SEARCH FOR NEW DRUGS

POTENTIAL FOR THE DEVELOPMENT OF A NEW GENERATION OF AMINOGLYCOSIDE ANTIBIOTICS

A. N. Tevyashova[1,*] and K. S. Shapovalova[1]

The present review summarizes recent publications devoted to aminoglycosides that study the main types of resistance to antibiotics of this class and the main directions of chemical modification aimed at overcoming the resistance or changing the spectrum of biological activity. Conjugates of aminoglycosides with various pharmacophores including amino acids, peptides, peptide nucleic acids, nucleic bases, and several other biologically active molecules and modifications resulting in other types of biological activity of this class of antibiotics are described. It is concluded that a promising research direction aimed at increasing the activity of antibiotics against resistant strains is the search for selective inhibitors of aminoglycoside-modifying enzymes. This would allow renewal of the use of antibiotics already meeting widespread resistance and would increase the potential of a new generation of antibiotics.

Keywords: aminoglycoside antibiotics, chemical modification, mechanism of action, drug resistance, structure–activity relationship.

The landmark discovery of antibiotics in the first third of the 20th century made a historic contribution to the improvement of human health and increased man's lifespan. Vaccination in addition to improved sanitary and hygiene systems and the use of antimicrobial drugs significantly reduced the mortality from infectious diseases. However, the microorganisms causing one disease or another sooner or later developed resistance to the antibiotics used for treatment. The development of antimicrobial resistance (AMR) meant that their effectiveness had a limited term so that inappropriate and unjustified use of them helped to generate and propagate antibiotic-resistant pathogens. This crisis brewed for decades so that antibiotic resistance is now a global problem. The WHO in 2017 published a list of priority antibiotic-resistant pathogens that included 12 species of bacteria presenting the greatest threat to human health. Gram-negative bacteria (*Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and Enterobacteriaceae resistant to third generation carbapenems and cephalosporins) were assigned to a critical group with respect to hazard level and the need to develop antimicrobial drugs. In general, 9 of the 12 most hazardous pathogens are Gram-negative bacteria. Therapy of Gram-negative bacteria is complicated mainly because of cell-wall structural features such as the presence of an additional membrane that acts as an additional impermeable barrier for exogenous chemical compounds, including antibiotics [1]. Colistin until recently remained the only drug of the last generation that was suitable for treating life-threatening infections caused by carbapenem-resistant enterobacteria (*Escherichia coli*, *Klebsiella pneumoniae*, etc.). However, colistin-resistant bacteria causing infections against which effective antibiotics are now lacking have already been found in several countries and regions [2, 3]. According to expert opinions, the epidemic of the coronavirus infection COVID-19 has muddled the problem even more because the overwhelming majority of patients, regardless of in-patient or out-patient status, receive antimicrobial drugs for prevention or treatment of bacterial complications [4, 5].

For these reasons, research on one of the first discovered classes of antibiotics, i.e., aminoglycosides (AG), which possess broad spectra of antimicrobial action (i.e., activity

---

[1] G. F. Gause Institute of New Antibiotics, 11/1 B. Pirogovskaya St., Moscow, 119021 Russia.

* e-mail: chulis@mail.ru

against Gram-positive and Gram-negative bacteria) and are active against mycobacteria, has recently become increasingly urgent. AG antibiotics are an important part of antibacterial therapy for treating serious infections caused by, e.g., Gram-negative bacteria such as *E. coli*, *Salmonella*, *Citrobacter*, and *Morganella*.

The present review summarizes recent publications devoted to AG, studies of the resistance mechanisms to this class of antibiotics, and the main directions of chemical modification aimed at overcoming resistance or changing the spectrum of biological activity.

Classification of AG antibiotics

AG represent one of the first discovered classes of antibiotics and received their name because of chemical structural features, e.g., the presence of several aminosugars bound to hexose (aminocyclitol) through glycoside bonds (Fig. 1). AG can be divided into two classes depending on the aminocyclitol structure. These are 2-deoxy-*D*-streptamine derivatives and other AG antibiotics, in particular, the streptomycin apramycin. In turn, aminocyclitols are divided into 4,5-disubstituted (neamine, neomycin B, paromomycin [6], paromamine, and ribostamycin) and 4,6-disubstituted AG (arbekacin, amikacin [7], dibekacin, gentamicin, geneticin, isepamicin, kanamycins A and B, nebramine, netilmycin, plazomicin, sisomicin [8], and tobramycin [9]) depending on the position of the substituents on the aminocyclitol ring. According to another generally accepted classification based on the spectrum of action and features of resistance development [10], four generations of AG are identified, i.e., generation I (streptomycin, neomycin, kanamycin, monomycin), II (gentamicin), III (tobramycin, amikacin, netilmicin, sisomicin), and IV (isepamicin) and a new generation (neoglycosides), to which plazomicin [11], which was approved by the FDA in 2018, now belongs.

Spectrum of activity and mechanism of action of AG antibiotics

Aminoglycosides are broad-spectrum bactericidal antibiotics. They are used to treat infections caused by Gram-positive (G+) bacteria including *Staphylococcus aureus* and *S. epidermidis* and Gram-negative (G–) pathogens, including the family Enterobacteriaceae that comprises *Escherichia coli*, *Klebsiella* spp., *Salmonella* spp., *Shigella* spp., *Proteus* spp., *Serratia* spp., and *Enterobacter* spp. [12]. In addition, AG are active against G– bacteria of other species, including *Acinetobacter* spp., *Moraxella* spp., and *Pseudomonas* spp., and mycobacteria [13]. AG are less effective against *Streptococcus* and *Pneumococcus*. Furthermore, anaerobic bacteria are usually resistant to AG because the antibiotics are transported into the cell via an oxygen-dependent process [14].

The mechanism of action of AG antibiotics is related to suppression of protein synthesis resulting from effects on 16S rRNA of the 30S ribosomal subunit [15, 16]. Ribosomal protein synthesis can be disrupted via several pathways. Binding of AG 2-deoxy-*D*-streptamycin derivatives to the ri-

bosomal A-site leads to a conformational change of rRNA in the decoding region and results in erroneous reading of information from mRNA and synthesis of defective proteins [17, 18]. The permeability of the cell membrane changes and the intracellular AG concentration increases as anomalous proteins accumulate in the cell membrane. Apparently, this plays an important role in the observed bactericidal effect of AG [19]. This class of AG antibiotics also inhibits ribosome translocation by immobilization of peptidyl-tRNA at the A-site [20]. Binding to the allosteric site of 23S rRNA of the 50S ribosomal subunit was also found for several AG, in particular, kanamycin, neomycin B, and gentamicin [21]. It was shown that the streptomycin binding site was located in the immediate vicinity of the ribosomal decoding center and differed from the binding site of the other AG [19]. Interaction of an antibiotic with a ribosome reduces the translation accuracy. Also, streptomycin was shown to disrupt the formation of the initiating 70S complex and to inhibit the protein-synthesis termination step [22, 23].

AG were demonstrated to have many binding sites with the 80S eukaryotic ribosome that change the ribosome intersubunit mobility and thereby disturb all protein-synthesis steps [24].

Common side effects of AG use are nephro- and ototoxicity [25, 26]. Therefore, special attention is paid to reducing these types of toxicity during development of new generations of drugs.

Main mechanisms of resistance to AG

Several mechanisms of resistance of bacteria to AG have been described and include inactivation of enzymes modifying amino or hydroxyl groups of antibiotics [27, 28]; modification of the active site of 16S rRNA methyltransferases [29]; modification of the target through mutations (e.g., mutations of ribosomal protein [30 – 33] or rRNA point mutation [34, 35]; altered penetration of AG into the cell [36], and enhanced active efflux of the antibiotic from cells [37]).

AG-modifying enzymes

Chemical modifications by AG-modifying enzymes (AME) are some of the most widespread resistance mechanisms to AG. This family of enzymes is divided into three main subclasses depending on the type of chemical modification: AG *N*-acetyltransferases (AAC, aac); AG *O*-nucleotidyltransferases (ANT, ant); and AG *O*-phosphotransferases (APH, aph) [19]. Furthermore, the position of the molecule modified by the antibiotic is differentiated within a subclass and is reflected in the name of the enzyme (Fig. 2). Enzyme-catalyzed transformation of an amino or hydroxyl group of the antibiotic reduces the affinity of the AG for the ribosome, which allows the bacterial cell to survive [38]. Greater than 85 AME occurring in G+ and G– bacteria have been described [39]. Some of them, especially ant(2′)-I, aac(6′)-I, aac(3′)- from I to IV, and aac(3′)-VI, are subject to
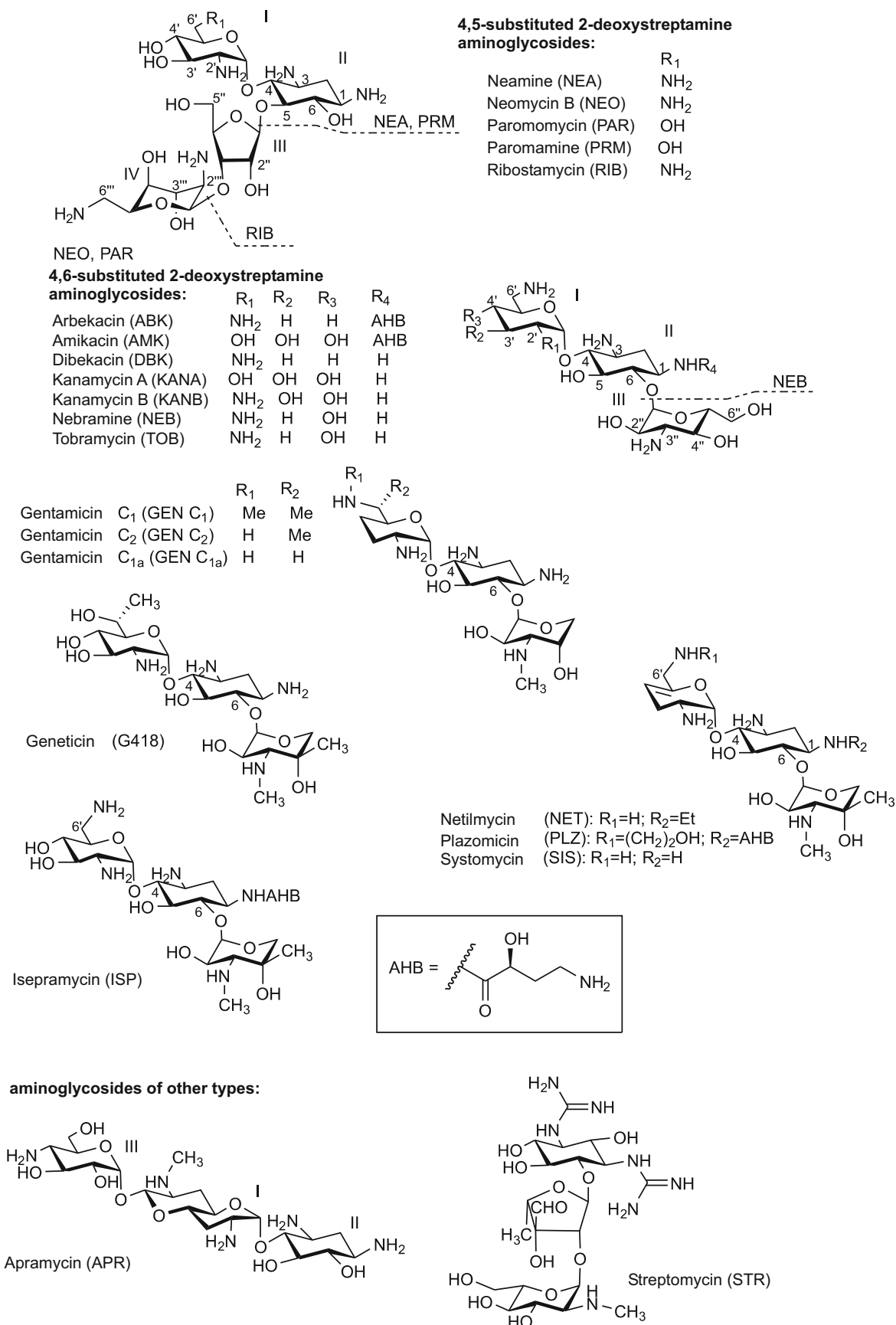
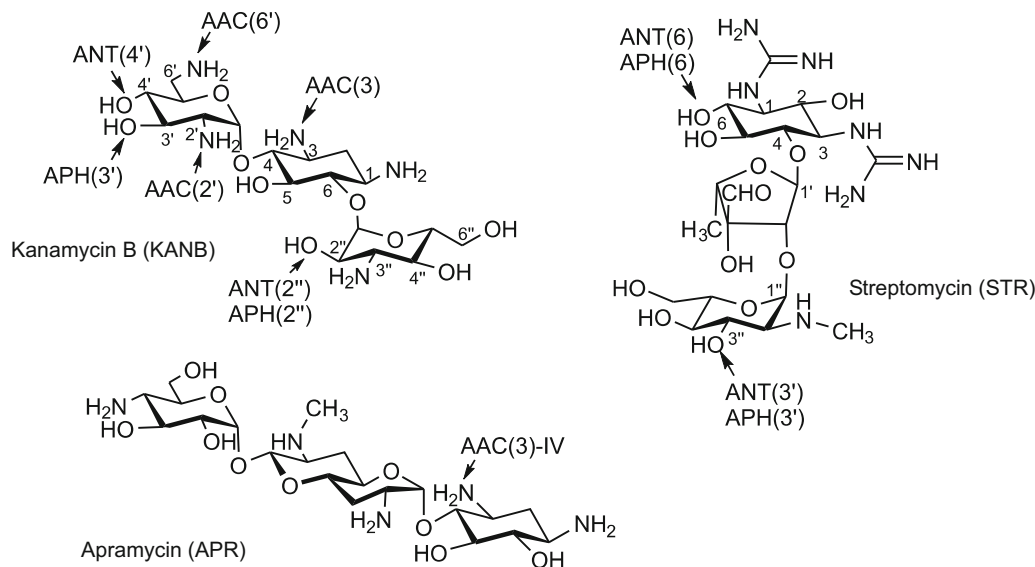**Fig. 1.** Structures of series of aminoglycoside antibiotics.

Fig. 2. Specificity of several aminoglycoside-modifying enzymes.

constant mutations leading to the emergence of new AME variants that are responsible for the spread of resistance to AG [40]. Recently, bifunctional enzymes, e.g., aac(6′)-Ie/aph(2″)-Ia that was isolated from *Enterococcus* spp., *S. aureus*, *S. agalactiae*, *S. mitis*, and *Streptococcus* [41, 42] or aac(3)-Ib/aac(6′)-Ib′, from *P. aeruginosa* [43], were characterized. Furthermore, proteins of enhanced intracellular survival (Eis) that could increase the intracellular survival of mycobacteria in macrophages via multiple acetylation of AG and via acetylation of dual-protein phosphatase 16/mitogen-activated protein kinase phosphatase-7 (DUSP16/MPK-7), which reduces innate *in vivo* immune protection, were observed in *Mycobacterium* and other species [44, 45]. Recently, the enzyme AAC(3)-IVa that is responsible for resistance to apramycin, which until recently avoided resistance via *N*-acetylation of the antibiotic, was reported [46].

AME are transferred by natural transformation or transduction of plasmids between bacteria, which is responsible for their broad distribution among pathogens [47]. Not only chemical modification of AG but also enzyme inhibitors, particularly those based on peptides, are being explored as approaches for battling resistance due to the presence of AME [48].

Ribosome modification

However, another mechanism for resistance to AG that is due to the presence of 16S rRNA methyltransferase (RMTase), an enzyme that methylates nucleic bases at the A-site of 16S rRNA with *S*-adenosylmethionine (SAM) as a cofactor, is of greatest clinical significance [49]. The binding affinity of several AG to methylated 16S rRNA is significantly reduced, which leads to high resistance to this class of antibiotics. The *armA* gene of 16S RMTase was first identified together with *blaCTX-M3* in the pCTX-M3 plasmid of

Citrobacter freundii, which was isolated in 1996 and documented in 2017 [50]. Later, this same gene was identified in the pIP1204 plasmid of *K. pneumoniae* in 2000 [51]. Genes *rmtB* and *rmtC* were observed in plasmids of *Serratia marcescens* and *Proteus mirabilis*, which were characterized in Japan in the first half of the 2000s [52, 53]. Since then, these and other 16S RMTases were identified around the world, mainly in Enterobacteriales including *E. coli*, *Klebsiella* spp., *Enterobacter* spp., and *Salmonella* spp. isolated from various sources including people, livestock, pets, and wastewater [54, 55]. The search for low-molecular-mass inhibitors of RMTases is one approach to overcoming resistance caused by this class of enzymes.

Another mechanism of resistance to AG is related to a structural change of their target associated with mutations of 16S rRNA (rrs) and S12 ribosomal protein of the 30S subunit (RpsL), which were encountered in resistant *Mycobacterium* strains [56].

Active transport (efflux pump)

Active transport of AG out of cells using transmembrane transporter proteins eliminating various substances from the cell (protein pumps) is another bacterial mechanism for resistance to AG. The main transporter protein eliminating AG from cells of G– bacteria is AcrAD, which belongs to the resistance/nodulation/cell-division (RND) family of transporters, i.e., antiporters that transfer substances using chemical osmotic energy and exchange xenobiotics for a proton [57]. AcrAD-TolC transporter proteins were identified in many resistant G– bacteria, including *E. coli*, *S. enterica*, *A. baumannii* (AdeABC and AdeDE), *P. aeruginosa* (MexXY-OprM), and *Burkholderia pseudomallei* (AmrAB-OprA and BpeAB-OprB) [58]. Homologs of AcrAD-TolC also exist in many Enterobacteriaceae and even in *Erwinia amylovora*, a

pathogenic bacterium of plants that is responsible for bacterial damage (blight) of apple, pear, and rose [59]. AG transporters, among other transporter proteins, were also reported from *M. tuberculosis* mycobacterium [60]. Resistance to AG caused by the RND family of proteins is battled by seeking structures that are not substrates for these proteins and developing inhibitors of these transporter proteins.

Change of membrane permeability

AG are thought to permeate into cells through porin channels because of their hydrophilic cationic structures and by simple diffusion through the phospholipid bilayer [61]. One of the mechanisms for developing resistance to AG could be structural changes in membrane transport proteins (porins) or the composition of cell-membrane lipopolysaccharides. However, it was shown in several instances that porin-deficit mutants were still capable of absorbing AG [62, 63]. It was hypothesized that self-maintained absorption of AG, during which the antibiotic interacts with the bacterial external membrane and disintegrates it, provides self-transport into the cell [64, 65].

Membrane proteases

Membrane proteases in several cases facilitated the survival of cells in the presence of AG although they are not considered a classical mechanism of resistance to AG. For example, the main determinant of the natural resistance of *P. aeruginosa* to AG was demonstrated to be proteolysis controlled by the membrane protease FtsH [66]. Membrane proteases are a part of the protein-biosynthesis internal quality control system that recognizes and destroys incorrectly folded and translated proteins. Accumulation of AG in cells can disrupt the cell-membrane integrity because one of their effects is the synthesis of aberrant (differing from normal) proteins. The protective mechanism diminishing this effect provides increased tolerance to the action of AG.

Adaptive resistance (biofilm formation)

Biofilm formation is one of the most important determinants of virulence in the pathogenesis of infections, especially those caused by *P. aeruginosa*. Biofilms help pathogens adhere to various surfaces and protect them from harsh environmental conditions (e.g., palpable forces, desiccation) and from the host immune system (e.g., natural killer cells, phagocytes, complement) [67]. Other adaptive resistance mechanisms of pathogens to AG were also described [68]. However, a detailed examination of them is beyond the scope of this review.

Chemical modification of AG antibiotics

Many pharmaceutical companies shunned the development of antibacterial drugs at the start of the 21st century. This was partially due to the shrinking market for antibiotics in developed countries and the fact that bacteria were acquiring antibacterial resistance faster than new drugs could be developed. This made investments for their development more difficult than for other drug classes. However, it is now recognized that antimicrobial resistance presents a serious threat to the global healthcare system so that the discovery of new antibiotics is a key issue for solving this problem. AG antibiotics will evidently play an important role as a source of lead compounds for the development of new antibiotics because pathogens such as Enterobacteriaceae, which produce the metallo-β-lactam NDM-1, and multi-drug resistant *P. aeruginosa* strains are still relatively sensitive to AG. Chemical modification of AG is also directed at reducing oto- and nephrotoxicity developed with prolonged use of this class of antibiotics.

Chemical modification of AG was attempted already in the 1970s. As a result. a whole series of semi-synthetic AG antibiotics was introduced into clinical practice, e.g., netilmicin, isepamicin, and plazomicin (2010s) [10].

A comprehensive review covering the main directions of chemical modification of AG antibiotics was published in 2018. These were 1) modification of the AG core (aminocyclitol or carbohydrate moiety); 2) introduction of alkyl or aryl substituents at various positions of the antibiotic; 3) synthesis of acyl derivatives; 4) production of AG conjugates with other pharmacophores; 5) synthesis of homo- and heterodimers of AG; 6) production of conformationally hindered AG molecules; 7) introduction into the antibiotic structure of carbohydrates of various structures; and 8) modification of AG using enzymatic reactions [69].

In general, the preparation of new semi-synthetic derivatives is far from simple because AG molecules include amino and hydroxyl groups of similar reactivities. However, success has recently been achieved in developing new synthetic strategies for selective modification of various positions of AG. An analysis of the literature devoted to chemical transformation of AG in general led to the conclusion that derivatives of the primary amino or hydroxyl are most often produced regardless of the AG class while modification of secondary functional groups remains relatively unstudied [69]. For example, introduction of a good leaving group, e.g., triisopropylbenzenesulfonyl (TIBS), in the 5″ (for NEO) or 6″ position (for ABK, AMK, KANA, KANB, or TOB) for further modification of the antibiotic is one of the most common strategies. TIBS is then easily replaced by various nucleophiles (e.g., amines and thiols) to form either intermediates or final products. Introduction of this leaving group presupposed two preliminary steps, i.e., protection of all amines using Boc or Cbz and introduction of TIBS on the primary hydroxyl.

The accent in this section of the review is aimed at chemical modification that enables production of compounds active against bacterial resistance and leads to a change in the type of biological activity of the AG.

Scheme 1
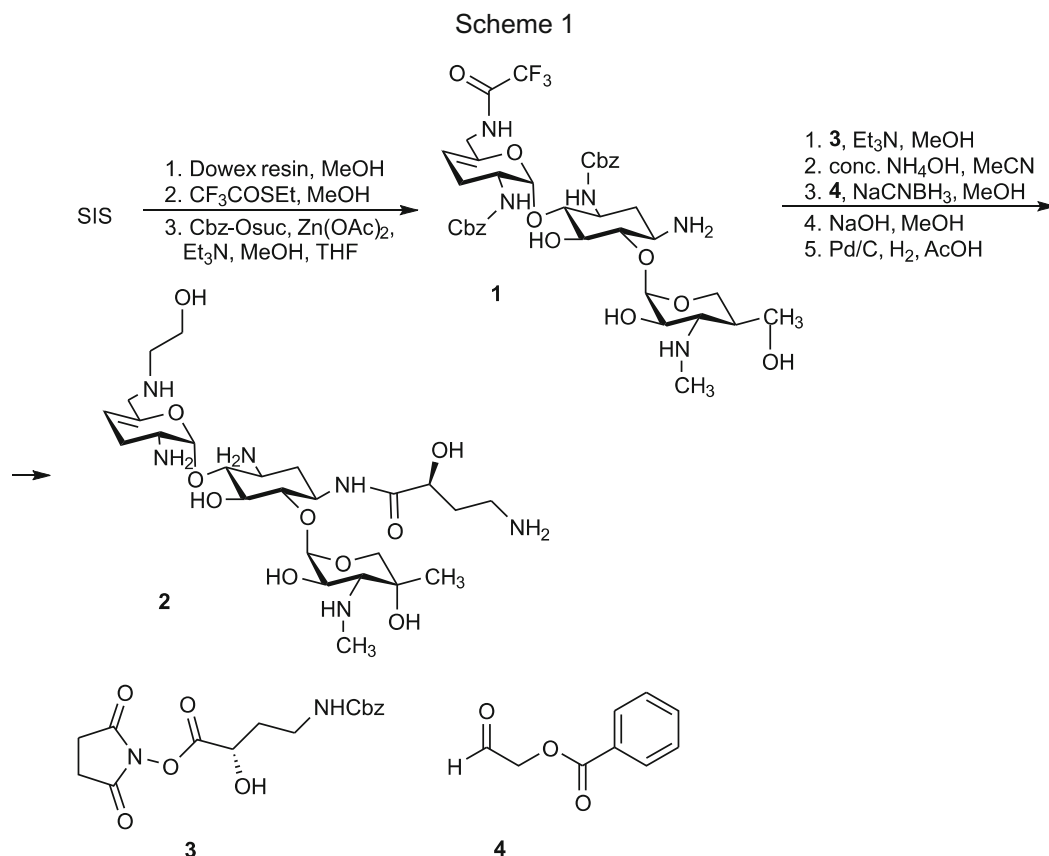
Synthesis of plazomicin starting from sisomicin.

N-Acylated AG

N-Acylation of AG is one approach for overcoming resistance due to the action of AME and increasing simultaneously the antibacterial activity. Syntheses of *N*-acylated AG can be classified according to the method used to produce them: one-step regioselective mono-*N*-acylation; multistep regioselective mono-*N*-acylation using activated esters or acids; multi-*N*-acylation. The one-step method for regioselective mono-*N*-acylation is used to modify 4,5-disubstituted 2-deoxy-*D*-streptamine AG (NEO and PAR). The use of RNA aptamers in combination with *N*-hydroxysuccinimide esters of the corresponding acids was reported for achieving high regioselectivity (from 89 to 99%) for NEO [69]. Several AG derivatives acylated at one of the amines demonstrated improved antibacterial activity as compared to the starting antibiotics. They were also active against several resistant strains expressing AAC(6′)- or AAC(3). However, rigorous structure–activity relationships have not yet been found. The biological activity of the new derivatives depends on both the structure of the starting AG and that of the added acyl radical [69]. Nevertheless, acylation at N1, especially with addition of *L*-(−)-γ-amino-α-hydroxybutanoic acid (ABH), can be cited as one of the most successful strategies for overcoming resistance to AG antibiotics. Thus, selective

acylation of kanamycin by ABH led to the emergence of the new antibiotic amikacin (Fig. 1) [7]. Another example confirming the urgency and potential of *N*-acylation of AG is PLZ (**3**, ACHN-490), a neoglycoside approved by the FDA in 2018 [11]. Plazomicin was selected during research on >400 semi-synthetic systomycin derivatives because it demonstrated a broad spectrum of antibacterial activity *in vitro* against Enterobacteriaceae, *P. aeruginosa*, and *Staphylococcus* spp., including methicillin-resistant *S. aureus* (MRSA)

The starting compound for PLZ synthesis was SIS sulfate, which was converted to the base by treatment with ion-exchange resin followed by reaction with ethyl trifluorothioacetate to form selectively the 6′-trifluoroacetamide (Scheme 1). Treatment of the 6′-trifluoroacetamide with Zn(II) acetate and carbonyl-(Cbz) succinimide led to selective protection of the 2′- and 3′-positions of the antibiotic with formation of intermediate **1**. Reaction of **1** with the activated ester of *L*-(−)-γ-amino-α-hydroxybutanoic acid (**3**) led to selective acylation of N1. Reductive alkylation after workup used *O*-benzoylglycolaldehyde (**4**). Alkaline hydrolysis in the last step, removal of protecting Cbz groups by catalytic hydrogenolysis, and purification by HPLC produced target plazomicin (**3**) [70].

A study of the antibacterial activity of PLZ using a panel of G– and G+ pathogens, many of which had resistance mecha-
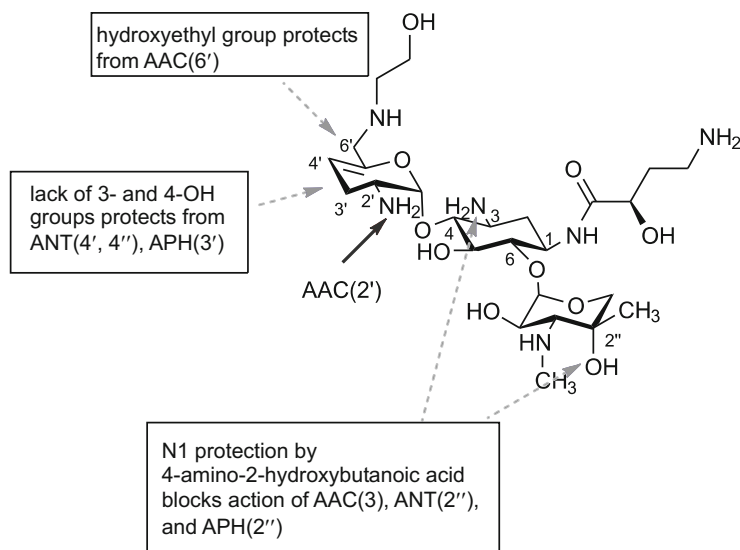
Fig. 3. Action of aminoglycoside-modifying enzymes on plazomicin.

nisms to AG, showed that PLZ suppressed the growth of AG-resistant strains of Enterobacteriaceae ($MIC_{50} \leq 4$ mg/mL) except for *P. mirabilia* and indole-positive Proteae strains (MIC_{50} 8 and 16 mg/mL, respectively) [70 – 73]. PLZ was shown to be active against strains expressing known AME, including the three most widespread of them found in Enterobacteriaceae, in contrast to first-generation AG [70]. Another advantage of PLZ was reduced nephrotoxicity. However, the activity of plazomicin against strains with resistance due to protein pumps was not as impressive. Plazomicin and starting antibiotic SIS lacked 3′- and 4′-OH groups in their structures, which protected them from the enzymes APH (3′) and ANT (4′), which were responsible for resistance to AG, in particular amikacin (Fig. 3). Introduction of an ABH moiety provided protection from AME AAC (3), ANT (2″), and APH (2″). The 6′-hydroxyethyl substituent blocked many AAC (6′) without reducing the antibacterial activity, which was important. This was observed for several other AG derivatives modified at the 6′-position (Fig. 3) [74].

Approval of plazomicin by the FDA in 2018 demonstrated again that AG have great potential as sources of new effective antimicrobial agents.

Conjugates of AG with biologically active molecules

Various series of conjugates of AG antibiotics with a variety of amino acids have been reported in the last decades [12]. Amino acids, in particular basic ones, are natural compounds capable of reacting with RNA through specific H-bonds with unpaired RNA bases and strong electrostatic interactions with the negatively charged skeleton. Conjugation with amino acids or incorporation of certain peptide sequences into an AG is intended to increase the affinity and more importantly the selectivity for an actual RNA target [75]. Conjugates of *L*-arginine with kanamycin and gentamicin were shown to be capable of binding to the HIV-I

transactivation response (TAR) element through a specific nucleotide sequence at the 5′-terminus of HIV-I RNA that is necessary for transactivation of the virus promotor and replication [76].

Interaction of TAR with transactivator transcription (Tat) protein, a regulatory protein that sharply increases the effectiveness of viral transcription, is important for virus replication. Therefore, compounds that bind to TAR and disturb these interactions are considered highly promising models for developing new antiviral drugs. The Tat sequence is rich in the basic amino acids arginine and lysine that are preferentially selected for producing AG derivatives [75]. The triarginine derivative of gentamicin C (**5**) (Fig. 4) and the tetraarginine derivative of kanamycin A (**6**) (Fig. 4) were shown to be capable of specific binding with TAR RNA. Compounds **5** and **6** had low toxicity. Analogously, the tetra-γ-guanidinobutanoic derivative of kanamycin A (**7**) (Fig. 4) and the hexasubstituted arginine derivative of neomycin (**8**) (Fig. 4) exhibited high affinity for TAR RNA and the ability to inhibit virus proliferation in infected cells [77, 78].

The triarginine derivative of gentamicin C and the hexasubstituted arginine derivative of neomycin were found to inhibit bacterial RNAase P [79 – 82]. Neomycin derivative **8** was also capable of selective binding with TAR RNA and RRE IIB and inhibiting strains HIV-1 R5 and X4. RRE IIB was RNA from a part of the HIV genome and formed a complex with transactivating protein Rev.

Later, conjugates of *L*-mono- and -diamino-acids with neomycin and kanamycin A were synthesized. Their biological activity was studied [83]. The obtained derivatives were demonstrated to be useful for specific targeting of microRNA (miRNA). The last were interesting biological targets because their deregulation, in particular overexpression, sharply correlated with a broad range of pathologies, e.g., cancer
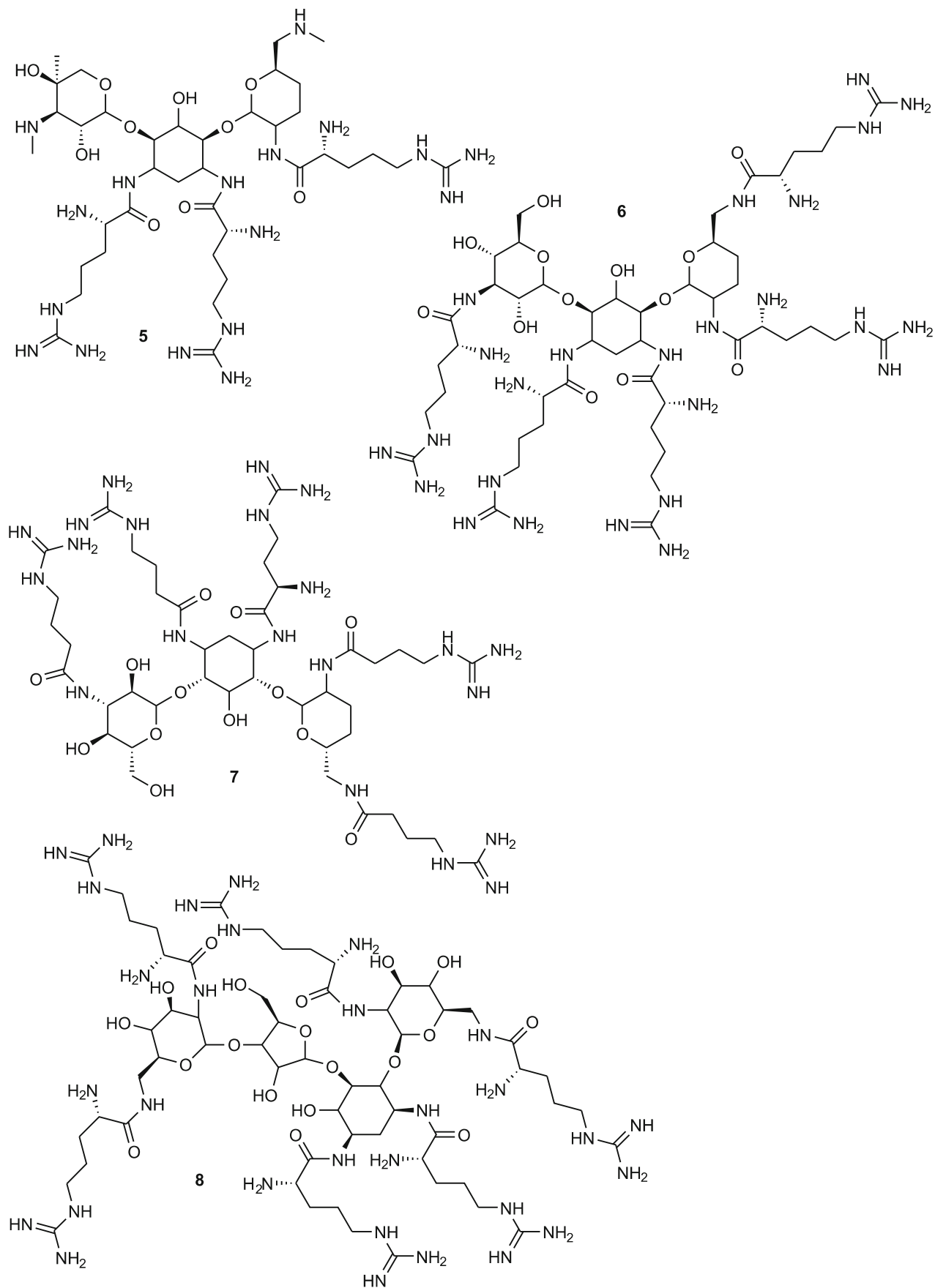
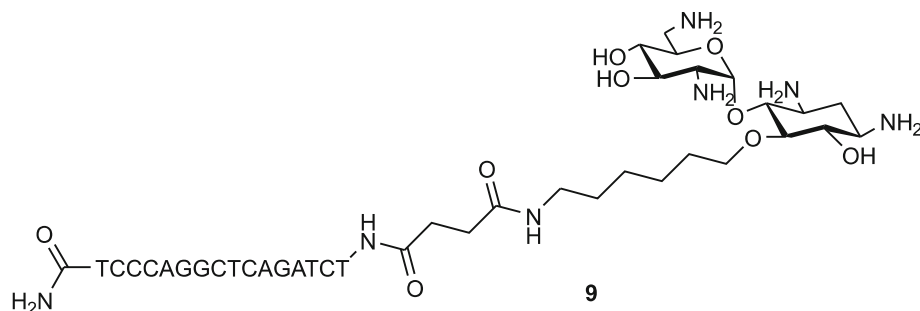**Fig. 4.** Structures of arginine–aminoglycoside conjugates.

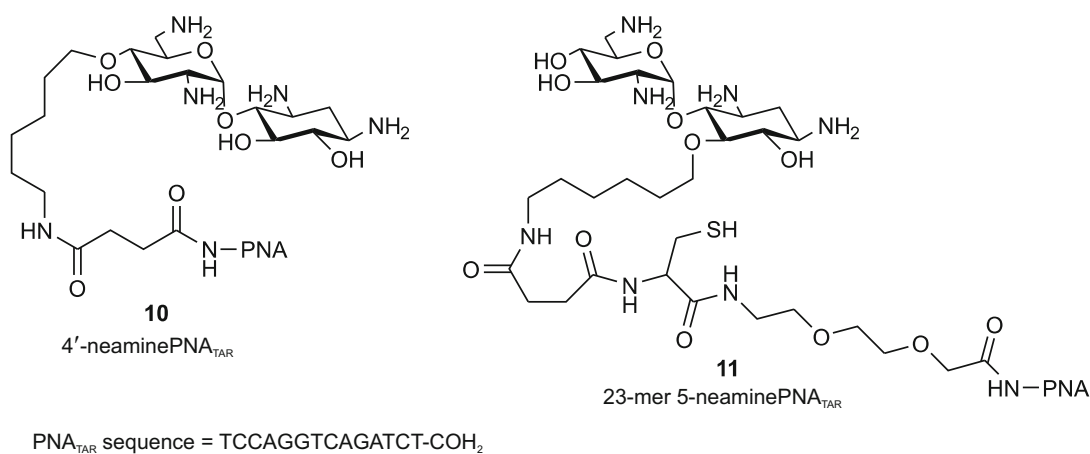**Fig. 5.** Structure of neamine–PNA conjugate.

PNA$_{TAR}$ sequence = TCCAGGTCAGATCT-COH$_2$

Fig. 6. Structures of neamine–PNA$_{TAR}$ conjugates.
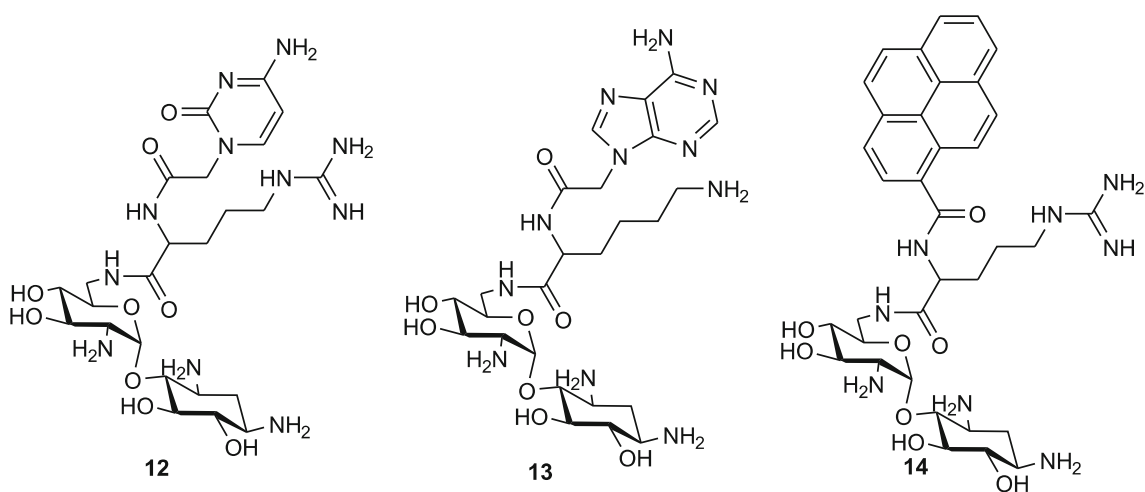

Fig. 7. Structures of neamine–amino-acid–nucleotide (**12**, **13**) and neamine–amino-acid–pyrene conjugates (**14**).

[84]. Thus, compounds capable of binding overexpressed miRNA or their precursors could potentially find interesting therapeutic applications [85, 86].

PNA (polyamide or peptide nucleic acids) are synthetic DNA mimetics without deoxyribophosphate in their struc-

tures although they can still specifically recognize nucleic acid sequences by forming pairs through the complementarity principle. The affinity of PNA for DNA and RNA is greater than the corresponding natural oligonucleotides. They are also significantly more stable [87]. Thus, PNA are

ideal tools for specific recognition of DNA and RNA sequences that are used in several cases to regulate gene expression [87]. Many methods enabling the production of this type of conjugates with AG of various structures were developed in the last decades [69, 75]. As expected, DNA conjugation with AG leads to increased biological activity. The first synthesis of this type of conjugate (**9**) (Fig. 5), which was used for targeting TAR RNA of HIV-1 [88], was reported.

The obtained compound was capable of binding not only TAR RNA but also inducing its cleavage and inhibiting viral proliferation in infected cells. Later, similar neamine–PNA$_{TAR}$ conjugates (**10**, **11**) were described (Fig. 6). It is noteworthy that the position of the PNA and the length of the spacer binding it to the AG antibiotic were critical factors for the activity of the obtained conjugates [88].

Another approach to producing new AG derivatives is their conjugation to natural and synthetic nitrogenous bases that is aimed at increasing the binding specificity of the antibiotics to RNA. Several conjugates of neamine and nucleotides using arginine or lysine as the linker (**12**, **13**) were recently reported (Fig. 7) [89].

These derivatives were developed as potential inhibitors of the TAR/Tat interaction. Neamine derivatives containing an arginine carrying a nitrogenous base showed higher inhibitory activity for TAR/Tat than conjugates containing lysine as the spacer between the antibiotic and the nucleic base. Also, the synthesis of neamine–arginine conjugate **14** (Fig. 7) with a pyrene intercalating group instead of the nitrogenous base was reported. The obtained compound **14** exhibited affinity for TAR–RNA and RRE–RNA [90].

Conjugates of neomycin with nitrogenous bases for targeting the dimerization initiation site (DIS) of HIV were also prepared [91]. The DIS is a spike with an internal asymmet-
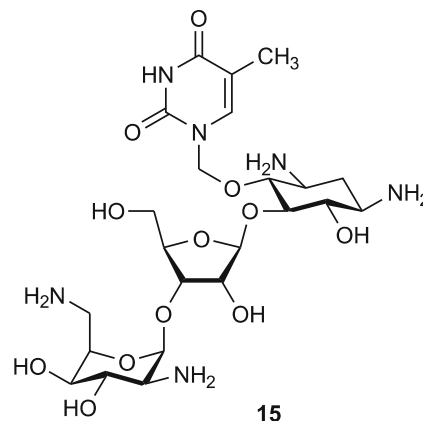

Fig. 8. Structure of neomycin–thymine conjugate.

ric loop located in the 5′-uncoding region of the viral genome. The DIS contains in the terminal loop a self-complementary sequence and is responsible for initiation of dimerization, a key stage of HIV replication. Inhibition of this stage is studied as a potential target for antiviral therapy. A structural study of DIS enabled the design of thymine–neomycin conjugates [91]. The resulting neomycin derivative **15** (Fig. 8) could bind DIS–RNA of HIV-1 subtypes A, B, and F with improved specificity as compared to neomycin itself. Moreover, conjugate **15** *in vitro* bound to the A-site of bacterial rRNA, which is structurally similar to DIS. This potentially opened a route to new classes of antibiotics with improved resistance to AME [91].

New conjugates of nitrogenous bases with neomycin (**16a-e**) and paromomycin (**17a-e**) (Fig. 9) that were potentially capable of selectively binding to the rRNA A-site were also described [75]. A study of the affinity and selectivity of these derivatives with respect to the A-site and TAR RNA
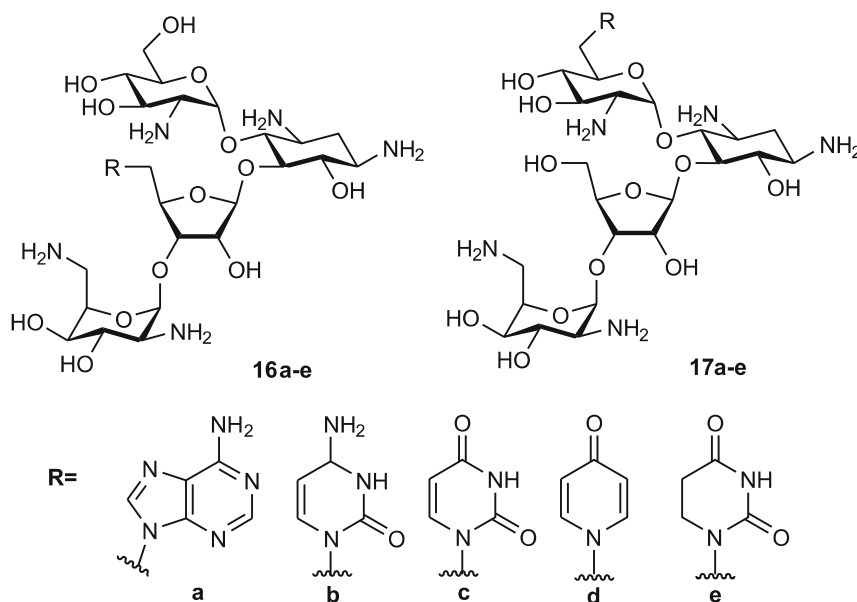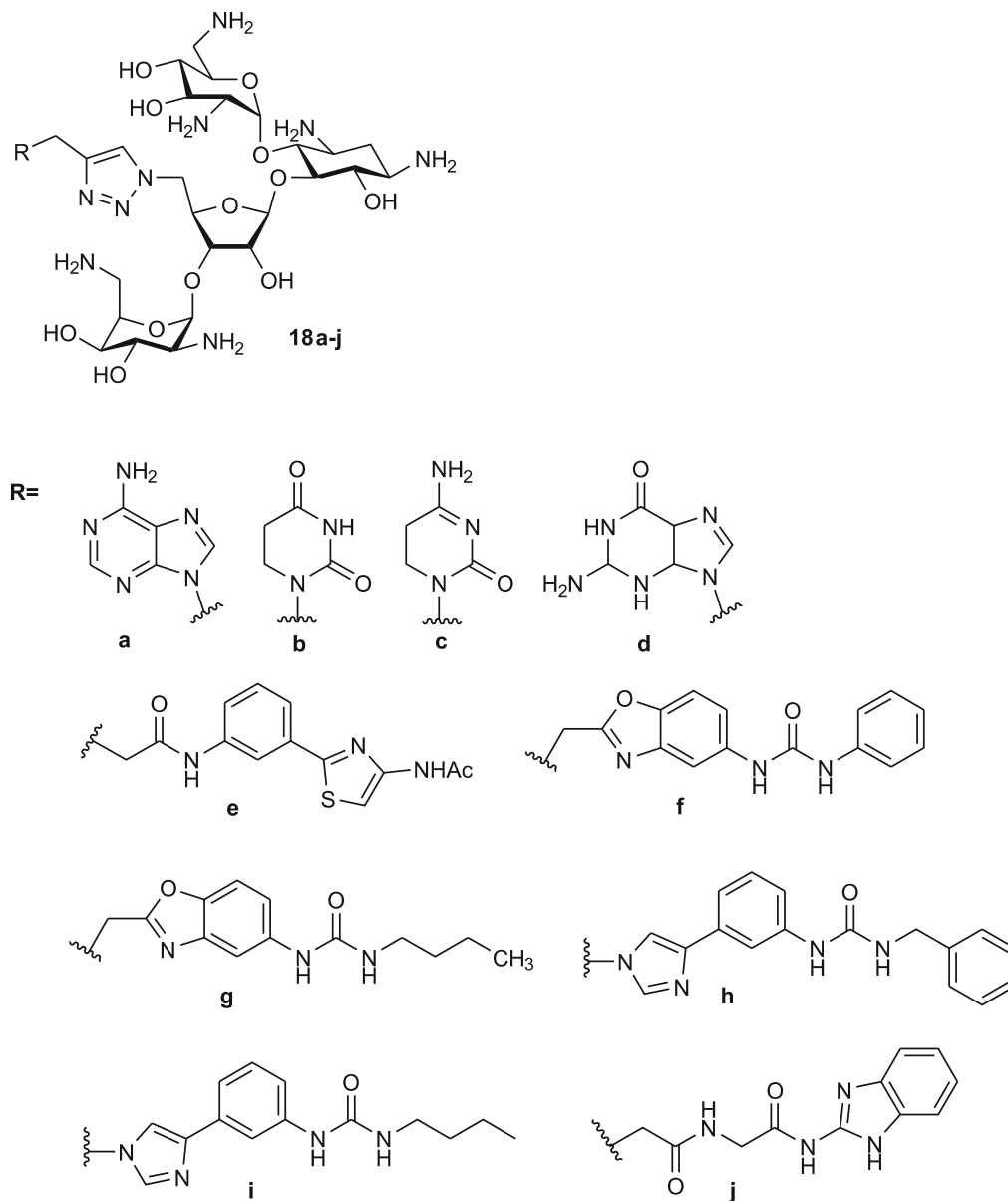

Fig. 9. Structures of neomycin (**16a-e**) and paromomycin conjugates (**17a-e**) with nitrogenous bases.
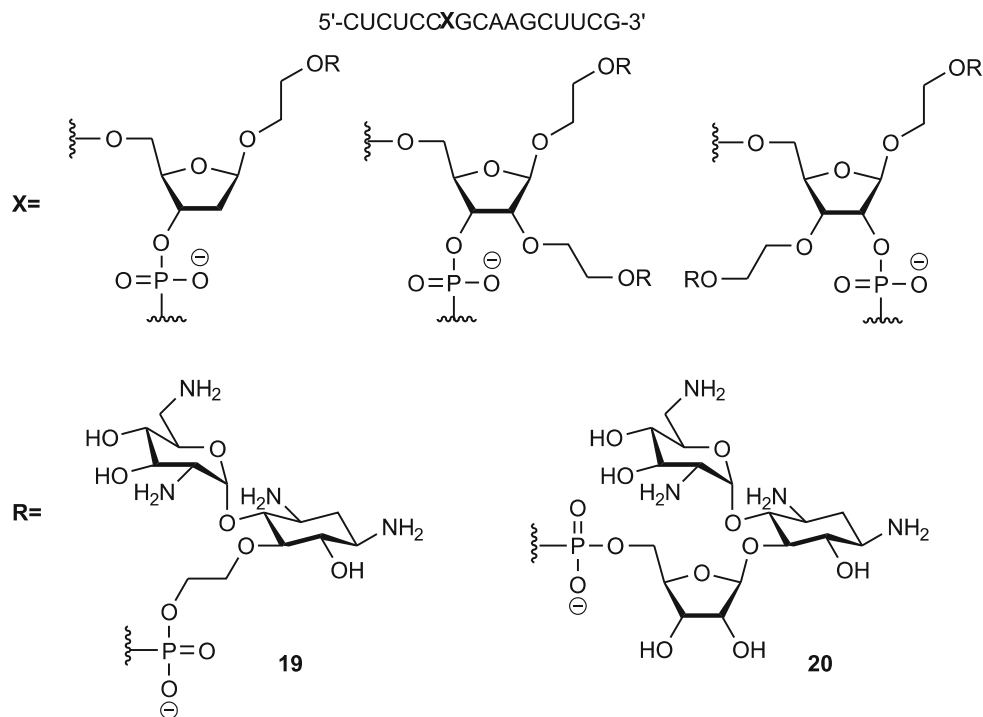
Fig. 10. Structures of neomycin conjugates with natural and synthetic nucleic bases.

showed that they had much better affinity for TAR RNA, despite the initial hypothesis. Apparently, this indicated that these two RNA targets differed in their ability to adapt to structurally different ligands and; therefore, possessed different selectivity. Some of the latest achievements in this area are AG–nitrogenous-base conjugates targeted at biogenesis of oncogenic miRNA [92]. miRNA precursors, called pri-miRNA and pre-miRNA, are RNAs that are studied as targets for inhibition of the biogenesis of overexpressed miRNA in cancer. Precursors pri-miRNA and pre-miRNA are cleaved by cellular ribonucleases (Drosha for pri-miRNAs in the nucleus and Dicer for pre-miRNAs in the cytoplasm) to form mature miRNAs.

Conjugates of neomycin with synthetic nitrogenous bases (**18a-j**) connected through a triazole spacer that could
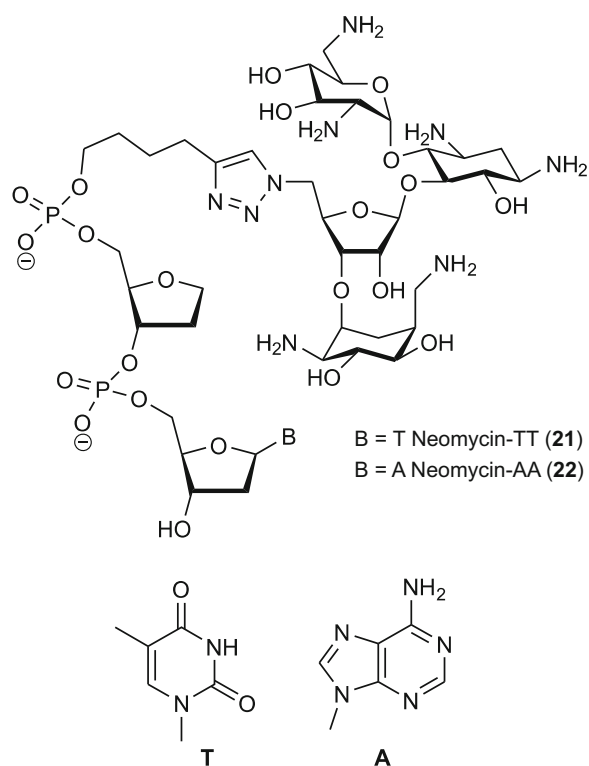
form triplets with AU and GC base pairs were reported (Fig. 10). The obtained constructs could selectively inhibit the production of oncogenic miRNA-372 and miRNA-373 upon binding to the corresponding precursors (pre-miRNAs) *in vitro* and in stomach adenocarcinoma cells overexpressing these two miRNAs.

Two years later, this series of conjugates was supplemented by a structure–activity relationship study that showed the biological activity increased as the aromaticity of the nitrogenous base increased while modification of the AG fragment or the linker decreased it [93]. Very recently, the chemical structures of these conjugates were expanded by an amino-acid fragment [94]. The resulting derivatives demonstrated a strong affinity and selectivity for targets and the highly promising ability to inhibit cancer-cell proliferation,

5'-CUCUCC**X**GCAAGCUUCG-3'

X=

R=

Fig. 11. Structures of neamine (**19**) and ribostamycin conjugates (**20**) with oligonucleotides.
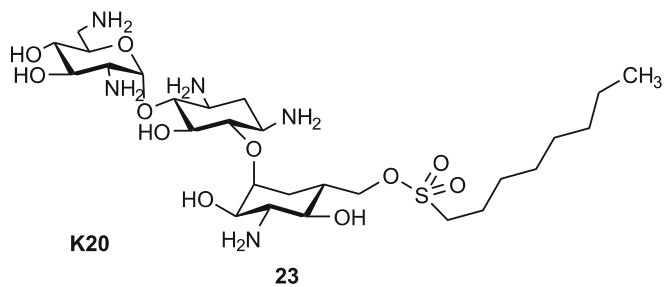
presumably because of the ability of the amino acid, especially histidine, to form additional bonds with the specific binding pocket of the pre-miR-372 target [94].

Examples of the preparation of various AG–oligonucleotide conjugates, mainly of neomycin, neamine, and ribostamycin, have been mentioned in the literature since the 2000s. The oligonucleotides, like PNA, can specifically bind to complementary RNA sequences. Correspondingly, their conjugation with AG can increase the affinity of the AG for the target and-or increase the penetration of the oligonucleotides into the cell. Data about this type of conjugates was first published in 2008 [95]. Solid-state synthesis was used to prepare conjugates of neamine and ribostamycin with 2′-O-methyloligoribonucleotides **19** and **20** (Fig. 11).

AG could in several instances cleave RNA in addition to binding to it. However, their catalytic activity remains very low in the absence of divalent metal ions. Therefore, conjugation of AG with oligonucleotides could potentially provide binding sufficient for metal-independent cleavage of RNA.

B = T Neomycin-TT (**21**)
B = A Neomycin-AA (**22**)

T   A

Fig. 12. Structures of neomycin and dinucleotide conjugates.

K20

23

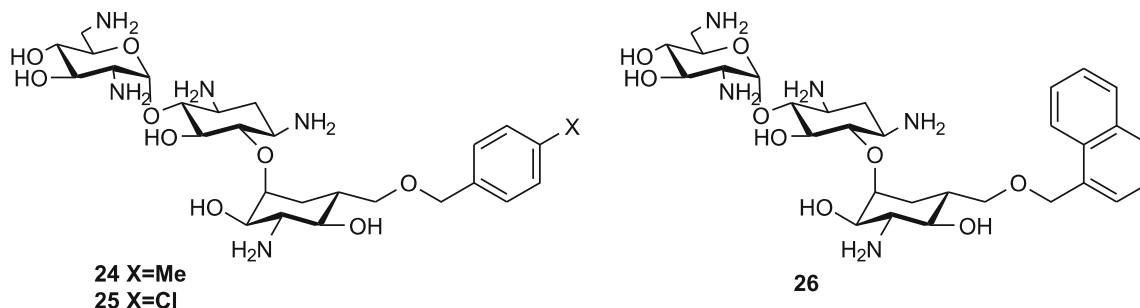**Fig. 13.** Structure of antifungal drug K-20.

Fig. 14. Structures of inhibitors of connexin HCs.

For this, conjugates of neomycin and a dinucleotide (**21**, **22**) were prepared by solid-state synthesis (Fig. 12) [96].

The interaction of the synthesized derivatives with human and bacterial ribosomal RNA was studied to show that they possessed submicromolar affinity for the bacterial rRNA A-site with a low capability to interact with eukaryotic rRNA.

It is worth noting that other types of activity than antibacterial have recently begun to be observed for AG derivatives. For example, comprehensive reviews in which the biological activity of AG was expanded to antifungal agents were published [69, 75, 97, 98]. The first reported type of AG derivatives with antifungal activity were amphiphilic kanamycin A derivatives although compounds of this type lacked antibacterial activity [99 – 101]. Thus, synthesized antifungal drug K-20 (**23**) could suppress various types of fungi, e.g., *Fusarium graminearum*, which causes Fusarium head blight in wheat [102, 103] (Fig. 13).

Recently, another target for AG was discovered, namely, half channels (HCs) formed by connexin. These are gap contacts or intercellular contacts that facilitate direct transfer of ions and small molecules among neighboring cells. Disruption of the operation of this type of channels not attached to the membrane or their anomalous opening can lead to cell damage and cause the development of diseases such as myocardial infarct, stroke, deafness, skin diseases, and cataracts [104, 105]. AG were recently identified as inhibitors of connexin HCs. However, the antibiotic effect typical of these compounds is a drawback for treating this type of disorders.
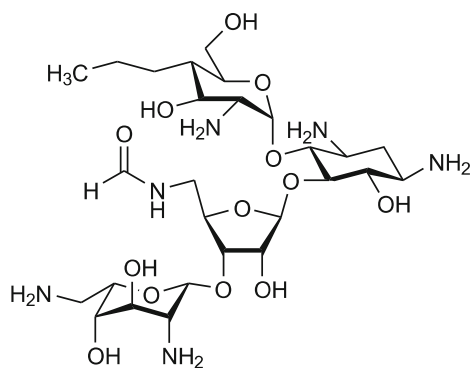
After the discovery of AG without antibacterial activity, several kanamycin A derivatives, e.g., compounds **24 – 26** (Fig. 14), were shown not to have bactericidal activity and to be nontoxic or moderately toxic for mammalian HeLa cells. However, they inhibited the formation of connexin HCs more effectively than the starting antibiotic [106].

Finally, another representative of the last generation of AG antibiotics, propylamycin (**30**, 4′-deoxy-4′-propylparomomycin), should be mentioned (Scheme 2) [107]. Propylamycin (**30**) was synthesized in several steps starting from paromomycin (**27**) via conversion of it sequentially into the pentatrifluoroacetamide and then into the benzylideneacetal and hexabenzoate **28**. Cleavage of the acetal formed the corresponding diol that was then selectively benzoylated at the primary OH and converted into the corresponding iodo-derivative **29** via intermediate production of the triflate. The final reaction formed the C–C bond by reacting intermediate **29** with allylphenylsulfone in α,α,α-trifluorotoluene in the presence of triethylborane. Then, the double bond was reduced and the benzoyl and trifluoroacetamide protection removed to afford the target propylamycin (**30**). The presence of the 4′-alkyl substituent in the new semi-synthetic paromomycin derivative produced high antibacterial activity against ESKAPE-panel pathogens and several resistant G– strains. The resistance was due to AME or rRNA methyltransferases. Propylamycin showed less ototoxicity than the starting antibiotic in experimental animal models.

Nevertheless, antibiotic **30** was shown to inactivate AG–phosphotransferase APH(3′)-Ia, which modifies the primary OH of ribofuranose (5″-position of propylamycin). The introduction of another amine in the 5″-position was studied in a search for new AG resistant to the action of this enzyme. However, this led to a significant reduction in the binding selectivity of the antibiotics with the bacterial ribosome as compared to eukaryote ribosomes [108]. Also, modification of the additional 5″-amine turned out to be extremely successful because 5″-deoxy-5″-formamidopropylamycin (**31**, Fig. 15) demonstrated high antibacterial activity at the level of the starting propylamycin while it was resistant to the action of AG–phosphotransferase and was more selective for bacterial ribosomes than starting propylamycin [108].

Fig. 15. Structure of 5″-deoxy-5″-formamidopropylamycin.

Scheme 2
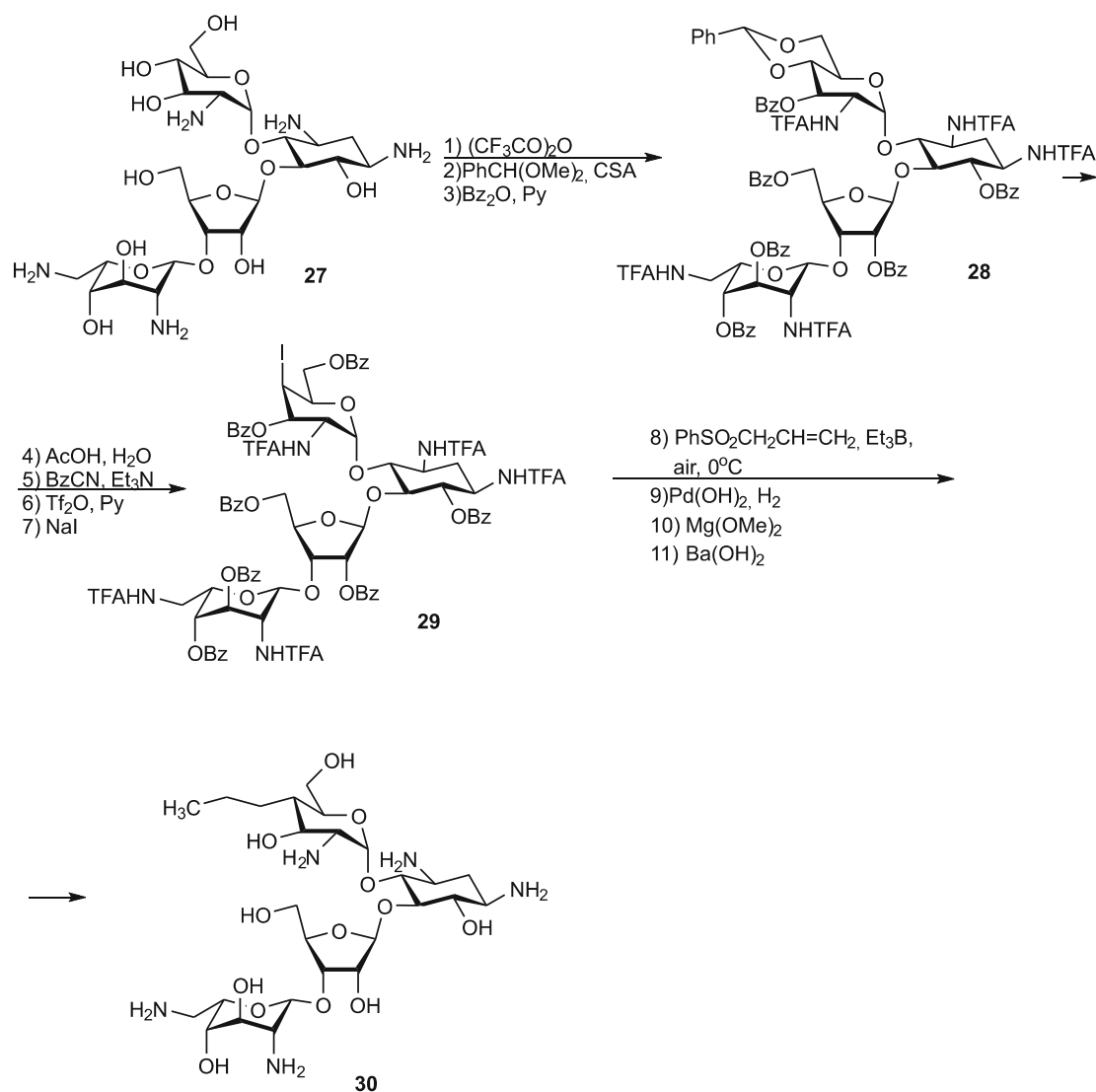
Apparently, propylamycin and its derivatives can be regarded as promising candidates for development of a new generation of AG.

CONCLUSION

AG antibiotics have in fact experienced a renaissance in the last two decades. The design and FDA approval of plazomicin again emphasized the potential for development of a new generation of antimicrobial agents against resistant bacteria. Various synthetic approaches were elaborated and described for modification of these complicated natural compounds. Several semi-synthetic AG were approved for clinical application. Transformation of primary amino or OH groups is one of the most studied approaches to producing semi-synthetic derivatives and was selective in several instances without the need to introduce and remove protecting groups. A strict correlation between the position of the added

substituent and its structure and antimicrobial activity has not yet been established despite the preparation of a rather expansive series of AG derivatives. Recent data indicate that addition of amino-acid residues, primarily basic ones, or the production of conjugates of AG with peptides increases the antibacterial activity of AG. Thus, increased affinity for other RNA targets, especially TAR RNA of HIV-I, was demonstrated in several instances. Conjugation of AG with PNA was effective for targeting the antibiotic to a certain RNA sequence or could help to increase the activity against strains with resistance due to the presence of AME. Apparently, this approach is highly promising with respect to increasing the selectivity against the selected target and expansion in this manner of the spectrum of biological activity of AG. Analogously, introduction of nucleic bases could potentially increase the number of specific interactions formed with RNA targets, which leads to more effective and selective ligands. The difficulties associated with the use of AG conjugates with PNA, oligonucleotides, or peptides are obvious. How-

ever, they are related to the permeation of such derivatives into the cell.

The search for selective inhibitors of AME is one of the highly promising research directions aimed at increasing the activity of antibiotics against resistant strains. This would renew the use of those AG, the resistance to which is already rather broad, and increase the potential of a new generation of antibiotics.

Thus, AG antibiotics, which were discovered almost a century ago, are still promising and used as effective and selective RNA ligands while the potential for development based on them of new highly effective biologically active agents, including via chemical modification, is obviously far from exhausted.

ACKNOWLEDGMENTS

REFERENCES

1. B. A. Pontefract, H. T. Ho, A. Crain, et al., *Open Forum Infect. Dis.*, **7**(7), ofaa276 (2020).
2. M. Hamel, J. M. Rolain, and S. A. Baron, *Microorganisms*, **9**(2), 442 (2021).
3. N. H. Hussein, I. M. Al-Kadmy, B. M. Taha, et al., *Mol. Biol. Rep.*, **48**, 2897 – 2907 (2021).
4. S. Ghosh, C. Bornman, and M. M. Zafer, *J. Infect. Public Health*, **14**, 555 – 560 (2021).
5. C. C. Lai, S. Y. Chen, W. C. Ko, et al., *Int. J. Antimicrob. Agents*, **57**, 106324 (2021).
6. R. N. Davidson, M. den Boer, and K. Ritmeijer, *Trans. R. Soc. Trop. Med. Hyg.*, **103**(7), 653 – 660 (2009).
7. H. Kawaguchi, T. Naito, S. Nakagawa, et al., *J. Antibiot.*, **25**(12), 695 – 708 (1972).
8. M. J. Weinstein, J. A. Marquez, R. T. Testa, et al., *J. Antibiot.*, **23**(11), 551 – 554 (1970).
9. R. Cella and L. C. Vining, *Can. J. Microbiol.*, **21**(4), 463 – 472 (1975).
10. N. O. Selizarova, *Obz. Klin. Farmakol. Lek. Ter.*, No. 2, 70 – 77 (2003).
11. G. Cox, L. Ejim, P. J. Stogions, et al., *ACS Infect. Dis.*, **4**(6), 980 – 987 (2018).
12. Y. Takahashi and M. Igarashi, *J. Antibiot.*, **71**(1), 4 – 14 (2018).
13. B. Beker and M. A. Cooper, *ACS Chem. Biol.*, **8**(1), 105 – 115 (2013).
14. L. E. Bryan, S. K. Kowand, and H. M. Van Den Elzen, *Antimicrob. Agents Chemother.*, **15**(1), 7 – 13 (1979).
15. A. P. Carter, W. M. Clemons, D. E. Brodersen, et al., *Nature*, **407**(6802), 340 – 348 (2000).
16. D. Fourmy, M. I. Recht, S. C. Blanchard, et al., *Science*, **274**(5291), 1367 – 1371 (1996).
17. S. Shandrick, Q. Zhao, Q. Han, et al., *Angew. Chem. Int. Ed.*, **43**(24), 3177 – 3182 (2004).
18. M. Kaul, C. M. Barbieri, and D. S. Pilch, *J. Am. Chem. Soc.*, **126**(11), 3447 – 3453 (2004).
19. S. Magnet and J. S. Blanchard, *Chem. Rev.*, **105**(2), 477 – 497 (2005).
20. M. B. Feldman, D. S. Terry, R. B. Altman, et al., *Nat. Chem. Biol.*, **6**(1), 54 – 62 (2010).
21. M. Misumi, T. Nishimura, T. Komai, et al., *Biochem. Biophys. Res. Commun.*, **84**(2), 358 – 365 (1978).
22. A. Okuyama, T. Watanabe, and N. Tanaka, *J. Antibiot.*, **25**(4), 212 – 218 (1972).
23. E. M. Scolnik and C. T. Caskey, *Proc. Natl. Acad. Sci. USA*, **64**(4), 1235 – 1241 (1969).
24. I. Prokhorova, R. B. Altman, M. Djumagulov, et al., *Proc. Natl. Acad. Sci. USA*, **114**(51), E10899-E10908 (2017).
25. E. Selimoglu, *Curr. Pharm. Des.*, **13**(1), 119 – 126 (2007).
26. J. M. Lopez-Novoa, Y. Quiros, L. Vicente, et al., *Kidney Int.*, **79**(1), 33 – 45 (2011).
27. M. E. Tolmasky, *Drug Resist. Updates*, **13**(6), 151 – 171 (2010).
28. M. S. Ramirez, N. Nikolaidis, and M. E. Tolmasky, *Front. Microbiol.*, No. 4, 121 (2013).
29. B. Springer, Y. G. Kidan, T. Prammananan, et al., *Antimicrob. Agents Chemother.*, **45**(10), 2877 – 2884 (2001).
30. L. C. Santos, *Open J. Med. Microbiol.*, **2**(1), 24 – 36 (2012).
31. C. E. Maus, B. B. Plikaytis, and T. M. Shinnick, *Antimicrob. Agents Chemother.*, **49**(8), 3192 – 3197 (2005).
32. S. B. Georghiou, M. Magana, R. S. Garfein, et al., *PLoS ONE*, **7**(3), e33275 (2012).
33. R. Nessar, E. Cambau, J. M. Reyrat, et al., *J. Antimicrob. Chemother.*, **67**(4), 810 – 818 (2012).
34. R. C. Cooksey, G. P. Morlock, A. Mcqueen, et al., *Antimicrob. Agents Chemother.*, **40**(5), 1186 – 1188 (1996).
35. T. Prammananan, P. Sander, B. A. Brown, et al., *J. Infect. Dis.*, **177**(6), 1573 – 1581 (1998).
36. M. E. Muir, D. R. Hanwell, and B. J. Wallace, *Biochim. Biophys. Acta*, **638**(2), 234 – 241 (1981).
37. X. Z. Li and H. Nikaido, *Drugs*, **69**(12), 1555 – 1623 (2009).
38. K. J. Shaw, P. N. Rather, R. S. Hare, and G. H. Miller, *Microbiol. Mol. Biol. Rev.*, **57**(1), 138 – 163 (1993).
39. K. V. L. Aishwarya, P. V. Geetha, S. Eswaran, et al., *J. Lab. Physicians*, **12**(1), 27 – 31 (2020).
40. S. B. Vakulenko and S. Mobashery, *Clin. Microbiol. Rev.*, **16**(3), 430 – 450 (2003).
41. J. W. Chow, V. Kak, I. You, et al., *Antimicrob. Agents Chemother.*, **45**(10), 2691 – 2694 (2001).
42. Y. H. Chen, S. Y. Lin, Y. T. Lin, et al., *J. Antimicrob. Chemother.*, **76**(7), 1689 – 1697 (2021).
43. B. F. Araujo, M. L. Ferreira, P. A. Campos, et al., *PLoS ONE*, **11**(5), e0155914 (2016).
44. Q. Pan, F. L. Zhao, and B. C. Ye, *Sci. Rep.*, **8**(1), 1 – 8 (2018).
45. A. Sowajassatakul, T. Prammananan, A. Chaiprasert, and S. Phunpruch, *Ann. Clin. Microbiol. Antimicrob.*, **17**, Art. No. 33, 1 – 7 (2018).
46. E. Bordeleau, P. J. Stogios, E. Evdokimova, et al., *mBio*, **12**(1), e02705 – 20 (2021).
47. R. A. Bonomo and M. E. Tolmasky, *Enzyme-Mediated Resistance to Antibiotics: Mechanisms, Dissemination, and Prospects for Inhibition*, ASM Press, Washington, D. C. (2007), pp. 267 – 270.
48. B. O. Costa, M. H. Cardoso, and O. L. Franco, *Curr. Protein Pept. Sci.*, **21**(10), 1011 – 1026 (2020).
49. J.-I. Wachino, Y. Doi, and Y. Arakawa, *Infect. Dis. Clin. North Am.*, **34**(4), 887 – 902 (2020).
50. M. Golebiewski, I. Kern-Zdanowicz, M. Zienkiewicz, et al., *Antimicrob. Agents Chemother.*, **51**(11), 3789 – 3795 (2007).

51. M. Galimand, P. Courvalin, and T. Lambert, *Antimicrob. Agents Chemother.*, **47**(8), 2565 – 2571 (2003).

52. Y. Doi, K. Yokoyama, K. Yamane, et al., *Antimicrob. Agents Chemother.*, **48**(2), 491 – 496 (2004).

53. J. Wachino, K. Yamane, K. Shibayama, et al., *Antimicrob. Agents Chemother.*, **50**(1), 178 – 184 (2006).

54. Y. Doi, J. I. Wachino, and Y. Arakawa, *Infect. Dis. Clin. North Am.*, **30**(2), 523 – 537 (2016).

55. J. Wachino and Y. Arakawa, *Drug Resist. Updates*, **15**(3), 133 – 148 (2012).

56. M. Wu, B. Li, Q. Guo, et al., *J. Glob. Antimicrob. Resist.*, **19**, 188 – 191 (2019).

57. F. Lv, J. Cai, Q. He, et al., *Microb. Drug Resist.*, ahead of print; http: // doi.org / 10.1089 / mdr.2020.0395 (2021).

58. K. Poole, *Ann. Med.*, **39**(3), 162 – 176 (2007).

59. N. Al-Karablieh, H. Weingart, and M. S. Ullrich, *Microb. Biotechnol.*, **2**(4), 465 – 475 (2009).

60. M. Laws, P. Jin, and K. M. Rahman, *Trends Microbiol.*, S0966 – 842X(21)00123 – 2 (2021).

61. H. Nikaido and J. M. Pages, *FEMS Microbiol. Rev.*, **36**(2), 340 – 363 (2012).

62. R. E. Hancock, S. W. Farmer, Z. S. Li, and K. Poole, *Antimicrob. Agents Chemother.*, **35**(7), 1309 – 1314 (1991).

63. S. Garneau-Tsodikova and K. J. Labby, *MedChemComm*, **7**(1), 11 – 27 (2016).

64. R. E. Hancock and A. Bell, *Eur. J. Clin. Microbiol. Infect. Dis.*, **7**(6), 713 – 720 (1988).

65. P. A. Lambert, *J. R. Soc. Med.*, **95**(41), 22 – 26 (2002).

66. A. Hinz, S. Lee, K. Jacoby, and C. Manoil, *J. Bacteriol.*, **193**(18), 4790 – 4797 (2011).

67. P. Behzadi, Z. Barath, and M Gajdacs, *Antibiotics*, **10**(1), 42 (2021).

68. J. A. Karlowsky, S. A. Zelenitsky, and G. G. Zhanel, *Pharmacotherapy*, **17**(3), 549 – 555 (1997).

69. N. T. Chandrika and S. Garneau-Tsodikova, *Chem. Soc. Rev.*, **47**(4), 1189 – 1249 (2018).

70. J. A. Clark and D. S. Burgess, *Ther. Adv. Infect. Dis.*, **7**, 1 – 15 (2020).

71. A. Bilinskaya, K. E. Linder, and J. L. Kuti, *Expert Rev. Anti-Infect. Ther.*, **18**(8), 705 – 720 (2020).

72. L. D. Saravolatz and G. E. Stein, *Clin. Infect. Dis.*, **70**(4), 704 – 709 (2020).

73. K. M. Shaeer, M. T. Zmarlicka, E. B. Chahine, et al., *Pharmacotherapy: J. Hum. Pharmacol. Drug Ther.*, **39**(1), 77 – 93 (2019).

74. K. Eljaaly, A. Alharbi, S. Alshehri, et al., *Drugs*, **79**(3), 243 – 269 (2019).

75. K. Aradi, A. Di Giorgio, and M. Duca, *Chem. Eur. J.*, **26**(54), 12273 – 12309 (2020).

76. T. Hermann, *Wiley Interdiscip. Rev. RNA*, No. 7, 726 – 743 (2016).

77. A. Litovchick, A. G. Evdokimov, and A. Lapidot, *Biochemistry*, **39**(11), 2838 – 2852 (2000).

78. A. Lapidot and A. Litovchick, *Drug Dev. Res.*, **50**(3 – 4), 502 – 515 (2000).

79. C. Cabrera, A. Gutierrez, J. Barretina, et al., *Antiviral Res.*, **53**(1), 1 – 8 (2002).

80. G. Borkow, V. Vijayabaskar, H. H. Lara, et al., *Antiviral Res.*, **60**(3), 181 – 192 (2003).

81. R. Hegde, G. Borkow, A. Berchanski, and A. Lapidot, *FEBS J.*, **274**(24), 6523 – 6536 (2007).

82. A. Lapidot, V. Vijayabaskar, A. Litovchick, et al., *FEBS Lett.*, **577**(3), 415 – 421 (2004).

83. L. Jiang, D. Watkins, Y. Jin, et al., *ACS Chem. Biol.*, **10**(5), 1278 – 1289 (2015).

84. K. B. Reddy, *Cancer Cell Int.*, **15**(1), 1 – 6 (2015).

85. D. Watkins, L. Jiang, S. Nahar, et al., *PloS One*, **10**(12), e0144251 (2015).

86. A. Ghosh, N. Degyatoreva, C. Kukielski, et al., *MedChemComm*, **9**(7), 1147 – 1154 (2018).

87. P. Muangkaew and T. Vilaivan, *Bioorg. Med. Chem. Lett.*, **30**(9), 127064 (2020).

88. E. Riguet, S. Tripathi, B. Chaubey, et al., *J. Med. Chem.*, **47**(20), 4799 – 4801 (2004).

89. B. Chaubey, S. Tripathi, J. Desire, et al., *Oligonucleotides*, **17**(3), 302 – 313 (2007).

90. K. Watanabe, T. Katou, Y. Ikezawa, et al., *Nucleic Acids Symp. Ser. (Oxf.)*, **51**(1), 209 – 210 (2007).

91. E. Ennifar, M. W. Aslam, P. Strasser, et al., *ACS Chem. Biol.*, **8**(11), 2509 – 2517 (2013).

92. D. D. Vo, C. Staedel, L. Zehnacker, et al., *ACS Chem. Biol.*, **9**(3), 711 – 721 (2014).

93. D. D. Vo, T. P. A. Tran, C. Staedel, et al., *Chem. Eur. J.*, **22**(15), 5350 – 5362 (2016).

94. D. D. Vo, C. Becquart, T. P. A. Tran, et al., *Org. Biomol. Chem.*, **16**(34), 6262 – 6274 (2018).

95. K. Ketomaki and P. Virta, *Bioconjugate Chem.*, **19**(3), 766 – 777 (2008).

96. J. Alguacil, J. Robles, C. Rafols, and E. Bosch, *J. Mol. Recognit.*, **29**(4), 142 – 150 (2016).

97. C. Dezanet, J. Kempf, M. P. Mingeot-Leclercq, and J. L. Decout, *Int. J. Mol. Sci.*, **21**(19), 7411 (2020).

98. K. C. Howard, E. K. Dennis, D. S. Watt, and S. Garneau-Tsodikova, *Chem. Soc. Rev.*, **49**(8), 2426 – 2480 (2020).

99. Y. P. Subedi, M. N. AlFindee, J. Y. Takemoto, and C. W. T. Chang, *MedChemComm*, **9**(6), 909 – 919 (2018).

100. Y. P. Subedi, P. Roberts, M. Grilley, et al., *ACS Infect. Dis.*, **5**(3), 473 – 483 (2019).

101. Y. P. Subedi, U. Pandey, M. N. Alfindee, et al., *Eur. J. Med. Chem.*, **182**, 111639 (2019).

102. J. Y. Takemoto, S. N. Wegulo, G. Y. Yuen, et al., *Fungal Biol.*, **122**(6), 465 – 470 (2018).

103. M. N. Alfindee, Y. P. Subedi, M. M. Grilley, et al., *Molecules*, **24**(10), 1882 (2019).

104. M. Delmar, D. W. Laird, C. C. Naus, et al., *Cold Spring Harbor Perspect. Biol.*, **10**(9), a029348 (2018).

105. M. A. Retamal, E. P. Reyes, I. E. García, et al., *Front. Cell Neurosci.*, No. 9, 267 (2015).

106. M. N. Alfindee, Y. P. Subedi, M. C. Fiori, et al., *ACS Med. Chem. Lett.*, **9**(7), 697 – 701 (2018).

107. T. Matsushita, G. C. Sati, N. Kondasinghe, et al., *J. Am. Chem. Soc.*, **141**(12), 5051 – 5061 (2019).

108. D. Lubriks, R. Zogota, V. A. Sarpe, et al., *ACS Infect. Dis.*, **7**(8), 2413 – 2424 (2021); https: // doi.org / 10.1021 / acsinfecdis.1c00158.