Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Going dual exonucleases to finely distinguish structural adjustment of aptamers for small-molecule detection

Lancheng Wang,[a] Huimin Zhou,[a] Kun Yan,[b] Peng Xu,[c] Bin Di,[b] Chi Hu,[a*] and Mengxiang Su[b*]

[a] Department of Pharmaceutical Engineering, School of Engineering, China Pharmaceutical University, Nanjing 211198, PR China

[b] Department of Pharmaceutical Analysis, School of Pharmacy, China Pharmaceutical University, Nanjing 211198, P.R. China

[c] Key Laboratory of Drug Monitoring and Control, Drug Intelligence and Forensic Center, Ministry of Public Security, No.18 Dongbeiwang West Road, Beijing 100193, P.R. China

E-mail: chihu@cpu.edu.cn; sumengxiang@cpu.edu.cn

Supplementary Figures

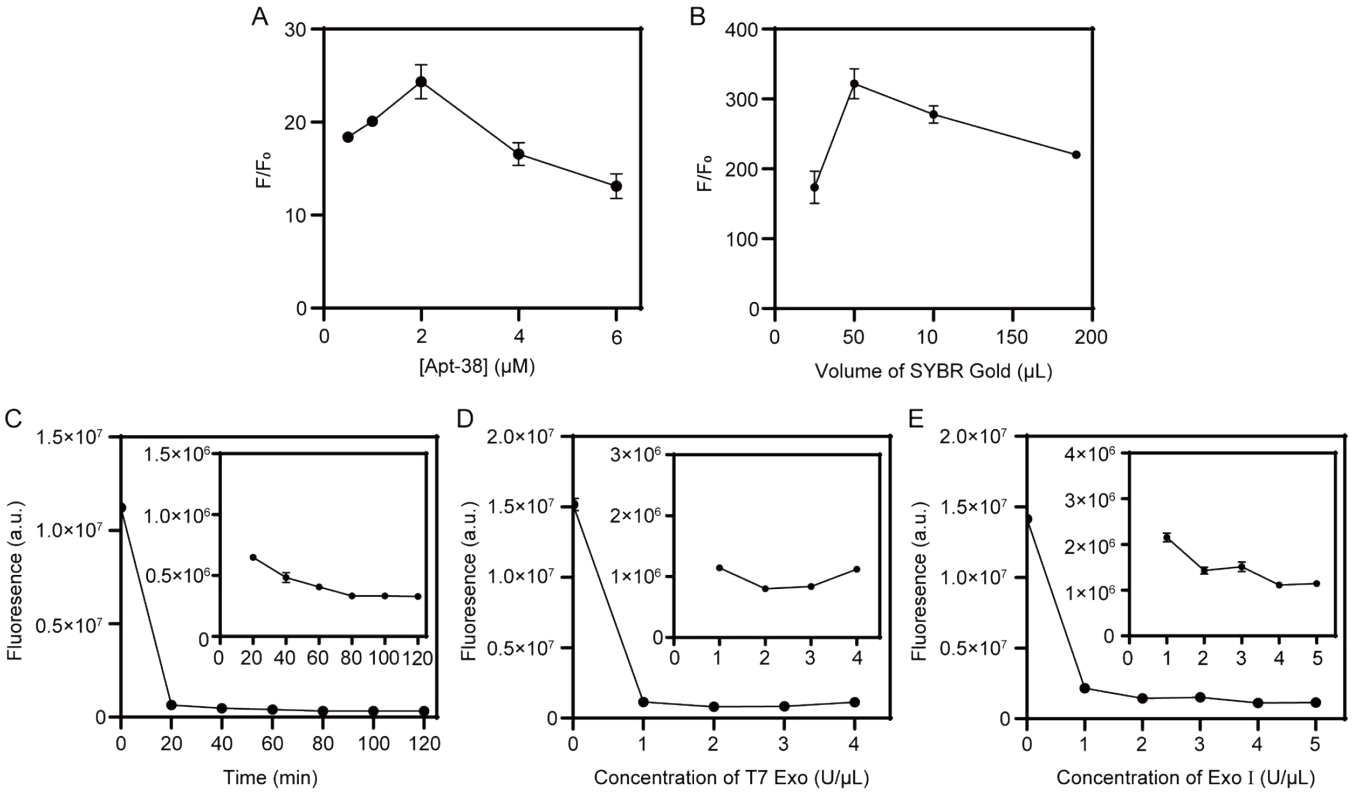

Fig. S1 (A) Optimizing of the concentration of aptamer; (B) Optimizing of the volumes of SYBR Gold (2×); (C) Optimizing of the enzymatic reaction time; (D) Optimizing of the concentration of T7 Exo and (E) Exo I for quinine detection. Error bars represent the standard deviations of three measurements.

In Fig. S1A, the aptamer concentration from 0.5 to 6 μM had been chosen to optimize the quinine detection, the concentrations in the reaction solution of the T7 Exo and Exo I was 2 U/μL and 4 U/μL. The time of enzymatic reaction was 90 min, and 50 μL 2× SYBR Gold was added to the reaction solution for further measurement. F and F_0 represent the fluorescence intensities in the before and after digestion, respectively.

In Fig. S1B, the SYBR Gold (2×) volume from 25 to 190 μL had been chosen to optimize the quinine detection, the concentrations in the reaction solution of the aptamer, T7 Exo and Exo I was 2 μM, 2 U/μL, and 4 U/μL. The time of enzymatic reaction was 90 min, and different volumes of 2× SYBR Gold

was added to the reaction solution for further measurement. F and F_0 represent the fluorescence intensities in the presence and absence of aptamer, respectively.

In Fig. S1C, the enzymatic reaction time from 0 to 120 min had been chosen to optimize the quinine detection, the concentrations in the reaction solution of the aptamer, T7 Exo and Exo I was 2 μM, 2 U/μL, and 4 U/μL. 10μL of the reaction solution was sampled at 20min intervals, respectively, and 50 μL 2× SYBR Gold was added to the reaction solution for further measurement. The inset is the local enlarged image of 20-120 min.

In Fig. S1D, the T7 Exo concentration from 0 to 4 U/μL had been chosen to optimize the quinine detection, the concentrations in the reaction solution of the aptamer and Exo I was 2 μM and 4 U/μL. The time of enzymatic reaction was 90 min, and 50 μL 2× SYBR Gold was added to the reaction solution for further measurement. The inset chart shows the enlarged curve in the T7 Exo concentration range of 1-4 U/μL.

In Fig. S1E, the Exo I concentration from 0 to 5 U/μL had been chosen to optimize the quinine detection, the concentrations in the reaction solution of the aptamer and T7 Exo was 2 μM and 2 U/μL. The time of enzymatic reaction was 90 min, and 50 μL 2× SYBR Gold was added to the reaction solution for further measurement. The inset chart shows the enlarged curve in the Exo I concentration range of 1-5 U/μL.

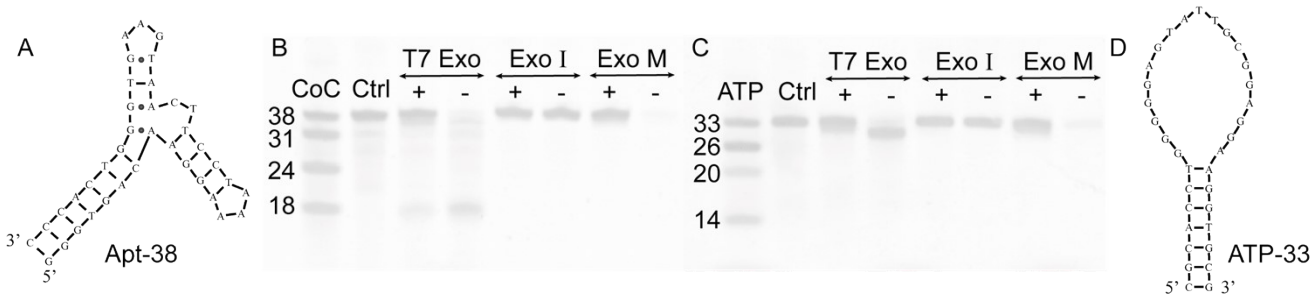

Fig. S2 Oligonucleotide structure and PAGE analysis of exonuclease digestion products of Apt-38 (A, B) and ATP-33 (C, D) with (+) and without (-) 2 mM cocaine or ATP, as generated by T7 Exo, Exo I, or a mixture of both.

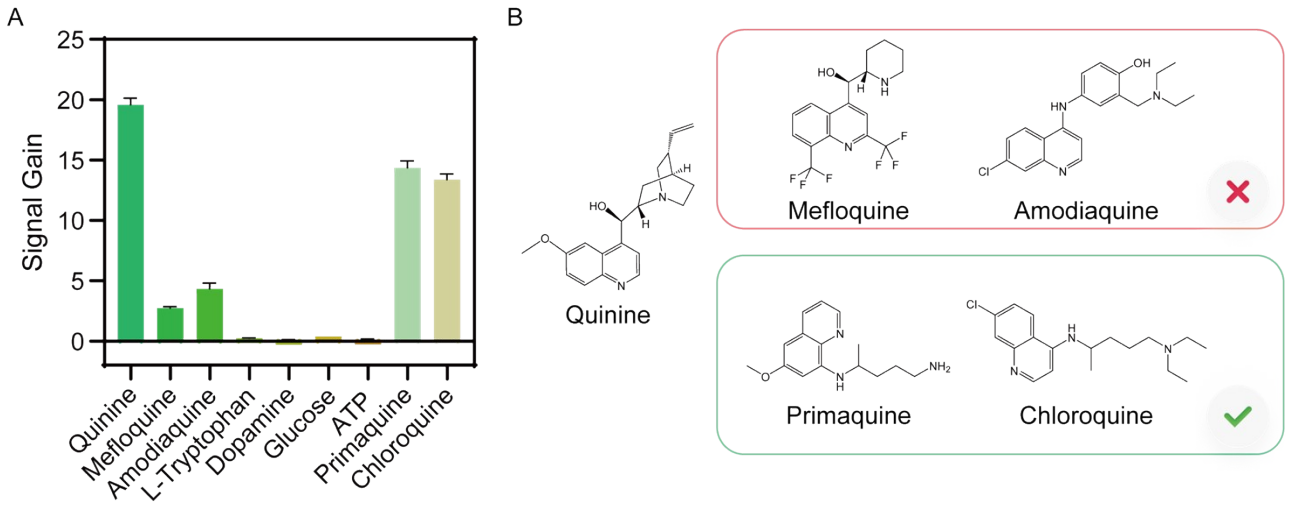

Fig. S3 (A) The dual-exonuclease-inhibition method cross-reactivity and selectivity to similarly structured antimalarial compounds and other disruptants. All small molecules were in 20 μM concentration. (B) Chemical structures of the quinine analogs.