

**New Dimeric Carbazole-Benzimidazole Mixed Ligands for the Stabilization of Human  
Telomeric G-Quadruplex DNA and as Telomerase Inhibitors. Remarkable Influence of  
Spacer**

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## Materials

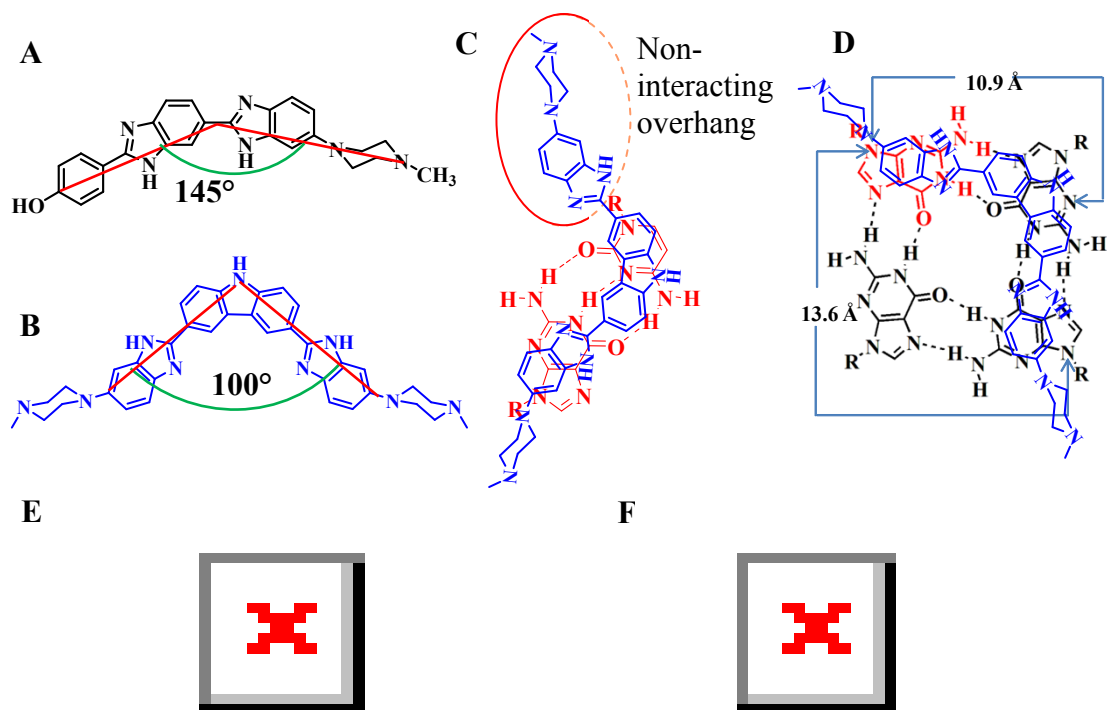
All the starting materials were procured from the best known commercial sources and used as received. All solvents were from Merck and they were distilled and/or dried prior to use whenever necessary.

## General Spectrometric Characterization

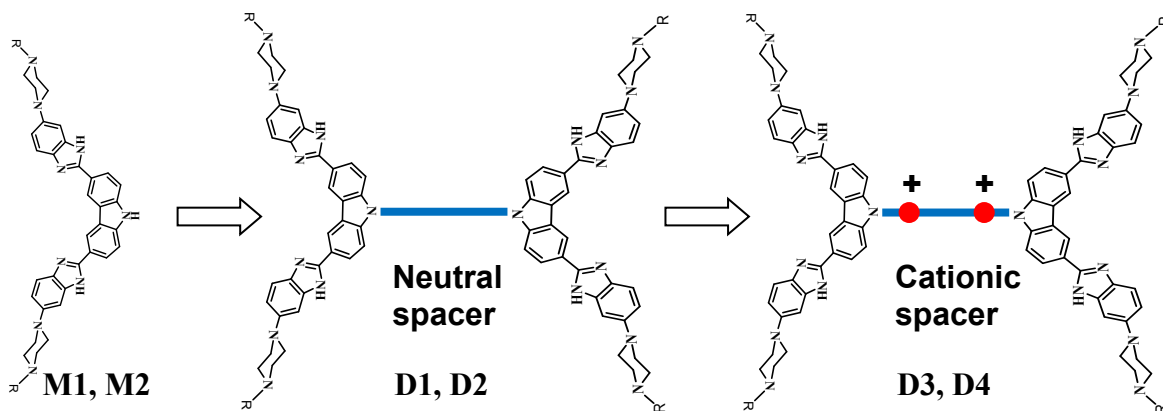
$^1\text{H}$  (300 MHz or 400 MHz) and  $^{13}\text{C}$  NMR (75 MHz or 100 MHz) spectra were recorded on Bruker AMX spectrometers. IR spectra were recorded on FT-IR Perkin Elmer Spectrum GX spectrometer. Melting points were taken on open capillaries inside a Buchi melting point B540 apparatus and are uncorrected. Mass spectra were recorded on Micromass Q-TOF Micro TM spectrometer. MALDI mass spectra were recorded on ultra flex TOF/TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) in positive ion mode, using  $\alpha$ -cyano-4-hydroxyl cinnamic acid as a matrix.

**Oligonucleotides.** HPLC purified oligodeoxyribonucleotides (ODN) d[5'-G<sub>3</sub>(T<sub>2</sub>AG<sub>3</sub>)<sub>3</sub>-3'], abbreviated as Hum<sub>21</sub>, and d[5'-GGGTTAGGG-3'], abbreviated as Hum<sub>9</sub>, were purchased from Sigma Genosys, Bangalore. Their purity was confirmed using high resolution sequencing gel. The concentration of each ODN solutions were determined from UV-absorption measurements at 260 nm based on their molar extinction coefficients ( $\epsilon_{260}$ ) 215000 and 104000 respectively for d[5'-G<sub>3</sub>(T<sub>2</sub>AG<sub>3</sub>)<sub>3</sub>-3'] and d[5'-GGGTTAGGG-3'].

## Design of ligands.



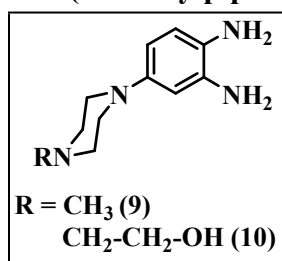
**Fig. S1.** Molecular structures of (A) Hoechst 33258 and (B) carbazole-benzimidazole conjugate. Superimposed structures of carbazole-benzimidazole conjugate with (C) G-C Watson-Crick duplex pair and (D) G-tetrad in a real scale. (E, F) Monomeric and dimeric ligands target via single to dual mode of binding with the G4 DNA.



**Fig. S2.** Dimeric G4 ligands with different kinds of spacers from their monomeric counterparts.

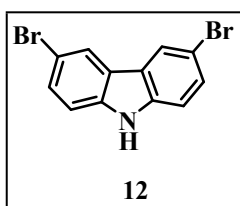
### Synthesis.

**4-(4-Methylpiperazin-1-yl)benzene-1,2-diamine (9)** and **2-(4-(3,4-Diamino phenyl) piperazin-1-yl)ethanol (10)**.



Compound **9** and **10** have been synthesized starting from 5-chloro-2-nitroaniline (**7**) according to the scheme 1 following our previously reported protocol.<sup>1</sup>

**3,6-Dibromo-9H-carbazole (12)**. Carbazole (7.046 g, 0.042 mol) was dissolved in dry DMF

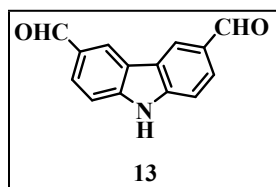


(80 mL) in a round bottomed flask (250 mL) and cooled to 0 °C. *N*-bromosuccinimide (NBS) (15 g, 0.084 mol) in DMF (20 mL) was added dropwise through a dropping funnel. The mixture was allowed to reach rt

and stirred for an additional 2 h. The mixture was then poured into water (400 mL). White precipitates that formed were collected by filtration and recrystallized from ethanol to afford colorless crystalline product (10.9 g, 80%).

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  ppm 8.13 (d,  $J = 2.0$ , 2H), 7.52 (dd,  $J = 8.4$ ,  $J = 2.0$ , 2H), 7.26 (d,  $J = 8.4$ , 2H); IR (KBr): 3405, 1561, 1472, 1459, 1430, 1285, 1126, 1050, 1018, 901, 869, 809, 805, 686, 640  $\text{cm}^{-1}$ ; mp 214-215  $^\circ\text{C}$  (lit. 215-216  $^\circ\text{C}$ ).<sup>2</sup>

**9H-Carbazole-3, 6-dicarbaldehyde (13).** To a suspension of potassium hydride (0.26 g, 6.5

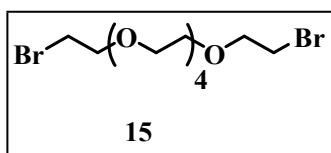


mmol) in dry THF (40 mL), compound **12** (2.03 g, 2.64 mmol) was added and stirred at 0  $^\circ\text{C}$  under nitrogen for 40 min before the temperature was lowered to -78  $^\circ\text{C}$ . To the mixture a solution of n-butyl

lithium (20 mL of a 1.7 M solution in hexane, 34 mmol) was added dropwise by a syringe over 3 min. The mixture was then allowed to reach rt over 1 h before being cooled again to -78  $^\circ\text{C}$ . Dry DMF (5 mL, 65 mmol) was introduced into the reaction mixture via a syringe and the mixture was allowed to reach rt. The mixture was stirred for additional 1.5 h at rt before it was poured into a 1 M  $\text{H}_3\text{PO}_4$  solution (200 mL) which afforded a fine precipitate. The precipitate was filtered through a bed of celite and the product was extracted in hot pyridine (100 mL). The solution was diluted with water (100 mL) to induce precipitation which was then suction filtered. The solid material was repeatedly washed with diethyl ether and the yellowish impurity was completely removed. This afforded a tan colored powder (0.38 g, 27%) which was adjudged pure by TLC (2% MeOH/ $\text{CHCl}_3$  on pre-coated silica gel).

$^1\text{H}$ -NMR (400 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  ppm 12.35 (br s, 1 H), 10.07 (s, 2 H), 8.86 (d,  $J = 1.5$ , 2 H), 8.00 (dd,  $J_1 = 8.5$ ,  $J_2 = 1.5$ ), 7.70 (d,  $J = 8.5$ ); IR (KBr): 1685  $\text{cm}^{-1}$ ; HRMS:  $m/z = 246.0533$ ; Calcd. = 246.0531 [ $\text{M}+\text{Na}$ ] $^+$ ; mp >300  $^\circ\text{C}$  (lit. >300  $^\circ\text{C}$ ).<sup>3</sup>

**1-(2-(2-(2-(2-(2-Bromoethoxy)ethoxy)ethoxy)ethoxy)ethoxy)-2-bromoethane (15).** To the

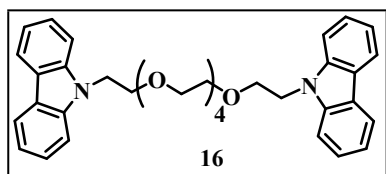


suspension of triphenylphosphine (5.6 g, 21.3 mmol) in acetonitrile (20 mL) bromine (3.4 g, 21.3 mmol) was added dropwise over 10

min under N<sub>2</sub> atmosphere at 0 °C. Hexaethylene glycol (3.0 g, 10.6 mmol) was dissolved in acetonitrile (10 mL) and added dropwise to the mixture and stirred for 48 h at ambient temperature. The white residue was filtered off and the solvent was evaporated under vacuum. The thick orange colored residue was extracted several times with petroleum ether and evaporated affording the pure dibromide as a colorless oil (3.0 g, 70%).<sup>4</sup>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ ppm 3.68 (t, *J* = 6.4, 4H), 3.54 (m, 16H), 3.35 (t, *J* = 6.4, 4H); HRMS: *m/z* = 428.9890, Calcd. = 428.9888 [M+Na]<sup>+</sup>.

**9-(2-(2-(2-(2-(2-(2-(9H-Carbazol-9-yl)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)ethyl)-9H-**

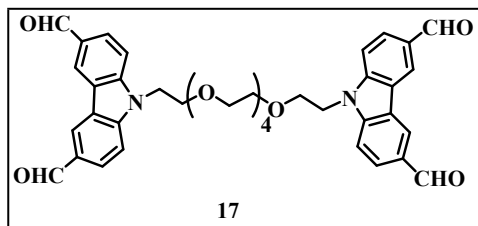


**carbazole (16).** Carbazole (1.98 g, 11.85 mmol) was taken with 25 mL of THF in a round bottom flask (100 mL) and to that 6 mL of 50% NaOH was added and stirred for 1 h. The dibromide **15**

(1.92 g, 4.74 mmol) and 160 mg TBAI were added to the mixture and stirred for 24 h at rt. Then 100 mL chloroform was added to the reaction mixture and the organic layer was separated and washed well with water followed by brine (~35%). The organic layer was separated and passed through a bed of dry Na<sub>2</sub>SO<sub>4</sub>. The resulting solution was evaporated under reduced pressure to get a crude product which was purified by silica gel column chromatography using 2% methanol/chloroform. Evaporation of the solvent from the pooled fraction afforded a sticky solid which was adjudged to be pure by TLC (2% MeOH/CHCl<sub>3</sub> on pre-coated silica gel) (1.5 g, 55%).

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ ppm 8.08 (m, 4H), 7.46 (m, 8H), 7.23 (m, 4H), 4.5 (m, 4H), 3.82 (m, 4H), 3.67-3.60 (m, *J* = 8.4, 8H), 3.53-3.45 (m, 8H); IR (KBr): 1683 cm<sup>-1</sup>; HRMS: *m/z* = 603.2837, Calcd. = 603.2835 [M+Na]<sup>+</sup>.

**9-(2-(2-(2-(2-(2-(2-(3,6-dicarbaldehyde-9H-carbazol-9-yl)ethoxy)ethoxy)ethoxy)ethoxy)**

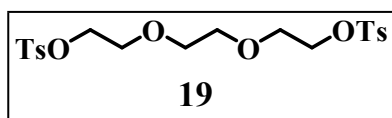


**ethoxy)ethyl)-9H-carbazole-3,6-dicarbaldehyde (17).**

Freshly activated anhydrous  $\text{ZnCl}_2$  (546 mg, 4 mmol) and compound **16** (580 mg, 1 mmol) were taken in 2.3 mL dry DMF and cooled in ice-bath followed by dropwise addition of  $\text{POCl}_3$  (1.9 mL, 20 mmol) while cooling on an ice-bath. The reaction mixture was refluxed for 24 h. The reaction mixture was then cooled and quenched with ice followed by neutralization with concentrated KOH solution. This was extracted with ethyl acetate and the organic layer was separated and passed through a bed of dry  $\text{Na}_2\text{SO}_4$ . The resulting solution was evaporated to dryness. The crude mixture was separated by alumina column chromatography and the product was isolated at 4% methanol/chloroform as eluent affording a yellowish solid (450 mg, 65%).

$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  ppm 10.15 (s, 4H), 8.67 (dd,  $J_1 = 1.2$ ,  $J_2 = 6.2$ , 4H), 8.09 (dd,  $J_1 = 1.2$ ,  $J_2 = 8.8$ , 4H), 7.62 (dd,  $J_1 = 8.8$ ,  $J_2 = 9.8$ , 4H), 4.76 (t,  $J = 8.4$ , 4H), 4.61 (t,  $J = 6.4$ , 4H), 3.964 (m, 8H), 3.6 (t,  $J = 5.6$ , 4H), 3.46 (t,  $J = 6$ , 4H); HRMS:  $m/z = 347.1442$ , Calcd. = 347.1445  $[\text{M}+2\text{H}]^{2+}$ ; mp 228 °C; Anal. (Calcd for  $\text{C}_{40}\text{H}_{42}\text{N}_2\text{O}_9$ ) C, 69.23, H, 5.82, N, 4.01; found: C, 69.15; H, 5.8; N, 4.02.

**Triethylene glycol ditosylate (19).** Triethylene glycol (3 g, 20 mmol) in THF (20 mL) and 5

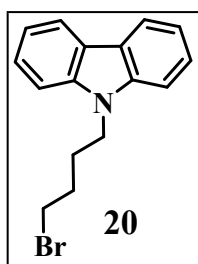


mL aqueous NaOH (2.0 g, 50 mmol) were mixed together and stirred for 1 h on a ice-bath. To that *p*-tosyl chloride (9.4 g, 50 mmol) dissolved in 15 mL of THF was added dropwise with cooling. The mixture was then allowed to reach rt and stirred for an additional 3 h. Reaction mixture was then evaporated and diluted with water (40 mL). The mixture was extracted with ethyl acetate and the collective

organic layer was passed through a bed of dry Na<sub>2</sub>SO<sub>4</sub> and evaporated under reduced pressure affording a crude mass. The off-white solid mass was purified using column chromatography (EtOAc/n-hexane) on silica gel (60-120 mesh size) and it was adjudged as pure by TLC (1% MeOH/CHCl<sub>3</sub> on pre-coated silica gel) and isolated as a white solid (9.2 g, 100%).

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ ppm 7.79 (d, *J* = 8, 4H), 7.34 (d, *J* = 8, 4H), 4.14 (t, *J* = 4.6, 4H), 3.66 (t, *J* = 5.0, 4H), 3.53 (br, 4H), 2.45 (s, 6H); IR (KBr): 2910, 2871, 1555, 1325, 1222, 1113, cm<sup>-1</sup>; HRMS: *m/z* = 481.0970, Calcd, 481.0967 [M + Na]<sup>+</sup>; mp 81-82 °C (lit. 81 °C).<sup>5</sup>

**9-(4-Bromobutyl)-9H-carbazole (20).** To a mixture of TBAI (277 mg, 0.75 mmol),



carbazole (3 g, 18 mmol) and 11.67 g (54 mmol) of 1, 4-dibromobutane, an aqueous solution of 50% NaOH (9 mL) was added at rt. The mixture was then stirred for 6 h at the same temperature and then poured into 100 mL of water and extracted with DCM (50 mL). The organic layer was then passed through a

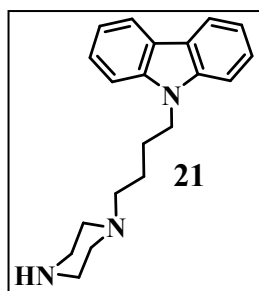
bed of dry Na<sub>2</sub>SO<sub>4</sub>. The resulting solution was evaporated to get a crude product which was purified by silica gel column chromatography using hexane/ethyl acetate mixture as eluent yielding a white solid (5.4 g, 72%).

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm 8.1 (d, *J* = 7.6, 2H), 7.47 (m, 4H), 7.24 (dd, *J* = 4.5, 7.8, 2H), 4.36 (t, 2H), 3.38 (t, 2H), 2.07 (m, 2H), 1.91 (m, 2H); HRMS: *m/z* = 302.0545 [M+H]<sup>+</sup>; Calcd. = 302.0544 [M+H]<sup>+</sup>; mp 101 °C (lit. 100 °C).<sup>6</sup>

**9-(4-(Piperazin-1-yl)butyl)-9H-carbazole (21).** Compound **20** (900 mg, 2.98 mmol), 596 mg (5.96 mmol) of piperazine and 823 mg of dry K<sub>2</sub>CO<sub>3</sub> were stirred together in 30 mL dry acetonitrile for 6 h. Then the reaction mixture was dried under reduced pressure and the residue was partitioned between water and chloroform. The organic layer was isolated and passed



through a bed of dry Na<sub>2</sub>SO<sub>4</sub>. The filtrate was collected, evaporated to get an off-white sticky solid product which was adjudged to be pure by TLC (3% MeOH/CHCl<sub>3</sub> on pre-coated silica

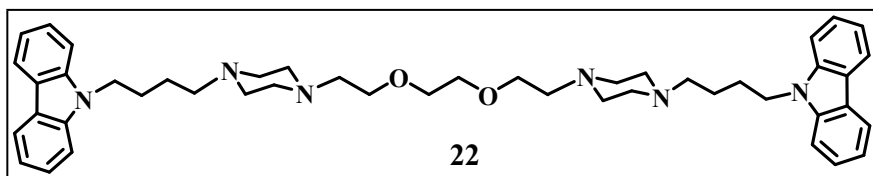


gel) (852 mg, 89%).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ ppm 8.1 (d, *J* = 7.8, 2H), 7.44 (m, 4H), 7.23 (dd, *J* = 4.5, 7.8, 3H), 4.33 (t, *J* = 5.6, 2H), 2.35 (m, 10H), 2.27 (s, 3H), 1.91 (m, 2H), 1.58 (m, 2); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ ppm 28.2,

29.4, 45.7, 54.2, 55.7, 61.2, 113.4, 119.2, 121.1, 125.1, 126.4, 141.3; HRMS: *m/z* = 322.2283 [M+H]<sup>+</sup>; Calcd. = 322.2283 [M+H]<sup>+</sup>.

**1,2-Bis(2-(4-(4-(9H-carbazol-9-yl)butyl)piperazin-1-yl)ethoxy)ethane (22).** The di-tosylate

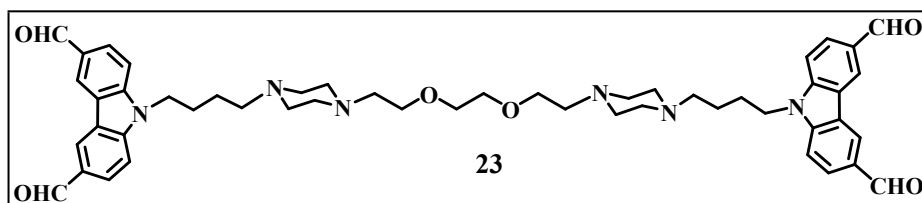


**19** (324 mg, 0.7 mmol) and the compound **21** (434 mg, 1.4 mmol) along with 966

mg K<sub>2</sub>CO<sub>3</sub> were taken together in 40 mL of dry acetone and refluxed for 24 h. The reaction mixture was then cooled to rt and filtered. The filtrate was passed through a bed of dry Na<sub>2</sub>SO<sub>4</sub> and the resulting solution was evaporated affording a crude gummy liquid which was purified by neutral alumina column chromatography using 6% methanol/chloroform as eluent. The product was isolated as a colorless gummy liquid (720 mg, 70%) which was adjudged to be pure by TLC (3% MeOH/CHCl<sub>3</sub> on pre-coated silica gel).

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ ppm 8.09 (s, 4H), 7.42 (m, 8H), 7.21 (t, *J* = 7.2, 4H), 4.29 (t, *J* = 6.8, 4H), 3.74-3.58 (m, 20H), 2.58-2.3 (m, 18H), 1.87 (t, *J* = 7.2, 4H), 1.55 (t, *J* = 7.6, 4H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ ppm 28.7, 29.5, 30.1, 46.2, 47.4, 56.1, 57.4, 58.1, 60.2, 61.9, 62.1, 68.5, 69.3, 70.4, 72.3, 113.1, 115.2, 120.1, 121.1, 125.7, 126.3, 130.5, 142.7, 144.3; HRMS: *m/z* = 751.4676, Calcd. = 751.4675 [M+Na]<sup>+</sup>.

**1,2-Bis(2-(4-(4-(9H-3,6-dicarbaldehyde-carbazol-9-yl)butyl)piperazin-1-yl)ethoxy)ethane**



(**23**). Freshly activated anhydrous  $\text{ZnCl}_2$  (280 mg, 2.056 mmol) and

compound **22** (374 mg, 0.514 mmol) were taken in 3 mL of dry DMF and cooled in ice-bath. To this  $\text{POCl}_3$  (0.96 mL, 10.27 mmol) was added dropwise while cooling and stirring. The reaction mixture was then heated at 100 °C for 48 h. The mixture was then cooled and quenched by addition of water and neutralized with concentrated KOH solution. The brown suspension was then extracted by ethyl acetate and evaporated under reduced pressure to afford a brown colored crude mass. The product was purified by neutral alumina column chromatography using 6% methanol/chloroform as eluent to furnish the tetra-aldehyde as a light yellowish solid (216 mg, 50%) which was adjudged as pure by TLC (8% MeOH/ $\text{CHCl}_3$  on pre-coated silica gel).

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  ppm 10.14 (s, 4H), 8.66 (s, 4H), 8.08 (d,  $J = 8.4$ , 4H), 7.58 (d,  $J = 8.4$ , 4H), 4.42 (t,  $J = 7.2$ , 4H), 3.59 (t,  $J = 5.6$ , 4H), 2.84 (s, 4H), 2.60-2.36 (m, 24H), 2.06 (s, 4H), 1.92 (t,  $J = 7.2$ , 4H), 1.58 (t,  $J = 7.2$ , 4H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 29.4, 29.8, 31.2, 45.3, 49.7, 57.3, 57.9, 59.3, 61.1, 62.1, 63.3, 67.5, 68.1, 69.7, 71.1, 72.6, 78.5, 115.7, 117.1, 122.3, 123.2, 126.8, 127.6, 132.1, 141.5, 143.2, 147.2, 190.2, 191.2, 192.5; IR (KBr): 1685  $\text{cm}^{-1}$ ; HRMS:  $m/z = 841.4655$ ; Calcd. = 841.4653  $[\text{M}+\text{H}]^+$ ; mp 283 °C.

**Circular Dichroism (CD) Spectroscopy.** The CD spectral experiments were performed in a Jasco J-815 CD spectropolarimeter equipped with a Peltier temperature controller in 1 cm quartz cuvette at 25 °C. To the preformed G4 DNA (4  $\mu\text{M}$ ) in 10 mM Tris-HCl (pH 7.4), 0.1 M NaCl/KCl and 0.1 mM EDTA, aliquots of ligand solutions (prepared in the corresponding buffer) were added and incubated for 15 min prior to recording of the spectra at a scan rate of 50

nm/min.

**DNA Melting Experiment.** The DNA melting experiments were performed in a Jasco J-815 CD spectropolarimeter equipped with a Peltier temperature controller in 1 cm quartz cuvette in the temperature range of 30 to 90 °C with change in temperature rate of 0.5 °C/min. All the experiments were repeated twice and an average value has been reported with a reproducibility of  $\pm 0.5$  °C. The data were plotted in origin 8.0 software and the CD spectral intensities were normalized within 1-0 scale. All the ligand solutions of 10 mM have been prepared in DMSO (biology grade) and diluted in the corresponding buffer related to the experiment. The final concentration of DMSO in the mixture did not exceed 1%.

**UV-vis Absorption Spectroscopy.** UV-vis spectroscopic titrations were performed using a Shimadzu UV-2100 spectrometer equipped with a temperature-controller in a quartz cuvette of 1 cm path length at 25 °C. Each ligand solution (5  $\mu$ M) in the specified buffer was titrated against increasing concentration of the preformed G4/duplex DNA. The data were plotted using origin 8.0 software and the respective binding constant was determined from Scatchard plot following our previously reported procedures.<sup>1</sup>

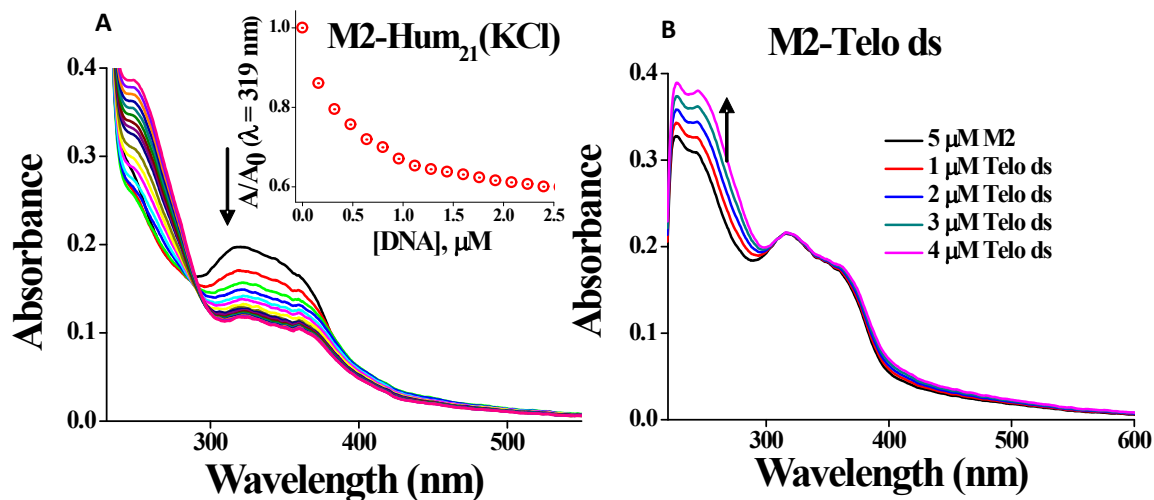
**Protocol for calculation of binding affinities.** Binding assays were performed with pre-formed d[5'-G<sub>3</sub>(T<sub>2</sub>AG<sub>3</sub>)<sub>3</sub>-3'] quadruplex in 10 mM Tris-HCl, having 0.1 M KCl/NaCl and 0.1 mM EDTA buffer, and with d[5'-G<sub>3</sub>(T<sub>2</sub>AG<sub>3</sub>)<sub>3</sub>-3']/[5'-(C<sub>3</sub>TA<sub>2</sub>)<sub>3</sub>C<sub>3</sub>-3'] duplex DNA or CT DNA in 10 mM Tris-HCl, having 40 mM NaCl and 0.1 mM EDTA buffer at pH 7.4. Ligand solution was titrated by stepwise addition of aliquots of DNA solution. Due to the ligand's low molar absorption coefficient values ( $\epsilon = 32,000$ - $45,000$  cm<sup>-1</sup>), the absorption spectral titrations were performed with moderate ligand concentration like 5  $\mu$ M. After each addition, the mixture was incubated at 25 °C for 15 min before measurement. The fractional decrease in absorbance at 320

(for **M1** and **M2**) or 350 nm (for **D1**, **D2**, **D3**, and **D4**) for each [DNA]/[ligand] ratio was normalized using,  $\Delta A = (A_{\text{free}} - A)/(A_{\text{free}} - A_{\text{sat}})$ , where  $A_{\text{free}}$  and  $A_{\text{sat}}$  are the absorbance for the free and fully bound ligands. The fraction of bound drug  $\alpha$  (on a 0-1 scale) at each intermediate titration position is given directly by the relative  $\Delta A$  hypochromicity term.<sup>7</sup> The concentration of free ligand is calculated using  $C_f = (1 - \alpha) \times C$ , where  $C$  is the total ligand concentration (fixed at 5  $\mu\text{M}$ ) and can be used to determine the binding ratio  $r$ , defined as  $(C - C_f)/[\text{DNA}]$ . Titration data were cast into the form of Scatchard plots of  $r/C_f$  versus  $r$  for analysis where  $K_a$  is the intrinsic equilibrium binding constant and  $n$  is an exclusion parameter that defines the number of ligand molecules bound per DNA quadruplex. Data were also fitted using the simpler, linear Scatchard equation, 1.

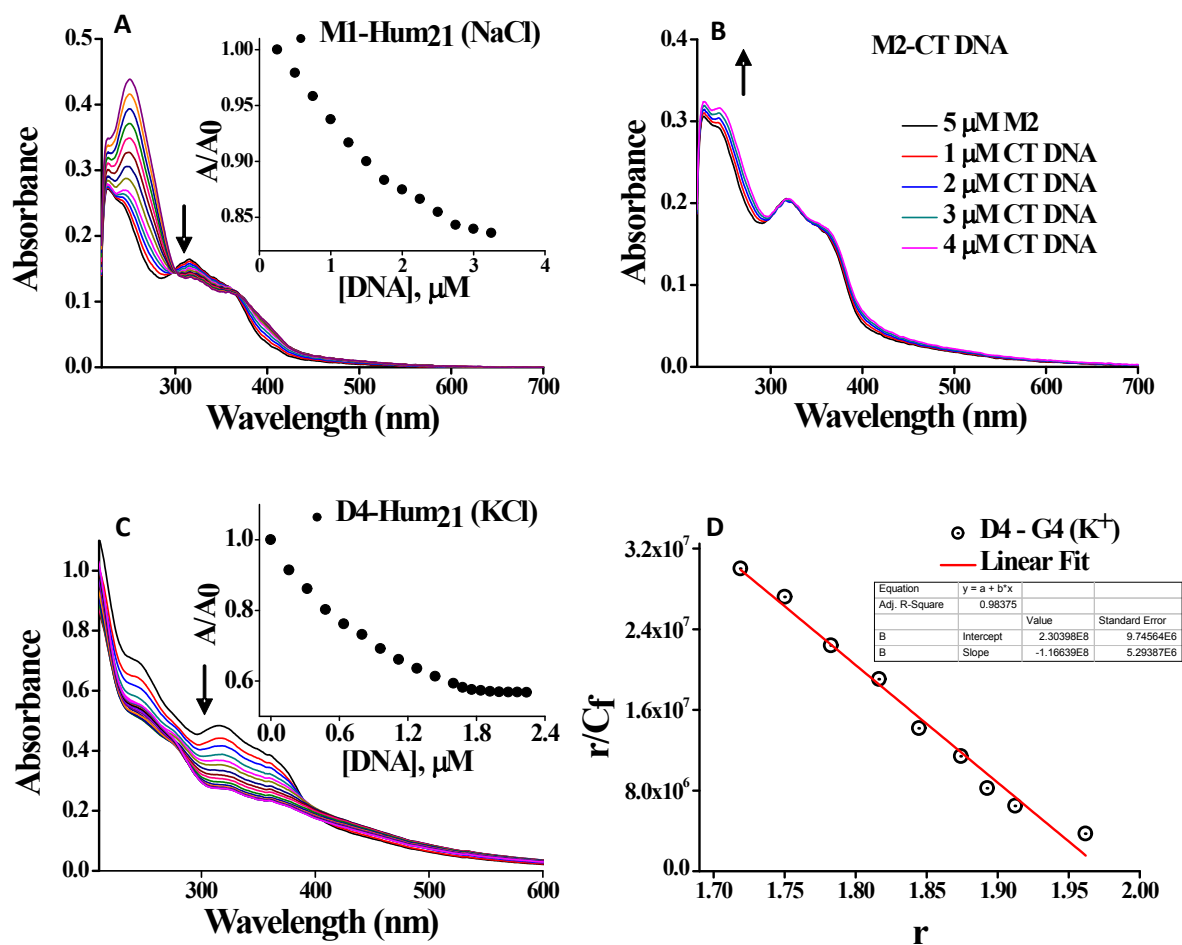
$$r/C_f = K_a(n - r) \dots\dots\dots 1$$

Dissociation constant ( $K_d$ ) has been calculated using equation,  $K_d = 1/K_a$

**UV-Vis titration of the ligands with pre-formed G-quadruplex/duplex DNA.**

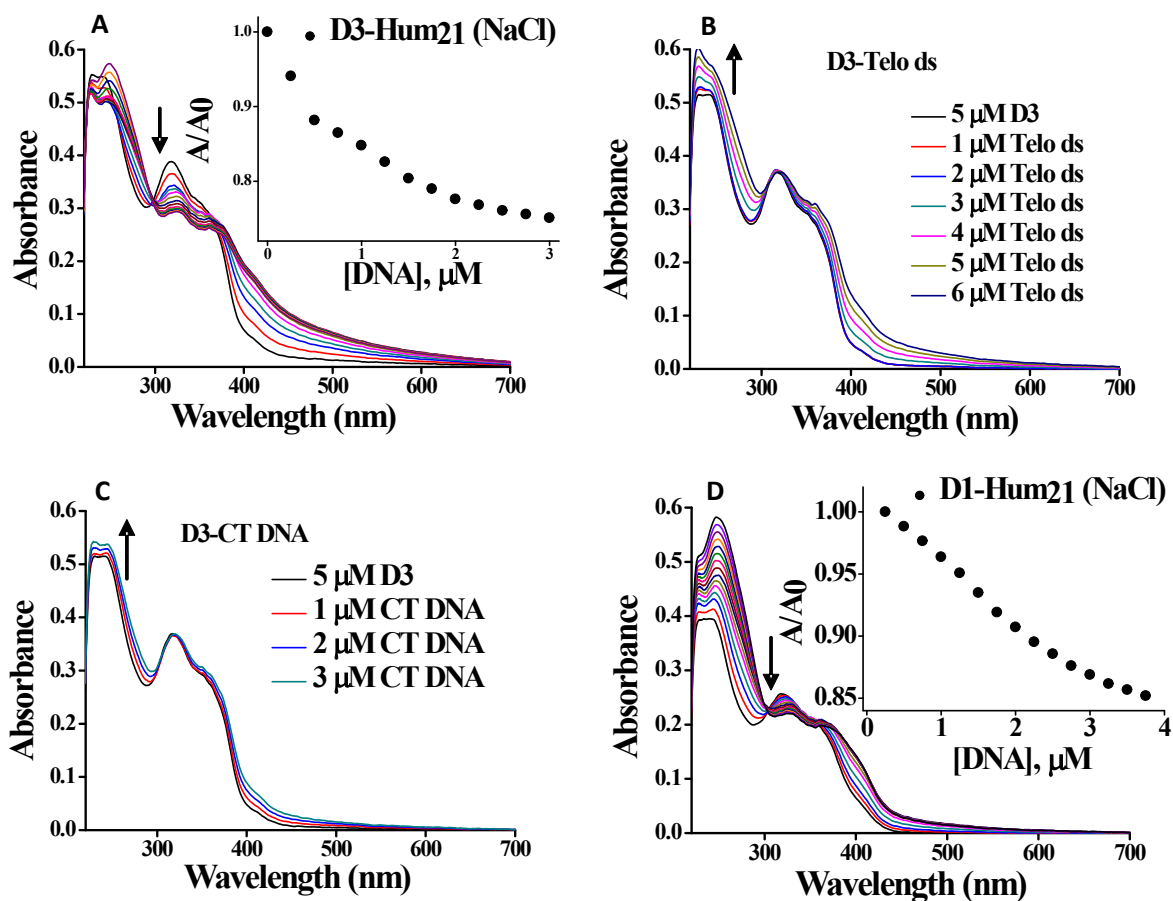


**Fig. S3.** UV-Vis titration of **M2** (5  $\mu\text{M}$ ) with (A) Hum<sub>21</sub> G-quadruplex DNA in 10 mM Tris-HCl (pH 7.4), having 0.1 M KCl and 0.1 mM EDTA. (B) UV-Vis titration of **M2** (5  $\mu\text{M}$ ) with the telomeric-duplex (Telo ds) DNA, d[5'-G<sub>3</sub>(T<sub>2</sub>AG<sub>3</sub>)<sub>3</sub>-3']/[5'-(C<sub>3</sub>TA<sub>2</sub>)<sub>3</sub>C<sub>3</sub>-3'] in 10 mM Tris-HCl (pH 7.4), having 40 mM NaCl and 0.1 mM EDTA.



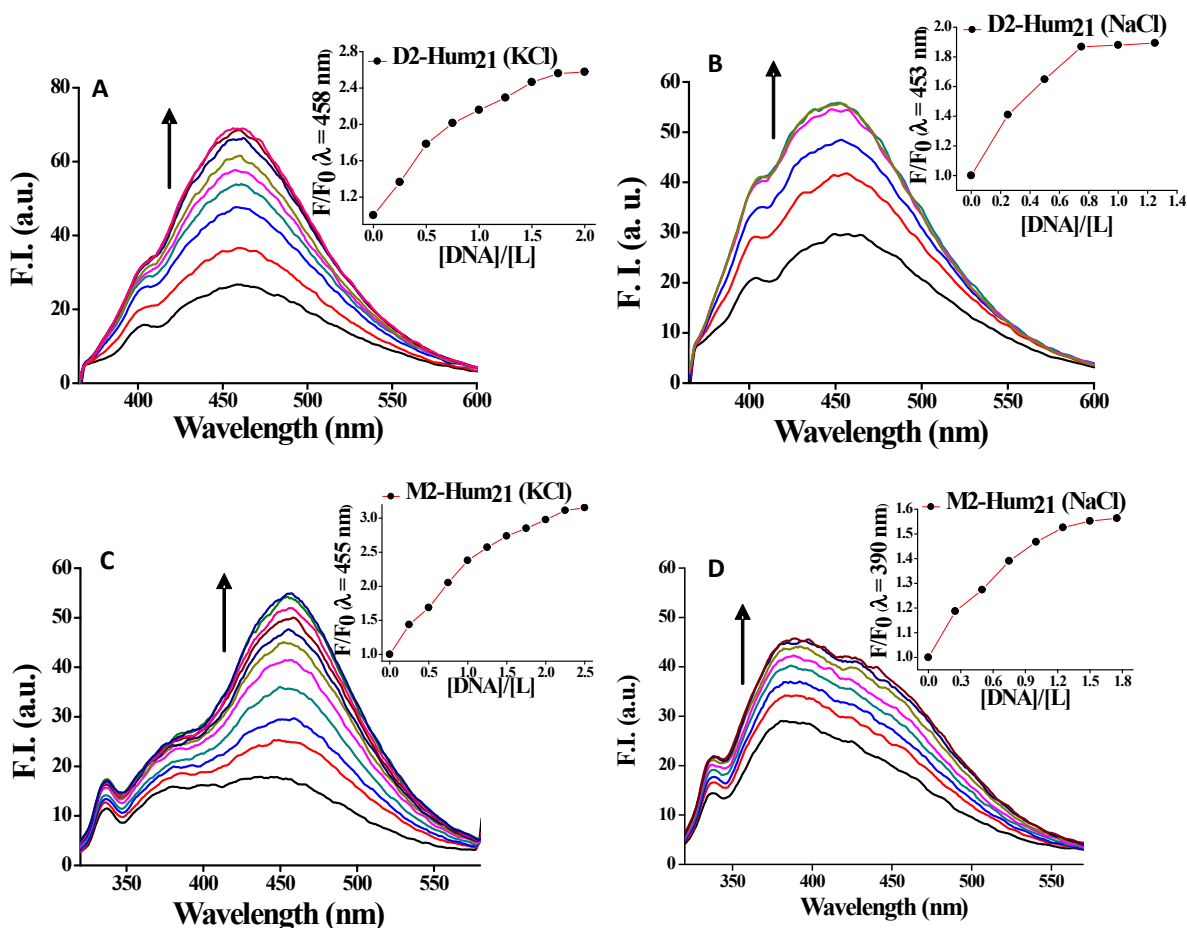
**Fig. S4.** UV-Vis titration of 5  $\mu\text{M}$  **M1** with Hum<sub>21</sub> G-quadruplex DNA in 10 mM Tris-HCl (pH 7.4) having 0.1 M NaCl and 0.1 mM EDTA. (B) UV-Vis titration of 5  $\mu\text{M}$  **M2** with CT DNA in 10 mM Tris-HCl (pH 7.4) having 40 mM NaCl and 0.1 mM EDTA. (C) UV-Vis titration of 5  $\mu\text{M}$  **D4** with Hum<sub>21</sub> G-quadruplex DNA in 10 mM Tris-HCl (pH 7.4) having 0.1 M KCl, 0.1 mM EDTA and (D) the corresponding Scatchard plot.

UV-Vis titration of the ligands with pre-formed G-quadruplex/duplex DNA.



**Fig. S5.** UV-Vis titration of 5 μM ligand (**D3**) with (A) Hum<sub>21</sub> G-quadruplex DNA, (B) telomeric-duplex (Telo ds) DNA, d{5'-G<sub>3</sub>(T<sub>2</sub>AG<sub>3</sub>)<sub>3</sub>-3'}/{5'-(C<sub>3</sub>TA<sub>2</sub>)<sub>3</sub>C<sub>3</sub>-3'} and (C) CT DNA in 10 mM Tris-HCl, 0.1 M NaCl/0.04 M NaCl, 0.1 mM EDTA, pH 7.4. (D) UV-Vis titration of 5 μM ligand (**D1**) with Hum<sub>21</sub> G-quadruplex DNA in 10 mM Tris-HCl, 0.1 M NaCl, 0.1 mM EDTA, pH 7.4.

## Additional Fluorescence spectral data

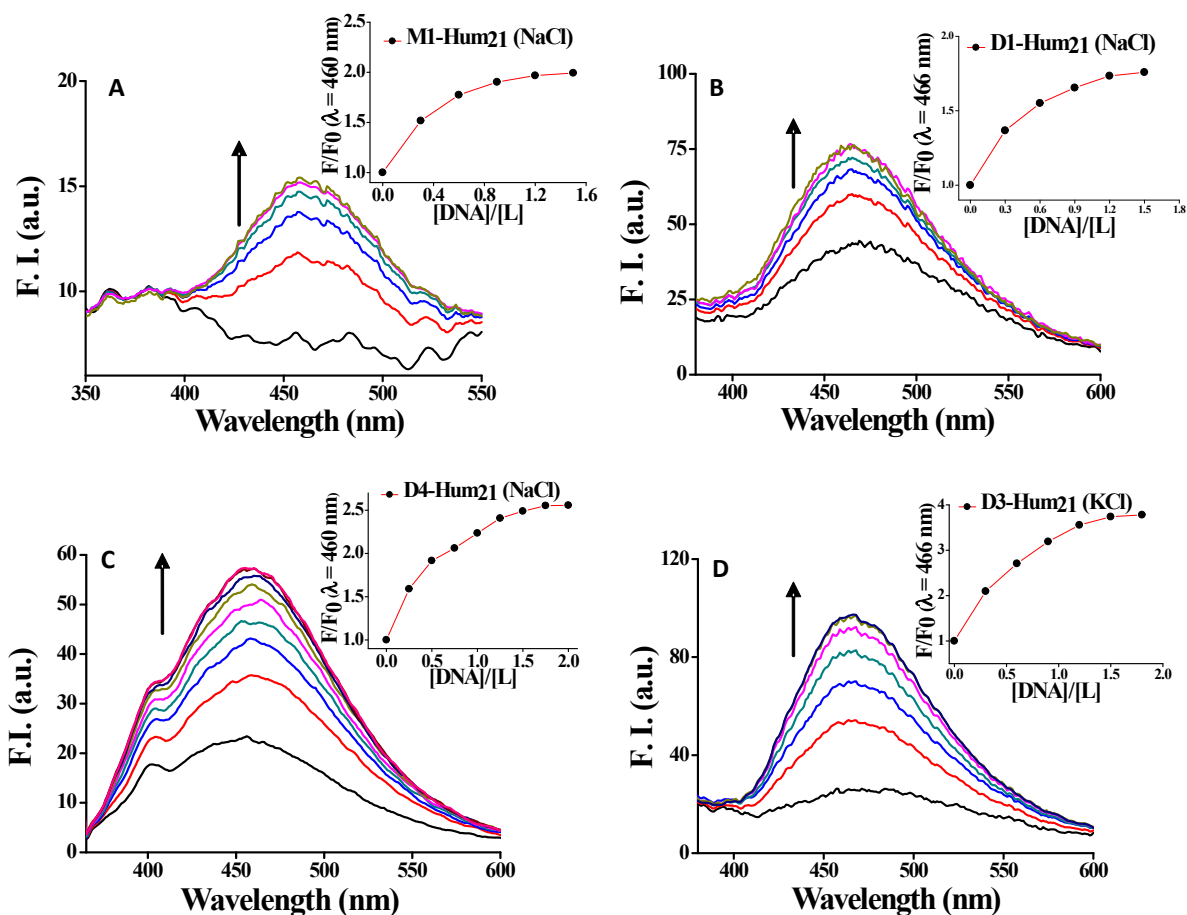


**Fig. S6.** Fluorescence titration of 0.8  $\mu$ M ligand **D2** in 10 mM Tris-HCl (pH 7.4) containing 0.1 mM EDTA and (A) 0.1 M KCl or (B) NaCl with pre-formed Hum<sub>21</sub> G-quadruplex DNA. Fluorescence titration of 0.8  $\mu$ M ligand **M2** in 10 mM Tris-HCl (pH 7.4) containing 0.1 mM EDTA and (C) 0.1 M KCl or (D) NaCl with pre-formed Hum<sub>21</sub> G-quadruplex DNA.

**Fluorescence Spectroscopy.** Fluorescence emission spectra were recorded on a Carey Eclipse Varian spectrophotometer using quartz cells with a path length of 0.5 mm. Temperature of the sample component was maintained at 25 °C using a Peltier controller. To 0.8  $\mu$ M of each ligand

in a specified buffer, the preformed G4 DNA (30  $\mu\text{M}$ ) in the corresponding buffer was added and incubated for 10 min prior to recording of each spectrum. The ligand solutions were excited at 320 nm (for **M1** and **M2**) or 350 nm (for **D1**, **D2**, **D3** and **D4**) with a slit width of 10/10 (nm). The data were processed using origin 8.0 software.

### Additional Fluorescence spectral data



**Fig. S7.** Fluorescence titration of 0.8  $\mu\text{M}$  ligand (A) **M1**, (B) **D1**, and (C) **D4** with pre-formed Hum<sub>21</sub> G-quadruplex DNA in 10 mM Tris-HCl (pH 7.4) containing 0.1 mM EDTA and 0.1 M NaCl. (D) Fluorescence titration of 0.8  $\mu\text{M}$  ligand **D3** with pre-formed Hum<sub>21</sub> G-quadruplex DNA in 10 mM Tris-HCl (pH 7.4) containing 0.1 mM EDTA and 0.1 M KCl.

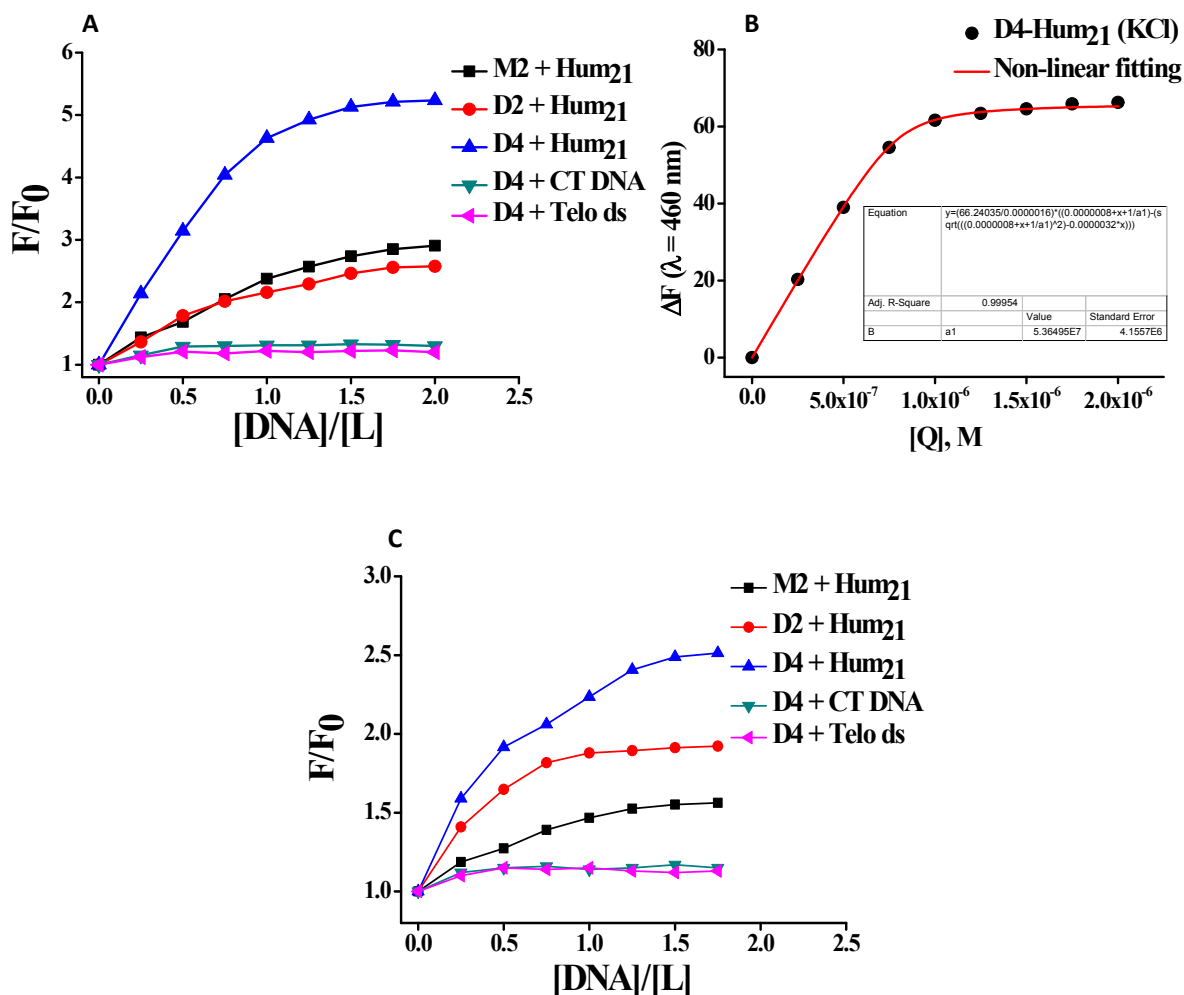


The binding affinities of the ligands with the G4 DNA were performed from the fluorescence emission studies at 25 °C using fixed ligand concentration of 0.8 μM and varying the G-quadruplex DNA concentration.

The binding affinity of the ligands was calculated following a reported protocol using the equation 2.<sup>8</sup>

$$\Delta F = (\Delta F_{\max} / 2[L]_0) \{ ([L]_0 + [Q] + 1/K_a) - ([L]_0 + [Q] + 1/K_a)^2 - 4[L]_0[Q] \} \dots\dots\dots(2)$$

where  $\Delta F = F - F_0$ ;  $\Delta F_{\max} = F_{\max} - F_0$ ;  $F$  and  $F_0$  are the initial and subsequent fluorescence intensities of the ligand (at 455 nm for **M1** and **M2**; 460 nm for **D1**, **D2**, **D3**, and **D4**) upon addition of the G4 DNA;  $K_a$  is the binding constant;  $[L]_0$  is the total ligand concentration;  $[Q]$  is the added quadruplex concentration. Dissociation constant ( $K_d$ ) has been calculated from the equation,  $K_d = 1/K_a$ . The dissociation constants ( $K_d$ ) were found to be quite similar with that calculated from the UV-Vis titrations (Table 1). The small difference in the magnitude in  $K_d$  may be due to the difference in the experimental procedure as reported earlier.



**Fig. S8.** (A) Relative increments in the fluorescence intensity for the ligands upon interaction with Hum<sub>21</sub> G-quadruplex DNA and the duplex DNA. (B) Binding affinity plot for **D4** ligand with the G-quadruplex DNA. (C) Relative increments in the fluorescence intensity for the ligands upon interaction with Hum<sub>21</sub> G-quadruplex DNA and the duplex DNA in 10 mM Tris-HCl (pH 7.4) having 0.1 mM EDTA and 0.1 M NaCl/0.04 M NaCl.

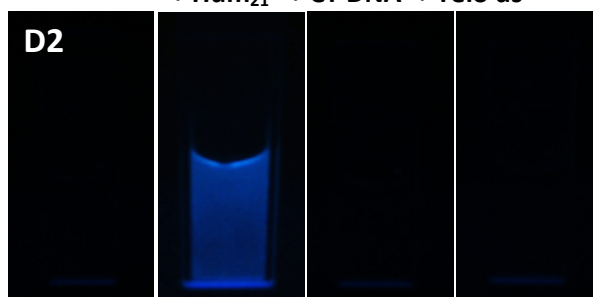
**Table S1.** Dissociation constants ( $K_d$ ) determined from fluorescence emission spectroscopic titrations for the ligands with the pre-formed Hum<sub>21</sub> G4 DNA.

Entry	Ligand	Dissociation constant, $K_d$ ( $\mu\text{M}$ )
		Hum <sub>21</sub> G4 DNA <sup>a</sup>
1	M1	2.21 $\pm$ 0.1
2	M2	1.12 $\pm$ 0.04
3	D1	0.04 $\pm$ 0.003
4	D2	0.11 $\pm$ 0.02
5	D3	0.03 $\pm$ 0.003
6	D4	0.02 $\pm$ 0.002

<sup>a</sup>Dissociation constants ( $K_d$ ) were calculated from the fluorescence emission spectral binding assays performed with the pre-formed Hum<sub>21</sub> G4 DNA in 10 mM Tris-HCl (pH 7.4) having 0.1 M KCl and 0.1 mM EDTA.

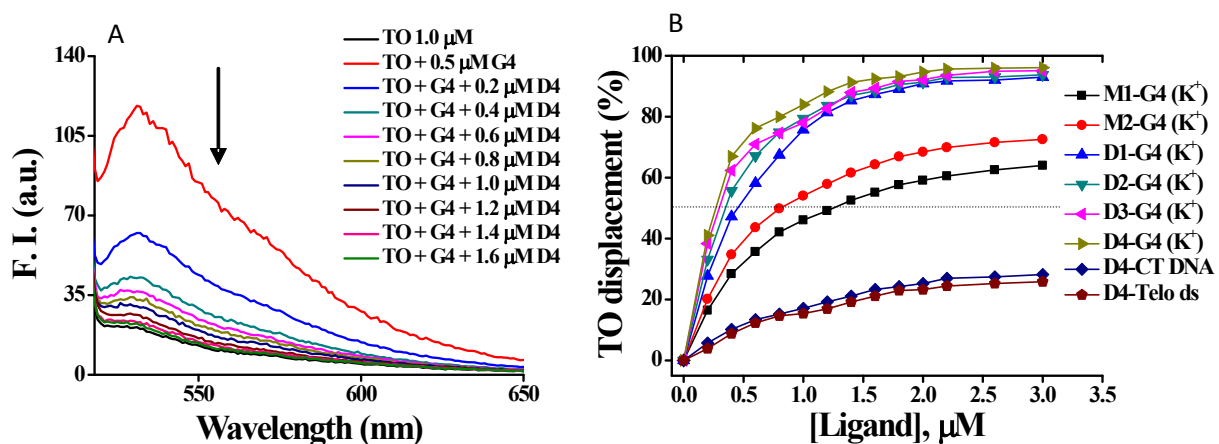
**Photographic images of ligand and ligand-DNA**

**complex in solution under UV light (~365 nm)**  
+ Hum<sub>21</sub> + CT DNA + Telo ds



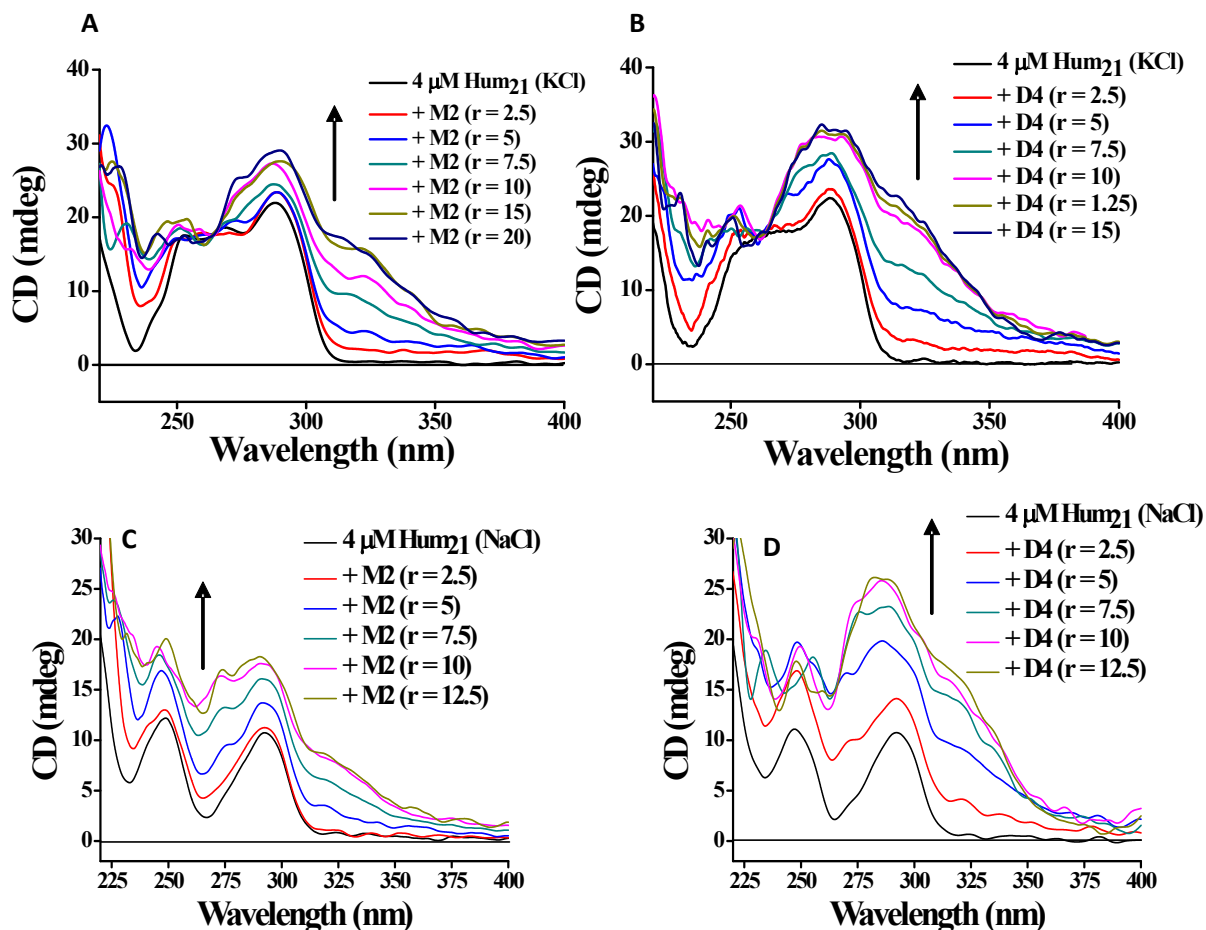
**Fig. S9.** Fluorescence images of ligand **D2** (20  $\mu\text{M}$ ) and ligand-DNA complexes (with 40  $\mu\text{M}$  of Hum<sub>21</sub>, CT DNA and Telo ds DNA) under UV light ( $\lambda_{\text{ex}} = 365 \text{ nm}$ ) in 10 mM Tris-HCl (pH 7.4) and 0.1 mM EDTA having either 0.1 M KCl (for G4 DNA) or 0.04 M NaCl (for CT DNA and Telo ds DNA).

**Fluorescent Intercalator Displacement Assays.** FID assays were performed on a Carey Eclipse Varian spectrophotometer using quartz cells with a path length of 0.5 mm. A solution of 1  $\mu\text{M}$  of TO in Tris.HCl (pH 7.4) buffer containing 0.1 M KCl and 0.1 mM EDTA was taken in a 500  $\mu\text{L}$  cell. The displacement assays were performed by adding increasing ligand concentration to various pre-formed DNA-TO complexes (1  $\mu\text{M}$  TO + 0.5  $\mu\text{M}$  G4 DNA; 1  $\mu\text{M}$  TO + 0.33  $\mu\text{M}$  CT DNA; 1  $\mu\text{M}$  TO + 0.33  $\mu\text{M}$  Telo ds DNA) followed by determining the emission spectra upon incubation for 5 min. The percentage of TO displacement is calculated from the fluorescence intensity at  $\lambda = 531 \text{ nm}$  ( $\lambda_{\text{ex}} = 501 \text{ nm}$ ) using the equation, percentage of displacement (DC%) =  $100 - [(F_t/F_0) \times 100]$  where  $F_t$  is the fluorescence intensity at each titration point and  $F_0$  is the fluorescence of TO bound to DNA without any added ligand.<sup>9</sup> The  $\text{DC}_{50}$  values have been calculated from the plot of percentage of fluorescent intercalator displacement (DC%) vs added ligand concentration.



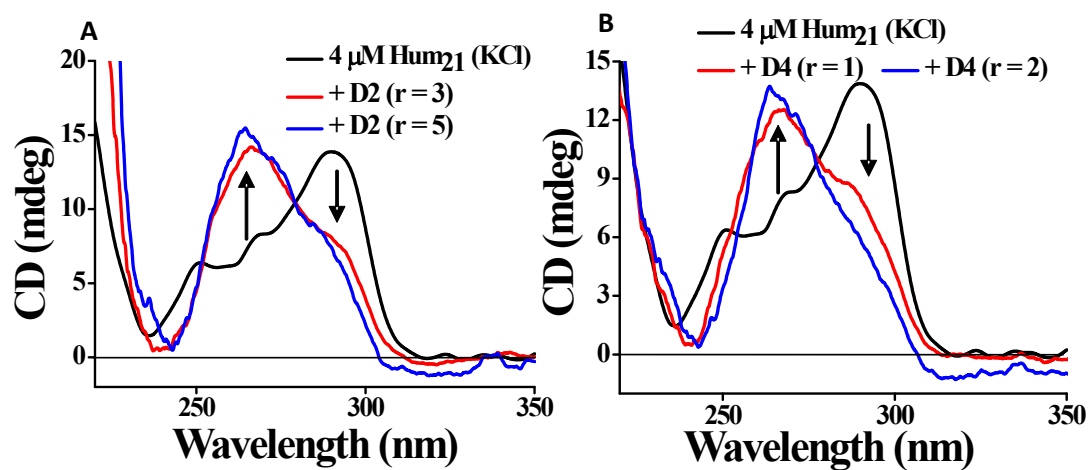
**Fig. S10.** (A) Representative G4 DNA-FID assay of the ligand **D4** as monitored by fluorescence emission spectroscopy and (B) the corresponding  $\text{DC}_{50}$  plot for all the ligands.

Additional CD titration data



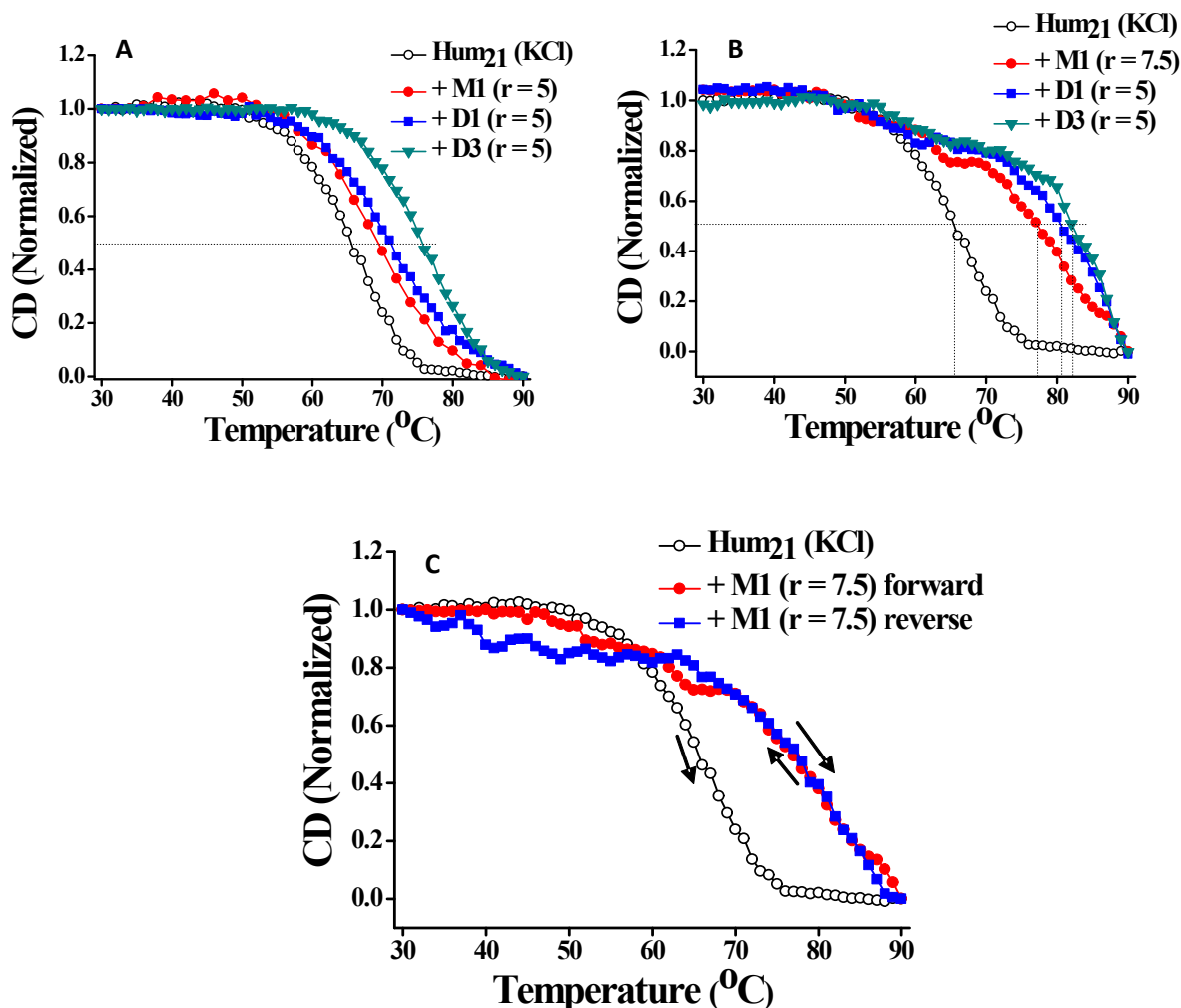
**Fig. S11.** CD titration of 4  $\mu\text{M}$  Hum<sub>21</sub> G-quadruplex DNA with (A) **M2** and (B) **D4** in 10 mM Tris-HCl (pH 7.4) containing 0.1 M KCl and 0.1 mM EDTA. CD titration of 4  $\mu\text{M}$  Hum<sub>21</sub> G-quadruplex DNA with (B) **M2** and (C) **D4** in 10 mM Tris-HCl (pH 7.4) containing 0.1 M NaCl and 0.1 mM EDTA.

## Formation of the G4 DNA in presence ligand.

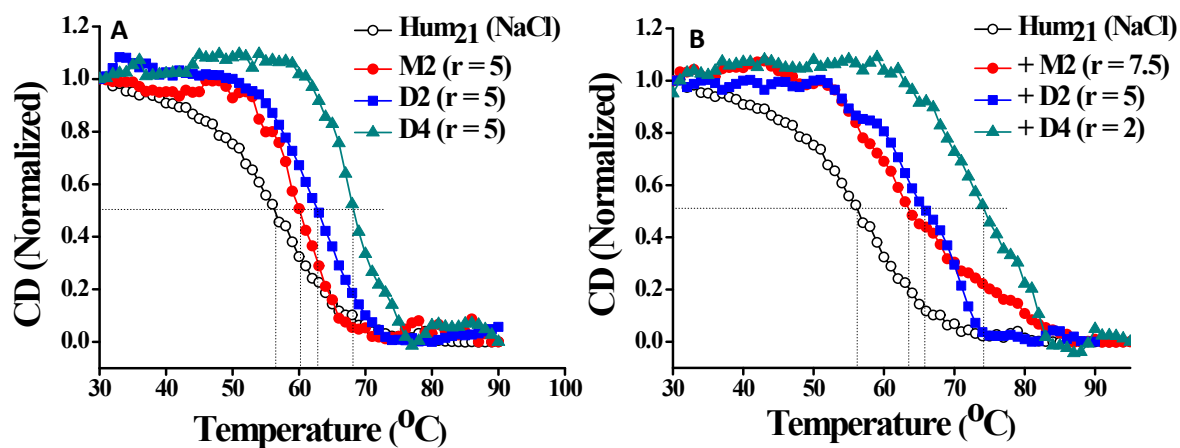


**Fig. S12.** Formation of the G4 DNA in presence of (A) **D2** and (B) **D4** in 10 mM Tris-HCl (pH 7.4) containing 0.1 M KCl and 0.1 mM EDTA.

## Thermal denaturation study



**Fig. S13.** (A) Melting profiles of the pre-formed G-quadruplex DNA and the G-quadruplex DNA incubated with ligands (**M1**, **D1** and **D3**) of specified concentration for 12 h (A) in 10 mM Tris-HCl (pH 7.4) containing 0.1 M KCl and 0.1 mM EDTA. (B) Melting profiles of the pre-formed G-quadruplex DNA and G-quadruplex DNA formed in presence with ligands (**M1**, **D1** and **D3**) of specified concentration in 10 mM Tris-HCl (pH 7.4) containing 0.1 M KCl and 0.1 mM EDTA. (C) Melting profile of the G-quadruplex DNA formed in presence with **M1** ( $r = 7.5$ ) in 10 mM Tris-HCl (pH 7.4) containing 0.1 M KCl and 0.1 mM EDTA showing no hysteresis in the melting path.



**Fig. S14.** Melting profiles of (A) G-quadruplex DNA incubated with ligands (**M2**, **D2** and **D4**) and (B) G-quadruplex DNA formed in presence of ligands (**M2**, **D2** and **D4**) of specified concentration in NaCl buffer.



**Table S2.** Thermal denaturation temperatures as probed using temperature induced changes in CD spectra of the G4 DNA-ligand complex<sup>a</sup> and the G4 DNA formed in the presence<sup>b</sup> of a ligand.

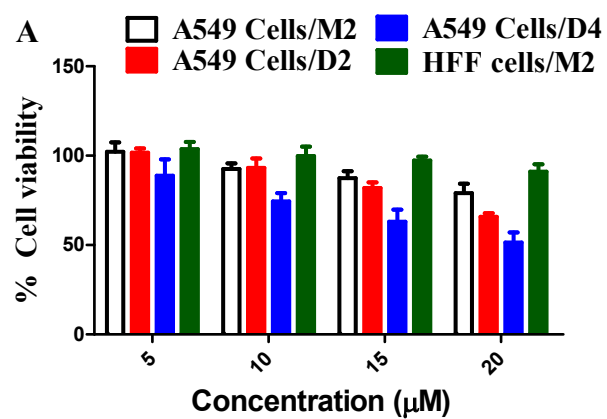
Entry	System	$T_m$	$\Delta T_m^c$	System	$T_m$	$\Delta T_m^c$
1	Hum <sub>21</sub> (NaCl)	57.0	---			
2	Hum <sub>21</sub> + <b>M1</b> <sup>a</sup>	60.0	3.0	Hum <sub>21</sub> + <b>M1</b> <sup>b</sup>	61.0	4.0
3	Hum <sub>21</sub> + <b>D1</b> <sup>a</sup>	62.0	5.0	Hum <sub>21</sub> + <b>D1</b> <sup>b</sup>	64.0	7.0
4	Hum <sub>21</sub> + <b>D3</b> <sup>a</sup>	66.0	9.0	Hum <sub>21</sub> + <b>D3</b> <sup>b</sup>	70.0	13.0
5	Hum <sub>21</sub> + <b>M2</b> <sup>a</sup>	60.0	3.0	Hum <sub>21</sub> + <b>M2</b> <sup>b</sup>	64.0	7.0
6	Hum <sub>21</sub> + <b>D2</b> <sup>a</sup>	63.0	6.0	Hum <sub>21</sub> + <b>D2</b> <sup>b</sup>	66.0	9.0
7	Hum <sub>21</sub> + <b>D4</b> <sup>a</sup>	68.0	11.0	Hum <sub>21</sub> + <b>D4</b> <sup>b</sup>	74.0	17.0

<sup>a</sup>The G4 DNA was incubated with a specified amount of ligand for 12 h at 25 °C in 10 mM Tris-HCl buffer (pH 7.4) containing 0.1 M NaCl and 0.1 mM EDTA. <sup>b</sup>The G4 DNA along with the specified amount of ligand was heated at 95 °C for 5 min and then subjected to slow cooling over 24 h in 10 mM Tris-HCl buffer (pH 7.4) containing 0.1 M NaCl and 0.1 mM EDTA.  $T_m$  and  $\Delta T_m$  values are reported in degree centigrade. <sup>c</sup>The difference in the melting temperature of DNA-ligand complex from that of the DNA alone. The results are average of two independent experiments and are within  $\pm 0.5$  °C of each other.

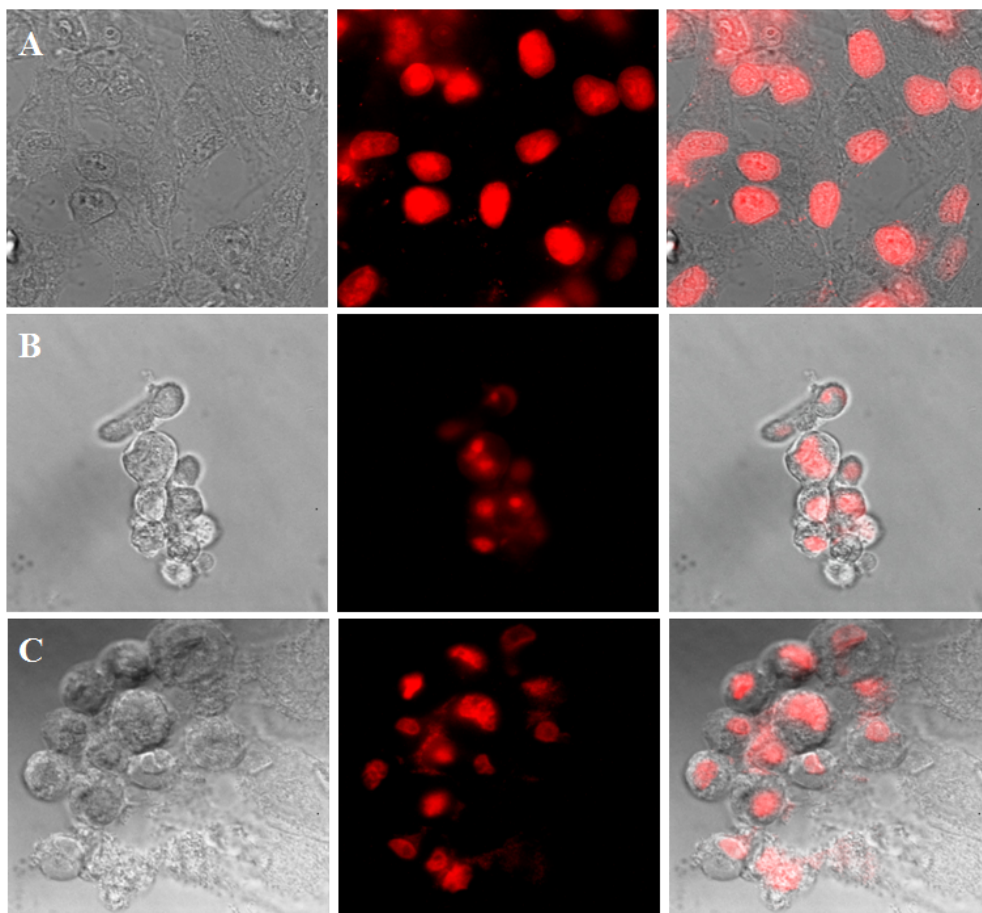
**Table S3.** Thermal denaturation temperatures as probed using temperature induced changes in CD spectra of the G4 DNA-ligand complex<sup>a</sup> and the G4 DNA formed in the presence<sup>b</sup> of a ligand.

Entry	System	$T_m$	$\Delta T_m^c$	System	$T_m$	$\Delta T_m^c$
1	Hum <sub>21</sub> (KCl)	67.0	---			
2	Hum <sub>21</sub> + <b>M1</b> <sup>a</sup>	70.0	3.0	Hum <sub>21</sub> + <b>M1</b> <sup>b</sup>	76.0	9.0
3	Hum <sub>21</sub> + <b>D1</b> <sup>a</sup>	72.0	5.0	Hum <sub>21</sub> + <b>D1</b> <sup>b</sup>	80.0	13.0
4	Hum <sub>21</sub> + <b>D3</b> <sup>a</sup>	76.0	9.0	Hum <sub>21</sub> + <b>D3</b> <sup>b</sup>	82.0	15.0
5	Hum <sub>21</sub> + <b>M2</b> <sup>a</sup>	72.0	5.0	Hum <sub>21</sub> + <b>M2</b> <sup>b</sup>	82.0	15.0
6	Hum <sub>21</sub> + <b>D2</b> <sup>a</sup>	80.0	13.0	Hum <sub>21</sub> + <b>D2</b> <sup>b</sup>	84.0	17.0
7	Hum <sub>21</sub> + <b>D4</b> <sup>a</sup>	83.0	16.0	Hum <sub>21</sub> + <b>D4</b> <sup>b</sup>	89.0	22.0

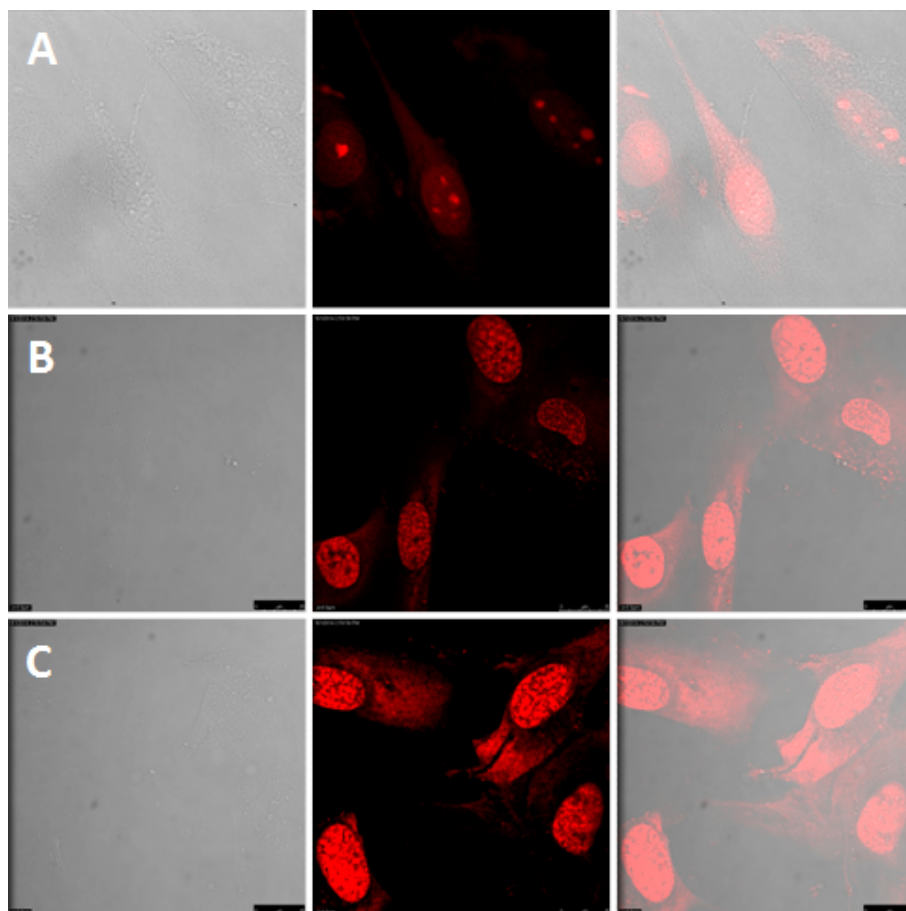
<sup>a</sup>The G4 DNA was incubated with a specified amount of ligand for 12 h at 25 °C in 10 mM Tris-HCl buffer (pH 7.4) containing 0.1 M KCl and 0.1 mM EDTA. <sup>b</sup>The G4 DNA along with specified amount of ligand heated at 95 °C for 5 min and then upon slow cooling over 24 h in 10 mM Tris-HCl buffer (pH 7.4) containing 0.1 M KCl and 0.1 mM EDTA.  $T_m$  and  $\Delta T_m$  values are reported in degree centigrade. <sup>c</sup>The difference between the melting temperature of DNA-ligand complex from the DNA alone. The results are average of two independent experiments and are within  $\pm 0.5$  °C of each other.



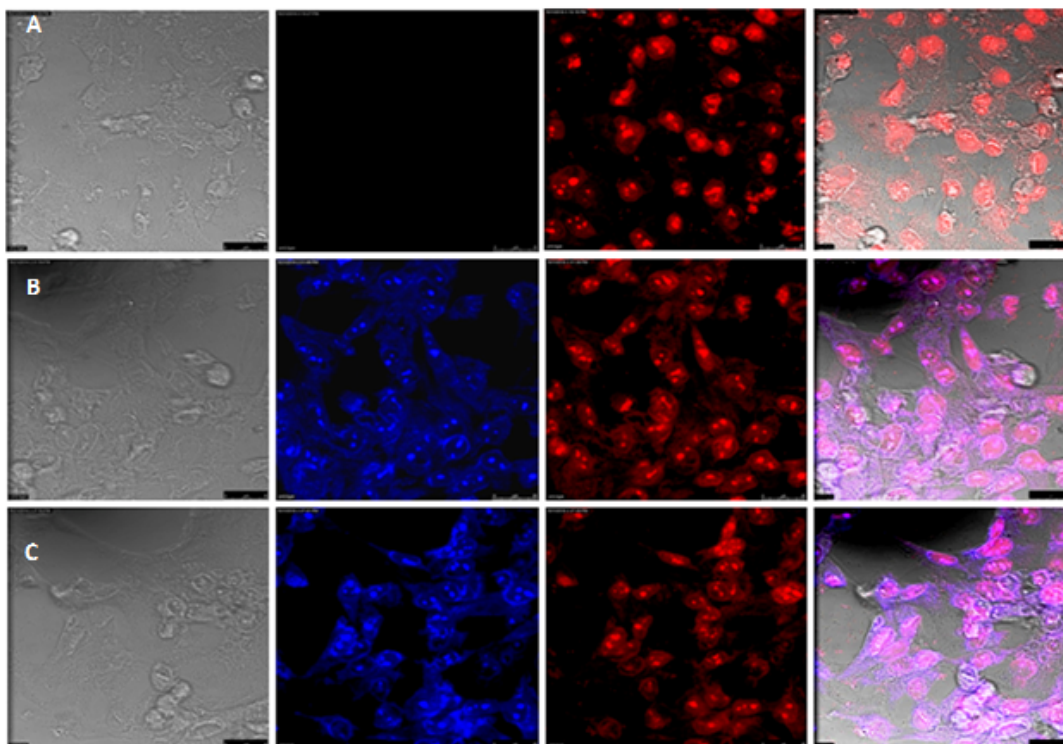
**Fig. S15.** Effect of ligand **M2**, **D2** and **D4** on the cell viability after a short-term exposure (72 h) exposure to HeLa cells.



**Fig. S16.** Representative bright field and fluorescence microscopic images of HeLa cells using PI as a nuclear counterstain. Untreated HeLa cells (A) and cells treated with 20  $\mu$ M of each of the ligands **M2** (B), and **D2** (C) for 48 h. Panel A-C (left to right) represent bright field, PI nuclear counterstain (red) and overlay of the previous two images.

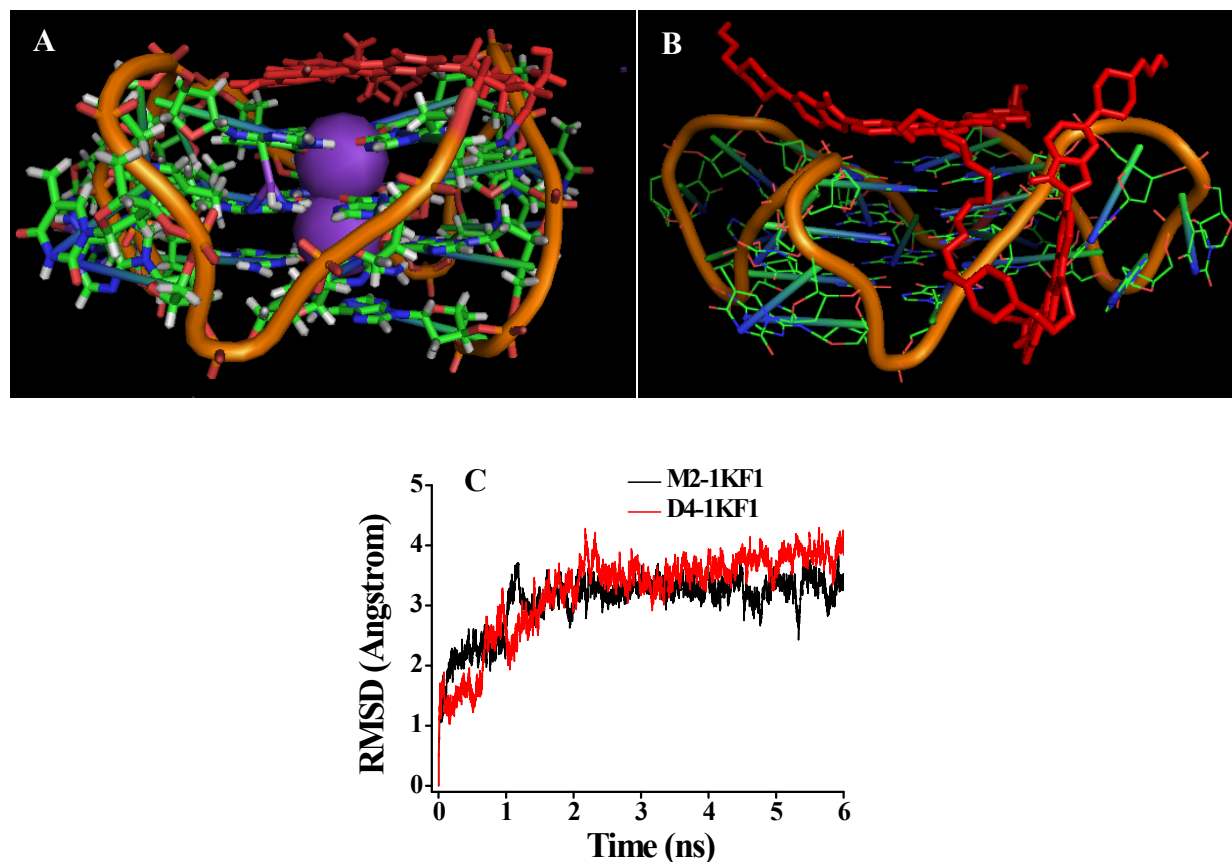


**Fig. S17.** Representative bright field and fluorescence microscopic images HFF of cells using PI as a nuclear counterstain. Panel A, B, and C (from left to right) represent untreated HFF cells, HFF cells treated with 20  $\mu$ M of ligand **D2** and **D4** for 48 h respectively.



**Fig. S18.** Representative confocal microscopic images depicting untreated cells (A) and cellular internalization of each of the ligands at a concentration of 10  $\mu$ M, **M2** (B) and **D2** (C) for 24 h in HeLa cells. PI was used as a nuclear counterstain. Panels A, B, and C represent (left to right) bright field, ligand fluorescence (blue), PI nuclear counterstain (red) and overlay of the previous three images.

**Computational Methods.** The ligand molecules have been energy optimized using *Gaussian 03* suite program at B3LYP/6-31G\* level of theory. The Mulliken atomic charges of the optimized conformation were obtained from the calculations and used for the docking studies using *AUTODOCK 4.0* program.<sup>[10]</sup>



**Fig. S19.** DNA ligand complex (A) M2-1KF1 and (B) D4-1KF1 after 6 ns MD simulation run and (C) RMSD plot. The complexes have been viewed in PyMOL/VMD software.

The most preferable binding conformation has been determined from a blind docking without any grid box restriction keeping the grid box at the center of the macromolecule. Docking calculations were carried out using the Lamarckian genetic algorithm (LGA). Initially,

we used a population of random individuals (population size: 150), a maximum number of 2500000 energy evaluations, a maximum number of generations of 27000, and a mutation rate of 0.02. Two hundred independent docking runs were performed for each ligand. The resulting positions were ascertained according to a root-mean-square criterion of 0.5 Å. Energetically minimized as well as maximum population in clustering structure has been taken for the next level of theory, MD simulations.

Molecular dynamics simulations have been performed using the ff99bsc0 force field in the AMBER 9.0 software package with *AmberTools* 1.3.<sup>11</sup> Partial atomic charges for each of the ligand molecule were derived using the HF/6-31G\* basis sets followed by RESP calculation, while the force-field parameters have been taken from the generalized Amber force field (GAFF)<sup>12</sup> using ANTECHAMBER module. Periodic boundary conditions were applied with the particle-mesh Ewald (PME) method used to treat long-range electrostatic interactions.<sup>13</sup> The G-quadruplex DNA and the ligand complexes were solvated in a truncated octahedral box of TIP3P water molecules with solvent layers of 10 Å thicknesses, and the potassium counter ions were added to neutralize the complexes as well. The hydrogen bonds were constrained using SHAKE to illustrate the non-bonded interactions and a residue-based cut off of 10 Å was used. The solvated structures were subjected to initial 1000 steps of minimization to equilibrate the solvent and the counter cations in two steps under force constant of 500 and 50 kcal mol<sup>-1</sup>. Then the minimization was carried out for the total system without any restraint for 2500 steps. The system was then heated slowly from 0 to 300 K in a 20 ps simulation keeping the volume constant with restraints of 50 kcal mol<sup>-1</sup> for the macromolecule. The temperature has been controlled using Langevin dynamics with a collision frequency of 1.0 ps<sup>-1</sup> and molecular dynamic run with a time step of 2 fs per step. Then it was allowed to equilibrate keeping the



pressure constant with gradual decrease of constraints (50, 25, 5 kcal mol<sup>-1</sup>) for 50 ps each step at a temperature of 300 K followed by 6 ns final production run without any constraint. The output and trajectory files were saved in every 2 fs for the subsequent analysis respectively. All trajectory analysis was performed using the Ptraj module in the Amber 9.0 suite and examined visually using the VMD software package.<sup>14</sup>

## References

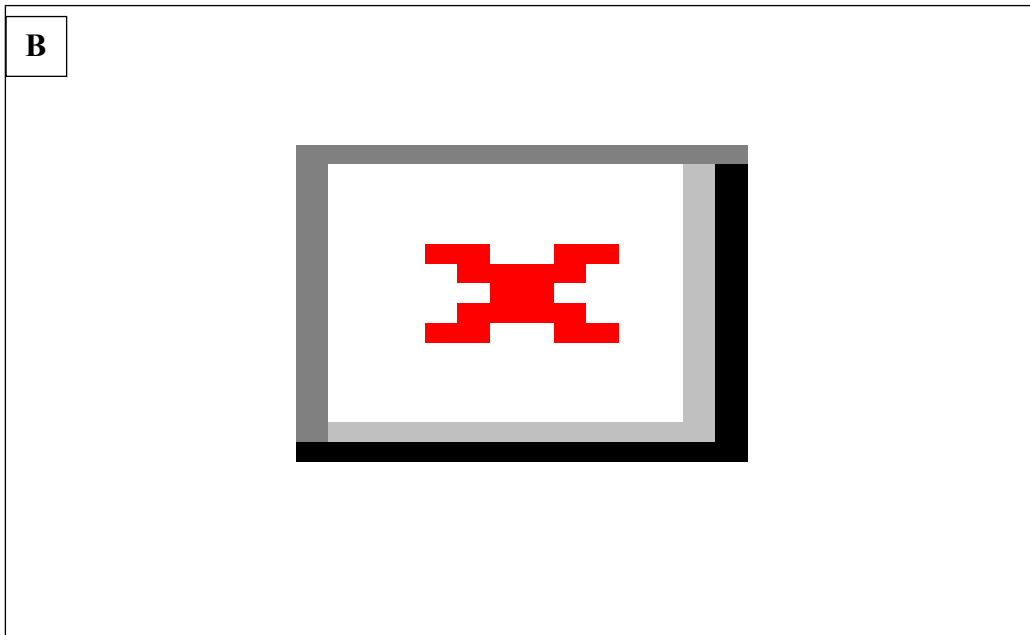
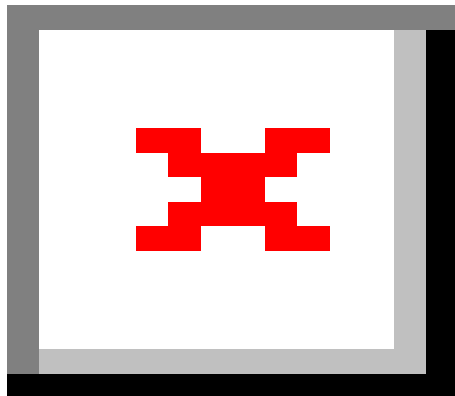
- [1] A. K. Jain, V. V. Reddy, A. Paul, K. Muniyappa and S. Bhattacharya, *Biochemistry*, 2009, **48**, 10693-10704.
- [2] Y. Li, J. Ding, M. Day, Y. Tao, J. Lu and M. D'iorio, *Chem. Mater.*, 2004, **16**, 2165-2173.
- [3] D. A. Patrick, D. W. Boykin, W. D. Wilson, F. A. Tanious, J. Sychalaz, B. C. Bender *et al. Eur. J. Med. Chem.*, 1997, **32**, 781-793.
- [4] E. Biron, F. Otis, J. C. Meillon, M. Robitaille, J. Lamothe, P. Van Hove, M. E. Cormier and N. Voyer, *Bioorg. Med. Chem.*, 2004, **12**, 1279-1290.
- [5] D. L. Mohler and G. Shena, *Org. Biomol. Chem.*, 2006, **4**, 2082-2087.
- [6] P. Taranekar, T. Fulghum, D. Patton, R. Ponnappati, G. Clyde and R. Advincula, *J. Am. Chem. Soc.*, 2007, **129**, 12537-12548.
- [7] A. R. Peacocke and J. N. H. Skerrett, *Faraday Trans.*, 1956, **52**, 261-279.

- [8] S. Maiti, N. K. Chaudhury and S. Chowdhury, *Biochem. Biophys. Res. Commun.*, 2003, **310**, 505-512.
- [9] D. Monchaud, C. Allain, H. Bertrand, N. Smargiasso, F. Rosu, V. Gabelica, A. De Cian, J. L. Mergny and M. P. Teulade-Fichou, *Biochimie*, 2008, **90**, 1207-1223.
- [10] G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew *et al.* *J. Comput. Chem.*, 1998, **19**, 1639–1662.
- [11] D. A. Case, T. Cheatham, T. Darden, H. Gohlke, R. Luo, K. M. Merz *et al.* *J. Computat. Chem.*, 2005, **26**, 1668-1688.
- [12] J. Wang, R. M. Wolf, J. W. Caldwell, P. A. Kollman and D. A. Case, *J. Comput. Chem.*, 2004, **25**, 1157-1174.
- [13] T. Darden, D. York and L. Pedersen, *J. Chem. Phys.*, 1993, **98**, 10089-10092.
- [14] W. Humphrey, A. Dalke and K. Schulten, *J. Mol. Graph.*, 1996, **14**, 33–38.

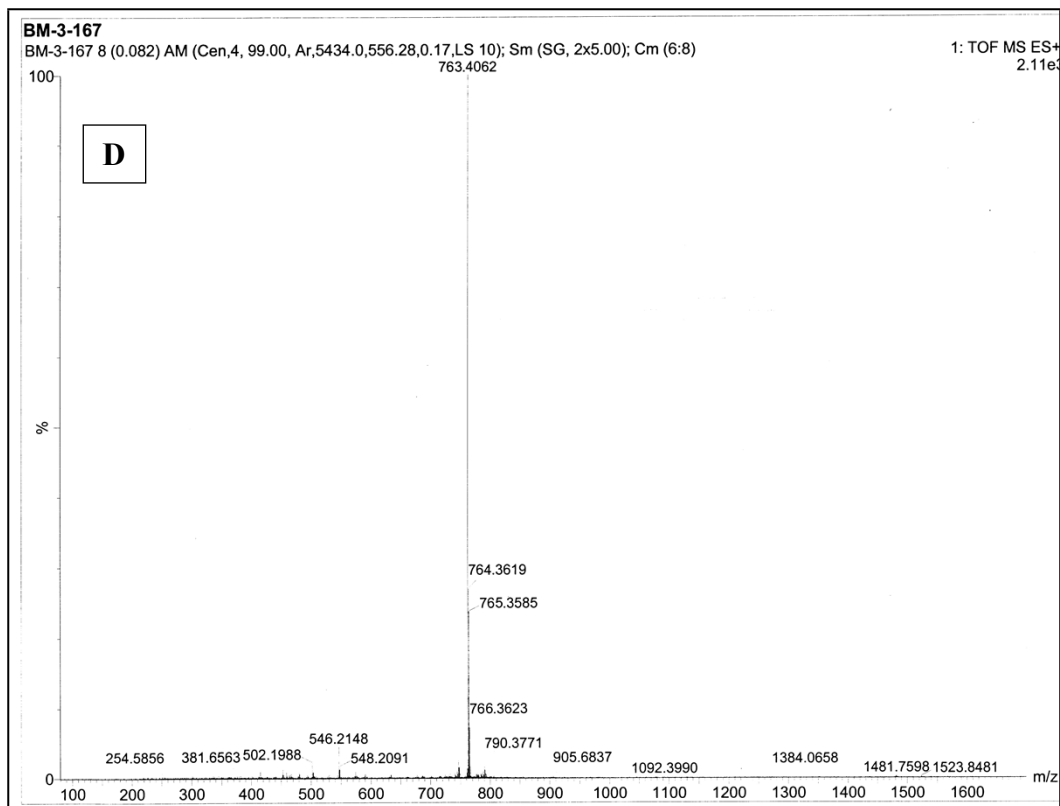
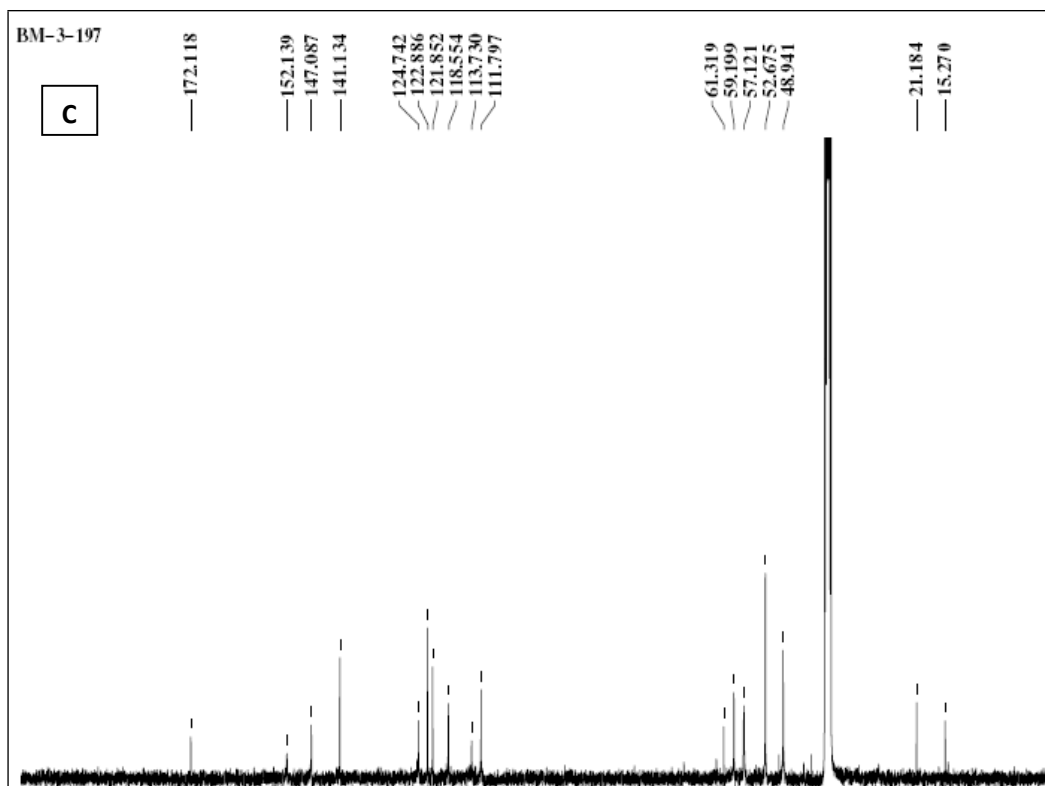
**Table S4.** Elemental Analysis Data for the Compounds **M1**, **M2**, **D1**, **D2**, **D3**, and **D4**.

<b>Compound</b>	<b>% of elements (Calculated)</b>	<b>% of elements (Found)</b>
<b>M1</b>	(Calcd for C <sub>36</sub> H <sub>37</sub> N <sub>9</sub> ) C, 72.58; H, 6.26; N, 21.16	C, 72.31; H, 6.28; N, 21.2
<b>M2</b>	(Calcd for C <sub>38</sub> H <sub>41</sub> N <sub>9</sub> O <sub>2</sub> ) C, 69.60; H, 6.30; N, 19.22	C, 69.73; H, 6.33; N, 19.18
<b>D1</b>	(Calcd for C <sub>84</sub> H <sub>96</sub> N <sub>18</sub> O <sub>5</sub> ) C, 70.17; H, 6.73; N, 17.54	C, 70.05; H, 6.75; N, 17.58
<b>D2</b>	(Calcd for C <sub>88</sub> H <sub>104</sub> N <sub>18</sub> O <sub>9</sub> .H <sub>2</sub> O) C, 67.07; H, 6.78; N, 16	C, 66.93; H, 6.8; N, 16.05
<b>D3</b>	(Cald for C <sub>94</sub> H <sub>116</sub> N <sub>22</sub> O <sub>2</sub> ) C, 71.18; H, 7.37; N, 19.43	C, 71.07; H, 7.39; N, 19.4
<b>D4</b>	(Calcd for C <sub>98</sub> H <sub>124</sub> N <sub>22</sub> O <sub>6</sub> .H <sub>2</sub> O) C, 68.27; H, 7.37; N, 17.87	C, 68.17; H, 7.36; N, 17.91

**A**



**Fig. S22.** Mass spectrum (A) and <sup>1</sup>H NMR spectrum (B) of **M2**.



**Fig. S23.** (C)  $^{13}\text{C}$  NMR spectrum of **M2** and (D) Mass spectrum of **D2**.

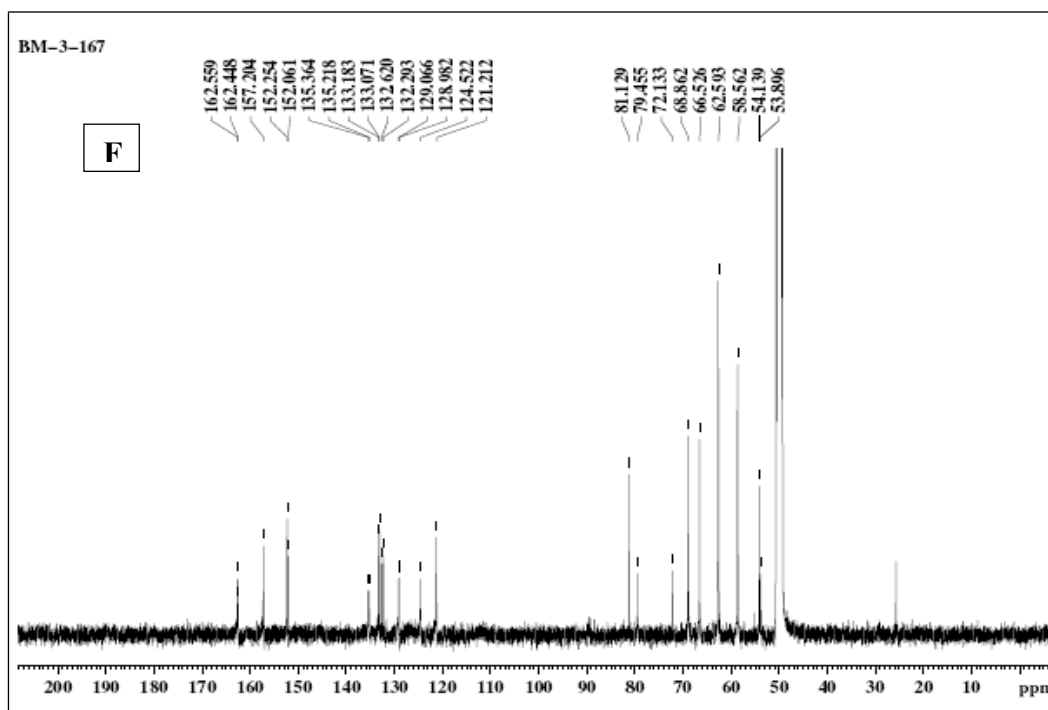
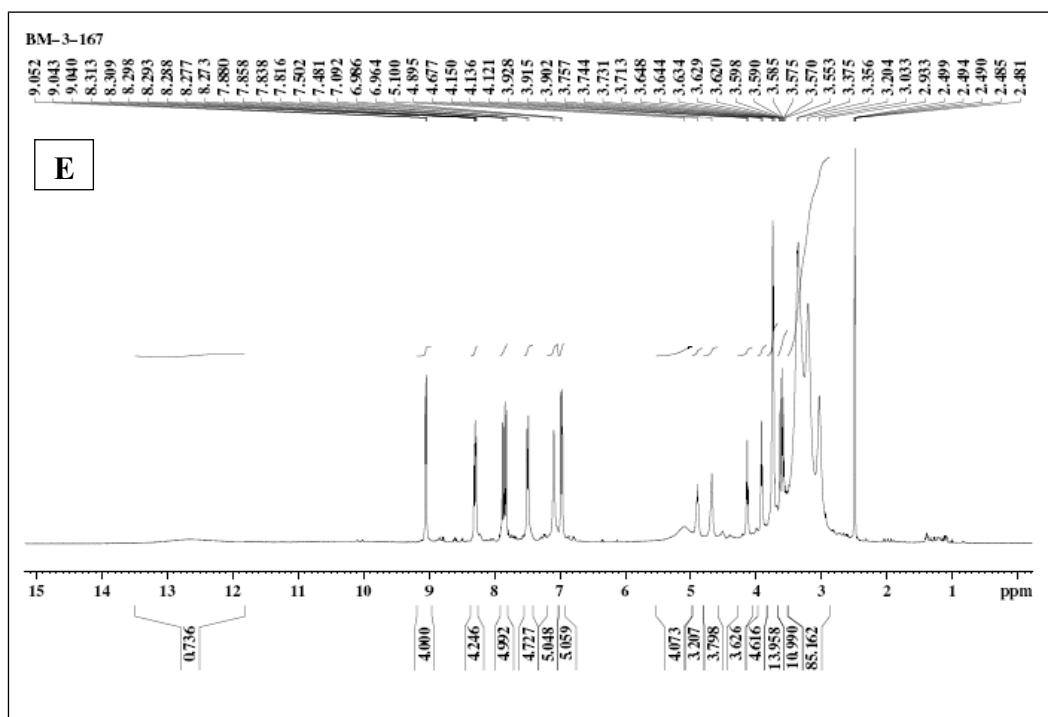
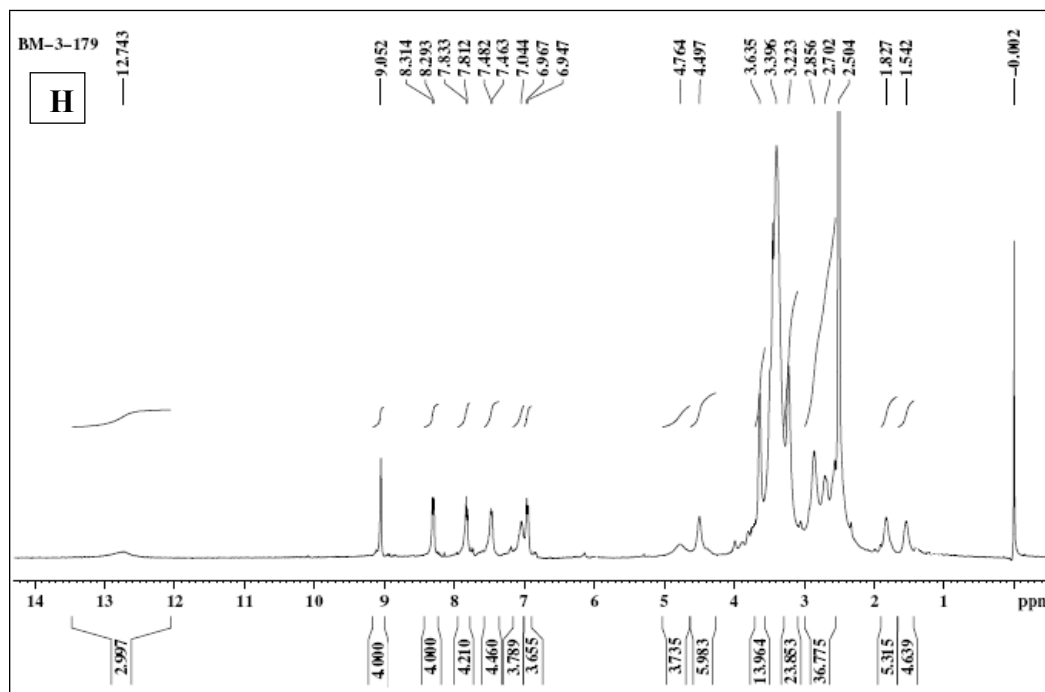
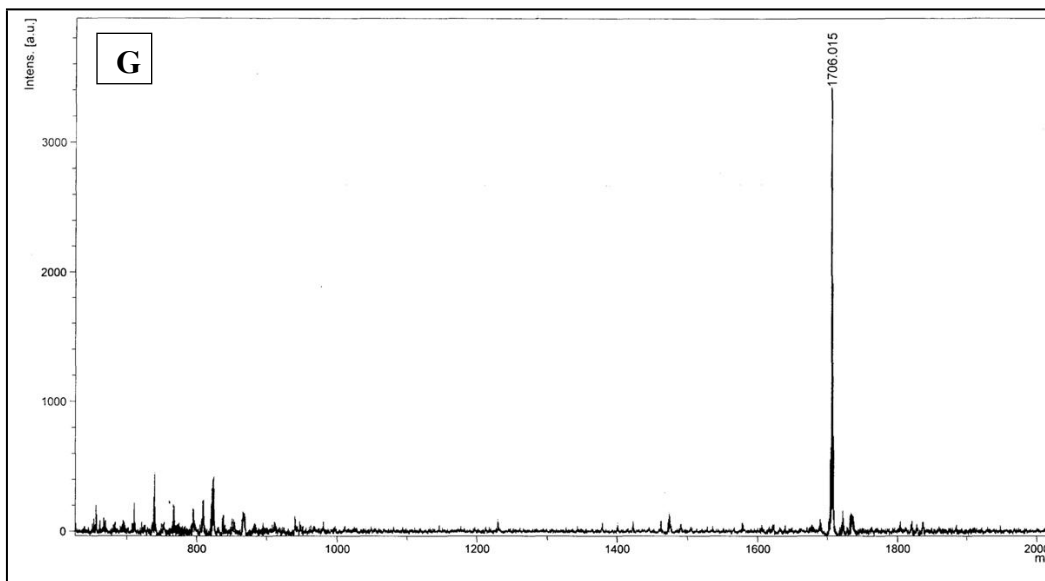
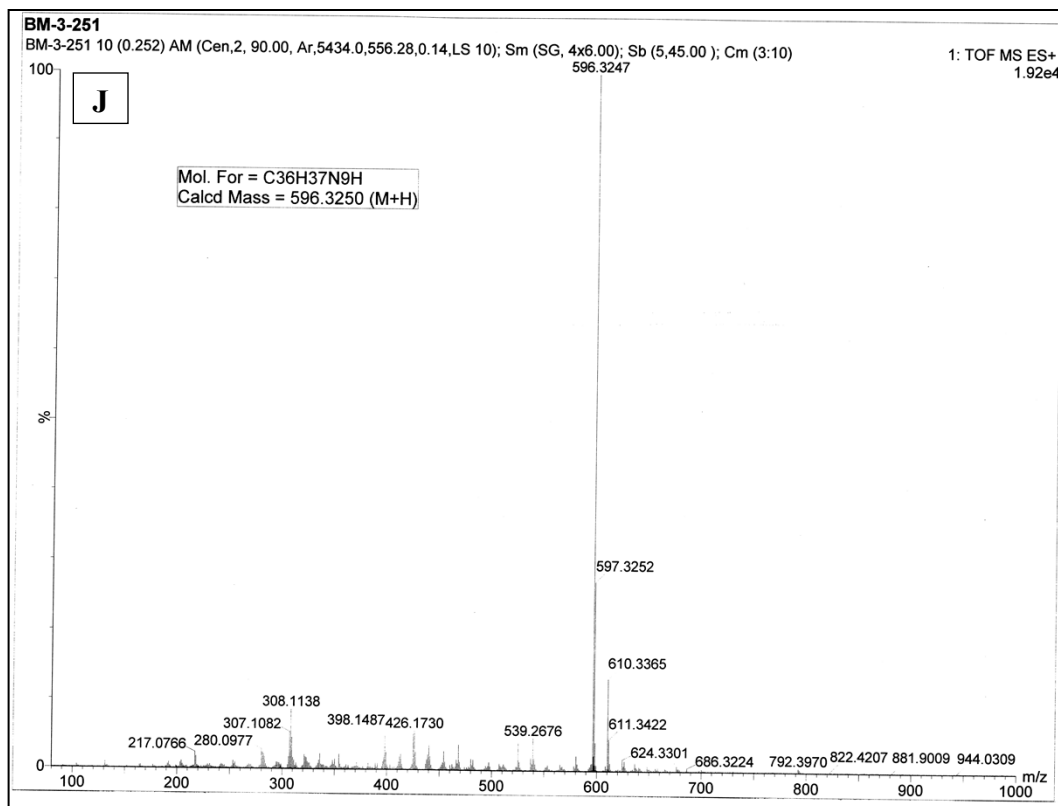
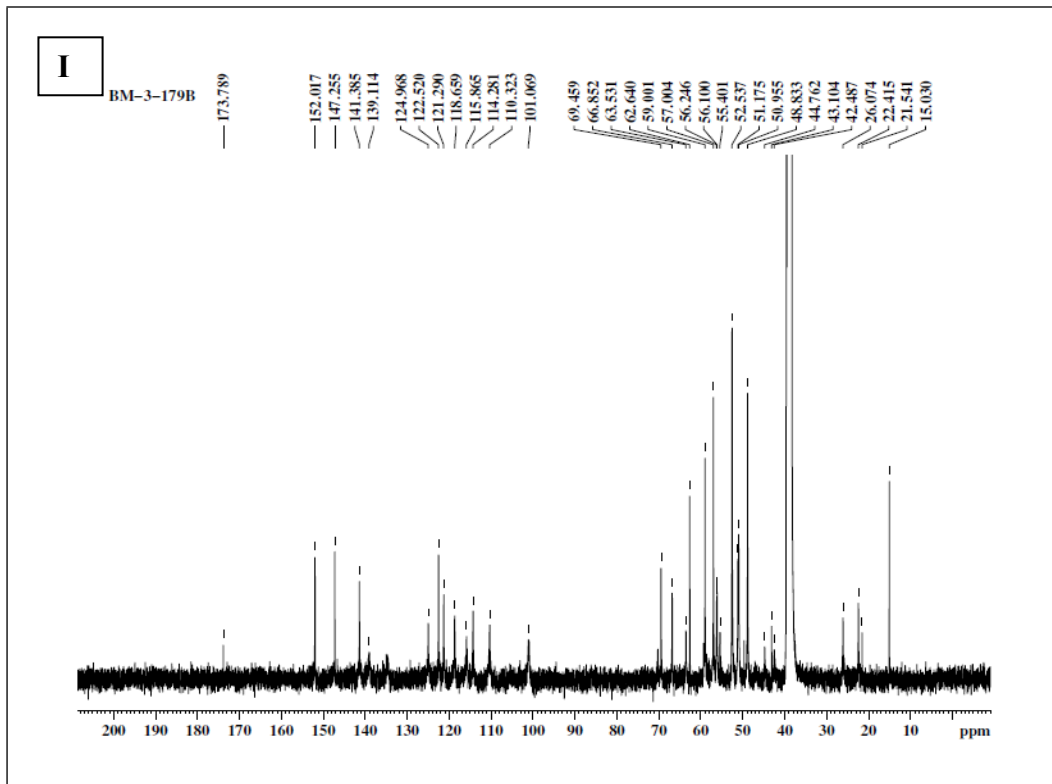


Fig. S24. (E)  $^1\text{H}$  NMR spectrum and (F)  $^{13}\text{C}$  NMR spectrum of **D2**.



**Fig. S25.** (G) Mass spectrum and (H)  $^1\text{H}$  NMR spectrum of **D4**.



**Fig. S26.** (I) <sup>13</sup>C NMR spectrum of **D4** and (J) Mass spectra of **M1**.



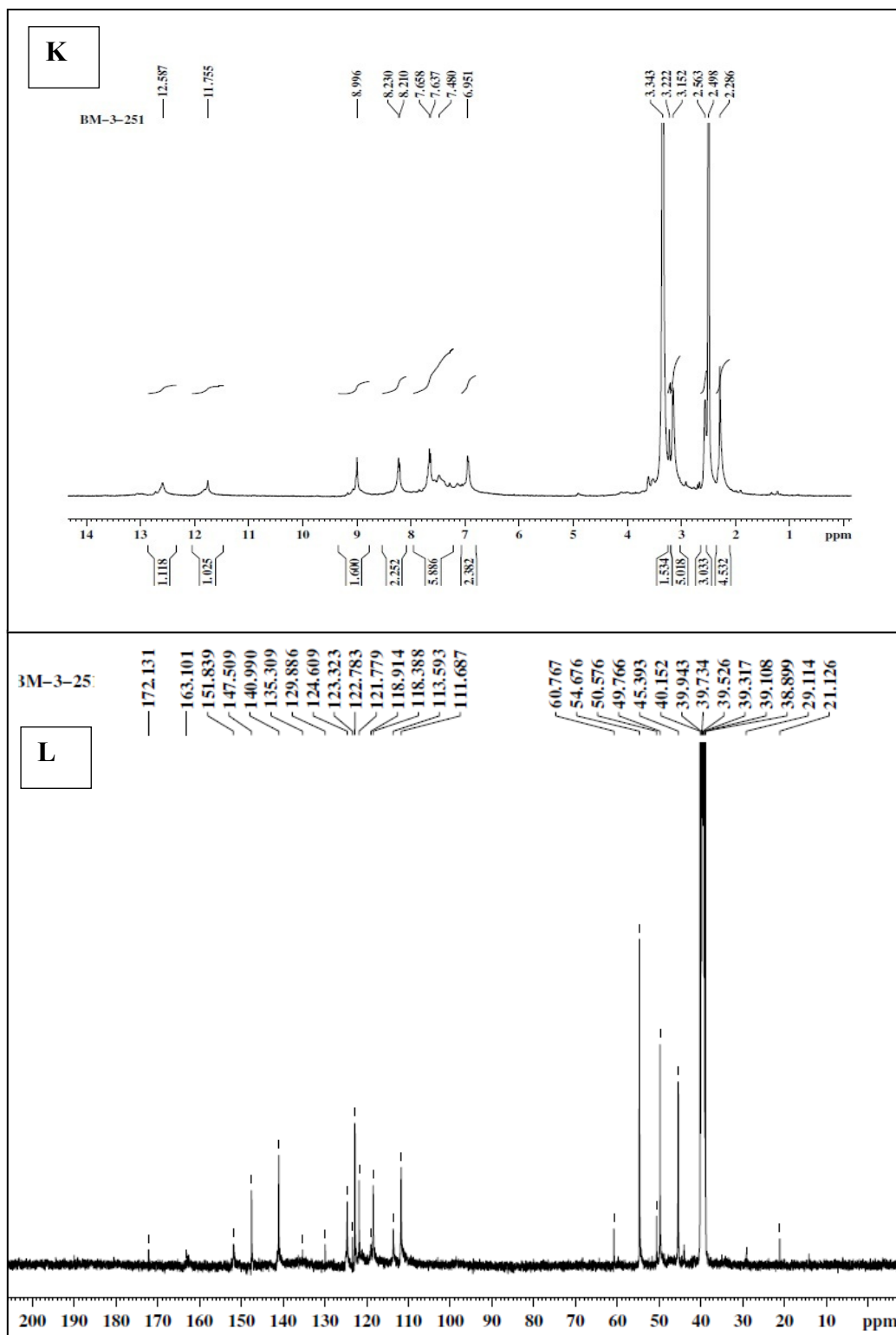
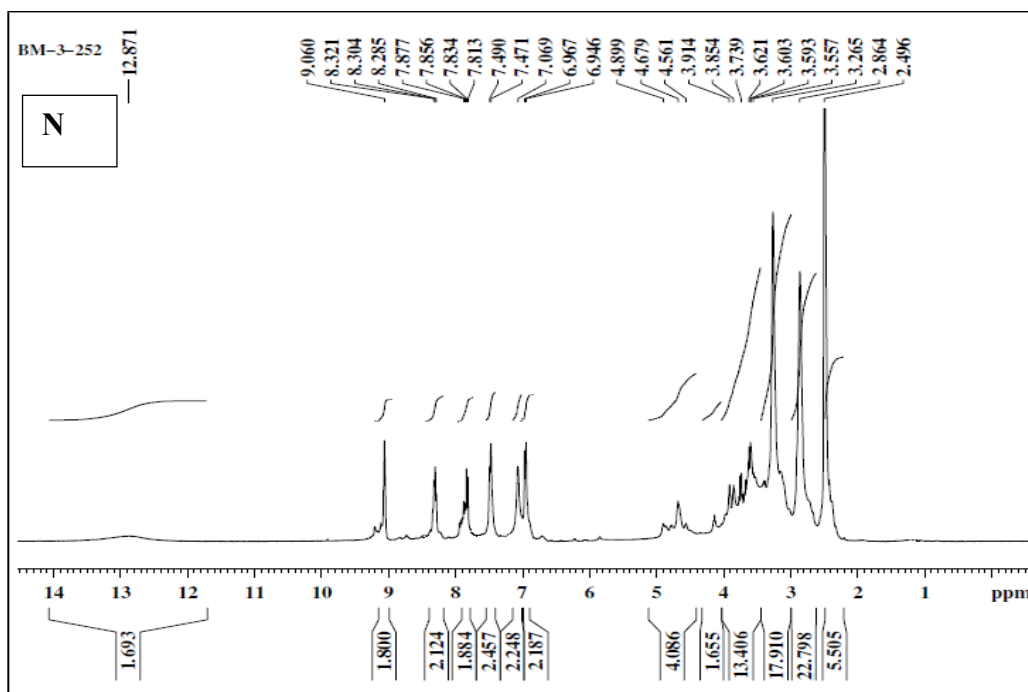
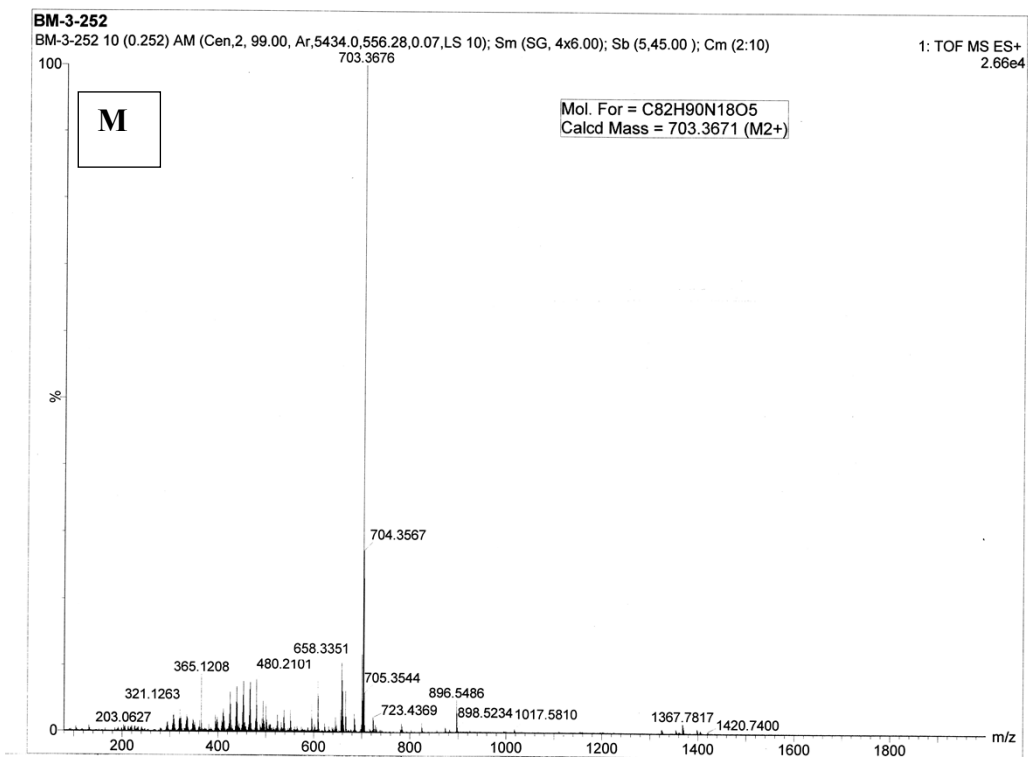


Fig. S27. (K)  $^1\text{H}$  NMR spectrum of **M1** and (L)  $^{13}\text{C}$  NMR spectrum of **M1**.



**Fig. S28.** (M) Mass spectrum of **D1** and (N) <sup>1</sup>H NMR spectrum of **D1**.

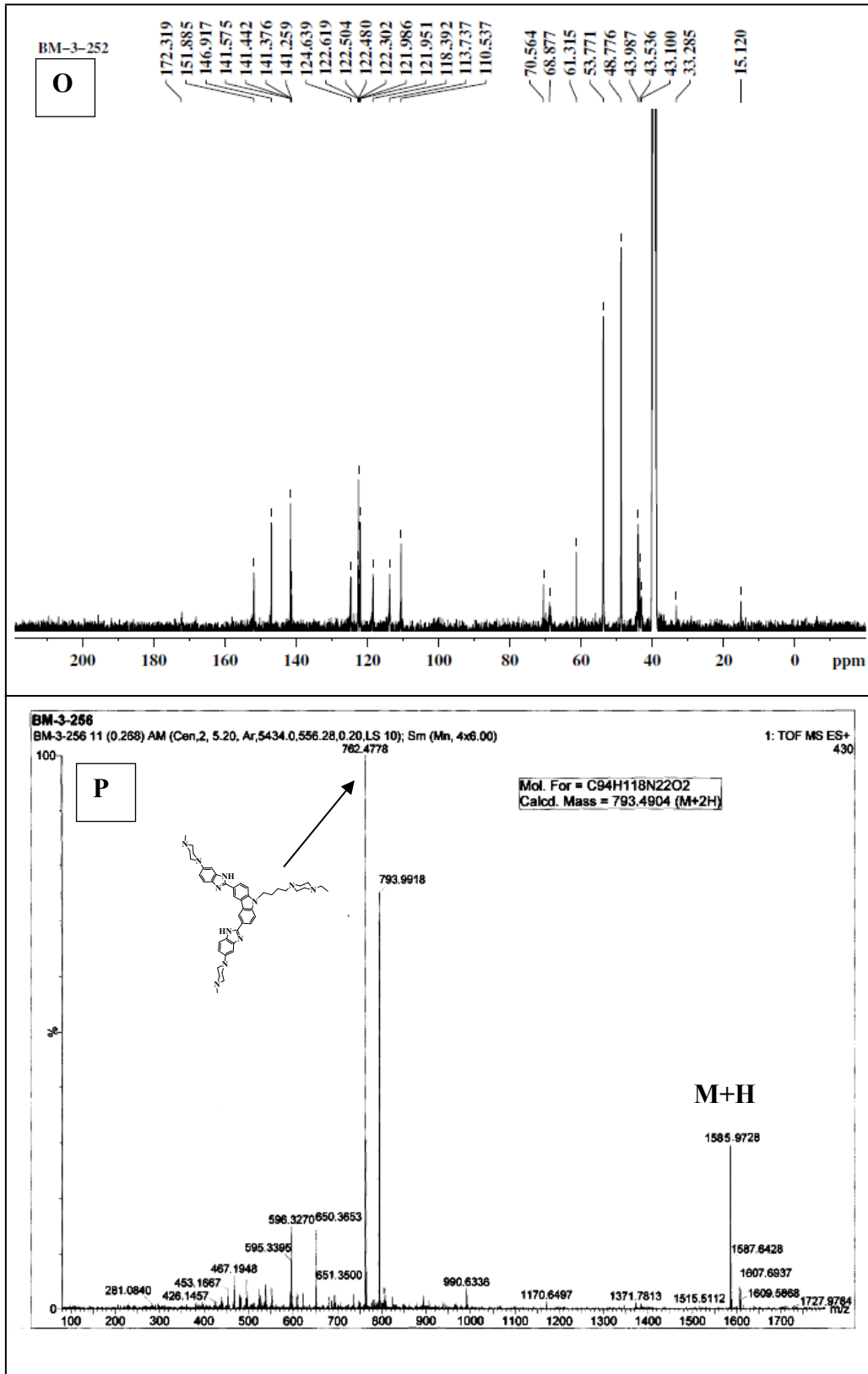


Fig. S29. (O)  $^{13}\text{C}$  NMR spectrum of **D1** and (P) Mass spectra of **D3**.

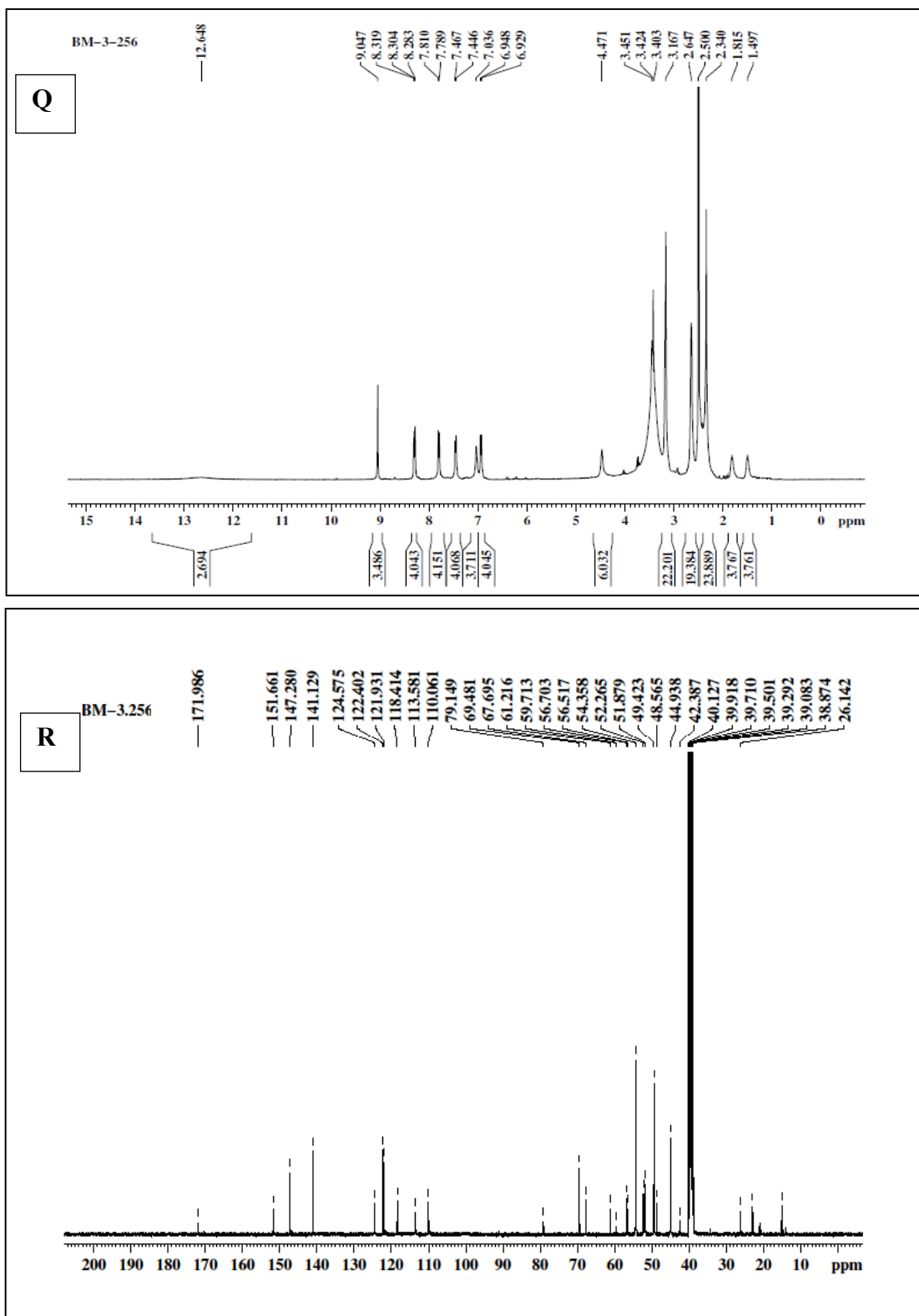


Fig. S30. (Q)  $^1\text{H}$  NMR spectrum of **D3** and (R)  $^{13}\text{C}$  NMR spectra of **D3**.