Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Electronic Supplementary Information

Hydrogen-bond-driven dimers of naphthyridine derivatives for selective identification of DNA G-quadruplex

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1. General conditions

NMR measurements

The NMR spectra were recorded on Bruker AV400 working at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. The spectra are referenced internally according to the residual solvent signals of DMSO-d_6 (^{1}H NMR; δ= 2.50 ppm). All shifts are given in ppm. All coupling constants (*J* values) are reported in Hertz (Hz). 2D ^{1}H-^{1}H NOESY (mixing time: 200 ms) and COSY were recorded at 80 ^{o}C. The 2D DOSY experiments were recorded with 8 scans a stimulated echo sequence with eddy current delay (Bruker sequence stegp1s) with a diffusion time Δ=200 ms and a total gradient pulse length δ=2.2 ms. The gradient strength was varied in 32 steps quadratically between 2% and 95% gradient strength (53.5 G/cm). Mass spectra were recorded on Bruker Autoflex III mass spectrometer in positive ion mode.

CD Melting Analysis.

CD melting analysis were recorded on Bio-Logic MOS-450 (BioLogic Science Instruments, France) using quartz cell of 1mm optical path length and scanning speed of 240nm/min with 0.5 s response time. CD measurements were collected from 230 nm to 320 nm. The concentration of Tel26 and ligands in CD experiments was 10 μM and 100μM, respectively. The ligands were added into Tel26 at 37 °C for 24 h before measurement.

Fluorescence Analysis

The fluorescence spectra were recorded on Perkin Elmer FL6500 (PerkinElmer Life Sciences, U.S.A.) spectrometer using a 1 cm quartz cuvette. The excitation wavelength of ligands was 315 nm with emission spectra collected from 350 nm to 550 nm. The concentration of Tel26 and ligands in fluorescence experiments was 10 μM and 100μM, respectively. The ligands were added into Tel26 at 37 °C for 24 h before measurement.

Gel Electrophoresis

Native gel electrophoresis was run on 20% polyacrylamide gel containing 100 mM KCl at room temperature, 12 V/cm in TBE buffer containing 100 mM KCl and visualized by gel imaging analysis system.

Cell Culture

Human cervical cancer cells (HeLa) were cultured at 37 °C in 5% CO_2 in DMEM high

glucose medium supplemented with penicillin-streptomycin and 10% fetal bovine serum. Cells were tested to confirm the absence of mycoplasma.

Confocal Fluorescence Microscopy

HeLa cells were seeded in 6-well plates (1.0×10^5 cells/ well) for 24 h followed by incubation with ligands for 2 h before washing with PBS buffer for five times. Then, cells were fixed paraformaldehyde for 10 min and incubated with DAPI for 30 min after washing with PBS buffer for five times. Localization of ligands were immediately analyzed by confocal microscope (Olympus Fluoview FV1000) with recorded emission at 405 nm.

Cell Cytotoxicity Assay

In the cytotoxicity assay, cells were seeded in 96-well plates (5×10^3 cells/well) and incubated for 24 h. Cells were treated with ligands at various concentrations for 48 h followed by addition of MTT (5 mg/mL, 20 μL) to the medium. After incubation at 37 °C in 5% CO_2 for 4 h, the supernatant was aspirated and 100 μL of DMSO was added to each well, followed by vibration for 30 min. The absorbance in the experimental wells was measured at 490 nm with a microplate reader.

Apoptosis

HeLa cells were seeded in 6-well plates (1.0×10^5 cells/ well) for 24 h. Cells in each 6-well plate were treated with ligands for 24 h in fresh growth medium. Cells were treated with medium alone as negative control. Cells were harvested with trypsinization and centrifuged at 1500 rpm for 3 min at room temperature. Cell pellets were resuspended in 400 μL binding buffer and then treated with Annexin V-FITC for 15 min and propidium iodide (PI) for 5 min at 4 °C in the dark.

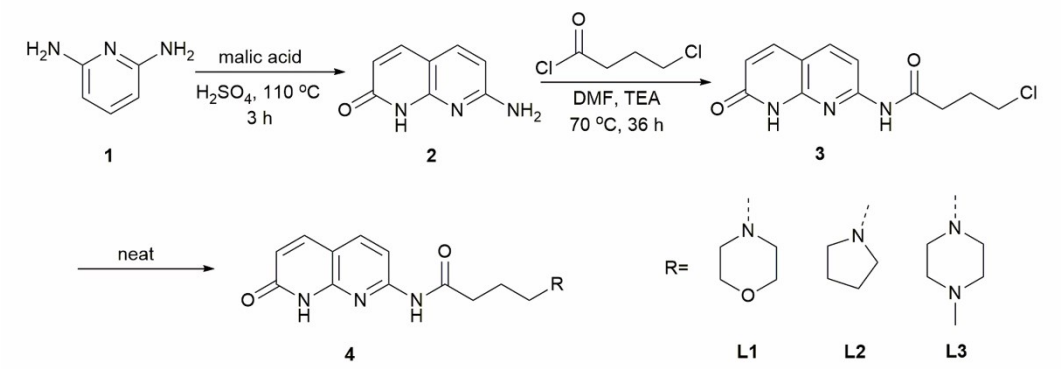
Cell Cycle Analysis

HeLa cells were seeded in 6-well plates (1.0×10^5 cells/ well) for 24 h. Cells in each 6-well plate were treated with ligands for 24 h in fresh growth medium. Cells were treated with medium alone as negative control. Cells were harvested by trypsinization, resuspended in cold PBS and fixed with 2 ml of ice-cold ethanol at 4 °C for overnight. Cell pellets were collected by centrifugation 1200 rpm for 3 min and washed with PBS solution. Cells were inbucated with RNase A (BestBio) at 37 °C for 30 min before stained with PI (BestBio) for 30 min in the dark at 4 °C.

Immunofluorescence microscopy

HeLa cells were seeded in 6-well plates (1.0×10^5 cells/ well) for 24 h. Cells in each 6-well plate were treated with ligands for 24 h in fresh growth medium. Cells were treated with medium alone as negative control. Cells were fixed with 4% paraformaldehyde solution for 10 min and treated with 1% Triton x-100 for 15 min at room temperature. Then cells were incubated with 1% BSA for 60 min at room temperature. TRF2 antibody (SANTA, sc-271710) was diluted with 1.5% BSA solution and BG4 (Absolute Antibody, Ab00174-1.1) was diluted with 0.1% BSA solution before added into petri dish. Cells were incubated with TRF2 antibody for 90 min at room temperature. Then, BG4 was added and incubated at 4 °C overnight. After that, cells were incubated with secondary antibody (Invitrogen, A28175SAMPLE), labeled by Alexa Fluor 488, which was diluted with 0.1% BSA solution for 45 min and then stained with DAPI for 20 min at room temperature in the dark. Cells were washed with PBS buffer for three times after each step.

2. Synthetic Route of Naphthyridine Ligands



3. Synthetic Data of Naphthyridine Ligands

2-amino-7-hydroxy-1,8-naphthyridine (**2**). Malic acid (20 g, 149 mmol) and **1** (2,6-diaminopyridine, 14 g, 128 mmol) were grinded into powder and added to 250 mL of round bottom flasks and cooled in the ice bath. Then, concentrated sulfuric acid (64 mL) was dropwise added. The solution was heated under argon atmosphere to 110 °C for 3 h. After the reaction was complete, the mixture was poured over ice and made pH to 8 with concentrated ammonium hydroxide. Subsequently, the precipitate was filtrated and washed

with H_2O for three times. Removed residual solvent from the vacuum oven to give as yellow solid (20 g, 124 mmol, 97%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.62 (d, J = 8 Hz, 2 H), 6.85 (s, 2 H), 6.34 (d, J = 8 Hz, 1 H), 6.11 (d, J = 8 Hz, 1 H).

4-chloro-N-(7-oxo-7,8-dihydro-1,8-naphthyridin-2-yl)butanamide (**3**). Triethylamine (11 g, 108 mmol) was added in DMF solution of 2-amino-7-hydroxy-1,8-naphthyridine (2.4 g, 15 mmol). 4-Chlorobutyryl chloride (10.6 g, 75 mmol) was dropwise added to the mixture in the ice bath. After the mixture had been stirred for 1 h at room temperature, gradually heated to 70 °C for 36 h under argon atmosphere. Quenching the mixture with methanol for 1 h at room temperature and removed residual solvent by evaporation. Subsequently, the mixture was washed with methanol for three times and evaporated to remove residual methanol to obtain yellow solid (2.4 g, 9 mmol, 60%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.88 (bs, 1 H), 10.62 (s, 1 H), 8.04 (d, J = 8 Hz, 1 H), 7.92 (d, J = 8 Hz, 1 H), 7.85 (d, J = 8 Hz, 1 H), 6.43 (d, J = 8 Hz, 1 H), 3.68 (t, 2 H), 2.62 (t, 2 H), 2.03 (m, 2 H).

4-morpholino-N-(7-oxo-7,8-dihydro-1,8-naphthyridin-2-yl)butanamide (L1). Morpholine (10 mL) and 4-chloro-N-(7-oxo-7,8-dihydro-1,8-naphthyridin-2-yl)butanamide (200 mg, 0.75 mmol) were mixed at 60 °C for 24 h in neat. Solvent was removed by evaporation and added moderate DMF to recrystallization. Precipitate was washed with methanol for three times and removed residual solvent in vacuo. The product was obtained as white solid (215 mg, 0.68 mmol, 91%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.79 (bs, 1 H), 10.46 (s, 1 H), 8.03 (d, J = 8 Hz, 1 H), 7.96 (d, J = 8 Hz, 1 H), 7.82 (d, J = 8 Hz, 1 H), 6.43 (d, J = 8 Hz, 1 H), 3.53 (t, 4 H), 2.47 (t, 2 H), 2.37-2.26 (m, 6 H), 1.75 (m, 2 H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.2, 162.7, 152.0, 148.3, 138.3, 137.8, 119.8, 109.9, 107.9, 65.6, 57.0, 54.3, 52.6, 33.7, 30.1, 21.2. ESI-HRMS: *m/z* 317.1618 ([$C_{16}H_{20}N_4O_3$+H^+] calcd for 317.1608)

4-(pyrrolidin-1-yl)-N-(7-oxo-7,8-dihydro-1,8-naphthyridin-2-yl)butanamide (L2). Pyrrolidine (10 mL) and 4-chloro-N-(7-oxo-7,8-dihydro-1,8-naphthyridin-2-yl)butanamide (300 mg, 1.13 mmol)were mixed at room temperature for 24 h in neat. Mixture was concentrated by evaporation and added moderate methanol to recrystallization. Precipitate was washed with methanol for one time and removed residual solvent in vacuo. The product was obtained as slight yellow solid (30 mg, 0.1 mmol, 9%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ

11.86 (bs, 1 H), 10.54 (s, 1 H), 8.03 (d, J = 8 Hz, 1 H), 7.95 (d, J = 8 Hz, 1 H), 7.85 (d, J = 8 Hz, 1 H), 6.42 (d, J = 8 Hz, 1 H), 2.47 (t, 2 H), 2.41 (m, 6 H), 1.74-1.65 (m, 6 H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 163.8, 153.0, 149.3, 139.4, 138.9, 120.9, 111.0, 108.9, 55.4, 53.9, 31.2, 23.4. ESI-HRMS: *m/z* 301.1660 ([$C_{16}H_{20}N_4O_2$+H^+] calcd for 301.1659)

4-(4-methylpiperazin-1-yl)-N-(7-oxo-7,8-dihydro-1,8-naphthyridin-2-yl)butanamide (L3). 4-methylpiperazine (5 mL) and 4-chloro-N-(7-oxo-7,8-dihydro-1,8-naphthyridin-2-yl)butanamide (500 mg, 1.9 mmol) were mixed at 60 °C for 72 h in neat. Mixture was concentrated by evaporation and added moderate DMF to recrystallization. Precipitate was washed with methanol for one time and removed residual solvent in vacuo. The product was obtained as white solid (63 mg, 0.19 mmol, 10%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.84 (bs, 1 H), 10.47 (s, 1 H), 8.03 (d, J = 8 Hz, 1 H), 7.96 (d, J = 8 Hz, 1 H), 7.82 (d, J = 8 Hz, 1 H), 6.42 (d, J = 8 Hz, 1 H), 2.44-2.27 (m, 12 H), 2.10 (s, 3 H), 1.72 (m, 2 H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.3, 163.8, 153.0, 149.3, 139.4, 138.8, 120.8, 110.9, 109.0, 57.6, 55.2, 53.0, 46.2, 34.8, 22.6. ESI-HRMS: *m/z* 330.1927 ([$C_{17}H_{23}N_5O_2$+H^+] calcd for 330.1925).

4. DNA Sequences

DNA Sequences used in this study were purchased from Come (Changchun, China).

Name	Sequence
Tel26	(5'-TTAGGG(TTAGGG)$_3$TT-3')
Tel26-G3T	(5'-(TTAGGG)$_3$TTTTTTTT-3')
Tel26-TG3	(5'-TTTTTT(TTAGGG)$_3$TT-3')
Tel23	(5'-TA(GGGTTA)$_3$GGG-3')
ssTel26	AAAGGGTTAGTGTTAGTGTTAGGGAA
c-MYC	TGAGGGTGGGAGGGTGGGAA
TBA	5'-GGTTGGTGTGGTTGG-3'
ds26	CAATCGGATCGAATTCGATCCGATTG
ss17	CCAGTTCGTAGTAACCC

Oligonucleotides Sequences were annealed by incubating at 95 °C for 10 min and then slowly cooled to room temperature (25 °C) following by incubation at 4 °C overnight before

use. Buffers were used in this study containing 10 mM Tris-HCl, pH 7.0, 1 mM EDTA with 100mM of KCl.

5. NOESY and COSY Spectrum of L1

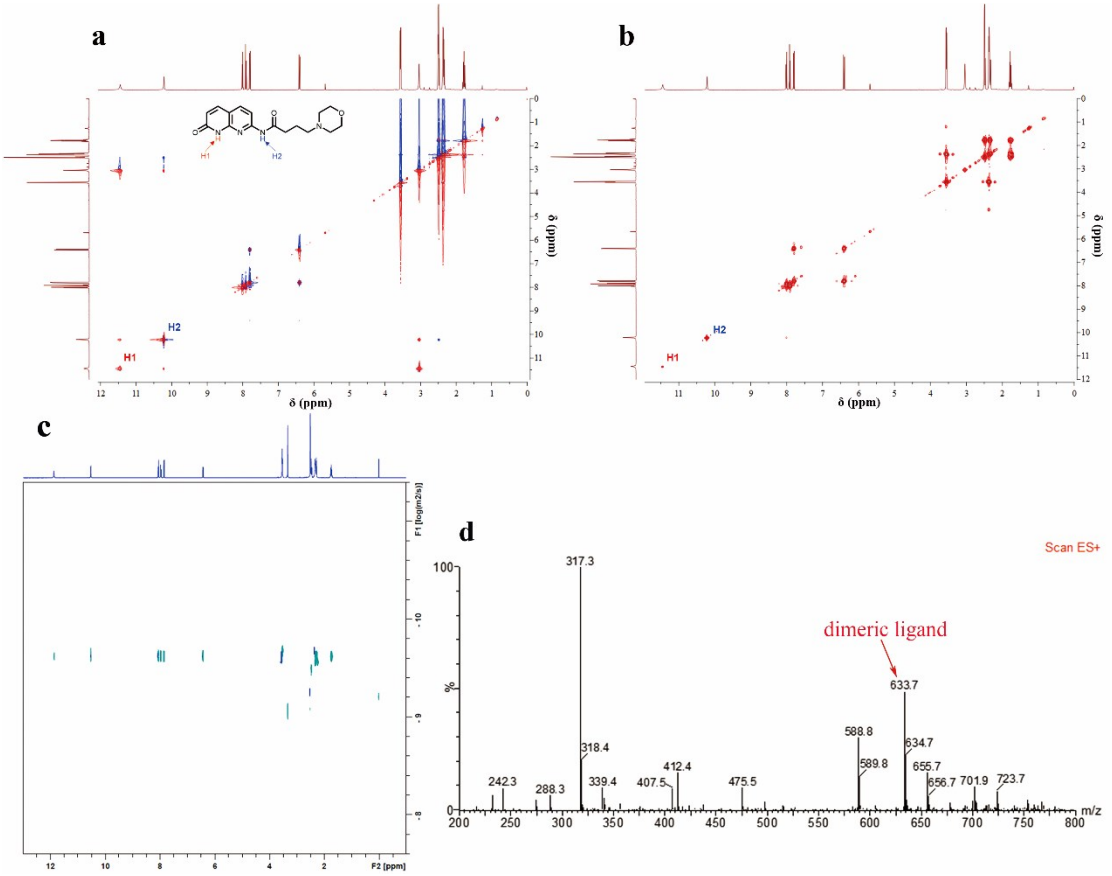


Fig. S1 (a) NOESY spectrum of **L1** in aqueous DMSO-d_6. (b) COSY spectrum of **L1** in aqueous DMSO-d_6. The spectra were recorded at 80 °C. (c) DOSY spectrum of L1 in aqueous DMSO-d_6. The spectra were recorded at 25 °C. (d) Mass spectroscopy profile of dimeric ligand of **L1**.

6. ^{1}H NMR Spectrum of L1 with Adenine

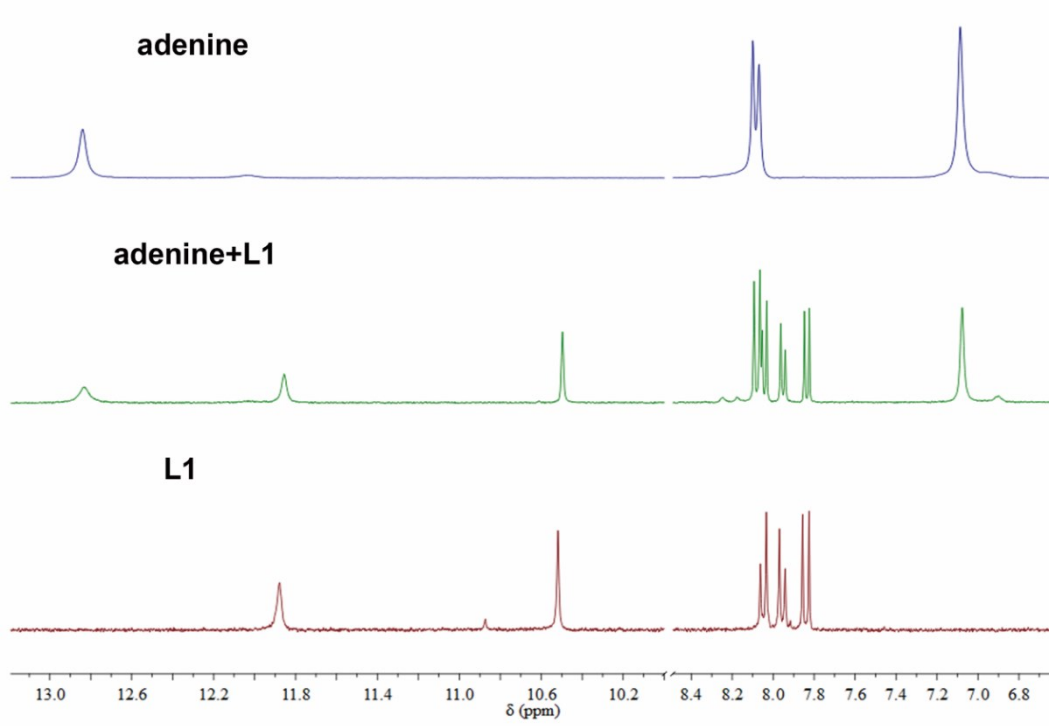


Fig. S2 ^{1}H NMR spectrum of **L1** with adenine in DMSO-d_6 at room temperature**.** From top to bottom are adenine(blue), **L1** with adenine(green), and **L1**(red), respectively.

7. ^{1}H NMR Spectrum of L1 with Cytosine

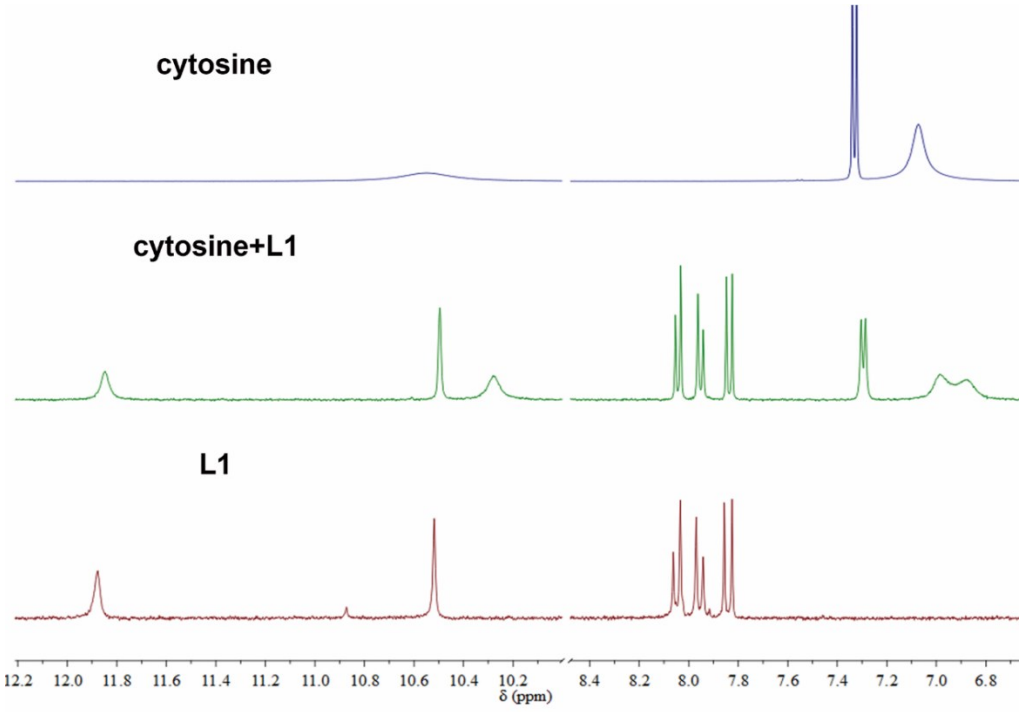


Fig. S3 ^{1}H NMR spectrum of **L1** with cytosine in DMSO-d_6 at room temperature. From top to bottom are cytosine(blue), **L1** with cytosine(green), and **L1**(red), respectively.

8. ^{1}H NMR Spectrum of L1 with Thymine

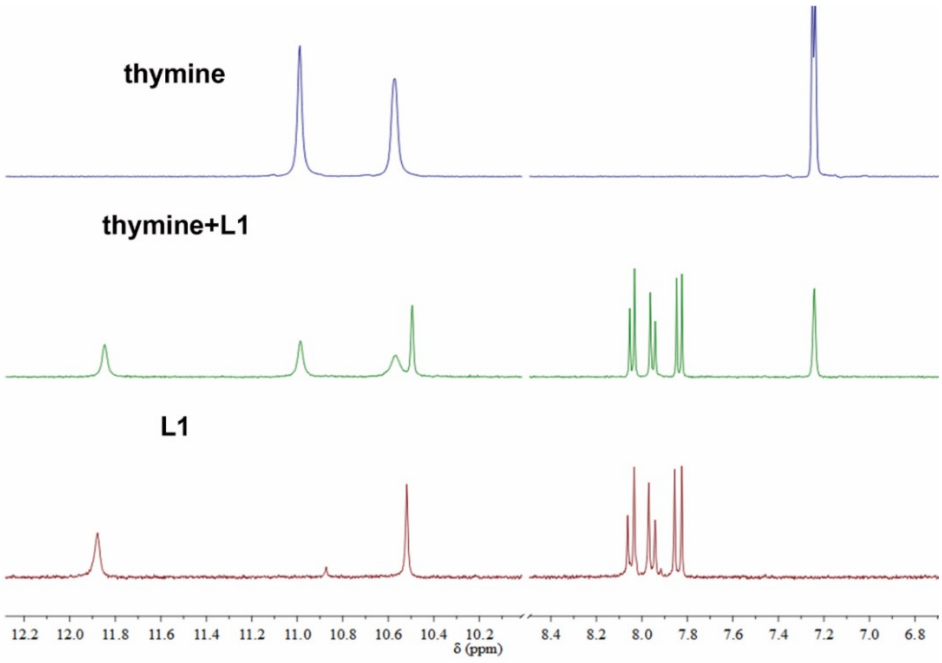


Fig. S4 ^{1}H NMR spectrum of **L1** with thymine in DMSO-d_6 at room temperature. From top to bottom are thymine(blue), **L1** with thymine(green), and **L1**(red), respectively.

9. ^{1}H NMR Spectrum of L1 with Guanosine

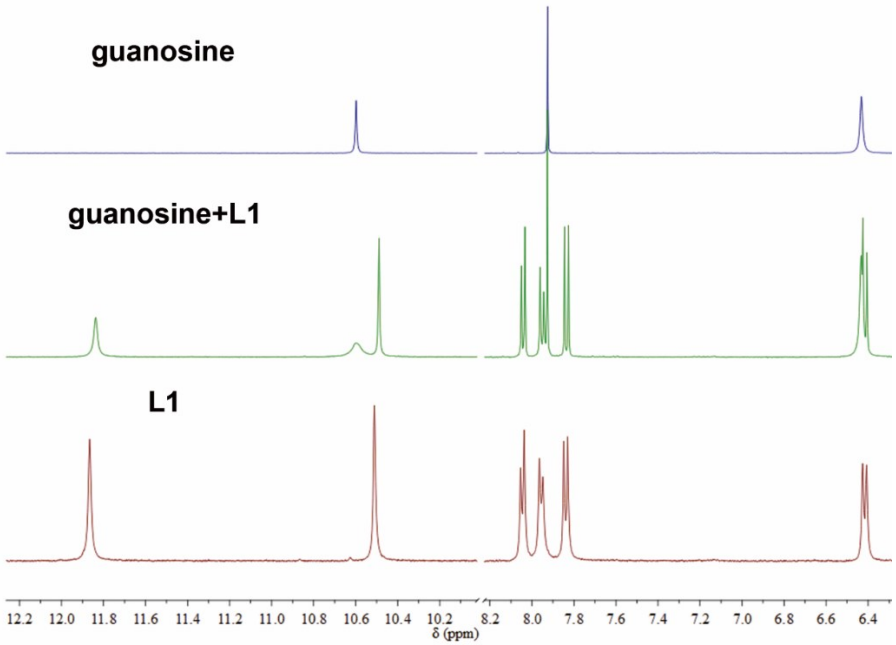


Fig. S5 ^{1}H NMR spectrum of **L1** with guanosine in DMSO-d_6 at room temperature. From top to bottom are guanosine(blue), **L1** with guanosine(green), and **L1**(red), respectively.

10. ^{1}H NMR Spectrum of L3 with Adenine

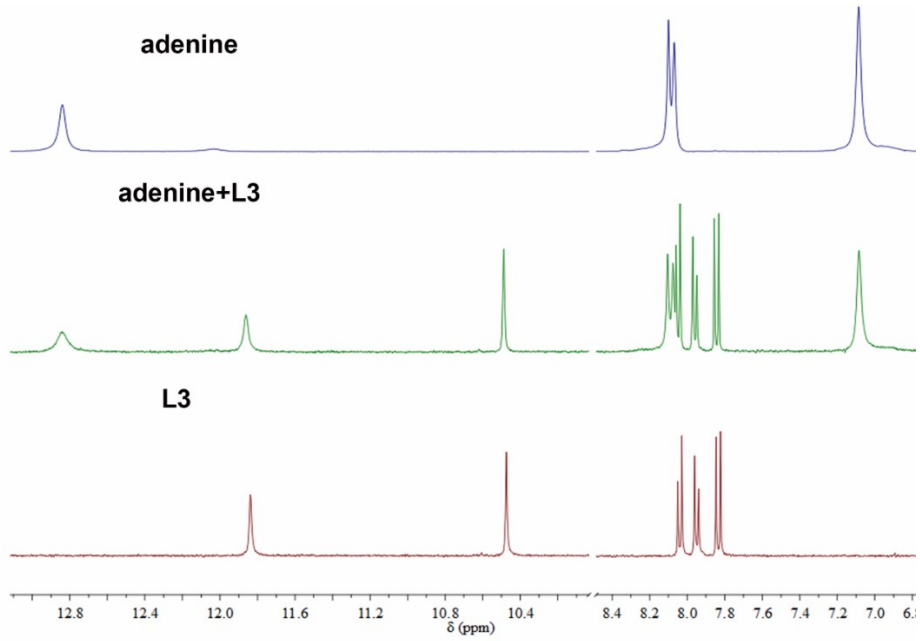


Fig. S6 ^{1}H NMR spectrum of **L3** with adenine in DMSO-d_6 at room temperature. From top to bottom are adenine(blue), **L3** with adenine(green), and **L3**(red), respectively.

11. ^{1}H NMR Spectrum of L3 with Cytosine

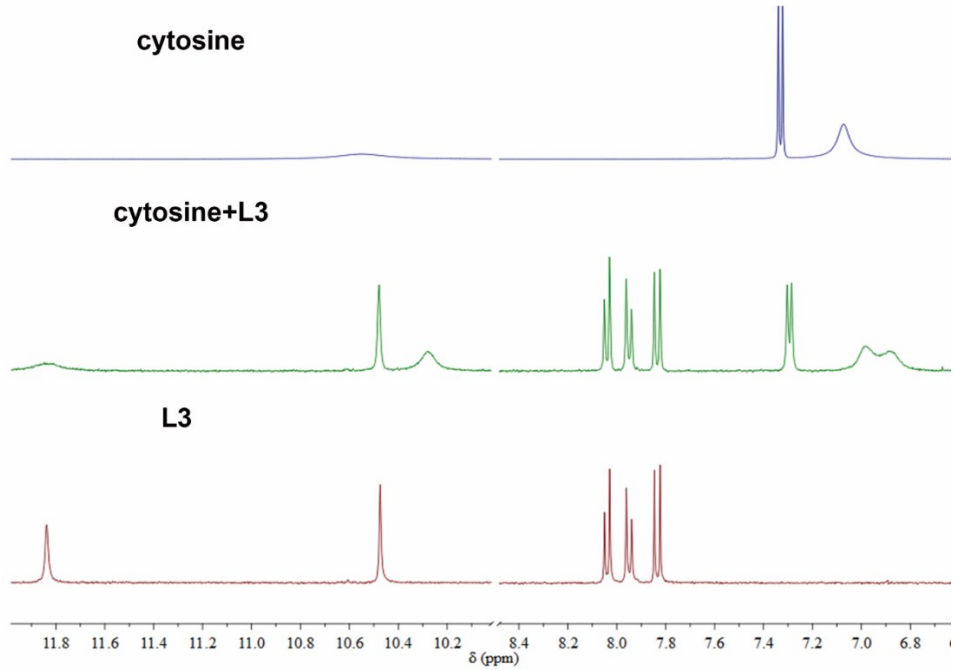


Fig. S7 ^{1}H NMR spectrum of **L3** with cytosine in DMSO-d_6 at room temperature. From top to bottom are cytosine(blue), **L3** with cytosine(green), and **L3**(red), respectively.

12. ^{1}H NMR Spectrum of L3 with Thymine

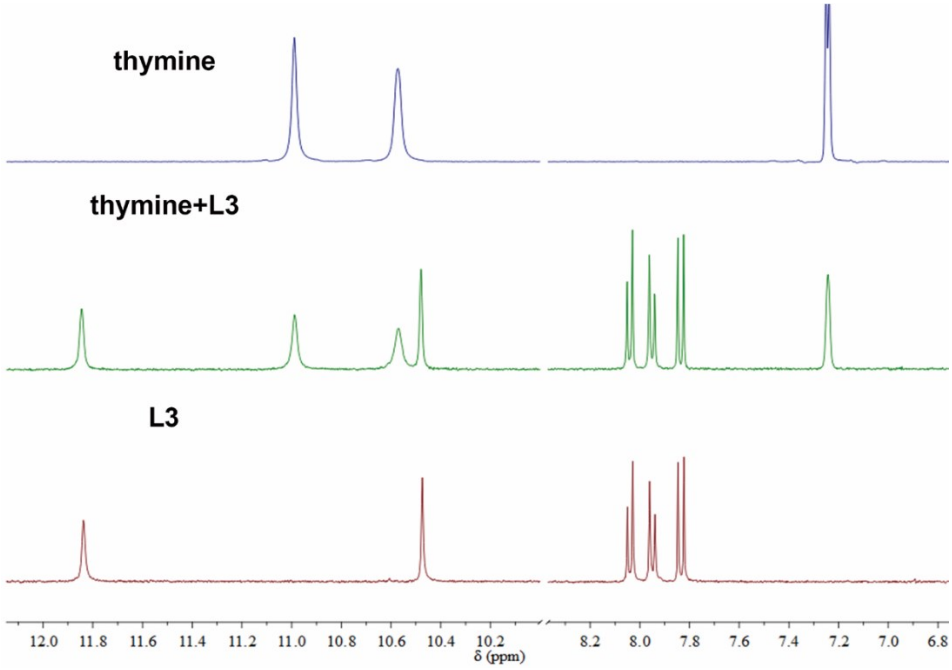


Fig. S8 ^{1}H NMR spectrum of **L3** with thymine in DMSO-d_6 at room temperature. From top to bottom are thymine(blue), **L3** with thymine(green), and **L3**(red), respectively.

13. ^{1}H NMR Spectrum of L3 with Guanosine

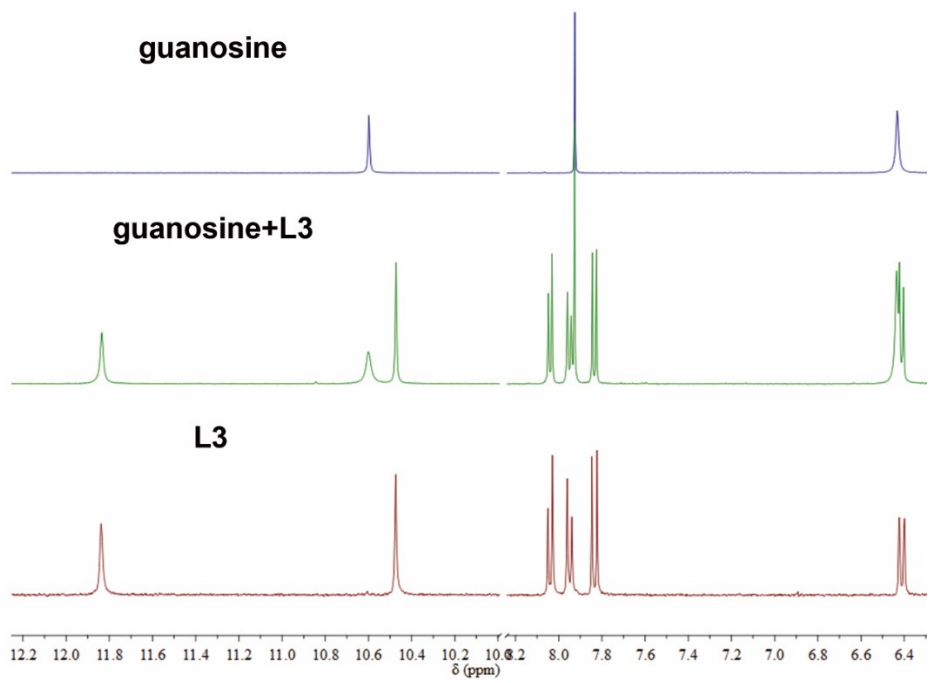


Fig. S9 ^{1}H NMR spectrum of **L3** with guanosine in DMSO-d_6 at room temperature. From top to bottom are guanosine(blue), **L3** with guanosine(green), and **L3**(red), respectively.

14. ^{1}H NMR Spectrum of L2 with Adenine

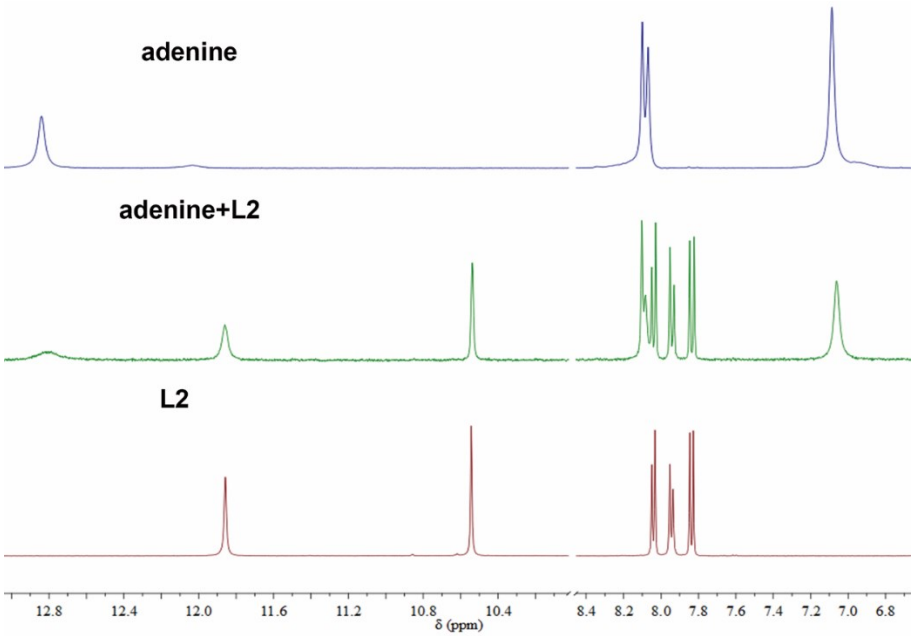


Fig. S10 ^{1}H NMR spectrum of **L2** with adenine in DMSO-d_6 at room temperature. From top to bottom are adenine(blue), **L2** with adenine(green), and **L2**(red), respectively.

15. ^{1}H NMR Spectrum of L2 with Cytosine

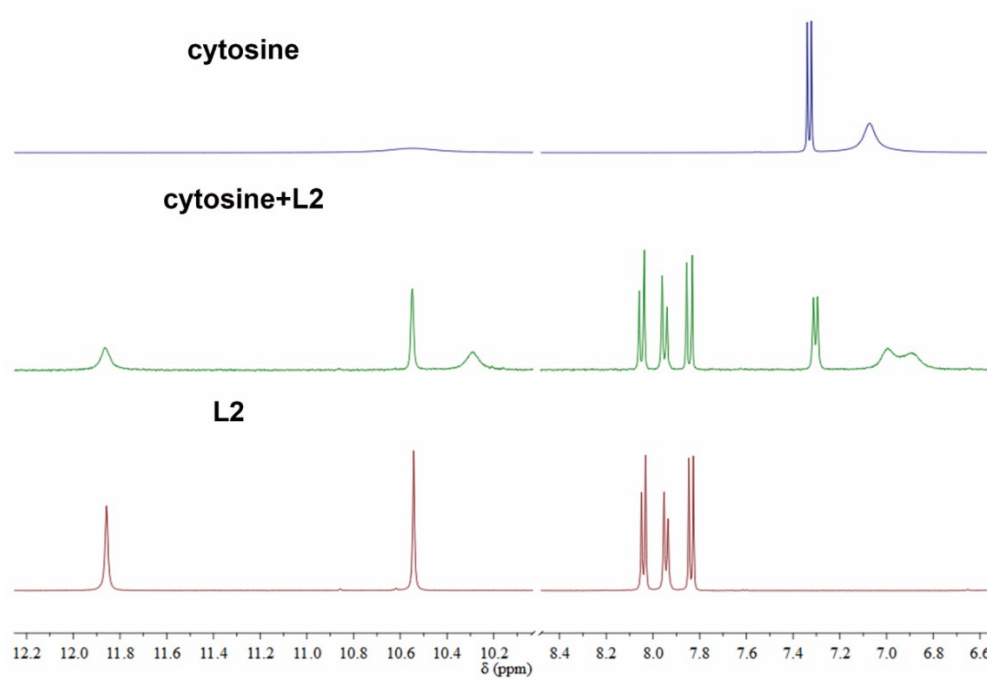


Fig. S11 ^{1}H NMR spectrum of **L2** with cytosine in DMSO-d_6 at room temperature. From top to bottom are cytosine(blue), **L2** with cytosine(green), and **L2**(red), respectively.

16. ^{1}H NMR Spectrum of L2 with Thymine

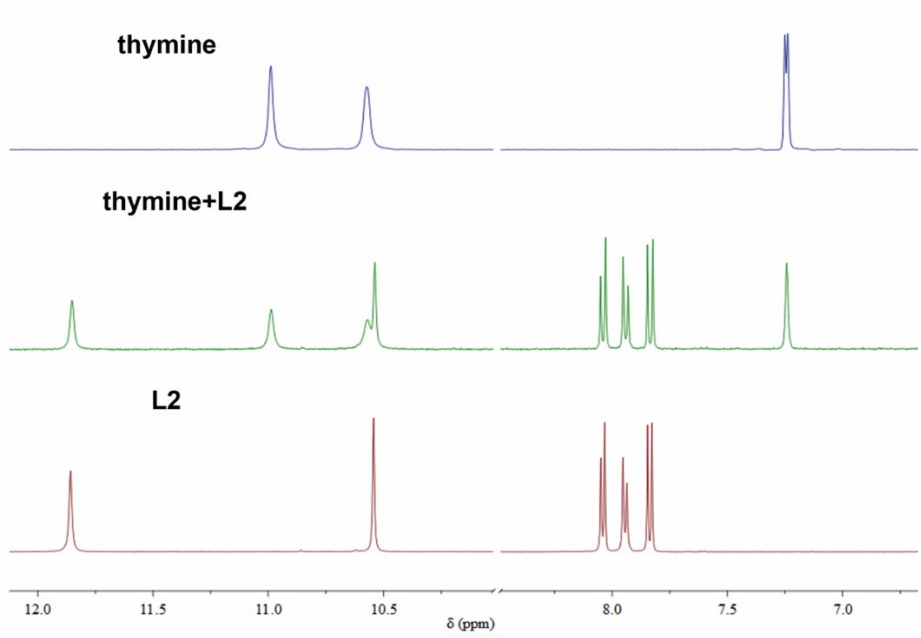


Fig. S12 ^{1}H NMR spectrum of **L2** with thymine in DMSO-d_6 at room temperature. From top to bottom are thymine(blue), **L2** with thymine(green), and **L2**(red), respectively.

17. ^{1}H NMR Spectrum of L2 with Guanosine

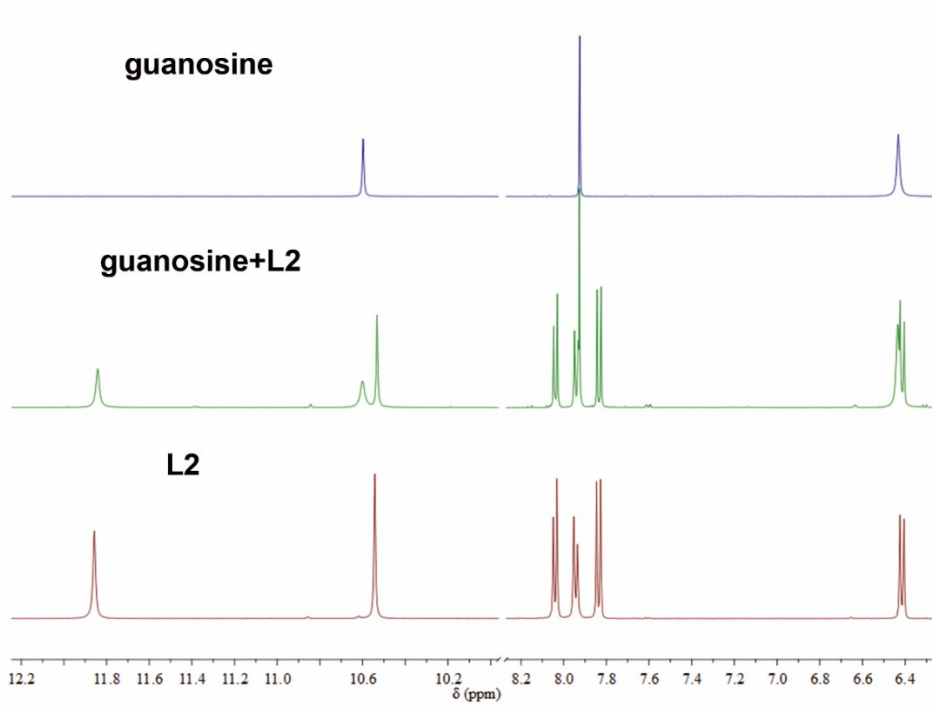


Fig. S13 ^{1}H NMR spectrum of **L2** with guanosine in DMSO-d_6 at room temperature. From top

to bottom are guanosine(blue), **L2** with guanosine(green), and **L2**(red), respectively.

18. ^{1}H NMR Spectrum of L3 in Different DMSO and H_2O ratios.

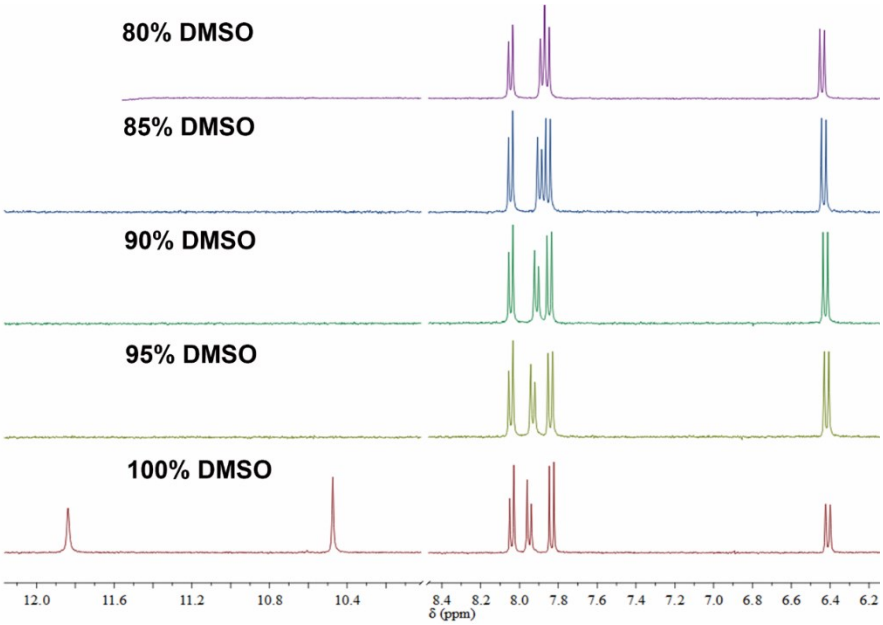


Fig. S14 Proportion of DMSO to water from top to bottom are 80%(purple), 85%(blue), 90%(green), 95%(yellow), and 100%(red), respectively.

19. ^{1}H NMR Spectrum of L2 in Different DMSO and H_2O ratios.

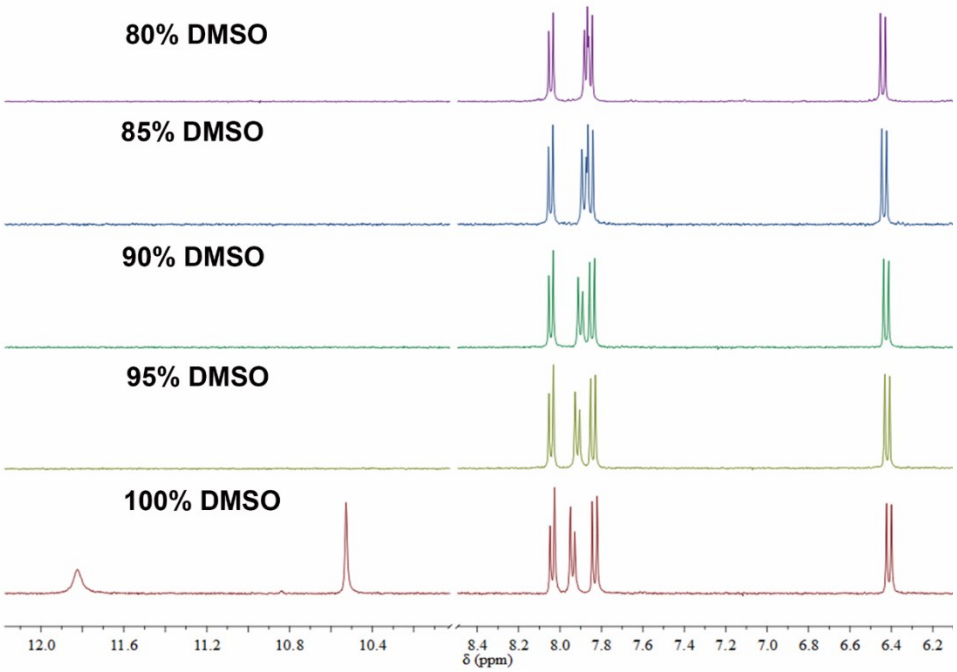


Fig. S15 Proportion of DMSO to water from top to bottom are 80%(purple), 85%(blue),

90%(green), 95%(yellow), and 100%(red), respectively.

20. Fluorescent Spectrum of L3 (20 μM) in Different DMSO and H_2O ratios

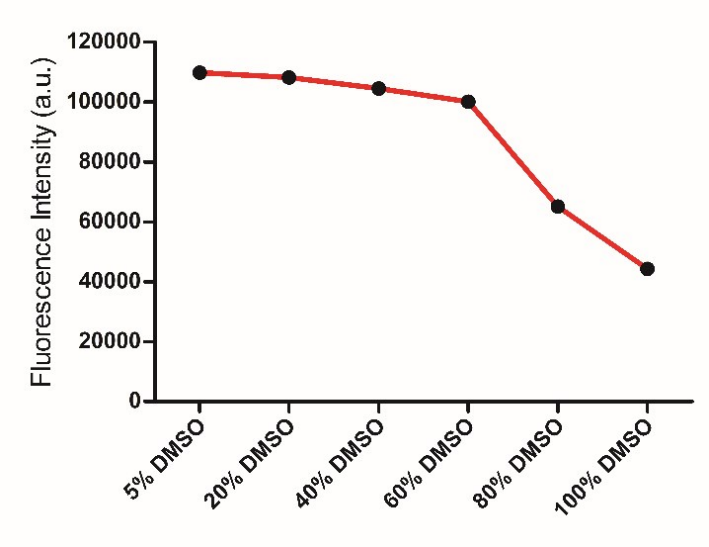


Fig. S16 Fluorescent spectrum of **L3** (20 μM) in different DMSO and H_2O ratios.

21. Fluorescent Spectrum of L2 (20 μM) in Different DMSO and H_2O ratios

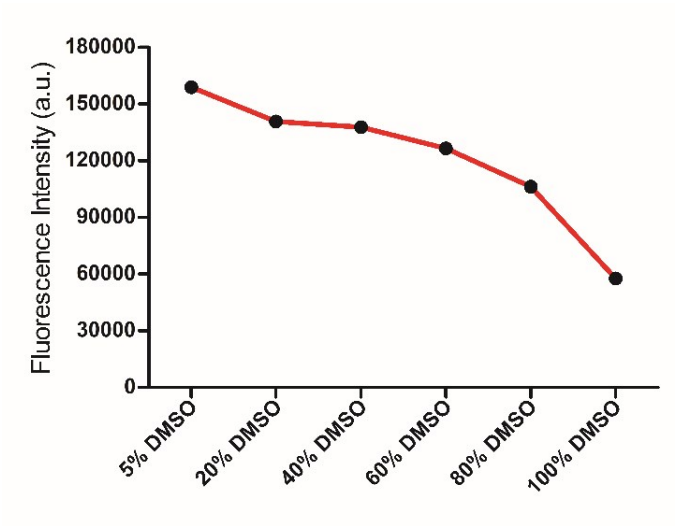


Fig. S17 Fluorescent spectrum of **L2** (20 μM) in different DMSO and H_2O ratios.

22. CD Spectra of Oligonucleotides upon the Addition of Ligands

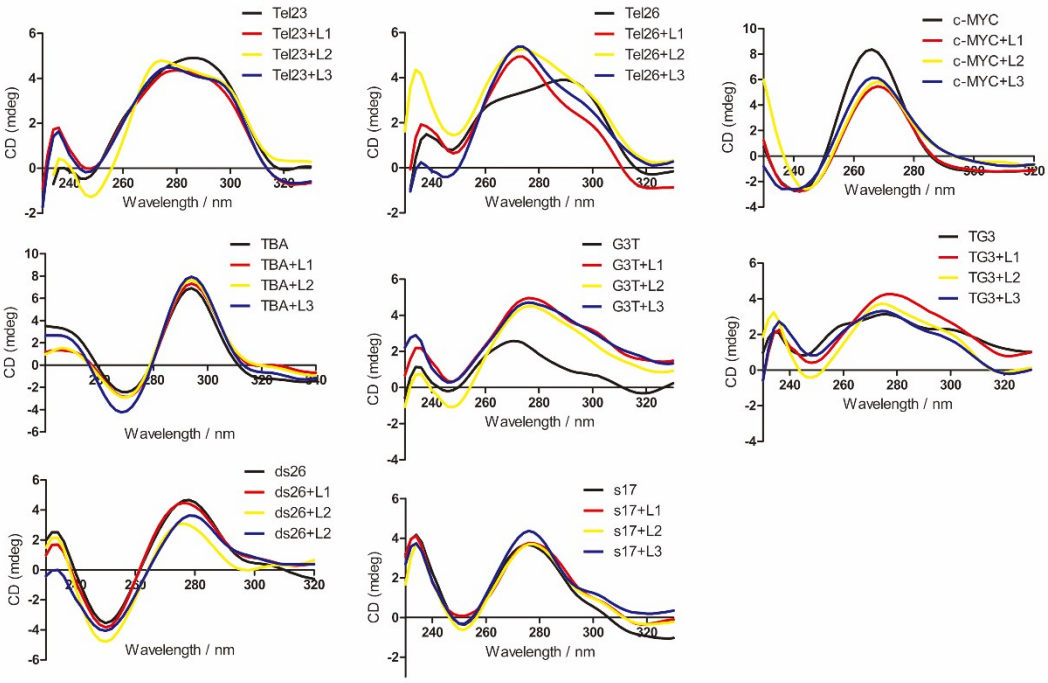


Fig. S18 CD spectra of oligonucleotides upon the addition of ligands

23. ^{1}H NMR spectroscopy of Tel26 and Tel23 in the presence and absence of L1

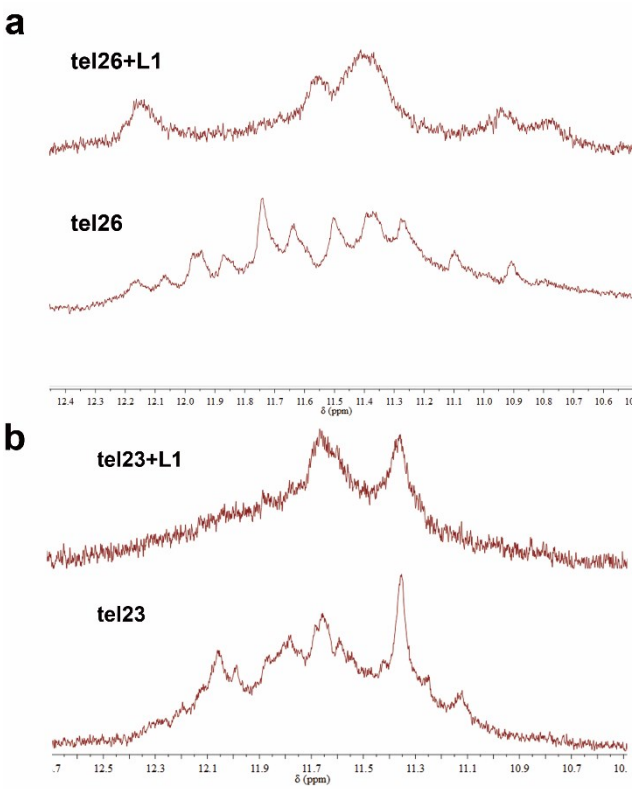


Fig. S19 The imino proton region of ^{1}H NMR spectroscopy of Tel26 (a) and Tel23 (b) in the presence and absence of L1. Conditions: 40 mM KCl, pH 7.0, 25 °C, 0.2 mM DNA, 2 mM **L1**.

24. Gel Electrophoresis for Oligonucleotides in the Presence or Absence of Ligands

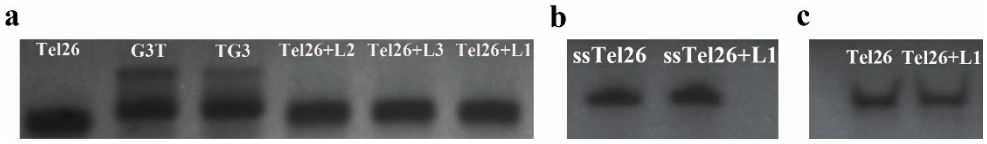


Fig. S20 Gel electrophoresis for oligonucleotides in the presence or absence of ligands. (a) The bonds from left to right represent Tel26, G3T, TG3, Tel26 with **L2**, **L3**, and **L1**, respectively. (b) The bonds represent ssTel26 with or without **L1** in the presence of K^{+}, respectively. (c) The bonds represent Tel26 with or without **L1** in the presence of Li^{+}, respectively.

25. Fluorescence Spectra of Ligands with DNA Sequences

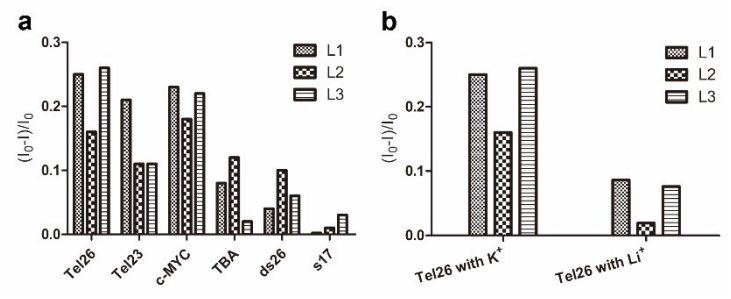


Fig. S21 (a) Fluorescent quenching of **L1**, **L2**, and **L3** (100 μM) upon addition of DNA sequences (10 μM). (b) Fluorescent quenching of **L1**, **L2**, and **L3** (100 μM) upon addition of Tel26 (10 μM) in the presence of K^+ and Li^+, respectively.

26. Jobs plot for complexes of ligands and Tel26

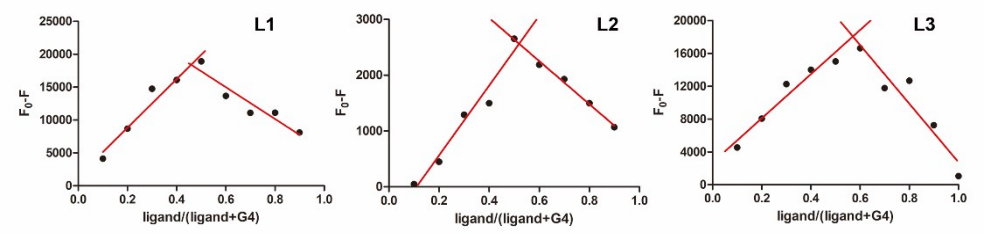


Fig. S22 Fluorescence titration for complexes of ligands and Tel26 in 100mM KCl, 10mM Tris buffer, pH=7.0. Total concentration of ligand and Tel26 was 20 μM for each complex.

27. Cell Cytotoxicity Assays of L2 and L3 for HeLa Cells

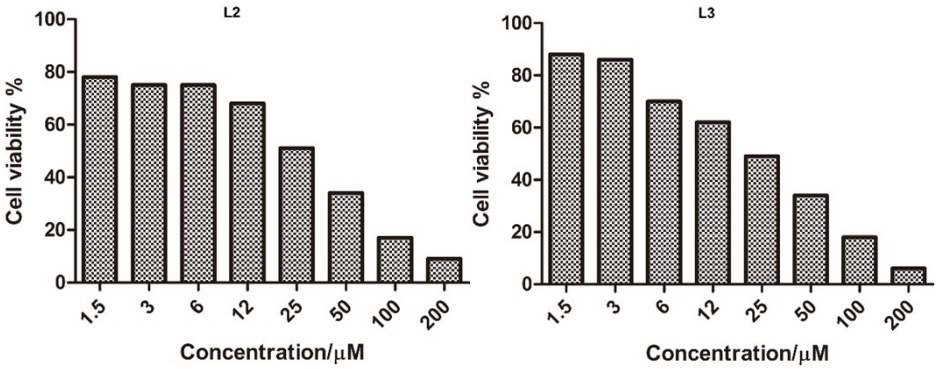


Fig. S23 Cell cytotoxicity assays of **L2**(left) and **L3**(right) for HeLa cells. The concentration of the ligands in the experiments is from 1.5 μM to 200μM. Cells were treated with ligands at various concentrations for 48 h followed by addition of MTT (5 mg/mL, 20 μL) to the medium.

28. Apoptosis Analysis of HeLa Cells Treated with L1

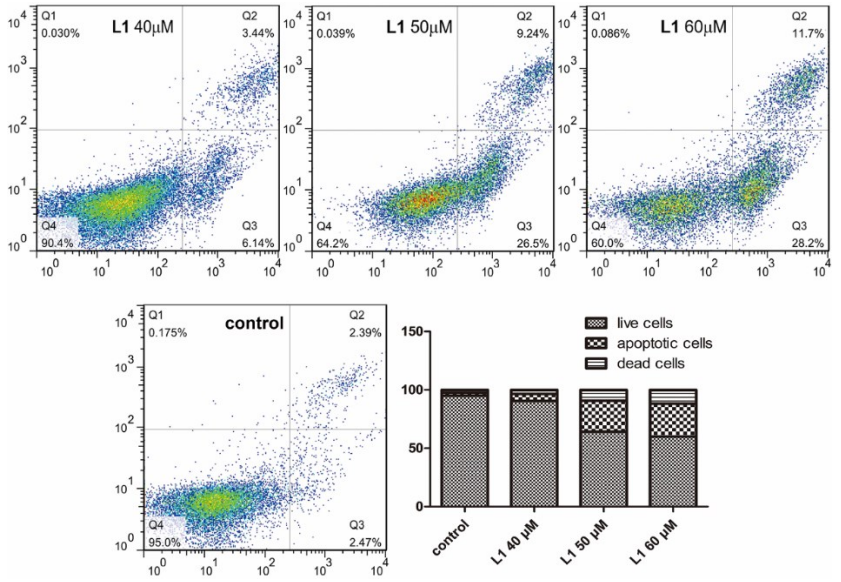


Fig. S24 Apoptosis analysis of HeLa cells treated with **L1** in different concentration for 24 h. Q1, Q2, Q3, and Q4 represent necrotic cells, dead cells, apoptotic cells, and live cells, respectively.

29. Apoptosis Analysis of HeLa Cells Treated with L3

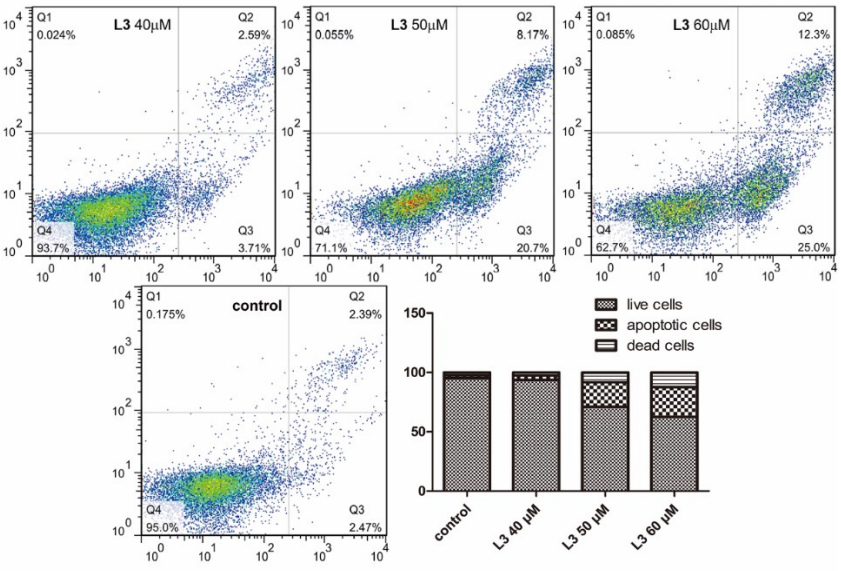


Fig. S25 Apoptosis analysis of HeLa cells treated with **L3** in different concentration for 24 h. Q1, Q2, Q3, and Q4 represent necrotic cells, dead cells, apoptotic cells, and live cells, respectively.

30. Cell Cycle Analysis of HeLa Cells Treated with L3

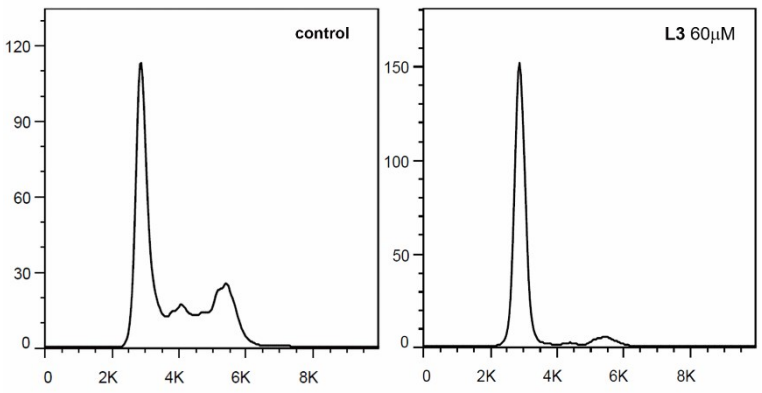


Fig. S26 Cell cycle analysis of HeLa cells treated with **L3** at 60 μM for 24 h.

31. Immunofluorescence microscopy of HeLa cells incubated with BG4 and TRF2 antibody

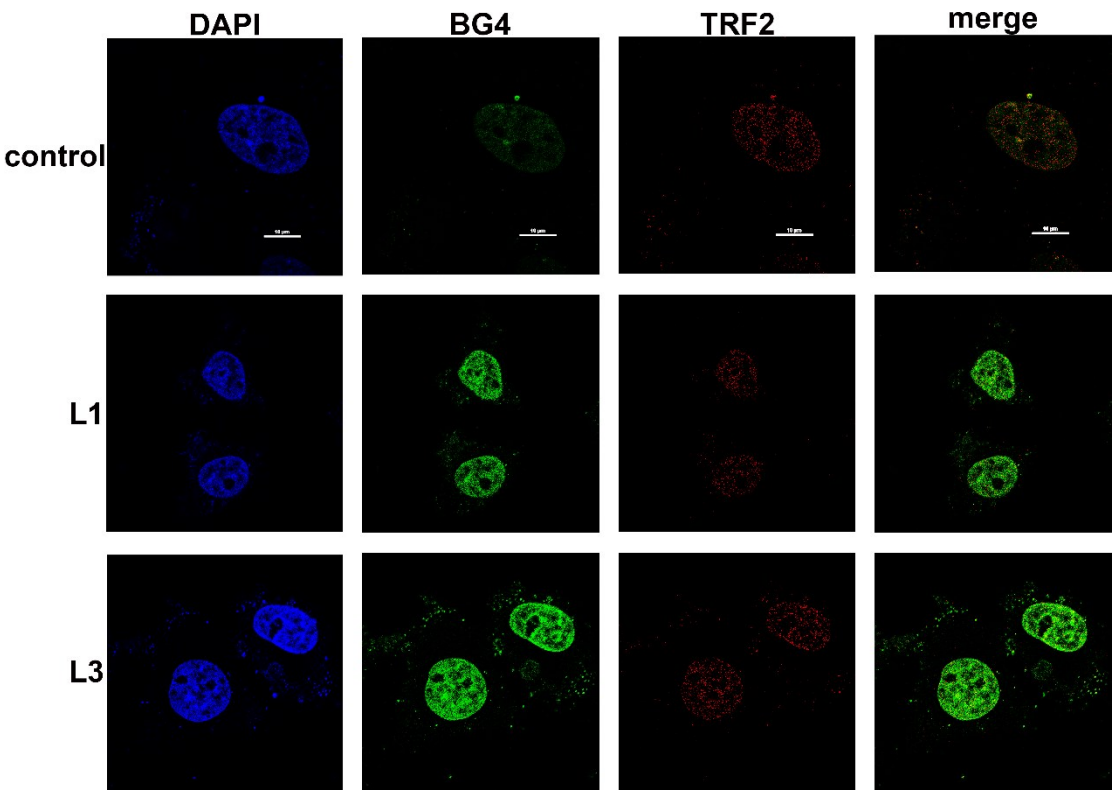


Fig. S27 Immunofluorescence microscopy of HeLa cells incubated with ligand **L1** and **L3** at 30 μM for 24 h. G-quadruplex structure-specific antibody BG4 and TRF2 antibody were colored green and red, respectively. Nucleus were coloured blue by DAPI. BG4 and TRF2 images were merged in the last column.

32. Melting curves of DNA sequences in the presence or absence of ligands

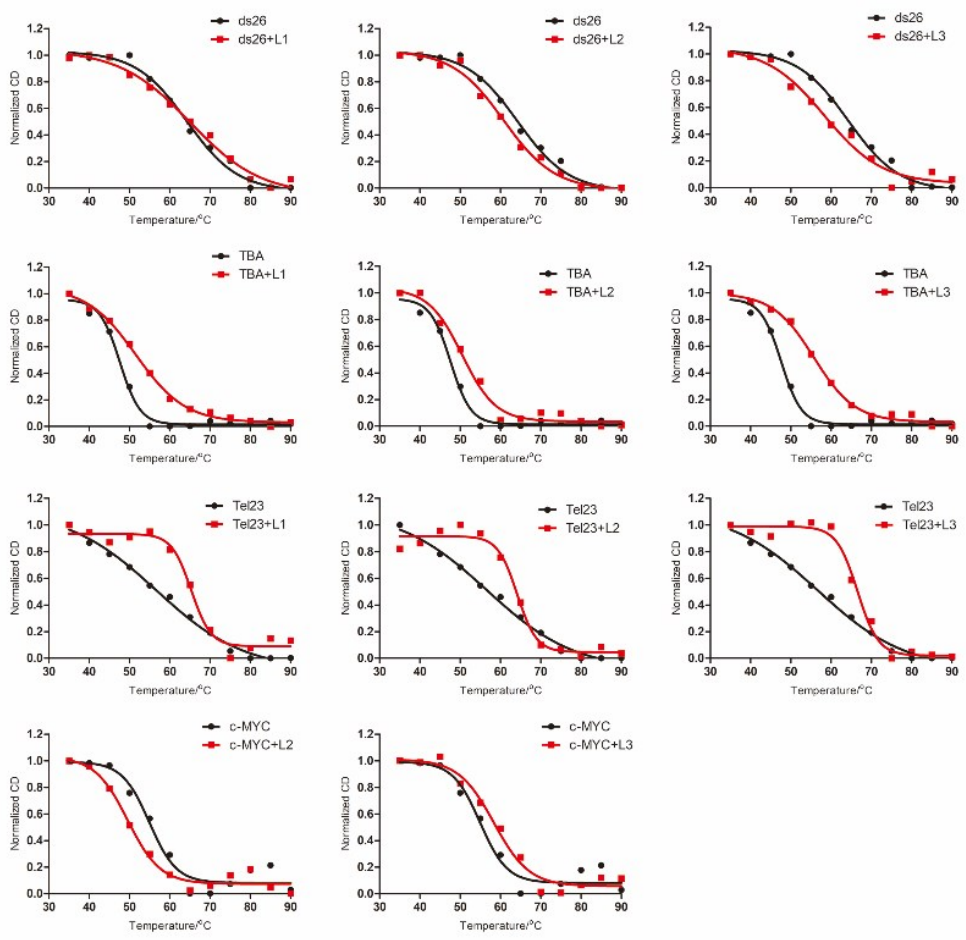


Fig. S28 CD spectra of melting curves of DNA sequences (10 μM) in the presence or absence of L1, L2, and L3 (100 μM) for 24 h. Ellipticity is normalized and normalization of each curve is independent.

33. Melting temperature of DNA sequences

Table S1 Melting temperature of GQs in the presence or absence of **L1**, **L2**, and **L3**.

	No ligand	L1	L2	L3
Tel26	**53 °C**	**65 °C**	**64 °C**	**65 °C**
Tel23	**56 °C**	**66 °C**	**64 °C**	**66 °C**
c-MYC	**56 °C**	**Not determined**	**51 °C**	**60 °C**
TBA	**48 °C**	**53 °C**	**52 °C**	**55 °C**
ds26	**63 °C**	**63 °C**	**62 °C**	**60 °C**

34. ^{1}H and ^{13}C NMR Spectra of L1, L2, and L3

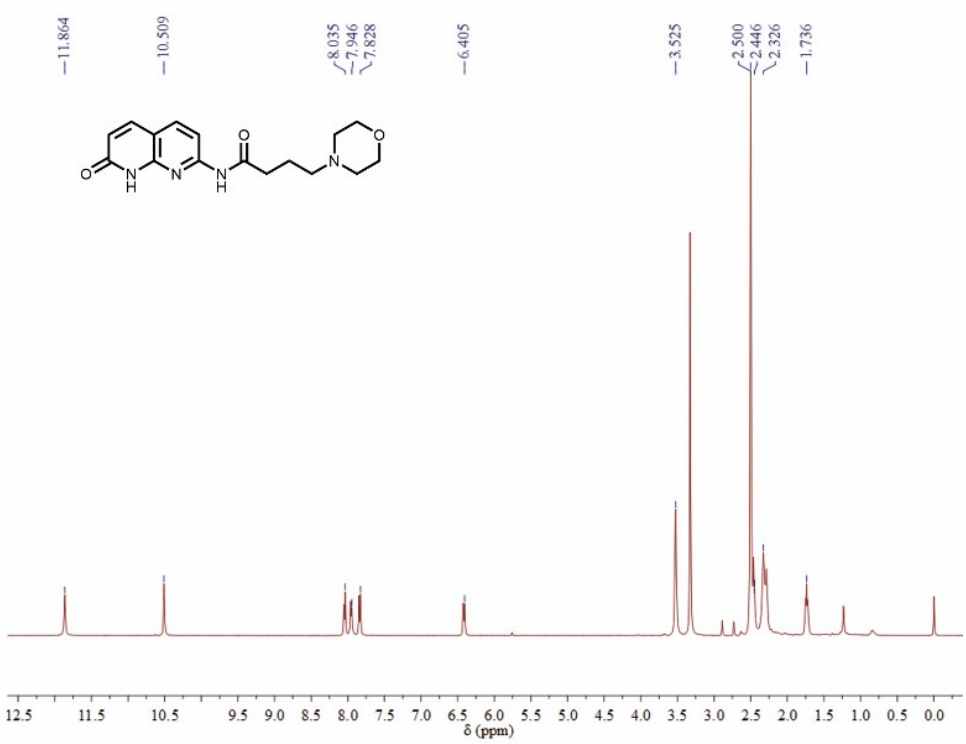


Fig. S29 ^{1}H NMR Spectra of L1 in DMSO-d_6

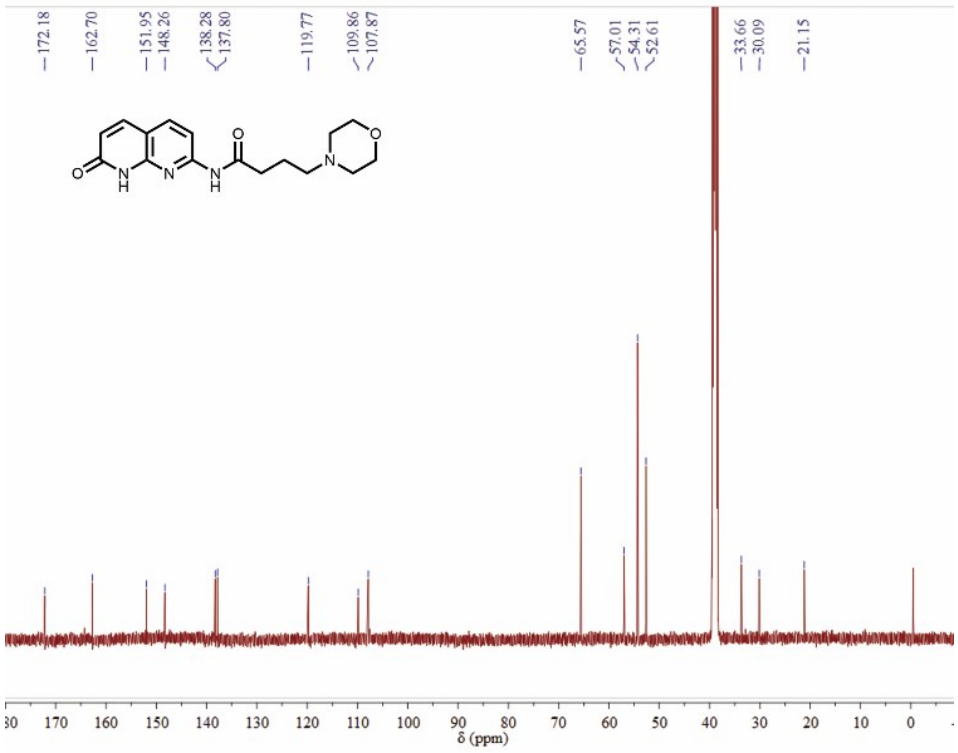


Fig. S30 ^{13}C NMR Spectra of L1 in DMSO-d_6

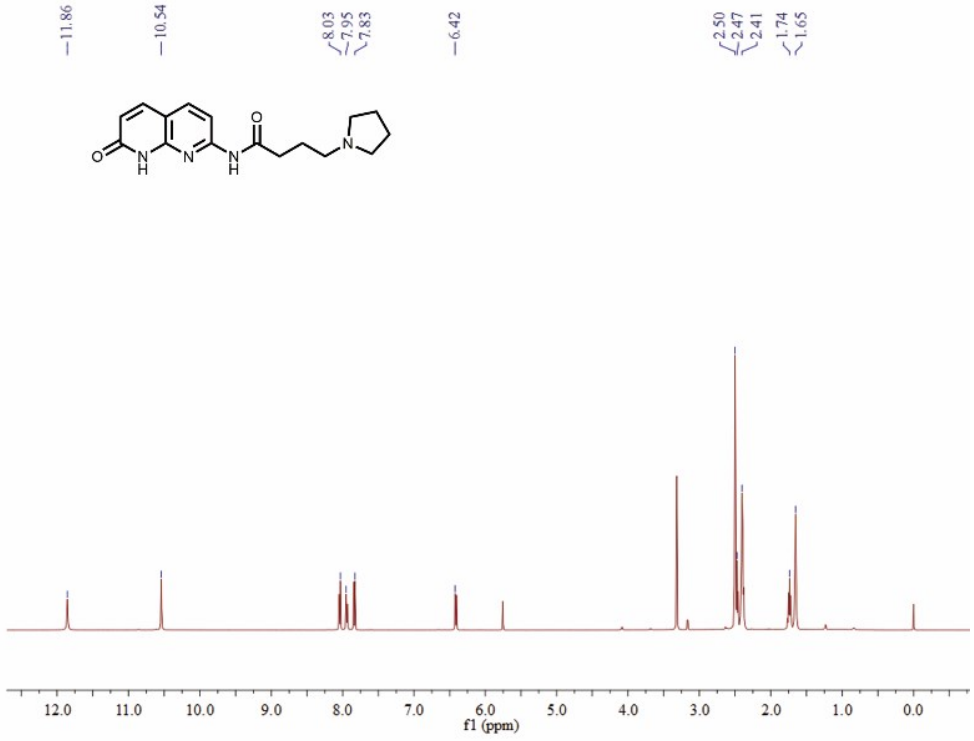


Fig. S31 ^{1}H NMR Spectra of L2 in DMSO-d_6

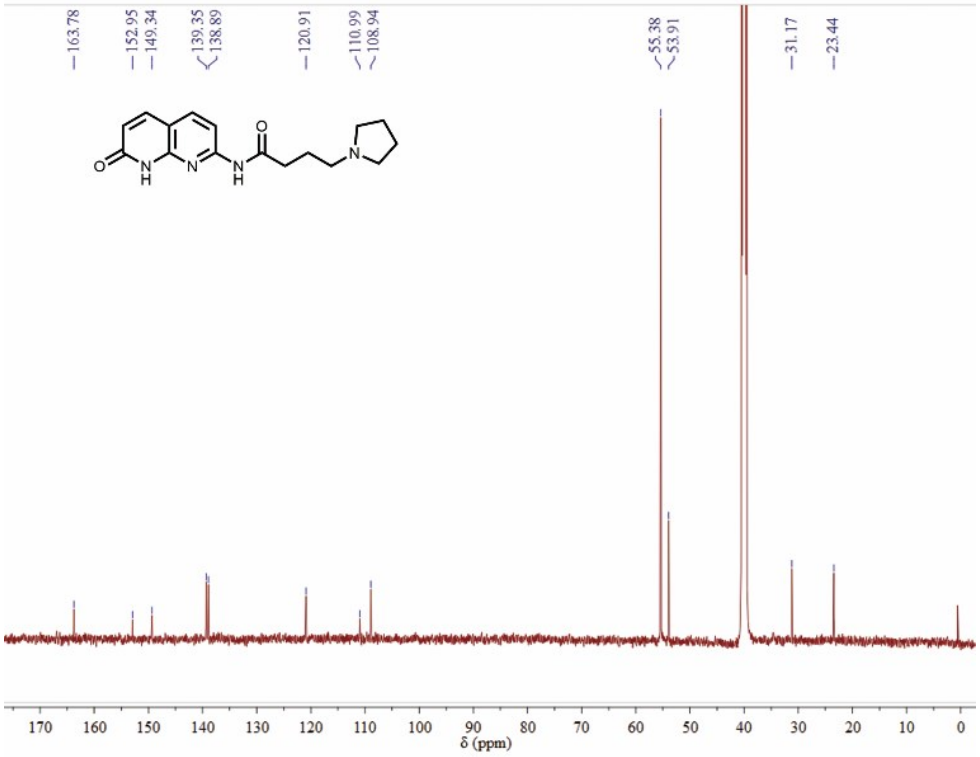


Fig. S32 ^{13}C NMR Spectra of L2 in DMSO-d_6

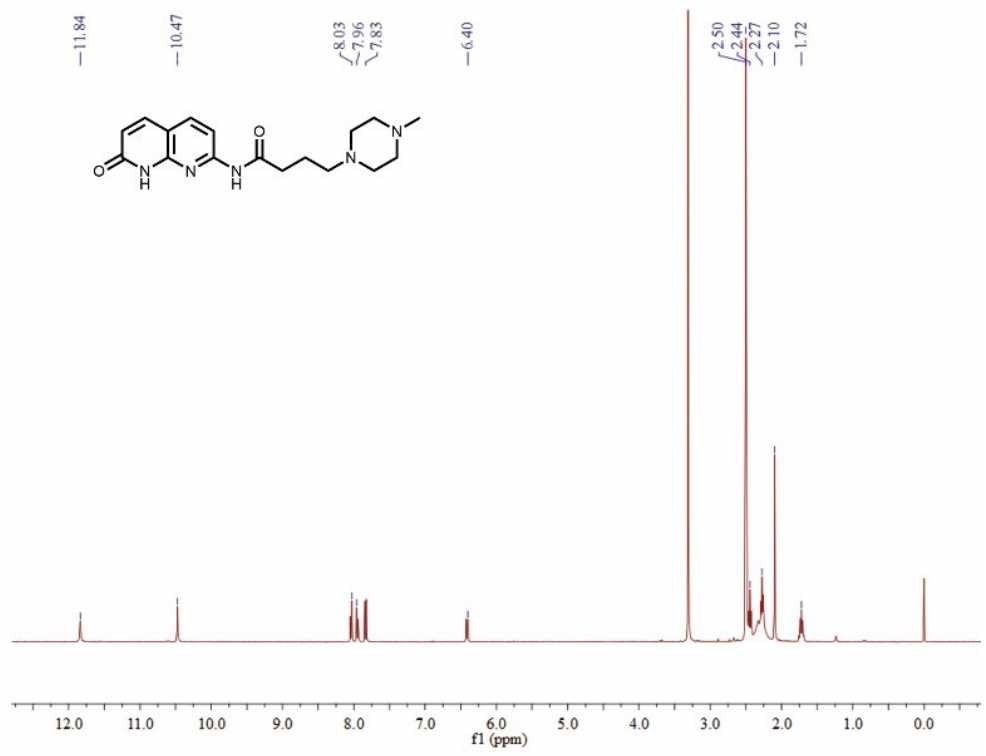


Fig. S33 ^{1}H NMR Spectra of L3 in DMSO-d_6

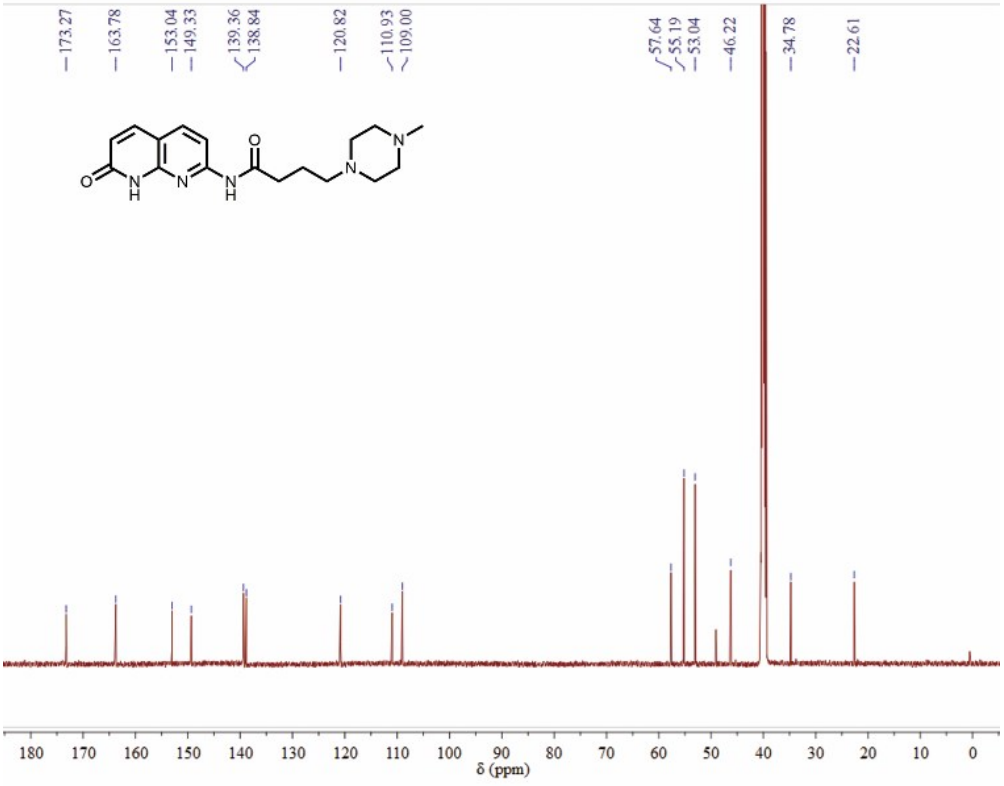


Fig. S34 ^{13}C NMR Spectra of L3 in DMSO-d_6