Screening of Aptamers that Bind to the Multivalent Aminoglycoside Amikacin

AHM Khurshid Alam1, Yoshiko Miura2, Aziz Abdur Rahman1, Md. Golam Sadik1, Mamunur Rashid1 and Toshifumi Tsukahara3

1Department of Pharmacy, University of Rajshahi, Rajshahi-6205, Bangladesh
2Department of Chemical Systems and Engineering, Graduate School of Engineering, Kyushu University 744 Motooka, Nishi-ku, Fukuoka-819-0395, Japan
3School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Nomi City, Ishikawa-923-1292, Japan

(Received: January 27, 2023; Accepted: March 30, 2023; Published (web): July 25, 2023)

Abstract
Increased awareness of the multiple roles of RNA molecules has led to the realization that, in addition to their structural and functional roles, RNAs can be drug targets for small molecular therapy. Amikacin, a member of the aminoglycoside group of antibiotics, binds to specific sites in bacterial 16S ribosomal RNAs (rRNAs) and interferes with protein synthesis leading to cell death. Here, we used the systemic evolution of ligands by exponential enrichment (SELEX) method to isolate high affinity RNA fragments (aptamers) that bind to amikacin. After five rounds of SELEX selection, in which a linear N25 DNA template was used for the first selection cycle, the resulting RNA was cloned and sequenced. Among the 38 clones generated, five groups of sequences (groups A through E) containing nine conserved motifs were identified. The sequences of groups A and B were almost identical, indicating that the selected RNA was enriched. Subsequently, the Basic Local Alignment Search Tool program was used to search for the conserved motifs in bacterial 16S rRNA sequences. Strikingly, no sequence homology was observed, suggesting that the conserved sequences (motifs) identified in this study may be novel target sites for amikacin.

Key words: SELEX, aptamer, amikacin.

Introduction
Aminoglycosides (AGs) are a class of antibiotics that contain a characteristic aminocyclitol ring, usually streptidine or 2-deoxystreptamine, and two or more amino sugars joined in a glycosidic linkage to a hexose nucleus (Liu et al., 2017). Characterizing the structure of AGs is important to understand their chemical and biological activities. AGs are basic and strongly polar compounds that are positively charged, which contributes to their antimicrobial activity (Kevin et al., 2016). Because they are polycationic, AGs show a binding affinity for nucleic acids (RNA and DNA); specifically, they possess high affinities for certain portions of negatively charged RNAs, particularly the A-site region on the 16S subunit of prokaryotic ribosomal RNA (rRNA) (Smritilekha et al., 2010). Thus, AGs exert their antibacterial effects by interfering with ribosomal function, which ultimately results in disruption of protein synthesis.

The most commonly used AGs in hospitals are amikacin, gentamicin and tobramycin (Lambert et al., 2015). Amikacin is a semi-synthetic AG antibiotic derived by acetylation of kanamycin A; it is particularly effective against bacteria that are resistant to other AGs because its chemical structure renders it less susceptible to inactivating enzymes (Kotra et al., 2000). Like other AGs, amikacin irreversibly binds to a specific A-site on the 16S rRNA subunit of the 30S
ribosome to prevent the formation of an initiation complex with mRNA, thereby inhibiting protein synthesis (Ramirez et al., 2010). More specifically, amikacin binds to four nucleotides of 16S rRNA and a single amino acid of protein S12 (Peloquin et al., 2004). Despite its wide ranging antibacterial effects, resistance to amikacin can be achieved via various mechanisms, including the production of amikacin-modified enzymes (acetyltransferase, nucleotidyltransferase, and phosphotransferase), the reduction of antibiotic penetration of the outer membrane protein, the acquisition of reduced affinity by changing nucleotides within the 16S rRNA, and augmented excretion by an efflux pump system (Kevin et al., 2016; Edson and Terrell, 1999; Poole, 2005). To overcome these problems, it is highly desirable to synthesize a modified amikacin molecule that has a higher RNA-binding affinity, better selectivity and antibacterial activity, and stronger resistance to AG-modifying enzymes than the parent molecule. Recently, Miura and her colleagues synthesized polymeric amikacin (multivalent amikacin) by chemoenzymatic esterification of the parent structure (Miura et al., 2003). Chemoselective reactions are important for the modification of AGs because they possess multiple amino and hydroxyl groups. The amino groups of amikacin play an important role in its antibacterial activity. One polymeric amikacin (denoted as amikacin (6)) has a stronger inhibitory effect on protein synthesis in vitro than other polymeric amikacins (8a and 8b) due to the modification of its side chains and amino groups (Miura et al., 2003). Similarly, a recent study developed a polymeric vancomycin that has a much stronger antibiotic activity than monomeric vancomycin (Ashraf et al., 2017).

Targeting RNA for drug development is not a new concept. An antisense drug approach has been intensively investigated for decades, resulting in multiple clinical trials. Recently, high-field NMR analyses of the structures of RNA molecules, including Group 1 intron of hammerhead ribozyme (Stage et al., 1995; Ahsen et al., 1991), HIV-1 mRNA Rev responsive element (Zapp et al., 1993), and a trans-activation response element, revealed new structural motifs in a three-dimensional manner (Hendrics et al., 1997; Mei et al., 1995). These advances in elucidating the structures and functions of RNAs led the pharmaceutical industry to regard them as new therapeutic targets (Noreen et al., 2017). Because AGs can generally bind strongly to any type of RNA motifs, they may be plausible candidates for new RNA-targeting drugs.

In vitro selection of RNAs that bind to target molecules is usually performed over iterative cycles, in a process known as systemic evolution of ligands by exponential enrichment (SELEX) (Ellington and Szostak, 1990; Kenan et al., 2016). This method has enabled the identification of unique high affinity RNAs (aptamers) that bind to ligands of interest within large random RNA libraries (Mariia et al., 2015; Hermann and Patel, 2000). Aptamers are single-stranded short oligonucleotide or peptide molecules that bind to a specific target, including proteins, carbohydrates and small molecules with high affinity and specificity. Since their initial discovery, numerous researchers have used aptamer selection as a means for widespread application and discovery of aptamers in diagnostic and therapy, as well as to approaches that could considerably expand the range of aptamer application (Lakhin et al., 2013). Efforts to identify specific RNA sequences that bind to certain AGs will enhance our understanding of the rules underlying RNA-AG recognition and will aid the development of sequence-specific RNA target therapeutics (Masayasu and Naoki, 2010). RNA aptamers against lividomycin have identical sequences to those of the Haemophilus influenza and Leishmania parasite rRNAs (Lato and Ellington, 1996), suggesting that the sequences could be new RNA target sites used to control these pathogens. An aptamer against neomycin was also reported to have a certain stem loop structure with wobble non-Watson-Crick base pairings (Julia et al., 2008). Lato and Ellington (1996) reported six RNA aptamers (sla 21, 26, 110, 150, 151, and 254) that recognized amikacin (Lato et al., 1995). Recently, three DNA aptamers (Aptamer 15, 16, and 41) and one modified truncated aptamer (Aptamer 8-2) that bind against amikacin have been reported (Soheili et al., 2016). Moreover,
an USA based world intellectual property organization published amikacin-binding five DNA aptamers (Nikolaus and Strehlitz, 2014). The most successful in vitro selection of RNAs was achieved for neomycin B and tobramycin as compared to other AGs, suggesting the differences in the specificity and affinity of aptamers in the AGs (Ann et al., 2010).

In the current study, we used SELEX to screen for RNA targets that bind to polymeric amikacin. After five cycles of selection, a characteristic sequence variation was observed among 38 clones. Overall, five groups of similar sequences (groups A, B, C, D, and E) were identified. The sequences of groups A and B were almost identical, suggesting that the selected RNA was enriched. Moreover, there was a significant sequence homology among different clones, and nine conserved motifs were identified. Subsequently, we used the Basic Local Alignment Search Tool (BLAST) program to search for similar motifs in bacterial rRNA sequences. Notably, we did not identify any sequence homology to bacterial rRNAs, suggesting that the RNA motifs (aptamers) identified here may be new target sites for amikacin.

Materials and Methods

**Immobilization of amikacin on epoxy-activated porous glass:** The procedure for immobilization of amikacin has been described previously (Abood et al., 1983). Briefly, amikacin solution (1 mg/ml) was prepared in 0.1 M NaHCO₃ buffer (pH 9.0) and incubated with epoxy-activated porous glass beads (genoglass PG-200-EP) at room temperature (RT) overnight. The solution was then removed and the glass beads were washed 5 times with buffer containing 0.1 M NaHCO₃ (pH 9.0) and 0.1 M acetic acid (pH 4.0). After soaking with 1M ethanolamine at RT overnight, the porous glass beads containing amikacin were washed 9 times with water and used for SELEX. Spectrophotometrically at a wavelength of 524 nm, the amount of immobilized amikacin on epoxy-activated porous glass was measured by dividing the immobilized amikacin on porous glass by the amount of amikacin applied initially to the column. Later, the % of immobilized amikacin on epoxy-activated porous glass was measured by subtracting the free amikacin obtained after washing from the amount of amikacin applied initially to the column.

**Polymerase chain reaction (PCR):** PCRs were carried out according to the method described by Alam et al., 2016. Briefly, the 50 µl reaction mixture contained 5 ng of oligo DNA 1× Ex Taq buffer, 0.2 mM MgCl₂, 2 μM T7 primer, 2 μM universal primer, and 0.5 U of Ex Taq polymerase (1 U/µl; TaKaRa). Thermal cycling was carried out in the GeneAmp PCR System 9700 under the following conditions: denaturation at 95°C for 3 min, followed by seven cycles of 95°C for 30 s, 54°C for 30 s, and 72°C for 30 s. A 1 µl aliquot of each PCR sample was analyzed via 8% polyacrylamide electrophoresis to confirm the size of the product. The remainder of each solution was concentrated by ethanol precipitation and resuspended in 10 µl of deionized water. Subsequently, a 9 µl aliquot of the concentrated DNA was digested with 0.5 U of BamHI (TaKaRa) in 1× buffer K (20 µl reaction volume) at 37°C overnight. The digested sample was purified by phenol-chloroform extraction, concentrated by ethanol precipitation, and then dissolved in 10 µl of deionized water.

**In vitro transcription:** In vitro transcription was performed according to the manufacturer’s (Ambion) instructions. Briefly, the 10 µl reaction included 1 µl of digested and purified DNA, 1× T7 MEGAscript buffer, 7.5 mM each NTP, and 1 µl of T7 MEGAscript enzyme. The solution was incubated at 37°C overnight. Subsequently, 1 U of RNase free Turbo DNase (2 U/µl; Ambion) was added and the mixture was incubated at 37°C for 15 min to remove residual DNA. A 1 µl aliquot of the resulting solution was analyzed by 8% polyacrylamide gel electrophoresis to detect the synthesized RNA. Next, a ProbeQuantG-50 microcolumn (GE Healthcare) was equilibrated using equilibrium buffer (0.3 M Na-acetate (pH 5.2), 0.5 M EDTA (pH 8.0), and 0.1% SDS) and the synthesized RNA was added to the column along with 92 µl of TEN buffers (40 mMTris-HCl (pH 7.5), 1mM EDTA
RNA purification and selection of RNA using a G-50 column: RNA purification was performed as described previously (Alam et al., 2009). A 50 µl sample of amikacin-immobilized epoxy beads was put into a fresh 1.5 ml tube and centrifuged at 10,000 g for 30 s at RT. The supernatant was removed, and 1 ml of binding buffer (20 mM HEPES-KOH (pH 7.9), 200 mM KCl, 5% glycerol, and 0.1% Triton X-100) was added to the tube. After centrifugation at 10,000 g for 30 s, the supernatant was removed. Subsequently, 100 µl of binding buffer, 1 µl of RNasin ribonuclease inhibitor, and 1 µg of in vitro transcribed RNA were added to a fresh 1.5 ml tube and the tube was rotated for 1 h at 4°C and centrifuged at 10,000 g for 30 s. The supernatant was removed, and the RNA was washed three times with 1 ml of binding buffer and centrifuged at 10,000 g for 30 s. For elution, 100 µl of binding buffer, 1 µl of RNasin, and 250 µg of free amikacin were added and the tube was rotated at 4°C for 5 min following a quick centrifugation at 10,000 g for 30 s. Subsequently, the solution was carefully transferred into a fresh 1.5 ml tube containing 1 µl of glycogen. The solution was then purified and concentrated (final volume: 10 µl) by phenol-chloroform extraction followed by ethanol precipitation. The concentration of amikacin-bound ssRNA was measured by subtracting the free ssRNA obtained after washing from the amount of ssRNA applied initially to the column at 260 nm using a UV spectrophotometer. Later, the % of binding was measured by dividing the amikacin-bound ssRNA by the amount of ssRNA applied initially to the column.

Reverse transcription: The cDNA was prepared as described by Alam et al., 2010. Briefly, the 13µl reaction mixture contained 1 µl of purified RNA, 2 µM T7 universal reverse primer, and 0.5 mM each dNTP. The solution was heated at 65°C for 5 min and then placed on ice for at least 1 min. Subsequently, 1× first strand buffer, 5 µM DTT, 2 U of RNaseOUT and 10 U of SuperScript III reverse transcriptase (Invitrogen) were added to make the 20 µl final reaction volume. The reaction was incubated for 1 h at 50°C and then inactivated by heating at 70°C for 15 min. The cDNA solution was then cooled, and a 1 µ l aliquot was used in 50 µl PCR samples.

In vitro selection (SELEX): In vitro selection experiments were carried out essentially as described by Abe et al., 1996. The starting RNA source was synthesized in vitro from a mixture of DNA templates containing a T7 promoter and a randomized 25 nucleotide region. The synthesized RNA was purified by adding 1 ml of binding buffer (20 mM HEPES-KOH (pH 7.9), 200 mM KCl, 5% glycerol, and 0.1% Triton X-100) into a fresh tube. After centrifugation at 10,000 g for 30 s, the supernatant was removed, and the RNA was washed three times with 1 ml of binding buffer and centrifuged at 10,000 g for 30 s. For elution, 100 µl of binding buffer, 1 µl of RNasin, and 250 µg of free amikacin were added and the tube was rotated at 4°C for 5 min following a quick centrifugation at 10,000 g for 30 s. Subsequently, the solution was carefully transferred into a fresh 1.5 ml tube containing 1 µl of glycogen. The solution was then purified and concentrated (final volume: 10 µl) by phenol-chloroform extraction followed by ethanol precipitation and selected using a G-50 column. Subsequently, the selected RNA was converted to single-stranded cDNA, which was amplified by PCR and then transcribed in vitro and used for the next round of selection. Five rounds of selection and amplification were performed (Figure 1).

Cloning and sequencing: In vitro cloning and sequencing were performed as described by Alam et al., 2009. The PCR products were purified using the LaboPass Gel Extraction Kit (Cosmogenetech), followed by phenol-chloroform extraction. The purified PCR products were inserted into the pGEM-T-Easy vector (Promega). The plasmid DNA containing the insert was purified using the QIAprep Spin Miniprep Kit (Promega) and sequenced using...
M13 forward primers and the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequences were determined using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems) and confirmed using BLAST software.

**Results and Discussion**

**Selection of RNA:** To select amikacin-specific RNAs, amikacin was immobilized on epoxy-activated porous glass beads (genoglass PG-200-EP) using ethanolamine under mild basic conditions (Abood et al., 1983). Approximately 50% of the amikacin applied was immobilized on the porous glass beads suggests that the amikacin was successfully immobilized on the porous beads (data not shown). A linear N25 DNA template underwent polymerase chain reaction (PCR) amplification in the first selection cycle, and a total of five rounds of selection and amplification were performed. Figure 1 provides an overview of the RNA selection process. To monitor the progress of the selection process, the quantity of the ssRNA was measured by a UV spectrometer. The percentage binding affinity of ssRNA on amikacin-bound beads was in the range of 22-31% in the five selection rounds (data not shown).

For the first selection cycle, seven rounds of PCR were performed, whereas 13 rounds of PCR were used for the subsequent selection cycles. Gel electrophoresis confirmed that a product of the expected size (103 bp) was generated in each cycle (Figure 2).
In each cycle, the PCR product was digested with BamHI to generate a 97 bp product. The digested product was then purified using the phenol-chloroform extraction method and concentrated by ethanol precipitation. Aliquots (1 µl) of each sample were run on an 8% polyacrylamide gel to confirm successful digestion and concentration of the 97 bp product (Figure 3).

Next, the digested and purified DNA was *in vitro* transcribed and the synthesized RNA was purified using a G-50 column. After each cycle, the purified RNA was run on an 8% polyacrylamide gel (Figure 4).

Subsequently, the synthesized RNA was incubated with amikacin-immobilized beads. Unbound RNAs and those that were weakly bound to the beads were removed by washing with binding buffer, and the remaining RNAs were eluted with free amikacin. The eluted RNAs were purified by ethanol precipitation, reverse transcribed, and then amplified by PCR for use in the subsequent cycles. The process was repeated for a total of five rounds. Overall, in this experiment, we obtained the expected size of DNA after PCR amplification and BamHI digestion and synthesized RNA that bound to immobilized amikacin.

**Sequence analysis of amikacin-specific aptamers:** Since *in vitro* selection was introduced in the early 1990s (Ellington and Szostak, 1990), the power of this combinatorial approach has been used to generate aptamers that bind targets ranging from organic molecules to proteins and even DNA (Beaudry and Joyce, 1992; Gold *et al.*, 1995; Pei *et al.*, 1991). Accumulating evidence suggests that a large number of generated aptamers can bind various target molecules, including AGs. The U.S. Food and Drug Administration approved the first aptamer-based drug, pegaptanib sodium (brand name: Macugen), for the
treatment for age-related macular degeneration (Siddiqui and Keating, 2005; Dombi et al., 2012).

After five rounds of SELEX, the resulting PCR product was cloned into the pGEM-T-Easy vector and transformed into competent *Escherichia coli* cells. Figure 5 provides an overview of the method used to identify and analyze the sub-clones. Briefly, 50 clones were sub-cultured; among them, 38 clones were positive for the inserted region and were confirmed by sequencing. There were marked sequence variations among different clones (Lakhin et al., 2013; Lato and Ellington, 1996), but five groups of similar sequences (groups A through E) were identified (Table 1).

From the five groups of sequences, nine conserved motifs were identified (Figure 6 and Table 2). The sequences in groups A and B were almost identical, suggesting that the selected RNA was enriched (Figure 6). Our data are consistent with those published previously (Kenan et al., 2016; Mariia et al., 2015 and Lakhin et al., 2013) where they reported that some of the identified sequences were more or less indistinguishable, indicates the enrichment of the selected RNA.

Table 1. Representative sequences of five groups.

| Group | Representative sequence |
|-------|-------------------------|
| A     | AGGUUAGCGGAUAAGAUCACCCUGA |
| B     | UUAGCCGACAGUUCAGAucagcuac |
| C     | acgGCCGAGCCaaGAGCAGc      |
| D     | gAGACCCCCCC(C)Ggy         |
| E     | yGACAGUCaUACUGACy         |

Lower-case letters indicate nucleotides that were not conserved among the clones. Y indicates a pyrimidine base.

Table 2. Motifs and their conserved sequences.

| Motif | Consensus sequence |
|-------|--------------------|
| Motif 1 | UUAAGCCGAU |
| Motif 2a (include1) | AGG(UUAGCCGAU)AAGAUCAC |
| Motif 2b | CAGUUUCAC |
| Motif 3 | CCUGA |
| Motif 4 | GAUGCUAC |
| Motif 5 | acgGCCGAGCCra |
| Motif 6 | aaGACAGC |
| Motif 7 | gAGACCCCCCC(C)Ggy |
| Motif 8 | yGACAGUCcua |

Lower-case letters indicate nucleotides that are not consistent with the respective motif. Y indicates a pyrimidine base.
Amikacin is the most effective AG used to treat severe bacterial infections (Lambert et al., 2015). As mentioned above, amikacin inhibits protein synthesis and causes bacterial cell death by binding to 16S rRNA (Ramirez et al., 2010). Despite its activity against a number of bacterial species, resistance to amikacin can be achieved through several mechanisms; hence there is a need to identify new target binding sites of this AG. In the current study, we identified a number of unique conserved motifs (aptamers) that bind to amikacin. This result is consistent with the data published by Strehlitz et al., 2012, who showed aptamers as specific recognition elements for the detection of pharmaceuticals. Subsequently, we performed a BLAST search to identify bacterial 16S rRNA sequences containing the conserved motifs identified here. Strikingly, we did not find any sequence homology with bacterial 16S rRNAs. These results suggest that amikacin has new binding sites that are not present in bacterial 16S rRNAs. It has been reported previously that AGs are potential antiviral (anti-HIV) agents (Cabrera et al., 2000). We suggest that the conserved motifs (aptamers) identified here not only could be novel target sites for amikacin but also could be used in targeted delivery mediated by these aptamers.

Future study is necessary to characterize the secondary structure of the identified aptamers, their binding and affinity as well as specificity or selectivity against targets, such as other aminoglycosides. Aptamers that would show specificity to other aminoglycoside antibiotics,
including amikacin could be used as target for the elimination of those pharmaceutical or their residues from environment samples.

**Funding**

This work was supported in part by grants-in-aid from the Japan Society for the promotion of science (17H02204 and 18K19288).

**Acknowledgment**

We gratefully acknowledge scholarships from Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

**References**

Abe, R., Sakashita, E., Yamamoto, K. and Sakamoto, H. 1996. Two different RNA binding activities for the AU-rich element and the poly(A) sequence of the mouse neuronal protein mHuC. Nuc. Acids Res. 24, 4895-4901.

Abood, L.G., Latham, W. and Grassi, S. 1983. Isolation of a nicotine binding site from rat brain by affinity chromatography. Proc. Natl. Acad. Sci. 11, 3536-3539.

Ahsen, U., Davies, J. and Schroder, R. 1991. Antibiotic inhibition of group 1 intron ribozyme function. Nature 353, 368-370.

Alam, A.H., Suzuki, H. and Tsukahara, T. 2009. Expression analysis of Fgf8a & Fgf8b in early stage of P19 cells during neural differentiation. Cell Biol. Int. 33, 1032-1037.

Alam, A.H., Suzuki, H. and Tsukahara, T. 2010. Retinoic acid treatment and cell aggrega‌itness independently regulate alternative splicing in P19 cells during neural differentiation. Cell Biol. Int. 34, 631-643.

Alam, A.H.M.K., Hossain, A.S.M.S., Khan, M.A., Kabir, S.R., Reza, M.A., Rahman, M.M., Islam, M.S., Rahman, M.A.A.R., Rashid, M. and Sadik, MG. 2016. The antioxidative fraction of white mulberry induces apoptosis through regulation of p53 and NFκB in EAC cells. PLoS ONE 11, e0167536.

Ann, E.S., William, D.G., Vendex, FA. and Paul, F.A. 2010. Binding of aminoglycoside antibiotics to helix 69 of 23S rRNA. Nuc. Acids Res. 38, 3094-3105.

Ashraf, Z., Heather-Rose, M., Dimitri, Y.C., Andrew, D.B., Andrew, R. and Hesketh, H. H. 2017. Zn(II) mediates vancomycin polymerization and potentiates its antibiotic activity against resistant bacteria. Sci. Rep. 7, 4893.

Beaudry, A. and Joyce, G.F. 1992. Directed evolution of an RNA enzyme. Science 257, 635-64.
Lato, S.M. and Ellington, A.D. 1996. Screening chemical libraries for nucleic-acid-binding drugs by in vitro selection: a test case with lividomycin. *Mol. Divers.* 2, 103-110.

Lato, S.M., Boles, A.R. and Ellington, A.D. 1995. *In vitro* selection of RNA lectins: using combinatorial chemistry to interpret ribozyme evolution. *Chem. Biol.* 2, 291-303.

Liu, Q., Li, J., Song, X., Zhang, M., Li, E., Gaob, F. and He L. 2017. Simultaneous determination of aminoglycoside antibiotics in feeds using high performance liquid chromatography with evaporative light scattering detection. *RSC Adv.* 7, 1251-1259.

Mariia, D., Silvie, R., Helena, G. and Tomas, R. 2015. Current approaches in SELEX: an update to aptamer selection technology. *Biotech. Adv.* 33, 1141-1161.

Masayasu, K. and Naoki, S. 2010. Molecular evolution of functional nucleic acids with chemical modifications. *Molecules* 15, 5423-5444.

Mei, H.N., Galan, A.A., Halim, N.S., Mack, D.P., Moreland, D.W., Sanders, K.B., Truong, H.N. and Czernik, A.W. 1995. Inhibition of an HIV-1 Tat derived peptide binding to TAR RAA by aminoglycoside antibiotics. *Bioorg. Med. Chem. Lett.* 5, 2755-2760.

Miura, Y., I. Wada, N. and Kobayashi, K. 2003. Chemoenzymatic synthesis of a multivalent aminoglycoside. *Macromol. Biosci.* 3, 662-667.

Nikolaus N. and Strehlitz B. 2014. DNA aptamers binding aminoglycoside antibiotics. *Sensors* 14, 3737-55.

Noreen, F.R. and Graham, F.S. 2017. RNA as a small molecule druggable target. *Bioorg. Med. Chem. Lett.* 27, 5083-5088.

Pei, D., Ulrich, H.D. and Schultz, P.G. 1991. A combinatorial approach toward DNA recognition. *Science* 253, 1408-1411.

Peloquin, C.A., Berning, S.E., Nitta, A.T., Simone, P.M., Goble, M., Huitt, G.A., Iserman, M.D., Cook, J.L. and Curran-Everett, D. 2004. Aminoglycoside toxicity: daily versus thrice-weekly dosing for treatment of mycobacterial diseases. *Clin. Infect. Dis.* 38, 1538-1544.

Poole, K. 2005. Efflux-mediated antimicrobial resistance. *J. Antimicrob. Chemother.* 56, 20-51.

Ramirez, M.S. and Tolmasky, M.E. 2010. Aminoglycoside modifying enzymes. *Drug Resist. Updat.* 13, 151-171.

Siddiqui, M.A.A. and Keating, G.M. 2005. Pegaptanib:in exudative age-related macular degeneration. *Drugs* 65, 1571-1577.

Bera, S., Zhanel, G.G. and Schweizer, F. 2010. Antibacterial activities of aminoglycoside antibiotics-derived cationic amphiphiles. Polyo1-modified neomycin B-, kanamycin A-, amikacin-, and neamine-based amphiphiles with potent broad spectrum antibacterial activity. *J. Med. Chem.* 53, 3626-3631.

Soheili, V., Taghdisi, S.M., Khayyat, M.H., Bazzaz, B.S.F., Ramezan, M. and Abnous, K. 2016. Colorimetric and ratiometric aggregation assay for streptomycin using gold nanoparticles and a new and highly specific aptamer. *Microchim. Acta* 183, 1687-1697.

Stage, T.K., Hertei, K.J. and Uhlenbech, O.C. 1995. Inhibition of hammerhead ribozyme by neomycin. *RNA* 1, 95-101.

Strehlitz, B., Reinemann, C., Linkorn, S. and Stoltenburg, R. 2012. Aptamers for pharmaceuticals and their application in environmental analytics. *Bioanal. Rev.* 4, 1-30.

Zapp, M.L., Stern, S. and Green, M.R. 1993. Small molecules that selectively block RNA binding of HIV-1 Rev protein inhibit Rev function and viral production. *Cell* 74, 969-978.