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DNA as a target for antimicrobials

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ABSTRACT

Resistance to antimicrobials is one of the biggest threats to our healthcare. However, in the last few decades very few truly novel antimicrobial compounds have been brought to market, creating the potential threat of a post-antibiotic era in which infections are very difficult to treat. Identification of novel compounds with antimicrobial activity is therefore paramount. Ideally, novel compounds should be designed that are active against targets that are not or barely used, as it is less likely that resistance already exists against such compounds. One example of an underexplored target in the treatment of infections is DNA. In this review we describe a number of DNA binding compounds and discuss potential opportunities and problems.

1. Introduction

Antibiotics play an essential role in the treatment of bacterial infections, but this has become more and more problematic due to the emergence of multidrug resistant pathogens. Indeed, the Chief Medical Officer of the UK, Dame Sally Davies, in 2013 called antibiotic resistance a health catastrophe, ranking similarly to terrorism and climate change [1]. Well-known examples of bacteria that create significant problems are extended spectrum beta-lactamase (ESBL) producing Gram-negative bacteria, multi-drug-resistant *Mycobacterium tuberculosis* and meticillin-resistant *Staphylococcus aureus* (MRSA). For instance, production of ESBL is the most common resistance mechanism for 3rd generation cephalosporins and, worryingly, resistance of *Escherichia coli* isolates to these cephalosporins in Europe has risen by 8-fold in the period from 2002 to 2011 (Fig. 1a). Some positives should however also be noted, with meticillin resistance in *S. aureus* gradually decreasing since its peak in 2003–2005 (Fig. 1b). Note however that the occurrence of MRSA is still high, with some European countries such as Portugal and Romania reporting around 50% of *S. aureus* isolates being meticillin resistant in 2011.

It is thus clear that it is crucial to develop new antibiotics and remain ahead of antibiotic resistance in pathogenic bacteria. Antibiotics that are currently used clinically have only a limited set of targets. These include the cell wall (targeted by penicillins, cephalosporins, glycopeptides), the cell membrane (polymyxins, daptomycin), ribosomes (aminoglycosides, macrolides and tetracyclines), as well as a small set of essential enzymes: RNA polymerase (rifamycins), DNA gyrase and topoisomerase (quinolones), and dihydropteroate synthetase (sulphonamides). A huge problem is that hardly any novel classes of antibiotics have been developed in the last few decades, with a few notable exceptions being daptomycin and linezolid. Most new antibiotics coming onto the market are variants of existing drugs, which carries the risk that (partial) resistance to such drugs already exists.

There are many potential antimicrobial targets in bacteria, several of which are not or hardly utilised in the treatment of infections. One target that has recently been addressed in a number of studies is DNA. This target may seem at first sight not very useful, as DNA-binding compounds would not only inhibit the growth of bacteria, but also that of eukaryotes. If, however, there is some way towards establishing specificity, then DNA may indeed be an attractive target. This issue is discussed towards the end of this review. Note also that there are already a few DNA-targeting antimicrobials on the market (see below), and further expansion of the repertoire of DNA-binding antimicrobials for clinical use thus seems most certainly conceivable.

Binding of compounds to DNA occurs through a number of mechanisms, which can be broadly divided into covalent and non-covalent interactions. Covalent interactions are the strongest and involve modification of DNA through alkylation. Non-covalent interactions mainly involve intercalation between bases, or binding to the major or minor groove of the DNA helix [2–4]. In some cases, interaction of compounds with DNA may also result in damage of the DNA, and lead to for instance single or double strand breaks. All of these interactions are likely to interfere with enzymes (e.g. polymerases, topoisomerases) and essential processes

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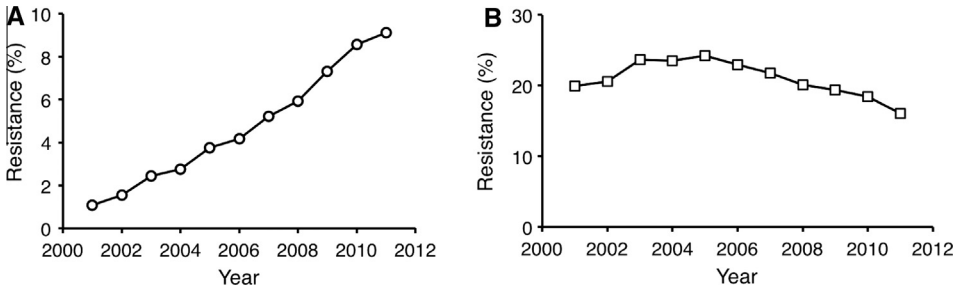

Fig. 1. Antibiotic resistance of clinical isolates in Europe. (A) Percentage of 3rd generation cephalosporin-resistant *E. coli* isolates, and (B) percentage of meticillin-resistant *S. aureus* isolates. Data were collected from the EARS-Net Database, http://www.ecdc.europa.eu/en/healthtopics/antimicrobial_resistance/database/Pages/table_reports.aspx).

of transcription, replication and repair, thus leading to cell death. In this review, we will consider a number of compounds of interest, with a particular focus on those with antimicrobial activity, and then discuss whether there is a future in the clinical use of DNA binding compounds as antimicrobial therapeutics.

2. DNA intercalators

Several organisms produce antimicrobial compounds as a means of competing with other organisms in their environment. Many of those that are used clinically originate from Gram-positive soil bacteria belonging to the genus *Streptomyces*, including streptomycin, vancomycin, daptomycin and tetracycline. Initial work on the isolation of compounds from *Streptomyces* spp. was conducted in the laboratory of Selman Waksman, who received recognition for his work with the Nobel Prize for Physiology or Medicine in 1952. Some of first compounds isolated were actinomycins [5], with most of the later research being performed on actinomycin D (compound **1**; also called dactomycin). This compound (Fig. 2) contains a planar tricyclic phenoxazone ring that intercalates double stranded DNA, and two cyclic pentapeptide lactone rings that interact with the minor groove above and below the intercalated phenoxazone ring. However, it has a strong preference for DNA that is partially unwound, such as found in transcription bubbles [6]. As a consequence, actinomycin D blocks RNA synthesis, leading to cell death. The molecule has both antibiotic and anticancer activity; because of the latter, the compound is too toxic for the treatment of infections. However, the compound has been used for many years as an anticancer agent, mainly in the treatment of paediatric cancers such as Wilms' tumour and rhabdomyosarcoma [7]. In addition, its activity as a transcription inhibitor has also been exploited as useful tool in molecular biology.

Another family of naturally produced compounds interacting with DNA are bis-intercalators [8] that includes for instance echinomycin (**2**), triostin A and sandramycin. Many of these are isolated from *Streptomyces* spp., but some are produced by other bacteria. All of these contain a peptide core that that is decorated with two intercalating planar aromatic groups, being quinoxaline-2-carboxylic acid in echinomycin and triostin A, or 3-hydroxyquinaldic acid in sandramycin [9].

Echinomycin (Fig. 3) has been tested in a number of phase I and II clinical trials against different types of cancer, but the majority of those did not show a significant effect [10–12]. One of the exceptions was a study on colorectal cancer, where a moderate activity was observed [13]. In more recent years there has been a renewed interest in echinomycin as an antimicrobial agent. For instance, it has been shown to be effective against clinical isolates of MRSA [14]. That study demonstrated that echinomycin (**2**) is more effective than vancomycin (one of the few antibiotics used clinically to treat MRSA infections), not only *in vitro* but also *in vivo* in a mouse model. Echinomycin has also been demonstrated to be active against other microbes, including vancomycin resistant enterococci (VRE) [15] and the parasitic protozoan *Entamoeba histolytica* [16]. A problem of echinomycin is that it is rather hydrophobic and not particularly soluble in water. For that purpose polar derivatives were designed [15]. Unfortunately, these were less effective *in vitro* than echinomycin; the minimal inhibitory concentration (MIC) of echinomycin against clinical isolates of enterococci was found to be in the range of 0.03–0.25 μg/mL, while that was 0.5–8 μg/mL for the better of the two derivatives (denoted YK2000). However, infections with VRE are particularly difficult to treat because of the multi-drug resistant nature of these bacteria, and YK2000 could still be considered for therapeutic use.

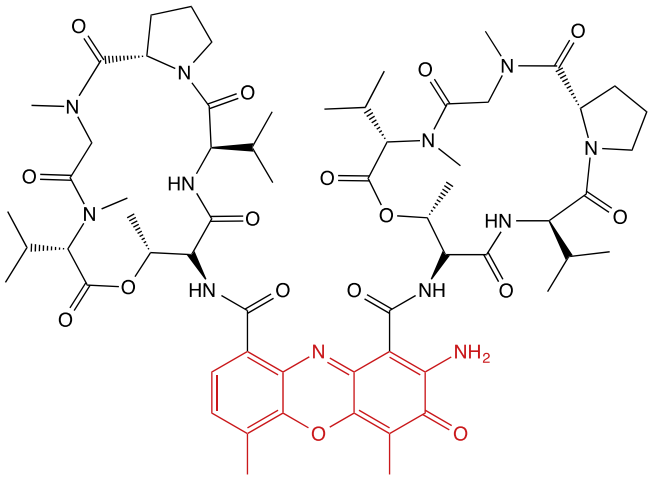

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Fig. 2. Structure of actinomycin D (**1**). The intercalating phenoxazone group is indicated in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

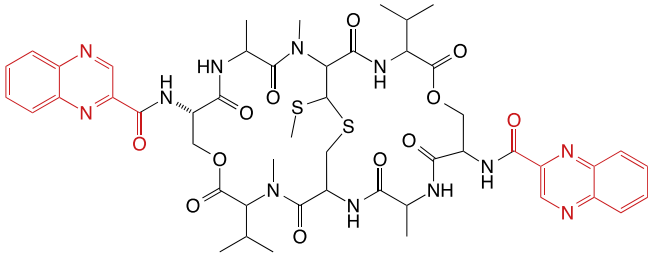

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Fig. 3. The structure of echinomycin (**2**) with the intercalating groups in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Antibiotics belonging to the anthracycline family form another large group of well-known DNA intercalators. These include the naturally produced compounds doxorubicin and daunorubicin (produced again by *Streptomyces* spp.), or (semi) synthetic derivatives thereof such as epirubicin. These compounds are antibacterial, but they also have a particularly strong cytotoxic activity. These anthracyclines are therefore not suitable in the treatment of bacterial infections, but several are used in the treatment of various types of cancer. More recently, amino-sugar functionalised anthracycline analogues have been made that were tested for antibacterial activity [17]. The compounds were active against a Gram-positive bacterium (*Bacillus atropheus*) but not against a Gram-negative bacterium (*E. coli*). The activity found correlated to a large extend with the DNA binding activity, but all of the compounds isolated had an antibacterial activity lower than that of daunorubicin or doxyrubicin.

While the compounds mentioned above are all produced naturally or analogues thereof, also a number of fully synthetic intercalators have been produced. Some of the earliest work on this was on a coordination complex of ruthenium with the ligand 1,10-phenanthroline (phen), $[Ru(phen)_3]^{2+}$ [Ref. 18], where this complex demonstrated activity against Gram-positive bacteria. The same group also performed more detailed studies with additional ligands (e.g. 2,2′-bipyridine) and metals (Ni(II), Cu(II), Fe(II) and Co(II) (compounds **3** and **4**, Fig 4), and tested these on a larger panel of bacteria [18,19]. These complexes were more active against Gram-positive bacteria than Gram-negative bacteria. Pathogenic bacteria such as *M. tuberculosis, Erysipelothrix rhusiopathiae*, *S. aureus* and *E. coli,* as well as the fungi *Candida albicans* and *Trichophyton mentagrophytes*, did not develop resistance of any significance to metal complexes of phenanthroline based ligands and the susceptibility of *S. aureus* and *E. coli* appeared to be independent of the antibiotic-resistance pattern of the organism [20]. *In vivo* (using mice or guinea pig models) the compounds were found not to be effective when administered parenterally, but some proved useful in the topical treatment of bacterial infections [19,20]. This was however not taken forward and these compounds have not been used clinically.

Recently, we and others revisited some of this work, where the antibacterial activity of a number of Ru(II) complexes (for examples, see Fig. 5) with proven DNA binding were evaluated [21–23, and references therein]. We used compounds with the general structure $[Ru(P_L)_2(I_L)]^{2+}$, where P_L is a peripheral ligand, and I_L the intercalating ligand. Of the compounds tested, the order of the antimicrobial activity followed the order of the affinity of the intercalating ligands for DNA, consistent with a DNA-binding mode of action. The compounds performed poorly against Gram-negative bacteria, but showed good activity against Gram-positive bacteria, including clinical multidrug resistant isolates such as MRSA. The most active compound that we identified was $[Ru(2,9\text{-}Me_2phen)_2(dppz)]^{2+}$ (where 2,9-Me_2phen = 2,9 dimethyl-1,10-phenanthroline, and dppz = dipyrido[3,2-a:2′3′-c]phenazine) (complex **5**, Fig. 5). Interestingly, the compound was also active in an infection model [21]. In the absence of compound, the soil nematode *Caenorhabditis elegans* was killed within a few days by MRSA, but the presence of $[Ru(2,9\text{-}Me_2phen)_2(dppz)]^{2+}$ rescued the nematodes from infection. This firstly demonstrated that the complex was active *in vivo*. In addition, it showed that the concentrations used were not toxic to the nematodes, although it should be noted that the lethal dose for nematodes was not determined. The nematodes served are only a very simple model system, but it should be noted that: (a) these organisms share several features with higher animals and have neurons, muscles, intestines, epidermis; (b) they have an innate immune response system; and (c) indeed they have been demonstrated to be a very useful model system for several human pathogens [24].

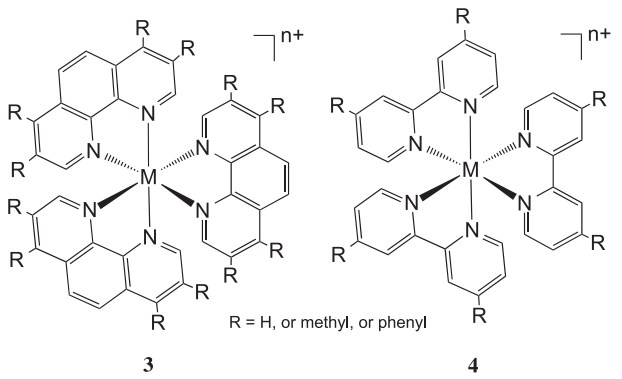

Fig. 4. Some of the transition metal complexes (**3** and **4**) investigated by Dwyer which demonstrated bacteriostatic and bacteriological activity.

Other mononuclear intercalating complexes like complex **6** (Fig. 5) have also shown antibacterial and antifungal activity against *E. coli* and *Neurospora crassa* with zones of inhibition (in disk sensitivity tests; 20 μg per disk) of 10 and 7 mm [23]. Chiral dinuclear compounds have also demonstrated antibacterial activity against Gram-positive bacteria, including MRSA (complex **7**, Fig. 5). Dinuclear complexes of this type have been reported to bind to bulge sites of DNA. More recently an example $[\{Ru(phen)_2\}_2\{u\text{-}bb_7\}]^{4+}$ (where bb_7 = bis[4(4′-methyl-2,2′-bipyridyl)]-1,7-alkane) has been shown to bind chromosomal DNA of *S. aureus* [22].

Cobalt complexes of phenanthroline and of multidentate ligands (complex **8**, Fig. 6) were also investigated by Dwyer and showed bacteriostatic and bactericidal properties. Other cobalt complexes (complex **10**–**13**, Fig. 6) that have been more recently developed and have demonstrated considerable activity against *S. aureus*, *Bacillus subtilis, E. coli* and *Pseudomonas aeruginosa.* While the hydrophobicity imparted by the alkyl chain may result in membrane damage, DNA binding was also suggested as a mode of action. Complexes of $[Co(en)_2I_{L2}]$ (where en = ethylenediamine and I_L = 2,2′-bipyridine (**9a**), 1,10 phenanthroline (**9b**), imidazole (**9c**), methylimidazole (**9d**), ethylimidazole (**9e**) or dimethylimidazole (**9f**)) (Fig. 6) have also been reported to show activity against several bacteria (*E. coli*, *Salmonella typhimurium*, *Proteus vulgaris, P. aeruginosa, S. aureus, Enterococcus faecalis* and *B. subtilis*). Their mode of action was not discussed but the parent complex $[Co(en)_3]^{2+}$ has been demonstrated by NMR to interact with DNA [25,26].

We also tested several coordination complexes of Cu(II), and found these to be not only active on Gram-positive bacteria such as MRSA, but also on Gram-negative bacteria. For instance, the activity of $[Cu\text{-}56MESS]^{2+}$ (56-MESS = (5,6-dimethyl-1,10-phenanthroline)(1*S*,2*S*-diaminocyclohexane); complex **14**, Fig. 7) was similar for both MRSA and *E. coli*, with an MIC of 8 μg/mL [27]. No activity against the Gram-negative bacterium *P. aeruginosa* was found. However, that is an organism with a high level of innate resistance against many compounds, in part due to its much lower outer membrane permeability (~8%) compared to that of *E. coli* [28]. A problem with some of the copper compounds tested was their toxicity, as confirmed by activity against a mammalian cell line (L1210) and toxicity against *C. elegans* nematodes [27]. However, some of the copper complexes had a low toxicity against nematodes, while still retaining a reasonable antimicrobial activity, suggesting that further development may lead to identification of compounds with low toxicity and high antimicrobial activity.

Other copper compounds that incorporate a 1,10-phenanthroline, cyanoguanidine, bipyridines, terpyridines and/or antibiotics have recently been evaluated for their activity. The complex $[Cu(phen)(cyanoguanidine)(H_2O)(NO_3)]NO_3$ (complex **15**, Fig 7) [29] was synthesised to explore the effect of combining the nitrogenase substrate cyanoguanidine with copper and phenanthroline

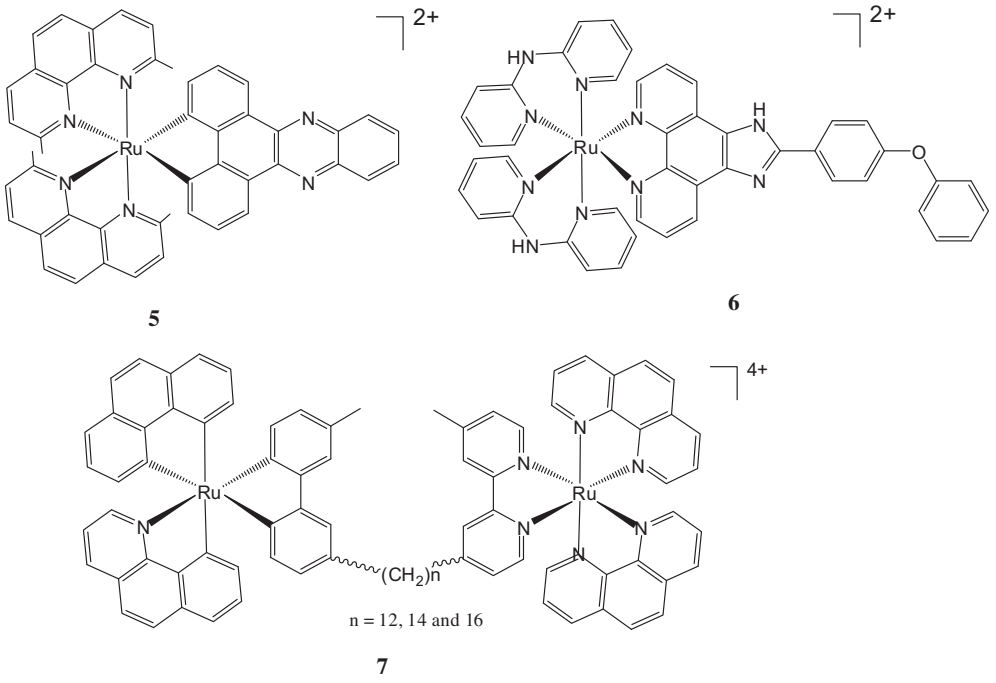

Fig. 5. Some of DNA binding antibacterial complexes (**5**, **6** and **7**).

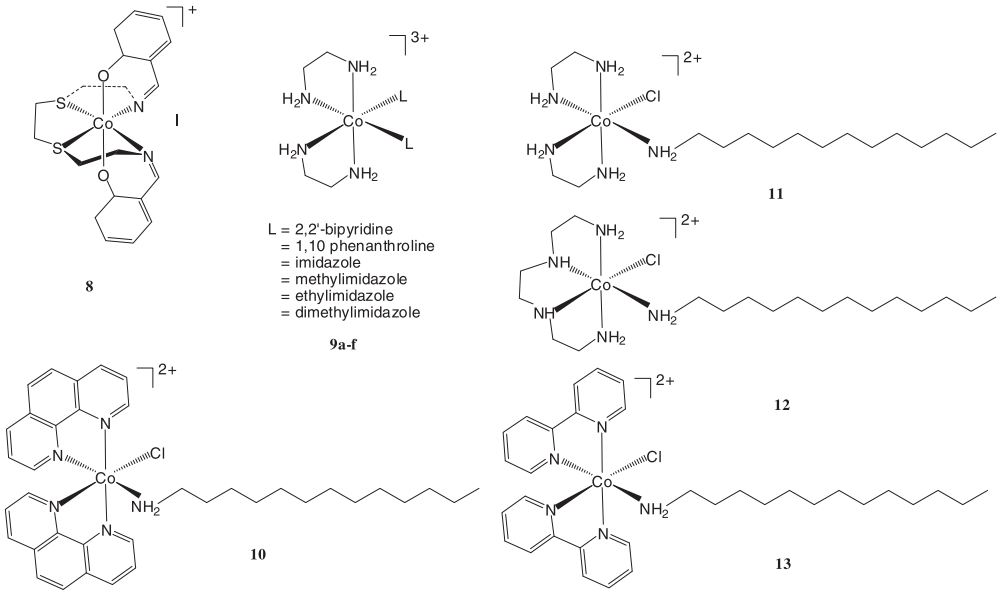

Fig. 6. Structures of Cobalt coordination complexes (**8–13**).

against *E. coli*, *S. aureus*, *E. faecalis* and *P. aeruginosa*. The most significant activity was observed against *S. aureus* and *E. faecalis* with MIC values of 24 μg/mL for both organisms, as compared to 375 μg/mL for $CuCl_2{\cdot}2H_2O$ (both organisms) and 24 (*S. aureus*) and 94 μg/mL (*E. faecalis*) for phenanthroline [29].

As a strategy to circumvent antibacterial resistance and improve solubility, the FDA approved broad-spectrum antibiotics (B-SA), iomefloxacin (complex **16**, Fig. 7) [30] or ciprofloxacin (complexes **17** and **18**, Fig. 7) [31–33]. These antibiotics, which function by inhibition of one step in DNA gyrase activity, were coordinated to copper complexes with the general structure $[Cu(B\text{-}SA)(I_L)(NO_3 \text{ or } Cl)]^+$, where B-SA is iomefloxacin (red) or ciprofloxacin (blue), and I_{Ls} with the potential to intercalate are 1,10-phenathroline [30], 4-(R-phenyl)-6-phenyl-2,2′-bipyridine [31,32], or 2,2′:6′,2″-terpyridine [33]. Examples of the resulting copper complexes **16**–**17**, shown in Fig 7, had demonstrated activity, with MIC values all in the range of 0.3–2.6 μM, against both Gram positive (*S. aureus* and *B. subtilis*) and Gram negative (*E. coli*, *Serratia marcescens* and *P. aeruginosa*) bacteria.

With these metal complexes it should be noted that the potential to bind DNA does not necessarily lead to antibacterial activity. For instance, complexes of $[Pt(I_L)Cl_2]$ where I_Ls are derivatives of phenanthroline such as 1,10-phenanthroline-5,6-dione (**19**, Fig. 8) or dppz = dipyrido[3,2-a:2′3′-c]phenazine (**20**) can intercalate or coordinate to DNA. However, antibacterial activity against *E. coli*, *B. subtilis* or *Streptomyces coelicolor* was not evident [34]. This suggests that DNA binding alone is not sufficient to induce antibacterial activity but that the mode of interaction is important.

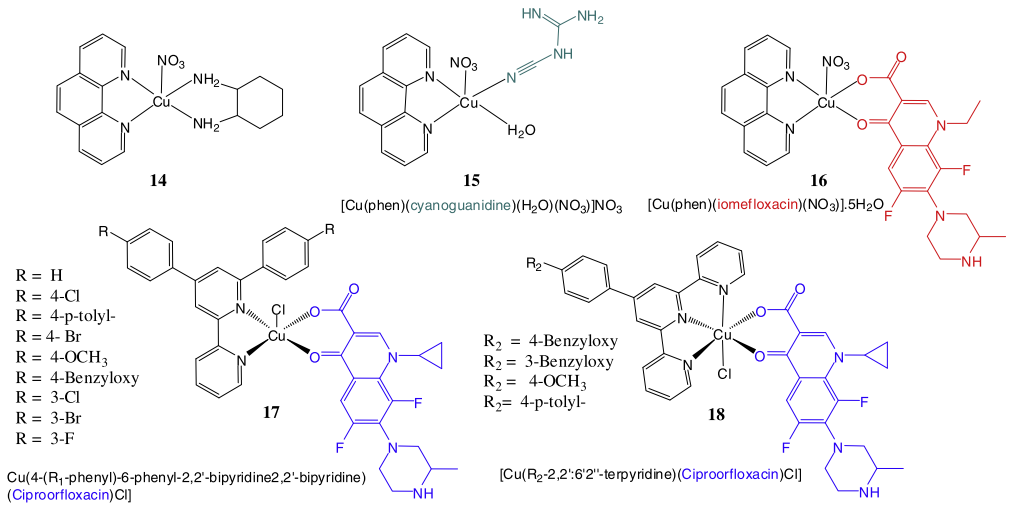

Fig. 7. Structures of copper coordination complexes (**14–18**).

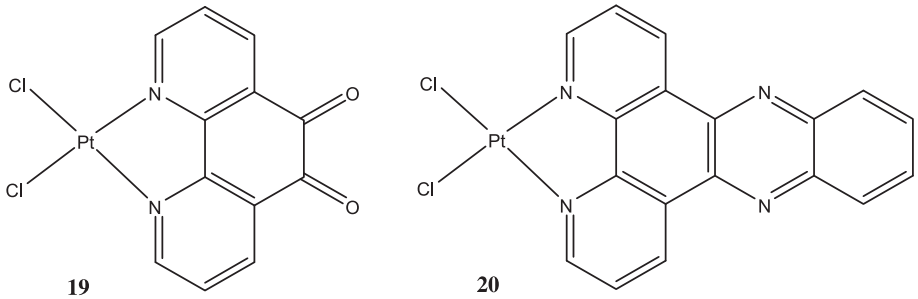

Fig. 8. Platinum complexes of the general structure of $[Pt(I_L)Cl_2]$] where I_Ls = 1,10-phenanthroline-5,6-dione (**19**) or dppz = dipyrido[3,2-a:2′3′-c]phenazine (**20**).

3. Minor groove binders

Another group of DNA-binding compounds are those that interact with the minor groove of DNA. A minor groove binder that has been used clinically since the 1940s is the bis-amide pentamidine (**21**), an important drug in the treatment against a number of protozoal diseases, including those that cause pneumocystis pneumonia, leishmaniasis and African trypanosomiasis (sleeping sickness). From studies with several analogues it was suggested that the isohelical shape of the molecule is important for interaction with the minor groove [35]. Pentamidine, however, is also capable of inducing DNA cleavage, which is observed specifically in kinetoplast DNA [36], a complex network of circular DNA molecules found only in the mitochondrion of flagellate protozoa. The selectivity of pentamidine is due to the specific accumulation of the drug in these protozoa [37], but toxicity (nephro- and hepatotoxicity) remains a significant issue and is the cause for several side effects.

Metals can greatly enhance the antibacterial activity of groove binders. For instance, the ligand bis(3-(4-dimethylaminophenyl)-allylidene)-1,2-diaminoethane, which is similar in shape to pentamidine, has MIC values of 250 and 125 μg/mL for *B. subtilis* and *E. coli*, respectively, but when complexed to Zn (complex **22**, Fig 9), the MIC for both bacteria is 0.49 μg/mL [38]. Complex **22** was, however, not active on *P. aeruginosa*.

Molecules that also have a similar crescent-shape as pentamidine are the naturally occurring antibiotics netropsin and distamycin A (**23** and **24**, Fig 10). These are polyamides belonging to the family of lexitropsins, which also includes several synthetic compounds. Netropsin (**23**) and dystamycin (**24**), both of which are

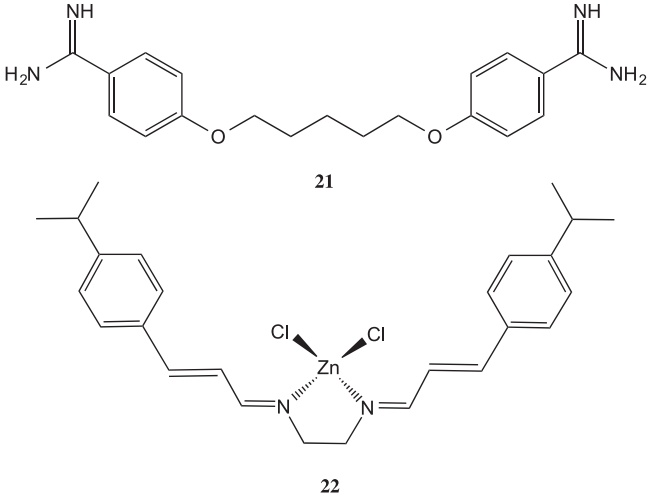

Fig. 9. The structure of groove binders pentamidine (**21**) and [Zn((3-(4-dimethylaminophenyl)-allyidene-1,2-diaminoethane)Cl_2] (**22**).

produced by *Streptomyces* spp., contain two or three N-methylpyrrole rings, respectively (Fig. 10). The compounds can, depending on the concentration, bind DNA either in a 1:1 stoichiometry, or in a 2:1 stoichiometry in which with two molecules bind to DNA in an antiparallel orientation [39]. Lexitropsins have a wide range of activities, including antiviral, antibacterial, antifungal, antiparasitic and anticancer activity. For instance, they have been shown to inhibit replication of the vaccinia virus, a virus closely related to

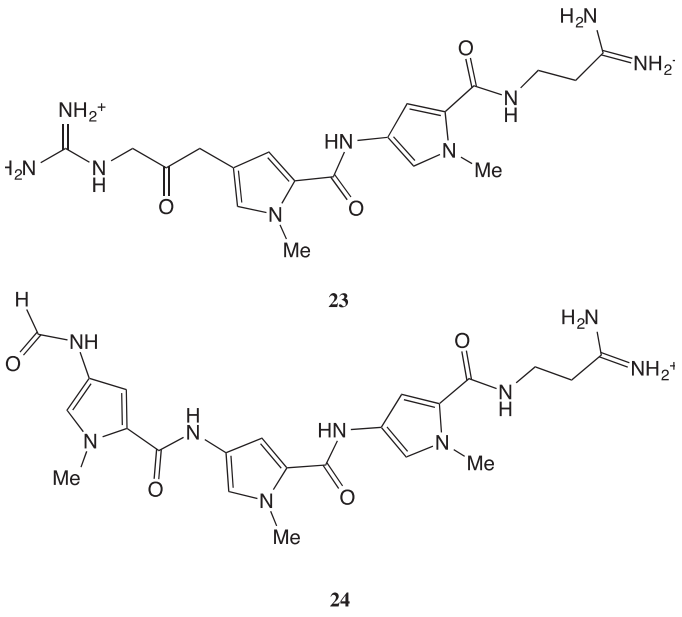

Fig. 10. Structures of netropsin (**23**) and distamycin (**24**).

cowpox virus [40], as well as HIV [41]. Other lexitropsins have been synthesised that are active against the fungi *Aspergillus niger* and *C. albicans* and the bacteria *S. aureus* and *Mycobacterium aurum* [42,43]. Netropsin and distamycin and are too toxic for clinical use, but it should be noted that some of their derivatives have been shown to have relatively low toxicity [44]. The latter study was focused on designing compounds with increased hydrophobicity, and it resulted in several compounds with high activity. It was not clearly established whether that was because of increased affinity or because of enhanced membrane permeability.

Of particular interest with lexitropsins is the potential for modulation of their sequence specificity. Netropsin and distamycin preferentially bind A/T rich DNA. However, this specificity can be altered by replacing the pyrrole rings (which preclude close contact with guanine) with imidazoles or hydroxypyrrole amino acids, thereby allowing interaction with G/C basepairs as well [45]. Further research allowed the design of a set of rules for interaction of lexitropsins with DNA, enabling the design of compounds interacting with specific DNA sequences [45]. Using this approach, lexitropsins have been designed inhibiting specific targets. An example of this is a number of compounds that target the sequence 5′-GGGACT-3′ and thereby inhibit binding of the eukaryotic transcription factor NF-kB [46], which regulates many genes involved in immune and inflammatory responses. Thus, even though this target sequence is rather short, specificity was sufficiently high to affect regulation of a specific set of genes.

4. Major groove binders

Major groove binders are less well researched than minor groove binders. There are a number of natural products known have major groove interacting elements (e.g. pluramycin and leinamycin), but the main contribution to their activity is through intercalation or alkylation (reviewed in Ref. [47]). A completely synthetic compound that was shown to bind specifically to the major groove is a dinuclear iron(II) supramolecular helicate $[Fe_2L_3]^{4+}$, where L is an imine based ligand (**25**, Fig. 11). Such helicates have diameters similar to α-helices and could thus be considered as peptide mimetics. The helicate $[Fe_2L_3]^{4+}$ is produced as a mixture of two enantiomers (M and P), with the M enantiomer strongly inducing coiling of DNA [48]. We showed that $[Fe_2L_3]^{4+}$ has activity

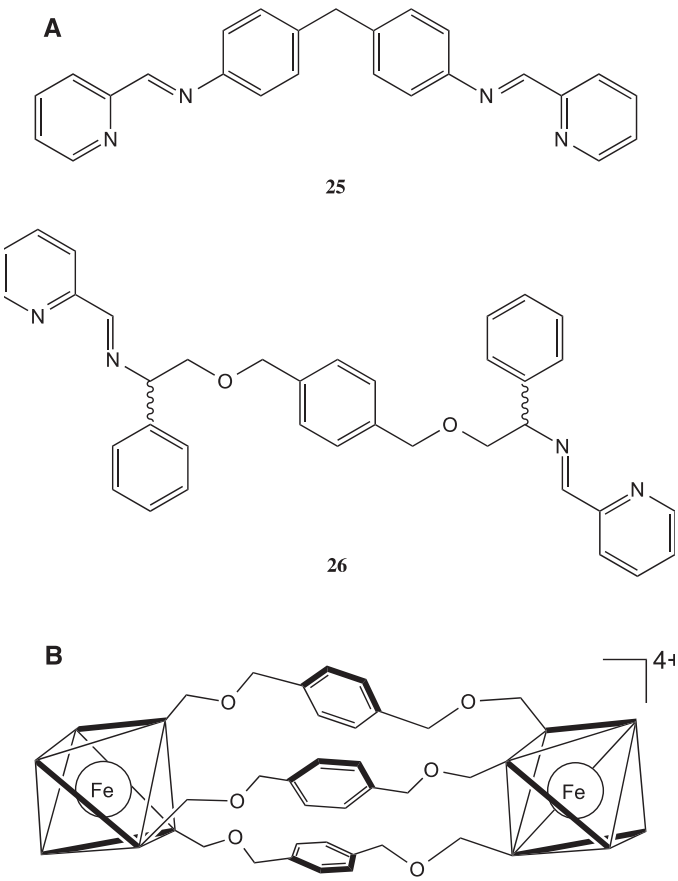

Fig. 11. (A) Ligands **25** and **26** used in supramolecular peptide mimetics. (B) Structure of a flexicate (from ligand **26**).

against both Gram-positive and Gram-negative bacteria, with the former being more susceptible [49]. Interestingly, a copper double helicate compound of similar shape and size was not active, possibly because of a different mode of action or a difference in uptake of the compounds [49]. The activity of $[Fe_2L_3]^{4+}$ was found to be rather moderate, with MIC values in the range of 32 μg/mL, and solubility of the helicate was rather poor. Much better activity was achieved with so-called flexicates [50]. These compounds have a number of notable advantages over the aforementioned helicates, such as a significantly better solubility in water, and the ability to synthesise the complexes as optically pure enantiomers using a highly adaptable self-assembly approach. Importantly, the most active flexicate (from ligand **26**; Fig. 11), was active against both Gram-negative as Gram-positive bacteria, with MIC values of 4–8 μg/mL, while displaying a low toxicity against nematodes (50% Lethal Concentration LC_{50} ~500 μg/mL).

5. DNA modifying compounds

This class of compounds includes those that modify DNA by for instance alkylation, crosslinking or inducing DNA cleavage. There are a number of clinically used compounds in this group, mainly for the treatment of cancer. Examples of these are: cisplatin, a DNA-crosslinking agent; bleomycin, a glycopeptide that induces breaks in DNA; and chlorambucil, an alkylating compound. Some of these have actually multiple targets, such as cisplatin (cis-$[PtCl_2(NH_3)_2]$), which was the first member of Pt(II) containing anticancer drugs. In mammalian cells, 95% of the drug binds to ribosomes or other targets [51], whereas in *E. coli* also several protein targets were identified [52].

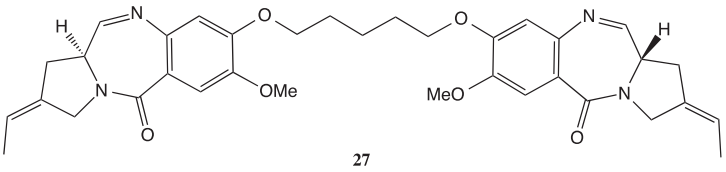

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Fig. 12. The structure of ELB-21 (**27**).

There are also a few DNA-modifying compounds that have been used for many years in the treatment of infections. One example is metronidazole, a nitroimidazole used particularly for treatment of infections with (micro)anaerobic bacteria (e.g. *Bacteroides fragilis*, *Clostridium difficile* and *Helicobacter pylori*) and protozoa (e.g. *Trichomonas vaginalis*, *Giardia lamblia* and *E. histolytica*) [53]. The compound itself is an inactive prodrug, but upon entry into cells it is reduced to a nitro radical that interacts with DNA, causing single and double strand breaks [54]. Only cells growing in anaerobic or microaerobic conditions have a redox potential sufficiently low to reduce metronidazole to its active form, explaining its activity on anaerobes in particular. A compound with (probably) a similar mode of action is nitrofurantoin, although this compound has been suggested to have other targets as well [55]. This is also a nitroheterocyclic compound that is mainly used in the treatment and prevention of urinary tract infections.

A compound named ELB-21 (Fig. 12, compound **27**) has much more recently been identified. This is a pyrrolobenzodiazepine (PBD) dimer related to the natural product anthramycin. It binds covalently with DNA, leading to inter- and intrastrand covalent cross-links with DNA [56]. The compound fits the minor groove, and then forms covalent bonds with guanine residues separated by three nucleotide basepairs [57], thus showing some sequence specificity. It is particularly active against Gram-positive bacteria, including (multidrug resistant) isolates of MRSA and VRE. The MIC values for ELB-21 are in the range of 0.008–0.06 μg/mL for MRSA [56] which is excellent, but toxicity of the compound is not yet clear. A similar compound, SJG-136, is in phase II clinical trials for testing as an antitumour compound [58]. Some clinical data on toxicity of this compound are thus available, suggesting that PDB dimers are effective at concentrations below the toxicity threshold [57]. A further development of the PBD dimers are PBD–biaryl conjugates; some of these also have excellent antimicrobial activity and, importantly, initial results indicate that these compounds are well tolerated in mice and that therefore their therapeutic index is much better than that of the PBD dimers [58].

6. Resistance

Several examples have been shown above on DNA-binding antimicrobial compounds. Yet only very few of these are used clinically, being metronidazole and nitrofurantoin. Do any of the other compounds mentioned, or any novel DNA-binding compounds have potential as therapeutics? For this several issues need to be considered, including the potential for resistance, toxicity and specificity.

As highlighted in the introduction, resistance is one of the major problems facing our healthcare system. In the initial stages of antibiotic discovery (1940s–1960s), many new classes were discovered but since that time most new antibiotics discovered are analogues of those same classes. Resistance to those appeared very quickly. Even when, after many years, a new class of antibiotic was being introduced in 2000 (linezolid, an oxazolidinone), the first reports on linezolid resistance in VRE or MRSA appeared within about a year [59,60]. Development of novel antibiotics is thus a continual arms race, and underexplored targets need to be utilised more.

One question that could be raised is whether microorganisms can develop resistance rapidly against antibiotics that target DNA. In general, antibiotic resistance develops through a number of common mechanisms, which include altering the target, degradation or modification of the antibiotic, or decreasing the intracellular concentration of an antibiotic (either by reduced permeability or increased efflux). There may also be additional mechanisms of resistance that depend on the specific mode of action of the antimicrobial.

The first of those options, altering the target, seems an unlikely mechanism for acquiring resistance against DNA-binding compounds, unless the target sequence is very specific (which is not the case for most DNA-binding compounds). The other mechanisms seem conceivable though and have indeed been observed. For instance, increased efflux can lead to resistance to the clinically used antibiotic metronidazole [61], and efflux is also used as a self-resistance mechanism for *Streptomyces* spp. producing daunorubicin and doxyrubicin [62] or netropsin (**23**) [63]. Similarly, intracellular accumulation can also be reduced through mutations in proteins involved in influx of compounds, as exemplified by pentamidine (**21**) resistance in the protozoan *Trypanosoma brucei* [64,65].

Some DNA-binding compounds lead to damage in DNA, and resistance to such compounds has been shown to arise through DNA-repair mechanisms. For instance, overexpression of the DNA repair protein RecA leads to increased metronidazole resistance in *B. fragilis* [66], whereas production of UvrA-like excision repair proteins provide *Streptomyces* spp. self-resistance against daunorubicin and doxorubicin [67] and echinomycin (**3**) [68].

The aforementioned metronidazole is a prodrug that is converted by specific reductases in anaerobic organisms to a DNA-damaging nitro radical. Mutations in these reductases [69], or production of alternative reductases [70] all can lead to metronidazole resistance. That is however a rather specific resistance mechanism, and in general the main modes of resistance to DNA-binding compounds thus appear to be through reduced intracellular accumulation (either reduced influx or increased efflux), or DNA-repair mechanisms.

7. Toxicity and selectivity

As with all drugs, toxicity and selectivity are important and related characteristics. Toxicity is naturally an obvious issue with DNA-binding compounds, as it might be difficult to discriminate between the DNA of the host and the pathogen. Some toxicity might have to be accepted though, in particular in the light of the ever-increasing threat of antibiotic resistance. For instance, there are already cases of totally drug resistant tuberculosis (TDR-TB), which are resistant against all first and second line drugs. That is something that may occur as well with other bacterial infections, and one option that than needs to be considered is to use toxic antimicrobials that display considerable side effects. That is a policy already in use as exemplified by colistin, a membrane-active polymyxin that was abandoned in the 1970s because of its toxicity. However, it is now back in use clinically in for example cystic fibrosis patients who often suffer from severe lung infections with multidrug resistant bacteria [71].

As shown above, many DNA-binding compounds are used in cancer therapy, but are still considered too toxic for use as antibacterials. The question is thus how increased selectivity can be achieved, thereby lowering host toxicity. Two general ways in which this can be established is either selective uptake of the compound in the pathogen, or selectivity of the compound for the target. More specific options are also possible, such as the activation of a prodrug as exemplified by the aforementioned antibiotic metronidazole (which is only activated in the strongly reducing conditions found in anaerobes [53]). Considering the transport of antibiotics, a significant amount of knowledge has been gathered on the efflux of these compounds and the resulting resistance. In contrast, very little is known about the uptake of antibiotics. If the compound is small and hydrophobic enough, compounds may enter through passive diffusion. In this case it is difficult to envisage selective uptake for such compounds, unless there is some specificity towards the type of lipids in the membrane, which is different between bacteria and eukarya. It is, however, conceivable to establish selective uptake for those compounds that require specific transporters. An example that has already been mentioned above for the minor groove binder pentamidine (**21**), which in the protozoa *T. brucei* is mainly taken up through the P2 aminopurine transporter [64] and aquaglyceroporin 2 [65]. For other antimicrobials, conjugation to specific molecules could ensure a more selective uptake. For instance, vancomycin conjugated to chitosan-nanoparticles has been shown to be effective against drug-resistant *S. aureus* [72], whereas fosmidomycin fused to the cell-penetrating peptide octa-arginine increased showed improved efficacy in both protozoal and bacterial species, being *Plasmodium* spp. and *Mycobacterium* spp., respectively [73].

Another strategy that has gained a fair amount of attention, and also called a "Trojan horse" approach, is to create conjugates with siderophores. In many bacteria siderophores play a key role in the uptake of iron, an essential nutrient for most bacteria [74]. They have a low molecular weight (<1500 Daltons), and are secreted by bacteria when availability of free iron is low. Siderophores have a very high affinity for iron and, after complexing insoluble or protein-bound iron, are imported back into the cell through receptor-specific transporters. Several conjugates of antimicrobials with siderophore-like compounds have been made, with mixed results. One successful example is the conjugation of the mycobacterial siderophore mycobactin to artimisinin [75]. Artimisinin is an antimalarial and by itself not active against *M. tuberculosis*, the causative agent of tuberculosis. However, a mycobactin–artimisinin conjugate was highly active against *M. tuberculosis*, including multidrug-resistant strains, and also retained activity against malaria. Other examples include siderophore–fluoroquinolone [76], and siderophore–beta-lactam conjugates [77], showing enhanced activity against Gram-positive and Gram-negative bacteria. From these examples it is clear that enhanced uptake can be achieved through various methods such as conjugation to peptides, nanoparticles or siderophores. A requirement is of course that, if specific transporters are utilised, these transporters should be fairly promiscuous to allow for the transport of conjugates. The examples above however demonstrate that this is feasible with e.g. the siderophores, as enhanced uptake has been demonstrated for a wide range of compounds ranging from fairly simple beta-lactams to the more complex artimisinin and vancomycin.

A second approach to enhance selectivity of DNA binding compounds is through increasing sequence specificity. Some DNA-binding compounds already show some degree of sequence specificity, such as the minor groove binders pentamidine (**21**), netropsin (**23**) and distamycin A (**24**), which have a preference for AT-rich DNA. *Plasmodium falciparum* DNA is very AT-rich (>80%), and it was speculated that that was the reason for the high activity of some of these compounds against malaria [78]. Also, as mentioned before sequence specificity of the lexitropsins can be manipulated, following a specific set of rules, by replacing the pyrrolo rings with imidazoles or hydroxypyrrole amino acids [45]. Through work in the group of Dervan in particular, it was demonstrated that hairpin-shaped polyamides, connecting two three-ringed lexitropsins with a γ-butyric acid linker could be designed to recognise sequences with high affinity and specificity [45]. Such molecules bind in the minor groove in an antiparallel fashion, with pyrrole (Py)–imidazole (Im) pairing targeting C–G, Im–Py targeting G–C, and Py/Py targeting A–T or T–A. The length of the sequences recognised is however still relatively short, being 6–7 nucleotides [45,79]. Such sequences do occur frequently in genomes of either humans or pathogens, but it is sufficient to target specific genes, as demonstrated by lexitropsins specifically inhibiting the eukaryotic transcription factor NF-kB [46] or RNA polymerase II in human cells [80].

Another approach recently taken in sequence-specific inhibition is by designing distamycin–peptide conjugates [81]. In that study, the authors focused on the DNA-interacting protein E2 from the human papillomavirus (HPV, the main causative agent of cervical cancer). This protein plays a key role in viral DNA replication and is thus attractive for developing antivirals. E2 is a transcription regulator that interacts with the major groove of DNA, but a peptide corresponding to the DNA-binding region of E2 has only low affinity. However, tethering that peptide to distamycin greatly enhanced its affinity [81], thus showcasing an interesting strategy for developing novel therapeutics. Similarly, intercalators have also been used to tether peptides. These include conjugates of the intercalator $[Rh(phi)_2(phen')]^{3+}$ (phi = 9,10-phenanthrenequinone diimine; phen′ = 5-(amidoglutaryl)-1,10-phenanthroline] with an α-helical peptide derived from the bacteriophage P22 repressor in order to explore peptide–DNA interactions [82]. It was demonstrated that these interacted strongly with DNA, and that changing the amino acid sequence of the peptide could also alter the recognition sequence. The aim in that study was not directly to develop bioactive compounds, but it did demonstrate the potential of such peptide–intercalator conjugates in developing sequence-specific molecules, which could be utilized to inhibit a particular, or a group of genes, in pathogenic microorganisms.

8. Concluding remarks

In order to stay ahead in the arms race against bacterial resistance there is a continual need to identify novel antimicrobials, and one strategy to achieve that is to go for targets that have not been utilised fully before. As is clear from this review, DNA as a target for antimicrobials is rather underexplored, with only a few antibacterials in clinical use (metronidazole, nitrofurantoin), plus a few compounds that are being used for protozoa, such as pentamidine. New compounds with good activity are however on the horizon such as the aforementioned ELB-21 (**27**). An interesting development is the addition of a metal to create DNA-binding complexes, which can provide additional geometric possibilities resulting from coordinate metal centres (4, 5 or 6 compared to 4-coordinate carbon). Compounds that contain hydroxyl, carboxylic acid, and amine groups offer excellent donor atoms to form coordination bonds. These can be classic polypyridyl ligands (e.g. 1,10-phenanthroline, 2,2 bipyridine), but also existing antibiotics such as ciprofloxacin may prove very useful in this. Coordination may also improve solubility, which in turn may increase biological activity. Toxicity and selectivity are issues that do play a role with DNA-binding compounds, but as shown above there are strategies to increase the therapeutic index, through either increasing selective uptake of compounds in microbes, or through development of DNA binding compounds that are sequence-specific. Rapid development of resistance is another factor that hampers the identification of novel

antibiotics generally, and that is something that also cannot be avoided with DNA-binding compounds. After all, the short generation time of some bacteria (20–30 min) provides plenty of scope for acquiring resistance genes or mutations that lead to resistance, in particular when that is compared to the speed at which novel antibiotics are introduced into the market (years). Expanding our arsenal with novel antimicrobials with barely utilised cellular targets may help us to stay ahead and prevent entering into a post-antibiotic era.

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