipophilic but the tetra-nuclear complexes were generally more active. Generation of non-linear tetra-nuclear variants yielded complexes that were slightly more lipophilic but they were consistently less active when compared to their linear counterparts [93]. The level of cellular uptake of the $Rubb_n$-tri and $Rubb_n$-tetra complexes was similar in both Gram-positive
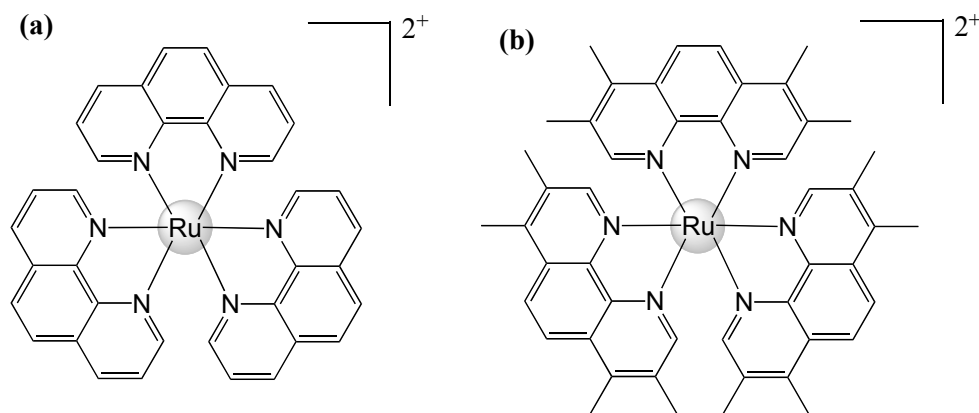
Fig. (5). (**a**) Structure of tris(phen)ruthenium(II) cation $[Ru(phen)_3]^{2+}$ and (**b**) structure of tris(3,4,7,8-tetramethyl-phen)ruthenium(II) cation, $[Ru(3,4,7,8-Me_4phen)_3]^{2+}$.
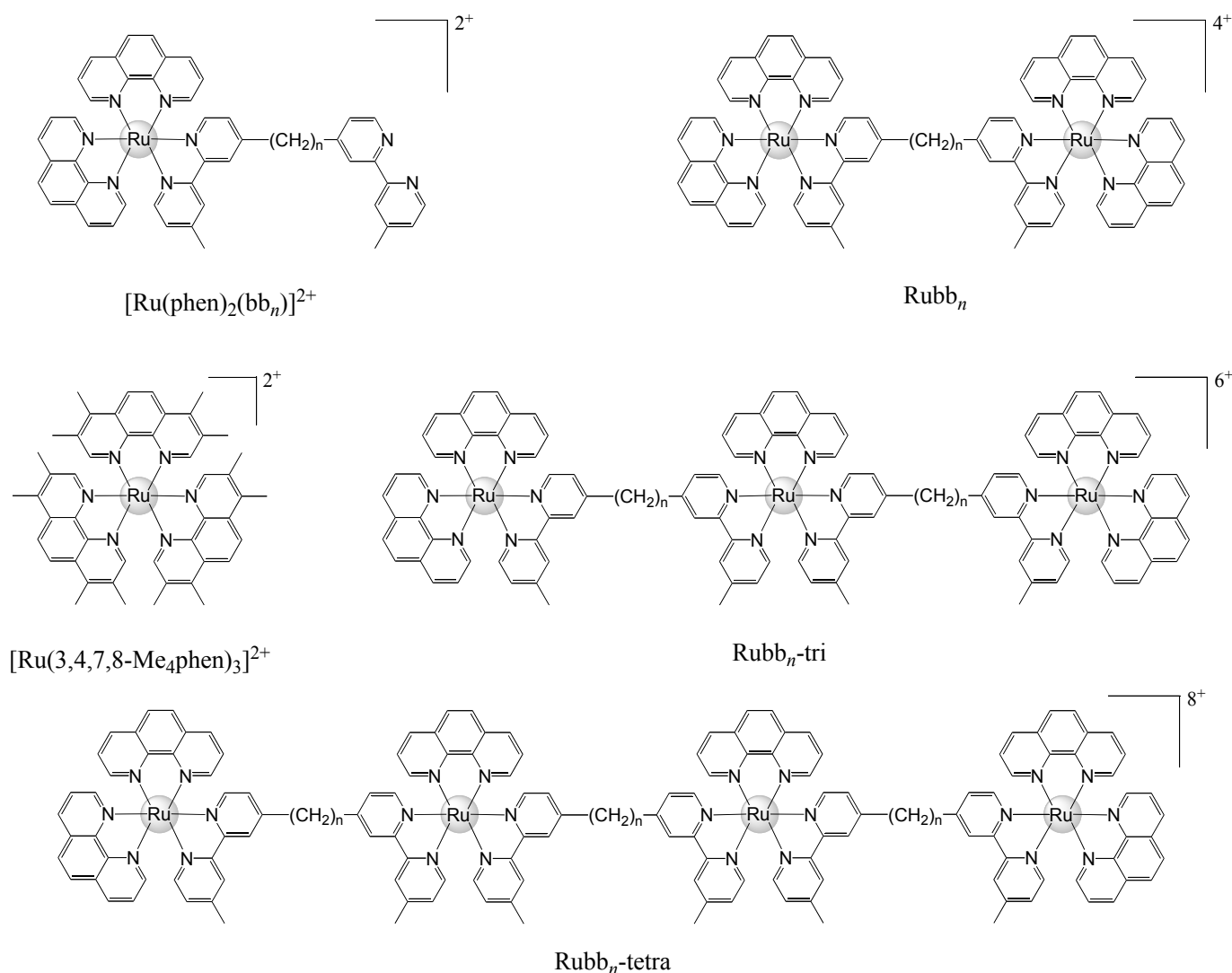
Fig. (6). Structure of [Ru(phen)$_2$(bb$_n$)]$^{2+}$, Rubb$_n$, [Ru(3,4,7,8-Me$_4$phen)$_3$]$^{2+}$, Rubb$_n$-tri and Rubb$_n$-tetra complexes.

and Gram-negative species, the complexes were considerably less active against the Gram-negative bacteria, an observation that the authors attributed to the fact that some Gram-negative bacteria, particularly *P. aeruginosa*, may have inherent resistance to inert polypyridyl ruthenium complexes [91].

In 2015, Keene and Collins *et al.* also reported the anti-*P. aeruginosa* (ATCC 27853) activities of the geometric isomeric complexes *cis*-α-[Ru(Phen)(bb$_n$)]$^{2+}$ and *cis*-β-[Ru(Phen)(bb$_n$)]$^{2+}$ (*n* = 10 or 12) (Fig. **7**) [99]. Whereas all of the [Ru(Phen)(bb$_n$)]$^{2+}$ complexes displayed excellent activity against the Gram-positive *S. aureus*, only the *cis*-α-[Ru(Phen)(bb$_{12}$)]$^{2+}$ exhibited activity against Gram-negative species (*E. coli* and *P. aeruginosa*; MIC = 8 μg/ml for each species), with its activity being two to four times that of its isomeric form, *cis*-β-[Ru(Phen)(bb$_{12}$)]$^{2+}$. Both *cis*-α-[Ru(Phen)(bb$_{12}$)]$^{2+}$ and *cis*-β-[Ru(Phen)(bb$_{12}$)]$^{2+}$ readily accumulate in the two bacteria, but significantly they displayed a much higher level of uptake in the *P. aeruginosa*. They also compared the activity and uptake profiles of the *cis*-α-[Ru(Phen)(bb$_{12}$)]$^{2+}$ and *cis*-β-[Ru(Phen)(bb$_{12}$)]$^{2+}$ with those of the previously studied mononuclear complexes

[Ru(Me$_4$Phen)$_3$]$^{2+}$ and [Ru(Phen)$_2$(bb$_7$)]$^{2+}$ (Fig. **6**) and concluded that the structural differences between the complexes are significant in terms of the interactions with the outer membrane of *P. aeruginosa*. They also postulated that it may be possible to modify this class of ruthenium complex to modulate their cell membrane interactive capabilities and essentially customize ruthenium complexes for activity against specific Gram-negative bacteria.

In 2015, Wang *et al.* reported the anti-bacterial activity of the mononuclear ruthenium complex cation, [Ru(Phen)$_2$(tip)]$^{2+}$ (Fig. **8**) [100]. [Ru(Phen)$_2$(tip)]$^{2+}$ exhibited significant activity against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria (33% and 42% reduction in cell viability at 5μM concentrations, respectively). Cellular uptake studies on this complex indicated that there was 2-3 time more accumulation of [Ru(Phen)$_2$(tip)]$^{2+}$ in *S. aureus* than in *E. coli*. SEM and AFM observations on the morphology of the two bacteria indicated that contact with [Ru(Phen)$_2$(tip)]$^{2+}$ caused cell membrane disruption and cytoplasm leakage, and agarose gel electrophoresis studies indicated that DNA and RNA damage were also key aspects of the anti-bacterial mechanism of action of [Ru(Phen)$_2$(tip)]$^{2+}$.

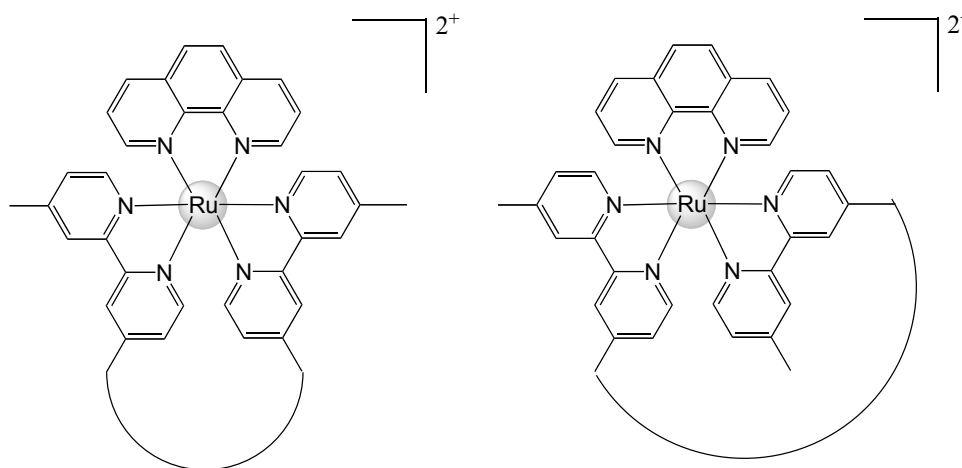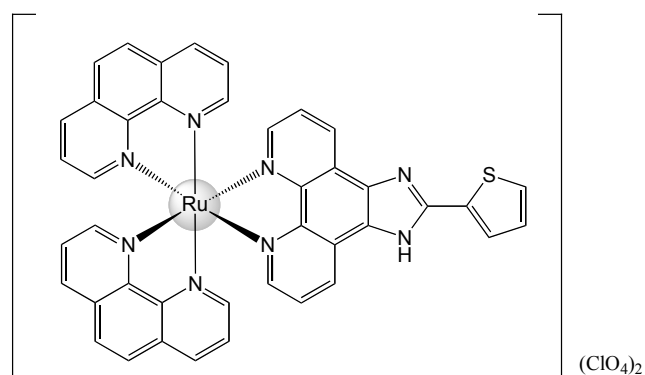**Fig. (7).** Structures of [Ru(phen)(bb$_n$)$_2$]$^{2+}$ complexes.

Fig. (8). Structure of [Ru(Phen)$_2$(tip)]$^{2+}$.

In 1982, Smit *et al.* reported evidence that the enzyme inhibitory action of [Cu^1(2,9-dimethyl-1,10-phenanthroline)$_2$]NO$_3$, associated with its antibacterial capability, is a consequence of the toxicity of free copper ions and not that of the phenanthroline ligand [101]. They observed, using uptake studies with radiolabled ^{67}Cu and [^{14}C] 2,9-dimethyl-1,10-phenanthroline, that significantly more copper than the 2,9-dimethyl-1,10-phenanthroline accumulated in the cell wall of *Mycoplasma gallisepticum* cells. They concluded that the [Cu^1(2,9-dimethyl-1,10-phenanthroline)$_2$]$^{+1}$ dissociated shortly after interaction with the cell membrane, that the copper ions were transported into the cytoplasm, and that the main function of the 2,9-dimethyl-1,10-phenanthroline appeared to be as a transport vehicle for the copper. The positive charge of the Cu^{2+} ion renders it hydrophilic and in nature uptake of copper ions relies on the presence of copper transporter proteins [102]. Such abnormal elevation of intracellular copper, in the presence of membrane-permeable phen ligands, has become well established [101].

More Li *et al.* have recently reported the antibacterial activity of the series of mixed amino acid/phen copper(II) complexes, [Cu(phen)(L-Ser)(H$_2$O)Cl], [Cu(phen)(Gly)(H$_2$O)]Cl, [Cu(phen)(L-Ala)(H$_2$O)]Cl, [Cu(phen)(L-Phe)(H$_2$O)]Cl and [Cu(phen)$_2$Cl$_2$] and CuCl$_2$ against *E. coli* [103, 104]. These workers showed that the presence of the lipophilic phen ligand in the complexes (which presumably dissociate into [Cu(phen)(amino acid)]$^+$ cations) can cause an elevation in the levels of intracellular copper when com-

pared to the CuCl$_2$ salt. Furthermore, they found that the bis-phen complex, [Cu(phen)$_2$]$^{2+}$, caused the greatest accumulation of intracellular copper in the bacterium and when compared to the antibacterial activities of the CuCl$_2$ (IC$_{50}$ = 120 µg/ml) and the [Cu(phen)(amino acid)]$^+$ complexes (IC$_{50}$ range = 24.3 - 31.4 µg/ml) it was found to be more active (IC$_{50}$ = 7.5 µg/ml). They also showed that the acellular DNA binding and cleaving capabilities of this series of compounds and the copper salt followed the order [Cu(phen)$_2$]$^{2+}$ > [Cu(phen)(amino acid)]$^+$ >> CuCl$_2$. Interestingly, they also observed that all of the complexes could stimulate the growth of the *E. coli* at lower concentrations. The three [Cu(phen)(amino acid)]$^+$ complexes, at a concentration of 16 µg/ml, enhanced growth by 17.61- 40.85%, [Cu(phen)$_2$]$^{2+}$ at concentrations < 5 µg/ml stimulated growth by approximately 10%, and the copper salt CuCl$_2$ had only a slight effect on growth of *E. coli* at low concentrations. The conclusion was that the presence of phen in the complexes increased their lipophilicity, improved the transport of copper into the cells, causing excessive intracellular accumulation of copper, and that the higher ratio of Cu: phen in [Cu(phen)$_2$]$^{2+}$ could explain its superior ability to induce the elevation of copper levels. Zou *et al.* also reported that phen ligands can cause abnormal elevation of intracellular copper [105].

In 2012, Aldrich-Wright *et al.* reported the results of a preliminary anti-bacterial structure-activity study of a series copper(II) and paladium(II) cationic complexes with the general formulae, [Cu(I$_L$)(A$_L$)(H$_2$O)]$^{2+}$ and [Pd(I$_L$)(A$_L$)]$^{2+}$ (where I$_L$ represents phen or 4-, 5-methylated or 4,7-, and 5,6-dimethylated 1,10-phenanthrolines, and A$_L$ represents 1,2-diaminoethane, 1*S*, 2*S*- or 1*R*,2*R*-diaminocyclohexane) [106]. The palladium(II) complexes displayed only minimal antibacterial activity, and whereas none of the complexes were active against *P. aeruginosa* the copper(II) complexes exhibited significant bactericidal activity against *B. subtilis*, *S. aureus* and *E. coli* (MIC ranges: 8-64 µg/ml; 8-32 µg/ml; 8-32 µg/ml, respectively).

These workers extended this study by examining the antibacterial activities (*S. aureus*, *E. coli* and *P. aeruginosa*) of a series of complexes, [M(I$_L$)(A$_L$)]$^{2+}$ (where M = Cu or Pt; where I$_L$ represents phen or 4-, 5-methyl, 4,7-, and 5,6-dimethyl-, 3,4,7,8-tetramethyl-, 5-chloro-, 3,8-dibromo-, 5-nitro- and 4,7-diphenyl- 1,10-phenanthrolines, and A$_L$ repre-

sents 1,2-diaminoethane, 1S, 2S- or 1R,2R-diaminocyclohexane) (Fig. **9**) [75]. Phen and the majority of the methylated-phen free ligands exhibited antibacterial activity with MIC values in excess of 20 μM. The exception was the 4,7-diphenyl-1,10-phenanthroline which had superior activity against *S. aureus* and *E. coli* (MIC's = 5-10 and 10-20 μM, respectively). With the exception of the complexes containing the 5-nitro-phen ligands, the copper complexes were most active against *S. aureus*, and where they contained the methyl-, chloro-, bromo-, and 4,7-phenyl-functionalized phen ligands they exhibited a significant increase in activity when compared to their unsubstituted phen containing analogues. In this series the most active complexes were the complexes containing the 3,4,7,8-tetramethyl- and 4,7-diphenyl- substituted phen ligands (MIC's = 1.25-2.5 μM). No significant differences were observed between the RR- and SS- enantiomers of the copper complexes. The platinum(II) complexes were found to be selective for *E. coli*. (Growth Inhibition GI_{50} = 10-20 μM), with no discernible added value accruing from replacing the unsubstituted phen with the 4- and 5-methyl, 4,7-, and 5,6-dimethyl- and , 5-chloro- functionalized phens. The complexes containing the 3,4,7,8-tetramethyl-phen ligand had the lowest activity against *E. coli* (GI_{50} > 20 μM). Furthermore, the mono-, di- and tetra-methylated phen derivatives exhibited decreasing anti-*E. coli* activity in that order, which is in contrast to the activity observed for the free ligands themselves.
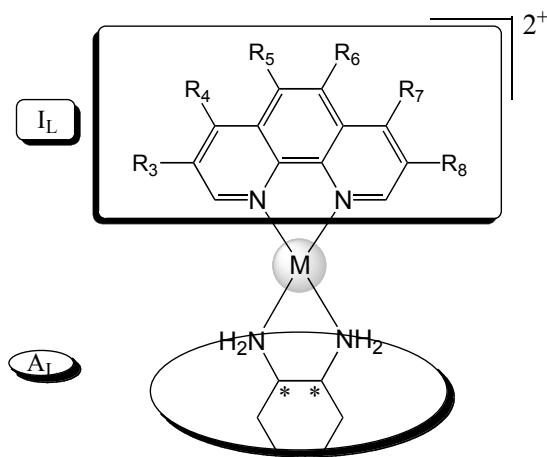
Fig. (9). The general structure of copper(II) and platinum(II) metal complexes where M = Cu or Pt. * Indicates a chiral centre, R_{3-8} represent functionalised positions [75].

To probe the different activity profiles of the copper(II) and platinum(II) complexes further these workers examined the cell membrane permeabilisation capabilities (over a short period - 25 mins) of the four most active complexes, $[Cu(4,7-diphenyl-phen)(A_L)]^{2+}$ and $[Pt(phen)(A_L)]^{2+}$ *(where A_L = 1S, 2S- and 1R,2R-diaminocyclohexane)*. The results suggested that the two copper complexes exert a substantial degree of membrane permeabilisation to *S. aureus* cells, possibly due to interaction with teichoic acid as a target. In contrast the platinum complexes did not exhibit significant disruption to *E. coli* membrane integrity. Interestingly, in a later report these worker showed that the $[Cu(I_L)(A_L)]^{2+}$ complexes (*where* I_L represents phen or 5-methyl, 5,6-dimethyl-,

3,4,7,8-tetramethyl- and 4,7-diphenyl-phen) did not induce lyses in red blood cells, and called into question their cell membrane activity [107]. These workers attribute the lack of activity of the copper and platinum complexes against *P. aeruginosa* to reduced cellular uptake due to its lack of cell membrane permeability (8% of that of *E. coli*) and the possible role of efflux mechanisms, provided by transport proteins associated with the 'intrinsic' antibiotic resistance of this microbe.

It is further postulated that compared to the stable platinum complexes that the copper complexes, $[Cu(I_L)(A_L)]^{2+}$, are labile and that their speciation products in solution are likely responsible for the variation in their mode of action. It is also proposed that their antibacterial activity is not simply a sum of their components (metals and ligands), noting that the mono-phen complexes such as $[Cu(5,6-dimethyl-phen)(A_L)]^{2+}$ are slightly more active against *S. aureus* (MIC = 2.5-5.0 μM) than bis-phen complex such as $[Cu(5,6-dimethyl-phen)_2]^{2+}$, reported earlier by Dwyer *et al.* (MIC = 4.0-7.9 μM) [43]. Furthermore, the metal-free phen and the majority of its functionalised derivatives were reported to primarily affect Gram-negative bacteria and upon coordination in the $[Cu(I_L)(A_L)]^{2+}$ complexes results in a reduction in activity against the Gram-negative species and an increase in activity against *S. aureus*. These workers suggest that the stable platinum complexes may be entering the *E. coli* cells through an, as yet, unknown mechanism.

DNA AS AN ANTIBACTERIAL TARGET FOR METAL-PHEN COMPLEXES

DNA offers an interesting, underexplored, target for potential antimicrobials, and this is an approach currently attracting significant attention [108]. In 1979 Sigman *et al.* reported the oxidative DNA cleavage capability of $[Cu(phen)_2]^{2+}$ in the presence of a reductant [109]. Since this discovery many metal complexes containing phen ligands have been reported to interact and cleave DNA, with the phen ligands increasing the intercalative properties of the complexes.

Several groups have reported the antibacterial activity of ternary metal(II)-phen complexes (metal = Cu^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}) containing known quinolone/fluoroquinolone antibiotics [110-130]. The structural features and the broad biological activity of such complexes containing Cu^{2+}, Ni^{2+}, Co^{2+} and Zn^{2+} have been described in a recent review [131]. The quinolone's are synthetic antibacterial agents which target the DNA-replication enzyme gyrase (type II topoisomerases) and topisomerase IV [132]. Upon formation of binary complexes with metals such as copper(II) the ability of quinolones, such as iomefloxacin (Fig. **10**), to bind DNA is significantly increased, but the complexes are not stable at physiological pH [131]. The addition of phen to $[Cu(quinolone)]^{2+}$ yields very stable ternary complexes (Fig. **10**), which are capable of binding DNA in an intercalative mode, are nuclease active, and possess a cellular uptake route different to that of the free quinolone drugs [128-130]. Furthermore, the use of a mutant strain of *E. coli*, devoid of porins, proteins responsible for the filtration of hydrophilic compounds in Gram-negative bacteria [76], have shown that

Lomefloxacin

[Cu(lomefloxacin)(NO$_3$)(phen)]
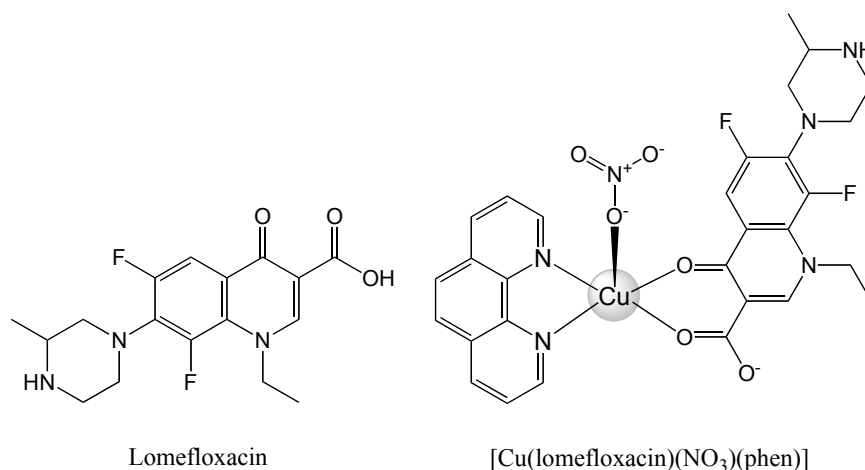
Fig. (10). The structure of lomefloxacin and its ternary copper(II) complex with phen [Cu(lomefloxacin)(NO$_3$(phen)].

[Cu(quinolone)(phen)]$^{2+}$-type complexes are hydrophobic and that they enter the cells in a porin-dependent manner [130]. Similar DNA binding and/or nuclease capabilities have been implicated in the antibacterial mechanism of action of a range of similar mono-phen complexes containing non-quinolone donor ligands and metal centres such as Ag^{1+} [133], Cu^{1+}[134, 135], , Cu^{2+}[114, 116, 136-141], Co^{2+}[136, 137], Ni^{2+}[142], Co^{3+}[143], Mn^{3+}[144], Pd^{2+} [116], Pt^{2+} [75], Fe^{3+}[145] and Zn^{2+} [146]. Furthermore, several cationic bis-phen complexes with the general formula [ML(phen)$_2$]$^{2+}$ (M = Cu^{2+}, Zn^{2+}, Ni^{2+},Y^{3+} Pd^{2+} and Dy^{3+}) have also been reported to bind/cleave DNA and they exhibit clinically relevant antibacterial activity against Gram positive and Gram negative bacteria [116, 147-152]. Cationic Cu(phen)$^{2+}$ and Cu(phen)$_2$$^{2+}$ moieties have also been incorporated into polymeric conjugates, which are also capable of binding and cleaving DNA and which also exhibit clinically relevant antibacterial activity against Gram positive and Gram negative bacteria [153, 154].

The ruthenium(II) polypyridyl complexes are well known for their ability to interact with DNA [155]. Keene and Grant *et al.* have reported the antibacterial activities of a range of lipophilic mono-, bi-, tri- and tetra-nuclear Ru-phen complexes (Fig. **6** and Fig. **7**) [92-99], and they attribute their biological activities to their ability to interact and damage DNA. Indeed the dinuclear complex, [{Ru(Phen)$_2$} (μ-bb$_7$)]$^{2+}$ has been shown to bind chromosomal DNA from *S. aureus* [96]. Aldrich-Wright *et al.* explored the antibacterial activity of the mononuclear ruthenium complexes, [Ru(2,9-dimethyl-phen)$_2$(I$_L$)]$^{2+}$ (I$_L$ = a strong intercalating ligand such as dppz) (Fig. **11**), which have proven DNA binding capabilities [156]. These workers found that there was a direct correlation between the antimicrobial activity profile of the complexes and the affinity of the intercalating ligands for DNA, and they concluded that the observation was consistent with a mode of action involving DNA-binding.

Although many metal complexes containing phen ligands have been shown to bind DNA it should be noted that this property does not necessarily bestow antibacterial capability on them. Reedijk and Wezel *et al.* have recently reported the platinum chloride complexes, [Pt(phendione)Cl$_2$] and [Pt(dppz)Cl$_2$] (Fig. **12**), which can intercalate DNA and ex-

hibit significant *in vitro* anticancer activity, but which are inactive against bacteria [60]. Starosta *et al.* reported the anti-*S. aureus* activity and plasmid DNA cleaving capabilities of a series of cuprous iodide complexes incorporating phen or 2,9-dimethyl-phen with three different tris(aminomethyl)phosphane ligands (Fig. **13**) [134]. They found that the complexes with the highest antibacterial activity exhibited the weakest ability to cleave the plasmid DNA. Chetna *et al.*, tested the DNA nuclease and antibacterial activities of the four binuclear copper(II) complexes, [Cu(oxpn)Cu(N-N)]$^{2+}$ (where oxpn = N,N′-bis[3-(methylamino)propyl] oxamide; N-N = bipy or phen or dpq or dppz) (Fig. **14**) [140]. Although the DNA cleaving capability was in the order of [Cu(oxpn)Cu(dppz)]$^{2+}$ > [Cu(oxpn)Cu(dqp)]$^{2+}$ > [Cu(oxpn)Cu(phen)]$^{2+}$ > [Cu(oxpn)Cu(bipy)]$^{2+}$ only the bipy containing complex exhibited appreciable antibacterial activity against the Gram-negative *E. coli*. (MIC = 20 μg/ml). In our laboratory we have recently reported the in-vitro antibacterial activity of the water-soluble silver complex, [Ag$_2$(phen)$_3$(udda)] (uddaH$_2$ = undecanedioic acid) (Fig. **15**) [157]. [Ag$_2$(phen)$_3$(udda)] displays appreciable activity against *E. coli* (IC$_{50}$ = 9.54 μM), *S. aureus* (IC$_{50}$ = 14.18 μM), and *P. aeruginosa* (IC$_{50}$ = 32.47 μM), and it is cytotoxic towards cisplatin-sensitive breast (MCF-7) and resistant ovarian (SKOV-3) cancer cell lines. It is an avid DNA binder with intercalative capability greater than that of the standard ethidium bromide, and yet it appeared to be incapable of inducing DNA damage in mammalian cells.

Zhao *et al.* have reported the DNA binding and antibacterial activities of the neutral ternary lanthanide complexes, [RE(sal)$_3$(phen)] and [RE(cin)$_3$(phen)] (RE = La^{3+}, Pr^{3+}, Nd^{3+}, Sm^{3+}, Eu^{3+}, Gd^{3+}, Tb^{3+}, Dy^{3+}, Ho^{3+}, Tm^{3+}, Yb^{3+}, Lu^{3+} ; salH = salicylic acid; cinH = cinnamic acid) [158, 159]. The complexes all bind to DNA in an intercalative mode and the strength of the binding is indirectly proportional to the size of the radius of the lanthanide cation in the complexes. Furthermore, these workers found that the complexes displayed antibacterial activity against *E. coli* comparable to that of the free phen ligands, and that the activity followed the same trend as the binding capabilities of the complexes, with the complexes of the heavier metals more active than those containing the smaller lanthanides.
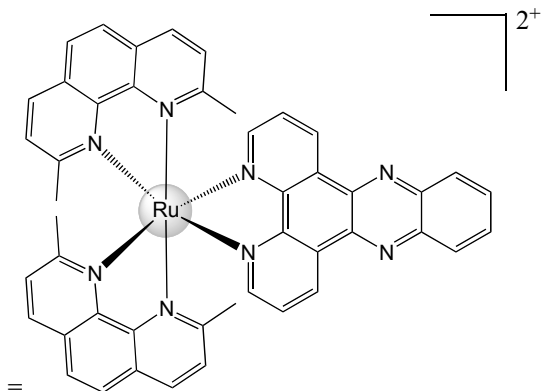
Fig. (11). The structure of[Ru(phen)$_2$(I$_L$)]$^{2+}$ (Where I$_L$ = dppz).
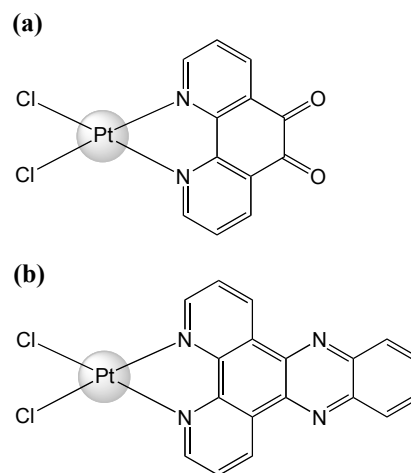
Fig. (12). Structures of (**a**)[Pt(phendione)Cl$_2$] and (**b**) [Pt(dppz)Cl$_2$].
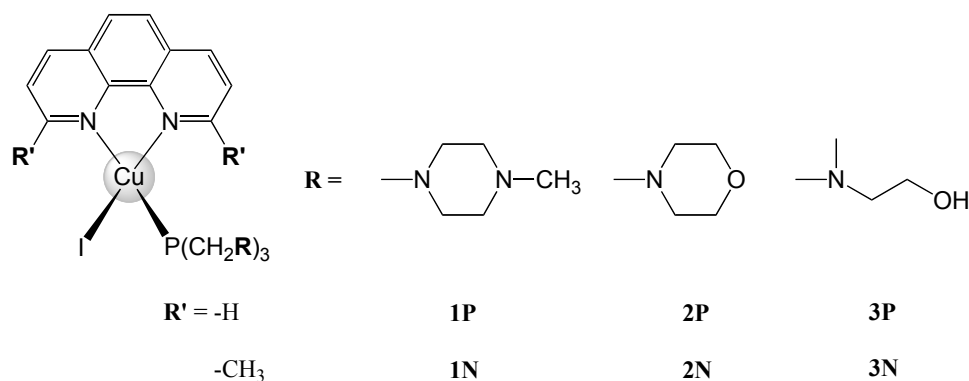
Fig. (13). Structure of the copper(I) mono-phen complexes.
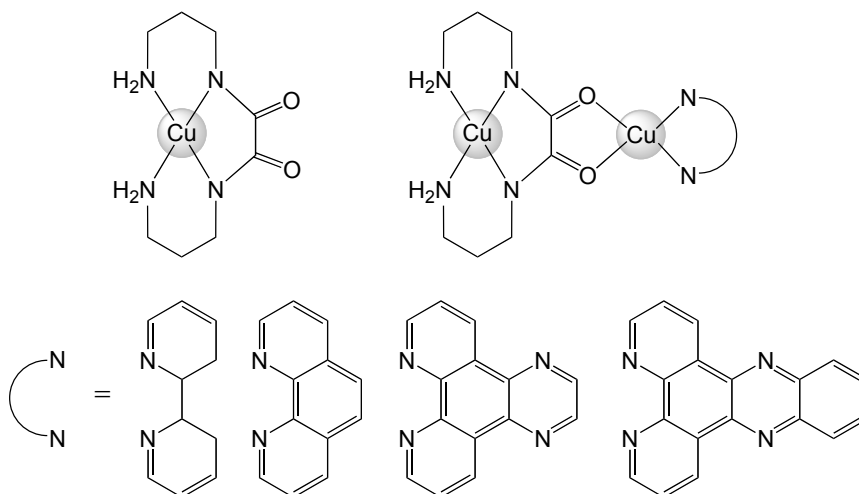
Fig. (14). Structure of the [Cu(oxpn)Cu(N-N)]$^{2+}$ complexes (N-N = bipy or phen or dpq or dppz, respectively).

Photocleavage of DNA

A number of papers have been reported in the literature that describes antibacterial active phen–based complexes that cleave DNA when irradiated with light. In 2015, Sudhamani *et al.* reported that the copper complexes [Cu(mqt)(N-N)H$_2$O]$^{1+}$ (mqt = 2-thiol-4-methylquinoline; N-N = phen, dpq or dppz) (Fig. **16**) bind DNA in a groove binding mode and that they exhibit photonuclease capability with the

photo-cleavage ability following the same order as the binding strengths of the three complexes, [Cu(mqt)(dppz)H$_2$O]$^{1+}$ > [Cu(mqt)(dpq)H$_2$O]$^{1+}$ > [Cu(mqt)(phen)H$_2$O]$^{1+}$ [160]. Mechanistic studies revealed the involvement of singlet oxygen (^{1}O$_2$) in the photo-cleavage process, the three complexes exhibited potential in photodynamic antimicrobial chemotherapy (PACT) as they were also found to kill *E. coli* upon photo-irradiation by a tungsten-halogen 500W lamp. N.

Fig. (15). Structure of the water-soluble silver complex, [Ag$_2$(phen)$_3$(udda)] (uddaH$_2$ = undecanedioic acid).

Fig. (16). Structure of [Cu(mqt)(N-N)H$_2$O]$^{1+}$.

Raman *et al.* have reported the DNA binding and photonuclease capability of the bis-phen copper(II) complexes [Cu(dbdppo)(phen)$_2$]$^{2+}$ and [Cu(hnbdppo)(phen)$_2$]$^{2+}$ {dbdppo = (4-(3′,4′-dimethoxybenzaldehyde)2-3-dimethyl-1-phenyl-3-pyrazolin-5-one; hnbdppo = (4- (3′-hydroxy-4′-nitrobenzaldehyde)2-3-dimethyl-1-phenyl-3-pyrazolin-5-one}[161]. The DNA cleavage reaction was found to be mediated by hy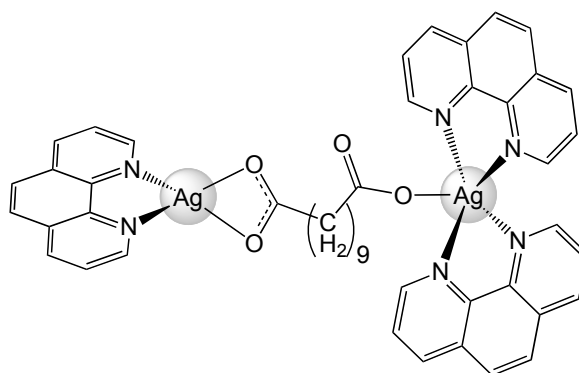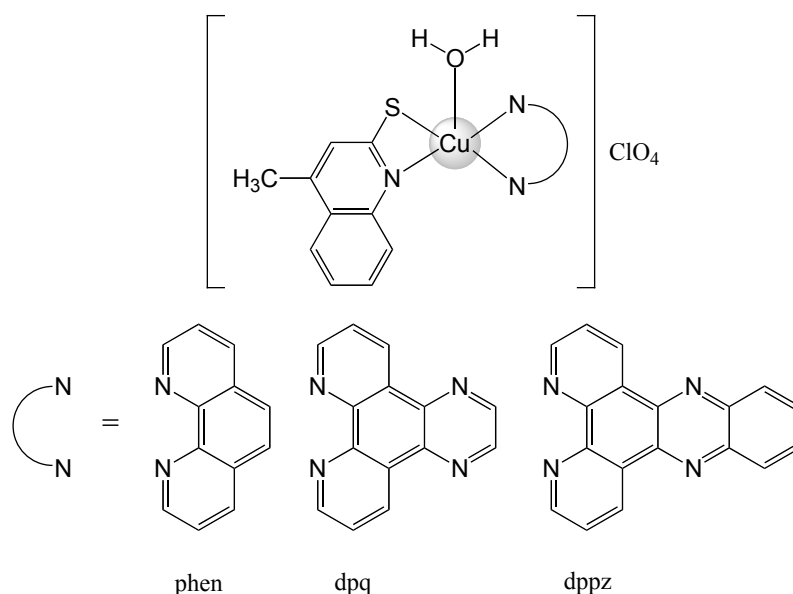droxyl radicals generated by a photo-redox mechanism. The complexes were found to exhibit significant antibacterial activity but their PACT potential under photo-irradiation was not investigated. Similarly, a number of other papers describe the photonuclease capability of bis-phen-based complexes with Co^{3+} [162-165] and Ru^{2+} [165-168], along with their antibacterial capabilities, independent of any PACT-type studies. In 2014, Frei *et al.* reported the results of a study on the PACT potential of the known stable neutral bis-phen ruthenium complex, [Ru(DIP)$_2$(bdt)] (DIP = 4,7-diphenyl-phen; bdt = 1,2-benzenedithiolate) (Fig. **17**) [169]. The phototoxicity of [Ru(DIP)$_2$(bdt)] was found to be considerably superior to the clinically approved photosensitizers porfimer sodium and 5-aminolevulinic acid, and the complex was efficient at killing Gram-positive bacteria. The DNA binding capabilities and the photochemical properties offered by metal complexes containing phen ligands presents sig-

nificant potential to contribute to the development of PACT. PACT is a relatively new avenue of research which offers an effective alternative approach in the era of antibiotic resistance, whereby DNA is only one target, which can be exploited in tandem with a number of other targets to deliver a multimodal therapeutic approach for which bacterial resistance has proved difficult [170].

INDUCTION OF OXIDATIVE STRESS

It has recently been shown that some bactericidal antibiotics induce microbial cell death by stimulating the production of destructive ROS, which can activate cellular respiration, causing superoxide production and the liberation of iron ions from iron-sulphur proteins [171-174] which, in turn, catalyses hydroxyl radical formation *via* Fenton chemistry. These HO˙ radicals can induce microbial cell death by oxidising proteins, lipids and DNA, [172-175] and can ultimately trigger mutations which promote antibiotic resistance [176]. In defence, bacteria respond to ROS by deploying antioxidant enzymes (*e.g.* superoxide dismutase and catalase) [175] and small antioxidant molecules like ascorbic acid and glutathione [177]. Additionally, microbially produced nitric oxide [178] and hydrogen sulphide [179] gas

molecules can each impede Fenton chemistry and promote antibiotic tolerance. There is also evidence to show that, under conditions of nutrient deprivation, some bacteria can endure antibiotics due to the limited manufacture of pro-oxidant metabolites and an increase in their antioxidant defences [180].
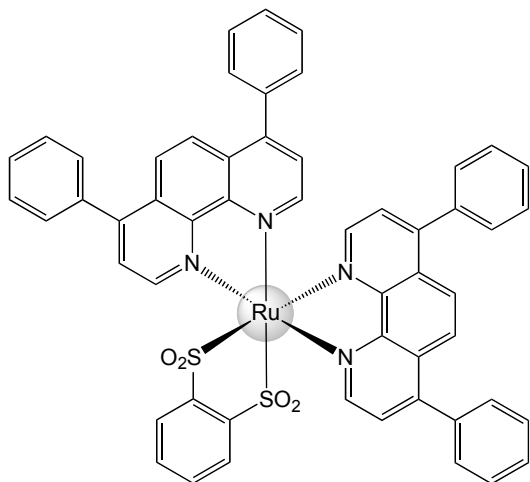

Fig. (17). Structure of [Ru(DIP)$_2$(bdt)].

Metal-phen complexes such as [Cr(phen)$_3$]$^{3+}$ are known to generate ROS in bacteria, contributing to their antimicrobial activity [181]. The use of metal-phen-based photo sensitizers in PACT also offers the potential to generate free radicals in bacteria leading to oxidative lethal damage and/or apoptosis [169]. It has been shown that upon the addition of hydrogen peroxide, the antibacterial activity of metal-free phen is significantly enhanced against *E. coli* [182]. It was concluded that the Fenton reaction, which generates highly cytotoxic hydroxyl and perhydroxyl free radicals, could potentially be catalysed through the *in vivo* formation of Fe^{3+}/Fe^{2+}-phen adducts. Furtado *et al.* later further examined this proposed pathway by repeating the exposure of *E. coli* to both phen and H$_2$O$_2$ in the presence of known Fe^{2+} and Fe^{3+} scavengers and, separately, in the presence of the free-radical scavenger, thiourea [183]. Interestingly, protection was achieved for the radical and Fe^{2+} scavengers (bipyridine, bipy), rather than the Fe^{3+} scavenger (desferal). It is noteworthy that these workers also found that neocuproine (2,9-dimethyl-phen, Fig. **4**), a known Cu^{1+} scavenger, also offered significant protection, supporting the notion that the known metallo-nuclease, [Cu(phen)$_2$]$^{1+}$, can play a role in bacterial cytotoxicity.

INHIBITION OF ENZYMES

According to the study of Zhu *et al.* in 2013, methyltransferase (MeTrCd) and acetyl-coenzyme A synthase (ACSCd) are two key enzymes in the acetylcoenzyme A synthesis pathway of the human pathogen *Clostridium difficile*, which is absent in humans and essential for the survival of the pathogen [184]. Screening inhibitors of the enzymes, the group has shown excellent inhibition effect of phen on ACSCd methyl group transfer and acetyl-coenzyme A synthesis activity at 1 mM concentration. This inhibitory effect was further examined by checking the antibacterial activity

of the inhibitor against *C. difficile* and, phen had an inhibitory effect on the pathogen at 25 mM. This study suggests that 1,10-phenantrholine targets and inhibits these key enzymes interfering with the pathogen survival pathway.

In 2009, Sharma *et al.* reported that the bacterial peptide deformylase (PDF) catalyses the removal of the N-terminal formyl group of proteins and it is essential for protein maturation, growth and survival of bacteria [185]. Thus PDF became a very attractive antimycobacterial drug target. In this study, various well-known PDF inhibitors, such as BB-3497, actinonin, 1,10-phenanthroline, hydroxylamine hydrochloride and galardin, were selected to evaluate their inhibitory activity against *Mycobacterium tuberculosis*. All compounds were active against *M. tuberculosis*, displaying a bacteriostatic mode of inhibition (MIC9 values ranging from 0.2-74 mg/L), but BB-3497 and 1,10-phenanthroline exhibited the most potent *in vitro* antimycobacterial activity with MIC's of 0.25 and 0.8 mg/L, respectively, suggesting the potential of these promising PDF inhibitors against *M. tuberculosis*.

In a study by Upadhye *et al.*, in 2009 the effects of serine, a metalloprotease inhibitor, have been evaluated on mycobacterial ES-31 serine protease, to assess the importance of this enzyme for bacterial cell growth [186]. 1,10-phenanthroline was tested and at a concentration of 0.5 mM it inhibited 78% of mycobacterial ES-31 serine protease activity *in vitro*. 1,10-phenanthroline also showed a decreased bacterial growth in 61% in axenic culture at 0.1mM and the inhibition was further confirmed by a decreased amount in 73% of ES-31 serine protease secreted in the culture filtrate. In human macrophage culture, 1,10-phenanthroline inhibited the infectivity of virulent and avirulent *M. tuberculosis* bacilli to macrophages by 68% and 63% growth inhibition of each respectively. It was observed that addition of mycobacterial ES-31 serine protease to macrophage culture enhanced the entry of bacilli and their multiplication in human macrophages, however, the addition of 1,10-phenanthroline strongly inhibited the mycobacterial growth as observed by decreased CFU count, showing the importance of mycobacterial ES-31 serine protease for pathogen infectivity.

In their pioneering work Dwyer *et al.* identified the enzyme inhibitory activity of polypyridyl metal complexes when they found that they could deactivate acetylcholinesterases [90], opening up the possibility of developing phen-based complexes that target enzymes in bacteria. In 2013, Tarushi and co-workers reported the antimicrobial activities of Zn(II) complexes with the general formula [Zn(quinolone)(phen)Cl] (quinolone = flumequine, oxolinic acid and enrofloxacin) [187]. The group reported that all complexes possessed a potent antibacterial activity against *B. subtilis*, *B. cereus*, *S. aureus* and *E. coli*, exhibiting very low MIC values in the range and 2–10 μg/mL. The complexes were also found to interact DNA in an intercalative binding mode, but their antibacterial activity was also attributed to their ability to inhibit DNA replication by targeting essential type II bacterial topoisomerases such as DNA gyrase and topoisomerase IV.

Recently, the antibacterial activities and the alkaline phosphatase (ALP) inhibitory capability of the metal-free phen and the ternary copper(II) complexes, [Cu(phen)

(meimzH)(H$_2$O)$_2$]$^{2+}$ and [Cu(phen)(cnge)(H$_2$O)(NO$_3$)$_2$] (meimzH = methylimidazol; cnge = cyanoguanidine) were examined [141, 188]. It was demonstrated that metal-free phen was an effective inhibitor of ALP and it was postulated that the mode of action was associated with the sequestration of Zn^{2+} ions from the active site of the enzyme. The two phen-based complexes, [Cu(phen)(cnge)(H$_2$O)(NO$_3$)$_2$] and [Cu(phen)(meimzH)(H$_2$O)$_2$]$^{2+}$ were also reported to exhibit an inhibitory effect with their mode of action attributed to a ligand-substitution mechanism involving the ALP protein.

ACTIVITY OF METAL-PHEN COMPLEXES ON BIOFILMS

1,10-phenanthroline-5,6-dione (phendione) (Fig. **4**), has been shown to be active against *Enterococcus*, with the ability to eradicate *E. faecalis* biofilms, and its activity appears to be related to its ability to sequester Zn^{2+} ions from metallo-enzymes [28]. The authors did not know exactly how phendione eradicated the *E. faecalis* biofilm, but they speculated that phendione may weaken the extracellular polymeric substances of the biofilm by disrupting the Zn^{2+} balance, and allowing the compound to get into bacterial cells harbored deep within the biofilm to exert its antimicrobial effects as a metalloprotease inhibitor. In 2014 Katzianer *et al.* reported the results of the antimicrobial activity of 5-nitro-phen against *Clostridium difficile* [189]. 5-nitro-phen showed potent bactericidal effects *in vitro* and it was shown to inhibit the growth of all three tested *C. difficile* strains efficiently (MIC ≈2 µM) at concentrations comparable with the first-line drug metronidazole. In the same study, 5-nitro-phen promoted *C. difficile* biofilm dispersal. For this assay, biofilm associated cells of *C. difficile* were incubated with the compound for different times and the remaining biofilm was quantified by crystal violet staining. The biofilm-associated biomass was significantly reduced in tests involving 6 h and 24 h exposures, suggesting that 5-nitro-phen may promote *C. difficile* biofilm dissolution. Additionally, the group examined the efficacy of 5-nitro-phen in a murine model and the compound displayed modest *in vivo* effects against *C. difficile* infection. For this test, mice were infected with *C. difficile* and were treated with the compound (5

mg/kg or 10 mg/kg doses). Although 100% of mice were moribund following 2 days of infection, treatment groups likewise survived significantly better, suggesting that 5-nitro-phen has a modest effect on *C. difficile* infection *in vivo*.

There are very few reports in the literature describing the anti-biofilm activity of phen-based complexes. In 2016, Viganor *et al.* also reported the antibacterial activity of phendione against *P. aeruginosa* and its biofilm, [190]. The phendione was found to be significantly more active against the pathogen than the parent phen (MIC$_{90}$ = 12.50 µg/ml and 200 µg/ml against susceptible and resistant isolates, respectively) Furthermore, pretreatment of the bacteria with phen, phendione and the Cu^{2+} and Ag^{1+} complexes, [Cu(phendione)$_3$]$^{2+}$ and [Ag(phendione)$_3$]$^+$ (Fig. **18**), at 0.5 x MIC values inhibited biofilm formation. [Cu(phendione)$_3$]$^{2+}$ and [Ag(phendione)$_3$]$^+$ were particularly active, reducing both biomass (by 48% and 44%, respectively) and viability (by 78% and 77%, respectively). Furthermore, phen, phendione, [Cu(phendione)$_3$]$^{2+}$ and [Ag(phendione)$_3$]$^+$ also disrupted mature biofilm, in a dose dependent manner, with the metal complexes being particularly active (IC$_{50}$ = 9.39 µM and 10.16 µM, respectively). These results are particularly significant given the resistance issues associated with *P. aeruginosa* which is a pathogen typically isolated from nosocomial infections, which is emerging as a particular global healthcare problem as the emergence of MDR isolates of this species is limiting the number of effective antimicrobials that are available to treat infected patients [190-193].

The anti-biofilm capabilities of the copper(II) complexes, [Cu(I$_L$)(A$_L$)]$^{2+}$ (where I$_L$ represents phen, 5-methyl, 5,6-dimethyl-, 3,4,7,8-tetramethyl-, and 4,7-diphenyl- 1,10-phenanthrolines, and A$_L$ represents 1,2-diaminoethane, 1*S*, 2*S*- or 1*R*,2*R*-diaminocyclohexane) (see Fig. **8** for the general structure), against *S. aureus* have been demonstrated recently [107]. Although the MIC values for the copper complexes (MIC range = 2-32 µg/ml) are significantly higher than that of the antibiotic vancomycin (MIC = 0.25 µg/ml), their anti-biofilm activities are significantly better. For vancomycin a minimum of 100 µg/ml (equivalent to 400
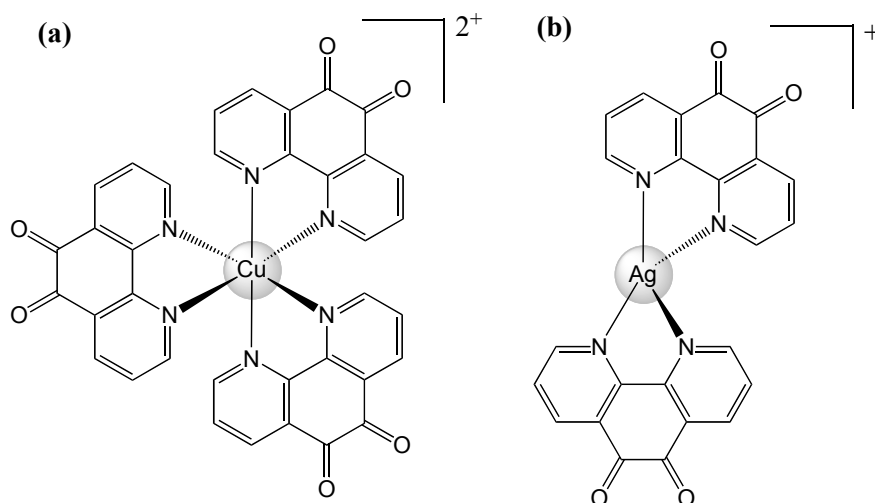

Fig. (18). Structure of (**a**) [Cu(phendione)$_3$]$^{2+}$ and (**b**) [Ag(phendione)$_3$]$^+$.

fold the MIC) was required to get an appreciable reduction (approximately 44%) in the biomass in the *S. aureus* biofilm, whereas for example 25 µg/ml of [Cu(5,6-dimethyl-phen)(*SS*-dach)]$^{2+}$ (equivalent to 3 fold the MIC) rapidly (2 h) reduced the biofilm by 68%. All of the copper complexes had similar activity against the biofilm. Replacing the Cu^{2+} in [Cu(5,6-dimethyl-phen)(*SS*-dach)]$^{2+}$ with Pt^{2+} and Pd^{2+} significantly reduces the antibacterial and anti-biofilm activities. It was suggested that the nuclease capability of the copper complexes (which is not a feature of the platinum and palladium complexes) may be a significant factor in their mechanism of action against both planktonic and biofilm growing cells, particularly given that the extracellular matrix of biofilms are known to contain significant levels of nucleic acids.

CONCLUDING REMARKS

Bacterial resistance to antibiotics now represents one of the greatest challenges to public health, globally. In an effort to address the diminution of the therapeutic options in this "era of resistance" there is a pressing need to identify antibacterial agents that can either target bacteria in ways that are different to the current arsenal of antibiotics or that can kill bacteria in a multimodal fashion, thereby preventing the rapid onset of resistance. In the 1950's, 1960's and 1970's work, pioneered by Francis Dwyer, on the antibacterial activity of metal complexes containing 1,10-phenanthroline ligands [90, 93], opened the door to the development of the diverse biological profile of this class of inorganic compound [44]. These early results clearly demonstrated the antibacterial chemotherapeutic potential of metal complexes of 1,10-phenanthroline, but the significance of the results was not embraced by the pharmaceutical sector as the "antibiotic era" was at its height and antibiotic resistance was not appreciated as an insurmountable problem.

As we are now aware the "antibiotic era" has been characterized by the overuse and misuse of antibiotics, culminating in the present critical "era of resistance". Consequently, there has been a recent surge in interest in the antimicrobial applications of metal-based compounds, with the application of metal complexes incorporating 1,10-phenanthroline ligands playing a significant role. 1,10-phenanthroline exerts *in vitro* antimicrobial activity against a broad-spectrum of bacteria, in its own right. The antimicrobial activity of 1,10-phenanthroline can be significantly modulated by modifying its structure. The development of metal-phen complexes affords the medicinal chemist an opportunity to exploit such structural diversity by introducing a variety of metal centres, offering the opportunity to control characteristics such as the geometry, lipophilicity, redox status, ability to generate beneficial reactive oxygen/nitrogen species and the ability to interact with biomolecules such as DNA and proteins. Metal complexes of phen-type ligands are generally more active than the metal-free ligand and the efficacy of such complexes can be further improved by introducing auxiliary ligands, such as the quinolones, which are themselves bioactive. The study of metal-phen complexes as potential antibacterials is in its infancy and although this review presents an encouraging perspective with respect to the potential for the deployment of such inorganic therapeutics in the fight against bacteria the transfer of the results of the *in vitro* stud-

ies into *in vivo* models remains largely unexplored. The toxicity profiles for phen and some metal-phen complexes in nematode [106], insect larval [32], and animal [58] models have been reported, with metal-free phen and some metal-phen complexes displaying favorable tolerance levels. Since Dwyer early reports of *in vivo* activity [64, 90] very few *in vivo* bacterial infection studies have been carried out on phen and its metal complexes. The complex, [Ru(2,9-dimethyl-phen)$_2$(dppz)]$^{2+}$ (Fig. **11**), was recently shown to be active in rescuing the soil nematode *Rhabditis elegans* post infection with MRSA [156]. Data derived from appropriate *in vivo* models are necessary to ensure that effective drug delivery, low toxicity and optimal drug concentrations preventing bacterial cell proliferation can be achieved. DNA is assumed as a key target for metal-phen-based antibacterials and to date the vast majority of the data relating to this aspect of their activity has been based on acellular assays. There is a need to study the effects that the complexes are having on DNA in a cellular context using biochemical and established cell-based spectroscopic techniques. Indeed, having researched this topic it is clear that there is dearth of knowledge in relation to how metal-phen complexes are interacting with a range of biomolecules within bacterial cells. Metal-phen complexes are clearly multimodal in their mechanism and there is a need to integrate what is currently known about the mechanisms into an advanced systems biology approach utilising novel 'omics' technologies with Bioinformatics. 'Omics' technologies involve global and high-throughput analytical methods such as genomic microarrays, 2D-gel and 2DLC/MS proteomics or biochemical reactions of metabolites (metabolomics), ions (ionomics) or metals (metallomics). These Omics technologies are supported by advanced Bioinformatic techniques and have been critical in providing information on key mechanistic targets and cellular pathways for many pharmaceutical agents. Integrated online databases with molecular networking of 'omics' data reveals relationships between novel complexes/drugs and functional molecules thus providing a widened biological perspective of the mechanisms of action. 'Omics' technologies are less-time consuming and more efficient than traditional biochemical techniques and are being widely adopted by many areas of biological and pharmaceutical research, however there are many recognised challenges which include data acquisition and analysis; modelling of multi-omics data sets for a systems biology approach and moreover the experimental preservation of an *in situ* state of metal-phen complexes in an already complex cellular system [194].

ABBREVIATIONS

AFM	=	Atomic force microscopy
ALP	=	Alkaline phosphatase
AMR	=	Antimicrobial resistance
BLM	=	Bleomycin
DNA	=	Deoxyribonucleic acid
EPS	=	Extracellular polysaccharides
ESBL	=	Extended spectrum *β*-lactamases
ESKAPE	=	Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter bau-

manii, Pseudomonas aeruginosa and Entero-
bacteriaceae

GBS	=	Group B *Streptococcus*
GI	=	Growth inhibition
HGT	=	Horizontal gene transfer
HUVECs	=	Human umbilical vein endothelial cells
MCF-7	=	(Michigan Cancer Foundation) Human breast adenocarcinoma cell line
MDR	=	Multidrug resitance
MeTrCd	=	Methyltransferase
ACSCd	=	Acetyl-coenzyme A synthase
MIC	=	Minimum inhibitory concentration
MRSA	=	Methicillin-resistant *S. aureus*
OmpC	=	Outer membrane porin C
OmpF	=	Outer membrane porin F
PACT	=	Photodynamic antimicrobial chemotherapy
PDF	=	Peptide deformylase
PHEN	=	1,10-phenanthroline
RNA	=	Ribonucleic acid
ROS	=	Reactive oxygen species
SEM	=	Scanning electron microscopy
SKOV-3	=	(Sloan Kettering) Human ovary adenocarcinoma cell line
SN	=	Streptonigrin
QS	=	Quorum sensing
VRE	=	Vancomycin-resistant *Enterococcus*
XDR	=	Extensively drug resistance

CONFLICT OF INTEREST

The authors confirm that the material and content presented in this article do not present any issues with respect to conflict of interest.

ACKNOWLEDGEMENTS

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