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Cellular and Molecular Life Sciences

Biomedicine & Diseases: Review

Aminoglycoside antibiotics: old drugs and new therapeutic approaches

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Abstract. Aminoglycoside antibiotics kill bacteria by binding to the ribosomal decoding site and reducing fidelity of protein synthesis. Since the discovery of these natural products over 50 years ago, aminoglycosides have provided a mainstay of antibacterial therapy of serious Gram-negative infections. In recent years, aminoglycosides have become important tools to study molecular recognition of ribonucleic acid (RNA). In an ingenious exploitation of the aminoglycosides' mechanism of action, it has been speculated that drug-induced readthrough of premature stop codons in mutated messenger RNAs might be used to treat patients suffering from certain heritable genetic disorders.

Keywords. Aminoglycoside antibiotics, cystic fibrosis, drug discovery, muscular dystrophy, ribosome, RNA.

Introduction

Aminoglycoside antibiotics were the first drugs discovered by systematic screening of natural product sources for antibacterial activity. The laboratory of Waksman reported the discovery and isolation of the aminoglycoside antibiotic streptomycin from soil bacteria in 1944 [1]. Streptomycin was the first antibiotic effective against *Mycobacterium tuberculosis*. In the following two decades, many other aminoglycosides were isolated from soil bacteria including *Streptomyces* and *Actinomycetes* species. Despite their long history as antibacterial drugs, the present aminoglycosides remain important antibiotics for the treatment of serious Gram-negative pathogens. Resistance development against the aminoglycosides as well as their relative toxicity have spurred medicinal chemistry approaches to develop improved aminoglycoside derivatives and mimetics [2, 3].

While the mechanism of action for the majority of aminoglycoside antibiotics had been elucidated over the past 50 years, it was only during the late 1980s that the molecular target was identified as the 16S ribonucleic acid (RNA) component of the bacterial ribosome [4]. And only over the past decade, high-resolution structures have become available for aminoglycosides in complex with their ribosomal RNA targets [5].

Knowledge of the mechanism of action and molecular target site of the aminoglycosides have led since the mid 1990s to an exciting new application of these drugs as an experimental treatment of genetic disorders that cause pathogenic nonsense mutations, including cystic fibrosis and Duchenne muscular dystrophy [6, 7]. In this article, I will review the lessons learned of the antibacterial properties and mode of action of the aminoglycosides and their application to emerging therapeutic concepts for the treatment of genetic disorders as well as potential non-ribosomal targets for these drugs. Traditional areas of aminoglycoside use as antibiotics, toxicity and resistance development have been covered in a number of excellent recent reviews [2, 8–10].

Aminoglycoside classes and target sites

Natural aminoglycoside antibiotics share a non-sugar 2-deoxystreptamine (2-DOS) scaffold connected to amino sugar substituents at the 4-, 5- and 6-positions (Fig. 1) [11]. The two most important classes of aminoglycoside antibiotics are the 4, 5- and 4, 6-disubstituted 2-DOS derivatives. The 4, 5-disubstituted 2-DOS compounds include neomycin B, one of the oldest aminoglycosides that is still in use, albeit only in topical applications due to its relative toxicity. The largest group of the 4, 6-disubstituted 2-DOS derivatives contains several antibiotics that are clinically important for the treatment of serious Gram-negative infections (gentamicin, tobramycin, amikacin). Apramycin, an aminoglycoside that is used only for veterinarian purposes, is the lone representative of a third class of 4-monosubstituted 2-DOS compounds with a somewhat exotic constitution that includes a bicyclic pyrano-pyranose sugar moiety. Aminoglycoside antibiotics of these three groups, 4, 5- and 4, 6-disubstituted 2-DOS derivatives and apramycin, share in common a target site at the decoding center (A-site) of bacterial 16S ribosomal RNA (rRNA) (Fig. 2). Three-dimensional structures of aminoglycosides bound to the bacterial decoding site have revealed the importance of the 2-DOS scaffold as the key pharmacophore required for the precise anchoring of the drugs at the RNA target [5].

Streptomycin, the first aminoglycoside antibiotic discovered, and hygromycin B bind to 16S rRNA at distinct sites in relative proximity to the ribosomal decoding center. Hygromycin B binds at a site that overlaps with the decoding site loop but involving only nucleotides that are conserved between bacteria and eukaryotes. Therefore, hygromycin B cannot be used as a selective antibiotic. Spectinomycin, a rigid tricyclic compound, targets 16S rRNA at the "head" region, removed from the binding site of the other aminoglycosides. Consequently, the mechanism of action of spectinomycin, as well as hygromycin B and streptomycin, is distinct from that of the majority of the 4, 5- and 4, 6-disubstituted 2-DOS derivatives [12]. Both streptomycin and spectinomycin are not strictly 2-DOS derivatives, as their non-sugar core scaffold is based on streptidine, which carries a hydroxyl group at the 2-position. Spectinomycin is sometimes classified outside the aminoglycosides since it does not contain a traditional sugar moiety. Compounds derived from streptidine, 2-DOS aminoglycosides as well as spectinomycin are collectively referred to as aminocyclitols [2].

Mechanism of antibiotic action

The mechanism of antibacterial action has been studied most thoroughly for the decoding site-targeting aminoglycosides of the 4, 5- and 4, 6-disubstituted 2-DOS derivatives. Many of the molecular details of aminoglycoside action have emerged from structural studies of ribosomal subunits and parts thereof [5, 13]. Aminoglycosides interfere with translational fidelity by binding to the ribosomal decoding site at an internal loop of the 16S rRNA that contains three unpaired adenine residues (Fig. 2). Two of the adenines (A1492 and A1493) are flexible and may adopt positions inside the RNA loop or project into a site at the ribosome that accommodates the hybrid between the messenger RNA (mRNA) and the A site-bound transfer RNA (tRNA). Decoding of the mRNA involves direct contacts between the flexible adenine "sensors" of the decoding site and the mRNA-tRNA codon-anticodon hybrid. Aminoglycoside binding at the decoding site displaces the flexible adenines from the RNA interior and locks them in a "flipped-out" state that closely resembles the conformation during mRNA decoding [13, 14]. It has been suggested that the aminoglycosides pre-organize the decoding site for accommodation of mRNA-tRNA hybrids by lowering the energy barrier between two conformational states of the flexible adenine sensors [15]. As a consequence, the discrimination between cognate and near-cognate tRNA-mRNA interactions is diminished, leading to reduced translational fidelity. Over time, accumulation of erroneous proteins that are truncated or incorrectly folded leads to bacterial cell death.

Among the decoding site-binding aminoglycosides, apramycin is the only antibiotic whose primary mechanism of action is not reduction of translational fidelity but blocking of ribosome translocation along the mRNA. This unique effect of apramycin is likely due to its unusual structure that allows for additional interactions with ribosomal protein S12, which is involved in the translocation process [16].

Specific contacts with nucleotides of the rRNA that are distinct between bacteria and human account, in part, for the selectivity of decoding site-binding aminoglycosides for the bacterial target. Crystal structure analyses of aminoglycoside complexes revealed that key hydrogen bonds occur to A1408 and G1491 of the bacterial decoding site [17]. Eukaryotic rRNA has a G at position 1408 and an A at 1491, which are incapable of forming critical interactions with aminoglycosides.

Streptomycin binds at the 16S rRNA in proximity of the decoding site but not at the internal loop and reduces translational fidelity by a complex mechanism

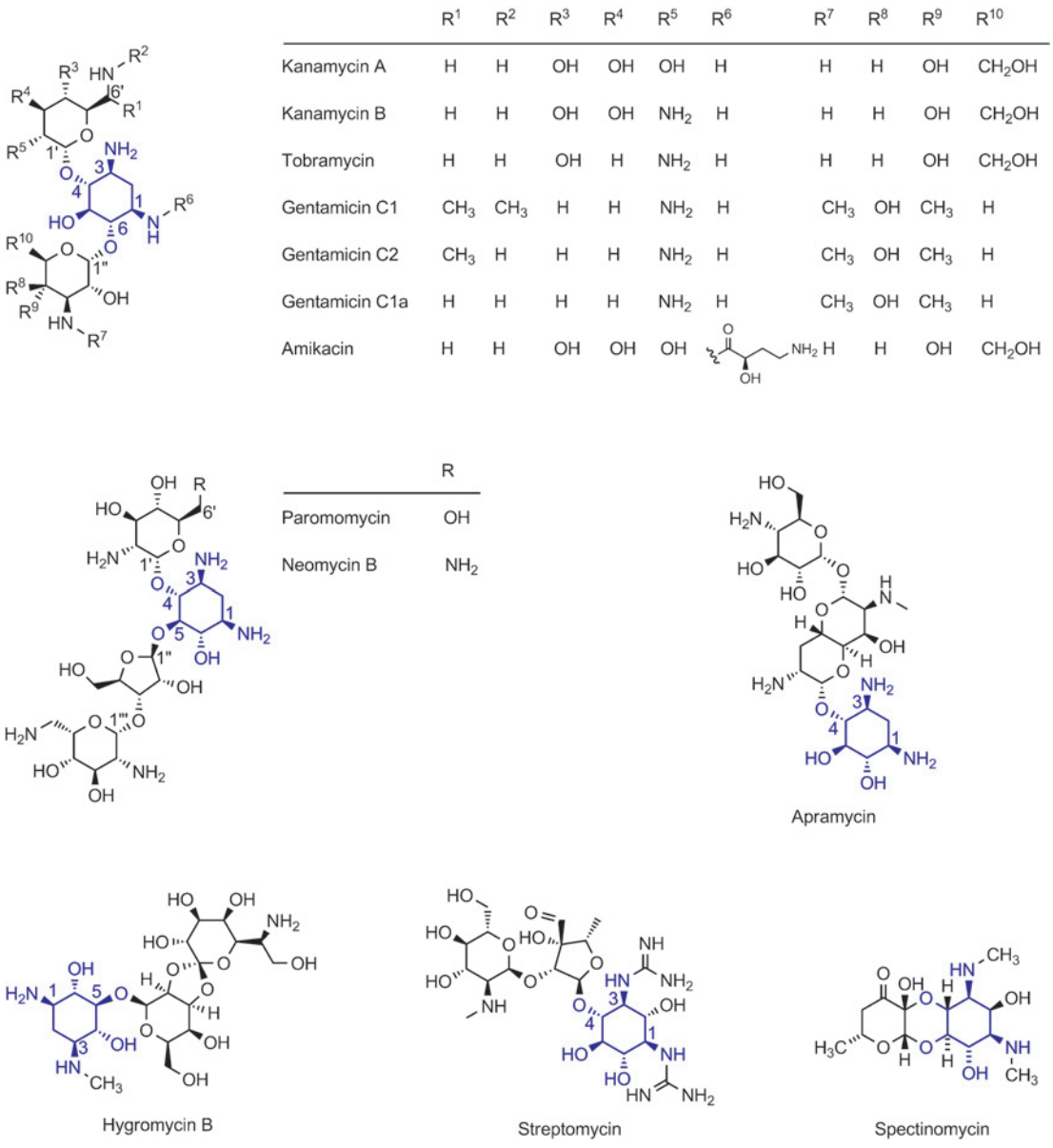

Figure 1. Structural families of aminoglycoside antibiotics. The 4, 6- and 4, 5-disubstituted 2-deoxystreptamine (2-DOS) derivatives of the kanamycin (top) and neomycin (middle) classes as well as apramycin bind to the decoding site of the bacterial 16S ribosomal RNA (rRNA). The aminoglycosides hygromycin B, streptomycin and spectinomycin bind at other sites of the 16S rRNA. The 2-DOS scaffold is highlighted in blue.

that might involve interference with initial tRNA selection and proofreading [18].

Apart from selectivity at the level of target recognition, the therapeutic utility of aminoglycosides also arises from the very low permeability of eukaryotic cells for drugs that are positively charged under physiological conditions. Uptake of aminoglycosides in bacteria is facilitated by an energy-dependent active transport process that depends on a membrane-bound respiratory chain of electron transporters. Thus, aminoglycosides lack activity in the absence of oxygen and specifically against anaerobes with deficient electron transport systems [2].

The primary antibacterial effect of aminoglycoside antibiotics that interfere with translational fidelity is compounded by secondary effects of the accumulation of erroneous proteins. Presumably, misfolded and truncated proteins that emerge within aminoglycoside-treated cells accumulate in the bacterial membrane and increase permeability for the drugs. The resulting increased intracellular aminoglycoside concentration is believed to play an important role in both

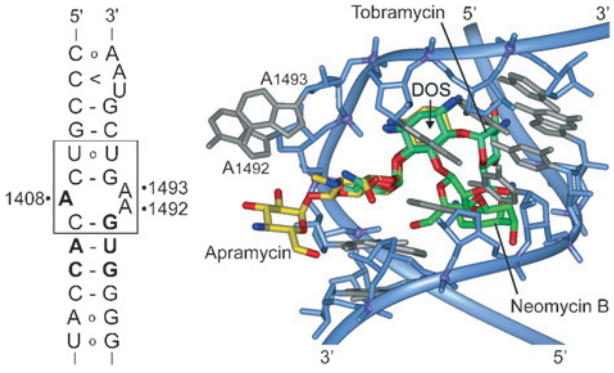

Figure 2. The decoding site in 16S ribosomal RNA. Secondary structure of the bacterial decoding site (left); the aminoglycoside-binding site is marked by a box. Superposition of the crystal structures of three aminoglycosides in complex with the bacterial decoding site RNA (right). The structures highlight the conserved docking site of the 2-DOS scaffold in the three aminoglycosides and the displacement of the flexible adenines A1492 and A1493, which play a key role in the decoding process.

the bactericidal and post-antibiotic effects of these antibiotics [2, 8].

Antimicrobial activity and clinical use

Aminoglycosides are the most commonly used antibiotics for the treatment of serious infections caused by Gram-negative bacteria, including bacilli such as *Escherichia coli*, *Enterobacter*, *Pseudomonas* and *Salmonella* species, and Gram-positive pathogens such as *Staphylococcus* and some streptococci as well as mycobacteria [2]. Broad-spectrum use of aminoglycosides is limited by drug-modifying enzymes and reduced uptake in Gram-positives, which have a distinct membrane composition that prevents aminoglycoside permeation, and in anaerobes, which lack the oxygen-dependent membrane transport mechanism. Differences in the spectrum of activity among aminoglycosides are related to the presence of drug-modifying enzymes that inactivate the antibiotics and efflux pumps.

The poor oral absorption of the highly polar aminoglycoside antibiotics, which are positively charged under physiological conditions, requires administration by parenteral injection. Intravenous injection of liposome-encapsulated aminoglycosides has been investigated in animal models [2]. Inhalation of aerosolized gentamicin and tobramycin solutions is used for the treatment of serious respiratory tract infections, including those caused by *Pseudomonas aeruginosa* in cystic fibrosis patients [19].

The concentration-dependent bactericidal activity of aminoglycosides favors once-a-day dosing [20]. Pharmacodynamic profiling suggests that bacterial killing by aminoglycosides is determined by the ratio of peak exposure to minimum inhibitory concentration (C_{max}:MIC) [20, 21]. High single doses per day are used to maximize peak levels while avoiding sustained high trough levels of the drugs. Aminoglycoside toxicity is related to uptake in the renal cortex and perilymph, a saturable process that is little affected by drug concentration [22]. Therefore, transient high aminoglycoside levels do not lead to excessive drug toxicity but improve bacterial killing. The post-antibiotic effect of the aminoglycosides further sustains their bactericidal activity at lower post-peak levels of the drugs.

Synergistic bactericidal potency is observed for aminoglycosides used in combination with antibiotics that inhibit cell wall biosynthesis, including β-lactams and vancomycin [2]. Presumably, inhibitors of cell wall synthesis lead to increased bacterial permeability and enhanced uptake of the aminoglycosides.

Resistance

Bacterial resistance against antibiotics is mediated by three distinct classes of mechanisms: decrease of intracellular drug concentration, target site modification and enzymatic drug modification. Intracellular aminoglycoside levels can be reduced in resistant bacteria by decreased uptake caused by mutations in components of transmembrane transport systems. Energy-dependent efflux is a major cause of antibiotic resistance [23]. In recent years, aminoglycosides have been shown to be substrates for a variety of multidrug efflux pumps [8].

Modification of the 16S rRNA target of aminoglycosides by mutation or nucleotide methylation has been observed in resistant bacteria. Target mutation is a rare mechanism of aminoglycoside resistance; except for few species of *Mycobacterium*, eubacteria carry multiple copies of ribosomal operons (seven in *E. coli*, for example), and at least half of the bacterial ribosome population must be in the mutant form to confer aminoglycoside resistance. Thus, clinically relevant ribosomal mutations have been found only during streptomycin treatment of *Mycobacterium tuberculosis* infections. The single ribosomal operon in this pathogen allows for the production of a homogenous population of aminoglycoside-resistant ribosomes after a single base change, regardless of the recessive nature of the mutation involved [8].

Along with efflux, enzymatic modification plays by far the most important role in aminoglycoside resistance in bacteria. The multiple functional groups in aminoglycosides render these antibiotics prime targets for

cofactor-dependent modifying enzymes that catalyze nucleotidyl-, phosphate- and acetyl transfer [2, 8, 24]. Aminoglycoside nucleotidyltransferases (ANT) and phosphotransferases (APH) regiospecifically modify hydroxy groups in the drugs by transferring AMP and the γ-phosphate of ATP, respectively. Acetyltransferases (AAC) catalyze acetyl-CoA-dependent N-acetylation of amino groups. Each class of modifying enzymes is comprised of many distinct members that show regio- and substrate specificity. Several aspects of molecular recognition of the aminoglycosides by the resistance enzymes show intriguing parallels to interactions of the drugs with their ribosomal RNA target [24].

While inhibition of resistance enzymes is a successful concept for overcoming resistance in other antibiotics classes, for example the β-lactam inhibitors of bacterial cell wall synthesis, little progress has been made in the development of clinically useful inhibitors of aminoglycoside-modifying enzymes. The difficulty of finding universal inhibitors for the large number of different enzymes is compounded by the fact that the biochemical mechanism of many aminoglycoside-modifying enzymes is not known in detail [2].

Bacterial biofilm formation is a distinct mechanism of antibiotics resistance that has emerged as a major concern in the treatment of chronic infections [25], in particular those caused by Gram-negative pathogens [26]. A recent study has shown that subinhibitory levels of aminoglycoside antibiotics can induce biofilm formation in *Pseudomonas aeruginosa* and *Escherichia coli* [27]. The aminoglycoside-triggered growth of biofilms was demonstrated to be a drug-specific defensive reaction to the presence of antibiotics that involves modification of bacterial cell surface adhesiveness.

Toxicity

The highly polar aminoglycosides are low protein-binding drugs that are excreted almost entirely by glomerular filtration without prior metabolization. Nephrotoxicity of aminoglycosides is related to small portions of the drug dose being accumulated in the renal cortex and leading to mostly reversible renal impairment [28]. In addition to nephrotoxicity, aminoglycosides can cause irreversible ototoxicity that occurs both in a dose-dependent and idiosyncratic fashion [29]. Dose-dependent ototoxicity manifests itself in damage of hair cells in the inner ear. While the mechanism of selective toxicity of aminoglycosides for auditory cells is only poorly understood, experimental evidence in animals has indicated that reactive oxygen species may be one factor responsible for the development of aminoglycoside ototoxicity [30]. Hence, the concomitant use of antioxidant drugs has been suggested to protect hair cells from aminoglycoside-induced oxidative damage [31]. Recently it has been suggested that the production of reactive oxygen species in the cochlea during aminoglycoside therapy triggers apoptosis of hair cells via a complex signaling pathway [32].

The idiosyncratic mechanism of ototoxicity has also been linked to genetic predisposition, related to an inheritable mutation (A1555G) in the mitochondrial 12S ribosomal RNA [29, 33]. Position 1555 in 12S mt-rRNA corresponds to position 1491 in the decoding site of bacterial 16S rRNA. Since the A1555G transition restores one of the two key bases that are required for aminoglycoside binding to the decoding site (Fig. 2), the mutated 12S mt-rRNA might have increased affinity for the drugs, leading to toxicity in the eukaryotic cell. Preventive screening of mitochondrial 12S rRNA mutations has been suggested to decrease the incidence of aminoglycoside-induced hearing loss [34, 35].

Discovery of new aminoglycoside derivatives and mimetics

Widespread resistance by compound modifying enzymes and drug toxicity are major shortcomings of aminoglycoside antibiotics. The poor oral absorption is a secondary concern since these antibiotics are used in clinical settings for the treatment of closely monitored patients suffering from serious infections. Numerous attempts have been made to decrease the resistance and toxicity profile of aminoglycosides by medicinal chemistry, focusing on modification of the natural products or synthesis of aminoglycoside mimetics [10, 12, 36]. The different approaches towards novel aminoglycoside-like compounds can be classified into five categories based on the design concept (see Fig. 3).

Historically, semi-synthetic derivatives of natural products have been the most fruitful source of new clinically useful antibiotics [37]. Amikacin, a clinically used antibiotic (Fig. 1), was one of the first semi-synthetic derivatives that overcame bacterial resistance caused by enzymatic modification [38]. A more recent example of an antibacterial aminoglycoside obtained by chemical modification of a natural product is the neamine derivative **1** (Fig. 3), which was prepared by Mobashery and coworkers [39]. Structure determination of the decoding site complex of **1** confirmed that this semi-synthetic derivative recognizes the RNA target in a similar fashion as the natural aminoglycosides [40].

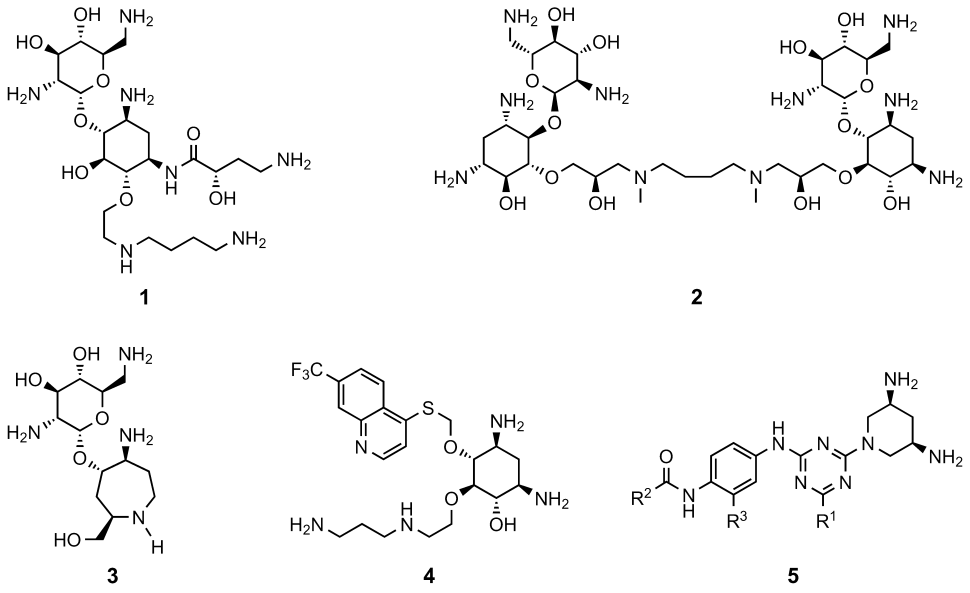

Figure 3. Structures of representative aminoglycoside derivatives and mimetics from the recent literature: **1**, a semi-synthetic derivative of the aminoglycoside neamine [39, 40]; **2**, a dimeric neamine derivative [41, 42]; **3**, an azepane glycoside mimetic in which 2-DOS is replaced by an aminoazepane [43]; **4**, a carbohydrate-free mimetic derived from 2-DOS [44]; **5**, carbohydrate-free aminoglycoside mimetics in which both 2-DOS and the aminosugars have been replaced by synthetic scaffolds [47].

Semi-synthetic approaches are limited by the structural complexity of the natural products along with the overabundance of similarly reactive functional groups. Many routes of chemical modification of aminoglycosides rely heavily on protection group strategies that quickly lead to uneconomic syntheses. To a lesser extent, these limitations also apply to a second class of aminoglycoside derivatives, dimers of the natural products, which can be considered semi-synthetic compounds [41]. A dimer of flexibly linked neamine, compound **2** (Fig. 3), which was also named OPT-11, showed activity against drug-resistant *Pseudomonas aeruginosa* [42]. The significantly increased molecular weight of dimers and the synthetic accessibility of useful connection sites in the natural products limit the scope of dimer approaches towards clinically useful aminoglycosides.

Total-synthetic mimetics of aminoglycosides were designed in which either the 2-DOS, the glycosidic substituents, or both were replaced by other scaffolds. The aim of replacement of 2-DOS by other amino group-carrying moieties has been the simplification or optimization of the key pharmacophore. For example, in the azepane derivative **3** (Fig. 3), 2-DOS was replaced by a synthetic seven-membered heterocycle that carries a strategically placed hydroxy methylene group to pick up a hydrogen-bond interaction with a highly conserved water molecule bound at the decoding site target [43].

The goal of sugar replacement by non-glycosidic substituents is the simplification of synthetic chemistry as well as avoidance of negative pharmacological properties introduced by the polar sugar moieties. For example, Swayze and colleagues have reported the synthesis of carbohydrate-free aminoglycoside mimetics such as compound **4** (Fig. 3) that show both binding to the decoding site target and inhibition of bacterial translation [44].

The partial replacement of either 2-DOS or the sugar components of aminoglycosides has the advantage of retaining some of the key structural features required for recognition of the decoding site target. It is hoped that novel aminoglycoside mimetics that show sufficient residual activity will be discovered; these can serve as useful starting points for further medicinal chemistry optimization. However, the resulting mimetics retain, along with structural features, some of the unwanted properties of the natural products, including stereochemical complexity and suboptimal pharmacology. Aminoglycoside mimetics that contain neither 2-DOS nor sugars would avoid these limitations. It is a formidable challenge for the design and development of such totally synthetic mimetics to deliver initial lead compounds that exhibit target affinity to an extent that allows medicinal chemistry optimization while being completely devoid of scaffolds of the natural products. *In silico* and affinity screening of the decoding site RNA target identified non-aminoglycoside ligands that might provide lead structures for the development of new antibacterial compounds [45, 46]. A structure-guided approach has been used to develop non-glycosidic mimetics **5**

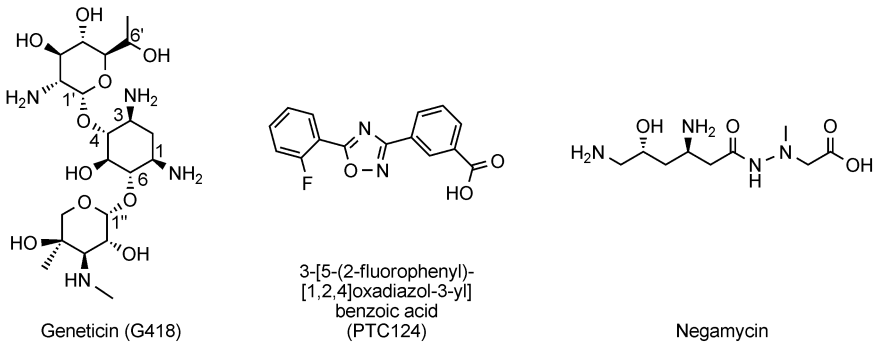

Figure 4. Structures of the aminoglycoside geneticin (also named G418) [63, 64], the [1, 2, 4]oxadiazole derivative PTC124 [87–89] and negamycin [83], which induce translational readthrough at premature stop codons and are thus candidates for the treatment of genetic disorders caused by mutations that introduce premature stop codons.

(Fig. 3) in which the 2-DOS pharmacophore was replaced by a diamino-piperidine scaffold that retained the cis-1, 3-diamino motif of the natural product while reducing stereochemical complexity. These diamino-piperidine aminoglycoside mimetics showed potent antibacterial activity both *in vitro* and in an animal infection model [47].

Experimental approaches to treat genetic disorders

Over the last decade, a new therapeutic approach to treat certain human genetic disorders, which relies on the ability of decoding site-binding aminoglycosides to induce translational miscoding, has emerged. More than 1000 distinct inheritable diseases are caused by single-base in-frame nonsense mutations that introduce premature stop codons and lead to the production of non-functional shortened proteins [6, 7]. Examples include two of the most common genetic disorders, namely cystic fibrosis (CF) [48], in which a transmembrane chloride channel (CFTR) is affected, and Duchenne muscular dystrophy (DMD) [49], in which expression of full-length dystrophin in muscle is impaired. While a variety of mutations may cause these inheritable diseases, roughly 10% of patients suffering from DMD or CF carry nonsense mutations in the affected gene [6]. Some genetic diseases, including lysosomal storage disorders such as mucopolysaccharidosis type I (MPS I), show an even higher prevalence of nonsense mutations (up to 70%) [50]. The concept of using decoding site-binding aminoglycosides to treat genetic disorders caused by nonsense mutations is based on the observation that these antibiotics can elicit readthrough of the ribosome past stop codons in eukaryotic cells, including yeast and human [51–55]. The mechanism of readthrough stimulation is linked to the binding of aminoglycosides to the ribosomal decoding site, which reduces translation fidelity. While eukaryotic decoding site RNA differs from the bacterial target by two key residues that impair the binding of many aminoglycosides (see above), certain compounds still retain affinity for human rRNA. Both apramycin (Fig. 1) and geneticin (Fig. 4), for example, are known to bind to the human decoding site with affinities comparable to those observed for aminoglycoside interactions with the bacterial rRNA [56]. Even before the emergence of detailed structural information on the molecular recognition of the human decoding site, it was suggested that aminoglycoside binding to the eukaryotic target contributes to toxicity of these antibiotics [57, 58]. Meanwhile, crystal structures of human decoding site RNA, free and in complex with apramycin, have revealed that alternative binding modes that might impact translational accuracy in eukaryotic cells are accessible to aminoglycosides [59–61].

Similar to binding in bacteria, aminoglycoside binding to the human decoding site reduces discrimination against near-cognate tRNAs. As a consequence, stop codons may be misinterpreted and a random amino acid incorporated. In a sufficiently large cell population, statistical incorporation of the correct wild-type residue will give rise to phenotypic repair of defective genotypes, a phenomenon described for bacteria in the 1960s [62]. However, it was not before 1996 that aminoglycoside-induced stop codon suppression was tested as a therapeutic approach in human genetic disorders. At that time it was demonstrated that treatment with gentamicin (Fig. 1) or geneticin (Fig. 4) restores function of defective CFTR in cell culture by stimulating readthrough of premature stop mutations [63, 64]. Since then, translational readthrough induction by aminoglycosides has been investigated for a variety of inheritable stop codon disorders [6, 48–50, 65–78].

While drug-induced readthrough induction appears to be an attractive method of therapy in genetic disorders, limitations arise from a number of factors. First, the efficacy of the suppression of premature translation termination by aminoglycosides is highly sequence context-dependent [55, 79, 80], ranging from as low as 1% to about 25% in human cell lines. Second, the random nature of amino acid incorporation at the

misinterpreted stop codon leads to production of full-length proteins that may still be nonfunctional due to a non-wild-type point mutation. Third, the consequences of nonspecific global suppression of correct stop codons during translation of cellular proteins are unknown. Fourth, toxicity of the aminoglycosides (see above) and their low cell penetration limit the selection of useful dosing ranges and routes in therapies of human genetic diseases.

Despite low efficiency of aminoglycoside-stimulated stop codon correction by incorporation of the wild-type amino acid, even a minimal increase in normal protein function may restore clinically less severe phenotypes, especially in autosomal recessive disorders [6, 81]. Positive results of phenotypic repair have indeed been reported mostly for recessive genetic diseases, although emerging studies indicate benefits of readthrough induction for correction of autosomal dominant defects [66, 71].

The problem of aminoglycoside toxicity might be addressed through the discovery of novel non-aminoglycosidic compounds that induce stop codon readthrough. The peptide antibiotic negamycin (Fig. 4), for example, affects ribosomal decoding with an accuracy similar to aminoglycosides [82]. Negamycin has been shown to bind to the decoding site target and restore dystrophin expression via stop codon readthrough in a mouse model of DMD [83]. In comparison to the aminoglycosides, negamycin is less cytotoxic and shows no ototoxicity. Further, its relatively simple chemical structure renders the negamycin scaffold amenable to medicinal chemistry optimization, which opens a promising route to future drug candidates for the therapy of genetic diseases caused by nonsense mutations [84–86].

Recently, a totally synthetic oxadiazole derivative, PTC124 (Fig. 4), that induces stop codon readthrough by an undisclosed mechanism was discovered [87]. Clinical testing of PTC124, a compound of low structural complexity and good potential for medicinal chemistry optimization, is underway for the treatment of CF and DMD [88, 89]. Unlike the aminoglycosides, PTC124 is orally bioavailable and shows low toxicity [90].

Non-ribosomal targets

The abundance of amino groups, which are positively charged under physiological conditions, predestines aminoglycosides to bind negatively charged nucleic acids. While aminoglycosides have little specific affinity for DNA, several distinct non-ribosomal RNA targets that bind these drugs with affinities in the high nanomolar to low micromolar range are known [9, 91]. It has been suggested that the distribution of positively charged amino groups on the relatively rigid oligosaccharide scaffolds provides a versatile network of spatially defined charges that facilitates docking of the aminoglycosides to negatively charged pockets in RNA folds [92, 93]. Displacement of RNA-bound metal ions by ammonium groups of the aminoglycosides is a hallmark of this structural electrostatic complementarity between the drugs and the RNA targets [94]. Thus, aminoglycosides bind relatively promiscuously to non-decoding site targets at distinct sites that represent electrostatic hot spots created by the folding of the RNA. The lack of complex three-dimensional architecture is likely responsible for the conspicuous absence of high-affinity binding sites for aminoglycosides in DNA.

Among the RNA targets that have been found to bind aminoglycoside antibiotics [9, 95] are transfer RNA (tRNA), transfer-messenger RNA (tmRNA) [96], RNase P, catalytic RNAs (ribozymes), regulatory elements of viral RNA genomes and a structured region of a human mRNA [97]. Highlighting the importance of structural electrostatic complementarity, binding of aminoglycosides to $tRNA^{Phe}$ proceeds via displacement of a divalent metal ion, which leads to conformational changes and inhibition of aminoacylation [98, 99]. Competition with metal ion binding at sites that are important for structural integrity or catalytic activity has also been implied for specific aminoglycoside binding and inhibition of catalytically active RNAs (ribozymes). Ribozymes that are inhibited by aminoglycosides include the self-splicing group I intron [100, 101], hammerhead motif [92, 102], the ribozymes from hepatitis delta virus (HDV) [103, 104] as well as ribonuclease P (RNase P) [105]. The catalytic function of bacterial RNase P, a ribonucleoprotein enzyme required for maturation of the 5'-termini of newly transcribed tRNAs, rests exclusively within an RNA component (M1 RNA) whose function is blocked by aminoglycosides. Inhibition of bacterial RNase P has been discussed as an attractive target for the discovery of new non-aminoglycoside antibiotics [106].

Aminoglycosides also bind to several structured RNA domains of the human immunodeficiency virus (HIV) that play key roles in viral replication [107, 108]. Interaction of neomycin B with the transactivator response element (TAR) induces, via an allosteric mechanism, dissociation of Tat protein, the main activator of viral gene expression [109]. Competitive binding between aminoglycosides and the viral Rev protein has been observed at the Rev-response element (RRE), which is involved in nuclear export of unspliced and partially spliced viral mRNA [110]. Following observations of aminoglycoside binding to

these RNA domains, both HIV TAR and RRE have attracted efforts to discover non-glycosidic ligands as potential leads for the development of antiviral drugs [111–118]. Two other RNA motifs within the HIV genome that bind aminoglycosides are the dimerization initiation site (DIS) [119, 120] and the packaging region (Ψ) [121]. Crystallographic structure determination and modeling studies suggest that aminoglycosides bind to the HIV DIS by docking to a metal ion-binding pocket of the RNA [120, 122].

Summary and Outlook

After 50 years of use, aminoglycosides continue to provide a mainstay in the therapy of serious Gram-negative infections. While the mechanism of action of these antibiotics has been studied for many decades, detailed molecular insight into the target sites has emerged only recently from structural studies of the bacterial ribosome and components thereof. Aminoglycosides have also provided a paradigm in RNA molecular recognition that promises to pave the way for novel therapies directed at non-ribosomal RNA targets. Recently, new therapeutic approaches have emerged that exploit aminoglycoside-induced read-through of premature stop codons in the treatment of certain human genetic disorders. Thus, aminoglycosides, while being "old" drugs, will continue to have impact on modern medicine as powerful antibiotics, experimental therapeutics and invaluable tool compounds for drug discovery.

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