Supplementary

Direct evidence of mitochondrial G-quadruplex DNA by using fluorescent anti-cancer agents

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Methods and Materials

Chemicals

Synthesis of compounds: The syntheses of 9-substituted o-BMVC derivatives were shown in Scheme S1. All Compounds (2-4C-P, 2-6C-P, 2-8C-P, 2-9C-P, 2-12C-P) were synthesized from 3,6-dibromocarbazole (1, 2 g, 6.15 mM, Aldrich) through 9-position substituting by sodium hydride (0.295 g, 12.3 mM, Aldrich) in DMF (20 mL) under nitrogen gas condition. Dibromoalkanes (Br-R-Br, 100 mM) were then added and the mixtures were refluxed for 12 h. After cooling and quenching the excess sodium hydride with methanol, the solution was extracted with H2O/ethyl acetate twice and the organic layer dried by MgSO4. The products (2-4C-P, 2-6C-P, 2-8C-P, 2-9C-P, 2-12C-P) were collected by flash column (silica, hexane/ethyl acetate, 2/1, v/v).

 Piperidine attaches to compound 3 was conveniently obtained by way of refluxing 2 (5.0 mM) and piperidine (0.5 mL, Aldrich) in ethanol (20 mL) for 6 h with trace of sodium iodide. The solvent was evaporated in vacuum and the residue purified via column chromatography (silica,hexane/ethyl acetate, 1/2, v/v) to collect the yellow products (3-4C-P, 3-6C-P, 3-8C-P, 3-9C-P, 3-12C-P). Then, the reactants 3 coupled with 2-vinylpyridine at mixed powders of Palladium (II) acetate/ tri-o-tolylphosphine under the triethylamine / acetonitrile solvent pairs in high-pressure system. The bottle was sealed after bubbling 10 min with nitrogen. After keeping the system under ~105 °C for two days, the precipitant was collected and then extracted with H2O/CH2Cl2 twice. The CH2Cl2 layer were dried by MgSO4 and then filtered. Crude products were purified by flash column chromatography with acetone/n-hexane as eluent gradient to collect the compounds 4-4C-P, 4-6C-P, 4-8C-P, 4-9C-P and 4-12C-P. The orange-red powders (9-substituted o-BMVC derivatives, o-BMVC-4C-P, o-BMVC-6C-P, o-BMVC-8C-P, o-BMVC-9C-P and o-BMVC-12C-P) were collected in very good yield after refluxing the compound 4 with excess CH3I in DMF.

3,6-Dibromo-9-(1-bromobuty1) carbazole (2-4C-P) (Yield: 58%, Mw: 462.02), 1H NMR (400 MHz, CDCl3): 8.07 (d, J = 1.8Hz, 2H), 7.55 (dd, J = 1.8, 8.4Hz, 2H), 7.21 (d, J = 8.4Hz, 2H), 4.20 (t, J = 6.2Hz, 2H), 3.35 (t, J = 6.6Hz, 2H), 1.95 (m, 2H), 1.84 (m, 2H).

3,6-Dibromo-9-(1-(piperidin-1-yl)butyl) carbazole (3-4C-P) (Yield: 92%, Mw: 464.03), 1H NMR (400 MHz, CDCl3): 7.86 (d, J = 1.89Hz, 2H), 7.35 (dd, J = 1.8,
8.7 Hz, 2H), 7.03 (d, J = 8.7 Hz, 2H), 3.95 (t, J = 7.2 Hz, 2H), 2.15 (m, 4H), 2.10 (t, J = 7.5 Hz, 2H), 1.63 (t, J = 7.2 Hz, 2H), 1.44 (m, 4H), 1.36 (m, 2H), 1.32 (m, 2H).

3,6-Bis-(2-vinylpyridine)-9-(1-(piperidin-1-yl)butyl) carbazole (4-4C-P) (Yield: 71%, Mw: 512.69), $^1$H NMR (400 MHz, CDCl$_3$): 8.59 (d, J = 4.5 Hz, 4H), 8.29 (d, J = 1.2 Hz, 2H), 7.82 (d, J = 16 Hz, 2H), 7.70 (dd, J = 2.0, 8.7 Hz, 2H), 7.52 (dd, J = 2.0, 7.6 Hz, 2H), 7.43 (d, J = 9.0 Hz, 2H), 7.40 (d, J = 4.8 Hz, 2H), 7.26 (d, J = 16.2 Hz, 2H), 7.06 (d, J = 7.2 Hz, 2H), 4.35 (t, J = 7.2 Hz, 2H), 2.32 (m, 8H), 1.93 (t, J = 7.5 Hz, 2H), 1.56 (m, 4H), 1.44 (m, 2H). EA (C$_{35}$H$_{36}$N$_4$): calc. (obs %) C: 81.99 (81.84), H: 7.08 (7.06), N: 10.93 (10.90)

3,6-Bis-(1-methyl-2-vinylpyridinium iodide)-9-(1-(1-methyl-piperidinium iodide)butyl) carbazole (o-BMVC-4C-P): (Yield: 82%, Mw: 994.61), $^1$H NMR (400 MHz, DMSO-d6): δ: 8.98 (s, 2H), 8.89 (d, J = 6.4 Hz, 2H), 8.56 (d, J = 8.4 Hz, 2H), 8.46 (t, J = 8 Hz, 2H), 8.16 (d, J = 16 Hz, 2H), 8.03 (d, J = 8.8 Hz, 2H), 7.87 (t, 2H), 7.86 (d, J = 8.4 Hz, 2H), 7.67 (d, J = 16 Hz, 2H), 4.55 (t, 2H), 4.45 (s, 6H), 3.37 (m, 6H), 2.99 (s, 3H), 1.85 (m, 4H), 1.77 (m, 4H), 1.52 (m, 2H). $^{13}$C NMR (500 MHz, DMSO-d6): 144.65, 144.37, 142.67, 128.36, 127.41, 124.85, 124.81, 123.23, 122.31, 115.11, 111.09, 60.46, 46.97, 26.29, 21.38, 21.13, 19.76.

EA (C$_{39}$H$_{45}$I$_3$N$_4$•H$_2$O): calc. (obs %) C: 48.17 (48.09), H: 4.89 (4.86), N: 5.91 (5.88).

3,6-Dibromo-9-(1-bromohexyl) carbazole (2-6C-P) (Yield: 72%, Mw: 490.02), $^1$H NMR (400 MHz, CDCl$_3$): δ: 8.08 (d, J = 2.0 Hz, 2H), 7.52 (dd, J = 2.0, 8.8 Hz, 2H), 7.21 (d, J = 8.4 Hz, 2H), 4.21 (t, J = 7.2 Hz, 2H), 3.35 (t, J = 7.2 Hz, 2H), 1.81 (m, 4H), 1.35 (m, 2H), 1.27 (m, 2H).

3,6-Dibromo-9-(1-(piperidin-1-yl)hexyl) carbazole (3-6C-P) (Yield: 76%, Mw: 492.03), $^1$H NMR (400 MHz, CDCl$_3$): δ: 8.11 (d, J = 2.0 Hz, 2H), 7.53 (dd, J = 2.0, 8.4 Hz, 2H), 7.25 (d, J = 8.4 Hz, 2H), 4.21 (t, J = 7.6 Hz, 2H), 2.34 (m, 4H), 2.24 (t, J = 8.4 Hz, 2H), 1.80 (t, J = 8.4 Hz, 2H), 1.61 (m, 4H), 1.50 (m, 4H), 1.39 (m, 2H), 1.25 (m, 2H).

3,6-Bis-(2-vinylpyridine)-9-(1-(piperidin-1-yl)hexyl) carbazole (4-6C-P) (Yield: 68%, Mw: 560.69), $^1$H NMR (400 MHz, CDCl$_3$): δ: 8.60 (d, J =4.8 Hz, 2H), 8.29 (d, J =2 Hz, 2H), 7.82 (d, J =16 Hz, 2H), 7.73 (dd, J = 2.0, 8.8 Hz, 2H), 7.65 (dd, J = 2.0, 7.6 Hz, 2H), 7.43 (d, J = 8 Hz, 2H), 7.36 (d, J = 8.8 Hz, 2H), 7.24 (d, J = 16 Hz, 2H), 7.12 (d, J = 7.2 Hz, 2H), 4.25 (t, J = 7.2 Hz, 2H), 2.35 (m, 4H), 2.25(t, J = 7.6 Hz, 2H), 1.87 (t, J = 7.2 Hz, 2H), 1.57 (m, 2H), 1.51 (m, 2H), 1.40 (m, 2H), 1.31(m, 2H).
3,6-Bis-(1-methyl-2-vinylpyridinium iodide)-9-(1-(1-methyl-piperidinium iodide)hexyl) carbazole (o-BMVC-6C-P) (Yield: 90%, Mw: 966.56), \(^1\)H NMR (400 MHz, DMSO-d6) \(\delta\): 8.96 (s, 2H), 8.89 (d, \(J = 8.4\) Hz, 2H), 8.58 (d, \(J = 8.4\) Hz, 2H), 8.49 (t, \(J = 7.6\) Hz, 2H), 8.18 (d, \(J = 16\) Hz, 2H), 8.06 (d, \(J = 8.8\) Hz, 2H), 7.87 (t, \(J = 6.8\) Hz, 2H), 7.83 (d, \(J = 8.8\) Hz, 2H), 7.65 (d, \(J = 16\) Hz, 2H), 4.53 (t, \(J = 6.8\) Hz, 2H), 4.43 (s, 6H), 3.23 (m, 6H), 2.93 (s, 3H), 1.83 (m, 2H), 1.74 (m, 4H), 1.59 (m, 2H), 1.51 (m, 2H), 1.30 (m, 2H), 1.24 (m, 2H). \(^1^3\)C NMR (500 MHz, DMSO-d6): 153.30, 146.30, 144.65, 144.38, 142.68, 128.37, 127.42, 124.86, 124.82, 123.25, 122.30, 115.1, 111.1, 60.40, 46.97, 28.89, 26.45, 26.19, 21.39, 21.13, 19.73.

EA (C\(_{40}\)H\(_{49}\)I\(_{3}\)N\(_{4}\)•H\(_{2}\)O): calc. (obs %) C: 49.71 (49.69), H: 5.11 (5.08), N: 5.80 (5.74).

3,6-Dibromo-9-(1-bromooctyl) carbazole (2-8C-P) (Yield: 72%, Mw: 516.11), \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\): 8.08 (d, \(J = 2.0\) Hz, 2H), 7.50 (dd, \(J = 2.0, 8.8\) Hz, 2H), 7.23 (d, \(J = 8.4\) Hz, 2H), 4.21 (t, \(J = 7.2\) Hz, 2H), 3.35 (t, \(J = 7.2\) Hz, 2H), 1.81 (m, 4H), 1.34 (m, 2H), 1.27 (m, 2H), 1.22 (m, 4H).

3,6-Dibromo-9-(1-(piperidin-1-yl)octyl) carbazole (3-8C-P) (Yield: 76%, Mw: 520.34), \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\): 8.12 (d, \(J = 2.0\) Hz, 2H), 7.53 (dd, \(J = 2.0, 8.4\) Hz, 2H), 7.25 (d, \(J = 8.4\) Hz, 2H), 4.21 (t, \(J = 7.6\) Hz, 2H), 2.34 (m, 4H), 2.25 (t, \(J = 8.4\) Hz, 2H), 1.80 (t, \(J = 8.4\) Hz, 2H), 1.62 (m, 4H), 1.51 (m, 4H), 1.42 (m, 4H), 1.27 (m, 4H).

3,6-Bis-(2-vinylpyridine)-9-(1-(piperidin-1-yl)octyl) carbazole (4-8C-P) (Yield: 68%, Mw: 568.79), \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\): 8.63 (d, \(J = 4.8\) Hz, 2H), 8.32 (d, \(J = 2\) Hz, 2H), 7.84 (d, \(J = 16\) Hz, 2H), 7.75 (dd, \(J = 2.0, 8.8\) Hz, 2H), 7.67 (dd, \(J = 2.0, 7.6\) Hz, 2H), 7.44 (d, \(J = 8\) Hz, 2H), 7.38 (d, \(J = 8.8\) Hz, 2H), 7.24 (d, \(J = 16\) Hz, 2H), 7.14 (dd, \(J = 4.4, 7.2\) Hz, 2H), 4.28 (t, \(J = 7.2\) Hz, 2H), 2.35 (m, 4H), 2.25 (t, \(J = 7.6\) Hz, 2H), 1.88 (t, \(J = 7.2\) Hz, 2H), 1.58 (m, 4H), 1.42 (m, 4H), 1.35 (m, 4H), 1.26 (m, 4H).

3,6-Bis-(1-methyl-2-vinylpyridinium iodide)-9-(1-(1-methyl-piperidinium iodide)octyl) carbazole (o-BMVC-8C-P) (Yield: 90%, Mw: 994.61), \(^1\)H NMR (400 MHz, DMSO-d6) \(\delta\): 8.96 (s, 2H), 8.80 (d, \(J = 8.4\) Hz, 2H), 8.49 (td, \(J = 2.0, 7.6\) Hz, 2H), 8.19 (d, \(J = 16\) Hz, 2H), 8.04 (d, \(J = 8.8\) Hz, 2H), 7.87 (t, \(J = 6.8\) Hz, 2H), 7.82 (d, \(J = 8.8\) Hz, 2H), 7.69 (d, \(J = 16\) Hz, 2H), 4.52 (t, \(J = 6.8\) Hz, 2H), 4.45 (s, 6H), 3.24 (m, 6H), 2.95 (s, 3H), 1.83 (m, 2H), 1.74 (m, 4H), 1.59 (m, 2H), 1.50 (m, 2H), 1.30 (m, 4H), 1.24 (m, 4H). \(^1^3\)C NMR (500 MHz, DMSO-d6): 153.30, 146.29, 144.66, 144.38,
142.69, 128.36, 127.38, 124.85, 124.81, 123.22, 122.31, 115.06, 111.07, 60.40, 47.00, 29.09, 28.93, 26.84, 26.25, 21.35, 21.16, 19.74.

EA (C_{42}H_{35}I_{3}N_{4}•H_{2}O): calc. (obs %) C: 49.38 (49.37), H: 5.52 (5.50), N: 5.48 (5.44).

3,6-Dibromo-9-(1-bromononyl) carbazole (2-9C-P) (Yield: 70%, Mw: 530.13), ^1H NMR (400 MHz, CDCl_3) δ: 8.13 (d, J = 2.0Hz, 2H), 7.54 (dd, J = 2.0, 8.8 Hz, 2H), 7.26 (d, J = 8.4Hz, 2H), 4.20 (t, J = 7.2Hz, 2H), 3.35 (t, J = 7.2Hz, 2H), 1.80 (m, 4H), 1.35 (m, 2H), 1.28 (m, 4H), 1.22 (m, 4H).

3,6-Dibromo-9-(1-piperidin-1-yl)nonyl carbazole (3-9C-P) (Yield: 75%, Mw: 534.37), ^1H NMR (400 MHz, CDCl_3) δ: 8.13 (d, J = 2.0Hz, 2H), 7.54 (dd, J = 2.0, 8.8 Hz, 2H), 7.26 (d, J = 8.8Hz, 2H), 4.22 (t, J = 7.2Hz, 2H), 2.35 (m, 4H), 2.23 (t, J = 8.0Hz, 2H), 1.81 (t, J = 8.0Hz, 2H), 1.57 (m, 4H), 1.43 (m, 4H), 1.29 (m, 4H), 1.23 (m, 6H).

3,6-Bis-(2-vinylpyridine)-9-(1-(piperidin-1-yl)nonyl) carbazole (4-9C-P) (Yield: 64%, Mw: 582.82), ^1H NMR (400 MHz, CDCl_3) δ: 8.62 (d, J = 4.8Hz, 2H), 8.31 (s, J = 2 Hz, 2H), 7.83 (d, J = 16 Hz, 2H), 7.74 (dd, J = 2.0, 8.8 Hz, 2H), 7.67 (td, J = 2.0, 7.6 Hz, 2H), 7.44 (d, J = 8 Hz, 2H), 7.38 (d, J = 8.4 Hz, 2H), 7.23 (d, J = 16 Hz, 2H), 7.13 (dd, J = 4.4, 7.2 Hz, 2H), 4.28 (t, J = 7.2Hz, 2H), 2.35 (m, 4H), 2.23 (t, J = 8.0Hz, 2H), 1.81 (t, J = 8.0Hz, 2H), 1.57 (m, 4H), 1.43 (m, 4H), 1.29 (m, 4H), 1.23 (m, 6H).

3,6-Bis-(1-methyl-2-vinylpyridinium iodide)-9-(1-(1-methyl-piperidinium iodide)nonyl) carbazole (o-BMVC-9C-P) (Yield: 92%, Mw: 1008.64), ^1H NMR (400 MHz, DMSO-d6) δ: 8.89 (d, J = 6.4Hz, 2H), 8.83 (s, 2H), 8.58 (d, J = 8.4Hz, 2H), 8.49 (td, J = 2.0, 7.6 Hz, 2H), 8.18 (d, J = 16Hz, 2H), 8.05 (d, J = 8.8Hz, 2H), 7.87 (t, J = 6.8Hz, 2H), 7.82 (d, J = 8.8Hz, 2H), 7.67 (d, J = 16Hz, 2H), 4.52 (t, J = 6.8 Hz, 2H), 4.43 (s, 6H), 3.26 (m, 6H), 2.95 (s, 3H),1.83 (m, 2H), 1.75 (m, 4H), 1.59 (m, 2H), 1.50 (m, 2H), 1.30 (m, 4H), 1.25 (m, 6H). ^13C NMR (500 MHz, DMSO-d6): 153.30, 146.33, 144.65, 144.42, 142.67, 128.05, 127.39, 124.85, 123.15, 122.35, 115.08, 111.12, 60.41, 46.78, 29.29, 29.27, 29.15, 29.00, 26.95, 26.28, 21.40, 21.16, 19.74.

EA (C_{43}H_{57}I_{3}N_{4}•H_{2}O): calc. (obs %) C: 50.31 (50.26), H: 5.60 (5.64), N: 5.46 (5.41).

3,6-Dibromo-9-(1-bromododecyl) carbazole (2-12C-P) (Yield: 69 %, Mw: 572.21), ^1H NMR (400 MHz, CDCl_3) δ: 8.14 (d, J = 2.0Hz, 2H), 7.55 (dd, J = 2.0, 8.8 Hz, 2H), 7.27 (d, J = 8.4Hz, 2H), 4.24 (t, J = 7.2Hz, 2H), 3.39 (t, J = 6.8Hz, 2H), 1.82 (m, 4H),
1.39 (m, 2H), 1.25 (m, 14H).

3,6-Dibromo-9-(1-piperidin-yl)dodecyl carbazole (3-12C-P) (Yield: 68%, Mw: 576.45), \(^1\)H NMR (400MHz, CDCl\(_3\)) \(\delta\): 8.14 (d, \(J = 2.0\)Hz, 2H), 7.55 (dd, \(J = 2.0, 8.4\)Hz, 2H), 7.26 (d, \(J = 8.8\)Hz, 2H), 4.24 (t, \(J = 8.0\)Hz, 2H), 2.36 (m, 4H), 2.26 (t, \(J = 8.4\)Hz, 2H), 1.80 (t, \(J = 8.0\)Hz, 2H), 1.73 (m, 4H), 1.61 (m, 4H), 1.45 (m, 4H), 1.24 (m, 12H).

3,6-Dibromo-9-(1-piperidin-yl)dodecyl carbazole (3-12C-P) (Yield: 68%, Mw: 576.45), \(^1\)H NMR (400MHz, CDCl\(_3\)) \(\delta\): 8.14 (d, \(J = 2.0\)Hz, 2H), 7.55 (dd, \(J = 2.0, 8.4\)Hz, 2H), 7.26 (d, \(J = 8.8\)Hz, 2H), 4.24 (t, \(J = 8.0\)Hz, 2H), 2.36 (m, 4H), 2.26 (t, \(J = 8.4\)Hz, 2H), 1.80 (t, \(J = 8.0\)Hz, 2H), 1.73 (m, 4H), 1.61 (m, 4H), 1.45 (m, 4H), 1.24 (m, 12H).

3,6-Bis-(2-vinylpyridine)-9-(1-piperidin-yl)dodecyl carbazole (4-12C-P) (Yield: 62%, Mw: 624.9), \(^1\)H NMR (400 MHz, DMSO-d6) \(\delta\): 8.62 (d, \(J = 4.8\)Hz, 2H), 8.31 (d, \(J = 2\) Hz, 2H), 7.83 (d, \(J = 16\) Hz, 2H), 7.74 (dd, \(J = 2.0, 8.8\)Hz, 2H), 7.67 (td, \(J = 2.0, 7.6\)Hz, 2H), 7.44 (d, \(J = 8\) Hz, 2H), 7.38 (d, \(J = 8.4\) Hz, 2H), 7.23 (d, \(J = 16\) Hz, 2H), 7.13 (dd, \(J = 4.4, 7.2\) Hz, 2H), 4.28 (t, \(J = 8.0\)Hz, 2H), 2.36 (m, 4H), 2.26 (t, \(J = 8.4\)Hz, 2H), 1.80 (t, \(J = 8.0\)Hz, 2H), 1.73 (m, 4H), 1.61 (m, 4H), 1.45 (m, 4H), 1.24 (m, 12H).

3,6-Bis-(1-methyl-2-vinylpyridium iodide)-9-(1-methyl-Piperidinium iodide)dodecyl carbazole (o-BMVC-12C-P) (Yield: 92%, Mw: 1050.72), \(^1\)H NMR (400 MHz, DMSO-d6) \(\delta\): 8.86 (d, \(J = 6.4\)Hz, 2H), 8.81 (s, 2H), 8.58 (d, \(J = 8.4\)Hz, 2H), 8.49 (td, \(J = 2.0, 7.6\) Hz, 2H), 8.19 (d, \(J = 16\)Hz, 2H), 8.05 (d, \(J = 8.8\)Hz, 2H), 7.87 (t, \(J = 6.8\)Hz, 2H), 7.82 (d, \(J = 8.8\)Hz, 2H), 7.67 (d, \(J = 16\)Hz, 2H), 4.52 (t, \(J = 6.8\) Hz, 2H), 4.44 (s, 6H), 3.26 (m, 6H), 2.95 (s, 3H), 1.81 (m, 2H), 1.75 (m, 4H), 1.61 (m, 2H), 1.51 (m, 2H), 1.28 (m, 4H), 1.20 (m, 12H). \(^{13}\)C NMR (500 MHz, DMSO-d6): 153.30, 146.33, 144.65, 144.42, 142.66, 127.93, 127.39, 124.84, 123.12, 122.37, 115.07, 111.14, 60.42, 46.70, 29.53, 29.49, 29.41, 29.33, 29.14, 29.03, 26.35, 21.42, 21.18, 19.73.

EA (C\(_{46}H_{65}I_{3}N_{4}•H_{2}O\)): calc. (obs %) C: 50.84 (50.80), H: 6.03 (6.0), N: 5.16 (5.13).

These compounds were dissolved in DMSO for stock solution 1 mM and stored at 4 \(^\circ\)C and light protection before use. These compounds are stable for several months.
Scheme S1: The syntheses of 9-substituted o-BMVC derivatives

(1) NaH/THF and BrBr, N_2 (ii) NaI/EtOH, piperidin, refluxes under ethanol.
(iii) Pd(OAc)_2 / (o-tol)_3P, 2-vinylpyridine,Et_3N / MeCN, N_2 (iv) MeI / DMF

Cell lines

CL1-0, human lung carcinoma cell line was kindly provided by Prof. P.-C. Yang (National Taiwan University). Human normal lung fibroblast MRC-5, human normal foreskin fibroblast BJ1, Human normal lung fibroblast IMR-90, human cervical adenocarcinoma HeLa, human lung carcinoma H1299 and human malignant melanoma A375 were obtained from American Type Culture Collection (ATCC). CL1-0 and H1299 were grown in RPMI1640 medium supplied with 10% fetal bovine serum (FBS) and 1% antibiotics. A375 was grown in DMEM medium supplied with
10 % FBS and 1 % antibiotics. HeLa, MRC-5, BJ1 and IMR-90 were grown in MEM medium supplied with 10 % FBS and 1 % antibiotics. Antibiotics concentration was 100 U/mL penicillin and streptomycin.

**Cell viability analysis**

Cells were grown in 96-well plates (2×10³ cells per well) in a 5% CO₂ incubator at 37 °C. To examine the short-term cytotoxic effect, cells were then treated with different concentrations (0.3125 to 20 μM) of BMVC-12C-P for 72 h. The cytotoxicity was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and analyzed by automatic microplate reader (Multiskan EX, Thermo electron corporation, Finland) at 595 nm. The error bars were calculated based on three independent experiments.

For colony formation, cells were seeded into 6 cm tissue culture plate with 1×10⁵ cells in each plate for 24 h, then each desired compound at different concentrations were added for 5 d. After that, cells were washed twice with PBS and fixed by 4 % formaldehyde for 2 h at room temperature. After fixation, cells were washed twice with PBS and stained by 0.01 % crystal violet (Sigma, USA) in 25 % methanol for 24 h. Cells were washed twice with PBS and counted.

**Population doubling**

Cells were treated with 0.5 μM BMVC-12C-P or DMSO (drug vehicle control) and grown in 6 cm tissue culture plates at 5×10⁵ cells per plate for 3 or 4 d and then trypsinized and counted. Each time, 5×10⁵ cells were replaced in the new culture plate with fresh BMVC derivatives compounds or an equivalent volume of DMSO. The experiments were continued until there were less than 5×10⁵ cells available for reseeding. The results were obtained from at least three independent experiments.
Flow cytometry

Cells were incubated with 1 μM o-BMVC derivatives for 24 h. Cells were collected by centrifugation and were resuspended in 300 μl PBS, adjusted to a density of approximately $1 \times 10^6$ cells per ml, and analyzed by flow cytometry (BD, FACSCalibur). The mean fluorescence intensity at 564-606 nm was measured upon excitation at 488 nm.

DNA preparation

All oligonucleotides were purchased from Bio Basic Inc. (Ontario, Canada) and used without further purification. The oligonucleotides were dissolved in 10 mM Tris-HCl (pH 7.5), followed by heat-denaturation at 95 °C for 10 min and cooled slowly at 1 °C/min to room temperature. The annealed oligonucleotides were stored at 4 °C over night before use. The DNA concentrations were determined by ultraviolet-visible absorption nanophotometer (Implen, Germany).

Absorption and fluorescence

Absorption spectra were taken on a ultraviolet-visible spectrophotometer (HELIOS α, Thermo Fisher Scientific, USA), and fluorescence spectra were recorded on a spectrofluorometer (LS-55, PerkinElmer, USA) with a 2 nm band-pass in a 1 cm cell length of quartz cuvette at room temperature.

Circular dichroism

The CD spectra were averaged 10 scans on a J-815 spectropolarimeter (Jasco, Japan), with a 2 nm bandwidth at 50 nm/min scan speed, and a 0.2 nm step resolution. The CD spectra were measured under N₂ over the range of 210-350 nm to ascertain the G4
structures. The melting curves were recorded at a set temperature controlled by a peltier thermal coupler chamber (PFD-425S/15, Jasco, Japan).

**NMR spectroscopy**

Experiments were performed using Bruker AVIII 800 MHz spectrometer equipped with a cryoprobe at 25 °C. The one-dimensional imino proton NMR spectra were measured in H$_2$O/D$_2$O (90%/10%) using a WATERGATE pulsed sequence for solvent suppression. The DNA samples were prepared at a strand concentration of 100 μM and specific salt condition with an internal reference of DSS (0.1 mM sodium 4,4-dimethyl-4-silapentane-1-sulfonate).

**Polyacrylamide gel electrophoresis (PAGE)**

The PAGE was conducted using 20% Polyacrylamide gels. Electrophoresis of the gels was carried out at 25 mA for 3 h at 4 °C. They were then photographed under ultraviolet light at 254 nm using a digital camera.

**Cyclosporin A (CsA) test**

For this experiment, HeLa cells were seeded in 12-well plated with coverslip incubated with 1 μM Cyclosporin A (CsA, Sigma, USA) for 24 h to block the mitochondria inner membrane permeability transition pore. After discarded medium, 5 μM BMVC-12C-P was added to incubate with cells for 4 h. Finally, cells were stained with Mitotracker Red and observed by Leica TC5 SP8 confocal microscopy.
Absorption and fluorescence of 12 μM BMVC-12C-P and its complexes with 6 μM each of LD12, calf thymus, mt6363, mt9438, mt8095, mt1015, and mt16250 in 150 mM K⁺ solution. The absorption maximum of BMVC-12C-P at ~450 nm is red-shifted to ~465 nm upon interaction with duplex DNA and further red-shifted to ~475 nm in the presence of G4s. Of importance is that the fluorescence of BMVC-12C-P increases ~70 times upon interaction with duplex DNA and 20-50 times in the presence of G4s.
The mean fluorescence intensity of 1 μM BMVC-12C-P incubated with various cancer and normal cell lines for 24 h measured by flow cytometry.
Colony formation assays of (a) BMVC-12C-P and (b) o-BMVC-12C-P on CL1-0 and HeLa cancer cell lines and MRC-5 and BJ1 normal cell lines. Cells (1x10^3) were seeded into each 6 cm culture plate for 24 h, and then added different concentrations of these molecules to corresponding plate for additional 5 days. The data show that o-BMVC-12C-P is slightly better anticancer agent than BMVC-12C-P.
Confocal images of HeLa cells incubated with 5 µM o-BMVC derivatives for 24 h. The images were taken under the same condition. Scale bar is 25 µm.
Absorption extinction coefficient and fluorescence quantum yield of each $o$-BMVC derivative and its interaction with LD12, mt6363, and mt9438 DNA.
Figure S6

(a) Molar ellipticity $[\theta]$ x $10^{-5}$ vs. Wavelength (nm) for mt377, tris, +150K(1h), and +150K(O/N).

(b) Molar ellipticity $[\theta]$ x $10^{-5}$ vs. Wavelength (nm) for mt714, tris, +150K(1h), and +150K(O/N).

(c) Molar ellipticity $[\theta]$ x $10^{-5}$ vs. Wavelength (nm) for mt1015, tris, +150K(1h), and +150K(O/N).

(d) Molar ellipticity $[\theta]$ x $10^{-5}$ vs. Wavelength (nm) for mt8095, tris, +150K(1h), and +150K(O/N).

(e) Molar ellipticity $[\theta]$ x $10^{-5}$ vs. Wavelength (nm) for mt10252, tris, +150K(1h), and +150K(O/N).

(f) Molar ellipticity $[\theta]$ x $10^{-5}$ vs. Wavelength (nm) for mt12086, tris, +150K(1h), and +150K(O/N).
The CD spectra of 4 μM mt377, mt714, mt1015, mt8095, mt10252, mt12086, mt15653, mt16250 (a-h) annealed in 10 mM Tris buffer, after the addition of 150 mM K\(^+\) for 1 h, and overnight.
Aromatic and methyl NMR spectra of 100 μM mt6363 annealed in 10 mM Tris buffer, after the addition of 150 mM K⁺ for 1 h, and overnight.
Figure S8

(a) The CD spectra of 4 μM mt9438 and (b) NMR spectra of 100 μM mt9438 annealed in 10 mM Tris buffer, after the addition of 150 mM K⁺ for 1 h, and overnight. (c) UV shadowing of gel assay of (1) LD12 + o-BMVC-12C-P, (2) mt9438 + o-BMVC-12C-P, (3) LD12 + mt9438 + o-BMVC-12C-P, (4) LD12 + o-BMVC-6C-P, (5) mt9438 + o-BMVC-6C-P, (6) LD12 + mt9438 + o-BMVC-6C-P (750 pmol per well). (d) The melting curves monitored at 265 nm of 4 μM mt9438 and their complexes with 8 μM o-BMVC-6C-P and o-BMVC-12C-P in 20 mM K⁺ solution.
Fluorescence titration of 1 µM o-BMVC-12C-P (a) and o-BMVC-6C-P (b) by adding mt9438 G4 and LD12 duplex DNA from 0.05 to 0.8 µM in 150 mM K⁺ solution. The inset shows the binding plots of $\gamma$ versus $C_t$ for the titration based on the multiple-equivalent-site model [Methods Enzymol. 340, 3 (2001)]. The binding ratio $\gamma$ is defined as $C_b/C_{DNA}$, where $C_t$, $C_b$, and $C_{DNA}$ are the molar concentrations of free ligand, bound ligand, and DNA, respectively. The difference between $C_t$ and $C_b$ gives the magnitude of $C_t$, where $C_t$ is the total concentration of ligand. Using $\gamma = nKC_t/(1+KC_t)$, one can obtain the equilibrium binding parameter K and the average number of ligands bound per each DNA structure.
The histograms of the fluorescence decay time of 0.2 μM α-BMVC-12C-P upon interaction with mt1015 G4, mt9438 G4, HT23 G4, and LD12 duplex DNA.
RT-PCR was used to evaluate the suppression of ND3 and COX I expression by TMPyP4. HeLa cells were incubated with TMPyP4 for 3 d. Here β-actin and a non-G4 forming gene sequence were used as controls.
### Table S1

Primers and sequences of mitochondria DNA studied in RT-PCR and PCR stop assay.

| Sequence (5’→3’) | Abbreviations | Location |
|------------------|---------------|----------|
| 5’-AAAATCCACCCCTTACGAGT | mtND3-F | 10139-10619 |
| 5’-TATTGGCTAAGAGGGGAGTGG | mtND3-R | |
| 5’-GCCTGACTGGCATTTGTATT | mtCOXI-F | 6899-7439 |
| 5’-GGTTCGATTCCTCTTCTTTT | mtCOXI-R | |
| 5’-ATCATAGCAGGCAGTTGAGG | mtND2-F | 4919-5399 |
| 5’-GGGTGGGTTTTGTATGTTCA | mtND2-R | |
| 5’-ATCATAGCAGGCAGTTGAGG | mtND5-F | 13019-13559 |
| 5’-GGGTGGGTTTTGTATGTTCA | mtND5-R | |
| 5’-CCCAGCAATCTCAATTACA | mtND6-F | 14159-14639 |
| 5’-CCCAGCAATCTCAATTACA | mtND6R | |
| 5’-CCCTGTATGCCCCCTTTCTCAA | mtCOXII-F | 7679-8159 |
| 5’-CCCTGTATGCCCCCTTTCTCAA | mtCOXII-R | |
| 5’-TACTACCGTATGCCCCACCA | mtATP8-F | 8379-8560 |
| 5’-GGGGCAATGAATGAAGCGAA | mtATP8-R | |
| 5’-CCCATTCCGATAAAAATCACC | mtCYT B-F | 15383-15876 |
| 5’-CCCATCCGATAAAAATCACC | mtCYT B-R | |
| 5’-GCACTCCTCCTCACCATT | mt nonG4-F | 9599-10179 |
| 5’-GCACTCCTCCTCACCATT | mt nonG4-R | |
| 5’-GTGGGCGGCCAGAGGCACCA | β-actin-F | Internal control |
| 5’-GTGGGCGGCCAGAGGCACCA | β-actin-R | |
| 5’-GGCGTAGGTGTTGGTCTAGGG | mt9438 | 7113-7132 |
| 5’-GGCGTAGGTGTTGGTCTAGGG | mt9438-mut | |
| 5’-TGTCCTAATGTGCACGCAGATTTTC | mt9438-R | |
| 5’-AGGGACCGCGGGGGGATAGGGT | mt6363 | 10182-10207 |
| 5’-AGGGACCGCGGGGGGATAGGGT | mt6363-mut | |
| 5’-CGAGGCTGCGCTTCGACCCTAT | mt6363-R | |