Electronic Supplementary Materials

Selection of DNA aptamers for detecting metronidazole and ibuprofen: two common additives in soft drinks

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Additional Methods

Chemicals

All DNA utilized were synthesized by Integrated DNA Technologies (Coralville, IA), with their sequences detailed in Table S1. Streptavidin-coated agarose resin possessed a loading capacity of 1-3 mg biotinylated BSA per milliliter was bought from Thermo Scientific (IL, USA). 6% BCL agarose bead standard (50-150 μM) was purchased Agarose Bead Technologies (Spain). Metronidazole, 2-methyl-5-nitroimidazol-1-ylacetic acid, 1,2-Dimethyl-5-nitroimidazole, hydroxydimethylimidazole, ornidazole, sycnidazole, 2-Methyl-5-nitroimidazole, ibuprofen, ibufenac, ketoprofen, flurbiprofen, acetaminophen, chlorphenamine maleate, dexamethasone, diclofenac sodium, diphenhydramine, erythromycin, meprednisone, phenacetin, phenylbutazone and piroxicam were purchased from Aladdin Scientific (Shanghai, China). The dNTP mix, Taq DNA polymerase with ThermoPol buffer, and a low-molecular-weight DNA ladder were obtained from New England Biolabs (Ipswich, MA). Micro Biospin chromatography columns and SsoFast EvaGreen supermix were procured from Bio-Rad Laboratories (Hercules, CA, American). Thioflavin T (ThT), amicon ultra-0.5 centrifugal filter unit (with 3K and 10K molecular weight cutoffs) were acquired from Millipore-Sigma (Oakville, ON, Canada). HEPES free acid, $MgCl_2 \cdot 6H_2O$, NaCl, $EDTA \cdot 2H_2O$, HCl and NaOH were obtained from Bio Basic (Toronto, ON, Canada).

Milli-Q water was used in the preparation of all buffers and solutions. The SELEX buffer contained HEPES (20 mM, pH 7.4) with 10 mM $MgCl_2$ and 100 mM NaCl. The strand separation buffer was HEPES (20 mM, pH 7.4) with 100 mM NaCl. Metronidazole was prepared in SELEX buffer at concentration 5 mM. Ibuprofen was prepared in NaOH (0.2 M) at concentration of 100 mM.

SELEX

For the first round, the single-stranded library (500 pmol) was annealed with biotinylated capture strand (1500 pmol) in SELEX buffer, the annealed DNA was stored at the room temperature for 30 min. Then, 100 μL of 6% BCL agarose bead standard (used to control the flow rate of the filter) and 100 μL of streptavidin agarose resin and were packed into a microchromatography column and washed with SELEX buffer to remove any preservatives. The annealed DNA underwent multiple cycles through the column to ensure optimal binding

via gravity flow. Subsequently, the column was washed with SELEX buffer to eliminate any non-bound or weakly bound library molecules, and the solution from the twelfth wash was subsequently collected, the solution was called background. Following this, 750 μL of targets were introduced, and the eluted DNA was collected via gravity flow. The collected DNA was subsequently concentrated and purified using a 3K spin column with Milli-Q water. Here, we employed a real-time polymerase chain reaction (RT-PCR, Bio-Rad Laboratories, Hercules, CA, American) step using unmodified primers to monitor the selection progress and to determine the PCR cycles for library amplification by assessing background and collected DNA. Then, the biotinylated reverse primer was used to obtain PCR products. The PCR products were mixed with 100 μL of streptavidin agarose resin and gently shaken for 10 min. 100 μL 6% BCL agarose bead standard was packed into a microchromatography column, then the mixture of PCR products and streptavidin agarose resin were added to the column. Similar to the earlier steps, the mixture was washed with separation buffer, and then 600 μL of NaOH (0.2 M) was added to the column and incubated for 15 min to elute the ssDNA library. 400 μL HCL (0.2 M). Then, the DNA was desalted with Milli-Q water using a 3K spin column. Finally, the purified DNA was dissolved in SELEX buffer. To maintain a consistent library concentration, the library was quantified using a spectrophotometer. For the subsequent rounds, the amount of DNA was adjusted to 100 pmol, while all other steps remained the same as the first round. Finally, the PCR products from the 14th round of the metronidazole selection and the 18th round of the ibuprofen selection were sequenced.

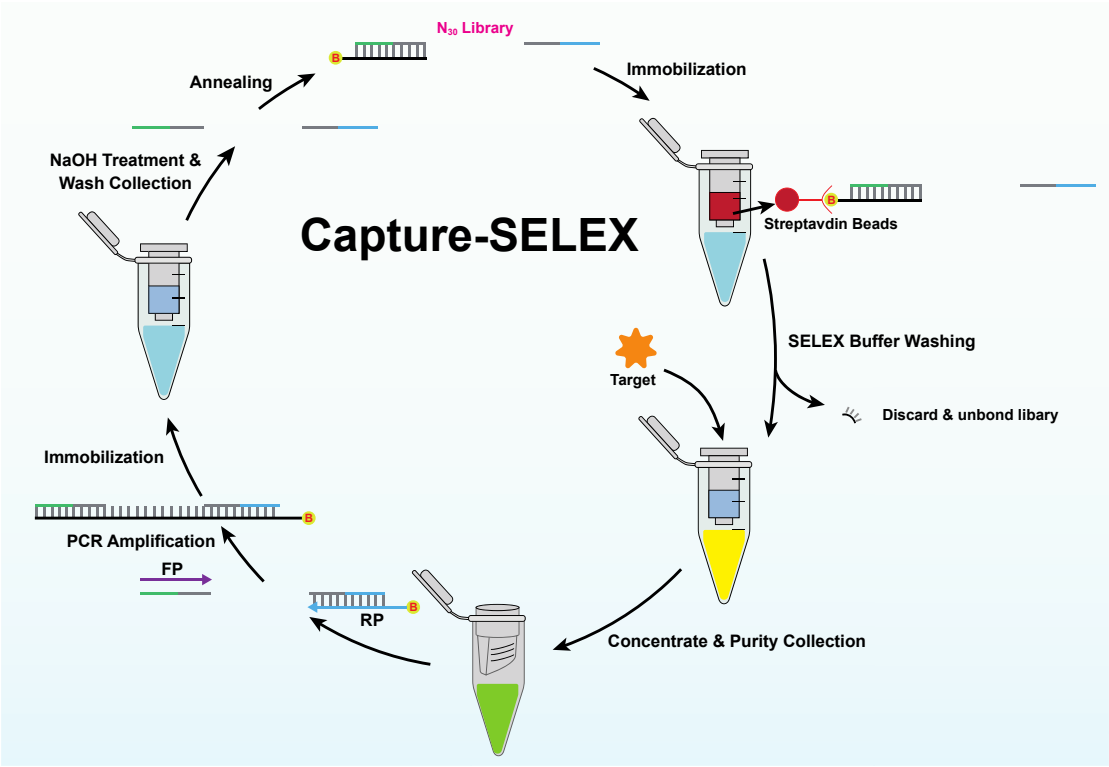

Figure S1. The SELEX scheme used in this study to obtain aptamers for targets. The library contained an N_{30} random region flanked by two constant regions for primer binding. Sequences eluted by targets were collected and amplified by PCR. The FP and RP are the two primers, and the biotinylated RP was used to separate the complementary strand.

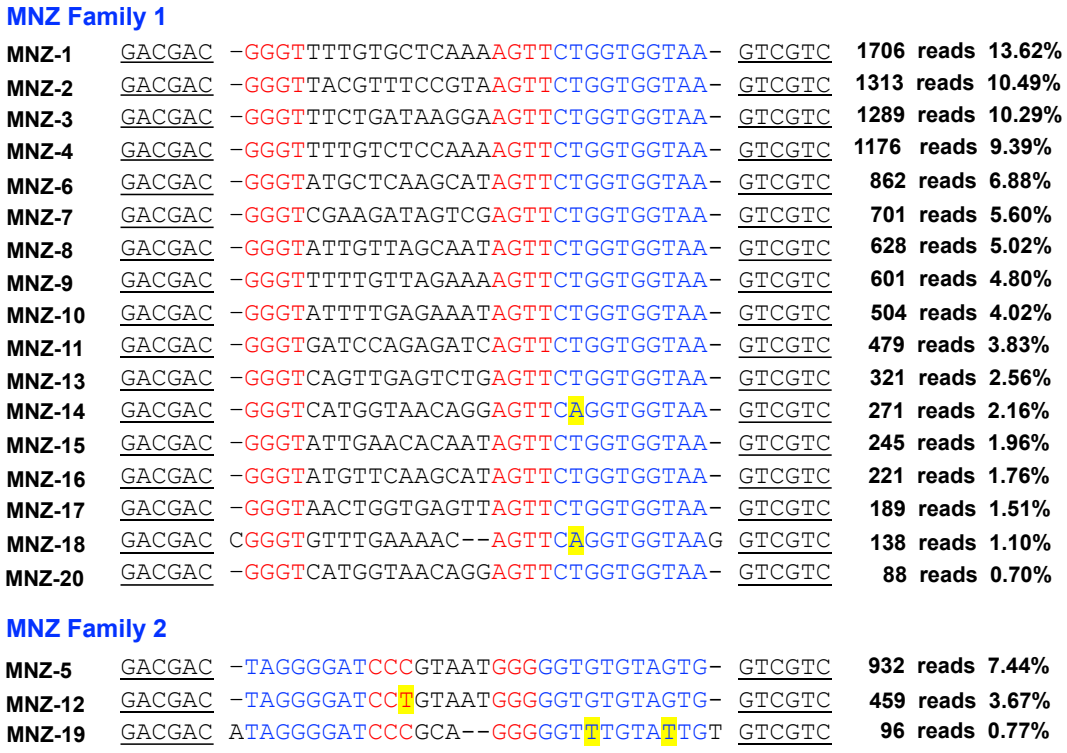

Figure S2. Alignment of the top 20 most abundant sequences from the round 14 selection of metronidazole. Two major families were identified. The nucleotides from the primer-binding regions are underlined.

Effect of metal ions on aptamer binding

To further understand aptamers binding, the effect of metal ions was studied.[1] To study the effect of Mg^{2+} on the metronidazole aptamer MNZ-1, we first fixed the NaCl concentration to be 100 mM and varied the Mg^{2+} concentration from 0 to 100 mM. To ensure that no free Mg^{2+} ions were present in the 0 mM Mg^{2+} sample, 5 mM EDTA was added to it. In the absence of Mg^{2+} in the buffer, no change in fluorescence intensity was observed. Conversely, when Mg^{2+} were present in the buffer, a decrease in fluorescence intensity was observed. This suggests that Mg^{2+} was required for the binding of aptamer MNZ-1. Moreover, with increasing concentrations of Mg^{2+}, the percentage decrease in fluorescence intensity rises. However, a decline was observed when the concentration surpasses 15 mM (Figure 3A, B). Overall, a good signal change was achieved when the Mg^{2+} concentration was higher than 5 mM. We then fixed Mg^{2+} concentration at 5 mM and changed sodium ion concentration from 1 to 200 mM. In these samples, a similar fluorescence drop was observed, suggesting that sodium was not important for aptamer binding.

Similarly, the salt-dependent experiments were performed for the ibuprofen aptamer IBF-1. In this case, an abrupt jump in the response was observed between 1 and 5 mM Mg^{2+}, and again sodium ions appeared unimportant for the binding (Figure 3C, D). The fact that both aptamers required Mg^{2+} for binding but not Na^{+} suggested that Mg^{2+} was playing a role beyond simple charge screening. It is likely that Mg^{2+} can bridge negatively charged groups in DNA (e.g. phosphate) with some metal binding groups in target molecules (e.g. the carboxyl group in ibuprofen and cation-pi interactions in metronidazole). It is also possible that Mg^{2+} was required to form certain secondary or tertiary structures in the aptamers.

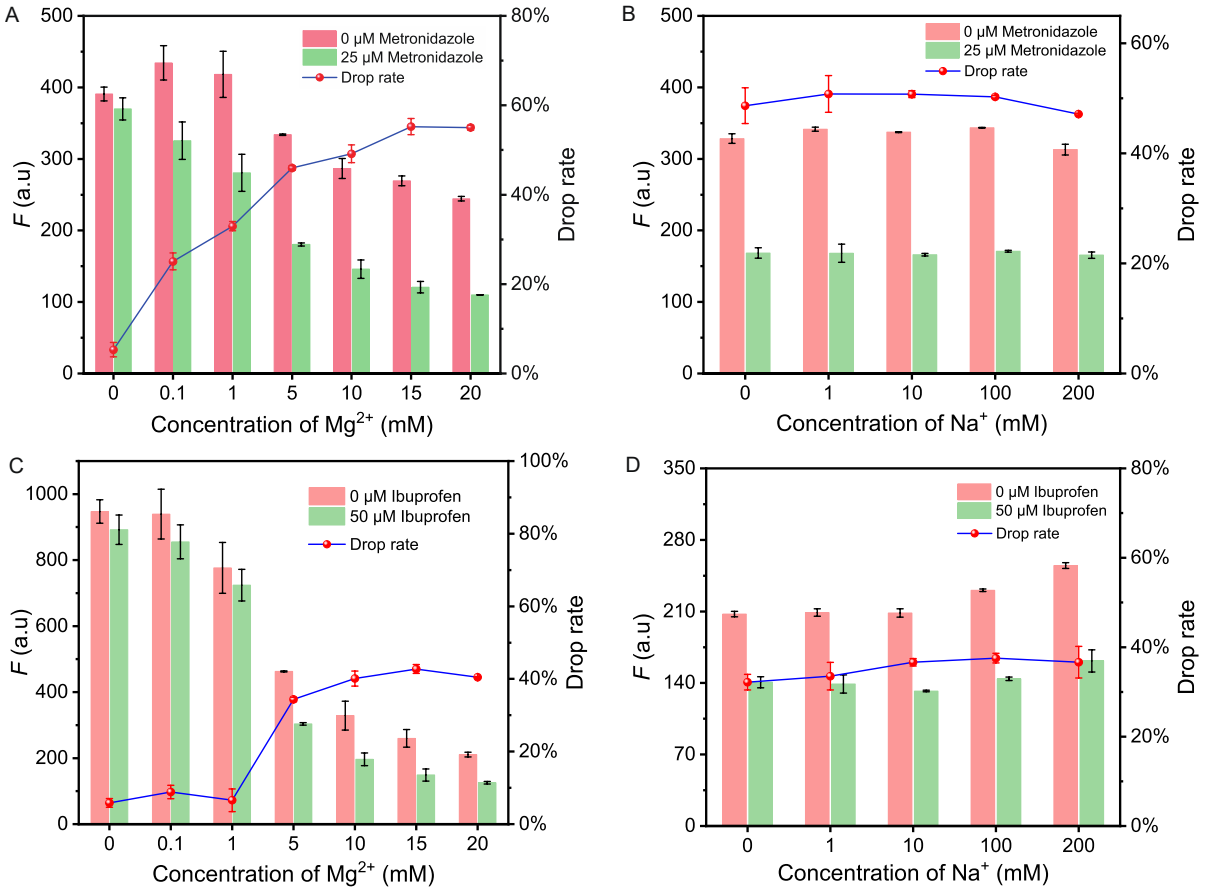

Figure S3. Effect of metal ions on aptamers binding. Effect of (A) Mg^{2+} and (B) Na^{+} concentration of for MNZ-1 binding. Effect of (C) Mg^{2+} and (D) Na^{+} concentration of for IBF-1 binding.

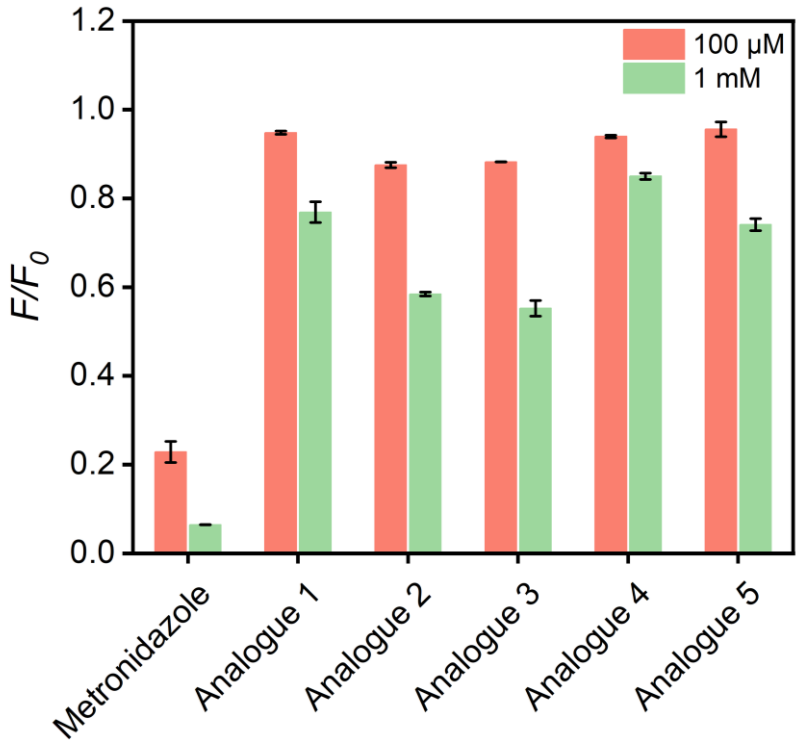

Figure S4. Selectivity testing of the MNZ-1 aptamer with metronidazole analogues.

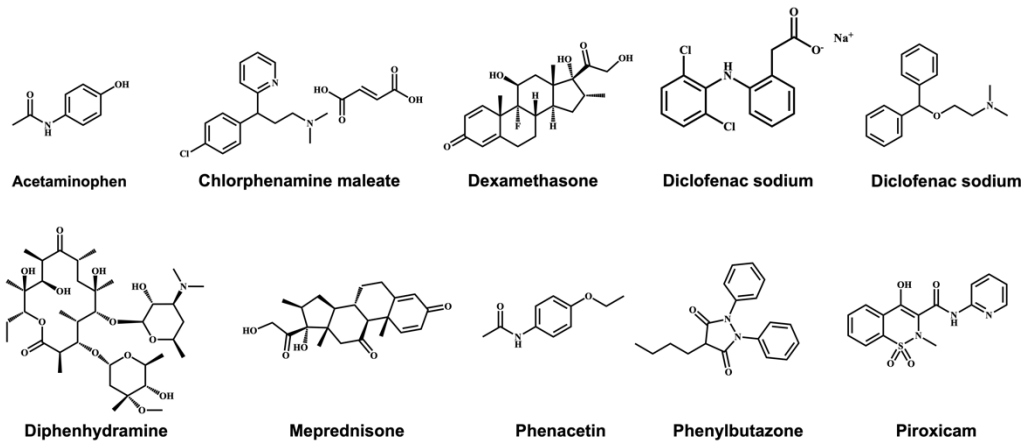

Figure S5. Structures of the other drugs tested for selectivity. These are also drugs found as additives in soft drinks.

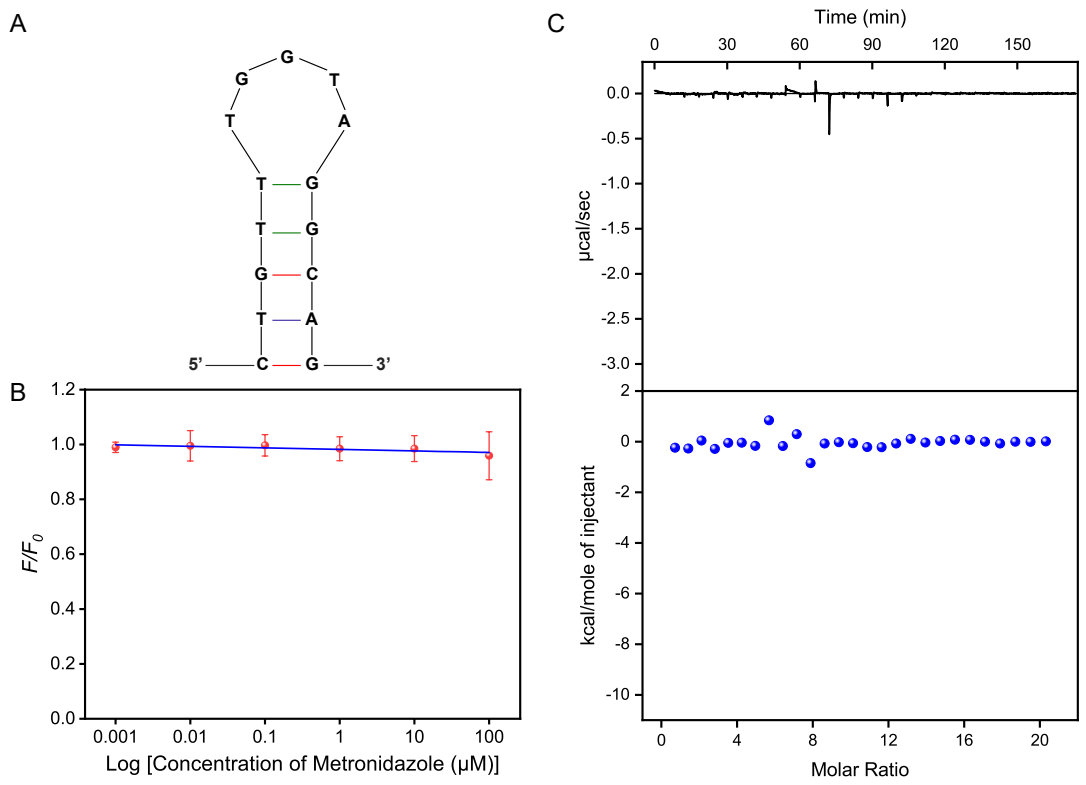

Figure S6. (A) The predicted secondary structure of aptamer AP32-4 reported in the literature. (B) Titration curves of metronidazole (0.001 μM to 100 μM) with aptamer AP32-4 (0.5 μM) using ThT (0.5 μM) fluorescence spectroscopy. (C) Titration of 1 mM metronidazole into 10 μM aptamer AP32-4 using ITC. Neither ThT fluorescence spectroscopy nor ITC showed binding.

Table S1. The DNA sequences used in this study.

DNA name	Sequences and modifications (5' to 3')
Library	GGAGGCTCTCGGGACGACN_{30}GTCGTCCCGATGCTGCAATCGTAA
Biotin-column	GTCGTCCCGAGAGCCATA/**3BioTEG**/
Forward primer	GGAGGCTCTCGGGACGAC
Reverse primer	TTACGATTGCAGCATCGGGACG
Biotin-reverse primer	/**5Biosg**/TTACGATTGCAGCATCGGGACG
P5-501	AATGATACGGCGACCACCGAGATCTACACTAGATCGCACACTCTTTCCCTACACGACGCTCTTCCGATCTTTACGATTGCAGCATCGGGACG
P7-701	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAGGCTCTCGGGACGAC
P7-702	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAGGCTCTCGGGACGAC
IBF-1a	GACGACCAAACCTAGCTTACGGTGGAGGTGGGTTTGGTCGTC
AP32-4	CTGTTTGGTAGGCAG
IBA4	ATACCAGCTTATTCAATTCCACAGACCCTTAGCTTTCCTATTATTCTGCGCGACGCTG AGATAGTAAGTGCAATCT

Note: /3BioTEG/ is biotinylation at the 3′-end with extended spacer and /5Biosg/ is biotinylation at the 5′-end. The highlighted nucleotides in IBF-1a indicate the sites of mutation.

Table S2. Thermodynamic Values of the Binding

Aptamer	Target	N^a	ΔH (kcal/mol)	ΔS （cal/K·mol）	K_d (μM)
MNZ-1	Metronidazole	0.85	-21.7	-51.0	17.0
IBF-1	Ibuprofen	0.75	-16.6	-36.6	66.7

[a]*N*: binding stoichiometry.