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Supplementary Information

Synthesis of quinolinium-based probes and studies of their effects for selective G-quadruplex DNA targeting

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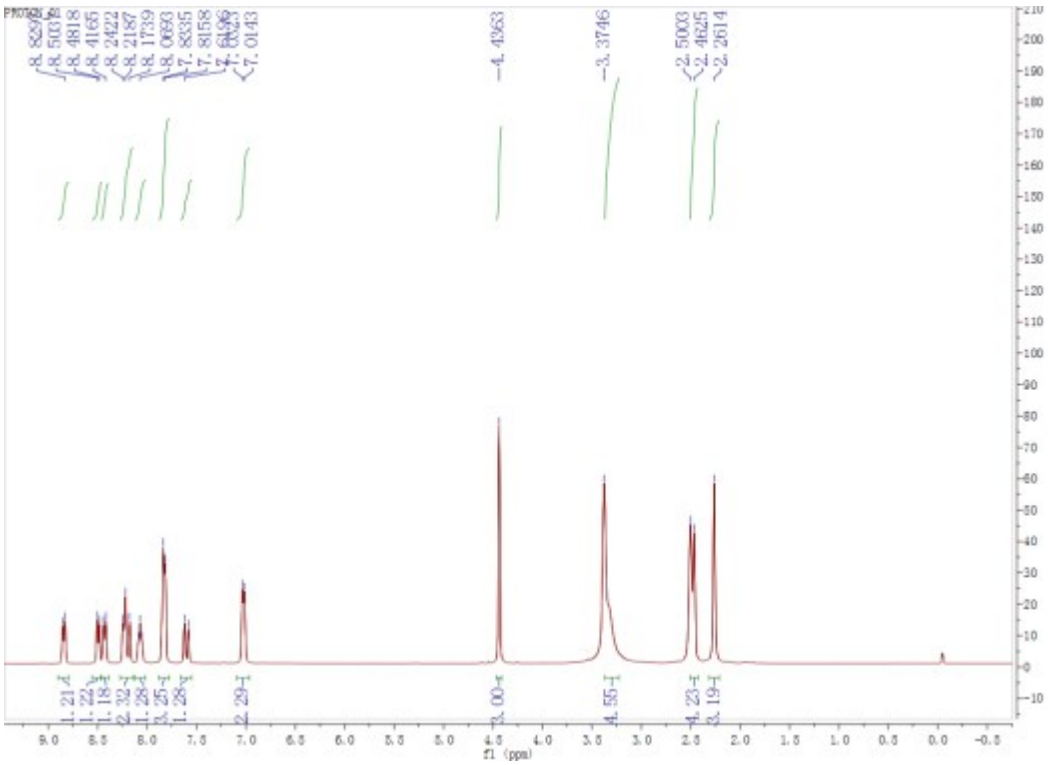
1. Sequence alignments of oligonucleotides

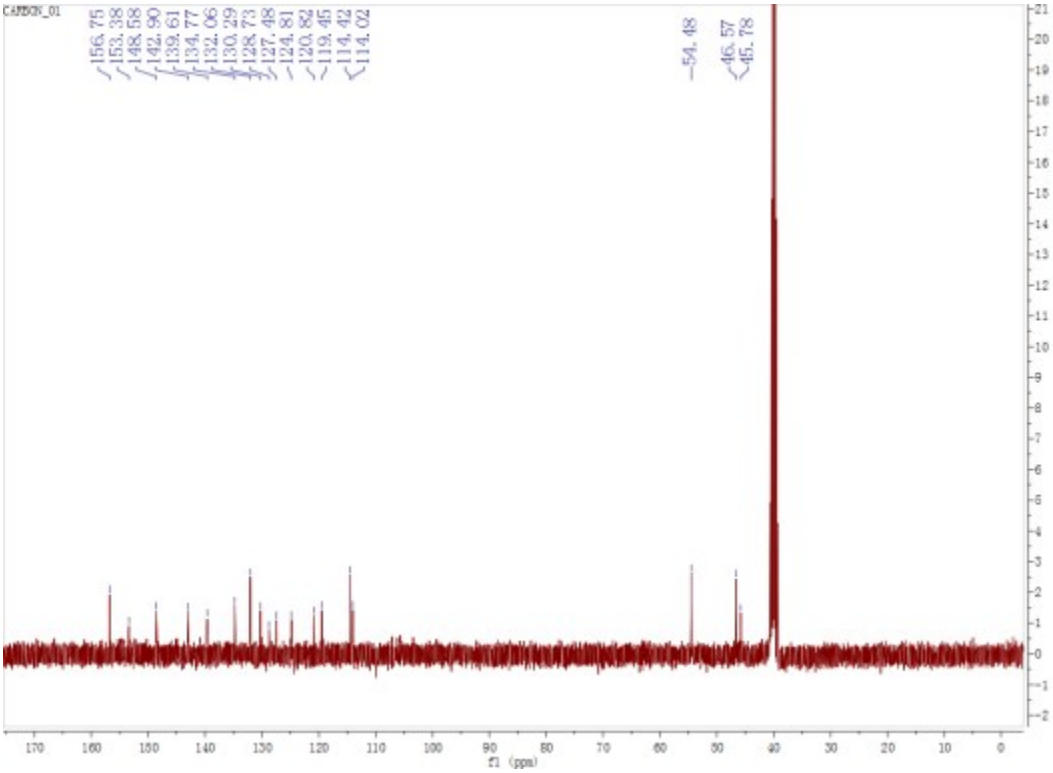
Name	Sequence (from 5' to 3')	Structure in K^+ solution
Ckit1	AGGGAGGGCGCTGGGAGGAGGG	G-quadruplex
HRAS	TCGGGTTGCGGGVGCAGGGCACGGGCG	G-quadruplex
G3T3	GGGTTTGGGTTTGGGTTTGGG	G-quadruplex
C-myc	TTGAGGGTGGGTAGGGTGGGTAAA	G-quadruplex
22AG	AGGGTTAGGGTTAGGGTTAGGG	G-quadruplex
CM22	TGAGGGTGGGTAGGGTGGGTAA	G-quadruplex
HTG-21	GGGTTAGGGTTAGGGTTAGGG	G-quadruplex
Ckit3	GGCGAGGAGGGGCGTGGCCGGC	G-quadruplex
ds18	GCGCGCGCGCGCGCGCGC	Duplex
dA21	AAAAAAAAAAAAAAAAAAAAA	Single-stranded
dT21	TTTTTTTTTTTTTTTTTTTTT	Single-stranded
ct-DNA	DNA from calf thymus	Duplex

Table S1*:* Sequence alignments of oligonucleotides used in the present study

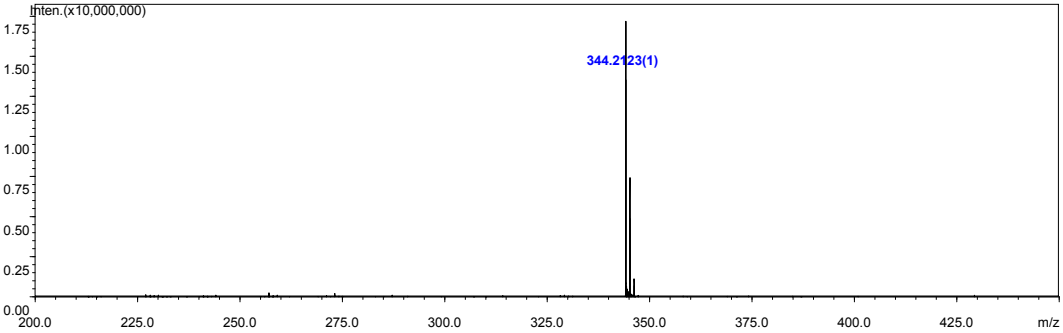
2. ^{1}H-NMR, ^{13}C-NMR and LC-MS spectra



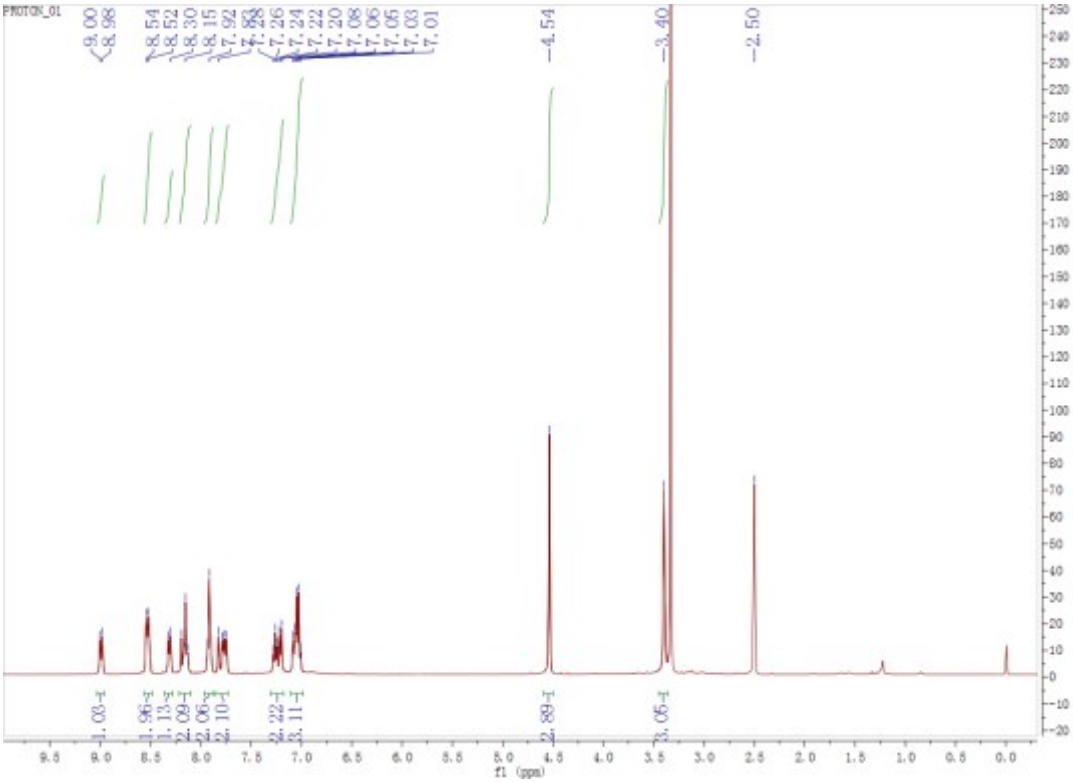
^{1}H NMR spectrum of **R-1**



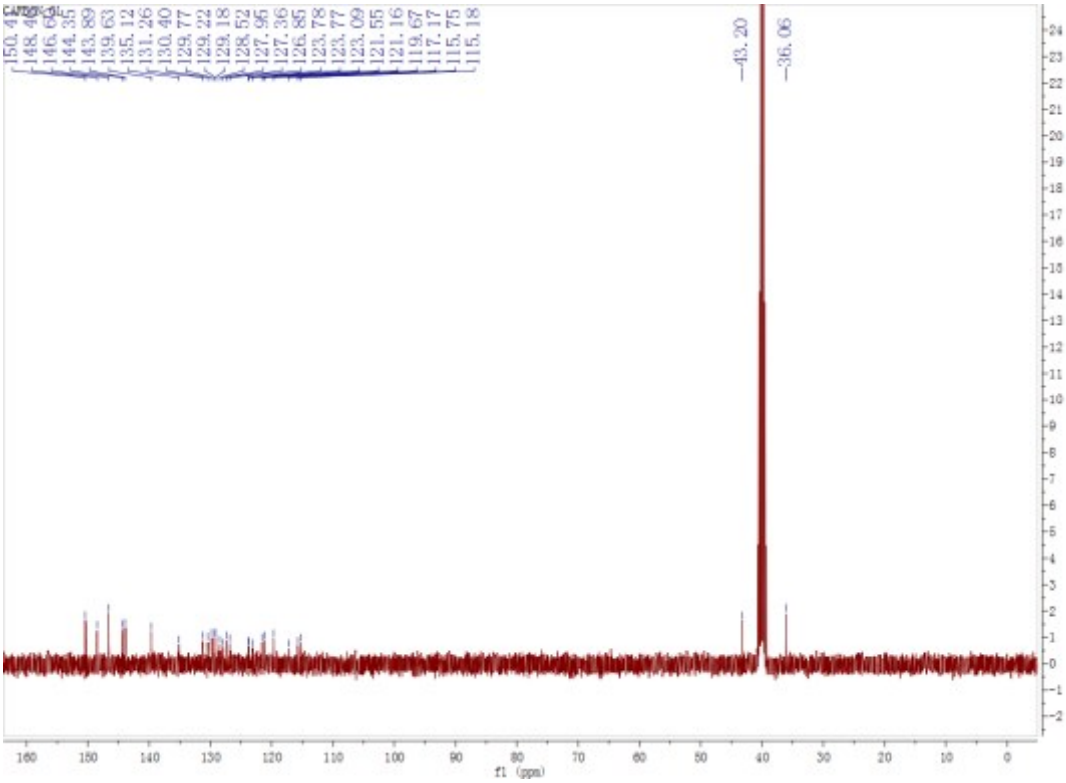
^{13}C NMR spectrum of **R-1**



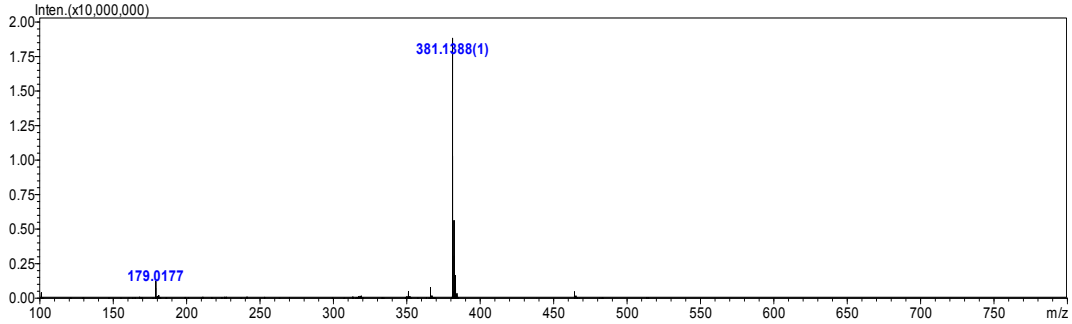
LC-MS spectrum of **R-1**



^{1}H NMR spectrum of **R-2**



^{13}C NMR spectrum of **R-2**



LC-MS spectrum of **R-2**

3. Fluorescence titrations of R-1 with DNAs

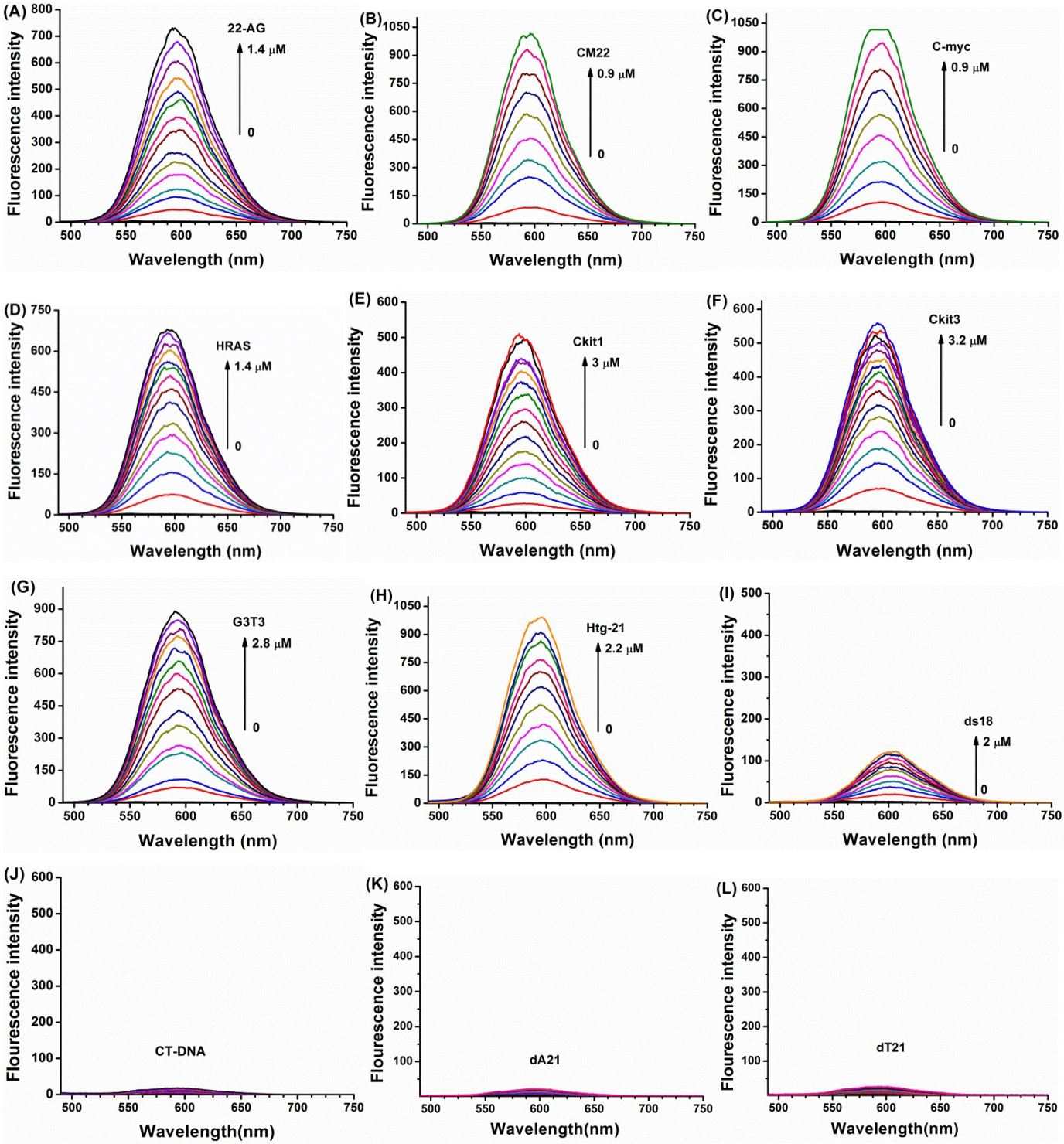


Fig. S1. Fluorescence titrations of **R-1** (2 μM) upon addition of increasing amount of G-quadruplex DNAs-Htg-21,Ckit1, 22AG, CM22, C-myc, G3T3, HRAS and Ckit3; double-stranded DNAs-ct-DNA and ds18; single-stranded DNA-dA21 and dT21. The arrows indicate the changes of fluorescence intensity.

4. Absorption titrations of R-1 with DNA oligomers

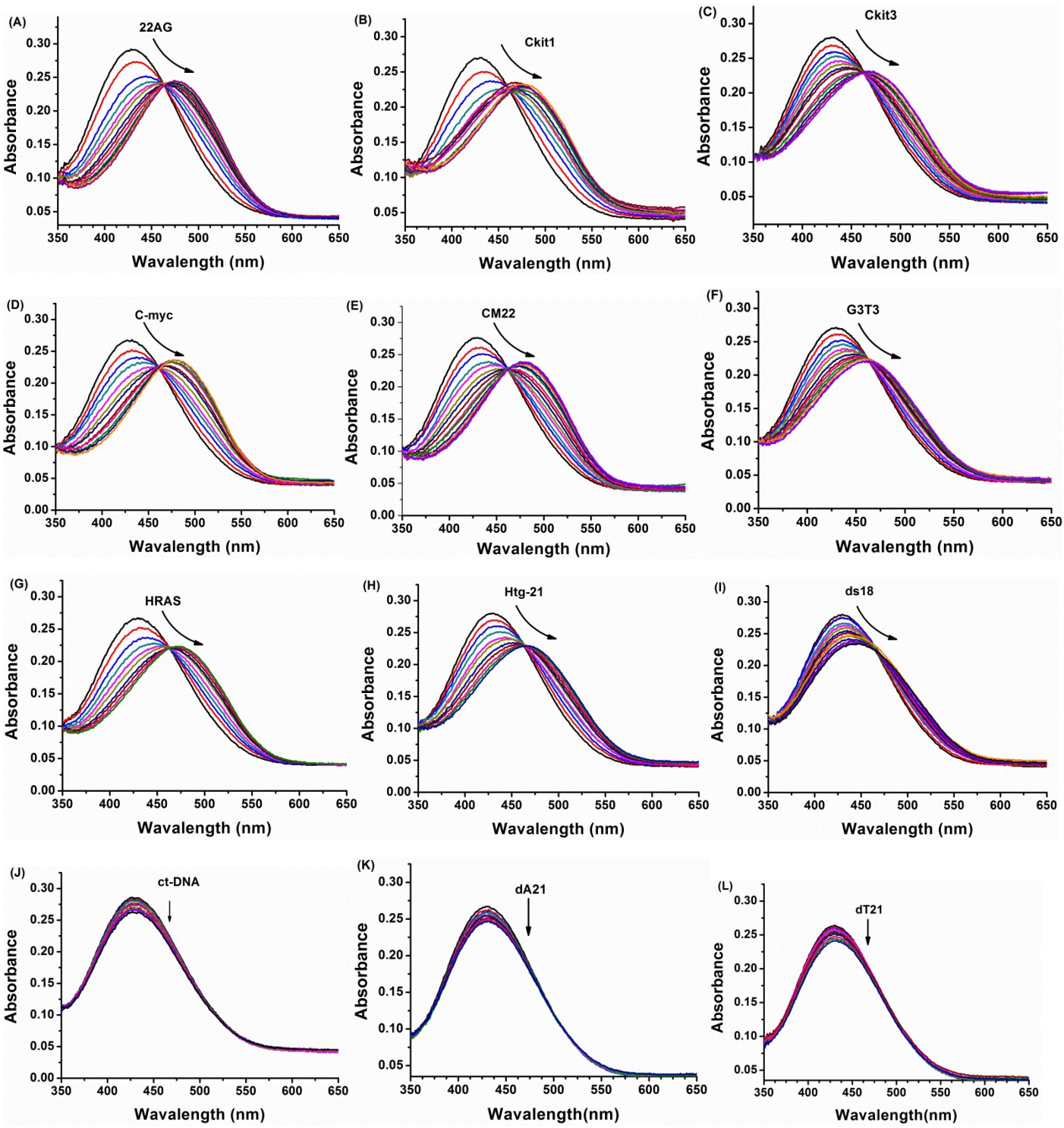


Fig. S2. The changes in the spectral profiles of **R-1** (8 μM) with increasing amount of DNAs (0-2 μM).

5. Detection limit of R-1 for G-quadruplex DNAs

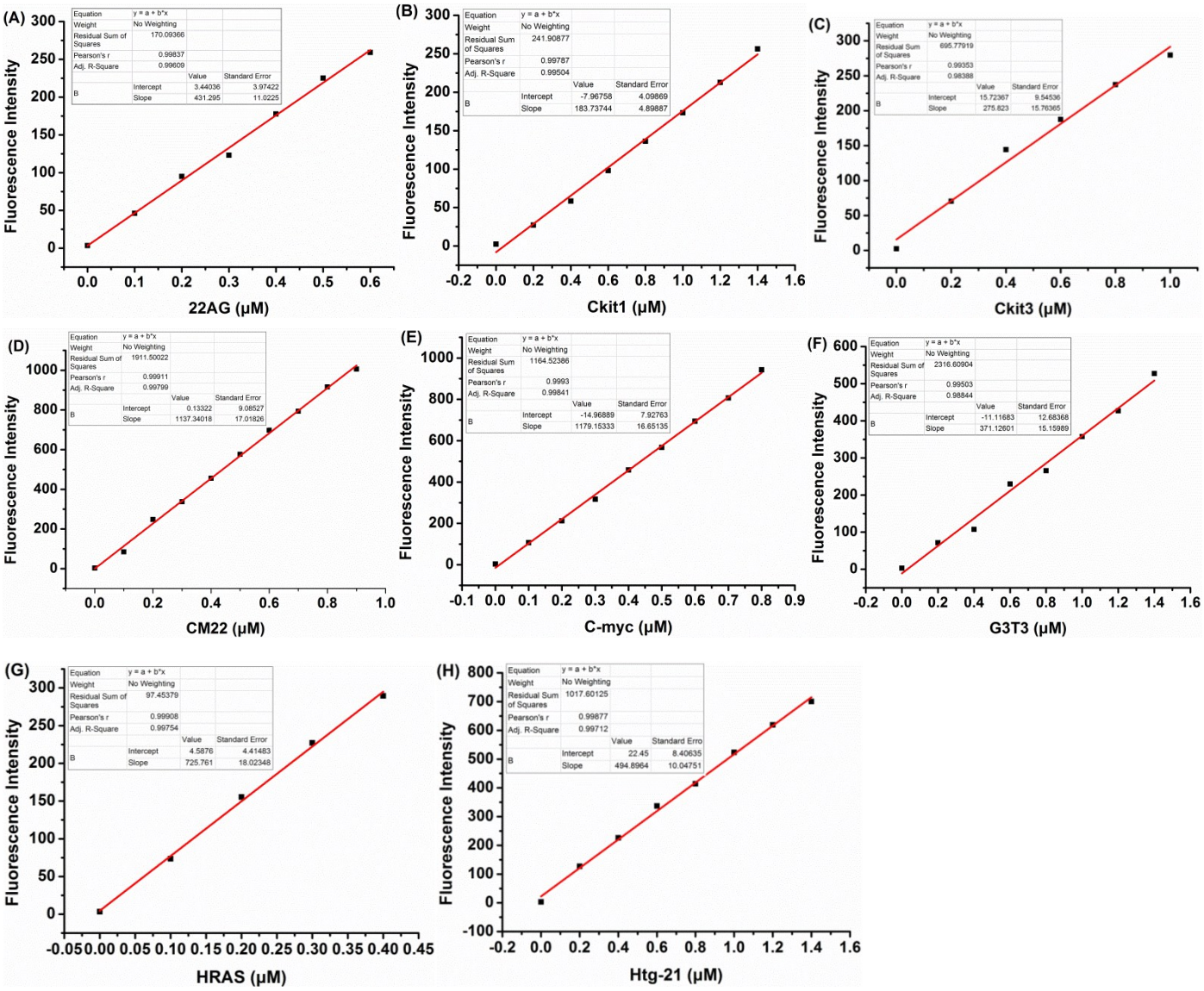


Fig. S3. The linear fit equation for calculating LOD values of **R-1** for G-quadruplex DNAs. The concentration of **R-1** was fixed at 2 μM in 10 mM Tris-HCl buffer, pH = 7.4, containing 60 mM KCl. Fluorescence intensities at 592 nm were collected.

6. Effect of R-1 on CD spectra of parallel G-quadruplex

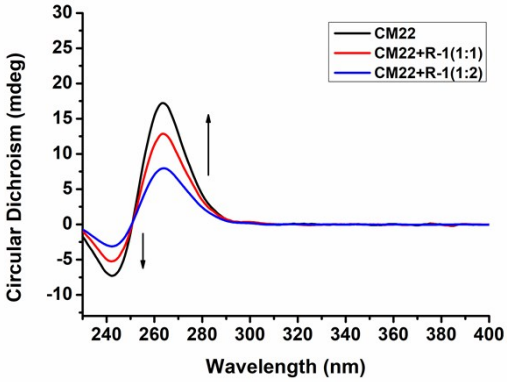


Fig. S4. CD titration spectra of **R-1** with CM22 in 10 mM Tris-HCl buffer (pH 7.4, 60 mM KCl)

7. Cytotoxicity of R-1

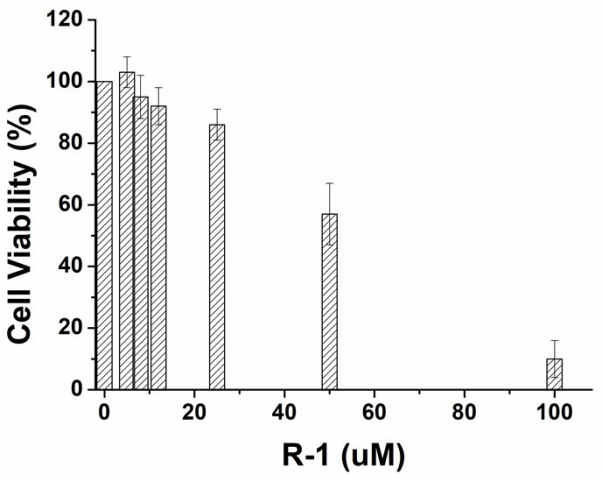


Fig. S5. Growth of Hep-G2 cells in the presence of different concentrations of R-1 after 24 h incubation.

6. Experimental methods

6.1. Materials and instruments

All the chemicals including organic solvents were obtained from commercial vendors and used as received without further purification. The oligonucleotides (HPLC purified) used in this work were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China) and the sequences were listed in **Table S1**. Oligonucleotides were dissolved in 10 mM Tris-HCl buffer (containing 60 mM KCl or NaCl, pH 7.4). Prior to use, all oligonucleotides were pre-treated by heating at 95 ^{o}C for 5 min, followed by gradual cooling to room temperature and kept at this temperature for 30 min. Calf thymus DNA which purchased from Sigma Aldrich was directly dissolved in water and the concentrations were determined using the molar extinction coefficient $\varepsilon_{260\ nm}$ = 6600 $cm^{-1}\cdot M^{-1}$.

The UV-vis spectra were recorded on an UV-2550 spectrophotometer and fluorescence spectra were measured on a Shimadzu RF-5301PCS spectrofluorophotometer. CD spectra were performed on a JASCO-J815 circular dichroism spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were measured on a 400 MHz spectrometer using TMS as internal standard in DMSO-d_6 solution. Mass spectra (MS) were recorded on a Shimazu LCMS-2010A instrument with an ESI detector.

6.2. Synthesis and characterization

*6.2.1. Synthesis of **R-1***

4-(4-Methylpiperazino)benzaldehyde (1.1 mmol) and 1, 2-dimethylquinolin-1-ium iodide (1.0 mmol) were added to a 50 mL flask with 20 mL anhydrous ethanol, followed by 5 drops catalytic piperidine. The resulting mixture was allowed to refluxed for 12 h under nitrogen with stirring. After cooling to room temperature, the solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel eluting with CH_2Cl_2/CH_3OH (20:1, v/v) to afford **R-1** as a brownish solid. Yield: 54.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.85 (d, J = 8.68 Hz, 1H), 8.50 (d, J = 8.76 Hz, 1H), 8.43 (d, J = 8.76 Hz, 1H), 8.24-8.17 (m, 2H), 8.08 (t, J = 7.2 Hz, 1H), 7.83-7.81 (m, 3H), 7.61-7.58 (m, 1H), 7.03 (d, J = 7.2 Hz, 2H), 4.43 (s, 3H), 3.37-3.31 (br, 4H), 2.50-2.46 (br, 4H), 2.26 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 156.75, 153.38, 148.58, 142.90, 139.61, 134.77, 132.06, 130.29, 128.73, 127.48, 124.81, 120.82, 119.45, 114.42, 114.02, 54.48, 46.57, 45.78; LC-MS: (positive mode, *m/z*) calculated 344.2121, found 344.2123 for $[M-I]^{+}$.

*6.2.2. Synthesis of **R-2***

Following the general procedure for **R-1**, the product **R-2** was obtained as a dark red solid (Yield: 43.8%).

^{1}H NMR (400MHz, DMSO-d6) δ: 9.00 (d, *J*=8.84 Hz, 1H), 8.54 (d, *J*=8.92 Hz, 2H), 8.30 (d, *J*=7.8 Hz, 1H), 8.19-8.13 (m, 2H), 7.92 (s, 2H), 7.83 (s, 1H), 7.76 (t, *J*=6.52 Hz, 1H), 7.28-7.20 (m, 2H), 7.08-7.01 (m, 3H), 4.54 (s, 3H), 3.40 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 150.41, 148.46, 146.60, 144.35, 143.89, 139.63, 135.12, 131.26, 130.40, 129.77, 129.22, 128.52, 127.95, 126.85, 123.78, 123.09, 121.55, 121.16, 119.67, 117.17, 115.75, 115.18, 43.20, 36.07; LC-MS: (positive mode, *m/z*) calculated 381.1420, found 381.1388 for $[M-I]^+$.

6.3. Measurements and methodology

6.3.1. Fluorimetric titrations

Stock solutions (5 mM) of the dyes were prepared in DMSO (**R-1**, **R-2**). Titrations of the dyes with DNAs were performed in aqueous buffer (10 mM Tris-HCl, 60 mM KCl, pH 7.4). The concentration of **R-1/R-2** was fixed at 2 μM in buffer, to which the DNA solution was added step by step. Dye-DNA solution was incubated for 1 min before fluorescence spectra recorded. The volume of the dye was 2.5 mL and the changes in the dye concentration caused by dilution at the end of each titration were negligible. The date from the fluorimetric titrations were analyzed according to the independent-site model by nonlinear fitting to equation [1], in which *n* is the putative number of molecules binding to a given DNA matrix, Q is the fluorescence enhancement upon saturation, $A = 1/[K_a C_{dye}]$ and $x = nC_{(DNA)}C_{(dye)}^{-1}$ The parameters Q and A were found by Levenberg-Marquardt fitting routine in Origin 8.5 software, whereas *n* was varied to obtain a better fit.

$$\frac{F}{F_0} = 1 + \frac{Q-1}{2}\left(A + 1 + x - \sqrt{(A+1+x)^2 - 4x}\right)$$

6.3.2. UV-vis spectral studies

Absorption titration experiments were carried out by varying the DNA concentration and maintaining the R-1 concentration constant (8 μM) in aqueous buffer (10 mM Tris-HCl, pH 7.4, 60 mM KCl). After each DNA addition, the solution was incubated for 1 min and then the spectra range of 370~650 nm were recorded.

6.3.3 Circular dichroism (CD)

CD spectra (230-400 nm) were performed on a JASCO-J815 circular dichroism spectrophotometer using a 10 mm path length quartz cuvette. The scanning speed of the instrument was set to 400 nm min^{-1}. The strand concentration of oligonucleotide 22AG used for measurement was 5 μM. The buffer condition is 10 mM Tris-HCl, pH 7.4 in the presence of 60 mM KCl or NaCl. Final analysis of the data was carried out using Origin 8.5.

6.3.4. Molecular docking

The crystal structure (PDB code: 1KF1, resolution: 2.10 Å) and the NMR structure (PDB code: 143D) of G4 DNA were downloaded from RCSB protein data bank. The redundant solvent molecules and copper ions were removed from the crystal structure while the first conformation was retained from the NMR structure by using UCSF chimera [2]. The dock prep module was then used to add hydrogens and assign AMBER ff14SB forcefield. The 3D structure of ligand compound was also built in chimera which was assigned AM1-BCC partial charges [3] and fully minimized. The DMS tool in chimera was employed to generate the molecular surface of receptor using a probe atom with a 1.4 Å radius. To avoid manual intervention, the whole structure of receptor was defined as binding sites. The sphgen module was used to generate spheres filling the active site, and the grid module generate the grid file, which was used in grid-based energy evaluation. Dock 6 program [4] was then utilized to conduct semi-flexible docking where 10000 different orientations were generated. van der Waals and electrostatic interactions were obtained between ligand and the binding site, based on which grid scores were calculated. After that, clustering analysis were performed (RMSD threshold was set 2.0 Å) to obtain the best scored poses.

References:

[1] Stootman FH, Fisher DM, Rodger A, Aldrich-Wright JR. Improved curve fitting procedures to determine equilibrium binding constants. Analyst 2006;131:1145-51.

[2] Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC. et al. Ucsf chimera–a visualization system for exploratory research and analysis. J Comput Chem 2004; 25(13):1605–12.

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[4] Lang PT, Brozell SR, Mukherjee S, Pettersen EF, Meng EC. Thomas V. et al. Dock 6: Combining techniques to model rna-small molecule complexes. RNA 2009; 5(6):1–12.