Supplementary Material (ESI) for The Analyst

A new method for the study of G-quadruplex ligands

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1. Experimental Section

1.1 Materials

The DNA oligonucleotides AG4: 5′-$TG_3TAG_3CG_3TTG_3AAA$-3′ and Hum24: 5′-$(TTAGGG)_4$-3′ were obtained from TianGen Biotech Co. Ltd (Beijing China), The G-quadruplex molecular beacon F22D: FAM-$AGGG(TTAGGG)_3$-DABCYL was synthesized and purified by Invitrogen Ltd. (Shanghai, China). All other chemicals were purchased commercially and used without further purification unless otherwise noted.

1.2 Screening studies of G-quadruplex binders

All experiments were performed in 10 mM Tris-HCl buffer solution, pH=8.0, that included 1.6 mM KCl, 0.8 mM $MgCl_2$, 0.0017% (v/v) Triton X-100, 0.90 μM AG4, 0.50 μM hemin, and different concentrations of candidate drugs. The reaction mixtures were held overnight in ambient temperature, then 1.28 mM of ABTS and 1.28 mM of H_2O_2 were added. The color of the reaction mixture was recorded by a digital camera. Densitometric quantitation of the photographed images was performed by Glyko BandScan software, Version 4.30.

1.3 G-quandruplex stabilizing abilities assays

Thermal melting studies of G-quadruplex in the presence or absence of the candidate drugs were carried out on Rotor-Gene 3000 (Corbett Research) with 100 μL of reaction mixture (10 mM Tris-HCl buffer (pH 8.0), 100 mM NaCl, 0.5 μM F22D and an appropriate concentration of the candidate drug). The temperature was increased in steps of 1 °C, from 30 °C to 95 °C, with the first step lasting 30 s and the remaining steps each lasting 5 s. Fluorescence was measured at each step.

2. Selection of hemin concentation

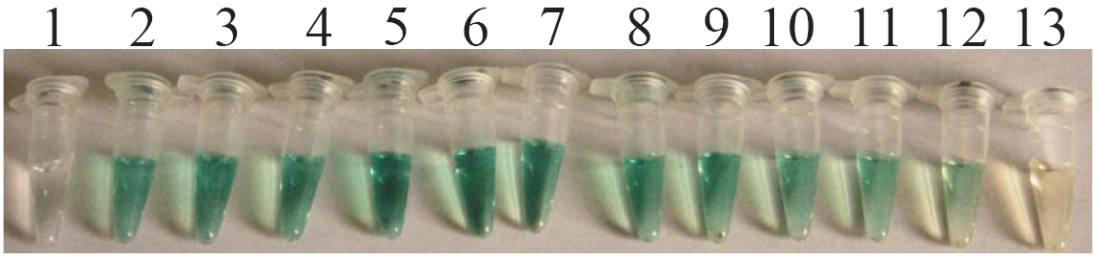

Figure S1. Oxidation of ABTS by H_2O_2 in presence of AG4 and different concentration of hemin. 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 2.0, 3.0 μM hemin was added in tube 1-13, respectively.

3. Selection of AG4 concentation

Figure S2. Oxidation of ABTS by H_2O_2 in presence of hemin and different concentration of AG4. 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, 1.7, 2.1 μM AG4

was added in tube 1-13, respectively.

4. Selection of ABTS concentation

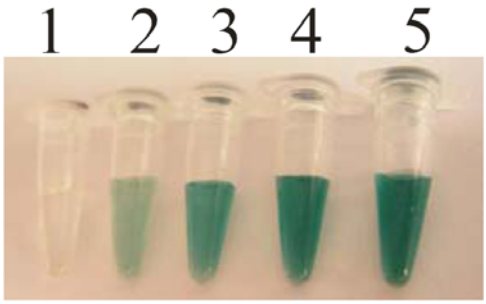

Figure S3. Oxidation of different concentration of ABTS by H_2O_2 in presence of AG4-hemin complex. 0, 0.32, 0.64, 1.28, 1.92 mM ABTS was added in tube 1-5, respectively.

5. Candidate drugs used for screening experiments.

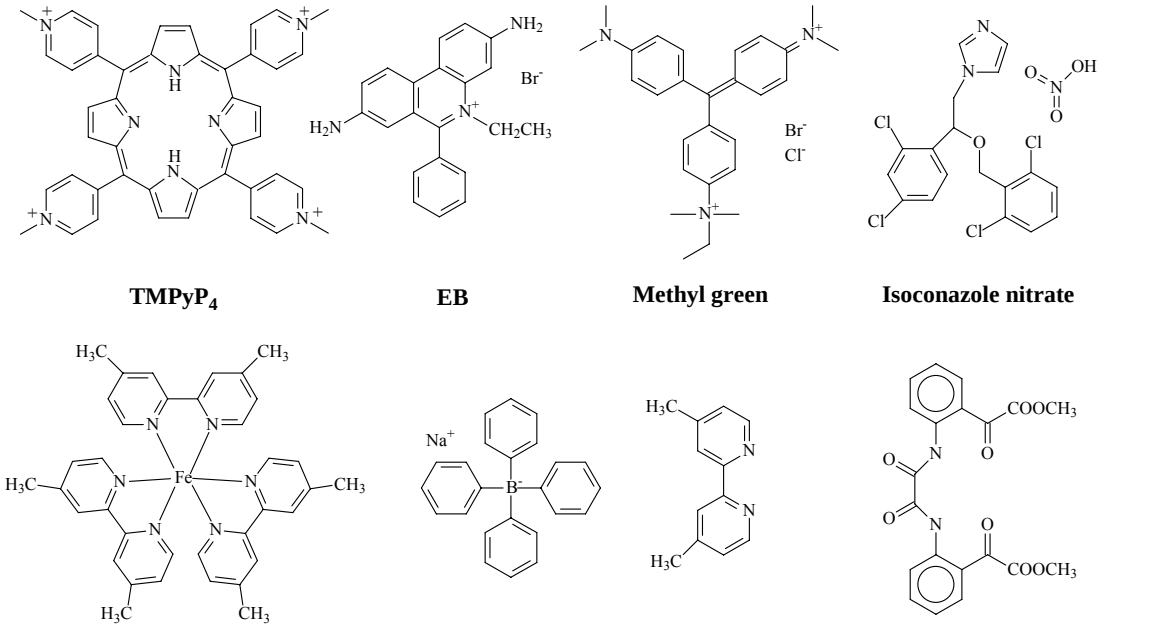

Figure S4. Candidate drugs used for screening experiments.

5. Respective results of fluorescence tests.

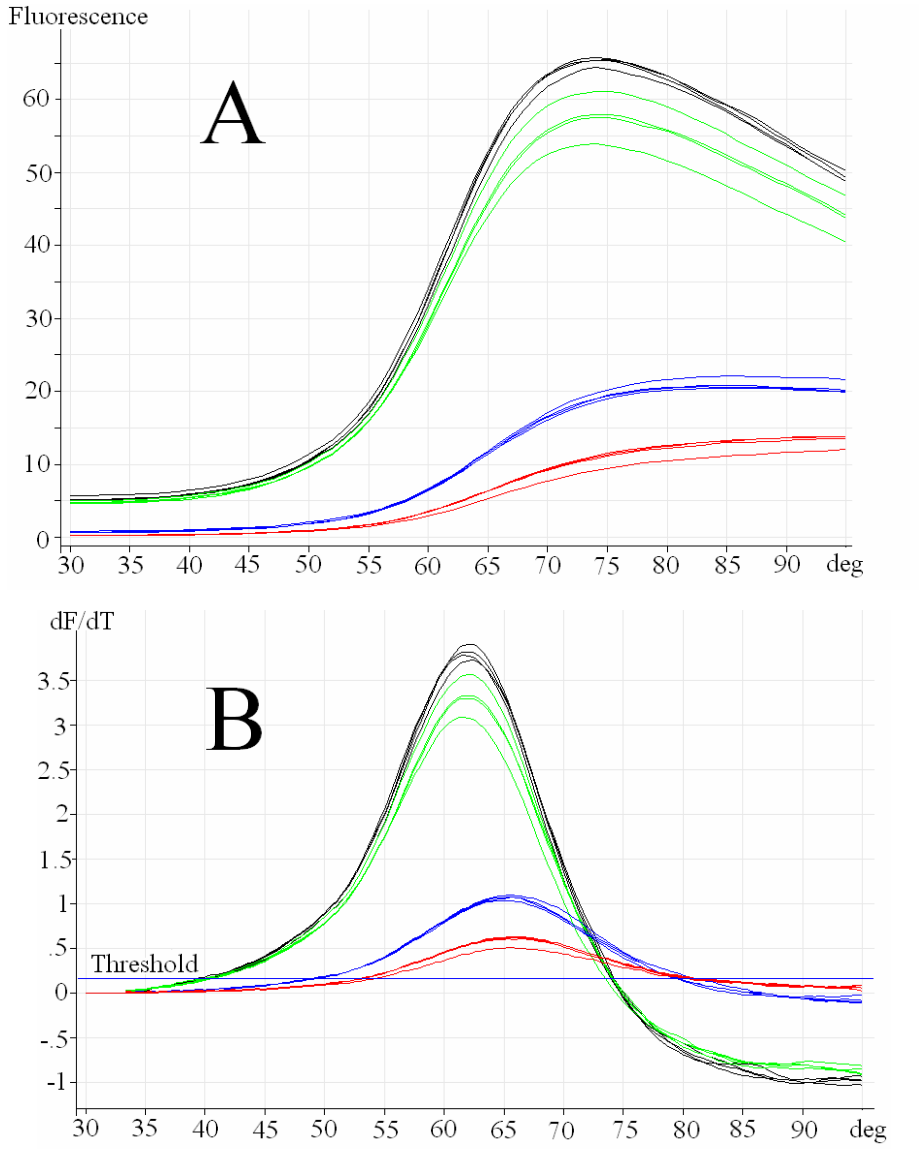

Figure S5. Stabilization of F22D in the presence of TMPyP4 or $Fe(dmbpy)_3(BF_4)_2$. (**A**) The thermal denaturation profile of F22D recorded alone (black) or in the presence of TMPyP4 (blue, 2 µM; red, 3 µM) or $Fe(dmbpy)_3(BF_4)_2$ (green, 25 µM). (**B**) First derivative of the melting curves.