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Targeting miRNA by tunable small molecule binders: peptidic aminosugar mediated interference in miR-21 biogenesis reverts epithelial to mesenchymal transition†

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Epithelial to mesenchymal transition (EMT) is a process in which epithelial cells lose cell polarity and cell-cell adhesion and gain migratory and invasive properties to become mesenchymal cells that are very vital for development, wound healing and stem cell behavior and contribute pathologically to fibrosis and cancer progression. miR21, a potent regulator of the tumor suppressor gene PTEN, can be silenced to reverse EMT, thereby providing an attractive target for abrogating the malignant behavior of breast cancer. Here, we report the design, synthesis and binding of a peptidic-aminoglycoside (PA) based chemical library against pre-miR21 that led to the identification of a group of small molecules that bind to pre-miR21 with high affinities and antagonize miR-21 maturation and function, thereby reversing EMT. The approach described here offers a promising miRNA targeting platform where such aminosugar conjugates can be similarly used to target other oncogenic miRNAs. Minor changes in the amino acid sequence allow us to tailor the binding effectiveness and downstream biological effects, thus making this approach a potentially tunable method of regulation of miRNA function.

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Introduction

Epithelial to mesenchymal transition (EMT) is a key cellular process that is highly regulated and occurs during embryogenesis and is a vital characteristic of stem cells that govern development.¹ Epithelial cells possess the features of cell-cell adhesion, attachment to the basement membrane, and apico-basal polarity, and are avascular and innervated in nature. In certain biological states, epithelial cells undergo a series of changes in their signaling pathway due to activation of regulated transcription factors. This leads to the loss of their epithelial properties that are replaced with invasive features observed as motility and migratory behavior – thereby acquiring mesenchymal characteristics.² Such a change in the nature of cells is known as epithelial to mesenchymal transition (EMT). EMT can broadly be seen in three different biological conditions. First, during the development of an embryo; this phe-

nomenon takes place to form the diverse organ systems originating from the different lineage of stem cells. Second, EMT occurs during wound healing and tissue regeneration where specific signaling pathways become activated due to inflammatory responses and some shut down due to a negative feedback mechanism upon reduction in inflammation. The loss of function of genes involved in the negative feedback mechanism leads to continued inflammation that leads to a pathological state of fibrosis finally resulting in organ destruction. The third and more pathologically chronic state driven by EMT happens in cancer.¹ Cancer in its preliminary stage is known to cause incessant growth of epithelial cells leading to the escalation of the primary tumor, followed by angiogenesis. During the later stages, the tumor milieu favors the cell transition from an epithelial to mesenchymal state, which advances to metastasis. This facilitates localization of the cancer cells from the primary tumor site to distant sites where they colonize into a new niche and revert to their epithelial characteristics *via* mesenchymal to epithelial transition (MET) and start forming secondary or tertiary tumors. The exact mechanism behind the onset of EMT in cancer is not yet deciphered.^{1–3}

It is known that in certain cancers, the cause and progression of EMT is marked by certain highly up-regulated and

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abundant microRNAs (miRs).^{4–15} miRs are small RNA sequences of about 22 nucleotides in length and they target their destined mRNA sequences and cause mRNA decay or translational repression that leads to reduction in the protein produced from it.^{16–20} miRs are known to govern ~60–70% of the regulation of normal cellular activities. However, specific up-regulated miRs in cancer target the tumor suppressor genes thereby allowing unprecedented proliferation of cells and thus acting as oncogenes. Hence, upregulated miRs in cancer are also called onco-miRs.^{4–6,8,11–15} MicroRNA-21 (miR21) is one such onco-miR that is highly up-regulated in breast cancer.^{21–24} miR21 is also known to target and repress the synthesis of proteins such as PTEN (phosphatase and tensin homolog),^{21,25} PDCD4 (programmed cell death 4),^{25,26} Bcl-2 (B-cell lymphoma-2), *etc.* miR21 has tumor suppressor properties and is involved in pathways inducing apoptosis. The loss of PTEN is also known to enhance the EMT mechanism.^{25,27}

Epithelial and mesenchymal properties of cells are distinguished by certain cellular markers. Epithelial to mesenchymal transitions are induced *via* up-regulation of transcription factors like Snail, Slug and Zeb1. The PI3K/Akt pathway governs the activation of Snail, which in the presence of PTEN is hindered as it inhibits the pathway. However, when PTEN itself is inhibited by miR21, this regulation is not maintained and thus Snail is transcribed which further downstream represses epithelial markers like E-cadherin present in the tight junctions of cells which is also responsible for cell–cell contact. The loss of epithelial markers gives rise to mesenchymal characteristics that is marked by upregulation of vimentin across the cytoskeleton of cells. Vimentin guides cytoskeletal rearrangements throughout the cell through formation of lamellipodia and filopodia, which helps the tumor cells exhibit their motility feature.^{1,25,28}

Small molecules are promising candidates for therapeutic application for inhibition of miRs involved in cancer. Small molecules have an advantage over antisense microRNAs (anti-miRs) in their ease of cellular uptake and have lower dissociation and diffusion rates inside the cell. Additionally, small molecules can be stable for longer durations within cells than anti-miRs and can result in lower toxicity.^{29–34} Small molecules have been shown to bind to the bulged loops of the precursor miR secondary structure and competitively inhibit the binding of Dicer from further processing it to mature miR.^{32,34,35} Here, we have developed an in-house miRNA targeted library of peptidic-aminosugar (PA) conjugates that consist of small peptides conjugated to the aminoglycoside kanamycin (PA-Kan) that bind to and cause inhibition of pre-miR21 processing. After screening for binding using our in-house developed screening method,³⁶ the PAs with high affinities were further screened *in cellulo* using the MCF-7 breast cancer cell line. Promising *in cellulo* hits were further processed to determine their efficacy in inhibiting miR21 and in up-regulating its cognate target protein levels. This was followed by binding site analysis using molecular docking and simulation. The most potent PA-Kan conjugate was further screened for its competence in impeding proliferation

and EMT. Inhibition of highly aberrant miR upregulation that also hinders EMT in cancer cells is expected to prevent metastasis and further aggravation of the disease.

Results & discussion

Syntheses of peptidic aminosugar-kanamycin conjugates

The six compounds identified from our preliminary screens containing diamino acid conjugated kanamycin were re-synthesized by modifying the standard 9-fluorenylmethoxycarbonyl (Fmoc)-based solid phase peptide synthetic strategy. Six L-amino acids (arginine, aspartic acid, tyrosine, threonine, cysteine, and phenylalanine) were studied in this library. Solid phase syntheses of kanamycin conjugates were carried out using Fmoc-PAL-PEG-PS resin as shown in Scheme 1. The growing peptide chain was attached to the MBHA resin *via* an amide linkage. The kanamycin acid derivative (R₃-COOH, as shown in Scheme 1) was coupled with different diamino acid conjugates on the resin beads using HCTU (2-(6-chloro-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylammonium hexafluorophosphate) as a coupling reagent. With the initial success, our last goal was to remove all the molecules from the solid support and achieve global deprotection to obtain a peptidic aminosugar-kanamycin-conjugate library. Successful global deprotection was achieved by using a TFA (trifluoroacetic acid)/phenol/water/TIPS (triisopropylsilane) (88/5/5/2 v/v) mixture. The detailed experimental procedures and characterization data (NMR and HRMS/MALDI-TOF) of all the compounds (DPA 1930, 1967, 2042, 2084, 2089, and 2093) are shown in the ESI.† The solution-phase synthetic procedure for the kanamycin acid derivative and characterization data for all the intermediate compounds are reported elsewhere (manuscript in review).

In vitro screening results

A variety of cationic amphiphilic peptides can transverse the cytoplasmic membrane to interact with internal targets. Aminoglycosides bind specifically to the A-site region of the bacterial 16S rRNA and can bind to other RNA moieties as well. Previously, we developed a fluorescent probe displacement assay using fluorescein conjugated to neomycin (F-neo) to screen various novel small molecules for binding nucleic acids in a high throughput format.^{37–42} We have used this fluorescence displacement assay to screen the PA-Kan conjugate library for binding to pre-miR21. The stoichiometry of F-neo binding to pre-miR21 was determined by addition of increasing concentrations of pre-miR21 with 100 nM F-neo. The emission intensity of F-neo decreased as the concentration of pre-miR21 increased indicating the binding of F-neo as observed by the quenching of fluorescence. The titration plot demonstrates the change of emission intensity as a function of [pre-miR21]:[F-neo] (Fig. 1). Graphical analysis estimated the approximate binding stoichiometry of 0.132 pre-miR21 for each F-Neo. This corresponds to approximately 7.6 (8) molecules of F-neo for one molecule of pre-miR21. The thermodynamically favored structure of the pre-miR21

Scheme 1 Solid-phase syntheses of peptidic aminosugar-kanamycin conjugates DPA 1930, 1967, 2042, 2084, 2089, 2093.

created by UNAFold (Fig. S25†) revealed that the pre-miR21 has several bulges that could serve as potential binding sites for PA-Kan conjugates and kanamycin.

PA-Kan conjugates were screened at a single point concentration against pre-miR21 by competitive displacement of F-Neo by PA-Kan conjugates and then checked using IC_{50} determination (Fig. 2). The percent binding to pre-miR21 was determined for each PA-Kan conjugate relative to kanamycin (Table S1†). The positively charged amino acid arginine at the first position of the PA-Kan conjugates (KanR*) assists the binding as reported in Table S1.† All the KanR* conjugates bind to pre-miR21 better (154–107%) than kanamycin (100%) alone. In addition, cysteine at the second position (Kan*C) significantly improves the binding properties of PA-Kan conjugates. For example, DPA 2084 (KanRC) (154%),

Fig. 1 A representative titration plot of 100 nM F-neo with pre-miR21 in 10 mM sodium phosphate, 25 mM potassium chloride, and 0.05 mM EDTA, pH 6.5. F-neo (100 nM) was titrated with increasing concentrations of pre-miR21. Relative fluorescence (RTF) emission intensity is plotted vs. [pre-miR21]/[F-neo] ratio.

DPA 2089 (KanFC) (140%), and DPA 2093 (KanYC) (133%) are among the top ten conjugates that bind to pre-miR21. Aromatic groups such as phenylalanine (F) and tyrosine (Y), without an arginine or cysteine (KanF* and KanY*), show reduced affinity towards pre-miR21 (70–5%), suggesting that small perturbations in the PA structure can have dramatic effects on binding to miR.

Determination of miR-21 transcript levels after treatment with test molecules

PA-Kan conjugates have a unique deoxystreptamine core with side chains consisting of amino acid arrangements conjugated to kanamycin that can enter the cell without the use of a transfecting reagent. It was hypothesized that the PA-Kan conjugates could target the miR21 at its precursor stage (pre-miR21) in cells and prompt inhibition of maturation. The level of mature-miR21 transcripts was therefore measured by quantitative RT-PCR. Low cellular levels of mature miR21 would indicate that treatment with PA-Kan conjugates inhibits processing of pre-

Fig. 2 A representative IC_{50} titration plot of pre-miR21:F-neo complex with kanamycin. Pre-miR21 at 12.5 nM was mixed with 100 nM F-neo and kanamycin was titrated by serial dilution to achieve a concentration ranging from 50 nM to 12 nM. Experiments were done in triplicate and the average emission intensity RTF (relative fluorescence) is plotted as a function of $\log[KAN]$, nM.

miR21 to the mature form. The cellular level of mature miR21 transcripts for cells treated with PA-Kan conjugates, kanamycin sulfate and kanamycin TFA was compared to the amount of mature miR21 produced in the presence of two positive controls known to bind to and inhibit maturation of pre-miR21 (streptomycin and antisense-microRNA-21 (LNA)). PA-Kan conjugates DPA 2089, 1930 and 1967 showed down-regulation of the mature transcript of miR21 with lower levels relative to the positive controls (Fig. 3). Thus, these three PA-Kan conjugates were chosen for further study.

Target protein (PTEN and PDCD4) levels and miR21 maturation inhibition after treatment with PA-Kan conjugates

miR21 is known to be up-regulated in cancer cells with inhibition of the primary proteins involved in EMT such as PTEN which is also involved in apoptosis and cell proliferation. To identify whether the down regulation of miR21 by PA-Kan conjugates could be observed at the translational level, western blots were performed on MCF7 breast cancer cells and the relative level of PTEN produced was determined. Streptomycin and antisense-microRNA (LNA) served as positive controls relative to untreated cells. The PA-Kan conjugate DPA 2089 consistently showed a considerable rise in protein PTEN as compared to the untreated cells and positive controls, indicating inhibition of action by miR21 (Fig. 4). These results corroborate the RT-PCR data. A western blot assay was also performed to check the protein levels of PDCD4, another cognate target of miR21, involved in proliferation and apoptosis pathways. Again, the PA-Kan conjugate DPA 2089 was observed to be very effective in enhancing the PDCD4 levels (Fig. S26†). PA-Kan conjugates DPA 1930 and DPA 1967 did show a rise in protein PDCD4 levels as compared to the other PA-Kan conjugates but were less effective than the PA-Kan conjugate DPA 2089.

In silico binding study of small molecules with hsa-pre-mir-21

An *in silico* approach was used to predict the binding position of PAs to the pre-miRNA (Fig. 5). The first step involved

Fig. 3 Fold change in miR 21 transcript levels in cells treated with kanamycin conjugates at 5 μ M concentration compared to the control samples chosen, measured through qPCR. Samples were normalized to U6 internal control. Data shown in the figures with error bars representing $\pm$ S.D., * $P < 0.1$; ** $P < 0.05$; *** $P < 0.005$ (Student's *t*-test).

Fig. 4 Western blot for PTEN. Fold change in the levels of PTEN (target protein for miR21 governing EMT) for cells treated with kanamycin conjugates at 5 μ M concentration compared to the control samples chosen as measured by the quantitative analysis done for western blots performed. Samples were normalized with the housekeeping gene GAPDH. Hits for the molecules are shown in the figures with error bars representing $\pm$ S.D., * $P \leq 0.05$, (Student's *t*-test).

the prediction of the secondary and tertiary structures of the pre-miR using the mc-fold mc-sym pipeline. The energy-minimized three-dimensional model was obtained. The molecules were built using ChemDraw, followed by OpenBabel and finally were minimized using Maestro9.8. To study the docking poses of the small molecules, we performed molecular docking using AutoDock. The results from the docking experiment are given in Table S2.† The docking results clearly show that the bulges on pre-miR21 act as potential binding sites for the PA-Kan conjugates and miR-21. The best-docked position for each molecule was chosen for an all-atomistic molecular dynamic simulation for 60 ns to check for conformational stability using GROMACS 4.6.1. All the molecules

Fig. 5 PA-Kan conjugates and kanamycin bind to groove-sites of pre-miR-21. (A) DPA 1930 (B) DPA 2089 (C) DPA 2093 and (D) DPA 1967. The miR21 is represented in grey. The highest affinity sites restricting the stoichiometry of complexes to 1 : 1 are shown here.

with high affinities showed stable interactions with the pre-miRNA. Specifically, we found that the aminosugar kanamycin imparts a very stable interaction with this pre-miR, and amino acids further impart a clear level of specificity in determining the sites of binding (Fig. S27†).

Binding affinity studies for miR21 with molecules

The binding analysis curves were obtained from Octet® RED96 for selected PA-Kan conjugates and were further validated with the data obtained from RT-PCR and western blot assays, yielding support for the role PAs play in the inhibition of miR21 maturation. Binding association and disassociation patterns and the K_D values observed for PAs and pre-miR21 were compared (Table S3†). DPA 1930 and DPA 2089 showed strong binding and a minimum dissociation rate over the first two concentrations (8 nM and 16 nM). The fold change in the dissociation rate increased for DPA 1930 (Fig. S28†) as compared to DPA 2089 (Fig. 6). A similar trend was reflected with the K_D values obtained, corroborating that DPA 2089 has a greater binding affinity for pre-miR21.

Checking the efficiency of molecules in controlling cell proliferation

The nucleolus is a dynamic region present within the nucleus of the cells that often undergoes degeneration as well as regeneration, reversibly in the case of cell division, and irreversibly in the case of cell apoptosis. There are many proteins that are involved which alter the fate of the nucleolus. Ki-67 is a major protein whose primary function is to transcribe ribosomal RNA (rRNA). Destruction of Ki-67 would lead to termination of rRNA production. Localization of Ki-67 within the nucleus changes as the cell cycle progresses. During the cell interphase, Ki-67 resides within the nucleolus but as the cell cycle progresses the nucleolus disintegrates and Ki-67 mobilizes to the surface of chromosomes during mitosis forming bright speckles that can be observed microscopically across the nucleus. MCF-7 breast cancer cell lines are known to have a very rich content of Ki-67 and its amount increases

as the cell cycle progresses from the interphase towards cell division. Throughout the mitotic phase, the amounts of Ki-67 remain the same which stays dispersed across the nucleus. These hallmark characteristics make Ki-67 a potent marker for cellular proliferation.

Given that PA-Kan conjugates (especially DPA 2089) appeared to inhibit the maturation of pre-miR in MCF7 breast cancer cells as observed by both transcription and translation assays, it was hypothesized that these compounds should affect the cell proliferation rate. Repressed levels of mature miR21 in cancer cells would lead to an increase in proteins involved in controlling cell proliferation. We monitored the production of Ki-67 in MCF-7 breast cancer cells exposed to the PA-Kan conjugate DPA 2089 *via* fluorescence microscopy (Fig. S29†). Untreated MCF7 cells and those treated with kanamycin sulphate showed a considerable amount of bright circular speckles dispersed all across the nucleus signifying a proliferative state of the cells. Another control used was the PA-Kan conjugate DPA 2093 that was least effective to cause down-regulation of miR21 as a negative control. From the set treated with the PA-Kan conjugate DPA 2089 the difference observed was quite stark as the presence of Ki-67 spots was dramatically reduced (Fig. S29†).

Cytotoxicity assay of the PA-Kan conjugate DPA 2089 on MCF-7 breast cancer cells

Cell viability is the amount of live cells present relative to dead cells. A decrease in viability indicates increased toxicity imposed on cells due to stress. Here, we used the MTT assay to assess the cytotoxicity of the PA-Kan conjugate DPA 2089. Compared to untreated cells, only a 2–4% reduction in cells treated with 2.5 μ M and 5 μ M PA-Kan conjugate DPA 2089 was observed (Fig. S30†).

Efficiency of the PA-Kan conjugate DPA 2089 to revert EMT in the MCF-7 breast cancer cell line

Epithelial to mesenchymal transition (EMT) is one of the major hallmarks of cancer. Due to this phenomenon, the cells lose their adherent capacity and gain the ability to translocate to different parts of the body and form secondary tumors. This process is known as metastasis. When cancer cells become more progressive (usually at later stages of the disease) cells become metastatic and spread to different organs. In breast cancer, miR21 is highly upregulated, blocking the action of many essential driver proteins in various important pathways. Several such target proteins of miR21 act as gatekeepers for the epithelial to mesenchymal transition of cells, such as PTEN. Inhibition of such proteins would lead to more mesenchymal characteristics of cells and thus greater metastasis. PTEN, as discussed earlier, is a direct inhibitor of the PI3K/Akt pathway and is involved in the upregulation of the Snail transcription factor that negatively regulates E-cadherin. The loss of E-cadherin leads to cell-cell contact inhibition and thus induction of

Fig. 6 Binding kinetics of DPA 2089 to pre-miR21 over the concentration range of 8–128 nM DPA 2089.

EMT. Since the PA-Kan conjugate DPA 2089 was shown to be a potent repressor of pre-miR21 maturation and inhibits proliferation, we postulated that it may also inhibit EMT. Some of the very vital characteristics of EMT are losing cell-cell contact and achieving more lamellipodia and filopodia-like structures that help the cells to travel to distant sites. Losing focal adhesion and cell-cell adhesion from down-regulation of the tight junction or desmosomal proteins and also rearrangement of the actin cytoskeleton gives the cells more motility features.

Given these motility features involved in EMT, two characteristic protein markers (E-cadherin and vimentin) were chosen for identifying EMT. E-cadherin is an essential Ca^{2+} dependent cell adhesion protein that is highly downregulated when cells undergo EMT. Similarly vimentin, a type III intermediate filament protein, is highly upregulated when cells undergo EMT. The most potent among the PA-Kan conjugates tested, DPA 2089, was screened for its ability to affect EMT. The results clearly show a higher level of E-cadherin in treated cells indicating that the epithelial characteristics were

Fig. 7 Images shown for EMT analysis done by immunofluorescence assays; (A) the comparative difference among the untreated cells, cells treated with Kan-TFA and with DPA 2089 for the E-cadherin marker. (B) The comparative difference between the untreated cells, cells treated with Kan-TFA and cells treated with DPA 2089 for the vimentin marker. The cells were viewed under 100 $\times$ magnification. Quantification of the image (done by ImageJ) has been provided to demonstrate the effect of the molecules on the inhibition of EMT by an increase or decrease in the percentage of intensity as compared to the control cells for the given markers. The experiment has been done in three replicate measurements and the quantified figures show error bars representing $\pm$ S.D., * $P < 0.1$; ** $P < 0.05$; *** $P < 0.005$ (Student's t -test).

restored whereas untreated cells were prone towards mesenchymal transition (MT) (Fig. 7a). In comparison, the vimentin marker was observed to be nearly absent in DPA 2089 treated cells but prominent in the cytoplasm of untreated cells as well as cells treated with Kan-TFA, indicating the potency of DPA 2089 in the restoration of epithelial characteristics and prevention of metastasis (Fig. 7b).

Effect of the PA-Kan conjugate DPA 2089 to check migration in the MCF-7 breast cancer cell line

miR21 is known to induce migration of cells. Inhibition of miR21 can reduce the migratory behaviour of cells. The MCF7 breast cancer cell line was used as a model to investigate this effect upon treatment with the PA-Kan conjugate DPA 2089. The wound healing assay or scratch assay was performed to observe this effect. After 48 hours of administration of the wound, it was evident in the untreated control cells as well as cells treated with the control Kan-TFA that closure of the wound was greater than for the cells treated with DPA 2089, as shown in Fig. S31.† Quantification of the images also showed clearly that 50% or more of the wound area was enclosed in the control cells as compared to DPA 2089 treated cells where the wounded area remained almost the same. Thus, DPA 2089 was quite effective in mitigating the migratory behaviour of the cells by suppressing the miR21 levels.

Initial antibacterial screening and minimal inhibitory concentration (MIC) determination of PA-Kan conjugates

Aminoglycosides such as kanamycin inhibit bacterial growth by binding to rRNA thereby interfering with protein translation machinery. Therefore the PA-Kan conjugates were assessed for their potential for bacterial growth inhibition. Antibacterial activity was determined for the six compounds and kanamycin controls (Table 1). Kanamycin is active against the aminoglycoside sensitive ATCC strains of *Escherichia coli* and *Staphylococcus aureus*. Except for MRSA, most compounds had limited and insignificant activity with the other strains. Because very little inhibition is exhibited by

these compounds against Gram positive and Gram negative strains, coupled with the relative non-toxicity of these compounds (5 μ M) for eukaryotic cells, it is suggested that translation inhibition is unlikely as the mechanism of action of these six compounds. It must be noted that at least for the Gram negative strain *E. coli*, the decrease in activity, compared to kanamycin, may be due to the effects of the Gram negative outer membrane. But the similar decrease in activity against the kanamycin sensitive *S. aureus* strain suggests that such permeability issues may not be key drivers in the lack of activity of these compounds.

Conclusions

MicroRNAs are being identified as unique drug targets, with various approaches being developed to target the specific micro-RNAs that lead to cancer. Our approach here focused on developing novel compounds that target upregulated microRNAs such as miR21 involved in cancer progression. The class of compounds studied here are peptidic-kanamycin aminosugars. Using a combination of RNA groove binding aminosugar (kanamycin) and potential amino acid contacts with the miR21 secondary structure, we have identified small molecules with a regulatory effect on microRNA maturation and inhibitory effect on cancer progression. Small perturbations in the amino acid chain composition lead to significant effects on miR21 binding and downstream effects associated with such binding. The work here demonstrates the effectiveness of such PAs in binding to pre-miR21 and inhibiting miR21 maturation, disrupting its ability to regulate expression of specific proteins involved in cancer progression such as PTEN, along with disruption of the epithelial to mesenchymal transition that can result in metastasis. Kan-FC (DPA 2089) was among the tightest binders, and the most potent candidate which inhibited pre-miR21 maturation making miR21 ineffective in regulating the expression of PTEN, effectively reducing the production of Ki-67 and reversing the epithelial to mesenchymal transition in MCF-7 breast cancer cells. Kan-FC was effective at low concentrations that resulted in significant optimum inhibitory effects without causing cellular death or toxicity. Importantly, the dissociation from the target miR21 was relatively slow, providing a relatively long-lasting effect on the inhibition of micro-RNA until the cells undergo apoptosis or until the microRNA degrades. These features of targeting regulation of miRNA biogenesis by small molecules that are relatively stable over the time course of treatment offer an alternative approach over antisense drug delivery in cancer therapy. EMT is the major cause of cancer at late stages that leads to higher mortality rates among patients. That these PA-Kan conjugates inhibit cell proliferation and metastasis by reducing the progression of EMT may offer immense therapeutic potential in reverting EMT by targeting specific microRNAs involved in cancer progression. Further studies elucidating compound tunability and ligand selectivity in other miR recognition processes are underway and will be reported in due course.

Table 1 Minimum inhibitory concentration of kanamycin against four bacterial strains and the percent growth inhibition of each bacterial strain with 64 μ M PA-Kan conjugates (NI = no inhibition)

DPA#	<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 25923	<i>P. aeruginosa</i> ATCC 27853	MRSA ATCC 1960649
Minimum inhibitory concentration (μ M)				
Kanamycin	4	4	>256	>256
Percent growth inhibition with 64 μ M kanamycin or conjugates				
Kanamycin	100	100	11 $\pm$ 5	41 $\pm$ 6
1930	25 $\pm$ 0	29 $\pm$ 9	11 $\pm$ 2	30 $\pm$ 3
1967	NI	NI	NI	NI
2042	NI	NI	NI	NI
2084	13 $\pm$ 5	20 $\pm$ 4	NI	25 $\pm$ 4
2089	NI	NI	NI	22 $\pm$ 2
2093	NI	34 $\pm$ 10	NI	38 $\pm$ 1

Conflicts of interest

DPA has an ownership interest in NUBAD LLC.

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