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In vitro selection of RNA aptamers that selectively bind danofloxacin

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ABSTRACT

Danofloxacin is a synthetic fluoroquinolone with broad spectrum antibacterial activity that is used for the treatment of respiratory diseases in animal husbandry. However, danofloxacin has many adverse reactions and is toxic to humans. Especially, it detrimentally affects muscle, central nerve system, peripheral nerve system, liver, and skin in those who ingest foods in which danofloxacin has accumulated. Prescreening and determination of the level of danofloxacin in foods or food products is necessary for human health. Aptamers are composing of oligonucleotides that specifically interact with target molecules. They are emerging as detection/diagnostic ligands. Here, we used the SELEX *in vitro* selection technology to identify specific and high-affinity RNA aptamers with 2′-fluoro-2′-deoxyribonucleotide modified pyrimidine nucleotides against danofloxacin. Selected RNA aptamers bound specifically to danofloxacin, but not to tetracycline. Truncation of RNA aptamer up to 36 mer did not comprise specificity and affinity. The truncated RNA aptamer specifically bound to target chemical, allowing the discrimination of danofloxacin from other fluoroquinolones. The isolated specific aptamer could be a potential agent used for the rapid and cost-effective detection and sensing of danofloxacin, replacing instrumental methods including the more expensive and time-consuming methods of high performance liquid chromatography and liquid chromatography/mass spectrometry.

1. Introduction

Fluoroquinolones are a class of synthetic and broad spectrum antibacterial drugs used for human and veterinary diseases. Danofloxacin is a fluoroquinolone used exclusively for animal husbandry. It internalizes via bacterial membrane porin protein, and interferes with bacterial DNA replication by inhibiting DNA gyrase. Danofloxacin activity includes gram-positive, gram-negative bacteria, and *Mycoplasma* [1]. However, use of fluoroquinolones including danofloxacin can leave residual drug in food animals, which can trigger development of bacterial resistance. Permissible and allowed maximum residue levels (MRLs) for fluoroquinolones range from 30 to 1500 μg kg^{-1} in many countries [2]. For example, the European Union permits MRLs of danofloxacin in fish ranging from 100 to 600 μg kg^{-1} [3]. Danofloxacin use is forbidden in milk-producing dairy cattle for human consumption and in laying hens. Fluoroquinolones cause adverse effects to human tissues in the gastrointestinal (GI) tract, skin, and central nervous system (CNS). GI effects include nausea and vomiting, and CNS reactions include severe and include dizziness, convulsions, and psychoses [4]. The United States Food and Drug Administration (FDA) forbids use of danofloxacin as a human medicine. The selective detection of danofloxacin from food animals is thus very important for human healthcare. Methods for the detection of fluoroquinolones including danofloxacin comprise liquid chromatography with fluorescence detection [4,5], mass spectrometry [6], and antibody-based immune assay [2,7]. Most of methods are time-consuming, require extensive sample preparation, and are cost-ineffective.

Aptamers are single-stranded nucleic acids that can bind specifically to target molecules [8]. The targets range from small molecules and proteins to cells and even tissues. Aptamers can be selected from a randomized combinatorial oligonucleotide library by the iterative *in vitro* selection techniques of systematic evolution of ligands by exponential enrichment (SELEX). Aptamers have many advantages as diagnostic agents including high affinity and specificity, reproducibility and batch-to-batch homogeneity through chemical synthesis, stability, and easy modifiability through conjugation of various functional molecules.

In this study, we identified RNA aptamers specific to danofloxacin with modified 2′-fluoro-2′-deoxyribonucleotide pyrimidines that confer RNase resistance for use as diagnostic agents. We characterized the selected aptamers and analyzed their optimal

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binding sequences and structures through an aptamer truncation experiment. The optimized aptamer bound to danofloxacin with high affinity and high specificity, allowing discrimination from other fluoroquinolones.

2. Materials and methods

2.1. Antibiotics and biotinylation

Danofloxacin, ciprofloxacin, enrofloxacin, difloxacin, marbofloxacin, kanamycin, and tetracycline were purchased from Sigma–Aldrich (St. Louis, MO). Danofloxacin (5.2 mg) was dissolved in 400 μl dimethylsulfoxide (DMSO) and incubated with 7.1 mg EZ-link iodoacetyl LC biotin in 800 μl 10 mM 2-(*N*-morpholino) ethanesulfonic acid (MES), pH 5.0, at 37 °C for 16 h. Tetracycline (12 mg) was dissolved in 400 μl DMSO and incubated with 11 mg EZ-link iodoacetyl LC biotin in 800 μl 10 mM *N*-cyclohexyl-2-aminoethanesulfonic acid (CHES), pH 12.5, at 37 °C for 16 h. Biotinylated antibiotics were then purified by high performance liquid chromatography (HPLC).

2.2. Selection

The initial RNA library was generated by *in vitro* transcription of synthetic DNA template with 2′-deoxy-2′-fluoro pyrimidine nucleotides (Epicentre Biotechnologies, Madison, WI), 2′-hydroxyl normal purine nucleotides, and T7 RNA polymerase (Epicentre Biotechnologies, Madison, WI). The sequence of RNA library was 5′-GGGAUACCAGCUUAUUCAAUN$_{60}$AGAUAGUAAGUGCAAUCU-3′ where the N_{60} represents equimolar incorporation of A, G, C, and U. The RNA library (3 nmole) was first incubated with biotinylated tetracycline (600 pmol) in SELEX binding buffer (30 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1.5 mM $MgCl_2$, 2 mM dithiothreitol, and 1% bovine serum albumin, BSA) for counter-SELEX. Tetracycline-bound RNA library was removed by streptavidin-coated agarose beads (Sigma–Aldrich, St. Louis, MO). The supernatant was further incubated with 20 μl streptavidin-coated agarose beads (Sigma–Aldrich, St. Louis, MO). The pre-cleared RNA pool was then incubated with biotinylated danofloxacin (150 pmol) in 200 μl SELEX binding buffer. Bound RNA was recovered, amplified by RT-PCR, *in vitro* transcribed, and used for next SELEX round, as previously described [9]. After the sixth selection round, the amplified cDNA pool was cloned and sequenced.

2.3. Fluorometer assay

Biotinylated danofloxacin or tetracycline (100 pmol) was immobilized on a streptavidin-coated 96-well black plate (Nunc, Penfield, NY) in 50 μl diethylpyrocarbonate (DEPC)-treated distilled water (dH_2O) for 30 min. After immobilization, non-immobilized danofloxacin or tetracycline was washed out twice with 200 μl DEPC-treated dH_2O. The 3′ terminal extended RNA aptamer (A16 tail, 10 pmol) was hybridized with 5-fluorescein (FAM)-conjugated oligo-dT at room temperature for 30 min. Hybridized RNA aptamer was incubated in an empty well (streptavidin control), danofloxacin-coated well, or tetracycline-coated well in 100 μl SELEX binding buffer at room temperature for 20 min. Unbound hybridized RNA aptamers were removed and washed three times. Each well was then blocked using 200 μl of blocking solution (3% BSA, 0.05% Tween-20, 1.5 mM $MgCl_2$, 1× phosphate buffered saline, PBS) at room temperature for 30 min. After blocking, anti-FAM-horseradish peroxidase antibody (Invitrogen, Carlsbad, CA) was added and incubated in antibody binding buffer (0.05% Tween-20, 1.5 mM $MgCl_2$, 1× PBS) at room temperature for 15 min. QuantaBlu peroxidase substrate solution was added and the level of bound RNA aptamer was measured using a fluorometer (Thermo Scientific, Pittsburgh, PA) with Excitation/Emission spectra of 325 nm/420 nm.

2.4. Aptamer truncation

Truncated RNA aptamers were constructed by PCR and *in vitro* transcription. Primers for the PCR were; dano-I 59 mer: forward (5′-GGTAATACGACTCACTATAGGGTGACTTTTCCCTCAGGCTCCTG-3′), reverse (5′-GGGCGGTTTCATTTCAGTCCATTCGGTTGCTTCACAGGAGCCTG-3′); dano-I 47 mer: forward (5′-GGTAATACGACTCACTATAGGGTCCCTCAGGCTCCTGTG-3′), reverse (5′-GGGTCATTTCAGTCCATTCGGTTGCTTCACAGGAGCCTG-3′); dano-I 36 mer: forward (5′-GGTAATACGACTCACTATAGGGTCAGGCTCCTGT-3′), reverse (5′-GTCAGTCCATTCGGTTGCTTCACAGGAGCCTGA-3′); dano-I 24 mer: forward (5′-GGTAATACGACTCACTATAGGGCTGTG-3′), reverse (5′-GGGCATTCGGTTGCTTCACAGCCCTAT-3′); dano-I 21 mer: forward (5′-GGTAATACGACTCACTATAGGGCTGTG-3′), reverse (5′-CATTCGGTTGCTTCACAGCCCTAT-3′); dano-II 64 mer: forward (5′-GGTAATACGACTCACTATAGGGTCGGCCCAATTTT-3′); reverse-1 (5′-ACACTAGATACTGAAGTCCGAAGGTAAAATTGGGC-3′), reverse-2 (5′-GGGTCTCGCTATGATTTAAAGCCACACTAGATA-3′); dano-II 43 mer: forward (5′-GGTAATACGACTCACTATAGGGTTTACCTTCGGACT-3′), reverse (5′-GGGTTTAAAGCCACACTAGATACTGAAGTCCGAAGG-3′); and dano-II 27 mer: forward (5′-GGTAATACGACTCACTATAGGGTCGGACT-3′), reverse (5′-GCCACACTAGATACTGAAGTCCGACCC-3′).

2.5. Competition assay

Dano-I 36 mer truncated RNA aptamer was extended at its 3′ terminus with A16 (10 pmol) and was hybridized with 5-FAM-conjugated oligo-dT at room temperature for 30 min. Hybridized dano-I 36 mer aptamer was then treated with competitor RNAs in a 96-well plate immobilized with biotinylated danofloxacin. Amount of bound dano-I 36 mer hybridized aptamer was measured using a fluorometer.

2.6. Surface Plasmon Resonance (SPR) assay

The SPR assay utilized a Biacore 2000 apparatus (GE Healthcare, Fairfield, CT). For immobilization of biotinylated antibiotics or biotin-conjugated oligo-dT hybridized RNA aptamer, the streptavidin-coated sensor chip (SA chip; GE Healthcare, Fairfield, CT) was used. The SA chip was activated with 50 mM NaOH. Biotinylated antibiotics or hybridized RNA aptamer was injected into the flow cells for the immobilization. For measurement of kinetic value, various concentrations of analytes were injected into the flow cells. Collected sensorgrams were then analyzed using the Biaevaluation program (GE Healthcare, Fairfield, CT).

3. Results

3.1. Selection of RNA aptamers against danofloxacin

The SELEX technology was used for the selection of specific RNA aptamers to danofloxacin. For the selection procedure, danofloxacin was conjugated with biotin (Supplementary Fig. S1), which could be precipitated with streptavidin-coated agarose beads. Biotinylated tetracycline was used as a non-specific antibiotic (negative control). Biotinylated tetracycline was first incubated with library RNA and then tetracycline-bound RNAs were removed by streptavidin-coated agarose beads. The remaining RNA library was incubated with biotinylated danofloxacin and bound RNA was amplified by RT-PCR and *in vitro* transcription. After 6

Sequence (Occurrence)

Dano-I	(4)	5′ GGGUGACUUUUCCCUCAGGCUCCUGUGAAGCAACCGAAUGGACUGAAAUGAAACCGCCC
Dano-II-1	(1)	5′ AAUCGGCCCAAUUUUACCUUCGGACUUCAGUAUCUAGUGUGGCUUUAAAUCAUAGC
Dano-II-2	(1)	5′ ----------U----------
III	(1)	5′ CUUUCCAUCCUAUAAAGUGAAAGGUAUGGACUCGUGAGGAGGAUUCGUUUAUAGGCGC
IV	(1)	5′ ACGCAUUAUCCUACUAGCAUUCCUUGGUUAUGCUCCCCGAUGGCUCCCAACGGGGUCUG
V	(1)	5′ AAUCGGCCCUUCACGUGUCACUUUCAGUCUACACACCGCGGUCUAUGACUGCUUCUC
VI	(1)	5′ AUGCGGCCCUUCACUGAUGCGCCUGUGAUAGACUGAACGGCGAUAAGUGUCUUGUUUC
VII	(1)	5′ AAUCGGCCCAUUCUGCACGCUCCCGCUGAGGUGGUAGCAGUACUGUGACUCUGUUAACC
VIII	(1)	5′ GGAUUGCGAACAGCGUGCCGGAAAACUGCCUUAGCUUGUUGUACGCUGUUUGCUUGUCCG
IX	(1)	5′ CAGUAUCAUAUUGAGUCUUCCCTTACACACCGAACGGGUCGUAGGAAGUAUAUGGUGCG
X	(1)	5′ UAGUUGCGUAGUUGAAAGACUUGCCGUGUUGUGGAGUACUGAAUACACCAUAGUCUUG

Fig. 1. The selected sequences. The lines indicate that nucleotides found at these positions are identical. The pyrimidine nucleotides (C/U) indicate 2′-fluoro-2′-deoxyribonucleotides.

selection rounds, amplified cDNA pool was cloned and sequenced (Fig. 1). Fourteen different clones were sequenced, and the same or similar sequences were found in multiple clones. Among 10 different groups, dano-I and -II RNA aptamers specifically and efficiently bound to danofloxacin, but not to tetracycline (data not shown).

3.2. Optimization and characterization of selected RNA aptamers

The selected dano-I and -II RNA aptamers were truncated for identification of optimal binding sequences. The secondary structure of these aptamers was predicted using the Mulfold program [10] (Fig. 2A). The sequences selected from the randomized sequence of the library in both aptamers were structured as a long stem-loop by intra base-pairs. Based on these configurations, we generated various truncated aptamers with stem loop structure from the dano-I and -II aptamers. Binding of the full size and truncated aptamers to danofloxacin was assessed by fluorometry (Fig. 2B and C). Most of the truncated dano-I aptamers bound to danofloxacin with similar avidity as the full-length dano-I aptamer. None bound to tetracycline (Fig. 2B). Dano-I 36 mer and 24 mer truncated aptamers bound to danofloxacin, but the 21 mer truncated aptamer did not (Fig. 2B, right panel). An apical hairpin-loop structure was disrupted in the case of the 21 mer truncated aptamer. The apical hairpin-loop structure of the 24 mer truncated aptamer was maintained by closing the 5′ and 3′ ends through the addition of three strong G-C base pairs to each end. This suggests that the apical stem-loop sequence and configuration is critical for binding of the dano-I aptamer to danofloxacin. In contrast, truncated aptamers of dano-II did not bind to danofloxacin, indicating the necessity of fixed sequences for binding to danofloxacin in this dano-II aptamer (Fig. 2C). To confirm the specific binding ability of truncated aptamer to danofloxacin, a competition assay between dano-I 36 mer truncated aptamer and non-specific RNAs was done (Fig 2D). Binding of the hybridized dano-I 36 mer truncated aptamer to danofloxacin was efficiently competed by non-3′ terminal extended dano-I 36 mer truncated aptamer. Other RNA aptamers including CEA-specific RNA aptamer, YJ-1 [11], and dano-II truncated aptamer could not disrupt the binding ability of dano-I 36 mer truncated aptamer. However, dano-I 21 mer was slightly competitive with dano-I 36 mer binding to danofloxacin. The reason for the low competition by dano-I 21 mer was due

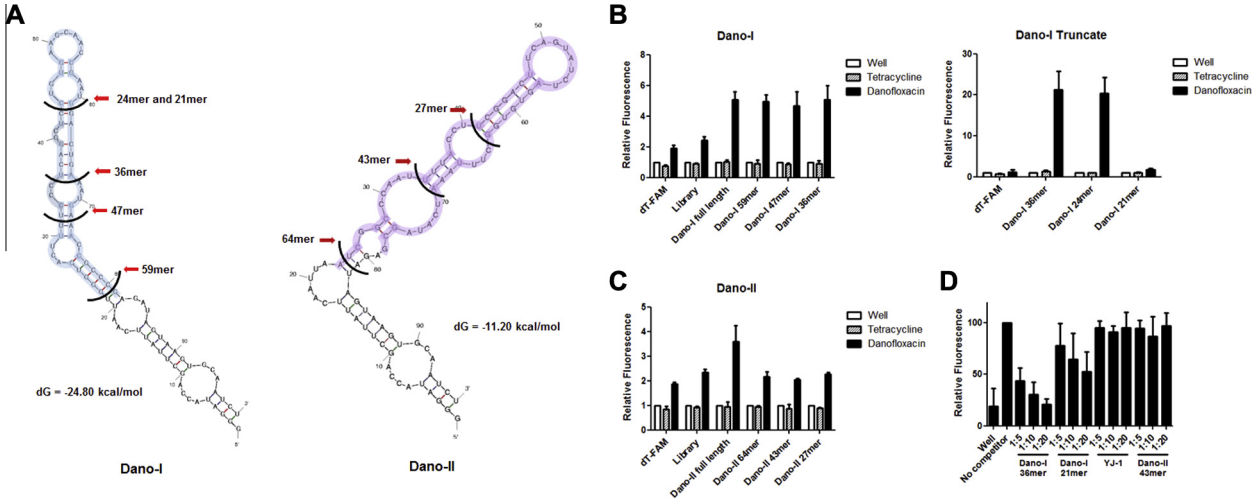
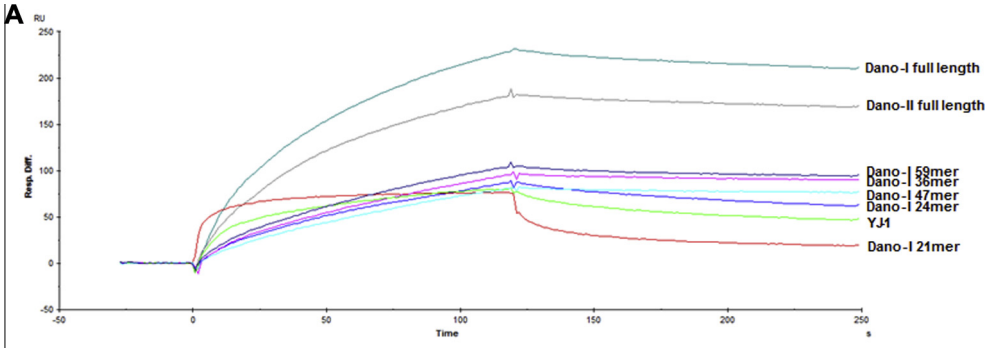


Fig. 2. Secondary structure of selected RNA aptamers and their binding ability to danofloxacin by fluorometer assay. (A) Sequence and predicted secondary structure of dano-I and dano-II RNA aptamers. Arrows indicate position of truncation of aptamer. Binding of dano-I (B) and dano-II (C) to danofloxacin. Full length or truncated aptamers were incubated with biotinylated danofloxacin or tetracycline. Binding ability of each aptamer to each antibiotic is presented as the relative fluorescence to the sample without any antibiotics (empty well). Values represent average of three independent experiments with standard deviation. (D) Specific binding of dano-I 36 mer truncated aptamer to danofloxacin. dT-FAM–dano-I 36 mer A16 hybridized aptamer was incubated with competitor RNAs (non-hybridized dano-I 36 mer truncated aptamer, dano-I 21 mer truncated aptamer, YJ-1, or dano-II 43 mer truncated aptamer) in danofloxacin-coated well. The amount of bound dT-FAM–dano-I 36 mer A16 hybridized aptamer was determined by fluorometry.

A



B

RNA	k_a (1/Ms)	k_d (1/s)	K_D (M)	Chi2
Dano-I full length	$4.28 \pm 0.07 \times 10^5$	$4.28 \pm 3.00 \times 10^{-4}$	$2.99 \pm 2.12 \times 10^{-9}$	4.88 ± 0.04
Dano-I 21 mer	$1.39 \pm 0.63 \times 10^3$	$5.04 \pm 0.06 \times 10^{-3}$	$3.64 \pm 0.21 \times 10^{-6}$	4.74 ± 0.13
Dano-I 24 mer	$3.22 \pm 1.77 \times 10^4$	$1.90 \pm 1.30 \times 10^{-3}$	$56.2 \pm 9.89 \times 10^{-9}$	4.42 ± 0.09
Dano-I 36 mer	$5.16 \pm 3.37 \times 10^4$	$1.77 \pm 1.25 \times 10^{-4}$	$3.36 \pm 0.24 \times 10^{-9}$	3.79 ± 0.63
Dano-I 47 mer	$1.94 \pm 1.88 \times 10^4$	$8.00 \pm 7.36 \times 10^{-5}$	$4.34 \pm 0.43 \times 10^{-9}$	4.78 ± 0.15
Dano-I 59 mer	$2.69 \pm 2.54 \times 10^4$	$1.06 \pm 1.00 \times 10^{-4}$	$3.81 \pm 0.30 \times 10^{-9}$	3.99 ± 1.29
Dano-II full length	$5.32 \pm 0.37 \times 10^4$	$4.06 \pm 0.58 \times 10^{-4}$	$7.71 \pm 0.30 \times 10^{-9}$	4.98
YJ-1	$6.50 \pm 6.20 \times 10^4$	$4.60 \pm 4.20 \times 10^{-3}$	$72.4 \pm 3.39 \times 10^{-9}$	3.45 ± 2.11
Library	$3.89 \pm 1.30 \times 10^4$	$3.82 \pm 1.66 \times 10^{-3}$	$96.5 \pm 10.2 \times 10^{-9}$	4.80 ± 0.02

Fig. 3. Binding affinity of RNA aptamers as assessed by the SPR assay. Biotinylated danofloxacin was immobilized on streptavidin-coated sensor chip. (A) Sensorgram of each RNA aptamer when injected with 100 nM concentration. (B) Affinity of each RNA aptamer to danofloxacin. Values were measured using the BIAevaluation program. At least five different concentrations (5–100 nM) of RNAs were used in each analysis. Chi 2, a value showing the difference between the calculation value by the BIAevaluation program and data obtained from actual experiment, which should be less than 10; k_a, concentration of analyte binding to the target per hour; k_d, concentration of analyte separating from the target per hour; K_D, equilibrium constant showing binding strength.

to its lower binding affinity than dano-I 36 mer (Fig. 3). These results indicated that selected aptamer binds to danofloxacin through a specific selected RNA sequence and structure formation.

3.3. Measurement of binding affinity and ability against danofloxacin and other fluoroquinolones

Binding affinity of the selected aptamers was measured by the SPR assay (Fig. 3A). Dano-I full-length and 59 mer, 47 mer, and 36 mer truncated aptamers, and dano-II full-length aptamer had high affinity to danofloxacin with 3–7 nM K_D (Fig. 3B). Dano-I 21 mer truncated aptamer showed very low affinity (3.64 μM), similar to the fluorometry results. However, dano-I 24 mer truncated aptamer had low affinity (56.2 nM), compared with other dano-I truncated aptamers, contrary to the fluorometry data. Taken together, we concluded that the optimal RNA aptamer that binds to danofloxacin is the dano-I 36 mer truncated aptamer. YJ-1 aptamer and library RNA had 21-fold and 29-fold higher K_D value, respectively, compared with dano-I 36 mer truncated aptamer.

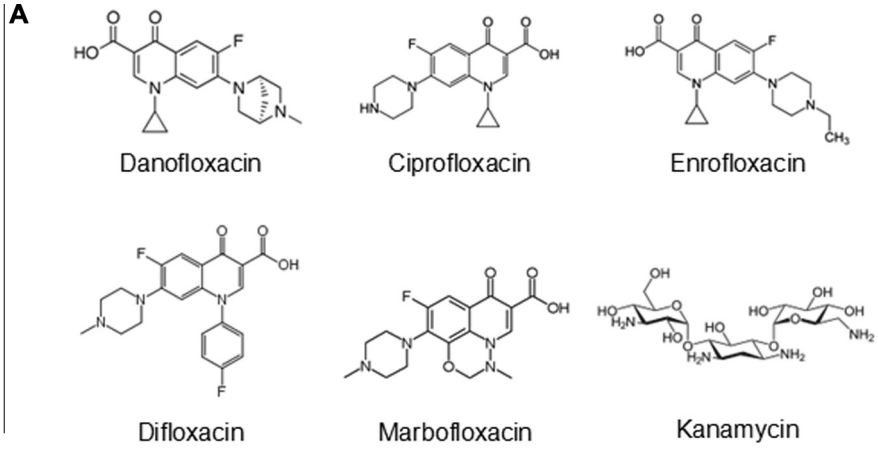
The binding ability of the dano-I 36 mer truncated aptamer to other classes of fluoroquinolones was assessed (Fig. 4A). The 3′ terminal extended aptamer (A16) was hybridized with dT(16)-biotin conjugated oligonucleotides. The hybridized aptamer was immobilized in an SA chip. Five different fluoroquinolones including danofloxacin or kanamycin as the non-fluoroquinolone antibiotic control were injected. Danofloxacin had approximately 14 to 26-fold higher affinity (1.81 nM K_D), compared with other antibiotics (25.5–48.1 nM K_D) (Fig. 4B). This result indicated the binding of the dano-I 36 mer truncated aptamer to danofloxacin with high affinity and specificity, and hence the ability to selectively discriminate danofloxacin from other fluoroquinolones.

4. Discussion

In this study, we identified and characterized RNase-resistant RNA aptamers to danofloxacin. The selected aptamer bound to danofloxacin with high affinity and specificity, compared with its binding ability to other fluoroquinolones, allowing discrimination of differences in chemical structure despite the similar configuration between various fluoroquinolones.

Several aptamers have been developed against synthetic antibiotics [12–15]. The theophylline RNA aptamer can discriminate the methyl group between theophylline and caffeine [12]. However, this aptamer has a low binding affinity to its target chemical (~μM). Streptomycin, tetracycline, and chloramphenicol binding aptamers were also reported through SELEX technology. One of the tetracycline aptamers consists of 2′-hydroxyl oligonucleotides [13] which would not be suitable for the application as a diagnostic agent. Contrarily, the aptamer selected in this study harbored nuclease-resistance and bound to the target chemical, danofloxacin, with high affinity (~2 nM K_D) and high specificity so as to distinguish it from other kinds of fluoroquinolones. Moreover, we optimized danofloxacin binding aptamer sequence and structure. The dano-I 36 mer truncated aptamer has sufficient nucleotide sequence and length and tertiary structure to enable binding to danofloxacin. It is unclear how the aptamer can discriminate

A



B

Antibiotic	*ka* (1/Ms)	*kd* (1/s)	K_D (M)	Chi2
Danfloxacin	$1.15 \pm 7.00 \times 10^{4}$	$2.15 \pm 1.48 \times 10^{-5}$	$1.81 \pm 0.18 \times 10^{-9}$	0.322 ± 0.16
Ciprofloxacin	$2.41 \pm 1.40 \times 10^{3}$	$6.00 \pm 3.15 \times 10^{-5}$	$2.55 \pm 0.20 \times 10^{-8}$	0.227 ± 0.008
Enrofloxacin	$3.48 \pm 2.10 \times 10^{3}$	$1.20 \pm 47.8 \times 10^{-4}$	$4.81 \pm 4.35 \times 10^{-8}$	0.238 ± 0.15
Difloxacin	$2.15 \pm 0.14 \times 10^{3}$	$7.43 \pm 1.80 \times 10^{-5}$	$3.49 \pm 1.07 \times 10^{-8}$	0.264 ± 0.21
Marbofloxacin	$4.08 \pm 1.83 \times 10^{2}$	$1.31 \pm 1.33 \times 10^{-5}$	$2.76 \pm 2.01 \times 10^{-8}$	0.305 ± 0.13
Kanamycin	$2.38 \pm 0.35 \times 10^{3}$	$1.15 \pm 1.09 \times 10^{-4}$	$4.53 \pm 3.93 \times 10^{-8}$	0.545 ± 0.12

Fig. 4. Determination of specific binding ability of dano-I 36 mer truncated RNA aptamer to danofloxacin. (A) Chemical structure of fluoroquinolones and kanamycin. (B) Biotinylated dano-I 36 mer aptamer was immobilized on streptavidin-coated sensor chip. Affinity of the truncated aptamer to fluoroquinolones and kanamycin was analyzed by SPR assay. Values are measured using BIAevaluation program and indicated as Fig. 3(B).

danofloxacin from other families of fluoroquinolones. 5-methyl-2,5-diazabicyclo residue is uniquely present at the terminus of danofloxacin, and hence the residue would be a specific site that the aptamer can use to discriminate between fluoroquinolones. Structural analysis of the complex between aptamer and danofloxacin will provide the clue of the specific interaction mechanism.

Antibody-based indirect competitive enzyme-linked immunosorbent assay was recently reported for the danofloxacin detection. This assay was designed to detect danofloxacin in food animal livers using anti-danofloxacin antibody [2]. Antibody-based detection has benefits in terms of simplicity, speed, and sensitivity. However, the approach has been hindered by cross-reaction with several other kinds of fluoroquinolones in contrast to the presently-developed aptamer that is specific to danofloxacin. In addition, aptamers have significant advantages with regard to availability in diagnosis/detection compared with antibody, which include low cost, heat-stability, and no occurrence of variation in the batch-to-batch through chemical synthesis [16,17]. In addition, aptamers can be easily modified, optimized, and fixed with various reporters depending on the types of detection platform. Therefore, the danofloxacin-specific RNA aptamer could be uploaded on sensor systems and useful as a rapid, selective, and sensitive monitoring, diagnostic, and detection ligand for danofloxacin in food animals.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.04.103.

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